THE BIOGEOGRAPHY, SYSTEMATICS AND CONSERVATION OF PHREATOICIDEAN ISOPODS IN SOUTH AFRICA

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Declaration

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and has not previously in its entirety, or in part, been submitted at any university for a degree. All references and help received have been fully acknowledged.

Signature:…….. Date:……

Abstract

Historically, isopods of the suborder Phreatoicidea were thought to be represented in southern Africa by four species belonging to the endemic genus *Mesamphisopus*. This taxonomy was based on poor collections and the extent of variation among and within populations were poorly understood. In the present study, intensive sampling was undertaken to determine the diversity, distribution and biogeography of phreatoicidean isopods within South Africa. Analyses of allozyme data and mitochondrial DNA sequences (from the 12S rRNA and protein-coding COI genes) were used to examine differentiation among populations, extricate species boundaries (in combination with morphometric and morphological data) and to elucidate the evolutionary relationships among taxa. Additionally, conservation units were identified among the sampled populations and conservation threats highlighted.

First, genetic and morphometric differentiation was examined among populations identified morphologically as *M. capensis*. Collection localities spanned two mountainous regions in the Western Cape and these were separated by a coastal plain remnant. Five morphometrically and genetically distinct species were identified. These taxa are also geographically partitioned in two regions, which were regarded as Evolutionarily Significant Units. Differentiation among populations of the two regions, and similar patterns in other taxa, was attributed to Cenozoic sea-level fluctuations.

Second, populations, variably assigned to *M. abbreviatus* or *M. depressus*, were examined to determine whether they were conspecific. A large geographic area was sampled to account for intraspecific differentiation. Limited morphometric discrepancies were observed, with individual populations being either similar to the *M. abbreviatus* or the *M. depressus* syntypes. Genetic support for the recognition of a cryptic species complex among the sampled populations was equivocal. Substantial genetic differentiation and a lack of gene flow were observed among all populations. Clear patterns of isolation by distance were not detected, and genetic structure appeared to be unrelated to geography or drainage systems. The mosaic pattern of relatedness among populations was best explained by stochastic demographic processes, such as extinction-recolonization events or population bottlenecks.

Thirdly, detailed taxonomic descriptions and illustrations of six new species, identified genetically and morphometrically among the populations included in the above analyses, were provided. These species were largely distinguished from each other, and the four original species, using a combination of setation, mouthpart, pleopod and uropod features.

Lastly, phylogenetic relationships among all ten recognized *Mesamphisopus* species, and an additional unresolved group of populations, were examined. MtDNA data partitions and a recoded allele frequency matrix were analysed independently and in combination. Topologies indicated unrecognized species-diversity within an unresolved group of populations. Evolutionary relationships, the identification of six biogeographic centres, and the dating of divergences using a relaxed Bayesian clock suggested that differentiation and speciation within *Mesamphisopus* was largely allopatric or vicariant and driven by Mesozoic sea-level and climate change. Chance long distance dispersal events would, in turn, explain spurious phylogenetic relationships and distributions.

This study contributes significantly to the understanding of the diversity and the conservation of the little-studied southern African freshwater invertebrates. Moreover, this study is the first to investigate genetic and morphometric differentiation, and phylogenetic relationships, below the generic level within the Phreatoicidea; thus establishing a methodological and theoretical framework for species delineation and the accurate determination of biodiversity within individual phreatoicidean genera.

Uittreksel

Isopoda van die suborder Phreatoicidea was histories in suidelike Afrika verteenwoordig deur vier spesies wat almal aan die endemiese genus *Mesamphisopus* behoort. Hierdie taksonomie is op ’n beperkte hoeveelheid versamelings gebaseer en die omvang van variasie tussen (en binne) bevolkings was swak verstaan. In die huidige studie is ekstensiewe versameling onderneem om die verspreiding, diversiteit asook biogeografie van dié Isopoda in Suid Afrika te bepaal. Analises van allosiem data en mitokondriale DNS volgorderbepalings (van die 12S rRNS en die proteïenkoderende COI geen) was gebruik om differensiasie tussen bevolkings te ondersoek, om (in kombinasie met morfometriese en morfologiese data) spesiesgrense te bepaal asook om die evolusionêre-verwantskappe tussen taksa te definieer. Benewens word bewaringseenhede binne die studie-bevolkings geïndentifiseer en moontlike bedreigings uitgelig.

Eerstens is genetiese en morfometriese differensiasie tussen bevolkings, wat as *M. capensis* geïdentifiseer is, ondersoek. Versamelingslokaliteite was versprei oor twee bergagtige streke in die Weskaap wat geskei word deur ’n voormalige kusvlakte. Vyf morfometries- en geneties-afsonderlike spesies is geïdentifiseer. Dié taksa was geografies geskei tot die twee streke, wat elk as ’n Evolusionêre Beduidende Eenheid (ESU) gesien kan word. Differensiasie tussen populasies van die twee streke en vergelykbare patrone binne ander taksa word aan Cenosoïese seevlak veranderings toegeskryf.

Tweedens is bevolkings wat as *M. abbreviatus* óf as *M. depressus* geïdentifiseer kan word ondersoek om te bepaal of hulle konspesifiek is. Bevolkings is oor ’n groot geografiese gebied versamel om intraspesifieke variasie in aanmerking te neem. Beperkte morfometriese verskille is waargeneem – enkel bevolkings was morfometries identies aan of die *M. abbreviatus* of die *M. depressus* sintipes. Genetiese getuienis vir die herkenning van ’n kriptiese spesieskompleks was dubbelsinnig. Bevolkings is gekenmerk deur merkbare genetiese differensiasie en die afwesigheid van geenvloei. Duidelike bewys van isolasie-met-afstand was nie waargeneem nie en genetiese struktuur was nie verwant aan geografiese ligging of riviersisteme nie. Die mosaïese patroon van verwantskappe is moontlik teweeg gebring deur stogastiese demografiese prosesse soos uitsterwing en hervestiging of deur afnames in bevolkingsgrootte.

Derdens is omvattende taksnomiese beskrywings en illustrasies van ses nuwe spesies wat deur bogenoemde analises geneties en morfometries uitgelig was, verskaf. Dié spesies is van mekaar, asook die ander vier spesies onderskeibaar deur ’n kombinasie van setasie-, monddeel-, pleiopoot- en uropooteienskappe.

Laastens is die filogenetiese verwantskappe tussen al tien herkende *Mesamphisopus*-spesies en ’n groep bevolkings waarvan verhoudings onseker was, ondersoek. MtDNS datastelle en ’n hergekodeerde alleelfrekwensie matriks is afsonderlike en in kombinasie geanaliseer. Topologië het onherkende spesies-vlak diversiteit binne die bogenoemde groep bevolkings aangedui. Evolusionêre verwantskappe, die herkenning van ses biogeografiese gebiede, en die bepaling van tye van divergensie (d.m.v. ’n ontspanne Bayesiaanse molekulêre klok) het aangetoon dat spesiasie binne *Mesamphisopus* grootliks allopatries was en deur Mesosoïese seevlak- en klimaatsveranderings teweeg gebring is. Toevallige lang-aftstand verspreiding kon dan eienaardige filogenetiese verhoudings en verspreidings verklaar.

Dié studie lewer ’n wesenlike bydrae tot die kennis van die diversiteit en tot die bewaring van die onbestudeerde Suid Afrikaanse varswater ongewerweldes. Daarenbowe, is hierdie studie die eerste om genetiese en morfometriese differensiasie benede die genusvlak binne die Phreatoicidea te ondersoek; sodoende word die metodologiese en teoretiese raamwerk vir die herkenning van spesies en die akkurate beskrywing van diversiteit binne afsonderlike genera van die Phreatoicidea geskep.

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Publications emanating from thesis

Of the chapters presented in this thesis, only Chapter 2 (dealing with differentiation and the delineation of species within the *Mesamphisopus capensis* complex) has yet been submitted for publication. This manuscript was accepted and has appeared in the February 2004 issue of in the *Biological Journal of the Linnean Society*. A reprint of this manuscript is included in the appendices (Appendix 12).

The individual chapters of this thesis have been prepared largely as stand-alone manuscripts to enable rapid submission for publication (following submission of the thesis). As a result, a degree of repetition, particularly with regards to taxonomic/systematic background provided in the individual chapter introductions, has been retained.

Chapter 1: General Introduction

South Africa is renowned worldwide for its biological diversity. Between 2 and 8% of the world's terrestrial mammal, reptilian, avian and amphibian fauna, as well as eight percent of all vascular plants, are supported within the borders of South Africa (0.8% of the total global land area), making it the third-most biologically rich country in the world (Siegfried, 1989; Gibbons *et al.*, 1999).

The Western Cape Province of South Africa encompasses both the Cape Floristic Region (CFR) and the Succulent Karoo biome. The CFR is the most species rich region in South Africa, owing to its remarkable floral diversity (Siegfried, 1989). Globally, this region has the highest plant species diversity at the subcontinental level (Taylor, 1978; Cowling *et al.*, 1989). This diversity is unsurpassed in regions of comparable size and climate, and is only matched in tropical forests (Cowling *et al.*, 1989; Hilton-Taylor and Le Roux, 1989; Cowling, Holmes and Rebelo, 1992). Levels of endemism are also remarkable (Taylor, 1978), with approximately seventy percent of species thought to be endemic (Hilton-Taylor and Le Roux, 1989; Rebelo, 1992; Wishart and Day, 2002). The Karoo biome has an unparalleled diversity of succulent plants, of which nearly thirty percent are endemic (Hilton-Taylor and Le Roux, 1989).

Although discrepancies exist in our knowledge of different taxonomic groups within the Western Cape (Picker and Samways, 1996), the floral diversity and endemism does not appear to be reflected in all faunal groups (Hilton-Taylor and Le Roux, 1989; Rebelo, 1992). For example, levels of endemism are high for the freshwater fish and amphibian fauna, but

low for the reptilian, avian and mammalian fauna (Jarvis, 1979; King and Day, 1979; O'Keeffe *et al.*, 1989; Rebelo, 1992). The CFR does, however, appear to have a very diverse invertebrate fauna with many endemics (Hilton-Taylor and Le Roux, 1989; Siegfried, 1989). Indeed, levels of endemism comparable to the floral endemism are to be found within terrestrial and freshwater invertebrate taxa (Wishart and Day, 2002). High levels of endemism are found among the insects (particularly endophagous insects, flies, bees, butterflies and beetles) and molluscs (Jarvis, 1979; Siegfried, 1989; Rebelo, 1992; Wright and Samways, 2000), while Wishart and Day (2002) reported that 64% of aquatic invertebrate species are endemic to the region.

Among the Crustacea specifically, groups show varying levels of diversity and endemism. Levels of endemism of freshwater and terrestrial crustacean species exceed those recorded in the marine environment and vary between < 10% and 100%, with a mean of 51% of freshwater species being endemic to South Africa as a whole (Griffiths, 1999). Likewise, within the Western Cape, to which distribution of freshwater phreatoicidean isopods is apparently restricted (Barnard, 1927, 1940), groups of considerable diversity and endemism can be identified. Of 25 species of amphipod belonging to the family Paramelitidae Bousfield, 1973, only one is known to occur outside the Western Cape (Griffiths, 1981; Stewart and Griffiths, 1995). Five species of freshwater crab (family Potamonautidae Bott, 1970) are found in rivers in the Western Cape, four of these appearing to be endemic (Barnard, 1935, 1950; Stewart, 1997a, b; Daniels, Stewart and Gibbons, 1998a; Daniels, Stewart and Burmeister, 2001). Simultaneously, there are groups, such as the freshwater shrimp of the genera *Macrobrachium* Bate, 1868 (family Palaemonidae Rafinesque, 1815) and *Caridina* H. Milne Edwards, 1837 (family Atyidae De Haan, 1849), that have no representatives in the Western Cape, although seven species and four species, respectively,

have been recorded for each genus from the rest of South Africa (Kensley, 1981). There appears to be a paucity of collection and distribution data for freshwater isopods and, consequently, levels of diversity and endemism are poorly understood. Of the approximately 17 freshwater species documented from South Africa, 95% of which are endemic to the country, seven species (including the members of the Phreatoicidea) appear to be restricted to the Western Cape, while another two more cosmopolitan species have been recorded from the region (Griffiths, 1999; Kensley, 2001).

Generally, invertebrate diversity is poorly documented (Bigalke, 1979; Picker and Samways, 1996). The lack of taxonomic work on this fauna has been a cause for concern (King and Day, 1979) and the current lack of available expertise on certain groups (e.g. Griffiths, 1999) remains an impediment to such work. Since the pioneering taxonomic monographs and cataloguing work completed in the early twentieth century, most invertebrate taxa have been poorly studied, including aquatic invertebrates (O'Keeffe *et al.*, 1989). Modern revisions and large-scale systematic studies, such as those recently undertaken for some freshwater Crustacea of the Western Cape (Griffiths, 1981; Cook, 1991; Stewart and Griffiths, 1995; Daniels, 1997), for example, are sadly lacking for many invertebrate groups. The phreatoicidean isopods are a case in point, with few publications dealing with any South African members of the group (Barnard, 1913, 1914, 1927, 1940; Nicholls, 1943). This is not only limited to systematic accounts, as only a few studies (e.g. Barnard, 1924; Dahl, 1954; Wirkner and Richter, 2003) investigating other aspects of their biology have been published. With about one hundred publications dealing with any aspect of phreatoicidean systematics or biology having been published worldwide, it appears that the group has attracted surprisingly little attention. Following a spell of fervent interest in the group in the first half of the

twentieth century, leading to the description of many species, during the latter half of the century the group has been little studied (Wilson and Keable, 1999).

1.1) Taxonomic history of Phreatoicidea

The first known species within the Phreatoicidea, *Phreatoicus typicus*, was described from subterranean waters in New Zealand by Chilton (1883). Individuals of this species possessed features typical of an array of different isopod groups (Chilton, 1883). The family Phreatoicidae was subsequently established for these isopods by Chilton (1891), as newly described species did not fit into recognized familial divisions. Stebbing (1893) substantiated the establishment of the family, but believed the specimens to be distinct enough from the remaining Isopoda Latreille, 1817 to be included in a new tribe, the Phreatoicidea, now recognized as a suborder.

Authors, such as Chilton (1891, 1918), Sheppard (1927) and Nicholls (1943), provided detailed diagnoses for the suborder. With hindsight, and with access to more representatives of various groups within the Isopoda, some of the characters used by these authors are indeed not strictly diagnostic. While earlier authors highlighted some of the more immediately apparent, idiosyncratic features of the suborder (see Appendix 1; and references therein), the key synapomorphies of the Phreatoicidea (Appendix 1) have been more accurately identified in more recent literature (Brusca and Wilson, 1991; Wilson and Ponder, 1992; Wilson and Keable, 2001; Poore *et al.*, 2002).

Prior to 1943, all but nine extant species described were placed in *Phreatoicus*. This genus contained species described from New Zealand (Chilton, 1883, 1894, 1906), both the Australian mainland (Chilton 1891; Sayce, 1900a; Nicholls, 1926) and Tasmania (Thomson, 1893, 1894; Smith 1909; Sheppard, 1927), and South Africa (Barnard, 1914, 1927, 1940). Three Western Australian forms, originally described as *Phreatoicus* species (Chilton, 1922; Glauert, 1924; Nicholls, 1924), were placed in Nicholls' (1926) genus *Amphisopus*, then moved to the newly established genus *Phreatomerus* in Sheppard's (1927) revision. The remaining species known prior to 1943 belonged to six monotypic genera (Spencer and Hall, 1897; Sayce, 1900b, 1902; Nicholls and Milner, 1923; Nicholls, 1926; Sheard, 1936).

Nicholls (1943, 1944) undertook the last comprehensive revision of the entire group, in which he established 14 new genera, and described some 34 new species and subspecies. He divided the Phreatoicoidea (*sic*) into two families: the Amphisopidae, individuals of which retain a secondary cutting edge (lacinia mobilis) on the right mandible, and the Phreatoicidae, in which the lacinia mobilis is lacking on the right mandible (Nicholls, 1943). Nicholls (1943) regarded the divergence of these two families to be ancient, dating to the Mesozoic. Further, the divergence of individual species took place early enough to lead to the presence of a large number of monotypic genera and the independent invasion of subterranean habitats by representatives of different subfamilies and families (Nicholls, 1943).

Nicholls (1943) recognized 12 genera, forming four distinct subfamilies within the Amphisopodidae (familial and subfamilial names recently having been changed by Wilson and Keable (1999) according to their correct generative roots). Amphisopodinae, Phreatomerinae, Phreatoicopsidinae and Mesamphisopodinae (including *Mesamphisopus* Nicholls, 1943 and *Hyperoedesipus* Nicholls & Milner, 1923) were recognized (Nicholls,

1943). A fifth subfamily, Hypsimetopodinae, including the poorly described, previously monotypic genera, *Hypsimetopus* Sayce, 1902 and *Phreatoicoides* Sayce, 1900, was hesitantly included in the Amphisopodidae (Nicholls, 1943). This family, recently characterized too by having an oblique compound terminal antennular article, the bases of the posterior pereopods produced to form plates, and vertically orientated pleotelson lateral lobes (Wilson and Keable, 2002a), has representatives in Tasmania and the Australian mainland, including the northern, central, southwestern and southeastern (Victoria) parts, and in South Africa (Nicholls, 1943).

Nicholls' (1944) family Phreatoicidae, restricted to Bassian Australia (southern and eastern mainland Australia and Tasmania) and New Zealand, comprised three distinct groups based primarily on the pleotelson shape. Ten genera and some thirty species were recognized within three subfamilies: the Phreatoicinae, the Mesocanthotelsoninae and the Paraphreatoicinae (Nicholls, 1944). The Paraphreatoicinae, Nicholls' (1944) largest subfamily, contained a third of all described phreatoicid species.

The family Nichollsiidae, originially recognized as a subfamily (Nichollsiinae) within the Amphisopodidae, was erected to accommodate two subterranean species (genus *Nichollsia*) described from India (Chopra and Tiwari, 1950; Tiwari, 1955a, b). These species appear to share primitive characteristics with subfamilies within both the Amphisopodidae and Phreatoicidae. They are, however, unique amongst the Phreatoicidea in that the outer uropodal ramus is longer than the inner and in having subequal posterior pleopods, while their mesially cleft pleopodal endites are unique amongst the Isopoda (Chopra and Tiwari, 1950, Tiwari, 1955b). The large number of unique characters (see Tiwari, 1955b) was taken to be evident of the fact that Nichollsiidae was derived very early.

Nicholls (1944) admitted that his subfamilial designations were, to a large extent, arbitrary, and authors have regarded particular subfamilies as being too broad and poorly defined (see Wilson and Ho, 1996; Poore *et al.*, 2002). Nicholls' (1943, 1944) familial designations, too, appeared to require revision. Within the Phreatoicidae, some species (*Notamphisopus flavius* Nicholls, 1944, *Colubotelson huonensis* Nicholls, 1944 and *C. gesmithi* Nicholls, 1944) appeared to that have retained structures resembling a vestigial lacinia mobilis on the right mandible (Nicholls, 1944). Other members of the Phreatoicidae, while apparently lacking the lacinia mobilis on the right mandible, exhibit characters typical of members of the Amphisopodidae (Nicholls, 1944). The systematic value of the right lacinia mobilis has, however, been questioned as it appears to be a plesiomorphic character, seen in other isopodan and non-isopodan groups (Wilson and Keable, 1999), and may have been lost independently in different species within the Phreatoicidae (Nicholls, 1944). Indeed, a subsequent revision (Poore *et al.*, 2002) dismissed the right lacinia mobilis as a useful character for defining families, as it is present in various forms in most phreatoicideans. Numerous recent cladistic analyses (Wilson and Johnson, 1999; Wilson and Keable 1999, 2001, 2002b; Wilson and Edgecombe, 2003) of morphological data have shown Nicholls' (1943) Amphisopodidae to be para- or polyphyletic. For example, the Phreatoicidae and Nichollsiidae have appeared nested within the group, while genera such as *Hypsimetopus*, hesitantly included in the Amphisopodidae by Nicholls (1943) and apparently lacking the lacinia mobilis on the right mandible, have appeared as sister-taxa to the Phreatoicidae. Wilson and Keable (1999) have suggested that the monophyly of the Amphisopodidae (and certain subfamilies) be established by affording some subfamilies familial status, and by rearranging their constituent species. For example, Wilson and Keable (2001, 2002b) proposed raising the Hypsimetopodinae to familial level, subsuming the family Nichollsiidae. Wilson and Edgecombe (2003) have also proposed reducing Nicholls' (1943) family to a

monophyletic Amphisopodidae *s. str.*, containing only seven genera. The most recent revision of the Australian phreatoicidean fauna (Poore *et al.*, 2002) incorporated and built upon the familial and subfamilial arrangements proposed by earlier workers (Knott, 1975; Bănărescu, 1995), who recognized seven phreatoicidean families. Under Poore *et al.*'s (2002) arrangement, most of Nicholls' (1943) amphisopodid subfamilies were elevated to families, as suggested by Knott (1975), and Wilson and Keable (1999). The constituent species of the families were rearranged and Nicholls' (1943, 1944) subspecies were recognized as species (Poore *et al.*, 2002). The extant families recognized (among the Australian fauna) under this classification were: the Phreatoicidae (in which the subfamily compositions were rearranged to exclude Australian taxa from the Phreatoicinae), the Amphisopodidae (including the single genus previously belonging to the Phreatomerinae), the Hypsimetopodidae (subsuming the Nichollsiidae), the Mesamphisopodidae (including *Mesamphisopus* and *Eophreatoicus* Nicholls, 1926), and the Phreatoicopsididae. More recently, Wilson and Keable (2004) have established an additional extant family (Ponderellidae) for a genus described from eastern Australia.

Subsequent to the description of the Indian species, six new monotypic genera have been established (Knott and Halse, 1999; Wilson and Keable, 1999, 2002a, b), three species have been added to existing genera (Wilson and Ho, 1996: Wilson and Keable, 2002b), and five new species included in two new genera (Wilson and Keable, 2002b, 2004). Numerous new species have been identified and await description (Wilson and Ho, 1996; Wilson and Johnson, 1999; Wilson and Keable, 2001, 2002a). These descriptions substantiate the high phreatoicidean diversity and endemism within Australia (Nicholls, 1943, 1944; Williams, 1966; Wilson and Johnson, 1999), but also illustrate that many taxa remain to be discovered through concerted collection effort and well-designed systematic studies.

Many authors (Chilton, 1883; Thomson, 1893; Sayce, 1902; Barnard, 1913, 1914, 1927; Chopra and Tiwari, 1950) had noted the apparent antiquity of the group. This was confirmed by the finds of fossil phreatoicids, morphologically similar to extant species, in Carboniferous, Permian and Jurassic sediments (Chilton, 1918; Glaessner and Malzahn, 1962; Schram, 1970, 1974, 1980; Rolfe *et al.*, 1982; Wilson and Edgecombe, 2003). These finds, in particular that of *Hesslerella shermani* Schram, 1970, predate all known isopodan and peracaridan fossils (Schram, 1970, 1974), and establish the existence of the Phreatoicidea as a distinct group since the Carboniferous (Rolfe *et al.*, 1982). These fossils were either placed within the Palaeophreatoicidae Birshtein, 1962 or within the Amphisopodidae (Nicholls, 1943), while *Hesslerella* appears to be intermediate to these two families (Schram, 1970, 1974, 1980).

1.2) Evolutionary biogeography of Phreatoicidea

The fossil record of Phreatoicidea reveals the sequential transition from marine to freshwater habitats seen in many Crustacea (Schram, 1974). Phreatoicidean fossils were found in Carboniferous near-shore marine sediments (Schram, 1980, 1981), Carboniferous-Permian, brackish, estuarine strata from the equatorial region of Laurentia (Schram, 1974, 1980), and in freshwater sediments from the Triassic (Chilton, 1918; Schram, 1974). All extant forms occur in freshwater habitats (Schram, 1974). In the Permian, Malacostraca, including the Phreatoicidea, which had probably had a global marine distribution within the Paleozoic (Brusca and Wilson, 1991), were no longer restricted to Laurentian tropical waters, but had spread to marine and freshwaters of Gondwana (Schram, 1977). With the formation of Pangaea in the Permo-Triassic, the distributions of many forms became more cosmopolitan

and included Gondwana (Schram, 1977). In the Early Triassic, the Phreatoicidea underwent a transition from marine Laurasian to freshwater Gondwanan habitats, from which they were forced, along with other primitive Paleozoic forms, into refugial habitats in the Gondwanan reaches of Pangaea by more advanced Peracarida and Decapoda (Schram, 1974, 1977). Barnard (1927) had earlier suggested that the present habitats of the Phreatoicidea were refugial.

Similarly, many authors (e.g. Sayce, 1902; Barnard, 1913, 1914, 1927; Chilton, 1918; Chopra and Tiwari, 1950) commented on the Gondwana distribution of phreatoicidean species, some (Barnard, 1913, 1914; see Hurley, 1990) alluding to the then only postulated connection of the southern continents. The Phreatoicidea remain one of the best examples of a Gondwanan relict (Newman, 1991; Bănărescu, 1995). Their distribution can only explained by the tectonic breakup of Gondwana, after its separation from Laurasia through the formation of the Tethys Sea (Newman, 1991; Bănărescu, 1995), rather than dispersal events (Wilson and Keable, 1999). Barnard (1927) had proposed that the breakup of Gondwana would have forced the separation, and independent diversification of an African group and an Australasian group. The major clades (families) were, however, probably in existence prior to the separation of East Gondwana (Antarctica, Australia, India and New Zealand) from West Gondwana (Africa and South America) (Wilson and Keable, 1999; Wilson and Johnson, 1999).

Presently, about 68 species within 30 genera are recognized within the Phreatoicidea, occurring with a typical Gondwanan distribution in a variety of freshwater habitats, including rivers, streams, temporary headwaters, swamps, lakes and in subterranean water, appearing at the surface through springs or wells (Kensley, 2001).

1.3) Phreatoicidean systematics

Due to the peculiar morphology, and the age of the group, coupled with the fact that no obvious marine relatives of the Phreatoicidea are known (Calman, 1918), various conflicting relationships have been proposed for the group.

In terms of general body facies, the Phreatoicidea resembles the Amphipoda Latreille, 1816, and appears to be intermediate to the Amphipoda and Isopoda, but these affinities are superficial (Chilton, 1883, 1891; Stebbing, 1893; Calman, 1918). Ironically, through a type-setting error (Chilton, 1891), *Phreatoicus typicus* was initially placed within the Amphipoda (Thomson and Chilton, 1886). Nicholls (1924, 1943), however, argued that the similarities between the Amphipoda and the Phreatoicidea reflected parallel descent from a shared marine ancestor, and were not necessarily brought about by convergent evolution.

Undoubtedly belonging to the Isopoda (Chilton, 1891), the phreatoicids were initially thought to be intermediate to the Anthuridae Leach, 1814 and the Idoteidae Samouelle, 1819 (Chilton, 1883). Chilton (1891) later regarded the Phreatoicidea to occupy a central position within the Isopoda. An additional, closer similarity to, or common ancestory with, the Asellidae Latreille, 1802 (suborder Asellota Latreille, 1802) was noted by Chilton (1891), Thomson (1893), Calman (1918), Barnard (1927) and Sheppard (1927). Others authors had documented similarities to, or postulated relationships with the Tanaidae Dana, 1849 (Thomson, 1893), the Flabellifera Sars, 1882 – the "typical" isopods (Calman, 1918: 279; Sheppard, 1927), Valvifera Sars, 1882, Epicaridea Latreille, 1831 and Cymothoidae Leach, 1814 (Sheppard, 1927).

In his revision, Nicholls (1943) chastised Barnard (1927) and earlier authors who accepted, as fact, the primitive nature of *Metaphreatoicus australis* (Chilton, 1891) (largely due its use as a reference specimen in taxonomic accounts) and who presented this as evidence of the relationship between the Phreatoicidea and Asellidae. According to Nicholls (1943, 1944), this relationship was a distant one, arising through parallel evolution from a common malacostracan ancestor, and the closest relative of the Phreatoicidea would be the Cirolanidae Dana, 1852 (within the Flabellifera). Dahl (1954) also suggested that the Phreatoicidea, from which the Asellota was derived, was, in turn, derived from Flabelliferan stock.

Although the phreatoicidean fossil record (Upper Carboniferous) predates that of other isopod groups, such as the Flabellifera (Jurassic) and Valvifera (Oligocene) (Chilton, 1918; Schram 1970, 1974; Brusca and Wilson, 1991; Wilson, 1996), Schram (1974) was the first to suggest, based on the fossil evidence and a proposed ancestral "groundplan", that the Phreatoicidea were ancestral within the Isopoda. Cladistic analyses (Wägele, 1989, 1990; Brusca and Wilson, 1991) of the isopodan suborders based on morphological data showed the Phreatoicidea to be, unambiguously, primitive to the other isopod groups. The Phreatoicidea, believed to be derived from a cirolanid-like ancestor by Wägele (1989), was placed next to a clade containing the Asellota, Microcerberidea Lang, 1961 and Calabozoidae Van Lieshout, 1983 in Wägele's (1989) analyses. In Brusca and Wilson's (1991) analyses, the Phreatoicidea was basal to a clade containing the Asellota and Microcerberidea, followed by the oniscidean clade, all these forms occurring in relictual habitats. Recent molecular phylogenies (using a combination of 12S and 16S rRNA mitochondrial gene fragments) have substantiated the basal position of the Phreatoicidea (Wetzer, 2002). Alternatively, the Asellota have been retrieved basally, with the Phreatoicidea the basal sister of the remaining isopod suborders (Dreyer and Wägele, 2002), for which the authors established the infraordinal group

Scutocoxifera. Subsequently, Brandt and Poore (2003) have further resolved relationships within the Scutocoxifera and, particularly, the Flabellifera. Although the authors proposed new subordinal, superfamilial and familial relationships and classifications based on their cladistic analysis of morphological data, they were confident enough of the basal position of the Phreatoicidea and Asellota to include representatives of these lineages as outgroups in their analysis (Brandt and Poore, 2003). Surprisingly, the Phreatoicidea have also erroneously been placed among the derived Scutocoxifera using molecular data (Wägele *et al.*, 2003).

1.4) Phreatoicidean isopods in southern Africa

The first phreatoicidean isopod collected from South Africa was noted in *Nature* by Barnard (1913). The specimens, collected from moss covering rocks on the bed of a swift-running stream on top of Table Mountain (Barnard 1913, 1914), were described as *Phreatoicus capensis* (Barnard, 1914). Of the twelve extant species then described from New Zealand, the Australian mainland and Tasmania (see Barnard, 1914), the South African species appeared to share few characters with *Phreatoicopsis* Spencer & Hall, 1897, *Phreatoicoides* and *Hypsimetopus*, and appeared to be similar enough to *Phreatoicus australis* Chilton, 1891 to warrant inclusion in the genus. These similarities included pleotelson shape, body proportions, and the fusion of the penial filament to the endopod of the second pleopod (Barnard, 1914). Barnard (1914) regarded the most distinguishing feature of this species to be the presence of a secondary cutting edge or surface (lacinia mobilis) on the right mandible, a feature later used to define the family Amphisopodidae (Nicholls, 1943).

Further collections led Barnard (1927) to extend the known range of *P. capensis* and to describe two varieties. Within *P. capensis*, variation is seen in the shape and setation of the telson, the length of the antennae, the shape of the propodus of the gnathopod, the degree of setosity of the body, and the coloration. Barnard (1927), however, felt that specimens from only two localities were worthy of varietal names (Barnard, 1927).

The variety *P. capensis* var. *depressus* was described from the Steenbras River valley in the Hottentot's Holland Mountains. The pereon was much more depressed than the typical form and the other variety. The pereon and cephalon were strongly setose laterally. The telson was not as abrupt as that of *P. capensis* var. *abbreviatus*, but more so than in the typical form. The propodus of the gnathopod was pyriform in shape. The coloration of the individuals was similar to the typical form (Barnard, 1927).

Phreatoicus capensis var. *abbreviatus* was described from Kogelberg, in the Hottentot's Holland Mountains (Barnard, 1927). The telson was also more blunt than in the typical form, and the appendages were pale, without any mottling. The propodus of the gnathopod was broad and ovate, with a straight posterior margin, and a distinct angle between it and the dactylus (Barnard, 1927). Depigmentation or albinism was reported (Barnard, 1927) for certain populations of this variety in the Hottentot's Holland Mountains and the Langeberge (Swellendam).

Later, Barnard (1940) described an additional variety, *Phreatoicus capensis* var. *penicillatus*, from a marshy basin, formerly a lagoon, near Hermanus. The variety was characterised by having the lateral margins of the pereon and cephalon strongly setose. The peduncular joints of the antennae were strongly setose, as was the telson. The telson carried two apical spines,

and often one pair laterally and a subapical pair dorsally (Barnard, 1940). The uropods were typical, but the outer ramus bore three apical spines, and the inner ramus three to four. The peduncles and rami were strongly setose, with the setae being longer than the spines (Barnard, 1940).

In the first revision of the group by Sheppard (1927), the South African species was retained in *Phreatoicus*, although the species did clearly not belong to the genus. Sheppard (1927) dealt very superficially with the South African forms, which, according to Nicholls (1943), have the coxae of the pereopods fused with the pleura of their respective pereonites, disagreeing with the generic diagnosis she proposed. In considering the relationship between *P. capensis*, the Australasian sub-alpine species of *Phreatoicus* and species from northern and western Australia (*Amphisopus*, *Paramphisopus* Nicholls, 1943, *Phreatomerus* and *Eophreatoicus*), Nicholls (1926) admitted that a new genus may be required to accommodate *P. capensis*. *Phreatoicus capensis* differed from the above-mentioned species in having plumose setae on the endopods of the pleopods, and a vestigial inner lobe on the second maxilla (Nicholls, 1926).

Subsequently, Nicholls (1943) established the genus *Mesamphisopus* for the South African forms and they clearly belonged to his newly established family Amphisopodidae. He considered *M. capensis* and two of Barnard's (1927) varieties, *M. depressus* and *M. abbreviatus*, as species. Nicholls (1943) did not mention Barnard's (1940) fourth variety, *P. capensis* var. *penicillatus*, nor Barnard's (1940) publication. In all probability, this publication was not seen by Nicholls (Kensley, 2001). Kensley (2001) was the first to regard *P. capensis* var. *penicillatus* as a species within *Mesamphisopus*.

Mesamphisopus is characterised by having setae on the endopods of all five pleopods, a primitive condition within the Phreatoicidea; by the presence of plumose setae on these endopods; by having a freely movable terminal spine on the uropodal rami; and in possessing a large simple spine at the end of the uropodal peduncle (Nicholls, 1943). Further, the second pleopods are modified in the males; the penial stylet is short and cylindrical; the pleopods have coupling hooks; and the antennula is short (Nicholls, 1943). Some of these characters are, however, found in species within the Amphisopodidae, as well as the Phreatoicidae (Nicholls, 1943, 1944).

In addition to the characters used by Barnard (1927) to define his varieties, Nicholls (1943) used the dimensions and proportions of the peduncles of the antennule and antennae, head, eyes and first pereon segment; relative length and armature of the uropodal rami; the degree to which the body is setose; the depth of the sutures between the gnathopod coxae and segments; the shape of the postero-inferior corners of the pleura of the pleon segments; the depth of the notch on the posterior margin of the fifth pleon segment; the shape of the telson; and the setation of the endopodite of the first pleopod to distinguish his species.

Barnard (1927) had reported that a pair of subapical spines is sometimes encountered on the dorsal surface of the telson of *Mesamphisopus*. Kensley (2001) identified the presence of the pair of subapical dorsal spines, or setae, as a character by which ***M. capensis*** can be identified, these spines being absent in the other species of *Mesamphisopus*. The remaining species were distinguished, somewhat arbitrarily, by the relative setosity of the antennal peduncles, lateral pereon, and cephalon (Kensley, 2001). Kensley's (2001: Fig. 3.8) illustrations and diagnoses, however, indicate that species may be distinguished by the setation of the pleotelson, in combination with that of the gnathopods.

1.4.1) Phylogenetic position of Mesamphisopus

When initially described, *Phreatoicus capensis* was regarded as being most closely related to *P. australis*, despite their geographical disjuncture (Barnard, 1927). *Mesamphisopus capensis* approaches *Metaphreatoicus australis* in terms of relative length of the cephalon-pereon to the pleon-pleotelson, and coloration, but differs in the structure of the uropods, with the inner dorsal margin being higher than the outer, and by lacking the two long spines on its lower apex as seen in *M. australis* (Barnard, 1927). *Mesamphisopus capensis* also differs from *M. australis* (as well as *P. typicus* and *Neophreatoicus assimilis* (Chilton, 1884)) in the shape and setation of the uropodal rami (Barnard, 1927).

Barnard (1927) regarded *M. capensis* and *M. australis* to be the most primitive of the species then known, and to represent the ancestral stock of the Phreatoicidea. From this form, the blind forms, such as *Crenoicus shepardi* (Sayce, 1900), could be derived, while a relative shortening of the pleon would give rise to the condition seen in *Notamphisopus kirkii* (Chilton, 1906), the lacustrine species *Onchotelson brevicaudatus* (Smith, 1909), and the burrowing species *Hypsimetopus* and *Phreatoicoides*, for example (Barnard, 1927).

Prior to his revision and the description of the two families within the Phreatoicidea (Nicholls, 1943, 1944), Nicholls (1924) believed *M. capensis* to be most similar to the species (*Amphisopus lintoni* (Nicholls, 1924), *Paramphisopus palustris* (Glauert, 1924) and *Phreatomerus latipes* (Chilton, 1922)) described from Western Australia, as these species all lacked a terminal spine/projection on the telson (Nicholls, 1924). Unsure of its position, Nicholls (1926) stated that *M. capensis* appeared to be intermediate to the sub-alpine, eastern Australasian *Phreatoicus* species and *Eophreatoicus kershawi* Nicholls, 1926, a Northern

Territory, Australian species. *Mesamphisopus* shared the possession of certain primitive characters, including the lacinia mobilis on the right mandible (the character later used by Nicholls (1943) to define the Amphisopodidae), with *Eophreatoicus*, *Amphisopus* (which then included *A. lintoni*, *P. palustris* and *P. latipes*) and the fossil *Protamphisopus wianamattensis* Chilton, 1918 (Nicholls, 1926). Certain features of *M. capensis*, specifically, were typical of the eastern Australasian (then) *Phreatoicus* species, including: the posterior, transverse groove of the cephalon; the short antennule; the distinct, pereopodal coxae; a subchelate fourth pereopod; the apparent absence of coupling hooks on the first pleopod; the short, curved penial filament, with terminal setae; the inner lobe of the first maxilla having six plumose setae; and the terminal telsonic projection (Nicholls, 1926).

Nicholls (1943) suggested that *Mesamphisopus* was, in many repects, the most primitive of the Phreatoicidea. He regarded *Mesamphisopus* (as well as *Synamphisopus* Nicholls, 1943) as occupying a central position within the Phreatoicidea (Nicholls, 1943). *Mesamphisopus*, while clearly belonging to the Amphisopodidae and retaining many primitive characters, showed clear affinities to the Phreatoicidae, and showed many similarities to widely scattered phreatoicidean groups (Nicholls, 1943, 1944), even with regard to "diagnostic" characters (Nicholls, 1943: 26). The free-articulating condition of the terminal spine of the uropodal rami of *Mesamphisopus* is restricted to certain genera within the Amphisopodidae (Nicholls, 1943). The presence of a simple seta on the uropodal peduncle at the base of the rami occurs in the Amphisopodidae and in the Phreatoicidae (*Phreatoicus* and *Neophreatoicus* Nicholls, 1944), while being dentate in certain other genera and species in both the Amphisopodidae and Phreatoicidae (Nicholls, 1943, 1944). Nicholls (1943, 1944) also discussed the similarity of *Mesamphisopus* to other genera and species, with regard to the prehensile nature of the fourth pereopod; the retention and arrangement of setospines on the proximal endite of the

maxillula; the cylindrical nature of the penial stylet; the cervical groove of the head; the freedom of the first pereon segment; and club-shaped antennule, the latter three characters being more typical of the Phreatoicidae.

Nicholls (1943) placed *Mesamphisopus* in the sub-family Mesamphisopodinae (within the Amphisopodidae), together with the Western Australian subterranean species, *Hyperoedesipus plumosus* Nicholls & Milner, 1923. Nicholls (1943), however, conceded that the inclusion of *Hyperoedesipus* (distinguished from *Mesamphisopus* by the setation of the uropodal peduncle and immovable terminal setae of the rami) deprived the subfamilial diagnosis of some accuracy. Subsequently, both Knott (1975) and Bănărescu (1995) have included *Mesamphisopus* in a single family (Mesamphisopidae) together with *Eophreatoicus* from northern Australia and the southwestern Australian genus *Mawbeyamphisopus* – a *nomen nudum* used by Bănărescu (1995) from Knott's (1975) unpublished thesis (see Poore *et al.*, 2002). Under Poore *et al.*'s (2002) most recent arrangement *Mesamphisopus* is included in the Mesamphisopodidae, with *Eophreatoicus* alone. The inclusion of *Eophreatoicus* was only provisional and the authors suggested that the family may need to be reconstituted in light of new species described from Western Australia (see Poore *et al.*, 2002; Wilson and Keable, 2002a).

Recent morphological cladistic analyses indicate the phylogenetic position of *Mesamphisopus*. Wilson and Keable (1999) regarded *M. capensis* as being the most primitive species within the subfamily Mesamphisopodinae, when choosing taxa for their cladistic analysis of the relationships among subfamilies within the Phreatoicidae and Amphisopodidae. Their analysis of nine species (each "least-derived" within their particular subfamily), rooted with a hypothetical, ancestral morphology, subsequently showed

Mesamphisopus capensis to be basal to the included phreatoicidean species (Wilson and Keable, 1999). *Mesamphisopus capensis* was also used as an outgroup in a subsequent cladistic analysis, due to the species being derived basally in the phreatoicidean phylogeny (Wilson and Johnson, 1999). Further phylogenetic studies have revealed *Mesamphisopus* to be no longer basal, but nested within the paraphyletic Amphisopodidae (Wilson and Keable, 2001, 2002b). *Mesamphisopus* has also been shown to be a sister taxon of *Eophreatoicus*, and *Eremisopus* Wilson & Keable, 2002, within the Amphisopodidae *s. str.* (with *Amphisopus*, *Phreatomerus* and *Paramphisopus*); with the Amphisopodidae *s. str.* being more derived than the former amphisopodid genera of Wilson and Keable's (2001) Hypsimetopodidae and the subfamily Phreatoicopsinae (Wilson and Edgecombe, 2003). Specific relationships among the species of *Mesamphisopus* have not been considered (Barnard, 1927, 1940; Nicholls, 1943, 1944) or have not been well resolved (Wilson and Keable, 2002b; Wilson and Edgecombe, 2003).

1.4.2) Distribution within southern Africa (Fig. 1.1)

Barnard (1927) maintained that phreatoicid isopods, together with the paramelitid amphipods, are abundant in the mountainous region of the southwestern Cape, South Africa, where they form an important and characteristic part of the fauna. Incapable of extensive active or passive migration, this fauna is more restricted, and it is generally expected that their distributions are dependent on the continuity of drainages and the evolution of river systems (Barnard, 1927).

When Barnard (1927) described *P. capensis* and its varieties, phreatoicideans were only known from Table Mountain, the Hottentot's Holland Mountains (from Landdroskop

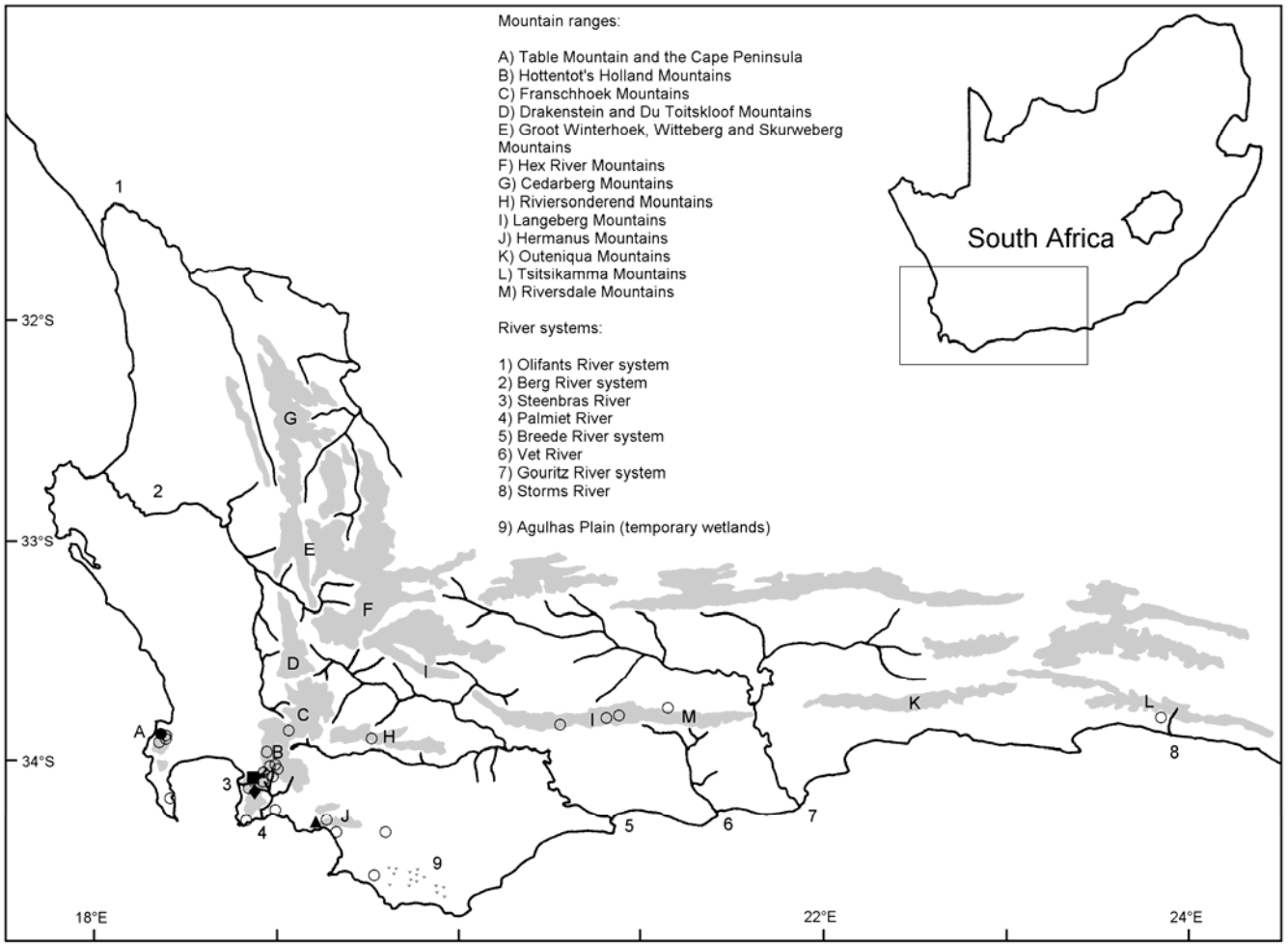


Figure 1.1: Known collection localities of *Mesamphisopus* within South Africa, based on museum and private collections. Filled symbols represent the type localities of *Mesamphisopus capensis* (circle), *M. depressus* (square), *M. abbreviatus* (diamond) and *M. penicillatus* (triangle). Open circles represent unidentified private collections or museum collections identified as *M. capensis* prior to the publication of the most recent key (Kensley, 2001). Some of the major topographical features (mountain ranges and drainage systems) referred to in the text are indicated on the map.

southwards to the Steenbras River valley and Kogelberg), the Riviersonderend Mountains and the Langeberge (in the vicinity of Swellendam, Tradouw Pass, and Riversdale). The animals were collected from much the same habitat at each locality, occurring in very narrow runnels and the upper reaches of rivers, often where the streams form a series of disconnected pools in the summer months (Barnard, 1927). They were restricted to portions of the streams where the flow was not too strong, and were found living in moss (*Chiloscyphus*, *Dicranum*, *Sphagnum*, and, specifically, *Scirpus fluitans*) and the upper layer of humid mud (Barnard, 1927).

On Table Mountain perennial streams are concentrated on the northern mountain proper (Barnard, 1927). Here phreatoicideans are found in the streams entering mature valleys, such as Waai Vlei and Kasteelspoort (Barnard, 1927).

Along the Hottentot's Holland Mountains, ancient, broad valleys and the remnants of plateaus separate the isolated peaks (Barnard, 1927). *Mesamphisopus* is typically found in these ancient valleys in the northern part of the range, along the narrow plateau south of Spitskop, and in the upper Steenbras River basin between Kogelberg and the Hottentot's Holland Mountains (Barnard, 1927). *Mesamphisopus abbreviatus* was described from the swampy headwaters of the Kogelberg stream, draining into the Steenbras River (Barnard, 1927). Interestingly, a pool containing *Mesamphisopus* was also noted to the west of the watershed near the source of a steep stream draining into the Lourens River, probably reflecting drainage capture (Barnard, 1927).

In the Swellendam vicinity of the Langeberge, phreatoicideans are found in high altitude boggy marshes (Barnard, 1927). Near Riversdale, specimens were found, on the dry northern

slopes where a small non-perennial stream flows out and dissipates on the northern plain (Barnard, 1940). The phreatoicideans collected from the Zonderend Mountains were collected from a small boggy, peaty valley on the southern slopes (Barnard, 1927). Although no phreatoicideans had been recorded from the mountains directly to the north of the type locality of *M. penicillatus*, Barnard (1940) regarded that population to have been established recently, by individuals washed down from the mountains during flooding. No phreatoicideans had yet been recorded from the western reaches of the Langeberge, the Stellenbosch and Franschhoek Mountains, the Winterhoeks Mountains, Witzenberge, Witteberge, Cedarberg, or mountains in the vicinity of Wellington or Ceres (Barnard, 1927).

Mesamphisopus appears to occur in broad, mature valleys, exclusively, as do Australasian sub-alpine species such as *Metaphreatoicus australis* and *Crenoicus shepardi*, once included in *Phreatoicus* with *Mesamphisopus* (Barnard, 1927). Barnard (1927) believed this high-altitude peneplain distribution to be ancient and refugial. From this distribution, and from these putatively primitive forms, other species and distributions could be derived (Barnard, 1927).

Within the South African Phreatoicidea, altitude does not appear be a factor directly influencing distribution, as *Mesamphisopus* occurs at various heights, from 450 m to 1 400 m (Barnard, 1927). Indirectly, in influencing the physical nature of the streams, altitude is, nonetheless, a factor (Barnard, 1927). The presence of sufficient moisture, however, appears to be the determining criterion. The precipitation on Table Mountain, and presumably other localities, is not enough to provide perennially flowing surface water, but is sufficient to keep the soil moist and cool during the dry summer months (Barnard, 1927). The presence of mist clouds, provided by the southeasterly winds, thus determines the distribution of

Mesamphisopus. The provision of moisture by this mist belt is variable, but *Mesamphisopus* (particularly *M. depressus*) is capable of aestivation in the moist soil, during exceedingly dry spells (Barnard, 1927). The southeast mists do not occur, or occur at a lower intensity, north of Table Mountain, Franschhoek, the Riviersonderend- and Langeberg Mountains, apparently limiting the distribution of *Mesamphisopus* to the afore-mentioned areas (Barnard, 1927). Even though apparently favourable habitat exists northwards, the regions are thought to be too dry to permit survival of populations, even those capable of aestivation (Barnard, 1927). Another factor influencing distribution is water temperature. Being eurythermal and generally found in cold water, Barnard (1927) failed to find phreatoicideans in water warmer than 20 °C.

The present, disjunct distribution of the Phreatoicidea within South Africa cannot be explained by extinction brought about by the Stormberg volcanic period, as the present distribution lies well outside the expanse of Drakensberg basalt (Barnard, 1927). Neither are the effects of the Pleistocene glaciation period seen within the Western Cape region of South Africa (Barnard, 1927). The present distribution of the phreatoicideans in South Africa is confined to areas of Table Mountain Sandstone, which have undergone comparatively less structural change during the formation of the Cape Fold Mountains. As a result, these strata have experienced less denudation, and have maintained vegetative cover and broad ancient plateaus, over which slow-flowing streams provide the marshy habitat for the phreatoicideans (Barnard, 1927). The overlying Bokkeveld beds, hard and dry during the summer months, with saline water, are unsuitable habitat for the phreatoicideans. These beds could have been inhabited, prior to the exposure of the Table Mountain Sandstones by erosion, if earlier climates were wetter (Barnard, 1927). The Bokkeveld beds are a barrier to the dispersal of the phreatoicideans, whose present occupation of habitats on Table Mountain Sandstone,

suggests that a colder, wetter period must have existed to enable the invasion of this habitat (Barnard, 1927). Table Mountain Sandstone outcrops in KwaZulu-Natal remain uninhabited by both paramelitid amphipods and phreatoicidean isopods, as suitable habitat has been eradicted by erosion and volcanic activity (Barnard, 1927). No mature valleys or perennial streams exist, and the water, when flowing, is far warmer than in the Western Cape (Barnard, 1927).

1.5) The problem

Jarvis (1979), while reiterating that the invertebrates of the Western Cape were an extremely diverse group, highlighted two specific problems preventing an accurate assessment of the diversity (and endemicity) of the invertebrate fauna. These problems extend to the phreatoicidean isopods and the genus *Mesamphisopus*.

Firstly, distribution records for most taxa are poor, and most are undersampled (Jarvis, 1979). For example, the South African Museum, situated in the most populous centre within the known distribution of the phreatoicidean isopods within South Africa (Barnard, 1927, 1940), carries collections from only fourteen localities. Additionally, three of the species (*M. abbreviatus*, *M. depressus* and *M. penicillatus*) are known from the type locality only. The need for an intensive collection program was highlighted by Barnard (1927), who wrote that "it is obvious that many more localities remain to be searched before we can state with certainty the limits of distribution of these Crustacea in the south-west mountains" (Barnard, 1927: 197). This sentiment was echoed by Kensley (2001), who had recommended that much

field work be undertaken to determine the diversity of the region, as considerable speciation may have taken place on the isolated mountain peaks of the Western Cape.

Secondly, there is a lack of taxonomic knowledge for many groups (Jarvis, 1979). As mentioned earlier, this is indeed the case for the phreatoicidean isopods. Inter- and intraspecific variation has not been studied (Kensley, 2001) and, consequently, the distribution and diversity of the group cannot be properly determined.

The morphological conservatism and homogeneity of species within the Phreatoicidea was noted by Barnard (1927), Nicholls (1943), Williams (1966), and Wilson and Ho (1996). Only under close scrutiny can characters be identified to discriminate species (Nicholls, 1943). Nonetheless, Barnard (1927) documented variation in the length of the antennae and shape of the telson between localities. Other characters, such as gnathopod shape, show considerable variation even within individual populations (Barnard, 1927: Fig. 5). As a result of this general conservatism, coupled with extensive intraspecific variation, often on a very small geographic scale, the delineation of species is very difficult (Wilson and Ho, 1996). A systematic study on such a group should then ideally use a combination of approaches, and independent data sets gathered by various techniques.

1.6) Study objectives

Broadly, the objectives of this study are:

1) To determine the distribution of the phreatoicidean isopod fauna by means of extensive collection within the Western Cape (and beyond), and by the examination of museum records and material.
2) To describe any new species or genera found.
3) To determine the extent of variation, morphometric, morphological and genetic, between recognized (as well as newly described or putative) species.
4) To determine the extent of genetic, morphometric and morphological variation between geographically separated populations within wide-spread species.
5) To determine the evolutionary relationships among species.
6) To identify populations with unique evolutionary trajectories and particular conservation worth.

In order to attain these goals, a number of key questions have been formulated:

1) Are there unidentified *Mesamphisopus* species (or even species warranting a new genus), differing from the four known species?
2) What are the distributions of the species, and are the distributions given by Barnard (1927) and his historical accounts accurate?
3) What is the extent of differentiation among known species?
4) Museum records and earlier collections reveal *M. abbreviatus, M. depressus* and *M. capensis* to be widespread: how differentiated are populations of these species over their distributions?

5) Can management units, evolutionary units or even separate species, be identified within these large distributions?
6) How do levels of genetic differentiation within and between species compare with those recorded for other isopod groups, Peracarida and Crustacea?
7) Are there characters that can be used to identify species easily and unambiguously?
8) What are the evolutionary relationships between these species?
9) How well are the species represented in conserved areas, and can potential threats be identified and recommendations made?

1.7) Some methodological and theoretical considerations

The following paragraphs, while not exhaustive discussions, provide some background and justification for the methodologies and concepts employed.

1.7.1) Allozyme electrophoresis

Since the 1960s, the use of allozyme electrophoresis to investigate population genetic and systematic questions has become widespread (Murphy, 1993; Leberg, 1996). Overviews of the biochemical, molecular and technical underpinnings of the methodology – the differential segregation, due to differences in molecular shape, size and nett charge reflecting underlying amino acid composition and, in turn, mutational changes at the DNA-sequence level, of enzyme variants (allozymes) representing allelic variants of a single nuclear locus – have been presented by Richardson, Baverstock and Adams (1986), Leberg (1996) and Murphy *et al.* (1996). The greatest appeal of the methodology lies in the fact that it is a robust, relatively

easy and inexpensive way of gathering large amounts of objective, phylogenetically informative data (Mabee and Humphries, 1993; Thorpe and Solé-Cava, 1994; Leberg, 1996). The objectivity arises from the fact that the proteins are the products of supposedly neutral, independent, single gene, autosomal loci and are unlikely to be modified by environmental factors; and the fact that alleles at a locus are co-dominant, enabling the identification of heterozygous individuals, and show Mendelian inheritance (Richardson *et al.*, 1986; Thorpe and Solé-Cava, 1994; Leberg, 1996). The strongest application of the technique, among a multitude of population and conservation genetics, paternity determination and forensic applications (see Richardson *et al.*, 1986), lies within the delimitation of taxonomic groups (α-systematics) (Thorpe and Solé-Cava, 1994), particularly at the species level (Mabee and Humphries, 1993). As such the technique has been widely applied in this regard, and to investigate population genetic questions, within isopod biology (e.g. Lessios and Weinberg, 1994; Piertney and Carvalho, 1994, 1995a; Garthwaite, Lawson and Sassaman, 1995; Messana *et al.*, 1995; Cobolli Sbordoni *et al.*, 1997; Gentile and Sbordoni, 1998; Wang and Schreiber, 1999; Ketmaier *et al.*, 2000).

The methodology is, however, not without its shortcomings. Primary among these is the fact that genetic variation detected in allozyme studies represents only a fraction of the variation present. It is largely unknown (in the absence of large-scale sequencing projects) what proportion of total genetic variation is represented by allozyme variation, as the variation in non-coding regions (such as introns), and in structural and regulatory genes (whose products are not expressed as proteins) remains unknown (Thorpe, 1982; Richardson *et al.*, 1986; Leberg, 1996). A large proportion of the variation underlying the allozymes themselves also goes undetected. Due to the redundancy in the coding of amino acids, many mutations do not result in amino acid substitutions and structurally different proteins (Richardson *et al.*, 1986).

Further, only 20 to 30% of actual amino acid substitutions are thought to result in electrophoretically detectable differences (Thorpe, 1982; Richardson *et al.*, 1986). Thus, while electrophoretically different proteins reflect amino acid substitutions, the true underlying allelic diversity still remains unknown (Richardson *et al.*, 1986).

Over and above the explicit practical reliance on fresh or frozen tissue (Richardson *et al.*, 1986; Thorpe and Solé-Cava, 1994) and the fact that tissue-specific enzyme expression often makes non-destructive sampling unfeasible (Leberg, 1996), there is an also an apparent trade-off to be considered when initiating an allozyme study rather than adopting a sequence-based approach (Hillis *et al.*, 1996). Whereas one or two sequenced gene loci may provide much detailed information, the assaying of many relatively information-poor allozyme loci may be required to provide equivalent data (Hillis *et al.*, 1996). The sampling strategies involved in allozyme studies themselves often require many individuals or loci to be screened and also involve a trade-off (Richardson *et al.*, 1986). In order to efficiently detect differences in allele frequencies in population genetic studies, the genotypes of many individuals need to be assayed at the expense of a larger number of loci. In these studies, it would be sufficient to examine only a few polymorphic loci (Richardson *et al.*, 1986). In systematic studies, by contrast, many more loci need to be assayed, albeit in very few individuals, to maximize the chance of detecting fixed allelic differences. These would be used to deduce specific status or be instructive of the evolutionary relationships among populations (Richardson *et al.*, 1986). When deducing estimates of genetic distance and heterozygosity in allozyme studies, sample sizes may be small, providing a sufficiently large number of loci are assayed, average heterozygosity is low and the genetic distances among populations are large (Nei and Roychoudhury, 1974; Nei, 1978; Gorman and Renzi, 1979; Hillis, 1987). Nei (1978) suggested examining as many as 50 loci for accurate estimates of genetic distance, but, as this

was seldom possible, the situation could be rectified through increased sample sizes (particularly if heterozygosity is low). It also bears considering that differences in sample sizes and the numbers of loci assayed may often lead to inaccurate genetic distance estimates and dendrograms in these studies (Archie, Simon and Martin, 1989).

1.7.2) Mitochondrial DNA sequencing

Since mitochondrial DNA (mtDNA) was first isolated and characterized from a crustacean (Komm *et al.*, 1982), the direct sequencing of genes or gene fragments situated on this molecule, and the analyses of these sequences, have been widely applied to address questions concerning the population genetic structure and phylogeography of, and the phylogenetic relationships within and among, many crustacean groups. Wetzer (2001) provides a comprehensive list of many of these studies published prior to 2001 and many more have appeared subsequently. The nuclear and mitochondrial genomes of Isopoda have been the subject of earlier study themselves (e.g. Choe *et al.*, 1999; Raimond *et al.*, 1999) and techniques such as RFLP surveys (e.g. Marcadé *et al.*, 1995) and DNA-fingerprinting (e.g. Piertney and Carvalho, 1995b) have been used earlier to address population genetic questions within isopod biology. Surprisingly, the first studies using nucleotide sequence data, and the phylogenetic analysis thereof, to address these or other questions of isopod phylogeny and evolution have been published only recently (Michel-Salzat and Bouchon, 2000; Held, 2000). Nonetheless, further sequence-based studies of isopods using genes/gene fragments of the mitochondrial (Held, 2001; Wares, 2001a; Wetzer, 2001, 2002; Hidding *et al.*, 2003; Ketmaier, Argano and Caccone, 2003; Rivera *et al.*, 2003) and nuclear (Mattern and Schlegel, 2001; Dreyer and Wägele, 2002; Wägele *et al.*, 2003) genomes have been published and, as

the use of sequence data to address these issues gains impetus, many more studies are likely to appear.

Mitochondrial DNA remains a popular and powerful marker for use in a range of molecular genetic studies (Avise, 2004). The popularity stems from the simplicity (e.g. the lack of introns, intergenic spacer regions or large repetitive DNA families) of the mitochondrial genome (Avise, 2004), the ease with which mtDNA is isolated and purified (Dowling *et al.*, 1996; Hillis *et al.*, 1996), resulting from a high copy number (Wilson *et al.*, 1985; Palumbi, 1996; Avise, 2000), and the fact that laboratory protocols are readily available and certain conserved primers for mtDNA amplification are universally applicable (Palumbi, 1996). Further, the structural features, gene content and conserved gene order are well documented (see Avise, 2000, 2004). The patterns and mechanisms of base substitution, length variation and gene rearrangement, as well as the various constraints on certain regions or changes are also relatively well understood (Wilson *et al.*, 1985; Moritz, Dowling and Brown, 1987). The power is due to the range of available structural features and gene and nucleotide characters, with varying evolutionary rates, that make mtDNA suitable for addressing questions at a myriad of hierarchical levels and evolutionary timescales (Wilson *et al.*, 1985; Moritz *et al.*, 1987; Hillis *et al.*, 1996).

Mitochondrial DNA has a very high rate of evolution at the sequence level (Dowling *et al.*, 1996; Avise, 2000, 2004). This high mutation rate, involving mostly point mutations and indels (Avise, 2004), results from relaxed functional constraints, high replicative turnover, inefficient repair mechanisms, and the molecule's high exposure to mutagenic free radicals in the mitochondria and its lack of protection by histone proteins (Avise, 2000). Further, the genome is maternally inherited, without intermolecular recombination, and is effectively

haploid – resulting in a four-fold lower population size (Dowling *et al.*, 1996; Hillis *et al.*, 1996; Avise, 2000, 2004). As a result, the genome is more prone to the effects of genetic drift, and the fixation of mutations and sorting of ancestral alleles and lineages are rapid (Dowling *et al.*, 1996; Avise, 2000). This leads to the rapid emergence of population structure and interpopulation differentiation (Dowling *et al.*, 1996; Avise, 2000). Coupled with the high mutation rate, intraspecific variation is high, making mtDNA a sensitive micro-evolutionary marker at the intraspecific level (Avise, 2000, 2004). As a result, mtDNA has found widespread use in epidemiology, in examining patterns of gene flow, in determining effective population sizes and historical demographic patterns, in determining parentage and relatedness, and in determining the maternal origin of parthenogenetic species (Hillis *et al.*, 1996; Dowling *et al.*, 1996). In conservation genetics, mtDNA has been used to detect inbreeding depression and reductions in heterozygosity (Hillis *et al.*, 1996), and has found numerous forensic applications (see Baker and Palumbi, 1996; Bowen and Avise, 1996). The more widespread applications have, however, been in the examination of patterns of geographic variation and relationships among populations or closely related species (and the tracing of patterns of hybridization and introgression), particularly through the construction and examination of allelic and organismal genealogies (phylogenies) (Hillis *et al.*, 1996; Dowling *et al.*, 1996). Although highly variable nucleotide characters (e.g. silent substitutions) are available, even conserved ribosomal genes often offer enough resolution to examine population genetic and phylogeographic patterns below the species level (Palumbi, 1996). At higher hierarchical levels, more conserved, slowly evolving gene regions and certain character changes (e.g. transversions and non-synonymous substitutions) open a different temporal window of resolution (Palumbi, 1996; Avise, 2004). At these inter- and supra-specific levels, matriarchal mtDNA phylogenies are often used to examine the macro-evolutionary patterns and processes involved in speciation, co-speciation and historical

biogeography (Hillis *et al.*, 1996). Further features of mtDNA that may be used to elucidate relationships at even higher taxonomic levels include: changes in genome size and sequence length variations, rearrangements of gene order, amino acid translations, and the secondary structure of the tRNAs and rRNAs (Palumbi, 1996; Dowling *et al.*, 1996; Avise, 2000, 2004).

There are, nonetheless, certain problems that may present themselves in a mtDNA study, possibly confounding analyses and conclusions, and these need to be considered. While mtDNA is largely homoplasmic, incidences of heteroplasmy have been documented (Dowling *et al.*, 1996; Avise, 2000, 2004). Cases of departure from strictly maternal inheritance, involving "paternal leakage" (the infrequent incorporation of male-derived mtDNA, by recombination, into otherwise female cytoplasmic lineages), have also been reported (Dowling *et al.*, 1996; Avise, 2000). A more commonly encountered and insidious problem involves gene duplications and horizontal gene transfer between the nuclear and mitochondrial genomes (Hillis *et al.*, 1996; Avise, 2004). Mitochondrial gene segments are transferred into the nuclear genome, where they remain as nonfunctional nuclear copies (pseudogenes) (Hillis *et al.*, 1996; Palumbi, 1996). These paralogous pseudogenes may be unintentionally amplified in mtDNA studies and misinterpreted as being orthologous to the true mtDNA gene regions, confounding phylogenetic analyses (Avise, 2004). Pseudogenes have been documented in Crustacea (Schneider-Broussard and Neigel, 1997; Williams and Knowlton, 2001) and these authors have suggested that they are more widespread than previously thought. Other problems relate directly to the temporal resolution offered by mtDNA: often the fast evolutionary rate of mtDNA may result in homoplasy and convergence that can obscure phylogenetic relationships, while incomplete lineage sorting and introgression can distort relationships among recently diverged species (Dowling *et al.*, 1996). Mitochondrial DNA's overt sensitivity to population size changes (e.g. bottlenecks and

founder events) may be also problematic on occasion (Wayne, 1996). Further, it should be remembered that phylogenies deduced from mtDNA represent the matrilineal phylogenies of the molecule (the "gene tree"), and not necessarily the organismal phylogeny (the "species tree") (Dowling *et al.*, 1996). Introgression, gene conversions and lineage sorting may obscure the true organismal phylogeny in some cases (Dowling *et al.*, 1996). Lastly, while many mtDNA gene fragments may be examined in a study, these gene regions are nonetheless linked as an effectively single locus due to the maternal transmission, without recombination, of the mitochondrial genome (Dowling *et al.*, 1996; Wayne, 1996).

Fragments of the 12S rRNA and protein-coding cytochrome oxidase *c* subunit I (COI) mitochondrial genes are used in the present study to examine differentiation and phylogenetic relationships within *Mesamphisopus*. These sequence data provide an independent and additional (perhaps alternative) perspective to that provided by allozyme data. The combination of mtDNA and allozyme (or other nuclear) markers has been an especially powerful approach. This is particularly due to the fact that genetic differentiation is structured at different evolutionary levels and the combination of multiple, unlinked markers, with differing temporal windows of resolution and resolving power, will enable the detection of shallow, as well as deep, genetic divergences (Avise, 1996; Baker and Palumbi, 1996). Due to the maternal and non-recombinational inheritance of mtDNA, mtDNA trees are non-amastomose, do not show reticulation, and are hierarchically branched even below the population level, unlike nuclear markers (Wayne, 1996; Avise, 2000). This, considered with the other properties of mtDNA discussed above, suggests that different evolutionary patterns may be detected with different markers (Baker and Palumbi, 1996) and the concordances or discordances revealed in a combined analysis may be instructive. The combination of allozyme/nuclear and mitochondrial markers has also been effective in the broad study of

hybridization (e.g. Quesada, Wenne and Skibinski, 1995; Dowling, Broughton and DeMarais, 1997; Kirby, Berry and Powers, 1997; Rawson and Hilbish, 1998), as it allows for the easier disentangling of the mosaic of characters typical of hybridization and the detection of directional or differential introgression (Dowling *et al.*, 1996). Similarly, reticulate lineages, evident of homoploid hybrid speciation, have been identified (e.g. Taylor, Hebert and Colbourne, 1996). The differences in transmission mode among molecular markers have also allowed the detection of differential, sex-biased dispersal and natal site philopatry, often in the face of apparent (nuclear) genetic homogeneity, as reviewed by Bowen and Avise (1996), Baker and Palumbi (1996) and Avise (2004). A combined approach has also highlighted the influence of balancing selection on particular markers (e.g. Piel and Nutt, 2000) – if viewed in isolation, these markers would lead to radically different conclusions. Lastly, perhaps most importantly, evidence of genealogical concordance among the independent markers (e.g. Castro *et al.*, 1999; Allendorf and Seeb, 2000; Gantenbein *et al.*, 2000; Cox and Hebert, 2001; Daniels, Stewart and Cook, 2002a), sometimes illustrating the different resolving powers and sensitivity of these markers, or the effects of population size fluctuations (e.g. Chenoweth *et al.*, 1998; Hughes *et al.*, 1999; Haavie, Særtre and Moum, 2000;) can only increase confidence in the conclusions drawn. This is particularly important in systematic studies (e.g. Taylor *et al.*, 1996; King and Hanner, 1998; Taylor, Finston and Hebert, 1998) where conclusions and taxonomic realignments based on mtDNA, an effectively single character set, may be misleading (Bowen and Avise, 1996).

1.7.3) Morphometric analyses

The apparent difficulty in collecting reliable, accurate morphometric data from phreatoicideans (see Wilson and Ho, 1996) almost necessitates that a morphometric data set

will always be secondary, and complementary, to the more easily obtained molecular data. It is, however, aimed, through the addition of a morphometric data set where required, to increase resolution and, with evidence of congruence among multiple data sets, provide increased confidence in conclusions. In this study, purely exploratory morphometric analyses are conducted using multivariate statistics: discriminant function analyses, in particular. This technique is primarily used to evaluate the ordination and morphometric distinctiveness of groups believed *a priori* to be different taxa, or to be morphologically differentiated (Thorpe, 1976; James and McCulloch, 1990; Lance, Kennedy and Leberg, 2000). Its few assumptions (James and McCulloch, 1990), that each group includes only one taxon and that variation is indeed categorical (taxonomically or geographically), are fairly robust and their violation does not easily negate results. While analyses are sensitive to sample size (the ratio of variables and cases examined) (James and McCulloch, 1990; Lance *et al.*, 2000), appropriate statistical (jackknifing) procedures in determining classification functions and examining the reclassification of individuals can greatly overcome many of these problems (Lance *et al.*, 2000). The statistical models employed in the analyses are also thought to be more rigorous and stringent than many other statistical approaches (Thorpe, 1976).

1.7.4) Species concepts

The conclusions of any systematic study, such as the present one, are directly contingent upon the species concepts used. While a critical evaluation of the multitude of species concepts appearing in the literature is beyond the scope of this study, a few comments as to the species concepts or operational definitions employed are warranted. Although most species concepts are in fundamental agreement as to the nature of a species, very few systematic studies place their findings within the framework of a particular concept or define what operational criteria

are used to delineate species (Wiens and Penkrot, 2002). In the present study, a multifaceted or multidimensional view is taken of species. This approach essentially follows the philosophies espoused by authors such as Sbordoni (1993) and Crowe (1999). Sbordoni (1993) believed that no single species concept could be applicable or be operational in all circumstances, or across all taxa. The author argued for a (pluralistic) multi-dimensional concept for the recognition of species, at least operationally (and suggested an exclusively phenetic approach, disregarded here). Under this concept, the available suite of unique characters would be sufficient to recognize a species, with the individual systematist best placed to evaluate the biological importance of (genetic, morphological, behavioural or ecological) characters in the taxa of interest. Crowe (1999) suggested that species concepts defined reproductively and bound by upper limits of reproductive isolation or cohesion, as postulated by the Biological (Mayr, 1942), "Ecological" (Bock, 1992), and Recognition (Paterson, 1985) Species Concepts, may either fail to recognize all the products of evolution or may define species too broadly, including para- or polyphyletic units. Likewise, concepts defining species phylogenetically or genealogically, e.g. the Phylogenetic Species Concepts of Cracraft (1989) or Nixon and Wheeler (1990), and the Genealogical Species Concept of Baum and Shaw (1995) and Shaw (1998, 2001), may diagnose taxa too narrowly (Crowe, 1999). Crowe (1999) suggested, as an operational alternative to these, that species (least-inclusive, biologically-meaningful, self-perpetuating evolutionary products) be recognized by congruent variation evidence from multiple, defensibly independent character sets, be they multiple unlinked molecular markers, morphology, behaviour or ecology.

The designation of Evolutionarily Significant Units (ESUs) and Management Units (MUs), according to the widely accepted criteria of Ryder (1986), Waples (1991) and Moritz (1994), remains an alternative to the recognition of species. The formulation of the ESU and MU

concepts and their application aimed to identify populations (or population groups) with independent and unique evolutionary trajectories for conservation purposes (Moritz, 1994). Initially, many of these concepts used molecular data exclusively as criteria (e.g. Moritz, 1994) and aimed to recognize unique, irreplaceable lineages and genetic diversity that was the product of historical isolation (Moritz, 1999, 2002). Adaptive (phenotypic and genetic) variation did not warrant any specific consideration under these concepts, as it could, conceivably, be maintained by conservation of the evolutionary processes that led to its creation and would enable its restoration, if lost (Moritz, 2002). Other concepts (e.g. Waples, 1995) advocated a more pluralistic approach (Moritz, 1999). Consequently, later concepts (e.g. Crandall *et al.*, 2000) aimed to recognize the component of (genetic) variation that was of adaptive significance by incorporating ecological and phenotypic criteria (e.g. ecological exchangeability) (but see Moritz, 2002). Despite differing criteria, these concepts all aimed to negate reliance on formal taxonomic designations, vague and inconsistent subspecies definitions (particularly in mammalian taxonomy), or reference to continually debated species concepts in identifying units worthy of conservation (Ryder, 1986; Bowen, 1998; Butlin and Tregenza, 1998; King, Pendleton and Villella, 1998; Roe and Lydeard, 1998). Conceivably, these concepts could also be applied to more accurately assess biodiversity, as biodiversity estimates may be directly dependent on the species concepts used (e.g. Peterson and Navarro-Sigüenza, 1999). Although the ESU concept was initially conceived to define conceptually different units to species and be more applicable at the intraspecific (population genetic) level, great conceptual overlap exists between species concepts and ESU concepts, with ESUs and species potentially representing equivalent entities as far as the criteria used to identify each is concerned (Moritz, 1994, 2002; Roe and Lydeard, 1998; Butlin and Tregenza, 1998). For example, Moritz's (1994) ESU criteria and the multifaceted species view of Crowe (1999), discussed above, are operationally very similar, while the independent and unique

evolutionary trajectories of individual ESUs (Moritz, 1994) implies that these are biologically-meaningful evolutionary products or species *sensu* Crowe (1999).

Depending on the particular species concept or ESU concept employed, units (species or ESUs) are usually delineated in a molecular context using distance criteria, or are based upon topologies and phylogenetic approaches. In phylogenetic and tree-based approaches (e.g. Wiens, 1999) units are typically identified on the basis of their constituent (mtDNA) haplotypes forming distinct monophyletic clades (e.g. Moritz, 1994), or their exclusivity relative to other included individuals (e.g. Wiens and Penkrot, 2002). Alternatively, units are defined by the possession of unique, diagnostic nucleotide characters, distinguishing them from other such units. These characters need not necessarily be fixed, as a statistically determined non-zero frequency cut-off can be used in cases of low levels of polymorphism (e.g. Wiens and Servedio, 2000). Similarly, in allozyme studies the presence of fixed allele differences among populations is routinely used to delineate species. Indeed, these are taken as evidence of reproductive isolation and Richardson *et al.* (1986), invoking the Biological Species Concept, have suggested that fixed allelic differences at more than two loci among populations collected in sympatry, or at more than 20% of assayed loci in allopatric populations, would warrant species recognition. Genetic distances (and sequence divergences) have also been touted as being ideally suited to species delimitation, as they are an objective measure of genetic divergence and are not tied to any species or speciation concept (see Ferguson, 2002; and references therein). Nonetheless, genetic distance is also often used within the framework of the Biological Species Concept, where genetic distance itself is taken as evidence of reproductive isolation (see Ferguson, 2002). Ferguson (2002) – who advocates a more indirect use of genetic distance and sequence divergences and the application of a Phylogenetic Species Concept (Cracraft, 1983), avoiding the invocation of the

conceptually intermediate evidence of reproductive isolation – suggested that demonstration of the lack of gene flow (by means of *F*-statistics or Analysis of Molecular Variance), and the presence of unique fixed genetic characters (fixed and unique allelic arrays or nucleotide characters), among populations would be sufficient for species delimitation. Here a holistic approach is adopted. As mentioned previously, concordance among the units identified through varied criteria enables greater confidence in the conclusions drawn.

Chapter 2: Cryptic species within the freshwater isopod *Mesamphisopus capensis* (Phreatoicidea: Mesamphisopodidae) in the Western Cape, South Africa: allozyme, 12S rRNA sequence data and morphometric evidence.

2.1) Introduction

The freshwater isopod *Mesamphisopus capensis* was initially described from Table Mountain (Cape Town, South Africa) by Barnard (1913, 1914) and placed in the genus *Phreatoicus*, which then included species described from Australia and New Zealand. *Phreatoicus capensis* was regarded to be widespread and morphological variation among populations from only three localities warranted the later description of varieties (Barnard, 1927, 1940). These varieties were subsequently afforded specific status and included, together with *P. capensis*, in the endemic South African genus *Mesamphisopus* (Nicholls, 1943; Kensley, 2001). Limited collection records (South African Museum, Cape Town) and sparse literature (Barnard, 1927, 1940) suggest that *Mesamphisopus capensis* is distributed across the south-western portion of the Western Cape province and extends eastwards towards the temperate forests, some 500 km east of Cape Town, along the South African south coast. The identification of specimens from many of the more eastern localities predates, and is questionable in light of, the most recently compiled key (Kensley, 2001). Harrison and Barnard (1972) had regarded populations of *M. capensis* from the mountains of the Cape Peninsula and the Hottentot's Holland Mountains, separated by the low-lying Cape Flats to be conspecific, although having been separated since the late Tertiary. These authors stated that slight, consistent morphological differences were observed, but provided no further information. Harrison, working from the late Keppel Barnard's notes, could possibly have

been referring to Barnard's (1927, 1940) varieties, and eventual species (Nicholls, 1943; Kensley, 2001).

Mesamphisopus capensis is defined in Kensley's (2001) key by the absence of a pair of dorsal sub-apical robust setae, Kensley's (2001: 70) "spines", on the pleotelson, typical of other species within *Mesamphisopus*. The typical morphological conservatism of the Phreatoicidea, however, coupled with intraspecific variation (Wilson and Ho, 1996), makes cursory identification of specimens problematic. For example, Barnard (1927) highlighted considerable variation with regard to pleotelson and gnathopod shape within individual *M. capensis* populations (e.g. Barnard, 1927: Fig. 5). Unrecognized diagnostic characters may possibly be obscured by this variation and geographically disjunct populations, initially identified as *Mesamphisopus capensis*, may represent a complex of cryptic species.

The paramelitid amphipods of the Western Cape provide an example of how (partly due to the unavailability of suitable, particularly genetic, methodologies) the failure to recognize the existence of cryptic species complexes has led to an initial inaccurate assessment of the biodiversity of the region. For example, *Paramelita capensis* (Barnard, 1916) and *P. nigroculus* (Barnard, 1916) were initially thought to be single widespread species (Barnard, 1927; Griffiths, 1981). Through intensive sampling regimes, coupled with genetic and morphometric analyses, *P. capensis* populations were instead found to represent a complex of five species (Stewart, 1992). This approach has led to the further identification and description of numerous new species, so that, entirely, 25 species belonging to three genera have been documented from the Western Cape (Stewart and Griffiths, 1992, 1995; Stewart, Snaddon and Griffiths, 1994; Griffiths and Stewart, 1996). Whether this diversity is reflected within the phreatoicidean isopods is hitherto unknown.

Against a backdrop of increasing anthropogenic threat to both fauna and habitat (see Barnard, 1927; Rebelo, 1992; Cowling, MacDonald and Simmons, 1996; Picker and Samways, 1996), it becomes imperative that the diversity within *Mesamphisopus capensis* (as well as other similarly unique, narrowly endemic, or poorly dispersing invertebrate species) be documented and conservation units identified. Accurate identification of biological diversity is paramount to its conservation (Roe and Lydeard, 1998). Genetic diversity is also increasingly being emphasized as a prerequisite for adaptation, evolutionary success and long-term survival of species (Mulvey, Liu and Kandl, 1998), a fact recognized in South African conservation policy (DEAT, 1997). Thus, the description of population differentiation serves to identify more populations to be conserved for the maintenance of sufficient variation for species survival (Newton *et al.*, 1999). Further, the geographic distributions, and demographic and ecological characteristics and requirements of widespread species are very different from those of the independent, constituent species of a species complex. The latter are more likely to be negatively affected by environmental perturbations and habitat destruction (Duffy, 1996).

In the present study, genetic differentiation, using both allozyme and mtDNA 12S rRNA sequence data, as well as morphometric variation, were examined within *M. capensis* across two mountain ranges, to determine whether disjunct populations were indeed conspecific. A further aim was to discern distinct lineages or identify units for conservation, in light of widely applied Evolutionarily Significant Unit (ESU) and Management Unit (MU) criteria (Ryder, 1986; Waples, 1991; Moritz, 1994). Lastly, collections made from Table Mountain were considered further to determine whether more than one taxon/species was present. When completing the last revision of the Phreatoicidea, Nicholls (1943, 1944) had examined numerous, presumably mature, individuals received from Barnard (see Nicholls 1943: 31), but

was hesitant to discuss or identify, a single specimen collected from Table Mountain (Nicholls, 1944: 154). Nicholls' (1943, 1944) hesitancy to comment on this specimen indicates that the specimen was immature, damaged, or represented an unknown morphotype to which he had no further access to material.

2.2) Materials and methods

2.2.1) Collections

Sampling focused, primarily, on the known collection localities of *M. capensis* on the Cape Peninsula and the Hottentot's Holland Mountains. Additional localities were sampled if they were accessible and if individuals collected from these localities could be identified as *M. capensis*, using the key compiled by Kensley (2001). Individuals were regarded as *M. capensis* if the pair of sub-apical robust setae was lacking dorsally on the pleotelson (Kensley, 2001). Due to the easier access to suitable collection localities, individuals were sampled from eight localities from the Cape Peninsula (including four from Table Mountain), while only three were sampled from the Hottentot's Holland Mountains (Fig. 2.1). Intermediate collection localities in the Hottentot's Holland Mountains were generally inaccessible, while individuals from geographically proximate localities could not be identified as *M. capensis* (see Chapter 3). It was initially aimed to collect approximately 50 individuals from each locality to provide large sample sizes (of 30 individuals or more) for the allozyme analysis and to retain enough individuals for DNA-sequencing, morphometric analyses and as voucher specimens. However, at certain localities, where similar collection effort suggested smaller population sizes, fewer individuals were caught. The sample sizes in the allozyme analysis

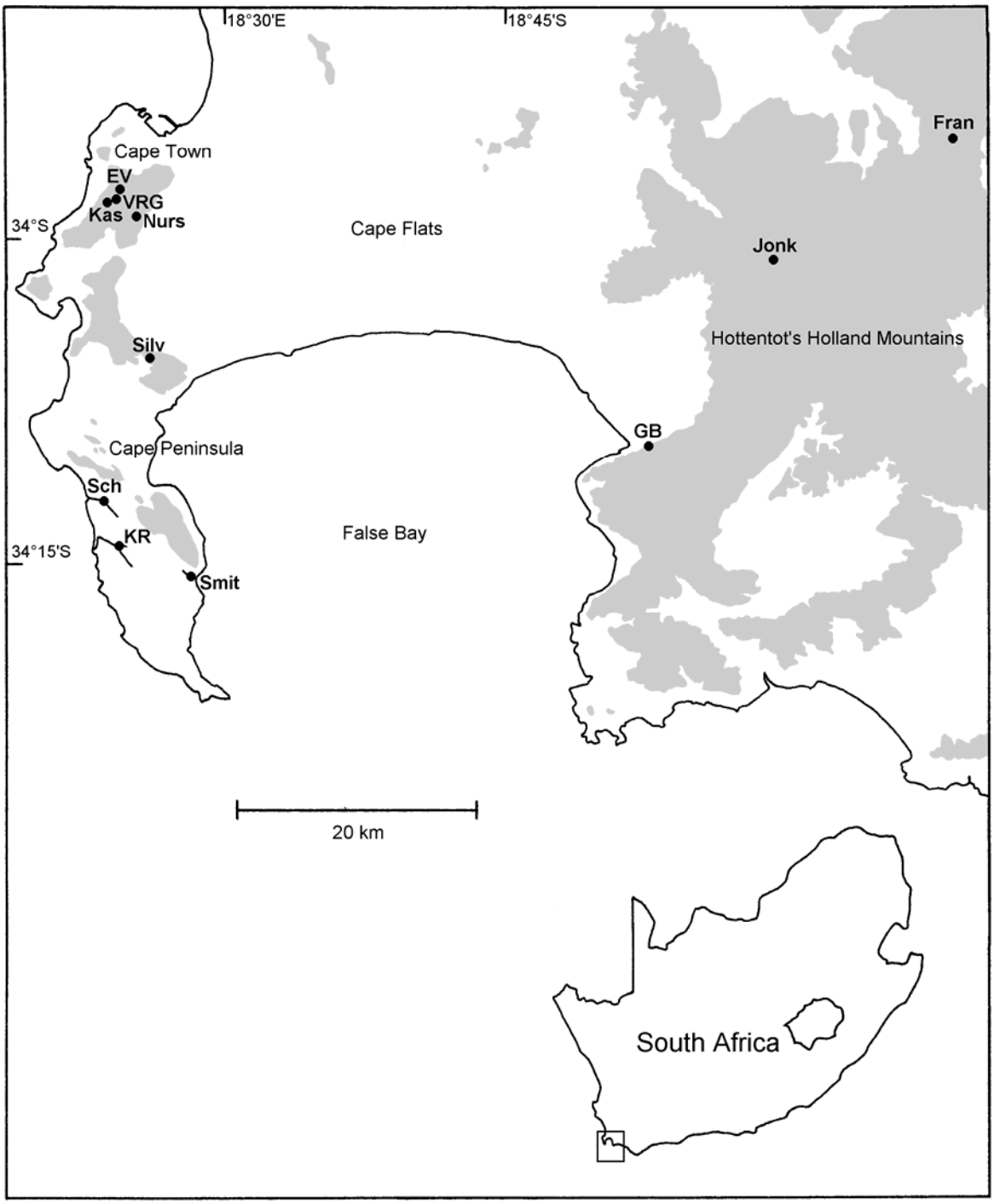


Figure 2.1: Collection localities of putative *Mesamphisopus capensis* populations from the Cape Peninsula and Hottentot's Holland Mountains in the Western Cape, South Africa: Echo Valley (EV), Valley of the Red Gods (VRG), Kasteelspoort (Kas), Nursery Ravine (Nurs), Silvermine (Silv), Smitswinkelbaai (Smit), Krom River (KR), Schusters River (Sch), Franschhoek (Fran), Jonkershoek (Jonk) and Gordon's Bay (GB). Shaded areas represent areas of greater than 300 m elevation.

were thus smaller for these populations. Isopods were collected from the shallow pools and slow-flowing seepage streams of these upper catchments, by sifting through the sand and mud sediment using hand-nets, or by picking individuals from matted plant material. Individuals to be used in genetic analyses were snap frozen, while remaining individuals were placed in absolute ethanol.

2.2.2) Allozyme electrophoresis

Between 19 and 70 individuals from each sampling locality were individually homogenized using a glass rod attached to a variable-speed, electric motor in 20 – 50 µL of 0.01 M Tris pH 8.0 extraction buffer. Prior to electrophoresis water-soluble proteins were separated from the homogenate by centrifugation at 13 000 $r.min^{-1}$ for three min. Filter paper wicks (Whatman #3) were dipped in the supernatant and inserted into the origin cut in the 13% hydrolysed starch gel (Aldrich Chemical Co., Milwaukee, U. S. A.; Fluka BioChemica, Steinheim, Switzerland)).

Gels were run (2 - 4 ºC) at 40 mA for five hours. Three electrophoretic buffer systems were used (Table 2.1): (A) a discontinuous Tris-citrate-borate-lithium hydroxide system, gel pH 8.7, electrode pH 8.0 (Ridgeway, Sherburne and Lewis, 1970); (B) a continuous Tris-borate-EDTA buffer system, gel and electrode pH 8.6 (Markert and Faulhaber, 1965); and (C) a continuous amine-citrate buffer, adapted from Clayton and Tretiak (1972), with a gel pH 6.5 and a electrode buffer pH 6.3. Staining for enzymatic activity followed standard protocols (Shaw and Prasad, 1970) with histochemical reagents being applied in a 2% agar overlay (Table 2.1). At each locus, the mobility of each electromorph was expressed relative to the mobility of the most common allele, designated a value of 100, in the Franschhoek

Table 2.1: Enzyme and buffer systems used in the investigation of allozyme differentiation among populations of *Mesamphisopus* studied. Consult text for further details of the electrophoretic buffer systems used.

Enzyme	Abbreviation	Loci	E.C. Number	Buffer
Aldehyde oxidase	*Ao*	1	1.2.3.1	B
Arginine kinase	*Ark*	1	2.7.3.3	A
Glucose-6-phosphate isomerase	*Gpi*	1	5.3.1.9	A
Hexokinase	*Hk*	1	2.7.1.1	B
Isocitrate dehydrogenase	*Idh*	1	1.1.1.42	C
Lactate dehydrogenase	*Ldh*	1	1.1.1.27	C
Malate dehydrogenase	*Mdh*	2	1.1.1.37	C
Malic enzyme	*Me*	1	1.1.1.40	B
Peptidase (leucine-tyrosine as substrate)	*Lt*	2	3.4.11.-	A
Phosphoglucomutase	*Pgm*	1	2.7.5.1	B

(Hottentot's Holland Mountains) population, arbitrarily chosen as the reference population. When more than one locus was expressed for a specific enzyme, the most anodally migrating locus was numbered one, with the remaining loci labelled sequentially.

Allozyme data were analysed numerically using the BIOSYS-1 package (Swofford and Selander, 1981). Allele and genotype frequencies were calculated for the 11 populations. A χ^2 goodness-of-fit test and an exact test of probability were used to test for significant deviation of observed genotype frequencies from those expected under Hardy-Weinberg equilibrium in each population for each case of polymorphism. Significance values were examined against table-wide significance using the sequential Bonferroni procedure (Rice, 1989) in order to eliminate false assignments of significance by chance in multiple tests of the same hypothesis. Observed (H_O) and expected (H_E) heterozygosities were calculated using Nei's (1978) unbiased estimates. The percentage of polymorphic loci was determined using a 95% criterion (loci were regarded as polymorphic if the frequency of the most common allele was less than 0.95). Nei's (1978) mean unbiased genetic identity (*I*) and genetic distance (*D*) were calculated among populations from the allele frequencies. The genetic identity values were used to construct a dendrogram of genetic similarity among populations using UPGMA (Sneath and Sokal, 1973). In the majority of cases, the combination of Nei's (1978) distance measure (and, hence, identity measure) and the UPGMA algorithm retrieves dendrogram topologies that are congruent to topologies derived by cladistic analyses of other data sets, for example morphological or sequence data (see Wiens, 1999). In addition, a principal components analysis was performed, with sampling localities as cases and the frequencies of alleles occurring at the polymorphic loci as variables. All principal components (factors) with eigenvalues > 1 were extracted, and preliminary ordination of populations visualized by

plotting cases according to their respective scores along the first three principal components extracted.

Partitioning of genetic variation was examined across the entire sample, and within regions, using Weir and Cockerham's (1984) θ-estimates. These were calculated for individual loci and across all loci, using FSTAT 2.9.3 (Goudet, 2001). Sampling localities were also pooled within regions, enabling a direct comparison between the Cape Peninsula and Hottentot's Holland Mountains.

2.2.3) DNA sequencing and sequence data analyses

Preliminary sequencing of the 12S rRNA gene-region of five individuals from each of the Echo Valley and Franschhoek populations revealed a single haplotype to be present within each of these sampling localities, while the near fixation of cytochrome oxidase subunit I (COI) haplotypes has been observed in several examined populations (Chapter 3). Similarly, Wetzer (2001) found, albeit with very limited sampling, single 12S rRNA and COI haplotypes to be present in individual phreatoicidean populations. Consequently, total genomic DNA was extracted from one individual per locality, as well as from one specimen of *M. penicillatus*, which was used as a outgroup, using a Qiagen DNEasy Tissue extraction kit and following the manufacturer's instructions. The choice of outgroup was determined by the species' basal position within a molecular phylogeny for *Mesamphisopus* (Chapter 5).

Polymerase chain reactions (PCRs) were set up in 25 μL volumes, including millipore H_20, ~5 ng.μL^{-1} template DNA, 10X Mg^{2+}-free buffer, 3 mM.μL^{-1} $MgCl_2$, 0.2 mM.μL^{-1} of each dNTP, 0.2 μM.μL^{-1} of each of the peracarid-specific 12S primer pair (12SCRF and 12SCRR;

Wetzer, 2001), and 0.5 units of super-thermal DNA polymerase (Southern Cross Biotechnologies). The PCR-regime included an initial denaturing step at 94 °C for 5 min, followed by 35 cycles of denaturing (94 °C) for 15 s, annealing (52 °C) for 1 min, and extension (72 °C) for 1.5 min. This was followed by a final cycle of annealing for 5 min and extension for 15 min. Each series of PCR reactions included a template-free negative control to test for contamination. PCR products were visualized under UV light after electrophoresis in a 1% agarose gel containing ethidium-bromide. Products were purified using a Qiagen QiaQuick purification kit, following manufacturer's directions. Purified products were cycle sequenced (both forward and reverse strands) following standard protocols, using 3 μL purified PCR product, 3 μL of a 1 μM solution of the respective primer, and 4 μL of flourescent-dye terminators (ABI PRISM Dye Terminator Cycle Sequencing Reaction Kit, Perkin Elmer). Samples were analysed using an AB 3100 automated sequencer.

Each sequence was visually inspected and checked for base ambiguity against its respective electropherogram using Sequence Navigator (Applied Biosystems) and a consensus sequence created for each sample. Sequences were aligned using Clustal X 1.81 (Thompson *et al.*, 1997) with the default parameters applied. Alignments were subsequently inspected manually.

Phylogenetic analyses were performed using PAUP*4b10 (Swofford, 2001). Parsimony (MP) analysis was performed regarding gaps (indels) as missing data, with a branch and bound search employed to find the most parsimonious tree. Characters were unweighted in all analyses. Phylogenetic support for nodes was determined by performing 1000 bootstrap replicates (Felsenstein, 1985) on the data set, using a random addition of sequences (1000 iterations).

To determine the appropriate model of nucleotide substitution for the maximum likelihood (ML) analysis, MODELTEST 3.06 (Posada and Crandall, 1998) was used. A neighbour-joining (NJ) tree was also constructed using "uncorrected p" sequence divergence obtained in pair-wise comparisons of representatives. In the ML and NJ analyses, bootstrap support was calculated using 100 and 10000 resampling replicates, respectively, together with a random addition of sequences (100 replicates) in the case of the ML analysis.

The potential monophyly of the two regions, given the apparent age of the separation of populations of each (Harrison and Barnard, 1972), was investigated. The SH (Shimodaira and Hasegawa, 1999) test was used to evaluate and compare the likelihood, given the data set and model, of a topology constrained to reflect the monophyly of each of the two regions to the likelihoods of other proposed topologies.

Further, the log-likelihood scores of the unconstrained ML tree and a ML tree with a molecular clock enforced (under the determined model) were compared, using a Likelihood Ratio Test (LRT; Felsenstein, 1981). This tests for overall rate constancy among lineages to determine whether a molecular clock can be applied to the data set.

2.2.4) Morphometric analyses

Morphometric analyses were conducted to determine the extent to which operational taxonomic units (OTUs) identified by genetic analyses could be ordinated or discriminated. From the limited morphometric and life history studies conducted on phreatoicideans, it is apparent that growth in these isopods is not linear or continuous (Barnard, 1927; Wilson and Ho, 1996) – not unexpected, given the ecdysis (moult) cycles of peracarid crustaceans. An

added consideration is the fact that population structure, in terms of the frequency representation of particular age cohorts and size classes (as well as sex ratio), has been shown to be seasonally determined and to vary substantially throughout the year (Barnard, 1927; Wilson and Ho, 1996; Wilson and Fenwick, 1999). Such differing age and size structures among sampled populations may be problematic in morphometric analyses and, if unaccounted for, can lead to the detection of differentiation among populations (Allegrucci *et al.*, 1992; Cumberlidge, 1993a, b; Daniels *et al.*, 1998b), aside from, and possibly obscuring, the morphometric patterns of interest. In an attempt to minimize these effects (by minimizing within-group variation attributable to immature individuals and by minimizing allometric differences among groups due to different size classes being sampled), only the largest male individuals in each population were examined. These individuals were also determined to be mature adults on the basis of the extent of development of the penes, as described in Wilson and Ho (1996), and Wilson and Fenwick (1999). Five of the largest ethanol preserved males from each locality were dissected and digitally photographed using a Leitz stereoscopic dissection microscope and a JVC TK-C1381 digital camera. In the case of the Valley of the Red Gods sample, two individuals were examined as only these were appreciably larger than the remaining males and were thought to belong to the largest size class. Following calibration using a micrometer-slide photographed under identical magnifications, absolute measurements of 47 variables (including 22 cephalon, pereon, pleon and pleotelson, and 25 pereopod dimensions) were taken from the captured images using Leica QWin and Leica Lida software (Leica Imaging Systems, 1996).

To eliminate possible confounding effects of asymmetry, insofar as was possible, only the right pereopods were measured. If these were missing, damaged or incomplete, they were substituted with the corresponding left limb. Although no evidence, as yet, suggests the

presence of heterochely, and substantial differences between right and left gnathopods were only observed where these limbs were damaged and regenerated, only the right pereopod I (gnathopod) was included in the analysis. Further missing data were substituted with the mean for the respective group, in order to maximize the number of cases.

Morphometric discrimination among the five identified units (OTUs) was investigated by means of standard discriminant function analyses, performed using the body and pereopod variables, independently. All data were log-transformed (common logarithms) prior to analysis and all analyses were performed using STATISTICA 6.0 (Statsoft, Inc., 2001).

For each analysis, classification functions (linear combinations of variables that optimally differentiate *a priori* determined groups) were calculated, using a jack-knife procedure. These classification functions were then used to reassign individuals to groups, based on *a posteriori* probabilities. Prior classification probabilities were kept equal for all groups. Scatterplots of scores for all individuals for the first two canonical (discriminant) functions were made to visualize the extent of differentiation between groups.

2.3) Results

2.3.1) Allozyme electrophoresis

Of an initial array of 29 enzyme systems screened, only 12 loci provided reliably interpretable zymograms and were included in the study. Eleven of the 12 loci were polymorphic, with *Lt-2* being monomorphic within and across all populations. Allele frequencies at the

polymorphic loci and genetic variability measures are presented in Appendix 2 and Table 2.2, respectively. The number of alleles per polymorphic locus varied between two (*Ao*, *Lt-1*, *Mdh-1* and *Mdh-2*) and ten (*Gpi*). While the mean number of alleles per locus varied between 1.083 ±0.289 (SD) (Nursery Ravine) and 1.667 ±1.155 (Silvermine), the largest number of alleles found at a locus in a single population was five at the *Gpi*-locus in the Silvermine population. Both observed (direct-count) heterozygosity (H_O) and expected heterozygosity (H_E) varied greatly among populations, ranging from 0.003 ±0.010 to 0.088 ±0.197, and from 0.003 ±0.010 to 0.133 ±0.218, respectively. The percentage of polymorphic loci (95% criterion) varied between 0% (Echo Valley and Nursery Ravine populations) and 25.00% (Silvermine and Jonkershoek populations). No loci were found to be polymorphic across all sampling localities, while the *Lt-1*- and *Mdh-1*- loci, although polymorphic within the entire data set, were monomorphic within individual populations.

Of 34 cases of polymorphism involving all populations and loci, five (14.71%) were found not to conform to Hardy-Weinberg expected frequencies (following Bonferroni correction), due to a deficit of heterozygous individuals (Appendix 2). Where more than two alleles were present at a particular locus within a population, the pooling of common/rare-allele heterozygotes, and rare-allele homozygotes with rare-allele heterozygotes, respectively, brought about conformance to Hardy-Weinberg expectations at the *Hk*-locus in the Schusters River population ($\chi^2 = 0.065$, $P = 0.799$), but failed to do so at the *Pgm*-locus in the Franschhoek population. Testing using exact probabilities revealed a single significant deviation from Hardy-Weinberg expectations: the *Ao*-locus ($P < 0.001$) in the Franschhoek population.

Table 2.2: Genetic variability measures for the 11 populations of *Mesamphisopus* studied. These include the mean number of alleles per locus (A), mean observed (H_O) and expected (H_E) heterozygosities, and the percentage of polymorphic loci ($P_{95\%}$) using a 95% criterion. Standard deviations are presented below the respective variability estimates. Population names are abbreviated as in Figure 2.1.

	Population										
	EV	VRG	Kas	Nurs	Silv	Smit	KR	Sch	Fran	Jonk	GB
A	1.417 ±0.515	1.167 ±0.389	1.167 ±0.389	1.083 ±0.289	1.667 ±1.155	1.333 ±0.888	1.333 ±0.492	1.417 ±0.669	1.333 ±0.651	1.417 ±0.669	1.167 ±0.389
H_O	0.008 ±0.011	0.011 ±0.032	0.011 ±0.031	0.003 ±0.010	0.088 ±0.171	0.063 ±0.161	0.058 ±0.113	0.088 ±0.197	0.013 ±0.030	0.085 ±0.151	0.017 ±0.043
H_E	0.011 ±0.016	0.011 ±0.031	0.010 ±0.030	0.003 ±0.010	0.096 ±0.177	0.095 ±0.224	0.078 ±0.161	0.087 ±0.180	0.038 ±0.105	0.133 ±0.218	0.016 ±0.041
$P_{95\%}$	0.00	8.33	8.33	0.00	25.00	16.67	16.67	16.67	8.33	25.00	8.33

The dendrogram (Fig. 2.2) constructed from the matrix of genetic identities (*I*) for among-population comparisons (Table 2.3) revealed a marked divergence between the Gordon's Bay population and the remaining populations. The Gordon's Bay population was separated from these by a mean genetic identity (*I*) of 0.454 ±0.059, with fixed allelic differences observed at the *Idh*- and *Mdh-1*-loci.

The remaining Hottentot's Holland Mountain populations (Franschhoek and Jonkershoek) were next separated from the Peninsula populations at a mean *I*-value of 0.491 ±0.067. These three populations from the Hottentot's Holland Mountains were separated by identity values between 0.367 and 0.703, while fixed allelic differences at the *Gpi*-, *Idh*-, *Ldh*-, *Lt-1*- and *Me*-loci identified individual populations or distinguished a pair of populations from the third.

Among the populations collected from the Cape Peninsula, the Silvermine population was shown to be genetically distinct, separated (I = 0.825 ±0.024) from the remaining Peninsula populations by a fixed allelic difference at the *Idh*-locus, and significant heterogeneity at the *Gpi*-, *Hk*-, *Ldh*-, *Mdh-2*- and *Pgm*-loci (all $P < 0.01$). Allele frequency differences, rather than qualitatively different sets of alleles, and the presence of unique rare alleles led to the distinction of the Smitswinkelbaai, Krom River, Schusters River and Table Mountain (Echo Valley, Valley of the Red Gods, Kasteelspoort and Nursery Ravine) populations. The Krom River and Schusters River populations, clustering together (I = 0.932), were separated from the remaining populations (I = 0.879 ±0.032) due to the high frequencies of the Hk^{95} and Ldh^{100} alleles in these two populations. The Hk^{85} and Ldh^{80} alleles were more abundant in the remaining populations. While the Smitswinkelbaai population clustered with the Table Mountain populations at an identity-value of 0.962 ±0.001, the populations collected from

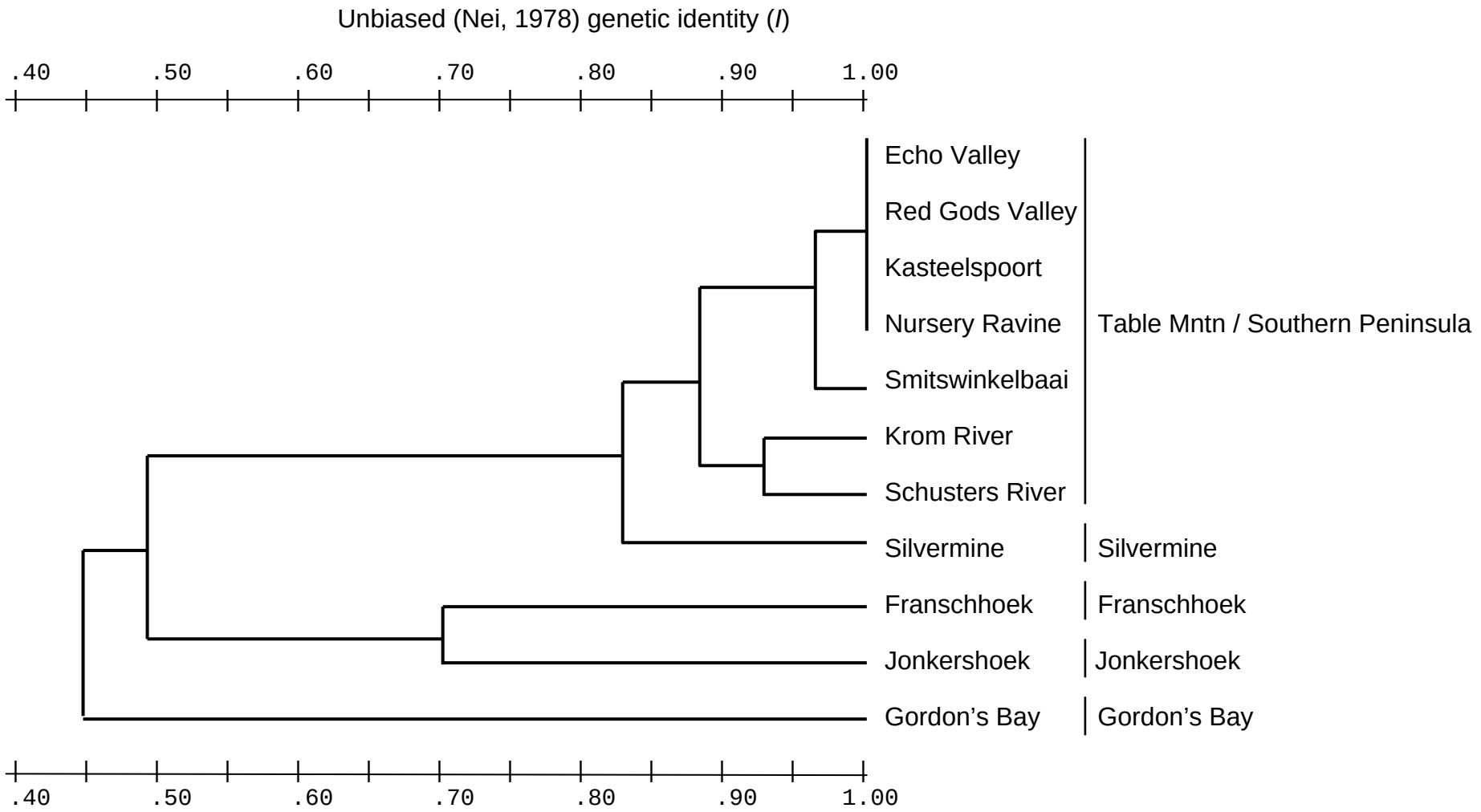


Figure 2.2: UPGMA-dendrogram of genetic similarity between 11 *Mesamphisopus* populations studied, constructed from the matrix of Nei's (1978) unbiased genetic identities obtained in pair-wise comparison among populations. The five genetically distinct geographic units identified on the basis of allele frequency and sequence data are indicated to the right of the dendrogram.

Table 2.3: Nei's (1978) unbiased genetic identity (above diagonal) and unbiased genetic distance (below diagonal) obtained from pair-wise comparison among the 11 *Mesamphisopus* populations studied.

	Populations										
	1	2	3	4	5	6	7	8	9	10	11
Echo Valley (1)	---	1.000	1.000	1.000	0.823	0.963	0.854	0.883	0.491	0.418	0.422
Valley of the Red Gods (2)	0.000	---	1.000	1.000	0.823	0.962	0.851	0.879	0.489	0.417	0.418
Kasteelspoort (3)	0.000	0.000	---	1.000	0.823	0.962	0.851	0.879	0.489	0.417	0.418
Nursery Ravine (4)	0.000	0.000	0.000	---	0.822	0.962	0.852	0.882	0.489	0.416	0.421
Silvermine (5)	0.195	0.195	0.195	0.196	---	0.869	0.830	0.787	0.503	0.445	0.445
Smitswinkelbaai (6)	0.038	0.038	0.038	0.039	0.141	---	0.954	0.906	0.551	0.447	0.482
Krom River (7)	0.158	0.161	0.161	0.160	0.186	0.047	---	0.932	0.629	0.492	0.523
Schusters River (8)	0.124	0.129	0.129	0.126	0.239	0.098	0.071	---	0.622	0.534	0.471
Franschhoek (9)	0.712	0.715	0.715	0.716	0.687	0.595	0.464	0.474	---	0.703	0.570
Jonkershoek (10)	0.872	0.876	0.876	0.877	0.809	0.806	0.710	0.627	0.352	---	0.367
Gordons Bay (11)	0.863	0.873	0.873	0.866	0.809	0.729	0.648	0.753	0.562	1.004	---

Table Mountain itself were genetically homogenous, with *I*-values of 1.000 obtained in all among population comparisons.

Comparison between the two regions (Cape Peninsula and Hottentot's Holland Mountains) resulted in a mean identity value 0.477 ±0.062. The two regions could be distinguished, primarily, by the *Ark*-locus. Populations of the Hottentot's Holland Mountains were fixed for the allele Ark^{100}, with Ark^{115} and the rare allele Ark^{130}, unique to the Echo Valley population, occurring in the Peninsula populations. Contingency χ^2-analyses revealed significant ($P < 0.001$) heterogeneity between the two regions at all polymorphic loci with the exception of *Mdh-2*.

In the principal components analysis of allele frequencies, seven factors were extracted from the 42 variables (alleles occurring at polymorphic loci). The first three factors, along which the populations were plotted, had eigenvalues of 12.732, 8.459 and 8.019, respectively, and accounted for 69.55% of the variation observed (30.32%, 20.14% and 19.09%, respectively). The scatterplot (Figure 2.3) revealed, firstly, the similarity of populations from Table Mountain (1 to 4), Smitswinkelbaai (6), Krom River (7) and Schusters River (8) along these three principal components. Secondly, the distinction between the Silvermine (5) population and the remaining Peninsula populations was substantiated. Thirdly, the three Hottentot's Holland Mountain populations were distinguished from the Peninsula populations by higher scores along the first principal component, while they were individually distinct.

Weir and Cockerham's (1984) θ-estimates (Table 2.4) indicated substantial structuring among individual populations across the entire sample. This was evident considering all loci ($\theta = 0.871$), as well as all individual polymorphic loci, with the exception of *Mdh-2* ($\theta = 0.000$).

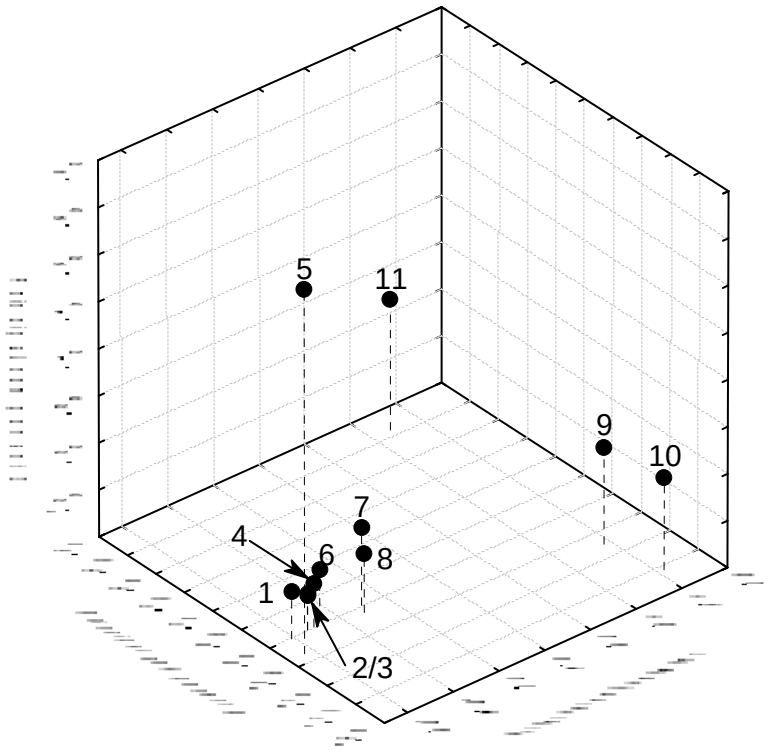


Figure 2.3: Populations plotted according to scores along the first three principal components extracted in the principal components analysis from the frequencies of 42 alleles occurring at 11 polymorphic loci. Populations are numbered as follows: (1) Echo Valley, (2) Valley of the Red Gods, (3) Kasteelspoort, (4) Nursery Ravine, (5) Silvermine, (6) Smitswinkelbaai, (7) Krom River, (8) Schusters River, (9) Franschhoek, (10) Jonkershoek, and (11) Gordon's Bay.

Table 2.4: Weir and Cockerham's (1984) θ-estimates for comparisons among (a) the eleven *Mesamphisopus* populations studied, (b) populations from the Cape Peninsula, (c) populations of the Hottentot's Holland Mountains, and (d) the two regions with populations pooled within each. Estimates are given over all loci, and at individual polymorphic loci. 95% Confidence intervals (determined by 1000 bootstrap replicates) are presented in parentheses for θ-estimates calculated over all loci.

		Weir and Cockerham's (1984) θ											
	Hierarchical level	Overall	*Ao*	*Ark*	*Gpi*	*Hk*	*Idh*	*Ldh*	*Lt-1*	*Mdh-1*	*Mdh-2*	*Me*	*Pgm*
(a)	All populations	0.871 (0.786 – 0.947)	0.239	0.994	0.822	0.742	0.991	0.904	1.000	1.000	0.000	0.941	0.793
(b)	Cape Peninsula	0.688 (0.532 – 0.833)	-0.006	-0.007	0.679	0.596	0.975	0.731	---	---	0.000	0.032	0.080
(c)	Hottentot's Holland	0.895 (0.724 – 0.991)	0.139	---	0.966	0.464	1.000	0.987	1.000	1.000	---	1.000	0.893
(d)	Two regions (pooled)	0.673 (0.544 – 0.798)	0.313	0.997	0.545	0.645	0.630	0.667	0.240	0.376	-0.002	0.805	0.347

While the overall estimate (θ = 0.688) and individual estimates at certain loci (e.g. *Gpi*, *Hk*, *Idh* and *Ldh*) indicated substantial differentiation among populations sampled from the Cape Peninsula (Table 2.4), estimates from other loci indicated only slight to moderate differentiation. Populations of the Hottentot's Holland Mountain region showed large population differentiation overall (θ = 0.895) and at all individual polymorphic loci (Table 2.4), with the exception of the *Ao*-locus, where differentiation was moderate. Direct comparison of these two regions, by pooling sampling localities within each, yielded an overall θ of 0.673 (Table 2.4). Individual loci showed θ-estimates typical of greatly differentiated populations, with the exception of the *Mdh-2*-locus (θ = -0.002).

In combination, these data supported the recognition of five OTUs or geographic populations (Fig. 2.2) for further examination. These included the individual Silvermine, Franschhoek, Jonkershoek and Gordon's Bay populations, and a large group (regarded as a "population" for the purpose of further discussion) formed by the Table Mountain (Echo Valley, Valley of the Red Gods, Kasteelspoort and Nursery Ravine) and Southern Peninsula (Smitswinkelbaai, Krom River and Schusters River) populations.

2.3.2) Sequence data analyses

A 328 bp region of the 12S rRNA gene could be unambiguously aligned (Appendix 3) for the ingroup and outgroup (*M. penicillatus*) specimens. Sequences, with individual lengths of 319 – 337 nucleotides, have been deposited in GenBank (accession numbers AY322172 – AY322183 inclusive). The average base frequencies (A = 0.406, C = 0.129, G = 0.112, T = 0.353) were characteristic of the 12S rRNA gene region in other isopods, and likewise the region was typically adenine and thymine rich (Wetzer, 2001).

The mean sequence divergence ("uncorrected p" distances; Table 2.5) between the outgroup and ingroup sequences was 16.85% ±1.31. Sequence divergence among the ingroup individuals ranged from 0.0% to 11.01%, with a mean sequence divergence of 9.79% ±0.74 separating representative individuals from the Cape Peninsula and Hottentot's Holland Mountains. Grouped according to the units identified by the allozyme analyses, a mean sequence divergence of 3.36% ±0.30 distinguished the Silvermine individual from the remaining Cape Peninsula individuals, while sequence divergences of 0.93 to 4.99% were found among the Hottentot's Holland Mountain individuals.

Thirty-four of 74 variable characters were parsimony informative and yielded a single tree of 52 steps (CI = 0.808, RI = 0.878, rescaled CI = 0.709). MODELTEST revealed that the use of the Tamura and Nei (1993) model of nucleotide substitution together with a gamma-distribution of among-site rate variation (TrN + Γ) resulted in a significantly improved likelihood score for maximum likelihood analyses over other less parameter-rich models. Estimated base frequencies (A = 0.417, C = 0.127, G = 0.108, T = 0.348) were inputted, together with the following rate matrix: $R_1 = R_3 = R_4 = R_6 = 1.000$, $R_2 = 3.586$, and $R_5 = 12.600$. The proportion of invariant sites was set to zero and the α-shape parameter estimated at 0.271.

Identical tree topologies were obtained in the MP and NJ analyses. Two monophyletic clades (Fig. 2.4), comprising individuals sampled from the Cape Peninsula, and Hottentot's Holland Mountains respectively, were identified. While the Hottentot's Holland clade received fair bootstrap support (≥ 68%), the clade comprising the Cape Peninsula representatives was supported by 100% bootstrap in both analyses. Within the Cape Peninsula clade, the Silvermine representative was placed as a sister taxon to the well-supported (≥ 75%) clade

Table 2.5: Sequence divergence ("uncorrected p") among representative individuals of eleven putative *Mesamphisopus capensis* populations and one outgroup (*M. penicillatus*) individual.

	Representative haplotype											
	Outgroup	1	2	3	4	5	6	7	8	9	10	11
M. penicillatus (outgroup)	---											
Echo Valley (1)	0.177	---										
Valley of the Red Gods (2)	0.177	0.006	---									
Kasteelspoort (3)	0.177	0.006	0.000	---								
Nursery Ravine (4)	0.177	0.006	0.006	0.006	---							
Silvermine (5)	0.170	0.034	0.034	0.034	0.034	---						
Smitswinkelbaai (6)	0.171	0.006	0.006	0.006	0.006	0.028	---					
Krom River (7)	0.180	0.016	0.016	0.016	0.016	0.038	0.009	---				
Schuster River (8)	0.173	0.009	0.016	0.016	0.016	0.031	0.009	0.019	---			
Franschhoek (9)	0.161	0.097	0.097	0.097	0.104	0.103	0.097	0.107	0.088	---		
Jonkershoek (10)	0.155	0.094	0.088	0.088	0.094	0.094	0.088	0.097	0.085	0.009	---	
Gordon's Bay (11)	0.136	0.107	0.107	0.107	0.107	0.094	0.101	0.110	0.098	0.050	0.043	---

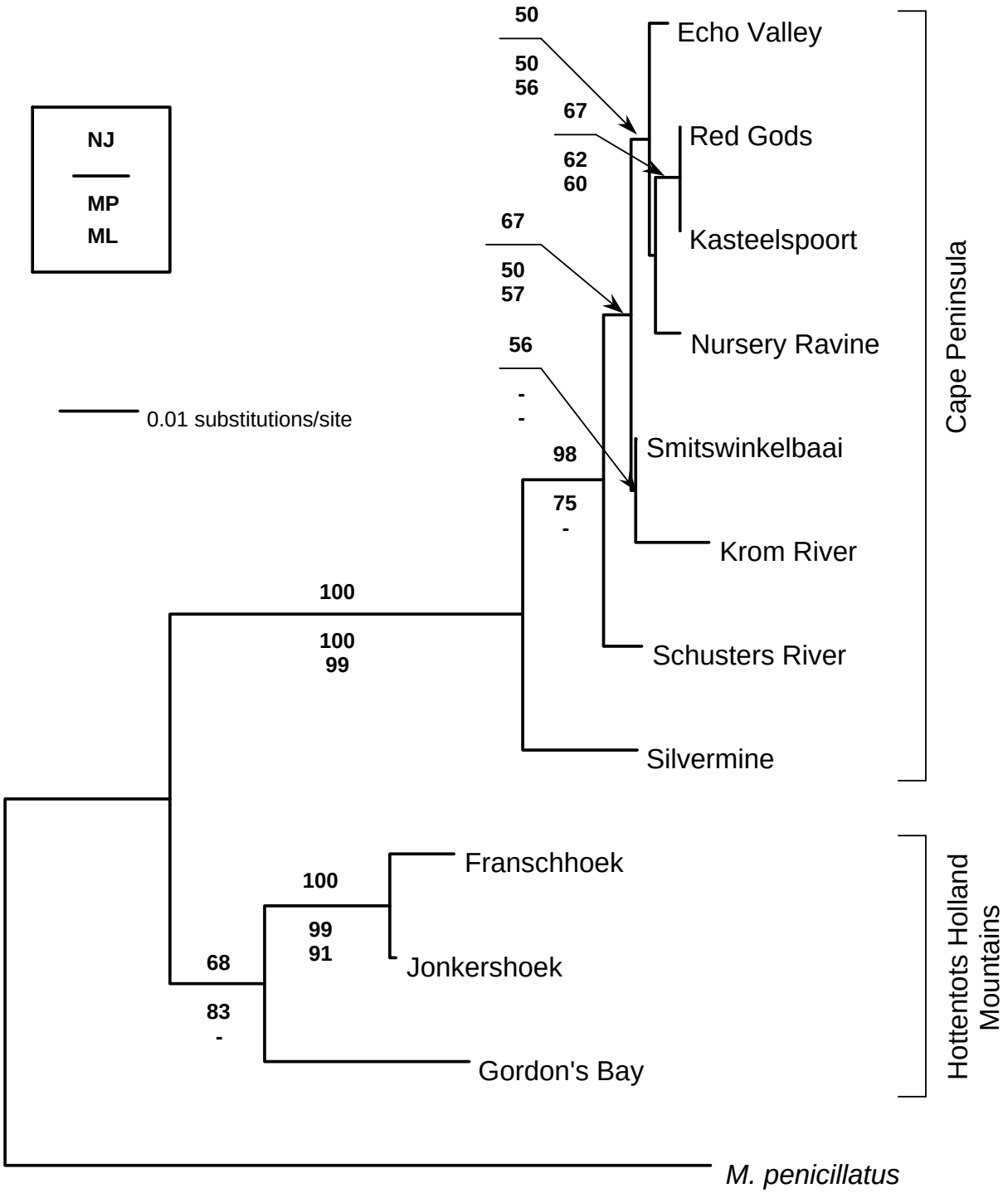


Figure 2.4: Neighbour-joining tree, based on "uncorrected p" sequence divergence, from an analysis of 328 bp of the 12S rRNA gene region from representative individuals from 11 putative *Mesamphisopus capensis* populations and one outgroup (*M. penicillatus*). Numbers above the branches indicate bootstrap support (10 000 replicates). Numbers below the branches represent bootstrap support from the MP (1 000 replicates) and ML (100 replicates) analyses. Bootstrap support < 50% is not indicated.

formed by the Table Mountain and remaining Peninsula representatives. Further relationships within the Cape Peninsula clade reflected those obtained in the allozyme analysis. Maximum likelihood retrieved a topology (not shown) largely congruent to the allozyme dendrogram, with the Gordon's Bay population occurring basally as a sister taxon to the clade (bootstrap support 77%; not shown) of remaining representatives. Within this clade, the relationship of the remaining two Hottentot's Holland Mountain representatives (Franschhoek and Jonkershoek) was well supported (91% bootstrap support). Again, the Peninsula representatives formed a strongly supported (99% bootstrap), monophyletic clade, with the individual relationships congruent to those revealed by the MP. A topology constrained to reflect the monophyly of representatives from the Hottentot's Holland Mountains had a higher log-likelihood score (-lnL = 872.325) than the unconstrained tree (-lnL = 871.429), but was not significantly less likely (SH test: $\ln L_1 - \ln L_0 = 0.896$; $P = 0.257$). The monophyly of the Hottentot's Holland Mountain individuals, supported in the MP analysis, could not be rejected.

No significant difference was observed (LRT: $2(\ln L_1 - \ln L_0) = 1.791$; df = 10; $P > 0.995$) between the log-likelihood scores of the unconstrained maximum likelihood tree and those obtained with a molecular clock enforced. A molecular clock could thus be tentatively applied.

2.3.3) Morphometric analyses

The 47 variables included in the morphometric analyses are indicated in Table 2.6. In the discriminant function analysis involving the body variables only (Table 2.6, variables 1 to 22), significant discrimination was obtained among the five defined populations (Wilks'

Table 2.6: The 47 body and pereopod variables used to examine morphometric differentiation among 11 putative populations of *Mesamphisopus capensis*. The factor structure (loading) matrices are summarized, providing correlations for the first two canonical variables, CV1 and CV2, from two independent discriminant function analyses, i.e. using body variables (variables 1 to 22), and pereopod variables (23 to 47), respectively.

Abbreviation		Measurement	Structure matrix	
			CV1	CV2
1)	BL	Body length	0.224	0.202
2)	HW	Head (cephalon) width	0.184	0.092
3)	HL	Head (cephalon) length	0.289	0.209
4)	HD	Head (cephalon) depth	0.322	0.256
5)	P1W	Pereonite 1 width	0.187	0.106
6)	P1L	Pereonite 1 length	0.243	0.156
7)	P1D	Pereonite 1 depth	0.225	-0.014
8)	P3W	Pereonite 3 width	0.180	0.221
9)	P3L	Pereonite 3 length	0.299	0.216
10)	P3D	Pereonite 3 depth	0.261	0.009
11)	P5W	Pereonite 5 width	0.190	0.249
12)	P5L	Pereonite 5 length	0.310	0.142
13)	P5D	Pereonite 5 depth	0.222	-0.029
14)	P7W	Pereonite 7 width	0.197	0.233
15	P7L	Pereonite 7 length	0.205	0.075
16)	P7D	Pereonite 7 depth	0.223	0.073
17)	PL4W	Pleonite 4 width	0.195	0.243
18)	PL4L	Pleonite 4 length	0.262	0.066
19)	PL4D	Pleonite 4 depth	0.301	0.246
20)	TW	Pleotelson width	0.263	0.188
21)	TL	Pleotelson length	0.016	0.226
22)	TD	Pleotelson depth	0.207	0.027
23)	Pe1L	Pereopod I (gnathopod) length	-0.314	0.136
24)	Pe1BL	Pereopod I (gnathopod) basis length	-0.267	0.014
25)	Pe1BW	Pereopod I (gnathopod) basis width	-0.165	0.050
26)	Pe1PL	Pereopod I (gnathopod) propodus length	-0.327	0.148
27)	Pe1PW	Pereopod I (gnathopod) propodus width	-0.291	0.252
28)	Pe3L	Pereopod III length	-0.317	0.003
29)	Pe3BL	Pereopod III basis length	-0.307	0.020
30)	Pe3BW	Pereopod III basis width	-0.175	0.138

31)	Pe3PL	Pereopod III propodus length	-0.312	-0.037
32)	Pe3PW	Pereopod III propodus width	-0.160	0.135
33)	Pe4L	Pereopod IV length	-0.184	-0.032
34)	Pe4BL	Pereopod IV basis length	-0.203	-0.079
35)	Pe4BW	Pereopod IV basis width	-0.140	0.051
36)	Pe4PL	Pereopod IV propodus length	-0.247	0.036
37)	Pe4PW	Pereopod IV propodus width	-0.213	0.064
38)	Pe5L	Pereopod V length	-0.232	-0.094
39)	Pe5BL	Pereopod V basis length	-0.273	0.040
40)	Pe5BW	Pereopod V basis width	-0.132	0.150
41)	Pe5PL	Pereopod V propodus length	-0.164	-0.232
42)	Pe5PW	Pereopod V propodus width	-0.061	-0.005
43)	Pe7L	Pereopod VII length	-0.296	-0.033
44)	Pe7BL	Pereopod VII basis length	-0.268	0.030
45)	Pe7BW	Pereopod VII basis width	-0.138	0.216
46)	Pe7PL	Pereopod VII propodus length	-0.237	-0.124
47)	Pe7PW	Pereopod VII propodus width	-0.089	0.207

Lambda = 0.012, $F_{(88, 105)} = 2.431$, $P < 0.001$). Similarly, all populations were significantly discriminated (Wilks' Lambda = 0.004, $F_{(100, 93)} = 2.913$, $P < 0.001$) using the 25 pereopod variables (Table 2.6, variables 23 to 47).

The five identified geographic populations appeared to be well differentiated in both analyses, as evident from the reclassification matrices (Table 2.7). In the analysis based on body variables, 96.88% correct reclassification was obtained for the Table Mountain – Southern Peninsula group, with one of the 32 individuals being incorrectly reassigned to the Silvermine population. The Silvermine, Franschhoek, Jonkershoek and Gordon's Bay populations all had 100% correct reassignment. In the analysis based on pereopod variables, all individuals were correctly reassigned to their respective populations.

Plots of individuals along the first two canonical variables in both analyses (Fig. 2.5) revealed the Gordon's Bay population to be markedly distinct from the remaining populations. This population was characterized by lower scores along the first canonical variable in the analysis of body variables, and higher scores along this variable in the analysis of pereopod variables. In the analysis of body variables, the Silvermine population overlapped the Table Mountain – Southern Peninsula, Franschhoek and Jonkershoek populations slightly. The first two canonical variables accounted for 85.18% of the variation among populations and had eigenvalues of 6.542 and 2.400, respectively. In the analysis of pereopod variables, the two canonical variables, with eigenvalues of 10.737 and 4.572, accounted for 87.28% of the between-population variation. Here, the Jonkershoek population overlapped the Table Mountain – Southern Peninsula and Franschhoek populations slightly, while the Silvermine and Table Mountain – Southern Peninsula populations too showed limited overlap.

Table 2.7: *A posteriori* reclassification of individuals to groups, based on classification functions determined in the discriminant function analyses of (a) body variables and (b) pereopod variables.

			A posteriori reclassifications				
		Percent correctly reclassified	Table Mntn – Southern Peninsula	Silvermine	Franschhoek	Jonkershoek	Gordon's Bay
(a)	Table Mountain – Southern Peninsula	96.88	31	1	-	-	-
	Silvermine	100.0	-	5	-	-	-
	Franschhoek	100.0	-	-	5	-	-
	Jonkershoek	100.0	-	-	-	5	-
	Gordon's Bay	100.0	-	-	-	-	5
(b)	Table Mountain – Southern Peninsula	100.0	32	-	-	-	-
	Silvermine	100.0	-	5	-	-	-
	Franschhoek	100.0	-	-	5	-	-
	Jonkershoek	100.0	-	-	-	5	-
	Gordon's Bay	100.0	-	-	-	-	5

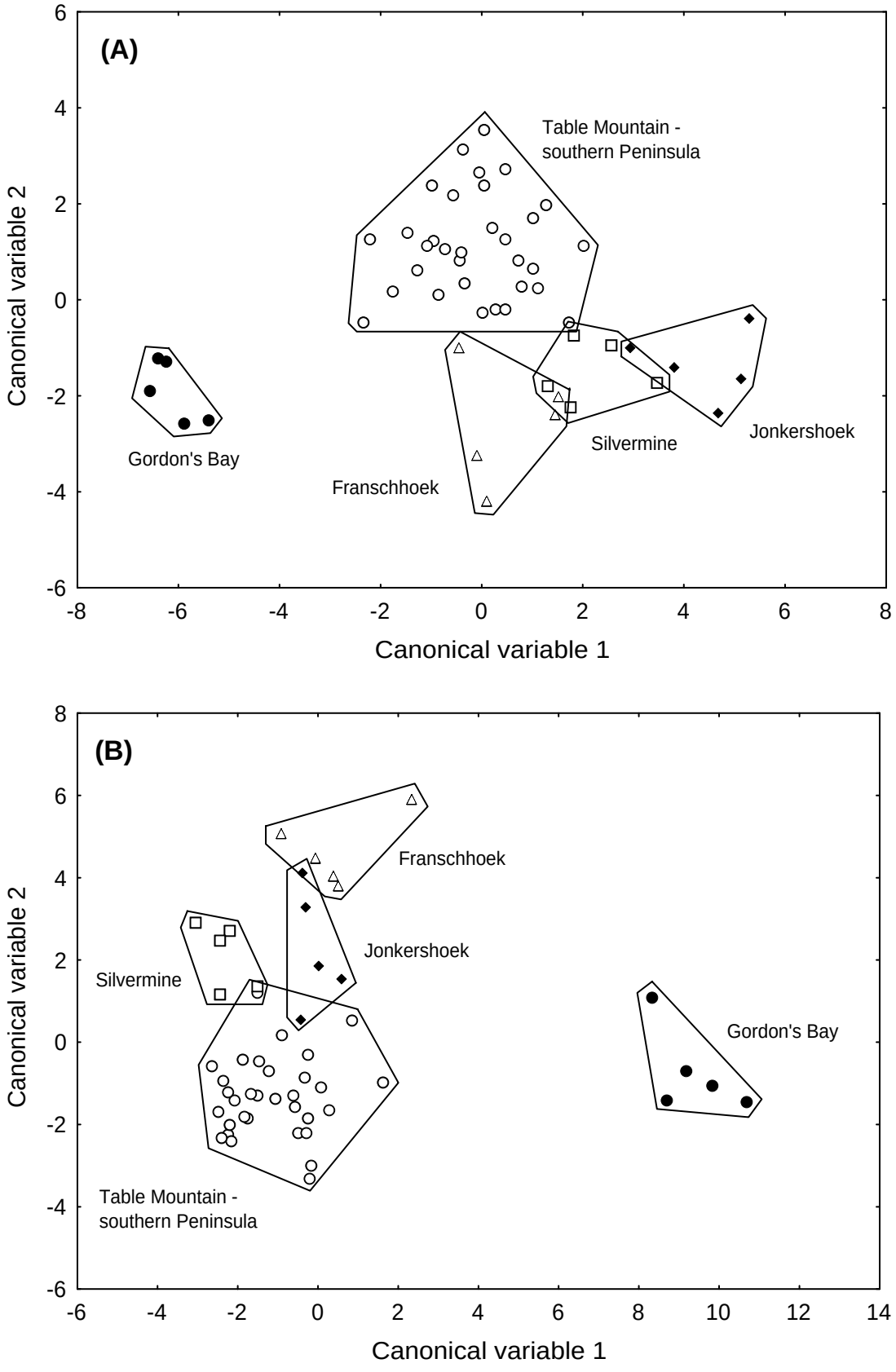


Figure 2.5: Individuals belonging to the five identified genetically distinct, geographic populations plotted along scores for the first two canonical variables derived from the discriminant function analyses of (A) 22 body variables and (B) 25 pereopod variables.

The factor structure (loading) matrices, representing the correlations between the variables and the functions, are summarized for the first two discriminant functions (canonical variables) in both analyses in Table 2.6. In the analysis of body variables, the first canonical variable had highest correlation with HD (4), P5L (12), PL4D (19) and P3L (9). For the second canonical variable HD, P5W (11), PL4D and PL4W (17) had the highest loadings. While it appeared as though dimensions of the fifth pereonite and fourth pleonite specifically contributed to the discrimination of the populations, the width and depth variables were generally less important in discriminating populations along the first and second canonical variables, respectively. The first canonical variable in the analysis of pereopod variables was correlated most highly (albeit negatively) with Pe1L (23), Pe1PL (26), Pe3L (28) and Pe3PL (31). The width of individual pereopod articles was less important in distinguishing populations than limb and article length, and thus generally carried the lowest loadings along this function. Along the second discriminant function the opposite was apparent, with width variables carrying the highest loadings. The highest correlations were observed with Pe1PW (27), Pe7BW (45) and Pe7PW (47), while Pe5PL (41) showed a high negative correlation.

The positive correlation of variables included in the analysis of the body variables and the simultaneous negative correlation of the variables included in the analysis of the pereon variables with their respective first canonical variables suggests that the separation along these variables may be related to size, with the Gordon's Bay individuals being smaller than the representatives of the other populations (Appendix 4). This size differences appears to be of systematic importance and does not appear to be due to the examination of immature and smaller individuals from this population. All examined individuals from this locality belonged to the largest size class sampled, and were determined to be adult on the basis of penes development (Wilson and Ho, 1996).

2.4) Discussion

Generally congruent patterns of population differentiation were observed in the two independent molecular markers examined. Additionally, five distinct geographic populations (Table Mountain – Southern Peninsula; Silvermine; Franschhoek; Jonkershoek and Gordon's Bay), distinguished on the basis of fixed allele differences, significant allele frequency heterogeneity, and the disassociation of genetic diversity and geographic proximity, were morphometrically distinct. Importantly, a large genetic divergence was seen between the Cape Peninsula and Hottentot's Holland populations in the allozyme data, while the 12S rRNA sequence data supported the monophyly of each of the two regions.

2.4.1) Genetic evidence of specific status

Genetically divergent populations occurring allopatrically are problematic where morphological or other criteria, which may be instructive of the taxonomic status of the populations, are absent (Thorpe, 1983) and species concepts based on reproductive compatibility cannot be tested (Butlin and Tregenza, 1998). Several authors have cautioned against the use of genetic distance measures in making taxonomic inferences, principally because such estimates are not equivalent at, or consistently partitioned among, equivalent taxonomic hierarchies within different classes (Avise and Aquadro, 1982; Sites and Crandall, 1997; Butlin and Tregenza, 1998; Johns and Avise, 1998; Avise and Johns, 1999). Although not an exact estimate, these divergence values can potentially be useful to provide corroborative evidence of taxonomic status (Bradley and Baker, 2001).

While no allozyme studies on phreatoicidean isopods have yet been published, identity values obtained in comparisons of valid congenerics or putatively new species of other freshwater, terrestrial, marine and troglobitic isopods ranged from 0.159 to 0.816 (Garthwaite, Lawson and Taiti, 1992; Viglianisi, Lombardo and Caruso, 1992; Lessios and Weinberg, 1994; Cobolli Sbordoni *et al.*, 1997; Ketmaier *et al.*, 1998, 2000). Intraspecific identity-values obtained in these studies varied between 0.656 and 1.000. Similarly, surveys of electrophoretic studies, involving a range of invertebrate taxa led Thorpe (1982, 1983), Skibinski, Woodwark and Ward (1993), and Thorpe and Solé-Cava (1994) to conclude that identity values for comparisons among congeneric species typically fell between 0.25 and 0.85, while intraspecific values were generally greater than 0.91. Further, allopatric populations with identity values less than 0.85 were considered unlikely to be conspecific.

Using these genetic distances as broad criteria, five putative species may be recognized from the allozyme data presented above: the Franschhoek, Jonkershoek, Gordon's Bay and Silvermine populations may be recognized as separate species, while the populations of the Table Mountain – Southern Peninsula group may be considered conspecific to each other. Mean identity values obtained in comparisons among these putative species ranged from 0.367 to 0.825, while (intraspecific) comparisons of populations within the Table Mountain – Southern Peninsula group resulted in *I*-values between 0.851 and 1.000.

From the sequence data, a mean sequence divergence of 7.90% (±3.08) was observed among these putative species. Individual comparisons among these different species ranged from 0.93% to 11.01%, while intraspecific sequence divergences (among Table Mountain and Southern Peninsula representatives) ranged from 0.0% to 1.88%. With the exception of the comparison between the Franschhoek and Jonkershoek sequences (0.93%), mean interspecific

sequence divergence estimates among any two identified geographic populations (between 3.36% and 10.52%) were greater than those reported for the 12S rRNA region in phreatoicidean isopods by Wetzer (2001), where congeneric phreatoicidean species showed approximately 2% sequence divergence. The values were, however, lower than those reported for interspecific comparisons within other isopod suborders, for example the Valvifera and Flabellifera (Wetzer, 2001).

Based on this data, only two putative *Mesamphisopus* species may be recognized from the Cape Peninsula. The diversity of the phreatoicideans on the Cape Peninsula appears to be considerably less than the region's 11 paramelitid amphipod species (Stewart and Griffiths, 1995), some of which were brought to light using a similar combination of techniques (e.g. Stewart, 1992). The presence of another species on Table Mountain is also not supported. Indeed, populations collected from Table Mountain were genetically identical in terms of allozyme data, with no evidence (significant deviations from Hardy-Weinberg expectations at polymorphic loci) suggesting separate, but sympatric, gene pools at any locality. The three 12S rRNA haplotypes from Table Mountain were similar and could be considered to be from conspecific individuals.

2.4.2) Evolutionarily Significant Units or species?

The five geographic populations initially identified above may qualify as ESUs under Ryder's (1986) initial broad definition. Under that definition, populations (subspecies) that showed significant adaptive variation, based on concordant data sets would be recognized as discrete units. Although the sampling regime for the mtDNA study was inappropriate to recognize ESUs, allozyme differentiation and morphometric separation of the five populations support

this designation here. While reproductive isolation, a criterion under Waples' (1991) expanded ESU definition, cannot be demonstrated empirically among allopatric populations, a lack of gene flow is apparent and reproductive isolation inferred between populations on the basis of fixed allele differences revealed by the allozyme data. However, great conceptual overlap exists between ESU and species concepts (see Chapter 1) and, as highlighted by Roe and Lydeard (1998), reproductive isolation may be invoked to argue for specific status under the Biological Species Concept (Mayr, 1963). Five species may thus be recognized with the acceptance of this concept.

Moritz (1994) defined ESUs as being reciprocally monophyletic for mtDNA alleles and showing significant divergence in allele frequency at nuclear loci. Significant differences in allele frequency have been identified at numerous loci between the five morphometrically distinct, geographic populations identified as putative species. However, the inclusion of only one individual per population in the DNA-sequence analyses precludes the identification of ESUs at the population (locality) level. Thus, only the two regions could be regarded as ESUs under Moritz's (1994) strictest definition, with the monophyly of each demonstrated by parsimony analysis, and not rejected with maximum-likelihood. Again, as highlighted by Roe and Lydeard (1998), diagnostic (nucleotide) characters bringing about monophyly of the two regions may be used to diagnose species under a Phylogenetic Species Concept. If accepted, only two species could be recognized with the sampling employed here. Significant differences in allele frequency at allozyme loci between the five identified geographic populations do, however, satisfy Moritz's (1994) criteria for each to be recognized as a MU; these being functionally independent populations with significantly different allele frequencies at nuclear or mitochondrial loci.

Although genetically divergent populations should be appropriate units of conservation regardless of taxonomy (Waits *et al.*, 1998), concern has been expressed over the use of molecular markers alone in the identification of ESUs (Newton *et al.*, 1999) and the lack of a "standard" of genetic differentiation whereby these units can be identified (Roe and Lydeard, 1998; Moritz, 2002). While a standard may be desirable for conservation and management authorities, a genetic distance criterion is likely to be fraught with the same problems as taxonomic designations based solely on genetic distance, discussed above. Butlin and Tregenza (1998) also recognized the need for evaluating ecologically relevant traits when defining ESUs. Subsequently, Crandall *et al.* (2000) explicitly used the rejection of ecological exchangeability as a criterion for population distinctiveness and ESU identification. Unfortunately, ecological data were not evaluated in this study. Aesthetic, economic, cultural, demographic and behavioural factors are additional considerations in defining conservation priorities, but do not yet provide an operational basis for defining ESUs (Waples, 1998) and are not considered further.

Despite the identification of ESUs and MUs in a number of South African taxa (e.g. Matthee and Robinson, 1999; Bloomer and Impson, 2000; Daniels *et al.*, 2003; Stewart *et al.*, 2004), these concepts have, as yet, found only limited application in South African conservation. These cases have typically involved only enigmatic taxa of economic importance (e.g. Matthee and Robinson, 1999). This is of concern as the best biological information is of little consequence if the legal framework does not exist to use this information in the implementation of sound conservation policy (Rohlf, 1991). Of greater concern is that only two of the presently used provincial ordinances within South African conservation include schedule provisions for invertebrate species (Bürgener, Snyman and Hauck, 2001).

2.4.3) Concordant patterns and historical narrative

Moritz (1994) alluded to a possible extension of the ESU concept whereby whole communities are examined and a comparative phylogeographic approach taken to define ESUs in terms of geographic areas, in which allopatric populations of different taxa, are distinct. In this regard, two genetic studies on freshwater invertebrates of the Western Cape provide useful comparison with the data presented above. Daniels *et al.* (2001) found marked divergence between freshwater crab populations initially regarded as *Potamonautes brincki* (Bott, 1960) collected from the Cape Peninsula and the Hottentot's Holland Mountains, respectively. Wishart and Hughes (2001) found an identical pattern of divergence between populations of the lotic, net-winged midge, *Elporia barnardi* Edwards. To a large extent, this divergence was also seen among populations of freshwater amphipods formerly believed to be *Paramelita capensis* conspecifics (Stewart, 1992). Direct comparison of patterns within each of the regions is difficult, however, as the sampling localities are not identical. However, both Wishart and Hughes (2001) and Daniels *et al.* (2001) found notably less differentiation within certain regions, attributable to the increased dispersal efficacy of the organisms examined. For example, the Silvermine population included by Daniels *et al.* (2001) was genetically identical to one of the Table Mountain populations, indicating probable migration of crabs across drainages along the eastern side of Table Mountain. In the present study, the Silvermine population is distinct and may represent a putative species.

The marked divergence among the freshwater fauna of the two regions can potentially be attributed to the Cape Flats. This coastal plain remnant stretches from False Bay to the west coast with elevations of less than 50 m, separating the Hottentot's Holland Mountains of the Cape Fold Belt from their outliers on the Cape Peninsula (Harrison and Barnard, 1972;

Lambrechts, 1979; Cowling *et al.*, 1996). Although exposed, gene flow between *Mesamphisopus* populations across the Cape Flats is unlikely, as present conditions have prohibited the establishment of viable populations (Harrison and Barnard, 1972). Indeed, Harrison and Barnard (1972) believed this current 'land bridge' to be as insurmountable as the marine transgressions. Although the sandy Cape Flats were periodically covered by forest during mesic periods in the late Pleistocene (Hendey, 1983a), they are presently dry, receiving less precipitation (400 mm) annually than the surrounding mountainous areas do from the mist belt alone (Fuggle and Ashton, 1979). Flowing water on the Cape Flats is also strongly alkaline or brackish, while the water of the mountain streams, in which the phreatoicideans are abundant, is highly acidic (Harrison and Barnard, 1972).

Although geologically stable throughout the Cenozoic (65 Myr), the Western Cape has experienced substantial and rapid climatic change (Hendey, 1983a,b; Cowling *et al.*, 1996). While tectonically induced sea level changes had occurred throughout the Cenozoic to middle Miocene, glacial and interglacial cycles became established during the Pliocene, during which marine transgressions and regressions exposed and inundated the coastal platform and low-lying areas (Deacon, 1983; Hendey, 1983b), including the Cape Flats and "gaps" interrupting the mountain range of the Peninsula (Cowling *et al.*, 1996). Repeated marine transgressions have been invoked to account for the general lack of invertebrates endemic to the southern Peninsula (Picker and Samways, 1996). While the magnitude of these transgressions and regressions is unknown, sea levels are thought to have dropped (through glacio-eustatic change) by 200 m towards the end of the Miocene, and may have risen substantially in the Tertiary (200 m), middle Miocene (150 m) and early Pliocene (100 m) (Hendey, 1983b; Linder, Meadows and Cowling, 1992). Sea levels have not risen more than 6 m during the more recent Pleistocene and Quaternary interglacials (Hendey, 1983b).

While the most important impact of these cycles is the inundation or exposure of coastal platforms, the changes between warm, mesic, interglacial conditions and cold, xeric, glacial conditions bring about concomitant changes in weathering, erosion and deposition regimes and can significantly alter river courseways, flow regimes and drainage patterns (Hendey, 1983a,b). These Pleistocene climatic oscillations (and induced environmental changes) have been cited as a major driving force in the speciation and differentiation of the flora of the region (Richardson *et al.*, 2001).

Applying a protein clock calibrated for isopods (Ketmaier *et al.*, 1999) to the mean allozyme divergence between populations of the two regions ($D = 0.748 \pm 0.123$) indicates a divergence time of approximately 14 Myr. This estimate would attribute the separation to a significant sea level rise occurring in the middle Miocene (see Hendey, 1983b: Fig. 2). Although no molecular clocks have been specifically calibrated for the 12S rRNA gene region in isopods, several mtDNA clocks calibrated for Crustacea (Cunningham, Blackstone and Buss, 1992; Knowlton *et al.*, 1993), including isopods (Ketmaier, Argano and Caccone, 2003), or other arthropods (Brower, 1994) have suggested a rate of sequence divergence of between 2.2 and 2.6% per Myr. Applying this to the mean maximum-likelihood corrected sequence divergence (17.67 ±2.03%) obtained in comparison among individuals of the two regions, suggests that the lineages of the two regions had diverged between approximately 6.8 and 8 Myr ago. This lends credence to the faunistic separation of the regions through marine trangressions and regressions, discussed above, and is entirely consistent with the view of Harrison and Barnard (1972), who believed that *Mesamphisopus capensis* existed as separate gene-pools in each of the regions since the late Tertiary. The differences in estimates of divergence times may well be due to differing evolutionary rates of the markers examined, specifically the allozyme loci included. The later divergence times estimated for other taxa

(e.g. Daniels *et al.*, 2001) could, in addition, reflect differences in dispersal capacity, possibly enabling a more recent divergence.

While the origin and nature of the Cape Flats may explain the differentiation between populations between the two regions, patterns of differentiation within each region may well be attributed to drainage evolution, and patterns of local extinction and recolonization. This narrative, however, remains to be tested with data from a wide variety of aquatic invertebrates from both regions.

While fixed allele differences and large sequence divergence values can be considered character differences, an essentially tree-based approach to species delimitation (see Wiens, 1999) has led to the identification of five geographic populations/genetic units within *M. capensis*, with four of these possibly representing undescribed species. Genetic distance and similarity data formed the basis of this delimitation, although morphometric analyses had also shown these putative taxa to be distinguishable. Wiens (1999) stated that the congruence (or incongruence) of multiple data sets is instructive of the extent of species boundaries. Thus, further work should focus on intensive morphological examination of individuals of the putative species identified above, as cryptic species are often revealed to be diagnosable by consistent differences in morphology, once initially identified using genetic or morphometric data (Duffy, 1996).

From a conservation point of view, prudence dictates the consideration of the five identified populations as management units. Due to the limitations of the mtDNA study, only two ESUs (the Cape Peninsula and Hottentot's Holland Mountain groups) could be defined using Moritz's (1994) criteria. As all populations sampled fall within existing conservation areas, it

is hoped that this study, in conjunction with further studies on endemic freshwater fauna, may contribute towards a management strategy for the conservation of aquatic invertebrates within the Western Cape.

Chapter 3: Intraspecific differentiation in an apparently widespread phreatoicidean isopod species (Phreatoicidea: Mesamphisopodidae: *Mesamphisopus*) from South Africa?

3.1) Introduction

The isopodan suborder Phreatoicidea is represented in southern Africa by the single, endemic genus *Mesamphisopus* (Kensley, 2001), among the most basal of the phreatoicidean genera (Wilson and Keable, 1999). The genus contains only four species: *M. abbreviatus* (described from the northern slopes of the Kogelberg, Hottentot's Holland Mountains), *M. capensis* (described from Table Mountain on the Cape Peninisula), *M. depressus* (described from the Steenbras Valley, Hottentot's Holland Mountains) and *M. penicillatus* (described from a type locality near Hermanus), all occurring within the Western Cape, South Africa (Kensley, 2001). Recent investigations (Chapter 2) have, however, identified an additional four cryptic species, morphologically similar to *M. capensis*.

Fine-scale morphological examination (see Chapter 2) of available material had suggested that many individuals, sampled from a wide geographic range across the Western Cape, and kept in museum collections (e.g. South African Museum A3992 – A3993, A4006, A4181 – A4183, A4186 – A4187, A6052, A6932 – A6934, A6950) had been incorrectly identified as *M. capensis,* particularly in light of the most recently compiled morphological key (Kensley, 2001). These specimens and newly collected material from proximate localities do not belong to *M. capensis,* the four cryptic species (all lacking a pair of sub-apical setae dorsally on the pleotelson) or *M. penicillatus* (possessing characteristically heavily setose antennal

peduncles), leaving only *M. abbreviatus* and *M. depressus* as possibilities for identification. However, these specimens could not be unambiguously assigned, using this key, to either of these two species. While earlier workers had noted subtle differences in pereon, telson and gnathopod shape, and coloration between *M. abbreviatus* and *M. depressus*, these two species were primarily distinguished in these works (Barnard, 1927; Nicholls, 1943) and the key (Kensley, 2001) by the degree of setation of the head and pereon, these being more setose in *M. depressus*. Characters such as gnathopod shape and telson shape are known to exhibit within-population variation (Barnard, 1927), while setation, in particular, is of only limited systematic importance (see Wilson and Keable, 1999, 2001). Hence, the identification of *M. abbreviatus* and *M. depressus*, described from geographically proximate type localities (Barnard, 1927) within the southern Hottentot's Holland Mountains (from the northern slopes of Kogelberg and the Steenbras Valley, respectively), as separate species is questioned in light of this morphological plasticity, while the use of "continuous" characters, such as the extent of setation, results in these specimens being equivocally identified as either species.

The use of molecular data to resolve such taxonomic difficulties and to identify and delineate cryptic species has become increasingly widespread in crustacean systematics (e.g. King and Hanner, 1998; Sarver, Silberman and Walsh, 1998; Schubart, Reimer and Diesel, 1998; Larsen, 2001). Genetic distance criteria, in addition to inferred reproductive isolation and evidence of morphometric differentiation, have already been presented as an argument for the delimitation of cryptic species within the genus *Mesamphisopus* (Chapter 2). However, the establishment of an interspecific "standard" for the genus or species of interest is critical in this regard. Underscoring this is an understanding of the extent of, and patterns of, intraspecific genetic differentiation present among individual populations. The identification of populations as either *M. abbreviatus* or *M. depressus* affords the opportunity to examine

intraspecific genetic differentiation, as these populations are likely to be either conspecific, or individual, albeit cryptic, entities – perhaps with wide distributions.

Classical population genetic theory predicts that taxa with limited dispersal capabilities will show great levels of genetic differentiation, resulting from mutation and genetic drift in the absence of gene flow and selection. Even sessile or relatively sedentary species, with narrow individual ranges, may show only limited genetic differentiation between geographically disjunct populations due to the occurrence of vagile life-history stages, where a negative relationship between the extent of genetic differentiation and the duration and dispersal efficiency of free-swimming larval stages has been documented or is expected (Burton and Feldman, 1982; Bohonak, 1999; Sponer and Roy, 2002).

While the marine environment has traditionally been thought to present few obvious physical barriers to gene flow (Bohonak, 1999) and genetic connectivity among conspecific populations is regarded as high (e.g. Bucklin *et al.*, 1997; Bahri-Sfar *et al.*, 2000; Rodriguez-Lanetty and Hoegh-Guldberg, 2002), freshwater environments are often more complex with habitats essentially existing as "islands" within the broader terrestrial environment (Michels *et al.*, 2003; Wishart and Hughes, 2003). Accordingly, more complex patterns of differentiation are expected among freshwater isopod populations than among populations of marine or terrestrial isopods, where high levels of gene flow and isolation by distance have often been documented (e.g. Beck and Price, 1981; Wang and Schreiber, 1999). General patterns of restricted gene flow and high levels of genetic differentiation among geographically separated populations have been reported for riverine organisms (Meyran, Monnerot and Taberlet, 1997; Woolschot, Hughes and Bunn, 1999), while genetic structure within riverine systems has been proposed to represent a nested hierarchy (Meffe and Vrijenhoek, 1988), with

populations nested within sub-catchments and larger catchments. As such, and given their predominant restriction to high-altitude streams in broad, mature valleys (Barnard, 1927), *Mesamphisopus* populations occurring within the same drainage system may be expected to be genetically more similar, with greater genetic differentiation observed among populations situated in different drainages systems. These among-drainages relationships, in turn, may be expected to reflect a pattern of isolation by distance, as chance genetic exchange (migrations) among adjacent drainage systems would be more likely than gene flow among geographically widely separated drainages. From the outset, however, genetic differentiation among populations is expected, and patterns perhaps complicated by the fact that within the Isopoda, as in other peracarid Crustacea, young are brooded within a marsupium and free-swimming larval stages do not occur (Kensley, 2001).

The aims of the present study are thus two-fold. First, to determine whether populations tentatively identified as either *M. depressus* or *M. abbreviatus* could reasonably be regarded as conspecific. To address this, specimens were collected from a large geographic range, encompassing the type localities of both species and the collection localities of the above-mentioned, misidentified museum specimens. Second, in light of the above expectations, the possible evolutionary processes underlying genetic differentiation are investigated.

3.2) Materials and methods

3.2.1) Collection

Isopods, assigned to either *Mesamphisopus abbreviatus* or *M. depressus* according to the single available key (Kensley, 2001) and limited species descriptions (Barnard, 1927; Nicholls, 1943), were collected from 14 localities across the Western Cape, and one locality in the Eastern Cape, South Africa (Fig. 3.1). The sampling strategy targeted known collection localities of *Mesamphisopus* and attempted to cover the known geographic extent of the above-mentioned, putatively misidentified populations. Individuals were also sampled from additional localities depending on the accessibility of suitable habitat. Approximately 50 isopods were collected at each locality, as in Chapter 2, by sifting through sediment and dense matted moss or roots using hand nets. Collection localities were typically high-altitude, first-order streams, seepage areas or springs. Collectively, the sampling localities are situated along a ~ 450 km transect, stretching from the Hottentot's Holland Mountains in the west, through the Riviersonderend and Langeberg Mountains, to the Tsitsikamma Forest in the east and represent seven separate drainage systems (Fig. 3.1). The scale of sampling, with populations being collected over such a large area, as well as within close geographical proximity (e.g. three localities were centred around the Steenbras Dam, while two localities from the Langeberg Mountains, Barrydale and Protea Valley, were within 1 km of each other) afforded the opportunity to examine the extent of genetic differentiation over large and local spatial scales.

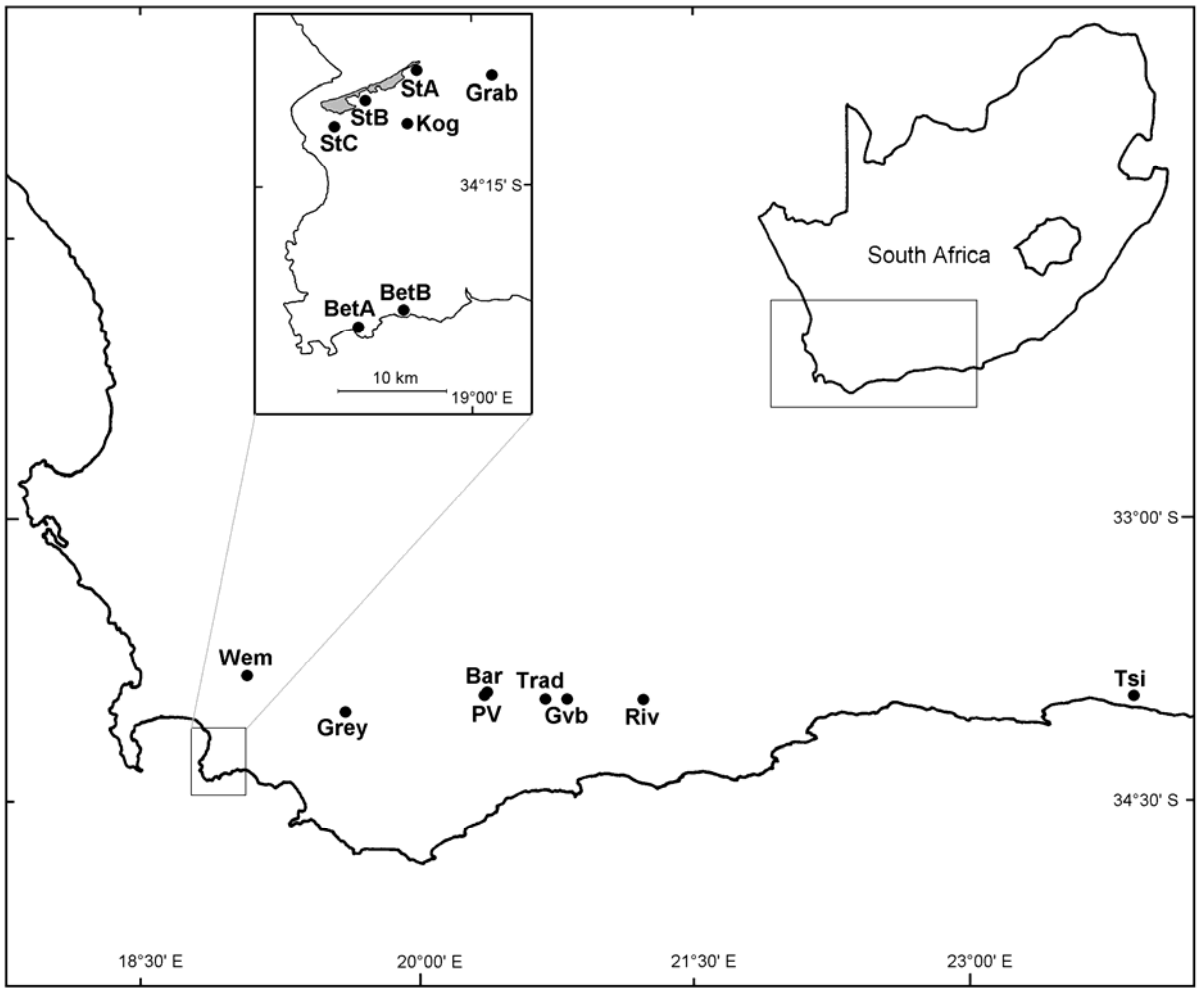


Figure 3.1: Collection localities of the 15 studied populations of *Mesamphisopus* in the Western Cape and Eastern Cape, South Africa. Localities included (with drainage systems in parentheses): (BetA) Betty's Bay A (Betty's Bay marshland), (BetB) Betty's Bay B (Disa Stream/Betty's Bay marshland), (Wem) Wemmershoek (Berg River), (StA) Steenbras A (Steenbras River), (StB) Steenbras B (Steenbras River), (StC) Steenbras C (Steenbras River), (Kog) Kogelberg (Palmiet River), (Grab) Grabouw (Palmiet River), (Grey) Greyton (Breede River), (PV) Protea Valley (Breede River), (Bar) Barrydale (Breede River), (Trad) Tradouw Pass (Breede River), (Gvb) Grootvadersbos (Breede River), (Riv) Riversdale (Vet River), and (Tsi) Tsitsikamma (Storms River). Individuals from these populations were tentatively identified as *Mesamphisopus abbreviatus* or *M. depressus*.

3.2.2) Morphometric analyses

Firstly, Barnard's type series (*Mesamphisopus abbreviatus*, South African Museum A5173; *M. depressus*, A4185) were examined to determine whether the two species could be distinguished morphometrically. All undamaged *M. depressus* males (N = 21) and an equal number of randomly chosen *M. abbreviatus* males were digitally photographed and measured following the procedures documented in Chapter 2. As the dissection required for the accurate measurement of pereopod variables was not possible for the type material, only the set of 22 body (cephalon, pereon, pleon and pleotelson) variables were included in the analysis. Variable details and abbreviations are presented in Chapter 2.

Morphometric discrimination between the two species was examined by means of a standard discriminant function analysis, using log-transformed (common logarithms) variables. Classification functions were determined for each of the two defined groups, using a jack-knifing procedure. Individuals were then reclassified to groups based on posterior probabilities. A frequency histogram of the scores of each group along the canonical (discriminant) function was compiled to visualize the possible differentiation between the two species. All analyses were performed using STATISTICA 6.0 (Statsoft, Inc., 2001).

Secondly, this analysis was extended to determine whether individuals sampled from the populations included in the genetic analyses could be identified as either of the two species using morphometric criteria. Five of the largest collected males from each of the populations were dissected and the same 22 variables measured as above. Due to unsuccessful attempts to recollect individuals from two of the localities, only two individuals were included from the Steenbras A population, while no individuals were included from the Betty's Bay B locality.

Individuals from each population were classified as *M. depressus* or *M. abbreviatus* based on posterior probabilities using the classification functions as determined above. A posterior probability greater than 0.95 was required for an individual to be identified as either species or the particular individual was regarded as unidentified.

3.2.3) Allozyme electrophoresis

Whole animals were prepared for electrophoresis following the procedures documented in Chapter 2. Gels were also run using identical buffer systems (Table 2.1) and running conditions (Chapter 2). The protocols of Shaw and Prasad (1970) were used to stain the sites of enzymatic activity of the allozymes of 12 loci, encoding ten enzyme systems (Table 2.1). Allelic mobilities were scored relative to the most common allele in a reference population (Franschhoek, see Chapter 2). Numbering of loci and alleles has been described in Chapter 2.

Numerical analyses of allozyme data were performed using the BIOSYS 1.7 (Swofford and Selander, 1981) and FSTAT 2.9.3.2 (Goudet, 2001) programs. Observed genotype frequencies were tested for deviation from frequencies expected under Hardy-Weinberg equilibrium, using chi-square goodness-of-fit tests and exact probabilities. Significance values were adjusted, as in Chapter 2, using the sequential Bonferroni technique (Rice, 1989). Genetic variability measures were examined as in Chapter 2. Nei's (1978) unbiased measure of genetic distance (*D*) was calculated from allele frequencies for pair-wise comparisons among populations. Genetic distance values were then used to construct a midpoint-rooted neighbour-joining tree (Saitou and Nei, 1987), as well as an UPGMA-dendrogram (Sneath and Sokal, 1973), using MEGA2.1 (Kumar *et al.*, 2001). The partitioning of genetic differentiation among populations across the entire sample was examined using Weir and

Cockerham's (1984) θ-estimates (see Chapter 2). Estimates of θ were also obtained for each pair-wise comparison of populations.

3.2.4) DNA-sequencing and analyses

Although Wetzer (2001) has recommended the use of the 12S and 16S rRNA gene regions for population- and species-level isopod studies, the cytochrome oxidase *c* subunit I (COI) mtDNA gene has recently found increasing use in isopod phylogeographic and phylogenetic studies. This latter protein-coding gene fragment has robustly resolved interspecific relationships and, albeit to a lesser extent, relationships among conspecific populations of isopods (e.g. Wares, 2001a; Rivera *et al.*, 2002; Ketmaier *et al.*, 2003). In order to assess whether populations could reasonably be regarded as conspecific across the range of collection localities, and to corroborate or refute the broad patterns seen from the allozyme data, a fragment of the COI region was amplified from a single representative individual from each population. An additional four to five individuals were sequenced from four populations (Barrydale, Betty's Bay A, Protea Valley and Wemmershoek) to broadly determine haplotype diversity within individual populations. These populations were selected to include genetically divergent populations, following the allozyme and preliminary sequence data analyses, and to include the two populations in closest geographic proximity (i.e. Barrydale and Protea Valley). A single *M. penicillatus* individual, as well as a morphologically distinct, undescribed species (*Mesamphisopus* sp. nov., collected from Ratel's River on the Agulhas Plain) was sequenced as outgroups.

Prior to DNA extraction, individuals (10 – 20 mm) were twice rinsed in distilled water by centrifugation (14 000 $r.min^{-1}$ for 2 min) to remove debris and epibionts. Total genomic DNA

was extracted using a commercial extraction kit (Qiagen DNEasy) or by means of conventional SDS – proteinase K digestion and phenol:chloroform-isopropanol extraction protocols (Hillis *et al.*, 1996; Sambrook and Russell, 2001).

Polymerase chain reactions (PCRs: Saiki *et al.*, 1988) were set up in 25 μL volumes, as in Chapter 2. The COI region was primarily amplified using the primer pair (LCO1490 and HCO2198) of Folmer *et al.* (1994). As amplification of certain individuals was problematic, two internal primers were designed; COI-intR (5'-GCW CCA AGA ATA GAA GAA GC-3') and COI-intF (5'-GTT GAA CTG TTT ATC CTC CTT-3'), which amplify ~ 420 bp and ~ 315 bp fragments in combination with LCO1490 and HCO2198, respectively. The thermal cycling regime included an initial denaturing step (94 ºC) for 4 min, followed by 33 cycles of denaturing (94 °C, 15 s), annealing (1 min) and extension (72 °C, 1.5 min). A final cycle included annealing for 5 min and extension for 10 min. Annealing was performed at 48 ºC for the Folmer *et al.* (1994) primer pair and at 55 ºC for combinations involving the internal primers. Purification and cycle-sequencing of amplicons, and automated sequencing, proceeded as in Chapter 2.

Sequences were checked for ambiguity against their respective chromatograms, using Sequence Navigator (Applied Biosystems). Due to ambiguity within the first ~ 15 bases, these were trimmed. If further ambiguities were present, the problematic region was re-amplified using the respective internal – Folmer *et al.* (1994) primer combination, sequenced, and a consensus sequence created. As the alignment of the sequences did not require the insertion of gaps (indels), this was done manually. Amino acid translations were examined, using MacClade 4.05 (Maddison and Maddison, 2000), to test for accuracy and functionality

of the sequences. No stop codons were detected in translations based on the *Drosophila* mitochondrial code.

Analyses were performed using PAUP*4b10 (Swofford, 2001). An initial neighbour-joining tree (Saitou and Nei, 1987), based on "uncorrected p" sequence divergence, was constructed using the entire data set. Phylogenetic relationships within a reduced data set, including all unique haplotypes, as well as a representative individual from each population, were examined further using parsimony (MP), maximum likelihood (ML) and neighbour-joining (NJ) approaches. In both the MP and ML analyses, heuristic tree searches were performed using the Tree-Bisection-Reconnection (TBR) branch-swapping algorithm with random additions of taxa (MP = 100 and ML = 10 replicates). Prior to ML analysis, the most appropriate model of sequence evolution was determined, and the nucleotide substitution parameters estimated, using MODELTEST 3.06 (Posada and Crandall, 1998). In the NJ analyses, the tree was constructed using sequence divergences corrected according to the substitution parameters estimated in the ML analyses. Confidence in the nodes was determined by bootstrapping of the data set (Felsenstein, 1985), with 1000, 100 and 10000 pseudoreplicates performed for the MP, ML and NJ analyses, respectively. Characters were unweighted in all analyses. The tree lengths of (alternative) topologies proposed by the allozyme, MP and ML analyses were determined and compared using MacClade 4.05. Additionally, an unrooted parsimony network of all unique haplotypes was constructed using Arlequin 2.000 (Schneider, Roessli and Excoffier, 2000).

3.2.5) Isolation by distance

To investigate the relationship between geographic distance and the extent of genetic divergence among populations (isolation by distance: Wright, 1943), the correlations between matrices of log-transformed straight-line geographic distances between collection localities and measures of genetic differentiation among populations, or sequence divergences among their representative haplotypes were examined. Due to the non-independence of data points, Mantel (1967) tests were used and executed using the Mantel for Windows (version 1.11) program (Cavalcanti, 2000) employing 10000 randomizations for each comparison. These relationships were explored using the mean genetic distance (*D*) among populations and measures of differentiation at individual loci, where Nei's (1978) genetic identities (*I*) were used, as *D*-values for single locus comparisons among populations approach infinity when fixed allelic differences are present. The sequence divergence matrix was comprised of "uncorrected p" sequence divergences among representative individuals. The correlation between sequence divergence among representative individuals and (allozyme) genetic distances among their source populations was also examined.

As patterns of isolation by distance may be biased by an uneven sampling strategy, with more intensive sampling in certain areas, and perhaps confounded by patterns of gene flow over these short geographic distances, correlations of genetic and geographic distances among regions were also examined. Populations were thus pooled by mountain range and geographic proximity, with populations separated by less than 30 km considered as belonging to the same region. Seven regions were identified: (1) Wemmershoek; (2) Hottentot's Holland Mountains (including the Betty's Bay A, Betty's Bay B, Grabouw, Kogelberg, Steenbras A, Steenbras B and Steenbras C populations); (3) Greyton; (4) west Langeberg

Mountains (Barrydale and Protea Valley); (5) east Langeberg Mountains (Grootvadersbos and Tradouw Pass); (6) Riversdale and (7) Tsitsikamma. Inter-region matrices were compiled by determining the mean geographic distances, sequence divergences and genetic distances (*D*) among populations belonging to these different regions.

Correlations among distance and sequence divergence matrices compiled for the constituent populations of the Hottentot's Holland Mountains region were also examined to determine whether patterns of isolation by distance were apparent over small geographic scales.

3.3) Results

3.3.1) Identification of specimens

Specimens were identified as either *M. abbreviatus* or *M. depressus* based on the presence of a robust pair of subapical dorsal setae on the pleotelson, as described and illustrated by Kensley (2001). However, it was apparent that this character was polymorphic within many populations (Table 3.1), with between ~ 3% (e.g. Barrydale) and 100% (e.g. Wemmershoek) of individuals possessing these setae. This was also observed in the museum specimens, previously identified as *M. capensis*, from proximate localities (counts not presented). All populations were retained in all analyses, however, as features of at least certain individuals of these populations (i.e. the presence of these setae and extent of the setation of the pereon, pleon and antennal peduncles) would diagnose them as *M. abbreviatus* or *M. depressus*, rather than *M. capensis, M. penicillatus* or the putatively new taxa (see above; Chapter 2). Further, the allozyme analysis did not suggest a genetic distinction within individual populations

Table 3.1: The number of individuals examined (*N*) possessing or lacking the pair of sub-apical dorsal robust setae on the pleotelson, as indicated by Kensley (2001), from each of the 15 studied populations. Percentages are given in parentheses. Examined material included presently unaccessioned voucher specimens, as well as accessioned museum specimens from the same collection localities, as indicated.

Population	*N*	Sub-apical dorsal pair of robust setae on pleotelson		
		Present	Absent	Indeterminable/damaged
Barrydale	108	3 (2.78%)	105 (97.22%)	---
Betty's Bay A[1]	45	45 (100.0%)	0 (0.0%)	---
Betty's Bay B	11	11 (100.0%)	0 (0.0%)	---
Grabouw[2]	43	37 (86.05%)	6 (13.95%)	---
Greyton	42	41 (97.62%)	1 (2.38%)	---
Grootvadersbos[3]	22	19 (86.36%)	3 (13.64%)	---
Kogelberg	13	13 (100.0%)	0 (0.0%)	---
Protea Valley	66	30 (45.45%)	35 (53.03%)	1
Riversdale[4]	19	13 (68.42%)	6 (31.58%)	---
Steenbras A	4	4 (100.0%)	0 (0.0%)	---
Steenbras B	58	58 (100.0%)	0 (0.0%)	---
Steenbras C	12	12 (100.0%)	0 (0.0%)	---
Tradouw Pass	3	2 (66.67%)	1 (33.33%)	---
Tsitsikamma[5]	12	6 (50.0%)	6 (50.0%)	---
Wemmershoek[6]	11	11 (100.0%)	0 (0.0%)	---

[1]South African Museum (SAM) A44932; [2]SAM A44931; [3]SAM A44934; [4]SAM A44941; [5]SAM A44935; [6]SAM A44938.

possibly underlying this morphological polymorphism. For example, there was no evidence of Wahlund (1928) effects, indicating the presence of independent, sympatric gene pools and thus multiple species, at any of the localities where this character was polymorphic.

3.3.2) Morphometric analyses

While distinguishing *M. abbreviatus* and *M. depressus* using qualitative, physical characteristics (i.e. setation) was largely equivocal, the syntypes of the two species could be reliably distinguished (Wilks' $\lambda = 0.023$, $F_{(22,19)} = 36.383$, $P < 0.001$) through the discriminant function analysis using the 22 body variables. All variables contributed to the discrimination of the species, with the width of pereonite 3 (P3W; Wilks' $\lambda = 0.035$, $P < 0.01$), telson depth (TD; Wilks' $\lambda = 0.032$, $P < 0.05$) and body length (BL; Wilks' $\lambda = 0.032$, $P < 0.05$) being the most significant discriminators within the discriminant functions. The reclassification of these individuals to groups based on posterior probabilities substantiated the distinction between the species with all individuals ($N = 21$) being correctly reassigned to their respective groups (Table 3.2). The frequency histogram of scores along the canonical variable (Fig. 3.2) revealed the large difference between the mean canonical scores for each species (6.334 and –6.334 for *M. abbreviatus* and *M. depressus*, respectively).

Using the classification functions determined for the two species, individuals from sampled populations were assigned to groups based on posterior probabilities (Table 3.2). Most examined individuals were determined to be morphometrically similar to *M. abbreviatus*. In only two populations, Steenbras B and Steenbras C, could all included individuals be classified as *M. depressus*. A measure of morphometric variability was observed within individual populations, such as the Betty's Bay A and Grootvadersbos populations, where

Table 3.2: Assignment of individuals to species based on posterior probabilities calculated from the classification functions[1] determined for the *M. abbreviatus* and *M. depressus* syntypes (South African Museum accession numbers and type localities in parentheses) in the discriminant function analysis using 22 cephalon, pereon, pleon and pleotelson variables. *N* = number of individuals included. Cases where posterior probabilities (< 0.95) prohibited the assignment of individuals to a species group are referred to as unclassified.

Population	*N*	Classification		
		M. abbreviatus	*M. depressus*	Unclassified
M. abbreviatus (SAM A5173. Northern slopes of Kogelberg, Hottentot's Holland Mountains)	21	21	---	---
M. depressus (SAM A4185. Steenbras Valley, Hottentot's Holland Mountains)	21	---	21	---
Barrydale	5	4	---	1
Betty's Bay A	5	4	---	1
Grabouw	5	5	---	---
Greyton	5	5	---	---
Grootvadersbos	5	4	---	1
Kogelberg	5	5	---	---
Protea Valley	5	5	---	---
Riversdale	5	5	---	---
Steenbras A	2	2	---	---
Steenbras B	5	---	5	---
Steenbras C	5	---	5	---
Tradouw Pass	5	3	2	---
Tsitsikamma	5	5	---	---
Wemmershoek	5	3	2	---

[1]*Mesamphisopus abbreviatus*: Y = 9373.180(BL) – 1511.315(HL) – 1055.443(HD) + 481.325(HW) – 2122.566(P1L) + 839.604(P1D) + 1320.926(P1W) – 1041.157(P3L) – 669.539(P3D) – 6152.109(P3W) – 397.806(P5L) – 465.202(P5D) – 1626.908(P5W) – 2724.097(P7L) – 332.462(P7D) + 4656.551(P7W) – 1360.636(PL4L) + 708.376(PL4D) – 130.131(PL4W) + 298.340(TL) + 1916.953(TD) – 314.259(TW) – 5404.434; *M. depressus*: Y = 8740.905(BL) – 1418.522(HL) – 1173.859(HD) + 301.994(HW) – 2003.568(P1L) + 787.382(P1D) + 1445.613(P1W) – 1074.747(P3L) – 658.538(P3D) – 5135.734(P3W) – 395.618(P5L) – 433.312(P5D) – 1586.701(P5W) – 2541.097(P7L) – 268.888(P7D) + 4323.975(P7W) – 1330.582(PL4L) + 786.825(PL4D) – 48.266(PL4W) + 61.171(TL) + 1601.399(TD) – 190.813(TW) – 4844.632.

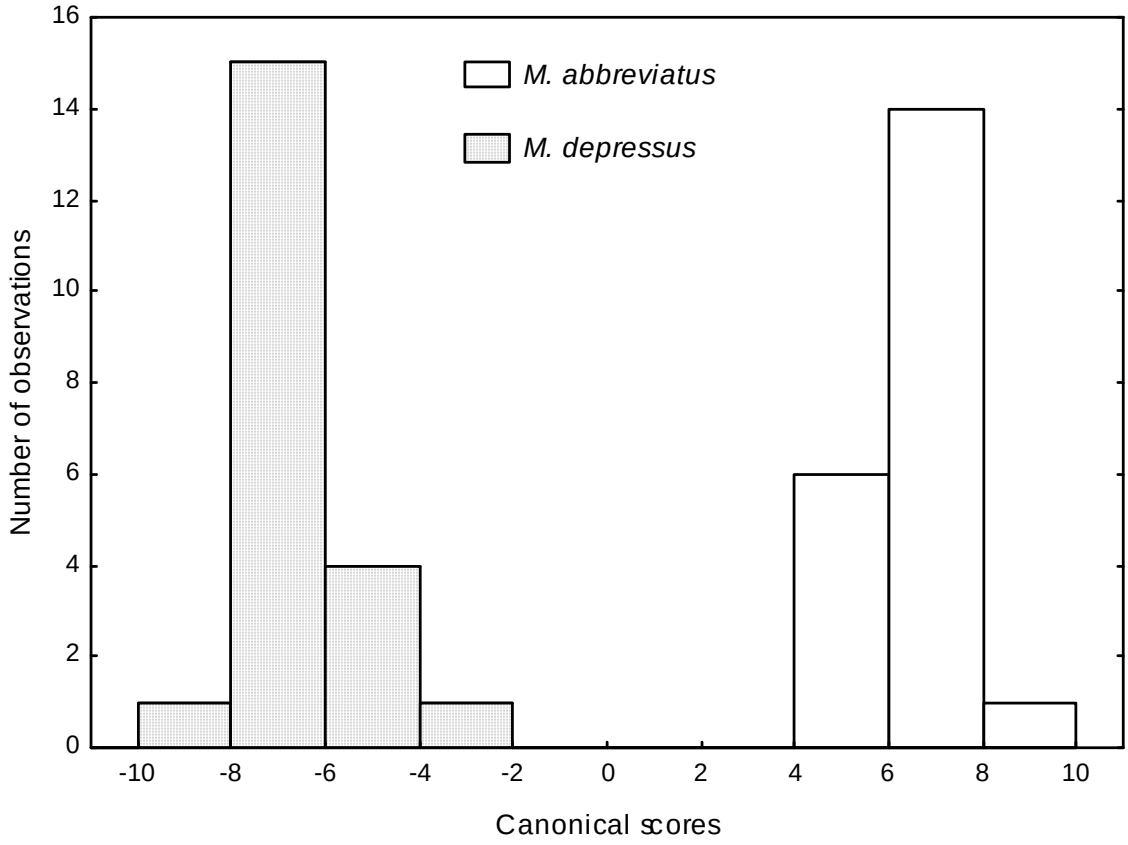


Figure 3.2: Frequency histogram of canonical scores for individuals of the types series of *Mesamphisopus abbreviatus* (open bars) and *M. depressus* (stippled bars) along the canonical (discriminant) variable calculated from a discriminant function analysis using 22 cephalon, pereon, pleon and pleotelson variables. The mean canonical scores were 6.334 and –6.334 for *M. abbreviatus* and *M depressus*, respectively.

single individuals could not be classified. Both morphotypes were encountered within the Tradouw Pass and Wemmershoek populations. This indicates a further morphological polymorphism that, like the polymorphism in pleotelson setation, does not appear to be supported by a genetic distinction or polymorphism in the individual populations.

3.3.3) Allozyme electrophoresis

All 12 loci included in the study were found to be polymorphic. Allele frequencies at each locus and genetic variability measures for each population are presented in Appendix 6 and Table 3.3, respectively. The *Lt-1*- and *Lt-2*-loci, although polymorphic across the entire sample, were monomorphic in individual populations. No loci were found to be polymorphic in all studied populations. From two (*Ao*, *Lt-1* and *Lt-2*) to 16 (*Gpi*) alleles were found per locus. Although a number of populations were determined to be fixed for null alleles at certain loci, these loci and populations were retained in further numerical analyses with the null alleles coded following the "minimizing" approach discussed by Berrebi *et al.* (1990), and Machordom, Doadrio and Berrebi (1995). This coding methodology was originally conceived to enable, mathematically, comparisons among taxa with differentially expressed loci resulting from gene duplication (polyploidy) events and subsequent inactivation of loci through "functional diploidization" (Berrebi *et al.*, 1990: 314). Here it was, however, applied to null alleles apparently fixed at a single locus (*Ldh*) in different populations (Barrydale, Greyton, Kogelberg, Protea Valley and Riversdale), with null alleles being coded identically in these populations for further analyses. This assumes a common evolutionary inactivation of expression in all populations and has the effect of minimizing genetic differentiation among these populations, while maximizing genetic distance between groups of populations fixed for null alleles and groups possessing alternate alleles, as documented by Berrebi *et al.*

Table 3.3: Genetic variability measures, determined from genotype data at 12 examined loci, for the 15 populations of *Mesamphisopus* studied. Measures include: the mean number of alleles per locus (A), the mean observed heterozygosity (H_O), the mean unbiased expected heterozygosity (H_E), and the percentage of loci that were polymorphic ($P_{95\%}$) using a 95% criterion. Standard deviations are presented below the individual variability estimates. Population names are abbreviated as in Figure 3.1.

	Population														
	BetA	BetB	Wem	StA	StB	StC	Kog	Grab	Grey	PV	Bar	Trad	Gvb	Riv	Tsi
A	1.167	1.333	1.500	2.083	1.333	1.083	1.167	1.500	1.250	1.583	1.083	1.333	1.500	1.083	1.250
	±0.389	±0.492	±1.000	±1.676	±0.651	±0.289	±0.389	±0.674	±0.622	±0.996	±0.289	±0.651	±0.522	±0.289	±0.452
H_O	0.008	0.031	0.048	0.138	0.059	0.022	0.021	0.064	0.071	0.087	0.023	0.061	0.070	0.028	0.042
	±0.021	±0.059	±0.104	±0.237	±0.118	±0.077	±0.058	±0.151	±0.179	±0.193	±0.080	±0.129	±0.108	±0.096	±0.115
H_E	0.008	0.037	0.062	0.132	0.089	0.041	0.020	0.077	0.083	0.097	0.027	0.084	0.083	0.036	0.064
	±0.021	±0.078	±0.147	±0.212	±0.177	±0.141	±0.054	±0.179	±0.194	±0.210	±0.093	±0.182	±0.140	±0.123	±0.133
$P_{95\%}$	0.00	16.67	16.67	33.33	25.00	8.33	8.33	16.67	16.67	16.67	8.33	16.67	25.00	8.33	16.67

(1990). Thus, genetic distances between certain populations are likely to be underestimated. The Tsitsikamma population was fixed for a null allele at the *Lt-2*-locus. The coding of this single locus does not bias estimates of genetic differentiation among populations.

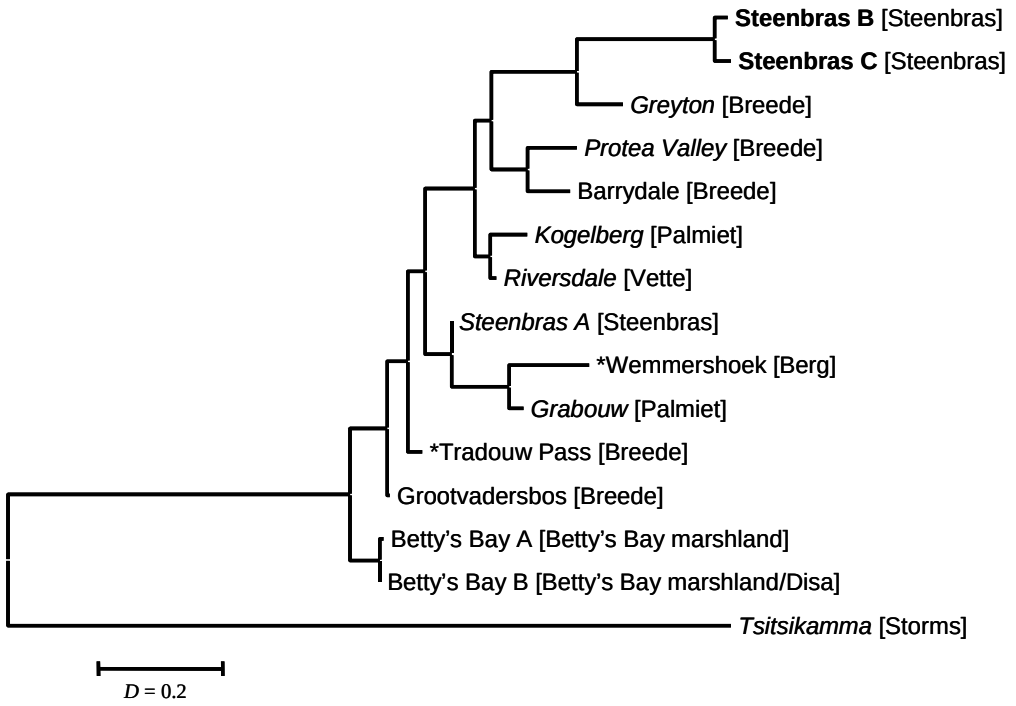
Estimates of genetic variability varied greatly between populations (Table 3.3). The mean number of alleles (A) per population varied between 1.083 ±0.289 (SD) (Barrydale, Kogelberg and Riversdale) and 2.083 ±1.676 at the Steenbras A population. Here seven alleles were found at the *Gpi*-locus, the most found at a single locus in a population. Mean observed heterozygosity (H_O) ranged between 0.008 ±0.021 (Betty's Bay A) and 0.138 ±0.237 (Steenbras A), with mean expected heterozygosity (H_E) ranging from 0.008 ±0.021 to 0.132 ±0.212, and the percentage of polymorphic loci ($P_{95\%}$) per population varying between 0% and 33.33% at the same two populations.

Deviations from Hardy-Weinberg expected genotype frequencies were observed (after Bonferroni correction) at four of 47 individual cases (8.51%) of polymorphism, considering all loci and populations. Although all deviations were due to a deficit of heterozygous individuals, these deviations were not restricted to specific populations or loci, and were not considered to be resulting from sampling artefacts, e.g. Wahlund (1928) effects. These deviations were observed at the *Mdh-1*-locus ($\chi^2 = 23.000$, $P < 0.001$) in the Steenbras A population, the *Idh*-locus ($\chi^2 = 13.405$, $P < 0.001$) in the Tsitsikamma population, and the *Pgm*-locus in the Grabouw ($\chi^2 = 36.000$, $P < 0.001$) and Tsitsikamma ($\chi^2 = 17.092$, $P < 0.001$) populations. Testing for deviation using exact probabilities showed only the *Idh*-locus in the Tsitsikamma population ($P < 0.01$) to be out of Hardy-Weinberg equilibrium.

The neighbour-joining tree and UPGMA-dendrogram (Fig. 3.3), constructed using genetic distances among populations (Table 3.4), both revealed a large genetic distinction between the Tsitsikamma population and the remaining populations. This population was separated from the remainder by a mean genetic distance of 2.020 ±0.336, primarily due to the occurrence of fixed allele differences at the *Ark*-, *Gpi*-, *Lt*- and *Mdh*-loci. Significant heterogeneity in allele frequency, as determined by χ^2-analyses, was further observed between the Tsitsikamma and the remaining populations at all remaining loci (all $P < 0.001$).

Neither topology revealed any distinct patterns relating to geographic locality. In some cases, genetically similar, geographically proximate populations clustered together, e.g. Betty's Bay A and Betty's Bay B samples, which were separated by a genetic distance of 0.002. In other cases, geographically proximate populations fell in separate clusters. For example, the Steenbras B and Steenbras C populations grouped together (D = 0.047), while the geographically proximate Steenbras A population was placed within a cluster containing the Wemmershoek and Grabouw populations in the neighbour-joining tree, and was placed within a larger cluster containing the Grabouw, Grootvadersbos, Tradouw Pass and Betty's Bay populations in the UPGMA-dendrogram. No clear patterns relating to drainage system were found either, as populations from the Palmiet, Steenbras and Breede River catchments clustered separately throughout the topologies (Fig. 3.3).

Further, geographically disjunct populations were often characterised by the shared fixation (or occurrence at high frequency) of alleles absent in other, geographically proximate populations. For example, the Wemmershoek and Grabouw populations were fixed for the Ao^{90} allele. This allele was present only at low frequencies in the Steenbras A population and absent from the remaining populations. Simultaneously, examination of allele frequencies



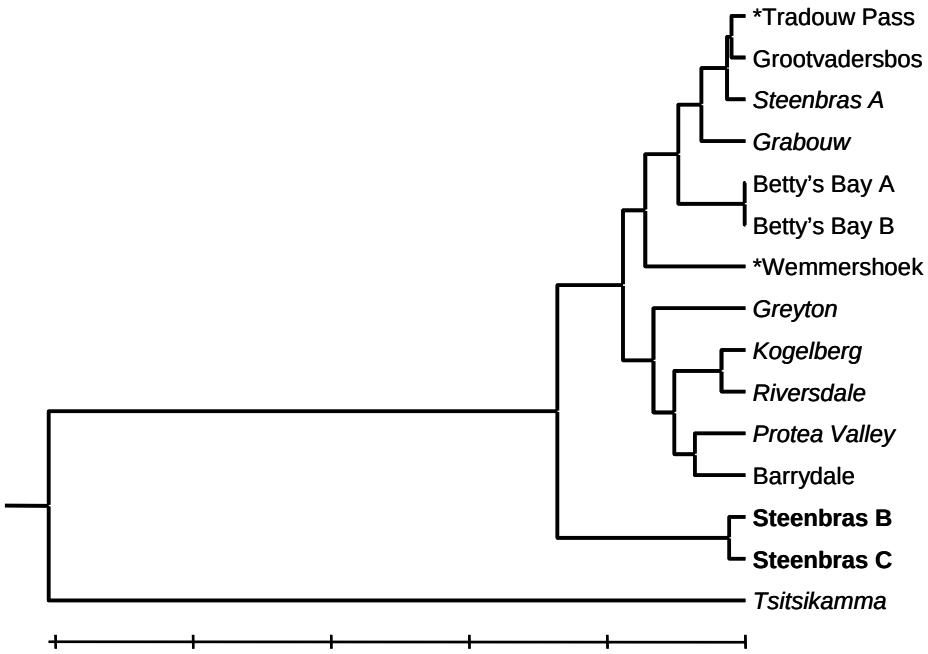


Figure 3.3: Midpoint-rooted neighbour-joining (Saitou and Nei, 1987) tree (above) and UPGMA (Sneath and Sokal, 1973) dendrogram (below) constructed from Nei's (1978) unbiased genetic distances obtained in pair-wise comparisons of the 15 populations of *Mesamphisopus* studied through allozyme electrophoresis of 12 loci. Drainage systems of each of the collection localities are presented in square parentheses. Populations where all examined individuals were morphometrically similar to *M. depressus* or *M. abbreviatus* are indicated in bold and italicized typeface, respectively. Asterices indicate populations where both morphotypes were observed, while normal typeface indicates populations where some individuals could not be assigned to either morphotype. The Betty's Bay B population was excluded from the morphometric analyses.

Table 3.4: Matrix of Nei's (1978) unbiased genetic distance (*D*; above diagonal) obtained in pair-wise comparison of populations and Weir and Cockerham's (1984) θ-estimate (below diagonal) of genetic differentiation among population pairs.

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
(1) Betty's Bay A	---	0.002	0.410	0.157	0.459	0.433	0.412	0.260	0.507	0.531	0.408	0.168	0.194	0.303	1.773
(2) Betty's Bay B	0.067	---	0.417	0.157	0.428	0.401	0.395	0.261	0.493	0.522	0.398	0.169	0.193	0.287	1.749
(3) Wemmershoek	0.903	0.863	---	0.205	0.723	0.718	0.312	0.152	0.482	0.456	0.475	0.257	0.302	0.394	2.832
(4) Steenbras A	0.667	0.625	0.638	---	0.515	0.522	0.199	0.074	0.318	0.303	0.263	0.042	0.064	0.125	1.801
(5) Steenbras B	0.882	0.838	0.861	0.767	---	0.047	0.525	0.741	0.317	0.477	0.547	0.545	0.635	0.502	2.138
(6) Steenbras C	0.931	0.886	0.904	0.822	0.393	---	0.585	0.715	0.322	0.648	0.625	0.542	0.608	0.559	1.938
(7) Kogelberg	0.964	0.912	0.852	0.678	0.864	0.930	---	0.360	0.259	0.161	0.297	0.274	0.290	0.070	1.762
(8) Grabouw	0.820	0.785	0.648	0.388	0.858	0.885	0.836	---	0.487	0.482	0.390	0.145	0.165	0.258	2.390
(9) Greyton	0.913	0.869	0.834	0.692	0.741	0.819	0.813	0.820	---	0.286	0.280	0.337	0.393	0.238	1.943
(10) Protea Valley	0.859	0.844	0.806	0.653	0.787	0.858	0.680	0.787	0.714	---	0.147	0.319	0.334	0.166	2.244
(11) Barrydale	0.930	0.912	0.888	0.730	0.872	0.921	0.923	0.837	0.822	0.672	---	0.224	0.290	0.201	2.399
(12) Tradouw Pass	0.772	0.708	0.743	0.262	0.816	0.865	0.799	0.614	0.762	0.734	0.775	---	0.040	0.181	1.868
(13) Grootvadersbos	0.748	0.733	0.769	0.322	0.833	0.867	0.809	0.615	0.784	0.736	0.799	0.302	---	0.196	1.696
(14) Riversdale	0.921	0.863	0.863	0.583	0.857	0.913	0.692	0.784	0.788	0.681	0.861	0.725	0.739	---	1.741
(15) Tsitsikamma	0.946	0.940	0.937	0.889	0.921	0.938	0.945	0.919	0.924	0.912	0.947	0.920	0.912	0.941	---

within the two sets of geographically most proximate populations revealed substantial fine-scale genetic differentiation. The Steenbras A population was separated from the Steenbras B and Steenbras C populations, which were genetically more similar, by a mean *D*-value of 0.519 ±0.005. Fixed allelic differences were present at the *Ldh-*, *Mdh-1-* and *Me*-loci distinguishing the Steenbras A population. Additionally, significant differences (all $P < 0.001$) in allele frequency were observed at the *Ao-*, *Gpi-* and *Pgm*-loci between these populations. The Protea Valley and Barrydale populations were separated by a genetic distance (*D*) of 0.147. This separation was attributable to a fixed allele difference at the *Gpi-*locus, and allele frequency differences at the *Idh-*, *Pgm-* (both $P < 0.001$) and *Mdh-2*-loci.

Genetic distances obtained in pair-wise comparisons of populations (Table 3.4) ranged from 0.002 to 2.832. Genetic distances between the Tsitsikamma population and the remaining populations ($1.696 \leq D \leq 2.832$) were substantially larger than distance values obtained in comparison of the remaining populations ($0.002 \leq D \leq 0.741$). A mean genetic distance of 0.569 ±0.606 separated all studied populations.

Estimates of θ across the entire sample were similarly indicative of substantial genetic structuring among populations (Table 3.4). The overall θ was estimated at 0.848 (95% confidence interval: 0.767 – 0.934), with estimates at individual loci varying between 0.577 (*Hk*) and 1.000 (*Lt-1* and *Lt-2*). With the exclusion of the Tsitsikamma population, a geographic outlier and phylogenetically distinct taxon (see Discussion), θ was estimated at 0.804 (95% confidence interval: 0.693 – 0.904). Individual estimates ranged from 0.046 (*Mdh-2*) to 0.984 (*Me*). With the exception of the pair-wise comparison of the two Betty's Bay populations (Table 3.4; $\theta = 0.067$), substantial differentiation was apparent among all

other populations, with θ ranging from 0.262 (the Steenbras A – Tradouw Pass comparison) to 0.964 (Betty's Bay A – Kogelberg).

3.3.4) Sequence data analyses

Following the removal of missing data at the end of sequences, a total of 600 bp were aligned (Appendix 7) for the 32 ingroup individuals and two outgroup specimens. The initial neighbour-joining tree (Fig. 3.4a) constructed using "uncorrected p" sequence divergence showed haplotype diversity to be low in populations from which more than one individual was sequenced. A single, unique haplotype was fixed within each of the Barrydale (N = 6), Betty's Bay A (N = 5) and Wemmershoek (N = 5) populations. The Protea Valley population possessed two haplotypes, with the unique Protea Valley 2 haplotype differing at one nucleotide (a transition) from the haplotype present in the remaining four individuals. On this basis, a reduced data set was compiled, including a single representative from each locality. While the haplotype of the Betty's Bay B representative was identical to those sampled in the Betty's Bay A population, the former individual was included as being representative of a different collection locality for the purpose of examining isolation by distance.

Within the reduced data set of 18 taxa, 172 characters were variable and 108 parsimony informative. Of the variable characters, 28 (16.3%) were found in first codon positions, with 15 (8.7%) and 129 (75.0%) occurring in second and third codon positions, respectively. Base frequencies, homogenous across taxa (χ^2 = 5.139, df = 51, P = 1.000), were adenine and thymine rich (A = 0.224, C = 0.127, G = 0.190, T = 0.459), reflecting a bias documented previously for *Mesamphisopus* (Chapter 2) and isopods in general (Wetzer, 2001; Ketmaier *et al.*, 2003).

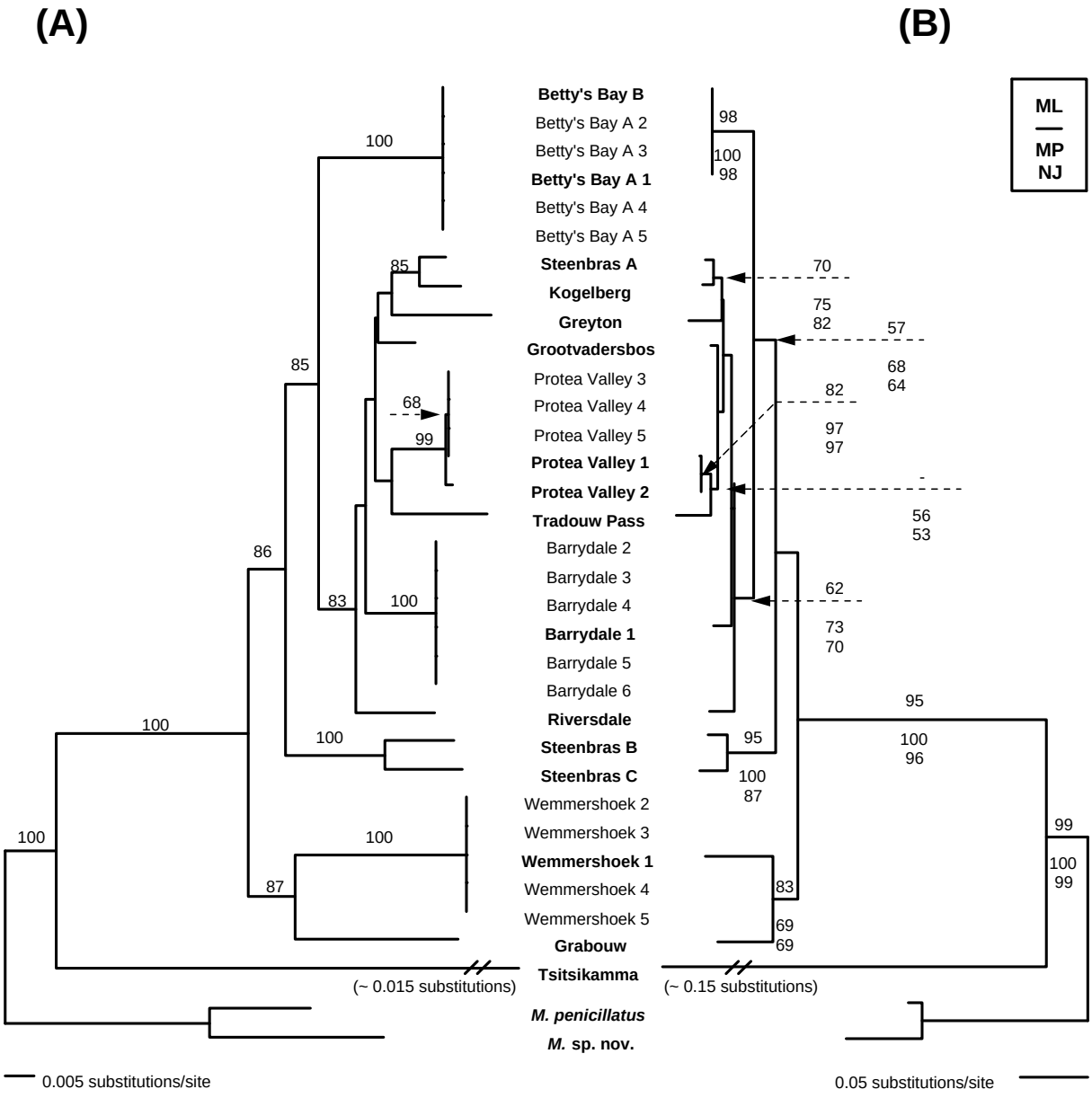


Figure 3.4: (A) Neighbour joining (Saitou and Nei, 1987) tree based on "uncorrected p" sequence divergences calculated from 600 bp of cytochrome oxidase I (COI) mtDNA for the 32 ingroup taxa (identified as *Mesamphisopus abbreviatus* / *M. depressus*) and two outgroup (*Mesamphisopus* sp. nov. and *M. penicillatus*) representatives. Numbers above the branches represent bootstrap support for the nodes calculated from 10000 pseudoreplicates. (B) Maximum likelihood tree (-lnL = 2268.579) of the reduced data set (bold typeface) of 16 ingroup taxa (individual unique haplotypes or representatives of different collection localities) and the two outgroup individuals. Analysis included 600 bp COI mtDNA and implemented a GTR + I + Γ (Rodríguez *et al.*, 1990) model of nucleotide evolution (individual substitution parameters presented in Chapter 3: Results). Numbers above the branches represent bootstrap support from 100 pseudoreplicates, while numbers below represent bootstrap support from the MP (1000 replicates) and NJ analyses (10000 replicates), respectively. Only bootstrap support > 50% is indicated.

The MP analyses retrieved six equally parsimonious trees of 240 steps (CI = 0.592, RI = 0.672, Rescaled CI = 0.398). MODELTEST suggested the use of a general time reversible model (Lanave *et al.*, 1984; Rodríguez *et al.*, 1990) of nucleotide substitution, together with a proportion of invariable sites and a gamma-distributed shape parameter (GTR + I + Γ), as the most appropriate to be implemented in the ML analyses. The following estimated base frequencies and substitution parameters were used in the ML analysis: base frequencies: A = 0.247, C = 0.113, G = 0.163, T = 0.477; rate matrix: R_1 = 0.222, R_2 = 15.196, R_3 = 1.493, R_4 = 0.523, R_5 = 4.727, R_6 = 1.000; proportion of invariant sites = 0.427; and a gamma distribution shape parameter (α) of 0.393. The resulting maximum-likelihood topology (-ln = 2268.579) is presented in Figure 3.4b. Generally, congruent topologies were obtained in the MP, ML and NJ analyses. While the sister-taxa relationships of certain individuals were supported in all analyses (e.g. Steenbras A + Kogelberg; Wemmershoek + Grabouw; Protea Valley 1 + Protea Valley 2), other relationships were usually not strongly supported or were retrieved only by certain analyses. All analyses, however, supported (with bootstrap ≥ 95%) the existence of a monophyletic ingroup clade to the exclusion of the distantly related Tsitsikamma representative. Although the two representatives of the populations identified through the morphometric analysis as *M. depressus* (Steenbras B and C) grouped together, they were nested among individuals identified morphometrically as *M. abbreviatus*.

Again no geographic patterns or patterns relating to drainage system were evident from the relationships among representatives. Representatives collected from geographically proximate localities were again widely separated, lying within different clades (e.g. Steenbras A, B and C), while the relationships among geographically more distant individuals were supported (e.g. Wemmershoek and Grabouw).

Topologies derived from the analyses of the sequence data were incongruent with those based on allozyme data. Indeed, topologies constrained to reflect the ingroup relationships proposed by the allozyme neighbour-joining tree and UPGMA-dendrogram were, respectively, 29 and 38 steps longer than the topologies retrieved in the MP and ML analyses (both 240 steps, autapomorphic characters excluded). While certain terminal relationships among closely related individuals/populations were consistently retrieved in all analyses, conflicting relationships were suggested for many of the populations/representatives. For example, while the relationship between the geographically proximate Protea Valley and Barrydale populations proposed by the allozyme analysis was not rejected in the analyses of sequence data, a sister-taxa relationship was proposed between the Protea Valley representative and the more distantly collected Tradouw Pass representative, with the Barrydale representative occurring basal to a larger clade including the former individuals.

Uncorrected sequence divergences among ingroup individuals and the outgroup specimens ranged from 12.00% to 16.50%. Despite the monophyly (bootstrap ≥ 99%) of the 16 ingroup taxa, the Tsitsikamma representative was less similar to the remaining ingroup individuals (distinguished from the remaining ingroup specimens by sequence divergences of 15.50% to 16.50%) than were the outgroup individuals (12.00% to 14.83% divergent). Sequence divergences among these remaining ingroup individuals were between 0.00% (the comparison of Betty's Bay A and Betty's Bay B representatives) and 7.83% (the comparison of the Grabouw and Greyton representatives), with a mean of 4.66 ±1.87%.

The unrooted haplotype network (Fig. 3.5) showed most representatives to be related, albeit distantly, to the central Grootvadersbos (13) representative. As in other analyses, no significant patterns related to geographic locality or drainage system could be detected. The

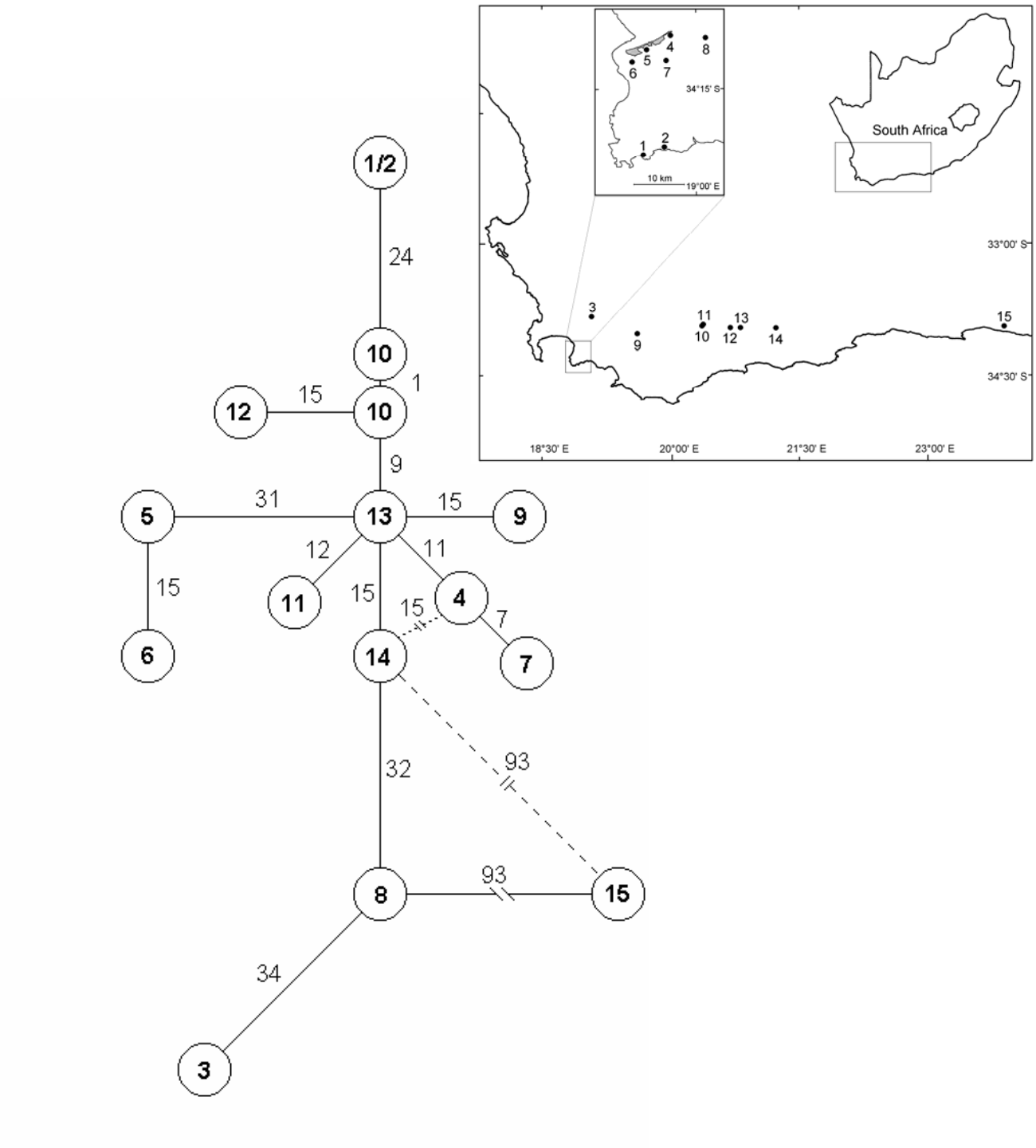


Figure 3.5: Unrooted parsimony network of the unique, representative haplotypes from the fifteen *Mesamphisopus* populations studied. Numbers above the branches indicate the number of mutational steps. Single haplotypes are numbered (in bold font) according to collection locality, following Figure 3.1 (inset). Dashed lines/branches indicate alternative, equally-parsimonious connections.

network did, however, substantiate the genetic distinctiveness of the representatives of certain populations, including the Grabouw, Wemmershoek and, particularly, Tsitsikamma populations. The long lengths of the branches connecting these representative haplotypes, in turn, indicates the extinction of, or the failure to sample, many haplotypes.

3.3.5) Isolation by distance

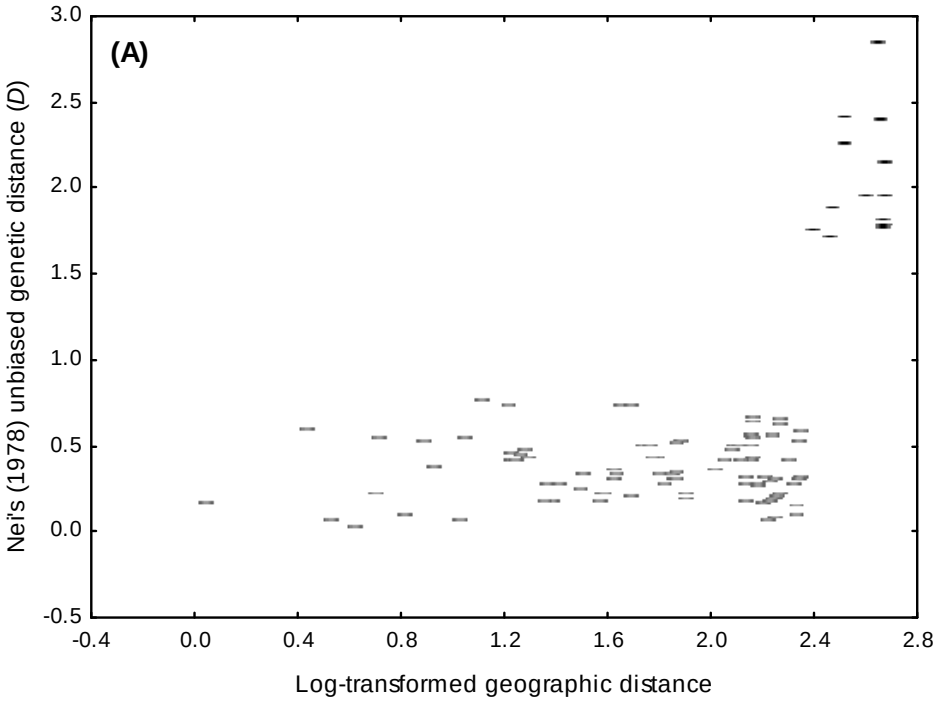
When considering individual populations, a significant correlation (r = 0.779, t = 3.561, $P < 0.05$; Table 3.5, Figure 3.6) was found between geographic distance between collection localities and the mean genetic distance (*D*) among populations, indicating isolation by distance. If comparisons involving the Tsitsikamma population were omitted, this relationship was, however, non-significant (r = -0.013, t = -0.109, $P = 0.484$). Correlations investigated for individual locus comparisons, using genetic identity (*I*) as a measure of differentiation, showed no evidence of isolation by distance (Table 3.5) with one exception; a significant relationship was evident at the *Mdh-2*-locus (r = 0.202, t = 1.462, $P < 0.05$) with the omission of the Tsitsikamma population.

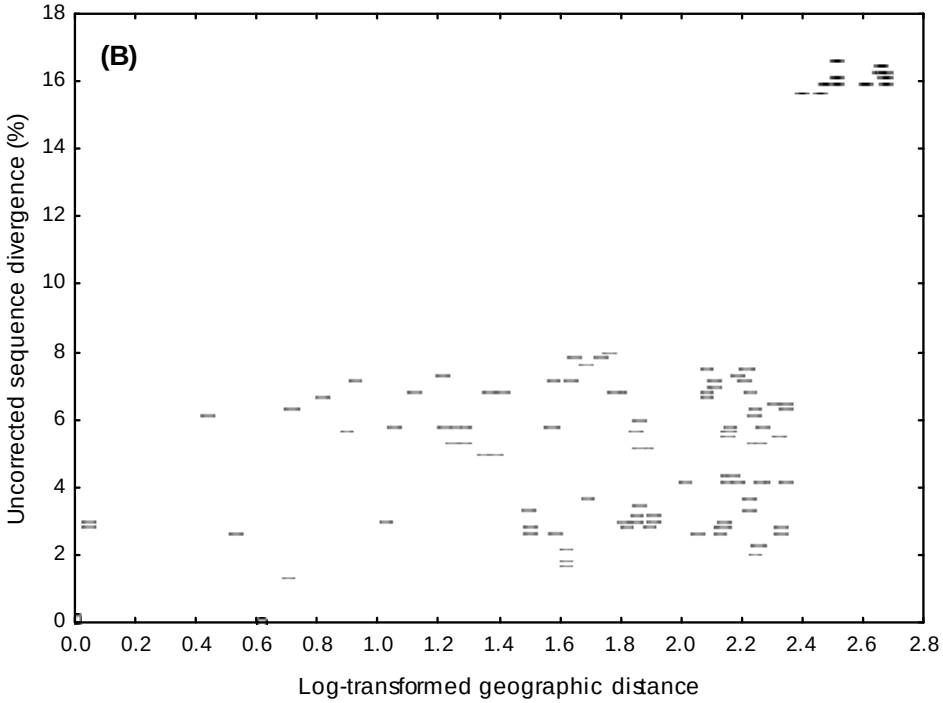
As in the allozyme analysis, a significant correlation was found between sequence divergence and the geographic distance between collection localities (Table 3.5, Fig. 3.6; r = 0.468, t = 3.249, $P < 0.01$), but the relationship again became insignificant (r = 0.131, t = 1.249, $P = 0.112$) upon exclusion of the Tsitsikamma representative.

Considering differentiation among regions, with populations pooled within regions, an identical pattern was observed. There were significant correlations (Table 3.5) among genetic distance (*D*) and geographic distance (r = 0.760, t = 2.369, $P < 0.05$), and sequence

Table 3.5: Correlations (r), t-values and significance values (*P*) from the Mantel (1967) tests examining correlations between geographic distance and genetic divergence. Comparisons involved (A) matrices of log-transformed geographic distances between collection localities of individual populations, genetic differentiation, measured over all loci (mean *D* values) and at individual loci (*I*-values), among individual populations, and sequence divergence ("uncorrected p") among representatives from each of the populations; or (B) matrices of mean log-transformed geographic distance, mean genetic distance (*D*) and mean sequence divergence calculated among defined regions (see Materials and Methods). Mantel tests were performed with all populations/representatives or regions, and excluding the Tsitsikamma population/representative or region, independently. Significant correlations ($P < 0.05$) are indicated in bold type font.

		Including all			Excluding Tsitsikamma		
		r	t	*P*	r	t	*P*
(A)	Mean *D* vs. geographic distance	**0.779**	**3.561**	**0.024**	-0.013	-0.109	0.484
	Ao (*I*) vs. geographic distance	0.044	0.278	0.517	0.003	0.026	0.580
	Ark (*I*) vs. geographic distance	-0.493	-2.871	0.995	-0.171	-1.199	0.930
	Gpi (*I*) vs. geographic distance	-0.261	-2.102	0.973	-0.202	-1.721	0.940
	Hk (*I*) vs. geographic distance	-0.056	-0.404	0.718	-0.133	-0.922	0.851
	Idh (*I*) vs. geographic distance	-0.490	-2.923	0.991	-0.155	-1.115	0.865
	Ldh (*I*) vs. geographic distance	-0.174	-1.639	0.938	-0.053	-0.499	0.747
	Lt-1 (*I*) vs. geographic distance	-0.493	-2.869	1.000	---	---	---
	Lt-2 (*I*) vs. geographic distance	-0.493	-2.869	1.000	---	---	---
	Mdh-1 (*I*) vs. geographic distance	-0.171	-1.134	0.851	0.108	0.839	0.182
	Mdh-2 (*I*) vs. geographic distance	-0.492	-2.866	0.935	**0.202**	**1.462**	**0.040**
	Me (*I*) vs. geographic distance	-0.240	-1.855	0.952	-0.066	-0.569	0.787
	Pgm (*I*) vs. geographic distance	-0.109	-0.845	0.811	0.061	0.521	0.331
	Sequence divergence vs. geographic distance	**0.468**	**3.249**	**0.007**	0.131	1.249	0.112
	Sequence divergence vs. mean *D*	**0.922**	**3.821**	**0.000**	**0.561**	**3.642**	**0.001**
(B)	Mean *D* vs. geographic distance	**0.760**	**2.369**	**0.025**	0.029	0.131	0.412
	Sequence divergence vs. geographic distance	**0.776**	**2.399**	**0.018**	0.224	1.199	0.148
	Sequence divergence vs. mean *D*	**0.948**	**2.391**	**0.001**	**0.672**	**1.810**	**0.016**


(A)
Nei's (1978) unbiased genetic distance (D)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-0.4
0.0
0.4
0.8
1.2
1.6
2.0
2.4
2.8
Log-transformed geographic distance


(B)
Uncorrected sequence divergence (%)
18
16
14
12
10
8
6
4
2
0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
Log-transformed geographic distance

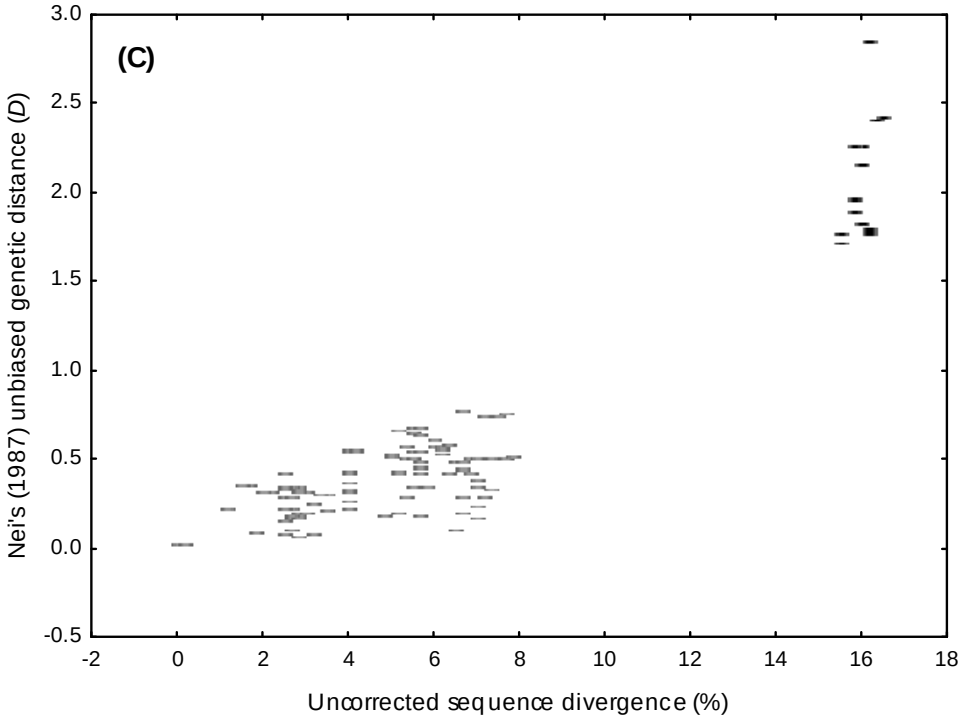


Figure 3.6: Scatterplots of (A) Nei's (1978) unbiased genetic distance among populations over the log-transformed geographic distance between their collection localities; (B) uncorrected sequence divergence (calculated form 600 bp of COI mtDNA) among representatives from each population over the log-transformed geographic distances between collection localities; and (C) genetic distance among populations over the uncorrected sequence divergences separating representatives from the same populations. Filled circles represent comparisons involving the Tsitsikamma population/representative, while comparisons between other ingroup populations/representatives are indicated by open circles.

divergence and geographic distance (r = 0.776, t = 2.399, $P < 0.05$), indicating isolation by distance. Again, these relationships became insignificant upon exclusion of the Tsitsikamma region (r = 0.029, t = 0.131, $P = 0.412$, and r = 0.224, t = 1.199, $P = 0.148$, respectively).

Over a smaller geographic scale, there was significant correlation between sequence divergence and geographic distance (r = 0.456, t = 2.024, $P < 0.05$) among individual populations of the Hottentot's Holland Mountain region, but not between genetic (*D*) and geographic distance (r = 0.147, t = 0.655. $P = 0.189$).

Mantel (1967) tests revealed a significant correlation between genetic distance and sequence divergence (Table 3.5, Fig. 3.6) in comparisons of distances matrices compiled to examine isolation by distance among individual populations and among regions. These significant relationships held, irrespective of the inclusion or exclusion of the Tsitsikamma population/representative or region. This correlation was also apparent when considering only the populations from the Hottentot's Holland region (r = 0.609, t = 2.707, $P < 0.01$).

3.4) Discussion

While the morphological characters presented in the published key (Kensley, 2001) and species descriptions (Barnard, 1927; Nicholls, 1943) appeared to be equivocal for identifying *Mesamphisopus abbreviatus* and *M. depressus*, the species appeared to be well separated using additional morphometric data. *Mesamphisopus depressus* individuals were separated from *M. abbreviatus* individuals in a discriminant function analysis, in which the body length, body width (at the third pereonite) and telson depth were among the most important variables

of those combined in the discriminant functions. Nicholls (1943) had suggested further differences among these putative species (e.g. the extent of the cervical groove, the shape of the posteroventral margins of the pleura of the pleonites, the setation of the pleopods, and the shape and setation of the uropodal peduncles), mostly through comparisons with *M. capensis*. Kensley (2001), however, did not consider these characters in compiling the key, or considered the characters to be of little importance. These characters thus need to be re-evaluated in these and other species within *Mesamphisopus* and may prove useful in distinguishing the two species.

A subsequent morphometric analysis, using classification functions determined from a discriminant function analysis and pereon and pleon dimensions of individuals, determined that individuals of most of the populations sampled for the genetic analyses were morphometrically identifiable as *M. abbreviatus*. In only two populations (Steenbras B and C) were all the examined individuals determined to be morphometrically similar to *M. depressus*. Interestingly, these two populations were grouped together (following the separation of the Tsitsikamma population (see below)) in the UPGMA dendrogram based on genetic distance, separate to a large cluster containing all the remaining populations. A level of uncertainty is inherent in these morphometric diagnoses, however, as certain individuals could not be assigned to either morphotype. Further, certain populations were shown to possess individuals of both morphotypes. The extent or patterns of morphometric differentiation among populations *per se* was not considered in the present analysis. It is possible, as an artifact of this classificatory approach, that intermediate or alternative morphotypes in these populations have not been identified, while differentiation among populations (all broadly morphometrically similar to *M. abbreviatus*) or their morphometric distinctiveness remain unknown. Further, dimensions of the pereopods and uropods, that may

differentiate these populations and the *M. abbreviatus* and *M. depressus* syntypes, were not considered. Given the apparently recent radiation of the group of studied populations (see below) and the morphological conservatism of the Phreatoicidea (e.g. Wilson and Ho, 1996), it is also probable that morphometric differentiation of populations has not proceeded to the extent that they are recognized as different morphotypes under the current approach.

Morphological and morphometric differentiation within Crustacea is often difficult to interpret. For example, while morphometric differentiation has been found to be consistent with species boundaries in some cases (Stewart *et al.*, 2004), substantial morphometric or morphological differentiation has also been observed in the absence of genetic differentiation or genetic evidence of species boundaries (e.g. Daniels *et al.*, 1998b; Finston, 2000; Schubart *et al.*, 2001), and vice versa (e. g. Baldwin *et al.*, 1998; King and Hanner, 1998). As a result, morphometric differentiation among populations is often seen to reflect morphological plasticity, in turn affected by factors such as diet and temperature (Hartnoll, 1982). Simultaneously, morphological and morphometric similarity among genetically differentiated populations or species is most often thought to result from convergence, hybridization and introgression, or shared ancestry (Taylor *et al.*, 1996; Harrison and Crespi, 1999; Remigio, Hebert and Savage, 2001).

The presence of both morphotypes in individual populations may similarly reflect morphological plasticity (and, thus, convergence in these different populations) in response to local environmental conditions. This explanation may too be extended to the variable presence or absence of the subapical robust setae on the pleotelson, although the geographic scale at which frequency differences are observed suggests that this is not the case. These polymorphisms, particularly with regard to morphotype, are unlikely to be related to

population age or size structures, as has been proposed to account for morphometric differentiation among populations elsewhere (e.g. Allegrucci *et al.*, 1992), as the male individuals chosen for the morphometric analyses were the largest in their respective population samples and were undoubtedly mature individuals. The further possibility of both of these polymorphisms (i.e. body shape/form and pleotelson setation) being due to the sympatric occurrence of two separate gene pools at each of the sampling localities cannot be discounted at present. Hybridization between these gene pools may obscure any genetic discontinuities within the "populations", while chance dispersal of individuals of each gene pool may maintain polymorphism in individual "populations". This will, however, need to be examined with more extensive sampling and genetic analyses of these particular populations. Alternatively, perhaps more likely, these may represent ancestral polymorphisms, with character states reaching various stages of fixation or loss in the individual populations. Stochastic demographic processes (as described below for the genetic patterns observed) could potentially determine the extent of fixation or loss of these features in individual populations.

Although the allozyme and sequence data analyses did not retrieve congruent topologies, all analyses supported the genetic distinctiveness of the Tsitsikamma population/representative, and its exclusion from a clade containing the remaining populations or representatives. Genetic distances between the Tsitsikamma population and the remaining populations were at least double and often an order of magnitude greater than distances among these remaining populations. These values are also substantially larger than those previously found among putative species within *Mesamphisopus* (Chapter 2). Further, the Tsitsikamma population was distinct from the remainder at all loci examined. Sequence divergences showed the Tsitsikamma representative to be more divergent from the remaining ingroup individuals than

were the two outgroup specimens, both distinct species (*Mesamphisopus* sp. nov. and *M. penicillatus*). In light of this, it must be concluded that the Tsitsikamma population represents a phylogenetically distinct, cryptic species. It is also likely that additional sampling within the poorly sampled Tsitsikamma region may reveal further undescribed species.

The delineation of further species is confounded by the lack of congruence among topologies, as consistently monophyletic clades, likely to represent species or species groups, were not retrieved. Genetic distances and sequence divergences among the remaining populations or representatives also present somewhat of a paradox as far as the possible delimitation of species based on distance criteria is concerned. While the two sets of divergence estimates were significantly correlated (regardless of the inclusion or exclusion of the Tsitsikamma population/representative), the implications of the differentiation suggested by each were different. Although Wetzer (2001) did not specifically include interspecific comparisons involving members of the Phreatoicidea for the COI gene fragment in her hierarchical investigation, interspecific sequence divergences between 32.9% and 34.9% were found within the Cirolanidae (Suborder Flabillifera). Remarkably high (corrected) sequence divergences (> 69%) also separated recognized species of *Stenasellus* Dollfus, 1897 (Ketmaier *et al.*, 2003), while (uncorrected) divergences of ~ 15 to 25% separated species within *Idotea* Fabricius, 1798 and *Hawaiioscia* Taiti & Howarth, 1997 (Wares, 2001a; Rivera *et al.*, 2002). Comparisons among the ingroup representatives yielded substantially lower (uncorrected) sequence divergences of 0.17% to 7.83% (mean 4.66% ±1.87). These values were more comparable to intraspecific sequence divergences presented by Wares (2001a) and Rivera *et al.* (2002), and the lower range of divergences presented by Ketmaier *et al.* (2003). Although these values were similar to interspecific sequence divergences (~ 3 to 11%) from a 12S rRNA gene fragment in *Mesamphisopus* (Chapter 2), it is important to note that this

fragment appears to be more conserved than COI for a range of comparisons at different taxonomic hierarchies within certain isopod groups (Wetzer, 2001). Thus, sequence divergences and the lack of strong resolution among populations did not strongly suggest the recognition of a species complex among the studied populations. However, genetic distances based on allozyme data appeared to be more ambiguous in this regard. The genetic distances among these populations (mean $D = 0.346 \pm 0.176$) were greater than presumably intraspecific distances hitherto documented within *Mesamphisopus* and were more comparable to some of the presented interspecific distances within *Mesamphisopus* ($D = 0.192 – 1.002$; Chapter 2) and other isopod groups (Garthwaite *et al.*, 1992; Viglianisi *et al.*, 1992; Lessios and Weinberg, 1994; Ketmaier *et al.*, 1998, 2001). However, the genetic distances were particularly comparable to those separating various identified subspecies within the Isopoda (see Cobolli Sbordoni *et al.*, 1997; Ketmaier *et al.*, 1999). Although Cobolli Sbordoni *et al.* (1997) highlighted the use of subspecies designations to recognise differentiated populations within species with poor dispersal ability and discontinuous distributions, it is premature to suggest such designations with the data at hand, given the discordance of patterns revealed by the data sets. That the examined populations are representative of a recently differentiated species complex is supported by only limited evidence: the two populations identified morphometrically as *M. depressus* and collected from near the presumed type locality of *M. depressus* in the Steenbras Valley were consistently retrieved as sister-taxa. These also formed a phylogenetically distinct lineage and were (with the exception of the UPGMA dendrogram based on the allozyme data) topologically nested among the remaining populations, all similarly differentiated. However, in the absence of additional data and topotypic samples to adequately resolve the status of *M. abbreviatus*, *M. depressus* and the above populations genetically, it would be most prudent to consider the populations studied here as potentially conspecific, at least for the sake of further discussion.

Genetic differentiation was evident among nearly all populations over all spatial scales. Broadly, this differentiation may be attributable to a lack of gene flow due to geographic barriers to dispersal, for example, or to stochastic demographic processes, such as random extinction and founder events or population crashes and bottlenecks. Considering the populations conspecific, a lack of gene flow was evident from the number of fixed allelic differences, resulting in high θ-estimates and distance values, and the great disparity in genetic variability estimates among local populations (e.g. those centred around the Steenbras Dam) and distant populations. Significant intraspecific genetic differentiation, evident as either significant allele frequency differences or fixed allele differences, and a lack of gene flow over small spatial scales have similarly been documented in cave-dwelling (Cobolli Sbordoni *et al.*, 1997; Gentile and Sbordoni, 1998; Ketmaier *et al.*, 1998) and intertidal isopods, where differentiation has been observed over less than a few kilometres (Lessios and Weinberg, 1993, 1994; Lessios, Weinberg and Starczak, 1994; Carvalho and Piertney, 1997) or metres (Piertney and Carvalho, 1994, 1995a, b). The patterns themselves appeared to be unrelated to the geographic distances between populations, resulting in non-significant relationships in tests for isolation by distance, especially over the larger spatial scales. This is unlike certain freshwater isopods, where (with certain exceptions) geography predicted genetic patterns (Ketmaier *et al.*, 2001), and terrestrial isopods in which isolation by distance has been demonstrated, often in the absence of clear geographic patterns (Wang and Schreiber, 1999). Patterns of relatedness among populations were apparently unrelated to drainages, as expected under a nested hierarchy of drainages (e.g. Woolschot *et al.*, 1999). The pattern observed, in analyses of both markers, is best described as a mosaic, with genetic differentiation on local scales being generally equivalent to differentiation over large scales and bearing no relation to geography. Similar patterns of genetic patchiness have been described for isopods (Lessios and Weinberg, 1993, 1994; Piertney and Carvalho, 1995a;

Bilton, Goode and Mallet, 1999) and, surprisingly, for marine decapods (e.g. McMillen-Jackson, Bert and Steele, 1994; Perez-Enriquez *et al.*, 2001).

In most of the above studies (Lessios and Weinberg, 1993, 1994; Lessios *et al.*, 1994; Piertney and Carvalho, 1995a, b; Carvalho and Piertney, 1997; Cobolli Sbordoni *et al.*, 1997; Gentile and Sbordoni, 1998; Ketmaier *et al.*, 1998, 1999) differentiation among populations has been explained by stochastic population crashes, due to habitat instability, leading to local extinction and recolonization (founder) events, or to population bottlenecks. In the absence of gene flow, small effective population sizes would then lead to the differentiation of populations through mutation, genetic drift, localized selection and inbreeding. This low gene flow, essentially isolating the populations reproductively, would be affected by the limited vagility and dispersal of adult individuals, the "direct" marsupial development of the young, the semi-isolated or patchy distributions of populations, the discontinuity of ecologically suitable habitat to aid dispersal, or the presence of geographic obstacles. These processes may lead to initial reductions in heterozygosity and variability (Lessios and Weinberg, 1994; Piertney and Carvalho, 1995b) and rapid, drastic and, often, frequent temporal changes in allele frequencies (Lessios *et al.*, 1994; Carvalho and Piertney, 1997), subsequently observed as spatial genetic differentiation.

Over and above the obvious restriction to drainages, *Mesamphisopus* is generally restricted to the high-altitude, slow-running portions of catchments in broad mature valleys, where the flow is low, and dark mud provides both a food source and refuge (Barnard, 1927). More broadly, the distribution of *Mesamphisopus* is also governed by water temperature and the presence of sufficient moisture from the mist belt in the dry months, which results in the typically patchy distribution of populations (Barnard, 1927). Although the observed patterns

seem to be indicative of migration and gene flow at some time in the past, continuous habitats through which dispersal would be possible are presently lacking, and in combination with low adult vagility and the peracarid characteristic of marsupial development of the young, recent gene flow or dispersal among populations would have been unlikely. While the ability to aestivate over long periods has been documented within *Mesamphisopus abbreviatus* (Barnard, 1927), the highly seasonal nature of their habitats (high-altitude, first-order streams/seepages) suggests that populations are exposed to frequent droughts during the summer months and would experience population crashes. Given the poor potential for dispersal and the large geographic distances involved, repeated population bottlenecks with sufficient numbers surviving through aestivation to enable population survival are favoured as an explanation for the differentiation among populations rather than extinctions and founder events. Similarly, frequent population crashes and bottlenecks, due to the nature of the habitat and climate, have been invoked as an explanation for the population differentiation and low variability observed within paramelitid amphipods (Stewart, 1992), often occurring sympatrically with *Mesamphisopus* in the Western Cape (Barnard, 1927; pers. obs.). These bottlenecks would lead to the differentiation of geographically proximate populations, through mutation, genetic drift and inbreeding, with geographically distant populations potentially remaining less differentiated, resulting in the observed mosaic pattern. Ancestral alleles could be retained in certain populations and lost in others, resulting in alleles being shared by geographically disjunct populations rather than proximate populations (Lessios and Weinberg, 1993; Cobolli Sbordoni *et al.*, 1997). It is interesting to note, however, that estimates of genetic variability, expected to be reduced by population bottlenecks, in the examined populations showed no clear relationship to altitude or to the present permanency of the freshwater habitat, as was found for amphipods from the same region (Stewart, 1992). While comparable levels of genetic variability were found in most populations, the population

with the lowest estimates (Betty's Bay A) inhabited the most permanent of the sampled water bodies. This latter case may indicate multiple, more recent bottlenecks, as repeated collections from this site have indicated large fluctuations in population size, related to flooding of this habitat. The patterns observed from the sequence data could result from stochastic lineage sorting and the random fixation (or near fixation) of different, albeit closely related haplotypes in these populations. Indeed, branch lengths, sequence divergences, and the parsimony network suggest that the radiation of most of the ingroup representatives was rapid and fairly recent, at least relative to the divergences of the Tsitsikamma lineage and the outgroup specimens. The parsimony network may provide additional evidence of these repeated bottlenecks, followed by drift or lineage sorting, as many extinct (or unsampled) haplotypes were identified. The processes of genetic drift, leading to differentiation, are additionally likely to be exacerbated by short generation times (Lessios *et al.*, 1994). *Mesamphisopus* individuals are thought to breed after one year, although it is likely (but improbable) that they can breed sooner (Barnard, 1927).

Discordances among patterns revealed by nuclear (allozymes) and mitochondrial markers have typically been explained by balancing selection (Piel and Nutt, 2000; and references therein). Given the small spatial scales over which differentiation is observed in the present study, local selection is unlikely to influence allele frequencies at these supposedly neutral loci. The discordance may, however, be explained in terms of the stochastic processes of lineage sorting and the fixation of haplotypes and alleles, discussed above. The apparent disparity between the extent of differentiation suggested by the mitochondrial (sequence divergences) and nuclear allozyme markers (genetic distances) is more difficult to explain. Mitochondrial DNA, due to its high mutation rate, haploid nature and uniparental inheritance, resulting in a four-fold smaller effective population size, is regarded as being a more sensitive

marker of population differentiation than nuclear markers (Moritz *et al.*, 1987), such as allozymes. Here the opposite appeared to be the case. This is not entirely improbable, in the context of founder events, particularly if a population is established by females bearing young. Lineage sorting may then proceed among a relatively small pool of closely related haplotypes, while genetic drift may lead to the fixation, or increased abundance of, any of the larger number of nuclear alleles. This hypothetical scenario would explain the differentiation among populations if they were established from a common source population (or genetically similar source populations). The above disparity may, however, be an artifact of the specific allozyme loci examined or the divergence estimates used (see Kalinowski, 2002). Although most loci were polymorphic or provided evidence of fixed allelic differences among populations, the choice of loci examined was not explicitly biased in this regard, as all loci that produced reliably interpretable zymograms were included in the analyses. The genetic distance (Nei, 1978) used may be vastly inflated or "saturated", as estimates at these individual loci approach infinity when fixed allele differences are present. Nei's (1978) distance measure also appears to be more sensitive than other measures to reductions in population sizes under modelled conditions (Kalinowski, 2002). Unfortunately, the sampling strategy for the mitochondrial DNA study also precluded the use of statistical analyses (e.g. Analysis of Molecular Variance) that would have provided estimates of among-population genetic differentiation that would be perhaps more appropriately comparable between markers. Nonetheless, these results, like those of Piel and Nutt (2000), indicate that conclusions based on topologies and genetic divergences derived from single markers may be spurious and again argue for caution in the use of genetic distances for making taxonomic decisions. Again, the use of multiple data sets would be most advantageous.

Chapter 4: New species within the endemic South African isopod genus *Mesamphisopus* (Isopoda: Phreatoicidea: Mesamphisopodidae).

4.1) Introduction

The suborder Phreatoicidea is the earliest derived and, phylogenetically, the most basal among the isopods (Wägele, 1989; Brusca and Wilson, 1991). Their fossil record, including among the earliest definitive peracarid crustacean fossils, extends to the Carboniferous (Schram, 1970, 1974, 1980) and indicates the occupancy of freshwater habitats since the Middle Triassic (Chilton, 1918; Wilson and Edgecombe, 2003). The present distribution of the group is Gondwanan, reflecting the fragmentation of the landmass, with representatives found in Australia, New Zealand, India and (South) Africa (Wilson and Johnson, 1999; Wilson and Keable, 1999; Kensley, 2001). Taxa are presently exclusively confined to freshwater habitats; including streams, springs, wells, marshes and lakes and subterranean waters (Wilson and Keable, 1999; Kensley, 2001). The extant fauna is represented by some 68 decribed species (some with identified subspecies and varieties), included in some 30 genera (Nicholls, 1943, 1944; Chopra and Tiwari, 1950; Tiwari, 1955a; Wilson and Ho, 1996; Knott and Halse, 1999; Wilson and Keable, 1999, 2002a, b, 2004; Kensley, 2001). The suborder's greatest diversity (including 25 genera) and endemism are to be found within Australia, particularly within the Bassian biogeographic province (south-eastern mainland Australia and Tasmania) (Wilson and Johnson, 1999; Knott and Halse, 1999; Wilson and Keable, 2001). The South African fauna, by contrast, is represented by a single, endemic genus.

The discovery of the first phreatoicidean isopod from South Africa was noted in 1913 (Barnard, 1913), with the species description appearing a year later (Barnard, 1914). The species, *Mesamphisopus capensis*, was initially placed in the genus *Phreatoicus*, which then contained eight species from New Zealand and Australia (from both the mainland and Tasmania) (see Barnard, 1914). These species, together with three additional monotypic genera (*Hypsimetopus*, *Phreatoicoides* and *Phreatoicopsis*), comprised the suborder Phreatoicidea at the time. The South African discovery was particularly noteworthy, hinting at both the Gondwanan distribution and the antiquity of the suborder (Barnard, 1913, 1914; Chilton, 1918); facts now widely accepted (see above; Newman, 1991; Bănărescu, 1995; Wilson and Johnson, 1999; Wilson and Keable, 2001).

More collections from across the Western Cape, South Africa, led Barnard (1927) to describe two additional varieties: *P. capensis* var. *abbreviatus* and *P. capensis* var. *depressus.* These varieties were distinguished from each other and from *P. capensis* by, primarily, the shape of the pleotelson and gnathopod, and the extent of dorsoventral compression and setation of the pereon (Barnard, 1927). Other features known to vary included the setation of the pleotelson, antenna length and coloration (Barnard, 1927). An additional variety, *P.* var. *penicillatus*, was later identified by Barnard (1940) and characterised by a head and pleotelson that were more strongly setose (but see Kensley, 2001) than the remaining varieties and an excessively setose antennal peduncle.

Nicholls (1926), prior to his major revision of the Phreatoicidea (Nicholls, 1943, 1944), had suggested that *P. capensis* appeared to be intermediate to *Phreatoicus*, *Phreatoicopsis*, *Eophreatoicus* and *Amphisopus*, and warranted inclusion in a new genus. Subsequently, he (Nicholls, 1943) established the genus *Mesamphisopus* for the South African members and

included three species: *M. abbreviatus*, *M. capensis* and *M. depressus*. The *P. capensis penicillatus* variety was not raised to the specific level, as were the others; Nicholls presumably being unaware of Barnard's (1940) publication (Kensley, 2001). The genus was included in the subfamily Mesamphisopodinae with the hypogean species *Hyperoedesipus*, and placed within the family Amphisopodidae (Nicholls, 1943). The subfamily was subsequently raised to familial level by Knott (1975). This arrangement is followed presently, although *Mesamphisopus* and *Eophreatoicus* (from the Northern Territory, Australia) remain the only genera included in the family (Poore *et al.*, 2002). The defining characters of *Mesamphisopus* have included the occurrence of setae on all five pleopodal endopods, and, in combination, the occurrence of plumose setae on these endopods, the retention of a vestigial innermost (medial) lobe on the maxilla, the retention of a fifth pair of (vestigial) oostegites, the presence of movable apical spines on the uropodal rami, simple spine(s) distoventrally on the uropodal penduncle, couplings hooks on the pleopods, prominent eyes, short antennules and short, cylindrical penes (Nicholls, 1926, 1943).

Since Nicholls' (1943, 1944) revision, however, the South African phreatoicidean fauna remained poorly collected and little studied. This is perhaps due to the difficulties in sampling (suitable sampling localities are often inaccessible) and difficulties presented by the morphological conservatism of the group, where subtle differences among species (Nicholls, 1943; Kensley, 2001) are coupled with extreme intraspecific and intrapopulational variation in certain features (Barnard, 1927). Although limited unaccessioned material and material in private collections exists, only one accessioned lot has been collected, and one published work (Kensley, 2001) dealing with the South African fauna released, since the publication of Barnard's 1940 paper. The South African Museum retains 26 collection lots from just 14 localities, although some localities are broadly defined (e.g. by mountain range). Most are

identified as *M. capensis*. Recently, Kensley (2001) briefly addressed the systematics and taxonomy of the group, affording specific status to the *penicillatus* variety and providing the only key for the genus. This key distinguished the species primarily by the presence or absence of a pair of robust sub-apical setae occurring dorsally on the pleotelson, and then by the extent of setation of the pereon, head, or antennal peduncles.

Kensley (2001) also highlighted the need for extensive systematic work on the group in South Africa, hinting at the possible presence of hitherto unknown species within the isolated mountainous habitats of the country. He also stressed the importance of intensive fieldwork, and the examination of the distributions of known species to meet this end. Subsequent collections have indeed highlighted the existence of additional taxa. Furthermore, recent molecular, morphometric and morphological studies have revealed the presence of additional cryptic species or species complexes among geographically disjunct populations of the same putative species (Chapter 2, Chapter 3).

This chapter presents detailed descriptions of six of these new taxa, some of which have been highlighted earlier (Chapter 2, Chapter 3), all belonging to the endemic genus *Mesamphisopus*. The newly described species are also compared to the four known species of the genus.

4.2) Materials and methods

Isopods described herein were collected during various field trips conducted since February 2000. Localities sampled included some already represented in museum collections, as well as unsampled localities. Isopods were preserved and fixed in absolute ethanol or 10% formalin.

Species descriptions were based on the undissected male holotype, and further dissected males and females of each species, or on dissected and undissected members of a syntypic series. Dissected individuals have been lodged (accession details provided below) as slide-mounted parts and as parts in micro-vials, together with the lot from which they were drawn. Dissections were made under a Wild M5 stereoscopic dissection microscope and observations made using the stereoscopic microscope and a Nikon compound microscope, where dissected parts were temporarily mounted in glycerine on cavity slides. Illustrations were made using a *camera lucida*. Measurements of individual features were made and ratios calculated under the microscope using a graticulated eyepiece, or were made directly from the *camera lucida* illustrations. Additional observations (e.g. counts of antennal articles) were made from digital images of up to five adult males of each species, dissected for use in previous morphometric analyses (Chapter 2, Chapter 3). In the case of *Mesamphisopus albidus* n. sp. (described below), dissected parts were measured, illustrated and then prepared and mounted for scanning electron microscopy following the procedures documented in Wilson and Keable (2002a, b), and Wilson (2003). Here additional measurements and observations were taken from the scanning electron micrographs.

Taxonomic descriptions were catalogued and generated using a modified DELTA (DEscriptive Language for TAxonomy) database (Dallwitz, 1980; Dallwitz, Paine and Zurcher, 2000a) developed for the Phreatoicidea by G. D. F. Wilson and S. J. Keable (Australian Museum, Sydney) (see Wilson and Fenwick, 1999; Wilson and Johnson, 1999; Wilson and Keable, 1999, 2001, 2002a, b). Intkey (Dallwitz *et al.*, 2000b) was used to generate diagnoses for each species, with taxa being diagnosed relative to other *Mesamphisopus* species included in the database with the diagnostic level set at 12. Only male characters were considered, with most ratios and setal counts being excluded. These were examined through data summaries, and included in the diagnoses where necessary. New species were further compared to the syntypes of the known species (*M. abbreviatus*: South African Museum (SAM) A5173; *M. capensis*: SAM A2257; *M. depressus*: SAM A4185; *M. penicillatus* SAM A8203), and published textual descriptions and illustrations of the species (Barnard, 1914, 1927, 1940; Sheppard, 1927; Nicholls, 1943; Kensley, 2001). Further to the discussions comparing newly described species to each other and the existing species, the morphological characters used to distinguish species are tabulated in Appendix 8. Here descriptions of representative individuals from the additional populations included in Chapters 2 and 3, examined for the same characters, are also presented.

For the sake of brevity, characters common to the six species described herein, and characters that are implicit within the Phreatoicidea, are presented as a preamble to the textual descriptions. These characters are, however, not to be interpreted as diagnostic or synapomorphic for the genus *Mesamphisopus* as a whole, as many are common to other genera and species within the Phreatoicidea, and the presence of certain characters or features within the four existing species has yet to be determined. The primary descriptions are based

on male individuals, with sexually dimorphic features, and the differences between females and males, particularly with regard to dimensions and ratios, presented afterwards.

4.3) Taxonomy

Suborder PHREATOICIDEA Stebbing 1893

Family **Mesamphisopodidae** Nicholls 1943

Genus *Mesamphisopus* Nicholls 1943

Common and implicit characters:

Head length shorter than width in dorsal view; lateral profile of dorsal surface smoothly curved; surface smooth and shiny, appears granular below cuticle; tubercles absent. Eyes present; ocelli distinguishable as clusters of units, not individually, pigmentation dark. Cervical groove smoothly curved. Mandibular notch present. Clypeal notch present. Antennal notch shallow, without posterior extension. Frontal process above antennula absent. Mouthfield angling ventrally, mandibular insertion axis in lateral view nearly level, line projected anteriorly along mandibular insertion passing below base of antenna; adjacent to the posterior margin of head and anterior margin of pereonite 1.

Pereon dorsal surface ridges absent; smooth; setae on dorsal surface scattered, fine. Pereonite 1 in dorsal view wider than medial length. Pereonites 2 – 7 in dorsal view wider than long. Coxal articulation of pereonites 2 – 7 free. Lateral tergal plates of pereonites 2 – 4 not extended over basis. Sternal processes absent. Typhlosole absent, gut round in cross-section; hindgut caecae absent.

Pleonites in lateral view much deeper than pereonites, with large pleurae, basal region of pleopods not visible; pleonite 1 pleura distinctly shallower than pleurae of pleonites 2 – 5. Pleonite 5 dorsal median ridge absent.

Pleotelson vaulted (lateral fields vertical); dorsal surface smooth; lateral ridges absent; ventral surface anterior to uropods strongly concave; ventral margin anterior to uropods posterior seta longer than anterior adjacent setae; postanal ventral surface present, unelaborated. Posterolateral margin uninterrupted (without major inflection in margin differentiating apex), unelaborated. Posterior apex

projecting in dorsal view, visible in lateral view, free (not strongly reflexed and flattened against dorsal surface with ventral surface exposed).

Antennula with more than 6 articles in male. Article 3 rudimentary second flagellum absent. Article 4 shorter than article 3. Terminal article vestigial, shorter than penultimate article. Penultimate article width approximately subequal to ante-penultimate article width.

Antenna flagellum proximal articles dense cover of cuticular hairs absent. Propodal article 1 absent. Propodal article 3 scale absent.

Mouthfield. Clypeus broad bar, rounded at mandibular fossae. Paragnaths with distolaterally rounded lobes; with dense mats of fine setae distomedially along lobes.

Mandible palp article 1 easily visible; 3rd article relatively linear, with more than 5 setae on medial-distal margins, coarsely spinulate setae absent, medial surface naked, lacking cuticular hair, cuticular combs absent. Incisor processes broad, width greater than thickness. Left incisor process with 4 distal cusps; lacinia mobilis with 3 cusps. Right incisor process with 4 cusps. Right lacinia mobilis large, well separated and distinct from remainder of spine row, with two dentate plates (smaller plate on anterior surface of larger plate). Spine rows with bifurcate spines, on projecting ridge between incisor and molar, forming strongly convex arc in ventral view, protruding medially; basal insertions crossing dorsally and then abruptly angling posteriorly. Left spine row with first spine not separated from remaining spines. Right spine row with first spine not separated from remaining spines. Molar process stout, heavily keratinised; triturating surface heavily ridged, with 1 tooth.

Maxillula medial lobe width less than lateral lobe; with 4 pappose setae. Lateral lobe distal margin narrow, with multiple denticulate robust setae.

Maxilliped epipod fine cuticular combs absent; ventral surface setae absent. Endite distal tip without subdistal biserrate setae on ventral surface; medial margin with multiple coupling hooks on left and right side. Palp insertion on basis medial margin without plumose setae; ventral surface without subdistal biserrate setae.

Pereopods I – VII coxae not laterally projecting.

Pereopod I subchelate. Dactylus dorsal margin dense group of elongate setae absent; lateral surface with row of fine setae along axis; ventral margin proximal projection absent; with 1 distal accessory claw; distal accessory spines absent. Propodus dorsal margin proximal region not protruding. Propodal palm convex to straight; simple spines absent; composite spines absent; stout robust simple setae basally inflated; setal ridge absent; elongate broad based setae present. Merus distodorsal margin in cross-section shelf-like and U-shaped, with numerous elongate simple setae. Basis ventrodistal margin with multiple elongate setae, setae shorter than ischium.

Pereopods II – III dactylus shorter than propodus; with 1 distal accessory claw; spines on ventral margin absent. Propodus articular plate present. Pereopod II dactylus lateral spine absent; basis dorsal ridge proximal knob absent. Pereopods II – IV basis lateral face ridge absent.

Pereopod IV prehensile in adult males, subchelate with major hinges on dactylus and propodus. Dactylus with distal accessory claw. Propodus distal width in male less than palm width; with multiple broad based setae on ventral margin; articular plate present on posterior side of limb. Carpus with multiple broad based setae on ventral margin. Ischium posterodistal margin with multiple setae. Basis dorsal ridge with multiple setae, positioned along ridge.

Pereopods V – VII dactylus with 1 distal accessory claw; spines absent. Propodus articular plate present on posterior side of limb. Pereopods V – VII ischium dorsal margin with multiple simple setae, including multiple robust setae. Basis lateral face central groove or ridge present. Pereopod VII basis dorsal ridge distal margin indented.

Penes curved posteriorly; extending to midline; cuticle smooth; distally tubular.

Pleopods. Exopods II – V biarticulate, I uniarticulate; II – V proximal article distolateral lobes shorter than distal article; lateral proximal lobes on II – V; medial proximal lobes on II – V. Endopods unilobed. Protopods medial margin II – V with epipods; I – IV with coupling hooks; with simple setae. Protopod I lateral epipod absent; protopod II lateral epipod absent; protopods III – V lateral epipods lobe-like. Pleopod I exopod distal margin rounded, lateral margin rounded, ventral surface flat. Pleopod II endopod appendix masculina curved; proximal half of shaft broadly concave in ventral cross-section, not forming tube; distal tip broadly rounded, margins smooth; with multiple setae on margin, occurring laterally and medially. Pleopod II endopod distal margin rounded; exopod distal segment longer than wide, lateral margin proximally rounded.

Uropod protopod dorsomedial ridge in dorsal view parallel to ventral margin, setae on margin robust and simple; dorsolateral margin setae robust and simple; distomedial margin without spinose setae; distoventral margin without robust spinose setae, with 3 robust simple setae. Rami distal tips rounded. Endopod longer than protopod; subequal-longer than exopod; straight-curving dorsally; dorsal margin with multiple setae, without spine; ventral margin convex-straight proximally. Exopod shorter than pleotelson; dorsal margin with multiple robust setae.

***Mesamphisopus albidus* n. sp.**

Figures 4.1 – 4.7

Type locality. Franschhoek Pass, Franschhoek – Villiersdorp road, Hottentots Holland Mountains, Western Cape, South Africa (33°55’44”S 19°09’34”E).

Material examined. Holotype: South African Museum (SAM) A45149, one adult male (body length (bl) 7.2 mm), Franschhoek Pass, Franschhoek – Villiersdorp road, Western Cape, South Africa (33°55’44”S 19°09’34”E), collected on 30/VIII/2001 by G. Gouws. SAM A45150, two males, four females, collection details as for holotype. SAM A44933, four males, five females, collection locality

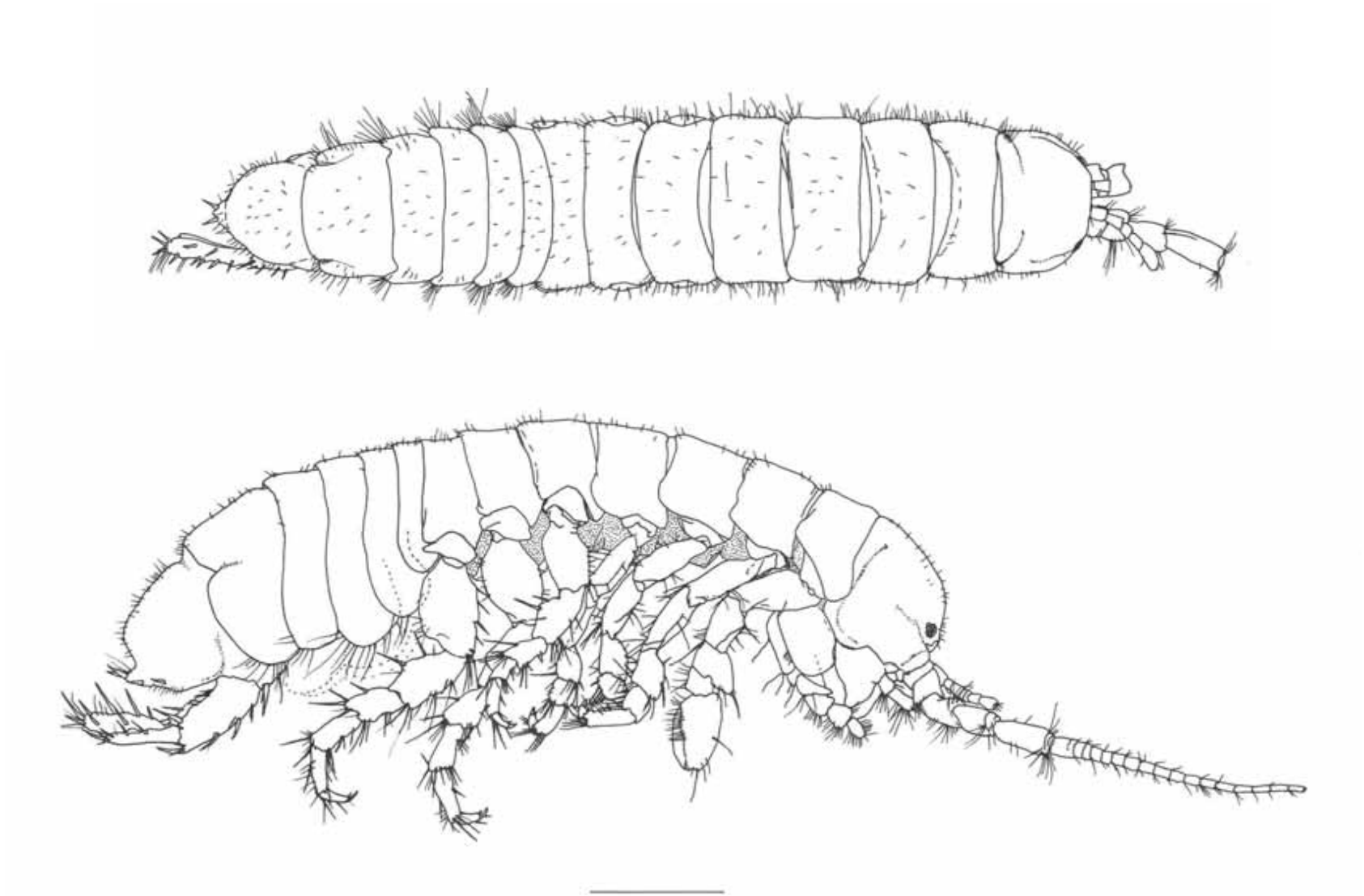


Figure 4.1: *Mesamphisopus albidus* n. sp., male holotype (South African Museum (SAM) A45149), dorsal view (above) and lateral view (below). Scale line 1 mm. Antennula, antenna and uropods incompletely figured in dorsal view.

as for holotype, collected by S. R. Daniels and G. Gouws (date unknown). Australian Museum P67144, mounted SEM stubs of parts of two adult males (stubs AW450 – 458 and AW459 – 463, respectively) and one preparatory female (AW461), Franschhoek Pass, Franschhoek – Villiersdorp road, Western Cape, South Africa (33°55.73'S 19°09.57'E) collected by S. R. Daniels and G. Gouws (date unknown).

Etymology. The species is given the Latin epitheton "albidus", meaning "white" or "light", in reference to the light pigmentation or complete lack of pigmentation of individuals. This adjective agrees in gender (masculine) with the generic name.

Diagnosis. Lightly pigmented or lacking pigmentation. Cervical groove smoothly curved, extending just above anterolateral margin of pereonite 1. Mandibular groove absent. Eyes small, maximum diameter 0.08 head depth. Pereon width in dorsal view near head width. Pleonite 5 dorsal length:maximum length of pleonites 1 – 5 0.63; pleonites 1 – 4 individual depths:pereonite 7 depth 1.09 – 1.56. Pleotelson dorsal surface sparsely covered with fine setae; lateral length less than depth; depth 1.33 pereonite 7 depth; ventral margin anterior to uropods with single row of simple robust setae grading anteriorly to fine setae; lateral uropodal ridge curving strongly and extending posteriorly from uropods on pleotelson margin; posterior apex with 1 or 2 pairs of simple robust setae. Antennula penultimate article length approximately subequal to length of other articles; distal articles in cross-section circular. Antenna article 5 length subequal to article 4. Mandibular palp article 3 with 25 setae. Maxillula lateral lobe distal margin with 5 smooth setae, ventral face with one plumose seta. Maxilla lateral lobes with bidenticulate setae on distal tips and medial margin. Maxilliped palp insertion on basis lateral margin with one plumose seta; palp article 4 elongate-oval. Pereopod I propodal palm stout denticulate setae bifid; basis dorsal ridge setae positioned proximally. Pereopod II propodus length:width < 2.00, with 4 broad based setae; basis length:width < 2.00. Pereopod III propodus with 3 broad based setae. Pereopod IV dactylus length subequal to propodal palm; propodus length:width approximately 1.40; basis length:width 2.30. Pereopods V – VII basis with no large setae, dorsal ridge distinctly separated from basis shaft, lateral face ventral ridge present. Pleopodal endopods setae plumose on I – IV, simple on V. Pleopod I exopod dorsal surface lacking setae. Pleopod II appendix masculina distal tip extending beyond distal margin of endopod. Uropod total length 1.86 pleotelson length; rami cross-sectional shape flattened dorsally and ventrally; endopod dorsal margin with 3 robust setae, placed midlength; exopod dorsal margin with 3 robust setae.

Description based on male. *Coloration.* Individuals lacking pigmentation, off-white to cream, or very lightly pigmented and light brown-grey to light slate-grey; most pigmentation occurs in longitudinal band dorsally with slight mottlings of pigment laterally, particularly on pereonites;

pereopods and uropods not pigmented. Pigmentation fades partially to light brown or completely to off-white or cream upon preservation, eyes remain black or fade to white in some individuals.

Head width 0.83 pereonite 1 width; setae sparse, fine. Eyes bulging dorsolaterally to projecting anteriorly; maximum diameter 0.08 head depth; approximately round. Cervical groove extending just above anterolateral margin of pereonite 1. Mandibular (genal or cheek) groove absent to weakly indented.

Pereon width near head width; setae on dorsal surface also forming rows along posterior pereonite margins, length of setae 0.11 body depth. Pereonite 1 length:width in dorsal view 0.58. Pereonite 2 length:width in dorsal view 0.43. Pereonite 3 length:width 0.47. Pereonite 4 length:width 0.42. Pereonite 5 length:width 0.37. Pereonite 6 length:width 0.31. Pereonite 7 length:width 0.27.

Pleonites in dorsal view 2 – 4 respective lengths less than half the length of pleonite 5, 1 – 4 relative lengths unequal, increasing in length from anterior to posterior; pleonites 1 – 4 width 1.00 composite length in dorsal view. Pleonites 1 – 5 dorsal length:maximum width of pleonites 1 – 5 respectively 0.19, 0.22, 0.30, 0.30 and 0.63. Pleonites 1 – 5 depth:pereonite 7 depth respectively 1.09, 1.40, 1.53, 1.56 and 1.40.

Pleotelson dorsal surface in lateral view inflected ventrally, sparsely covered with fine setae, length 1.00 width; lateral length less (0.75) than depth; depth 1.33 pereonite 7 depth; ventral margin anterior to uropods with single row of 5 – 6simple robust setae grading anteriorly to fine setae; lateral uropodal ridge curving strongly and extending posteriorly from uropods on pleotelson margin, lacking setae. Posterior apex with 1 – 2 pairs of robust setae.

Antennula (Fig. 4.2A) length 0.13 – 0.14 body length, with 7 – 8 articles. Articles 4 and 5 divisible into one large or two small articles. Article 5 (undivided) length:width 2.00. Article 6 length:width 2.46. Six fine aesthetascs on article 6, below terminal article. Terminal article length:width 0.67; length:antennular length 0.01. Penultimate article length approximately subequal to length of other articles. Distal articles in cross-section circular.

Antenna (Fig. 4.2B) length 0.49 – 0.55 body length. Flagellum length 0.58 total antenna length, generally with 19 – 20 articles (sometimes 16 – 28 articles). Article 5 length subequal to article 4; article 6 shorter than articles 4 and 5 combined.

Mouthfield. Clypeus width 0.72 head width. Labrum (Fig. 4.2C) roughly ventrally semicircular in anterior view, distal margin finely hirsute; asymmetrical, with notch along right margin. Paragnaths (Fig. 4.2D) with dense mats of fine setae medially on lobes, longer setae laterally; setal row on thickened proximal medial margins.

Mandible (Figs 4.2E,F, 4.3A) palp length 1.10 mandible length; 3rd article with 25 – 29 finely setulate setae on medial-distal margins; article 2 with longitudinal row of 16 – 23 elongate simple setae along dorsolateral margin, separate distal group of 4 – 5 elongate setae more medially; article 1 with group of elongate simple setae on dorsodistal margin. Left spine row with 14 spines, 4 of which bifurcate. Right spine row with 12 – 13 spines, 4 of which bifurcate. Molar process length subequal to width.

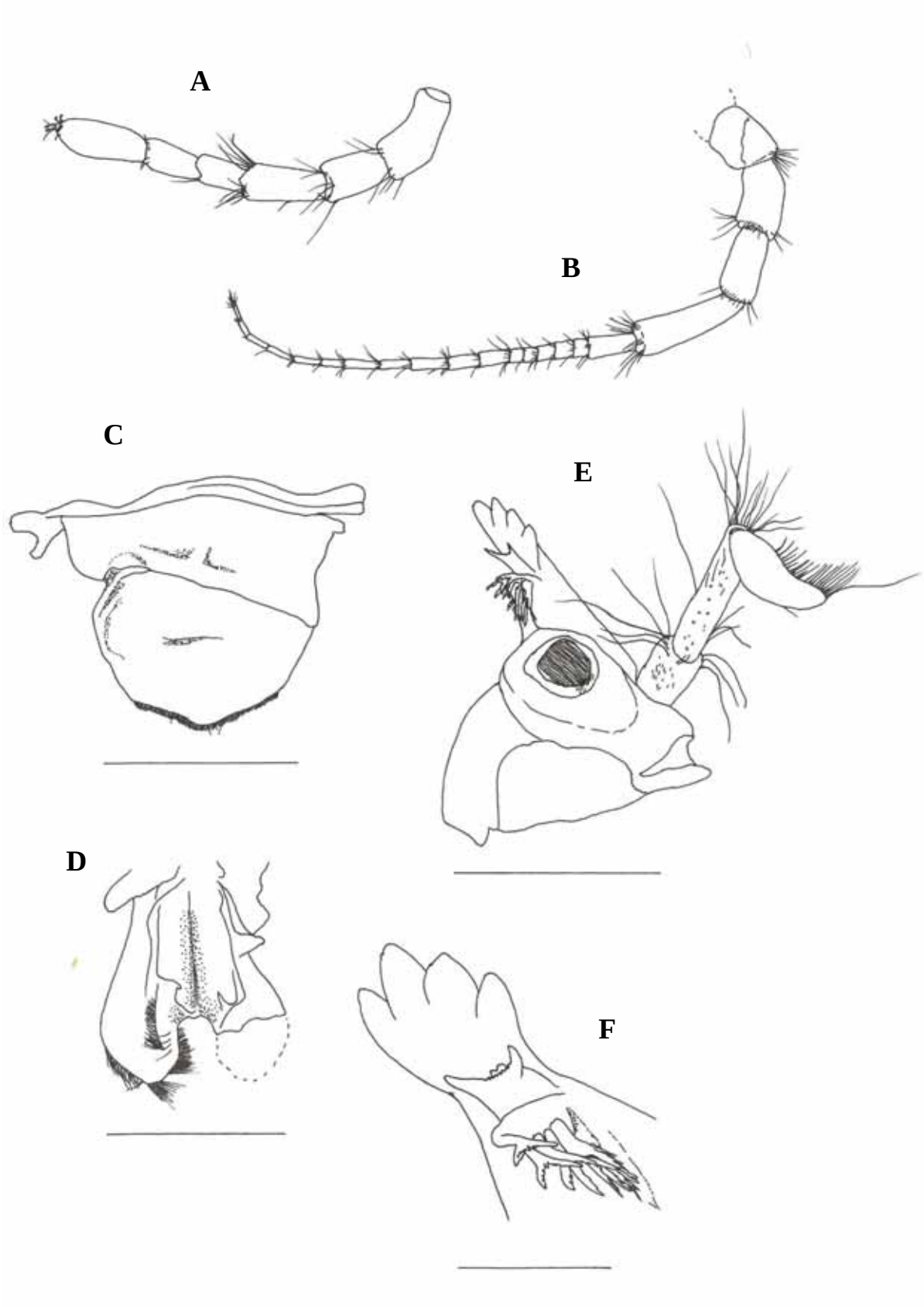


Figure 4.2: *Mesamphisopus albidus* n. sp., dissected male and female parts (Australian Museum (AM) P67144). A, antennule; B, antenna; C, labrum; D, paragnath; E, right mandible; and F, right mandible incisor process and spine row. Scale lines represents 0.5 mm, except for F, where it represents 0.1 mm.

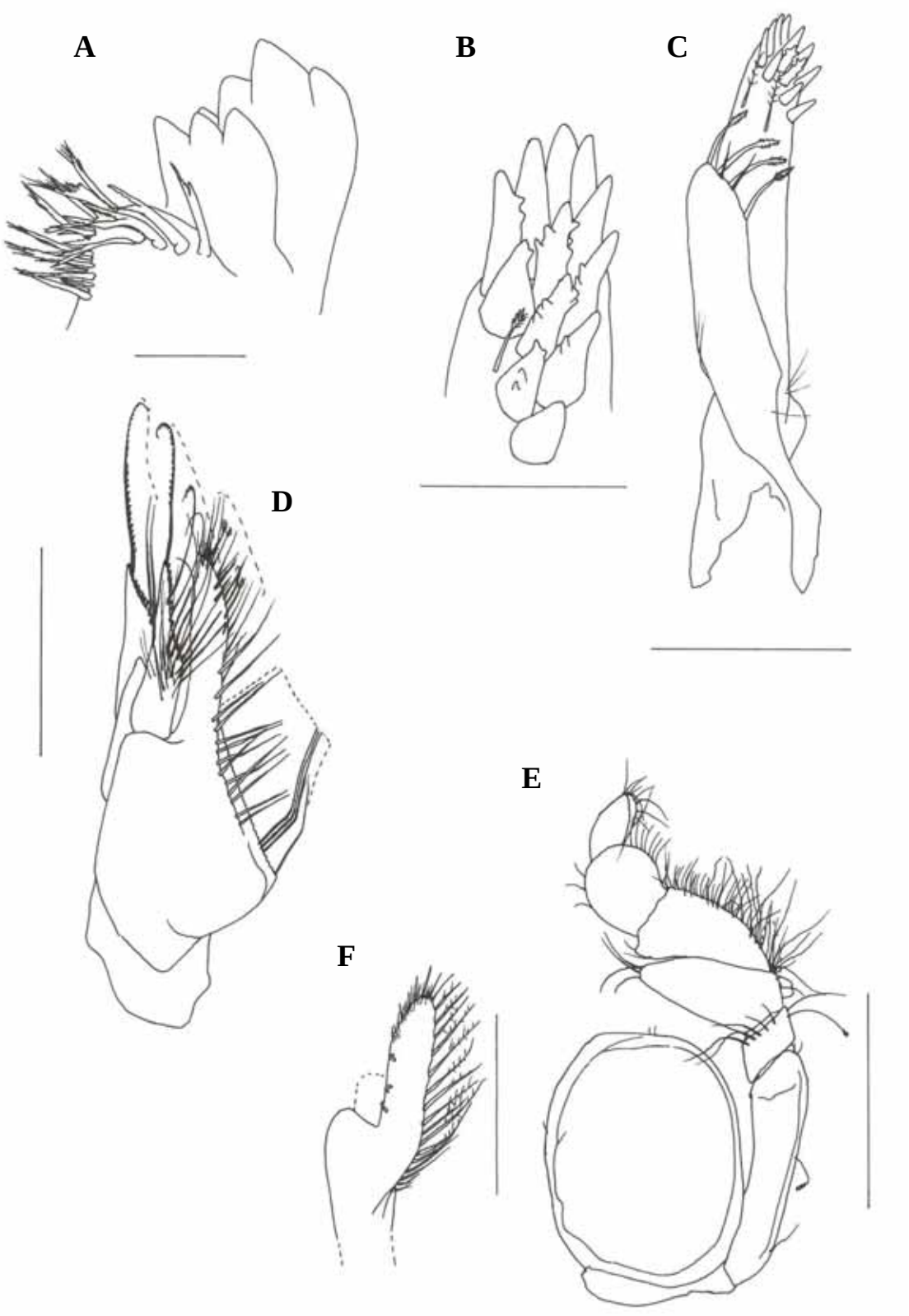


Figure 4.3: *Mesamphisopus albidus* n. sp., dissected male and female parts (AM P67144). A, left mandible incisor process and spine row; B, maxillula (lateral lobe distal margin); C, maxillula; D, maxilla; E, right maxilliped (ventral view); F, maxilliped basal endite. Scale lines 0.1 mm (A, B, and F) and 0.5 mm (C, D and E).

Maxillula (Figs 4.3B,C) medial lobe length 0.54 lateral lobe length; width 0.38 lateral lobe width; with 2 'accessory' setae, one on distolateral margin and one between central pappose setae, or one between central pappose setae, and one between medial and central pappose setae, 'accessory' setae simple. Lateral lobe distal margin with 7 denticulate robust setae, 5 smooth robust setae, distal setal row 4 robust setae; ventral face with 2 plumose/pectinate setae, setae widely spaced, additional shorter plumose seta among distal robust seta.

Maxilla (Fig. 4.3D) medial lobe proximal portion distinctly angled to distal portion; proximal and distal setal rows separated by gap; short fine setulate/plumose setae becoming more elongate proximally in single dorsal basal row; 13 – 15 simple elongate setae with distinct base and smooth shaft in single ventral basal row; evenly spaced equally long distally setulate setae, numerous simple setae in distal rows. Outer lateral lobe longer than inner lateral lobe, wider than inner lateral lobe; with 16 long bidenticulate setae. Inner lateral lobe with 21 long bidenticulate setae. Lateral lobes with bidenticulate setae on distal tips and on medial margin.

Maxilliped (Figs 4.3E,F) epipod length:width 1.22; distal tip rounded to truncate; distal margin setae absent. Endite length:total basis length 0.44; medial margin with 3 coupling hooks on left side, 2 on right side; dorsal ridge with 16 large distally denticulate plumose setae. Palp insertion on basis lateral margin with 1 plumose seta; medial margin with 1 simple seta; ventral surface with 1 subdistal smooth seta, 3 simple setae more proximally on ventral surface; palp length:basis length 0.92; width across articles 2 – 3:endite width 1.31; article 4 elongate-oval, length:width 1.11; article 5 length:width 1.67, article 5 length:article 4 length 1.00.

Pereopod I (Figs 4.4A,B) dactylus length subequal to palm, length:palm length 1.03; ventrodistal margin with row of thin scale-like spines, along 0.05 – 0.24 total length; claw length:dactylus length 0.10; distal accessory claw ventrolateral to primary claw, 0.20 length of primary claw. Propodus length:pereopod length 0.25; length:width 0.92; dorsal margin setae in several groups between proximal and distal margin, 6 along margin, 14 in distal group. Propodal palm cuticular fringe weakly developed; stout denticulate setae bifid, 4 – 5 altogether; 4 basally inflated stout robust simple setae altogether, 3 – 5 elongate broad based setae present. Basis length:width 2.08; dorsal setae positioned proximally and lateral to margin distally, 4 altogether; ventrodistal margin with 4 elongate setae. Ischium dorsal margin with 3 simple setae, none robust.

Pereopods II – III (Figs 4.4C,D). Pereopod II dactylus length:propodus length 0.45; primary claw length:dactylar length 0.26. Propodus length:pereopod length 0.13; length:width 1.71. Carpus length:pereopod length 0.15; length:width 1.75. Basis length:pereopod length 0.24; length:width 1.92. Pereopod III dactylus length:propodus length 0.61; primary claw length:dactylar length 0.28. Propodus length:pereopod length 0.14; length:width 2.00. Carpus length:pereopod length 0.13; length:width 1.50. Basis length:pereopod length 0.28; length:width 2.36. Pereopods II – III dactylus distal accessory claw ventral to primary accessory claw. Propodus broad based setae present, respectively 4, 3 on pereopods II and III; on pereopod II broad based setae 0.20 – 0.36 propodus

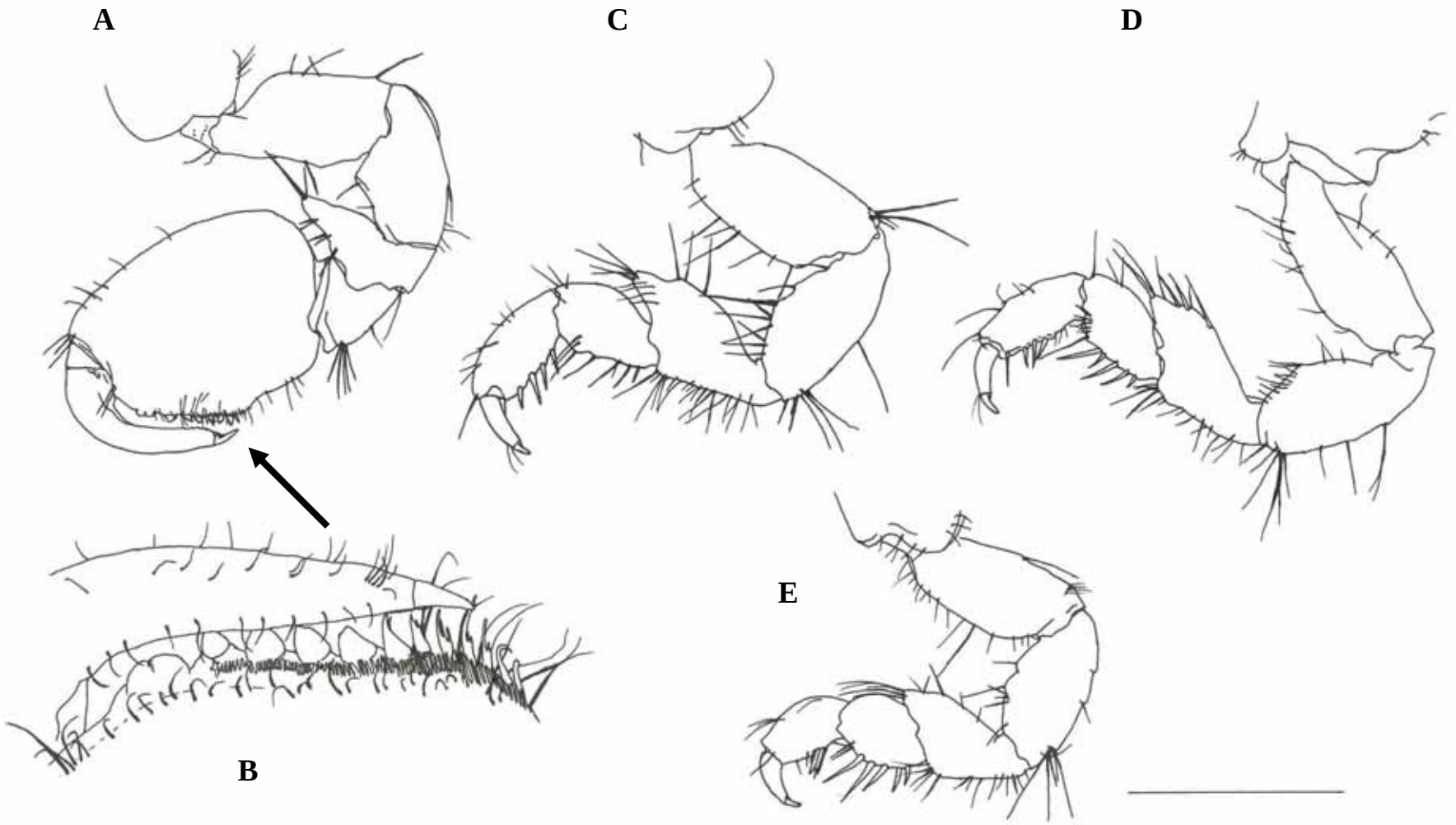


Figure 4.4: *Mesamphisopus albidus* n. sp., dissected male specimen (AM P67144). A, pereopod I; B, pereopod I propodal palm; C, pereopod II; D, pereopod III; E, pereopod IV. Scale line approximately 1 mm.

length, evenly spaced along ventral margin; on pereopod III broad based setae 0.18 to 0.36 propodus length, evenly spaced along ventral margin. Carpus broad based setae present, respectively 5, 6 on pereopods II and III; on pereopod II broad based setae 0.22 – 0.62 carpus length, evenly spaced along ventral margin; on pereopod III broad based setae 0.15 – 0.76 carpus length, evenly spaced along ventral margin. Basis dorsal ridge in cross-section angular and produced but not forming distinct plate, with approximately 13 elongate simple setae, along length of margin or just lateral to margin, includes single plumose seta distally on pereopod III. Pereopods II – IV ischium dorsal margin with 11 – 12 simple setae, including 1 robust.

Pereopod IV (Fig. 4.4E). Penicillate setae present on dorsal margin of basis. Dactylus length subequal to propodal palm; distal accessory claw approximately 0.25 – 0.33 length of primary claw. Propodus length:pereopod length 0.12, length:width 1.46; distal width:palm width 0.69; with 6 broad based setae on ventral margin, 3 distinctly larger than remainder; articular plate subequal in length to dactylar claw. Carpus length:pereopod length 0.13; with 5 – 7 broad based setae on ventral margin, none distinctly larger than others. Ischium posterodistal margin with 10 setae. Basis length:width 2.30; dorsal ridge in cross-section angular and produced but not forming distinct plate, with 9 setae.

Pereopods V – VII (Fig. 4.5). Pereopod V dactylus claw length:dactylar length 0.26. Propodus length:pereopod length 0.13. Carpus length:pereopod length 0.16. Basis length:width 1.63. Pereopod VI dactylus claw length:dactylar length 0.24. Propodus length:pereopod length 0.15. Carpus length:pereopod length 0.17. Basis length:width 1.50. Pereopod VII basis length:width 1.45. Pereopods V – VII penicillate setae on dorsal ridge of basis. Dactylus distal accessory claw ventral to primary claw. Propodus distal margins with 6 elongate robust setae. Basis dorsal ridge distinctly separated from basis shaft, in cross-section angular on V, produced and forming distinct plate on VI – VII, with no large setae; lateral face central ridge present; lateral face ventral ridge present, setae absent. Pereopods V – VII ischium dorsal margin with 11 – 17 simple setae, including 4 – 6 robust. Pereopod VII ischium dorsal ridge flange absent.

Penes length 0.38 body width at pereonite 7; with setae on shaft; distal tip broadly rounded.

Pleopods (Fig. 4.6). Pleopod I exopod length:width 3.16. Endopod length:width 2.45; endopod length:exopod length 0.90. Pleopod II exopod length:width 1.94; length of distal article:exopod length 0.31. Endopod length:width 2.36; endopod length:exopod length 0.74. Pleopod III exopod length:width 2.06; length of distal article:exopod length 0.28. Endopod length:width 2.00; endopod length:exopod length 0.81. Pleopod IV exopod length:width 1.73; length of distal article:exopod length 0.28. Endopod length:width 1.53; endopod length:exopod length 0.77. Pleopod V exopod length:width 1.67; length of distal article:exopod length 0.33. Endopod length:width 1.56; endopod length:exopod length 0.60. Endopods I – V with setae on margins, setae plumose on I – IV, simple on V. Protopods medial margins/epipods I – IV with coupling hooks, respective counts 4, 2, 2, 1; with 2, 5, 7 and 6 elongate simple setae on II, III, IV and V respectively; with 2, 2 and 8 fine short simple setae on III, IV and V respectively. Protopods with 17 (5 lateral, 1 apical, 11 medial), 17 (8 medial, 1

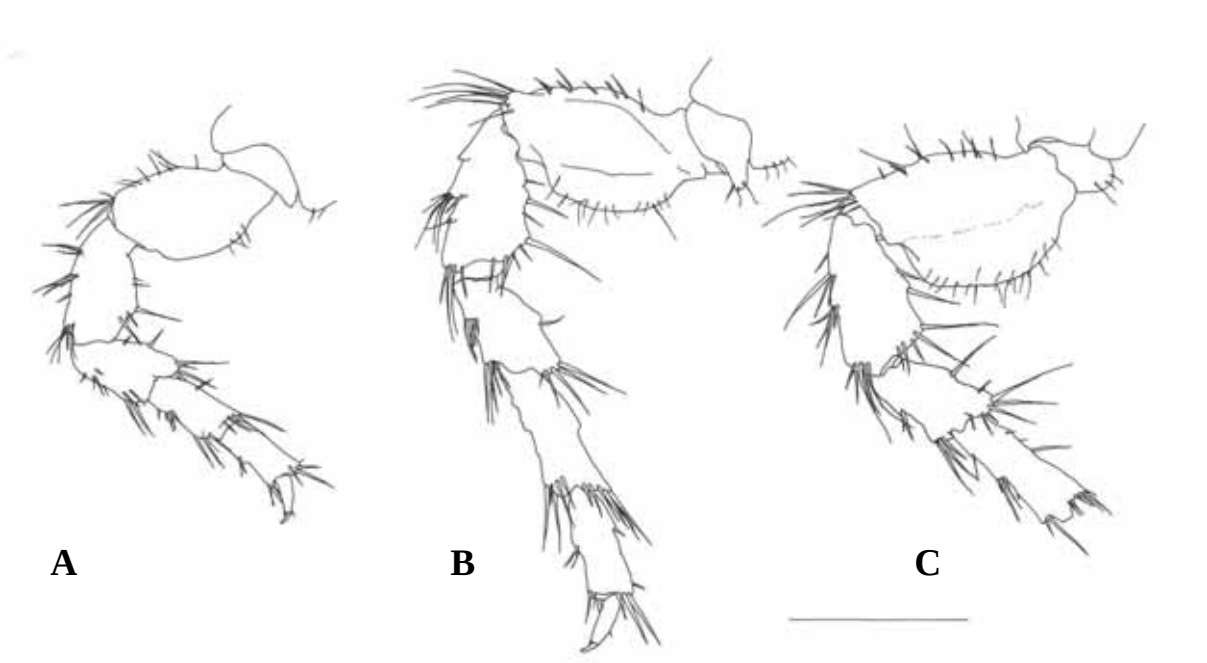


Figure 4.5: *Mesamphisopus albidus* n. sp., dissected male specimen (AM P67144). A, pereopod V; B, pereopod VI; C, pereopod VII. Scale line approximately 1 mm.

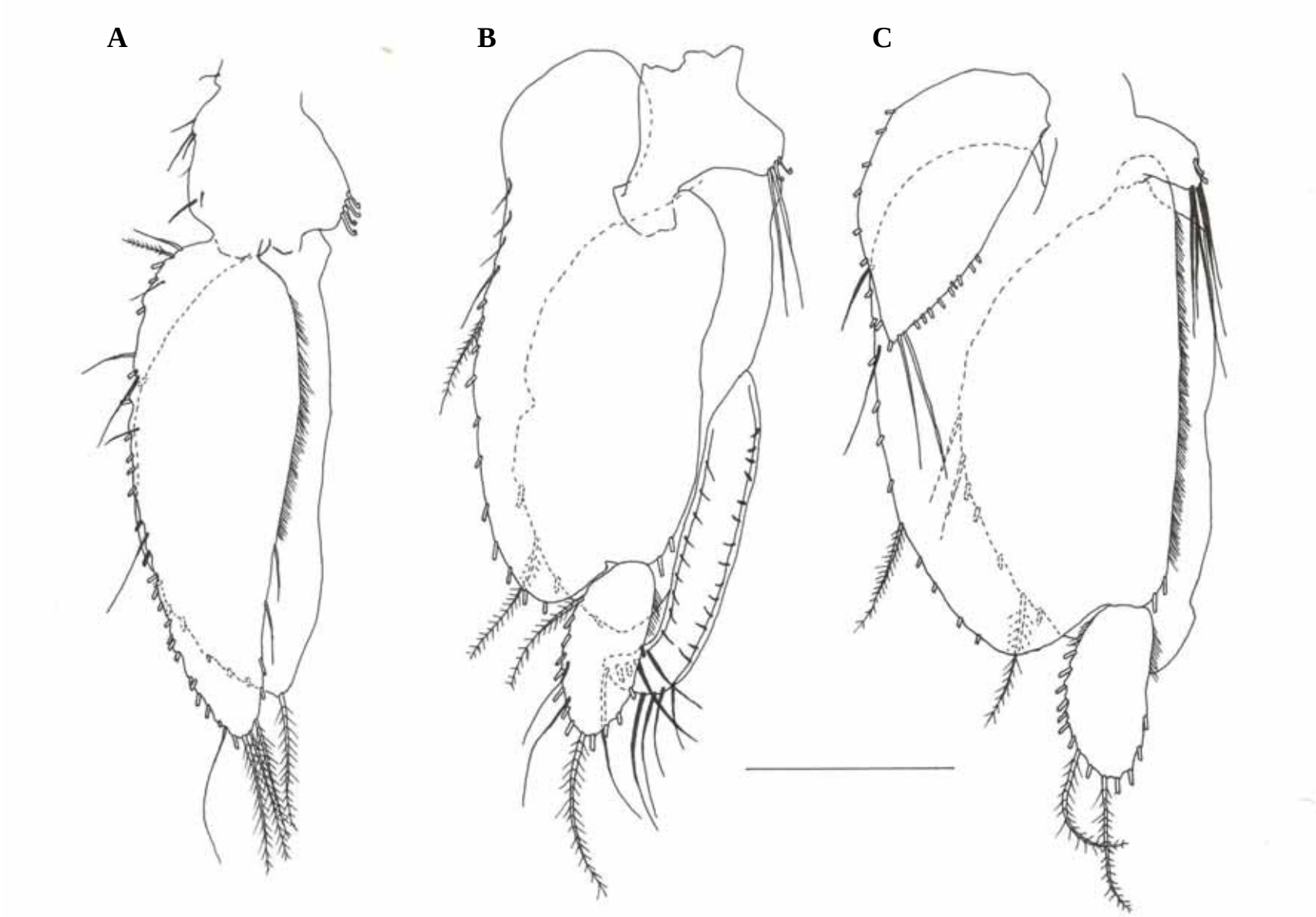


Figure 4.6: *Mesamphisopus albidus* n. sp., dissected male specimen (AM P67144). A, pleopod I; B, pleopod II; C, pleopod III. Scale line 0.5 mm.

apical, 8 lateral) and 16 (8 medial, 1 apical, 7 lateral) simple elongate setae on margins of lateral epipods of pleopods III, IV and V respectively. Lateral epipod III margin with 2 plumose setae. Pleopod I exopod broadest proximally, medial margin straight (convex in proximal half, concave in distal half), dorsal surface lacking setae; protopod length subequal to that of other pleopods, width subequal length. Pleopod II endopod appendix masculina basal musculature pronounced; with 33 – 34 setae on margin; length 0.47 pleopod length; distal tip extending beyond distal margin of endopod.
Uropod (Fig. 4.7) total length 1.86 pleotelson length. Protopod length:width 3.27; length 0.41 uropod total length; extending posteriorly subequal to pleotelson apex; dorsomedial ridge produced, plate-like, margin smooth, in lateral view approximately straight, ridge length:endopod length 0.41. Rami cross-sectional shape flattened dorsally and ventrally. Endopod dorsal margin robust setae starting at midlength, with 9 – 10 robust setae. Exopod length 0.83 endopod length; dorsal margin with 7 – 8 robust setae.

Sexual dimorphism, female differences from male. *Pereon.* Pereonite 1 length:width in dorsal view 0.35. Pereonite 2 length:width 0.39. Pereonite 3 length:width 0.39. Pereonite 4 length:width 0.39. Pereonite 5 length:width 0.37. Pereonite 6 length:width 0.30. Pereonite 7 length:width 0.21.
Antennula length 0.14 body length, with 7 articles.
Antenna length 0.54 body length. Flagellum length 0.65 total antenna length, with 25 articles.
Pereopod I length:body length 0.28. Dactylus length:palm length 1.07; ventrodistal margin with row of thin scale-like spines, along 0.39 – 0.48 dactylus length; claw length:dactylus length 0.17. Propodus length:pereopod length 0.19; length:width 1.24. Propodal palm concave; cuticular fringe poorly developed; low, cuticular process or projection lacking distally; bifid and denticulate stout setae present, 1 bifid, 3 – 4 denticulate; basally inflated stout robust simple setae absent; 9 – 10 elongate broad based simple setae present. Basis length:width 2.32; dorsal setae positioned along ridge, 9 altogether, 4 elongate.
Pereopods II – III. Pereopod II length:body length 0.36. Dactylus length:propodus length 0.64; primary claw length:dactylar length 0.32. Propodus length:pereopod length 0.12; length:width 1.83. Carpus length:pereopod length 0.11; length:width 1.18. Basis length:pereopod length 0.30; length:width 2.04. Pereopod III length:body length 0.33. Dactylus length:propodus length 0.73; primary claw length:dactylar length 0.31. Propodus length:pereopod length 0.12; length:width 1.75. Carpus length:pereopod length 0.10; length:width 1.33. Basis length:pereopod length 0.27; length:width 1.74. Propodus broad based setae present, respectively 4, 4 on pereopods II and III; on pereopod II broad based setae 0.17 – 0.30 propodus length, evenly spaced along ventral margin; on pereopod III broad based setae 0.19 – 0.29 propodus length, evenly spaced along ventral margin. Carpus broad based setae present, respectively 4, 5 on pereopods II and III; on pereopod II broad based setae 0.20 – 0.32 carpus length, evenly spaced along ventral margin; on pereopod III broad based setae 0.16 – 0.52 carpus length, evenly spaced along ventral margin.

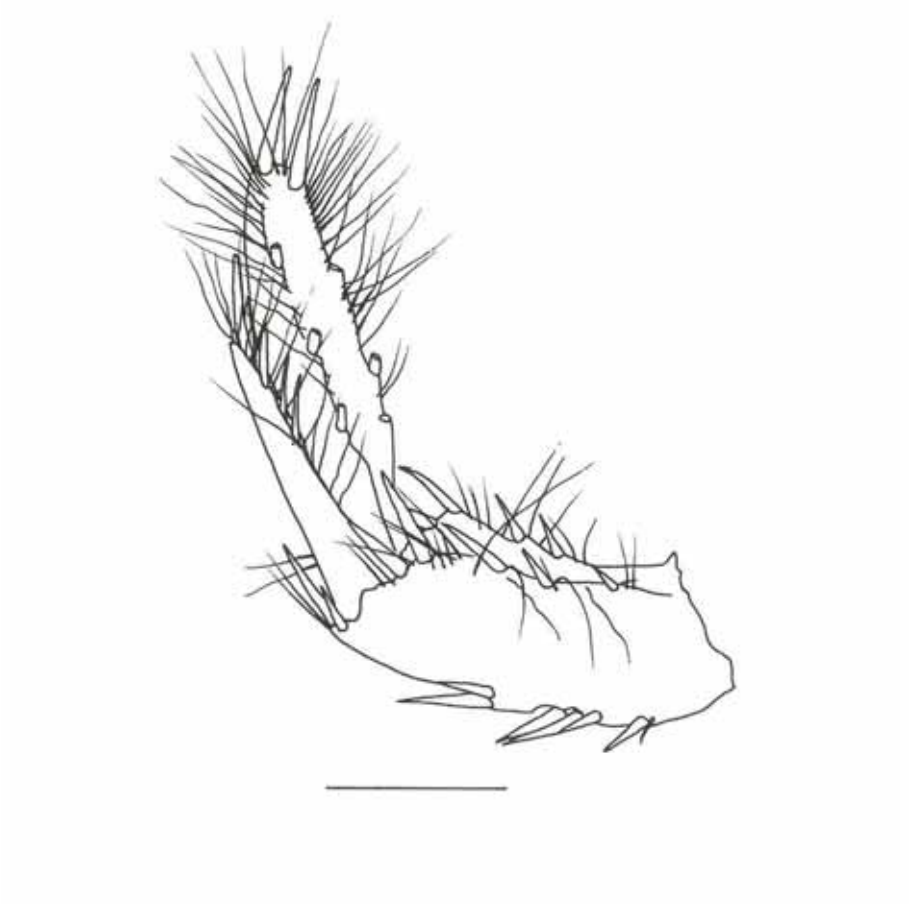


Figure 4.7: *Mesamphisopus albidus* n. sp., dissected male (AM P67144). Uropod. Scale line 0.5 mm.

Pereopod IV simple. Length:body length 0.23. Dactylus distal accessory claw approximately 0.25 length of primary claw. Propodus length:pereopod length 0.11; length:width 1.53; without broad based seta on ventral margin. Propodus articular plate shorter than dactylar claw. Carpus length:pereopod length 0.11; with 3 broad based setae on ventral margin, medial seta substantially smaller than remainder. Ischium posterodistal margin with 7 setae. Basis length:width 2.22; dorsal ridge with 5 setae.

Pereopods V – VII. Pereopod V length:body length 0.28. Dactylus claw length:dactylar length 0.25. Propodus length:pereopod length 0.17. Carpus length:pereopod length 0.13. Basis length:width 1.30. Pereopod VI length:body length 0.38. Dactylus claw length:dactylar length 0.24. Propodus length:pereopod length 0.15. Carpus length:pereopod length 0.15. Basis length:width 1.19. Pereopod VII length:body length approximately 0.42. Propodus length:pereopod length approximately 0.14. Carpus length:pereopod length approximately 0.14. Basis length:width 1.55.

Pleopods. Pleopod I length:body length 0.14. Exopod length:width 2.86. Endopod length:width 2.22; endopod length:exopod length 0.95. Pleopod II exopod length:width approximately 1.67. Endopod length:width 2.25; endopod length:exopod length approximately 1.20. Pleopod III length:body length 0.16. Exopod length:width 1.68; length of distal article:exopod length 0.33. Endopod length:width 1.68; endopod length:exopod length 1.00. Pleopod IV length:body length 0.16. Exopod length:width 1.52; length of distal article:exopod length 0.34. Endopod length:width 1.32; endopod length:exopod length 0.74. Pleopod V length:body length 0.15. Exopod length:width 1.40; length of distal article:exopod length 0.42. Endopods I – V with plumose setae on margins. Protopods medial margins/epipods with coupling hooks on I – III, respective counts 3, 1 and 2; with 4, 5, 6 and 6 elongate simple setae on II, III, IV and V respectively. Lateral epipod of pleopod III with 17 (10 medial, 3 apical, 4 lateral) simple elongate setae.

Uropod total length 1.68 pleotelson length. Protopod length:width 3.31, length 0.43 uropod total length; dorsomedial ridge length:endopod length 0.68. Endopod 6 – 7 robust setae. Exopod length 0.84 endopod length; with 3 robust setae.

General Distribution. Known only from type locality, near Franschhoek, in the Hottentots Holland Mountains.

Remarks. An immediately distinguishing feature of *M. albidus* n. sp. is the light, or complete absence of, pigmentation of individuals. This feature is however not entirely diagnostic, as individuals of two species, *M. setosus* n. sp. and *M. tsitsikamma* n. sp., may occasionally show a lack of pigmentation. Earlier, Barnard (1927) had also documented depigmentation in several populations of *M. capensis* collected in the Hottentots Holland Mountains and Langeberg Mountains (more specific collection localities were not provided). In the extent of the setation of the head, pereon and pleotelson, *M. albidus* n. sp. approaches the condition seen in *M. capensis*, *M. tsitsikamma* n. sp., and perhaps *M.*

abbreviatus, although individual setae are longer in *M. tsitsikamma* n. sp. The species is thus more setose than *M. penicillatus* and *M. paludosus* n. sp., and less setose than *M. depressus*, *M. baccatus* n. sp. and *M. kensleyi* n. sp., particularly with regards to the pleotelson. The eyes of *M. albidus* n. sp. are remarkably reduced and are the smallest within *Mesamphisopus*. This feature, in combination with the depigmentation, may suggest an early adaptation to hypogean lifestyle — individuals of this species were collected and dug out of the sandy bottom of the small seepage stream in which they occurred, beneath a considerable depth of matted root fibres, through which light is unlikely to penetrate. The species appears to be unique with regards to the mid-length occurrence of the robust setae on the uropodal exopod and the dorsally and ventrally flattened endopod (these setae occur more along the length of the exopod and endopod, which is dorsally flattened, in other species). While the setation of the pleopodal endopods is more typical of *Mesamphisopus* (plumose on I – IV, simple on V), *M. albidus* n. sp. is the only species, thus known within *Mesamphisopus*, where plumose setae have been observed on the margins of the lateral pleopodal epipods. While apparently lacking the pair of sub-apical robust setae dorsally on the pleotelson, as described by Barnard (1927) for some individuals, and used as a diagnostic characteristic for certain species by Kensley (2001), the setation of the posterior apex of the pleotelson is known to vary. One or two pairs of robust setae are common on the apex (or one pair on the apex, with one pair more ventrally), although five setae have been observed in one individual.

***Mesamphisopus baccatus* n. sp.**

Figures 4.8 – 4.15

Type locality. Above dam, east of road, Silvermine Nature Reserve, Western Cape, South Africa (34°05’33”S 18°25’22”E).

Material examined. Holotype: SAM A45151, one adult male (bl 9.0 mm), above dam, east of Silvermine Nature Reserve, Western Cape, South Africa (34°05’33”S 18°25’22”E), collected on 10/XI/2000 by S. R. Daniels and G. Gouws. SAM A44937, one dissected adult male (bl 8.6 mm) and one dissected preparatory female (bl 7.5 mm) parts slide mounted and in microvials, additional three males, three females, collection details as for holotype.

Etymology. The species epitheton is the Latin adjective “baccatus” meaning “adorned, ornamented or set with pearls”. This is in reference to the distinct round or globular flagellar articles of the antenna, which can be seen to resemble a string of pearls.

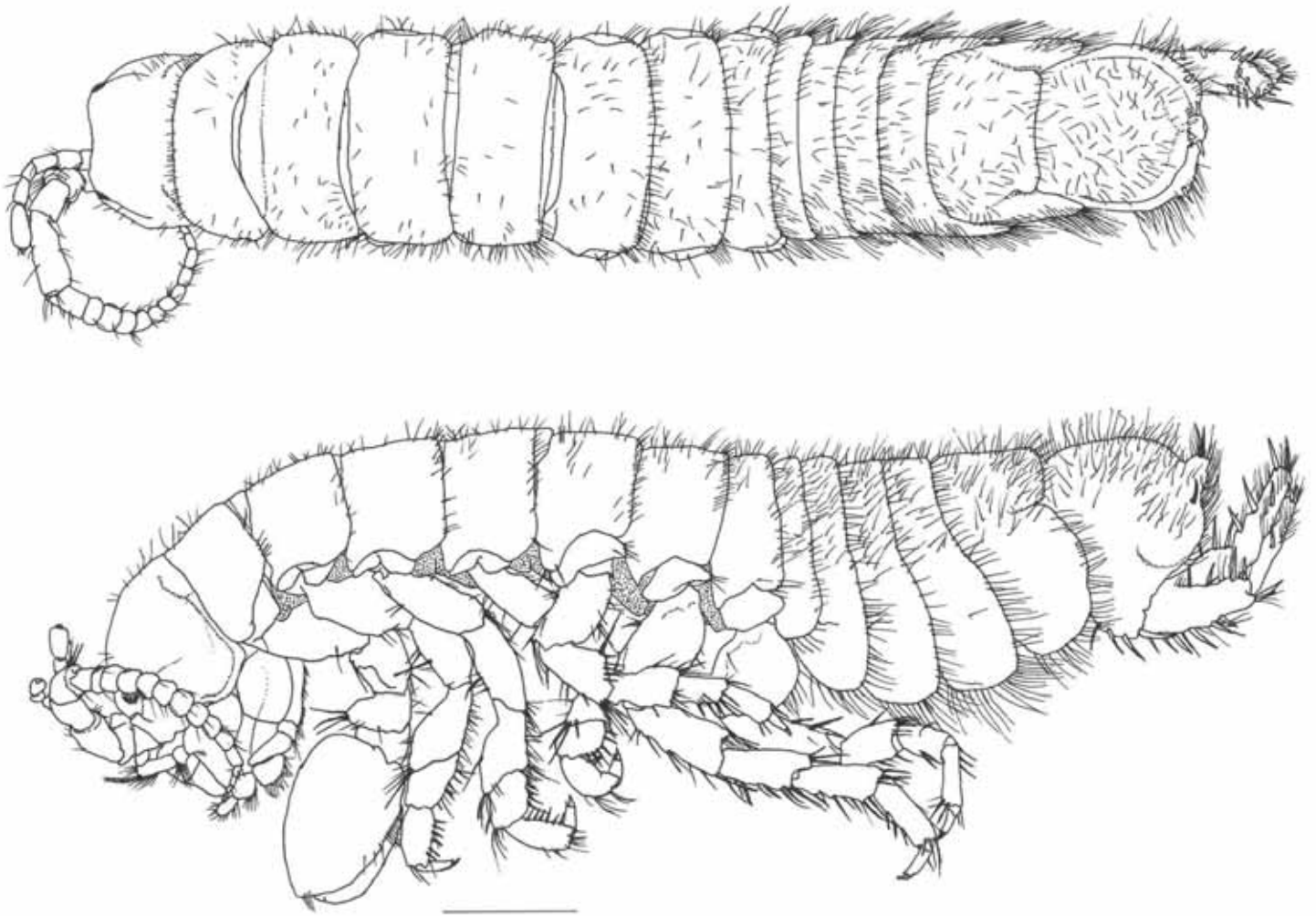


Figure 4.8: *Mesamphisopus baccatus* n. sp., male holotype (SAM A45151), dorsal view (above) and lateral view (below). Scale line 1 mm. Single antennule, antenna and uropod figured in dorsal view

Diagnosis. Mandibular groove smoothly indented. Pleonites 1 – 4 width 1.30 – 1.45 composite length in dorsal view; pleonites 1 – 4 individual dorsal lengths:maximum width of pleonites 1 – 5 0.10 – 0.25; pleonites 1 – 4 individual depths:pereonite 7 depth 1.20 – 2.05. Pleotelson dorsal surface covered with abundant elongate setae; lateral length less than depth; depth 1.45 – 1.55 pereonite 7 depth; ventral margin anterior to uropods with single row of simple robust setae; lateral uropodal ridge absent; posterior apex with one pair of robust setae. Antennula penultimate article length approximately subequal to length of other articles; distal articles rounded and inflated, in cross-section oval. Antenna article 5 length subequal to article 4, flagellum articles broad, rounded and globular. Mandibular palp article 3 with 19 setae. Maxillula medial lobe width 0.77 lateral lobe width; lateral lobe distal margin with 2 smooth robust setae. Maxilliped palp insertion on basis medial margin without simple setae. Pereopod I dactylus ventrodistal margin with row of thin scale-like spines, along 0.05 total length; propodus dorsal margin setae in several groups between proximal and distal margin; propodal palm stout denticulate setae bifid and serrate; basis dorsal margin setae positioned along ridge. Pereopods II – III penicillate setae absent; dorsal ridge in cross-section rounded. Pereopod II propodus length:width < 2.00; carpus length:width < 1.50, with 6 broad based setae; basis length:width approximately 2.40. Pereopod III propodus length:width < 2.00. Pereopod IV dactylus longer than propodal palm; propodus length:width approximately 1.40; carpus with 3 broad based setae on ventral margin; basis length:width approximately 2.60. Pereopods V – VII penicillate setae distodorsally on carpus; basis lateral face ventral ridge absent. Pereopod VII ischium dorsal ridge forming flange subequal to shaft width. Pleopodal endopods setae plumose on I – V. Pleopod II appendix masculina distal tip extending near to distal margin of endopod. Uropod total length 1.35 pleotelson length; protopod ventral margin with long laterally projecting setae; endopod dorsal margin with 4 robust setae; exopod length 1.20 endopod length, dorsal margin with 2 robust setae.

Description based on male. *Coloration.* Body strongly pigmented, brown/slate-grey to black-grey; dorsal band most strongly pigmented, lighter pigmentation towards ventral margins of pleonites; unpigmented patches give mottled appearance to lateral cephalon, pereonites and pleotelson; slight pigmentation on uropodal protopods; pereopods generally lack pigmentation, white to off-white; pigmentation fades to lighter brown-grey upon preservation.

Head width 0.85 – 0.86 pereonite 1 width; setae sparse, fine. Eyes projecting anteriorly, bulging slightly dorsolaterally; maximum diameter 0.14 – 0.16 head depth; round to oval, with orientation of longest axis horizontal, or between horizontal and vertical. Cervical groove extending nearly to dorsal margin of head. Mandibular (genal or cheek) groove smoothly indented. Maxillipeds insertion from posterior margin of head 0.09 head length.

Pereon width exceeding head width; setae on dorsal surface concentrated along posterior pereonite margins, length of setae 0.18 – 0.21 body depth. Pereonite 1 dorsal margin in lateral view shorter than on pereonite 2; length:width in dorsal view 0.33. Pereonite 2 length:width in dorsal view 0.32 – 0.39.

Pereonite 3 length:width 0.45 – 0.55. Pereonite 4 length:width 0.43. Pereonite 5 length:width 0.47. Pereonite 6 length:width 0.41. Pereonite 7 length:width 0.20 – 0.24.

Pleonites in dorsal view 2 – 4 respective lengths less than half the length of pleonite 5, 1 – 4 relative lengths unequal, pleonite 4 length greater than pleonites 1 – 3; pleonites 1 – 4 width 1.28 – 1.46 composite length in dorsal view. Pleonites 1 – 5 dorsal length:maximum width of pleonites 1 – 5 respectively 0.12 – 0.16, 0.16, 0.17, 0.23 and 0.49. Pleonites 1 – 5 depth:pereonite 7 depth respectively 1.19 – 1.40, 1.59 – 1.89, 1.75 – 2.07, 1.73 – 2.06 and 1.47 – 1.74.

Pleotelson dorsal surface in lateral view inflected ventrally, covered with abundant elongate setae, length 1.07 – 1.10 width; median ridge absent; lateral length 0.13 – 0.14 body length, less (0.86 – 0.88) than depth; depth 1.46 – 1.53 pereonite 7 depth; ventral margin anterior to uropods with single row of 2 – 3 simple robust setae; lateral uropodal ridge absent. Posterior apex with one pair of robust setae.

Antennula (Fig. 4.9A) length 0.17 body length, with 6 – 9 articles. Articles 4 and 5 divisible into one large or two small articles (articles 4 – 5, 6 – 7 potentially single articles). Article 3 with rudimentary antennule scale. Article 5 length:width 1.07. Article 6 length:width 0.83. Tiny aesthetascs, 3 to 4, around terminal article. Terminal article vestigial, distally oblique; length:width 0.25 – 0.33; length:antennular length 0.01 – 0.02. Penultimate article distinctly longer than any other article. Distal articles in cross-section oval.

Antenna (Fig. 4.9B) length 0.54 body length. Flagellum length 0.62 total antenna length, with 18 articles. Article 5 length subequal to article 4; article 6 shorter than articles 4 and 5 combined.

Mouthfield. Clypeus rounded laterally, asymmetrically at mandibular fossae; width 0.72 head width. Labrum (Fig. 4.9C) semi-circular (oblong along dorsoventral axis) to broadly triangular (pointed ventrally) in anterior view; asymmetrical, with invagination along right margin; dorsal margin approximately same width as clypeus. Paragnaths (Fig. 4.9D) with distally rounded lobes; dense mats of fine setae on distomedial margins and in multiple rows on surfaces; lateral margins of lobes with scattered simple setae.

Mandible (Figs 4.9E,F,G, 4.10A,B,C) palp length 1.10 mandible length; on medial-distal margins, 3rd article with 19 finely setulate setae on medial-distal margins, 7 – 8 additional medial surface setae present; 2nd article with elongate simple setae scattered along length of medial margin, separate distal row of 4 closely-set elongate simple setae distally on both medial and lateral surfaces; article 1 with elongate simple setae distomedially. Left spine row with 11 – 13 spines, 4 of which bifurcate. Right spine row with 8 spines, 5 of which bifurcate. Molar process longer than wide or length subequal to width; spines absent.

Maxillula (Figs 4.10D,E) medial lobe length 0.85 lateral lobe length; width 0.77 lateral lobe width; with 2 'accessory' setae, one on distolateral margin and one at base of medial central pappose seta, 'accessory' setae distally denticulate; short weakly setulate seta on distal tip absent. Lateral lobe distal margin narrow or subequal to medial lobe, with 10 denticulate robust setae, 2 smooth robust setae,

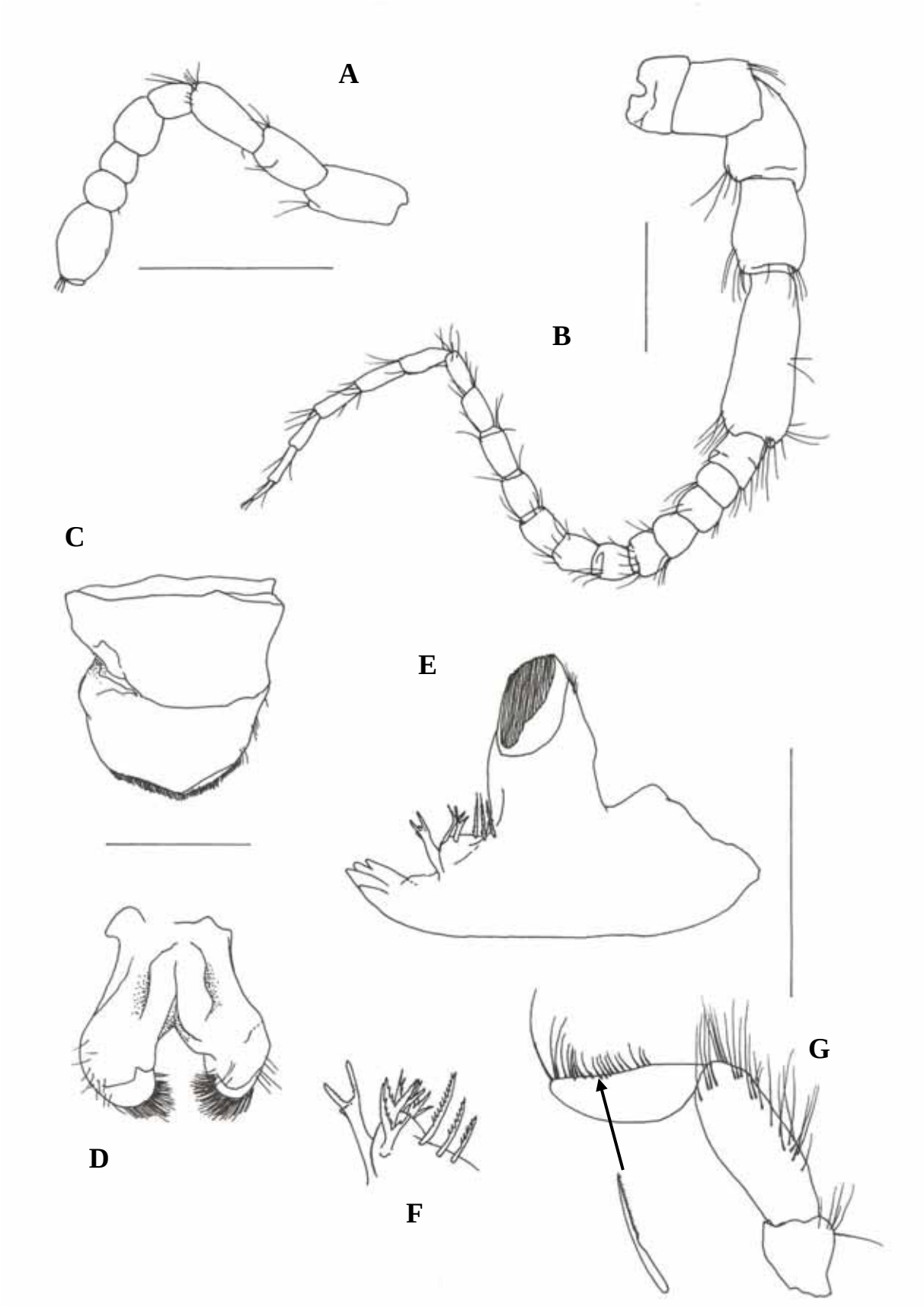


Figure 4.9: *Mesamphisopus baccatus* n. sp., dissected male (SAM A44937). A, antennule; B, antenna; C, labrum; D, paragnath; E, right mandible; F, right mandible lacinia mobilis and spine row; G, right mandibular palp. Scale lines 0.5 mm.

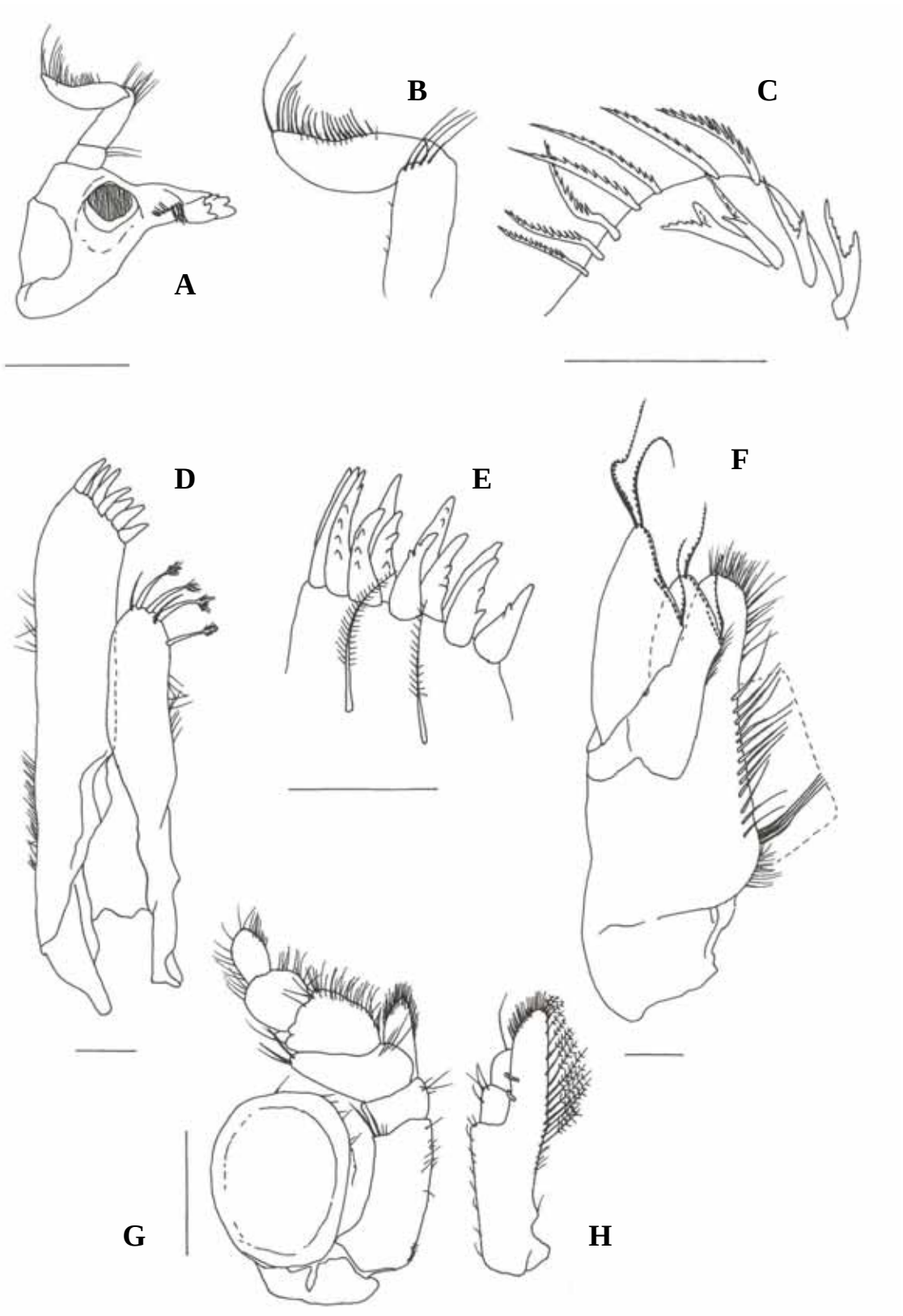


Figure 4.10: *Mesamphisopus baccatus* n. sp., dissected male (SAM A44937). A, left mandible; B, left mandibular palp; C, left mandible spine row; D; maxillula; E, maxillula lateral lobe distal margin; F, maxilla; G, right maxilliped (ventral view); H, maxilliped basal endite (dorsal view). Scale lines 0.1 mm, except A and G (0.5 mm).

distal setal row with 3 robust setae; ventral face with 2 plumose setae, setae widely spaced; additional plumose seta absent.

Maxilla (Fig. 4.10F) medial lobe width 0.80 outer lateral lobe width; proximal portion smoothly continuous with distal portion; proximal and distal setal rows separated by gap; 13 simple, fairly broad-based elongate setae in single ventral basal row; 40 – 42 elongate, closely-set setae, with distinct base, smooth shaft, slightly plumose distally in dorsal basal row; 16 – 17 broad-based, distally plumose elongate setae with distinct base and smooth shaft grade into apical cluster of multiple pectinate, simple and plumose setae (approximately 24) in 3 distal rows. Outer lateral lobe length subequal to inner lateral lobe, wider than inner lateral lobe; distal margin with 23 long bidenticulate setae. Inner lateral lobe with 14 long bidenticulate setae. Lateral lobes with bidenticulate setae only on distal tips.

Maxilliped (Figs 4.10G,H) epipod length:width 1.28; distal tip truncate; simple setae scattered along mediodistal margin. Endite length:total basis length 0.44; medial margin with 3 coupling hooks on left side, 2 on right side; dorsal ridge with 19 – 20 large distally denticulate plumose setae. Palp insertion on basis lateral margin without plumose setae, with 3 elongate fairly stout simple setae; medial margin without simple setae; ventral surface with approximately 8 subdistal smooth setae towards medial margin, without subdistal biserrate setae; palp length:basis length 0.98; width across articles 2 – 3:endite width 1.75; article 4 subcircular, length:width 0.85; article 5 length:width 1.25, article 5 length:article 4 length 0.91.

Pereopod I (Figs 4.11A,B) length:body length 0.53. Dactylus length subequal to palm or longer than palm, length:palm length 1.10; ventrodistal margin with row of thin scale-like spines, minute cuticular hairs, along 0.05 total length; claw length:dactylus length 0.10; distal accessory claw ventrolateral to primary claw, 0.25 – 0.33 length of primary claw. Propodus length:pereopod length 0.27; length:width 1.14; dorsal margin setae in several groups between proximal and distal margin, forming group distally, 8 – 15 setae altogether, including 4 in distal group. Propodal palm cuticular fringe weakly developed; serrate and bifid stout denticulate setae present, 3 serrate, 1 bifid; 4 basally inflated stout robust simple setae 4 altogether; 8 elongate broad based setae present along margin or lateral to margin. Ischium dorsal margin with 7 – 8 simple setae, including 1 robust. Basis length:width 2.08; dorsal setae positioned along ridge, 2 – 7 altogether; ventrodistal margin with 3 elongate setae.

Pereopods II – III (Figs 4.11C,D). Pereopod II length:body length 0.43. Dactylus length:propodus length 0.70; primary claw length:dactylar length 0.26. Propodus length:pereopod length 0.14; length:width 1.80. Carpus length:pereopod length 0.13; length:width 1.39. Basis length:pereopod length 0.28; length:width 2.39. Pereopod III length:body length 0.40. Dactylus length:propodus length 0.79; primary claw length:dactylar length 0.24. Propodus length:pereopod length 0.13; length:width 1.60. Carpus length:pereopod length 0.13; length:width 1.35. Basis length:pereopod length 0.28; length:width 2.27. Pereopods II – III penicillate setae absent. Dactylus distal accessory claw ventrolateral to primary claw, 0.25 – 0.33 length of primary claw. Propodus broad based setae

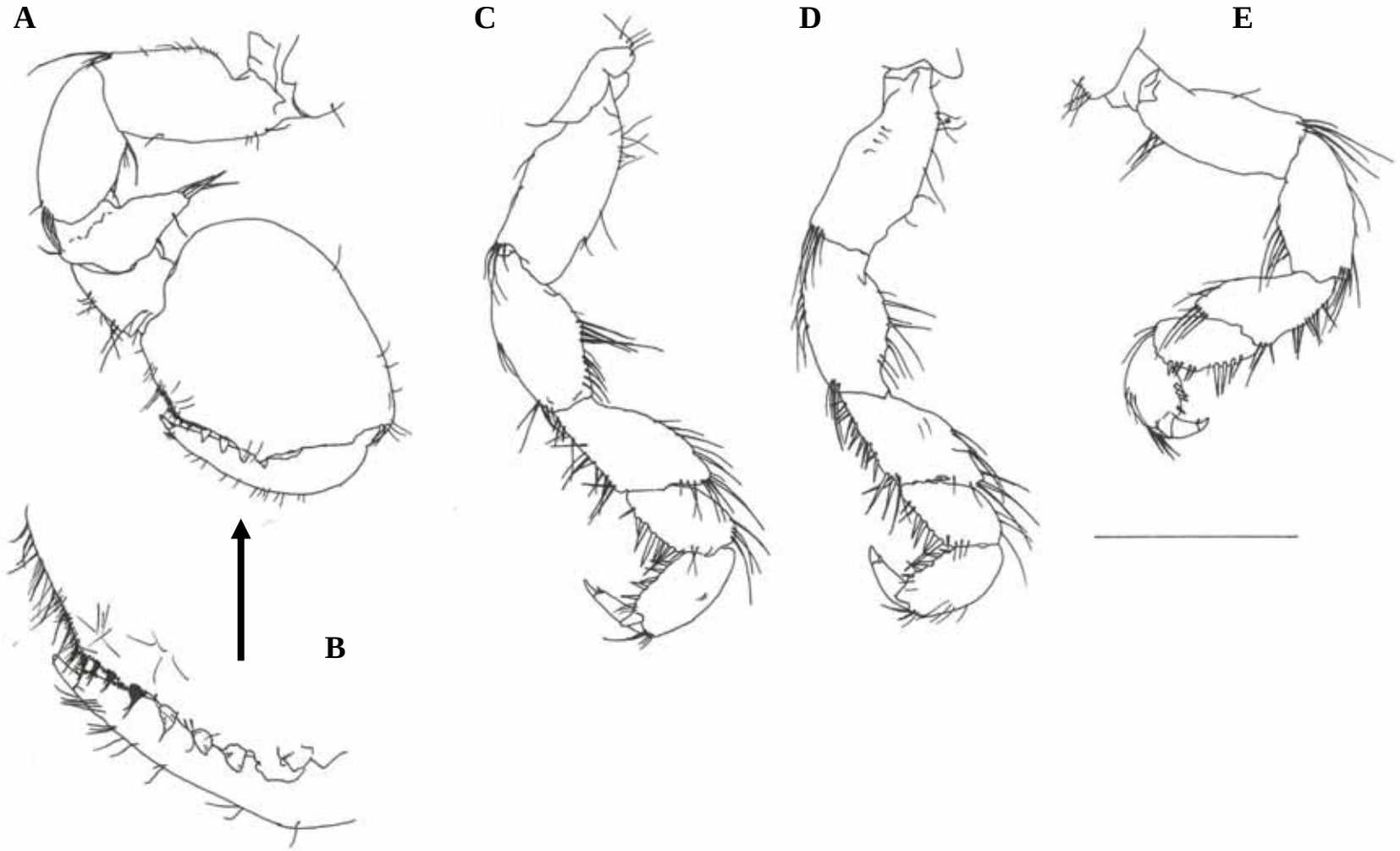


Figure 4.11: *Mesamphisopus baccatus* n. sp., dissected male (SAM A44937). A, pereopod I; B, pereopod I propodal palm; C, pereopod II; D, pereopod III; E, pereopod IV (left). Scale line 1 mm.

present, respectively 5, 5 on pereopods II and III; on pereopod II first (proximal) and second setae 0.17 propodus length, third longest (0.26 propodus length), fourth and fifth (distal) 0.19 propodus length, evenly spaced along margin; on pereopod III increasing in length from proximal (0.08 propodus length) to third (0.27 propodus length) setae, decreasing in length to fifth (0.15 propodus length), evenly spaced along margin. Carpus broad based setae present, respectively 8, 6 on pereopods II and III; on pereopod II generally increasing in length from proximal seta (0.20 carpus length) to sixth/distal seta (0.52 carpus length), third and fourth shorter (0.24 carpus length), evenly spaced along margin, with 2 setae along distolateral surface; on pereopod III increasing in length from proximal (0.20 carpus length) to distal (0.54 carpus length) setae, evenly spaced along margin. Basis dorsal ridge in cross-section rounded to angular and produced but not forming a distinct plate, with approximately 9 – 10 elongate simple setae (up to 0.25 basis length) along margin, with some clustering in proximal group. Pereopods II – IV ischium dorsal margin with 8 – 11 simple setae, including 1 – 3 robust setae.

Pereopod IV (Fig. 4.11E) length:body length 0.36. Penicillate setae absent. Dactylus longer than propodal palm; distal accessory claw approximately 0.33 length of primary claw. Propodus length:pereopod length 0.12, length:width 1.41; distal width:palm width 0.70; with 4 broad based setae on ventral margin, 2 distinctly larger than remainder; articular plate longer than dactylar claw. Carpus length:pereopod length 0.15; with 3 broad based setae on ventral margin, 2 distinctly larger than others. Ischium posterodistal margin with 5 – 8 setae. Basis length:width 2.54; dorsal ridge in cross-section rounded to angular and produced but not forming plate, with approximately 8 setae.

Pereopods V – VII (Fig. 4.12). Pereopod V length:body length 0.35. Dactylus claw length:dactylar length 0.33. Propodus length:pereopod length 0.13. Carpus length:pereopod length 0.16. Basis length:width 1.59. Pereopod VI length:body length 0.45. Dactylus claw length:dactylar length 0.26. Propodus length:pereopod length 0.12. Carpus length:pereopod length 0.15. Basis length:width 1.83. Pereopod VII length:body length 0.47. Dactylus claw length:dactylar length 0.29. Propodus length:pereopod length 0.16. Carpus length:pereopod length 0.18. Basis length:width 1.64. Pereopods V – VII penicillate setae dorsodistally on carpus and on dorsal margin of basis. Dactylus distal accessory claw ventrolateral to primary claw, 0.33 – 0.45 primary claw length. Propodus distal margins with 4 – 6 elongate robust setae. Pereopods V – VII ischium dorsal margin 5 – 10 simple setae, including 2 – 4 robust setae. Basis dorsal ridge not distinctly separated from basis shaft, in cross-section angular on V, produced and forming distinct plate on VI – VII, with elongate fine setae positioned along entire margin; lateral face central ridge present; lateral face ventral ridge absent. Pereopod VII ischium dorsal ridge forming flange subequal to shaft width.

Penes length 0.32 body width at pereonite 7; with setae on shaft and tip; distal tip rounded.

Pleopods (Figs 4.13, 4.14). Pleopod I length:body length 0.17. Exopod length:width 2.99. Endopod length:width 2.68; endopod length:exopod length 0.98. Pleopod II length:body length 0.19. Exopod length:width 1.95; length of distal article:exopod length 0.32. Endopod length:width 2.21; endopod

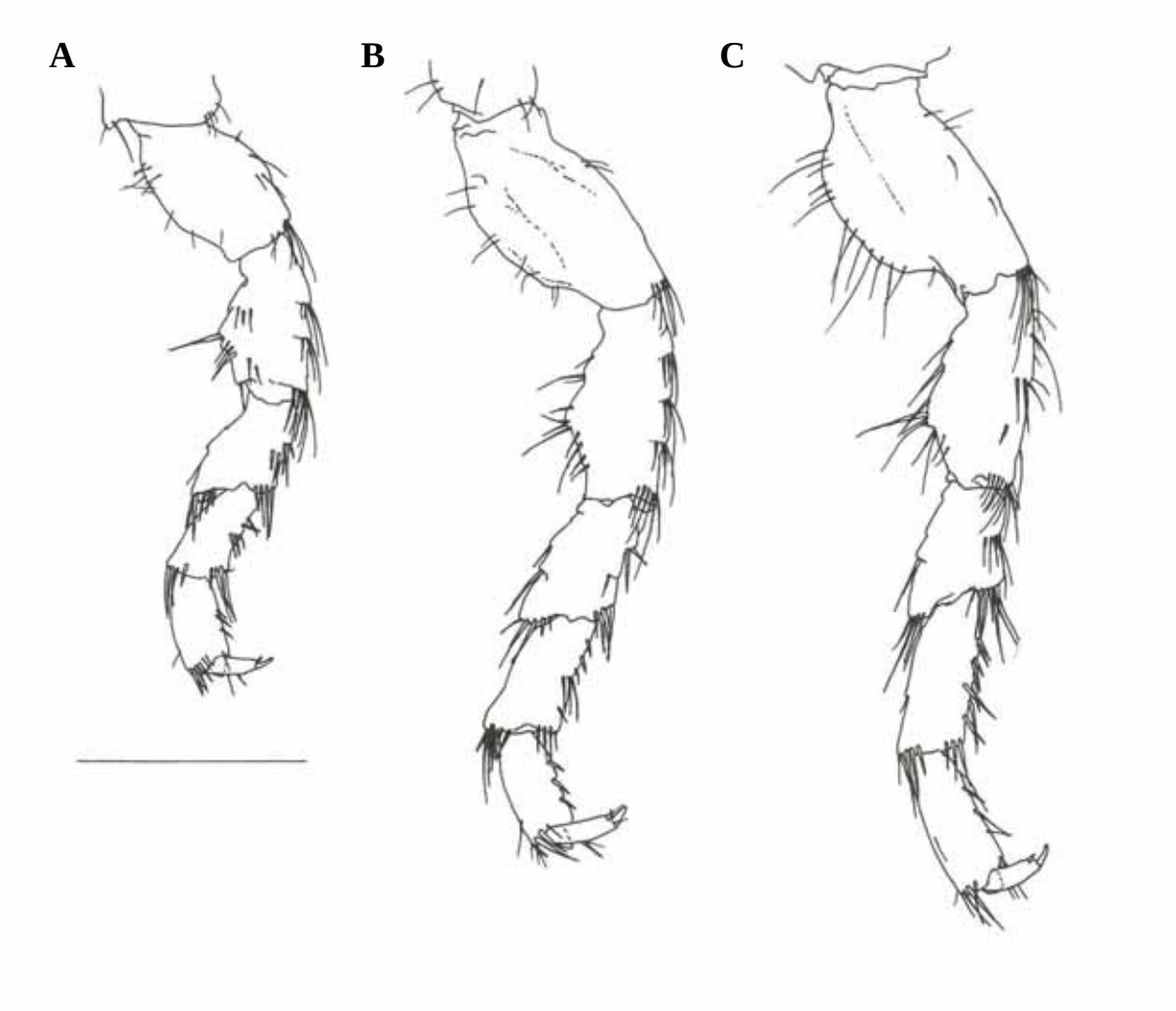


Figure 4.12: *Mesamphisopus baccatus*, n. sp., dissected male (SAM 44937). A, pereopod V; B, pereopod VI; C, pereopod VII. Scale line 1 mm.

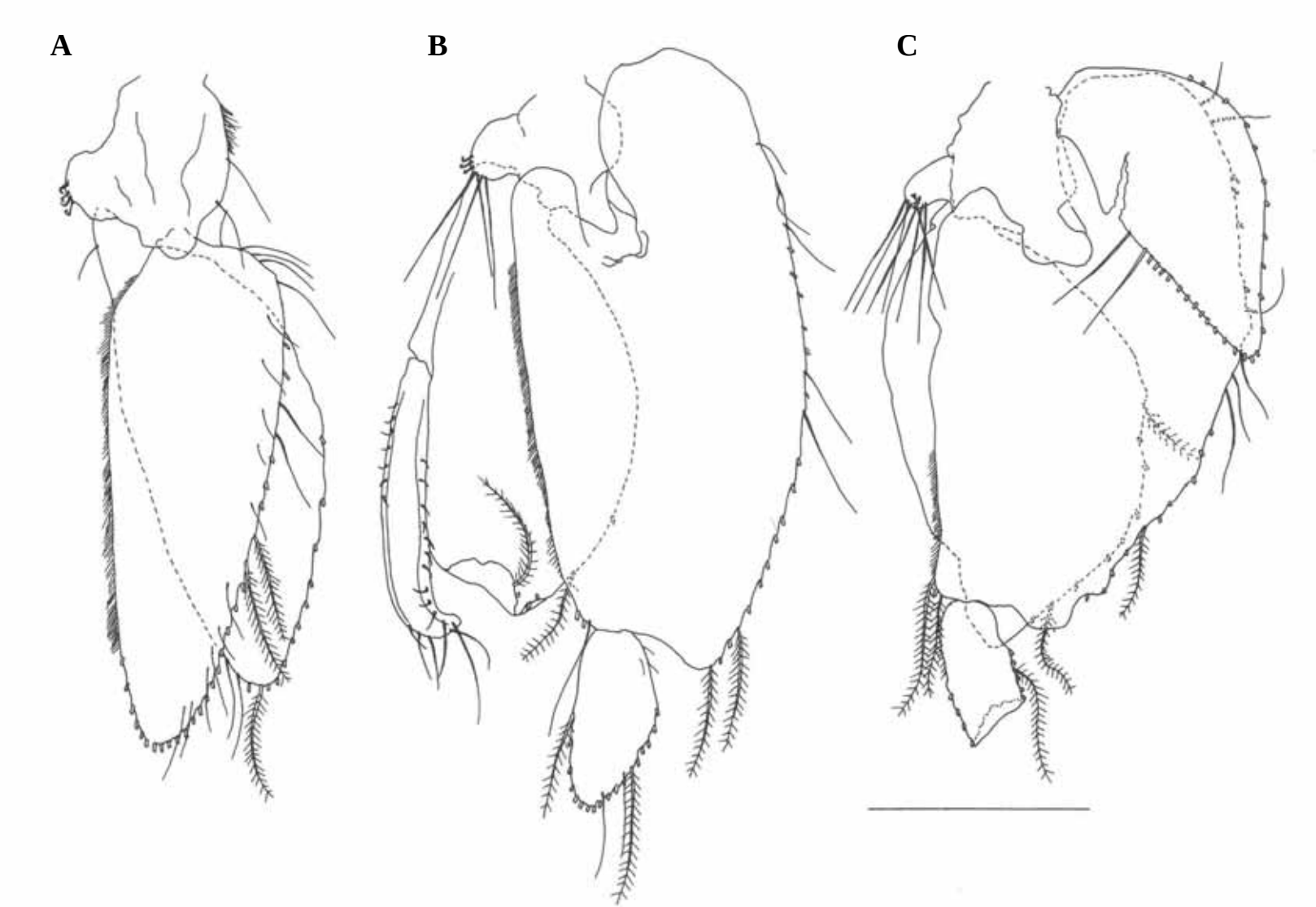


Figure 4.13: *Mesamphisopus baccatus* n. sp., dissected male (SAM A44937). A, pleopod I; B, pleopod II; C, pleopod III. Scale line 0.5 mm.

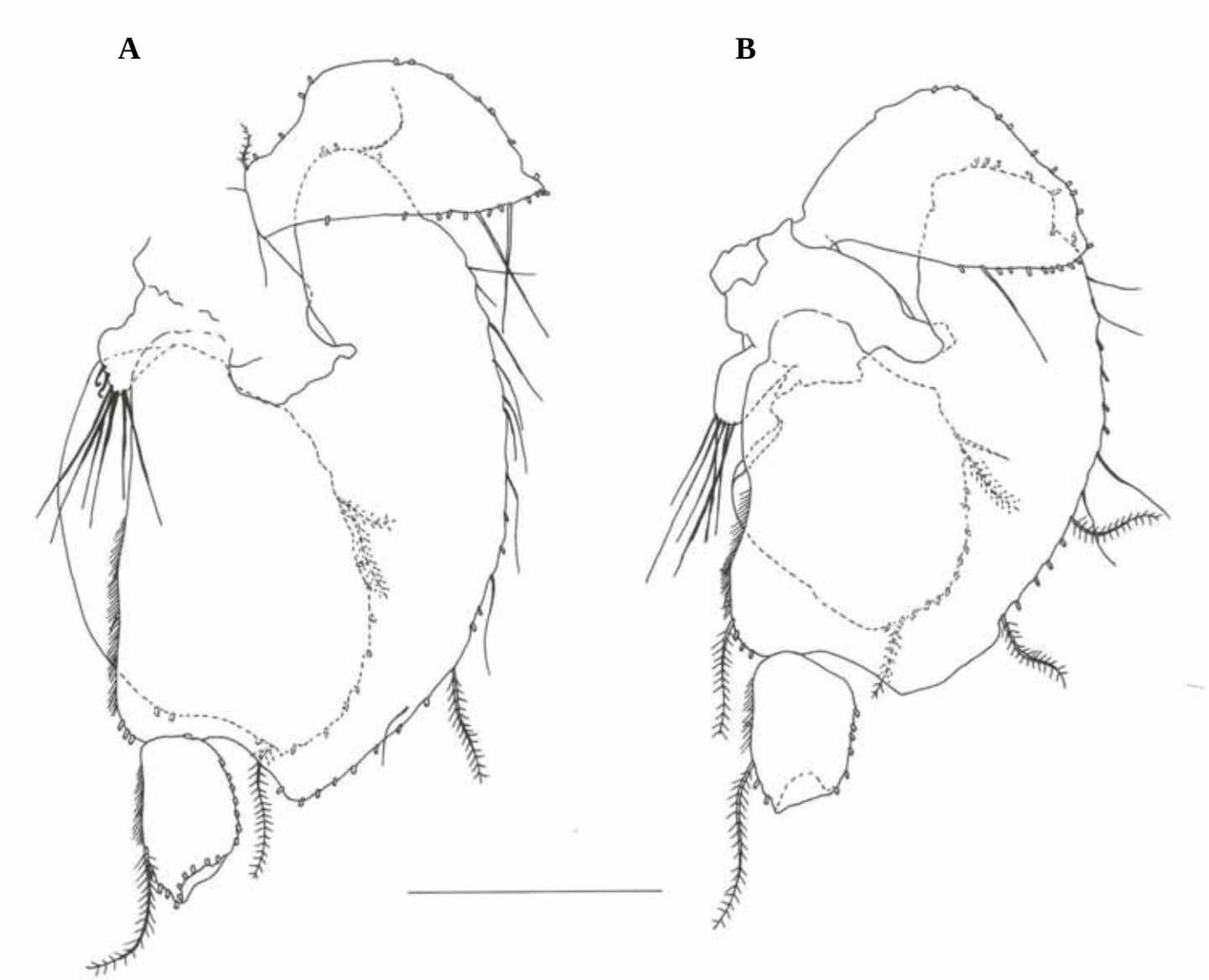


Figure 4.14: *Mesamphisopus baccatus* n. sp., dissected male (SAM A44937). A, pleopod IV; B, pleopod V. Scale line 0.5 mm.

length:exopod length 0.82. Pleopod III length:body length 0.17. Exopod length:width 1.44; length of distal article:exopod length 0.30. Endopod length:width 1.76; endopod length:exopod length 0.96. Pleopod IV length:body length 0.16. Exopod length:width 1.35; length of distal article:exopod length 0.32. Endopod length:width 1.51; endopod length:exopod length 0.81. Pleopod V length:body length 0.13. Exopod length:width 1.40; length of distal article:exopod length 0.35. Endopod length:width 1.06; endopod length:exopod length 0.51. Endopods I – V with plumose setae on margins. Protopods medial margins/epipods I – IV with coupling hooks, respective counts 4, 3, 2, 2; with 4, 7, 6, and 6 elongate simple setae on II, III, IV, and V respectively; lateral epipod III length 2.25 – 2.29 width, lateral epipod V length 1.64 – 1.93 width. Protopods with 2 elongate simple setae on lateral margin of pleopod I; with 28 (15 medially, 1 apically, 12 laterally), 25 (11 medially, 1 apically, 13 laterally) and 20 (8 medially, 1 apically, 11 laterally) elongate simple setae on margins of lateral epipods of pleopods III, IV and V respectively. Pleopod I exopod broadest proximally, medial margin straight to slightly convex — divergent from lateral margin proximally, dorsal surface with setae; protopod length subequal to that of other pleopods, width subequal length to longer than wide. Pleopod II endopod appendix masculina basal musculature pronounced; with 24 setae on margin, occurring along lateral margin and proximally and most distally along medial margin; length 0.38 pleopod length; distal tip extending near to distal margin of endopod.

Uropod (Fig. 4.15) total length 1.34 pleotelson length. Protopod length:width 2.90; length 0.44 uropod total length; extending posteriorly subequal to pleotelson apex; dorsomedial ridge produced, plate-like, margin smooth, in lateral view approximately straight, ridge length:endopod length 0.50; ventral ridge with long laterally projecting setae. Rami cross-sectional shape flattened on dorsal surface only. Endopod dorsal margin robust setae along length, with 6 robust setae, 2 medially, 2 laterally, 2 apically. Exopod length 1.21 endopod length; dorsal margin with 2 robust setae, excluding 2 apical setae.

Sexual dimorphism, female differences from male. *Head.* Mandibular (genal or cheek) groove more acutely indented than in male.

Pereon. Pereonite 1 length:width in dorsal view 0.31 – 0.35. Pereonite 2 length:width in dorsal view 0.41 – 0.43. Pereonite 3 length:width 0.38 – 0.46. Pereonite 4 length:width 0.42 – 0.51. Pereonite 5 length:width 0.36 – 0.40. Pereonite 6 length:width 0.43. Pereonite 7 length:width 0.17.

Antennula length 0.13 body length, with 6 articles. Article 5 (penultimate article) length:width 2.40. Penultimate article length approximately subequal to length of other articles.

Antenna length 0.49 body length. Flagellum length 0.61 total antenna length, with 16 articles.

Pereopod I length:body length 0.42. Dactylus projecting beyond palm, length:palm length 1.12; ventrodistal margin with row of thin scale-like spines, along 0.31 total length; claw length:dactylus length 0.13. Propodus length:pereopod length 0.21; length:width 1.23. Propodal palm concave; cuticular fringe well developed; stout denticulate setae bifid, 7 altogether; stout robust simple setae

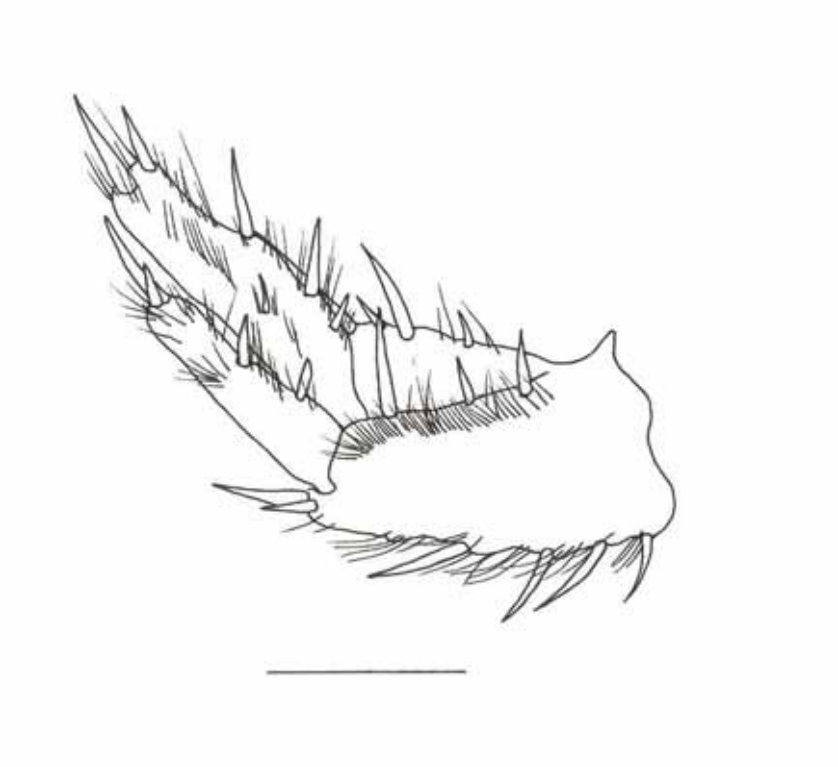


Figure 4.15: *Mesamphisopus baccatus* n. sp., dissected male (SAM A44937). Uropod. Scale line 0.5 mm.

absent; 7 elongate broad based setae present, additional broad-based simple setae occur laterally to margin. Ischium dorsal margin with 3 – 6 simple setae, none robust. Basis length:width 2.00; dorsal setae clustered proximally and positioned along ridge, 6 – 8 altogether; ventrodistal margin with 3 elongate setae.

Pereopods II – III. Pereopod II length:body length 0.38. Dactylus length:propodus length 0.86; primary claw length:dactylar length 0.33. Propodus length:pereopod length 0.12; length:width 1.46. Carpus length:pereopod length 0.13; length:width 1.63. Basis length:pereopod length 0.28; length:width 2.30. Pereopod III length:body length 0.34. Dactylus length:propodus length 0.72; primary claw length:dactylar length 0.35. Propodus length:pereopod length 0.13; length:width 1.64. Carpus length:pereopod length 0.12; length:width 1.36. Basis length:pereopod length 0.23; length:width 1.64. Propodus broad based setae present, respectively 5, 5 on pereopods II and III; on pereopod II increasing in length from proximal seta (0.31 propodus length) to third (0.43 propodus length), decreasing in length to most distal seta (0.20 propodus length), evenly spaced from proximal third to distal margin; on pereopod III most proximal 0.31 propodus length, median 0.33 propodus length, most distal 0.19 propodus length, evenly spaced along margin from proximal third to distal margin. Carpus broad based setae present, respectively 4, 6 on pereopods II and III; on pereopod II increasing in length from most proximal (0.36 carpus length) to most distal (0.56 carpus length), evenly spaced along margin; on pereopod III increasing in length from most proximal seta (0.18 carpus length) to most distal (0.71 carpus length), evenly spaced along margin; pereopods II and III with respectively additional 3, 2 broad based setae on distolateral margins. Pereopod II setation includes 2 plumose setae on basis dorsal ridge.

Pereopod IV simple. Length:body length 0.32. Propodus length:pereopod length 0.14; length:width 1.80; with 3 broad based setae on ventral margin; articular plate shorter than dactylar claw. Carpus length:pereopod length 0.11; with 5 broad based setae on ventral margin, 3 broad based setae on lateral/posterior surface. Ischium posterodistal margin with 4 setae. Basis length:width 1.78; dorsal ridge with approximately 10 setae.

Pereopods V – VII. Pereopod V length:body length 0.31. Dactylus claw length:dactylar length 0.35. Propodus length:pereopod length 0.13. Carpus length:pereopod length 0.15. Basis length:width 1.54. Pereopod VI length:body length 0.43. Dactylus claw length:dactylar length 0.32. Propodus length:pereopod length 0.16. Carpus length:pereopod length 0.15. Basis length:width 1.51. Pereopod VII length:body length 0.46. Dactylus claw length:dactylar length 0.31. Propodus length:pereopod length 0.13. Carpus length:pereopod length 0.16. Basis length:width 1.71.

Pleopods. Pleopod I length:body length 0.13. Exopod length:width 2.68. Endopod length:width 3.05; endopod length:exopod length 1.03. Pleopod II length:body length 0.16 – 0.17. Exopod length:width 1.90 – 2.10; length of distal article:exopod length 0.26 – 0.27. Endopod length:width 1.57 – 2.69; endopod length:exopod length 0.76 – 0.94. Pleopod III length:body length 0.15 – 0.18. Exopod length:width 1.64 – 1.83; length of distal article:exopod length 0.30 – 0.31. Endopod length:width

1.59 – 1.89; endopod length:exopod length 0.88 – 0.94. Pleopod IV length:body length 0.15 – 0.16. Exopod length:width 1.38 – 1.40; length of distal article:exopod length 0.28 – 0.30. Endopod length:width 1.53 – 1.67; endopod length:exopod length 0.80 – 0.92. Pleopod V length:body length 0.13 – 0.16. Exopod length:width 1.16 – 1.50; length of distal article:exopod length 0.27 – 0.34. Endopod length:width 1.45 – 1.47; endopod length:exopod length 0.71 – 0.73. Endopods I – V with setae on margins, setae plumose on I – IV, simple on V. Protopods medial margins/epipods with coupling hooks on I – IV, respective counts 4, 3, 2, 1; with 4, 6, 6, and 7 elongate simple on II, III, IV, and V respectively. Protopods with 3 elongate simple setae on lateral margins on pleopod I; with 21 (13 medial to apical, 8 lateral), 19 (12 medial to apical, 7 lateral) and 18 (12 medial to apical, 6 lateral) elongate simple setae on margins of lateral epipods of pleopods III, IV and V respectively.

Uropod total length 1.64 pleotelson length. Protopod length:width 3.25, length 0.40 uropod total length; dorsomedial ridge length:endopod length 0.46. Endopod with 6 robust setae. Exopod length 1.20 endopod length; with 4 robust setae.

General Distribution. Known only from type locality, lying within the general distribution of *Mesamphisopus capensis*.

Remarks. *Mesamphisopus baccatus* n. sp., the type locality of which lies within the general distribution of *M. capensis*, is distinguished from the latter species by the abundant setation of the pleon, and particularly the elongate setation of the pleotelson. In this regard, the species approaches the condition seen in *M. kensleyi* n. sp. and perhaps *M. depressus* and *M. abbreviatus*. *Mesamphisopus baccatus* n. sp. individuals lack the sub-apical pair of robust setae occurring dorsally on the pleotelson of *M. abbreviatus*, *M. depressus* and *M. penicillatus*, as discussed Kensley (2001). Additionally, *M. baccatus* has a relatively short antenna (of approximately 20 articles) in comparison with *M. capensis* and certain other species. While *M. albidus* n. sp. and *M. kensleyi* n. sp. have similarly short antennae, the articles of these, and of the antennules, are more rectangular in shape or more slender, lacking the globular or inflated bulbous appearance of those of *M. baccatus* n. sp. *Mesamphisopus baccatus* n. sp. is also unusual in having plumose setae on the endopods of all five pleopods, a condition seen only in *M. tsitsikamma* n. sp. and the dissected female individual of *M. albidus* n. sp. (this perhaps giving an indication of the variability of pleopodal setation). Among the six species described herein, *M. baccatus* n. sp. and *M. paludosus* n. sp. are apparently unique in lacking a well-developed lateral uropodal ridge on the pleotelson, this ridge being figured for *M. abbreviatus*, *M. depressus* and *M. capensis*, where the ridge has been illustrated as bearing setae.

***Mesamphisopus kensleyi* n. sp.**

Figures 4.16 – 4.23

Type locality. Along stream, trail above Aurora Drive (off Chapman's Road), Gordon's Bay, Western Cape, South Africa (34°09'49"S 18°52'38"E).

Material examined. Holotype: SAM A45152, one adult male (bl 9.0 mm), along stream, trail above Aurora Drive (off Chapman's Road), Gordon's Bay, South Africa (34°09'49"S 18°52'38"E), collected 17/XI/2000 by S. R. Daniels and G. Gouws. SAM A44940, one dissected adult male (bl 8.9 mm) and one dissected preparatory female (bl 7.7 mm) parts slide mounted and in microvials, additional four males, four females, collection details as for holotype. SAM A45153, one male, one female, two juveniles, stream above Chapman's Road, Gordon's Bay, South Africa (34°08'S 18°52'E), collected on 19/X/1989 by C. L. Griffiths and P. le Roux.

Etymology. The species is given the epitheton "kensleyi" in memory of Brian Kensley and in recognition of his contribution to the systematics and taxonomy of southern African Isopoda.

Diagnosis. Mandibular groove smoothly indented. Pereonite 1 dorsal margin in lateral view subequal-longer than on pereonite 2. Pleonites 1 – 4 individual depths:pereonite 7 depth 1.45 – 2.10. Pleotelson dorsal surface covered with abundant elongate setae; lateral length less than depth; depth 1.35 – 1.70 pereonite 7 depth; ventral margin anterior to uropods with single row of simple robust setae, posterior seta longer than anterior adjacent setae; lateral uropodal ridge terminating at pleotelson margin above uropods; posterior apex with one pair of robust setae. Antennula penultimate article distinctly longer than any other article; distal articles in cross-section circular. Antenna article 5 length subequal to article 4. Mandibular palp article 3 with approximately 20 setae. Maxillula medial lobe width 0.54 lateral lobe width; lateral lobe distal margin with 5 smooth robust setae. Maxilliped palp insertion on basis medial margin with multipe simple setae. Pereopod I dactylus ventrodistal margin smooth; propodus dorsal margin setae in several groups between proximal and distal margin; propodal palm stout denticulate setae bifid; ischium dorsal margin with singular robust simple seta; basis dorsal margin setae positioned proximally. Pereopod II propodus length:width < 2.00; carpus length:width < 1.50, with 7 broad based setae. Pereopod III propodus length:width < 2.00, with 4 broad based setae; carpus with 7 broad based setae. Pereopods II – III basis dorsal ridge cross-section rounded. Pereopods II – IV ischium dorsal margin with > 12 simple setae, one robust. Pereopod IV dactylus longer than propodal palm; propodus length:width approximately 1.40, ventral margin with 2 broad based setae; basis length:width < 2.20. Pereopods V – VII basis lateral face ventral ridge absent. Penes with setae on tip. Pleopodal endopods setae plumose on I – IV, simple on V; shallowly cleft distomedially on III – V. Pleopod II appendix masculina distal tip extending beyond distal

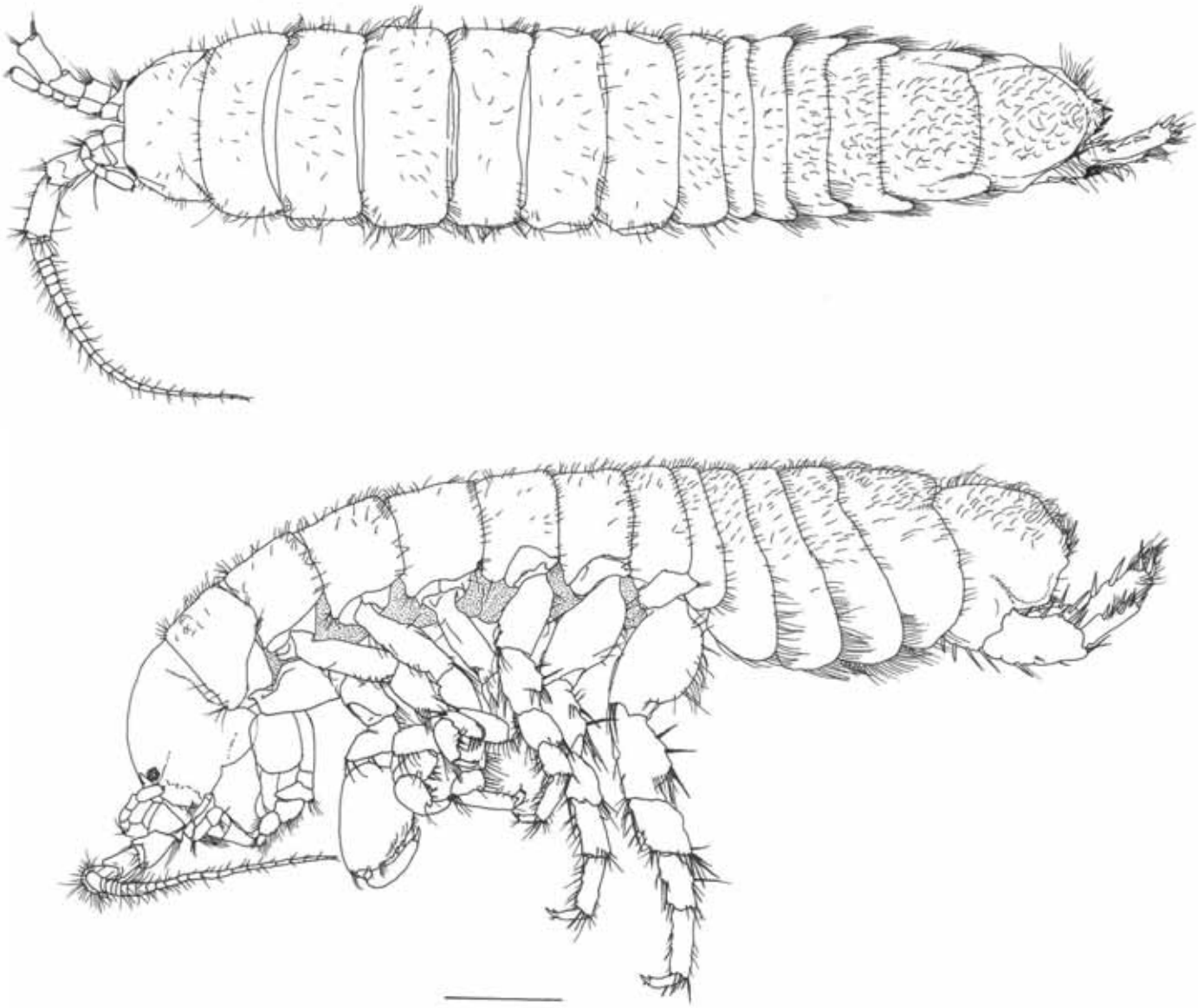


Figure 4.16: *Mesamphisopus kensleyi* n. sp., male holotype (SAM A45152), dorsal view (above) and lateral view (below). Scale line 1 mm. Only one antenna and uropod figured completely in dorsal view.

margin of endopod. Uropod total length 1.80 pleotelson length; protopod extending posteriorly subequal to pleotelson apex; endopod dorsal margin with 5 robust setae, along length; exopod dorsal margin with 3 robust setae.

Description based on male. *Coloration.* Body pigmented, slate grey to dark brown grey, fades to brown or almost completely to light brown, yellow-brown upon preservation. Unpigmented parts white to off white, turning off-white to yellow-brown upon preservation. Darker pigmentation forms longitudinal dorsal band along pereon. Unpigmented parts give mottled appearance to lateral parts of pereon, and dendritic pattern to head and pleotelson. Pereopods generally unpigmented, bases may be lightly pigmented. Uropods lack pigmentation. Pleonites lightly pigmented towards ventral extent of pleura, more heavily coloured along posterior margins of pleonites.

Head width 0.76 – 0.86 pereonite 1 width; setae common, fine. Eyes projecting anteriorly; maximum diameter 0.11 head depth; approximately round. Cervical groove extending nearly to dorsal margin of head. Mandibular (genal or cheek) groove smoothly indented. Maxillipeds insertion from posterior margin of head approximately 0.2 head length.

Pereon width exceeding head width; length of setae on dorsal surface 0.16 – 0.24 body depth. Pereonite 1 dorsal margin in lateral view subequal-longer than on pereonite 2; length:width in dorsal view 0.36 – 0.40. Pereonite 2 length:width in dorsal view 0.42 – 0.45. Pereonite 3 length:width 0.44. Pereonite 4 length:width 0.39. Pereonite 5 length:width 0.40 – 0.42. Pereonite 6 length:width 0.37 – 0.39. Pereonite 7 length:width 0.25 – 0.27.

Pleonites in dorsal view 2 – 4 respective lengths less than half the length of pleonite 5, 1 – 4 relative lengths unequal, pleonite 4 length greater than pleonites 1 – 3 (increasing in length from anteriorly to posteriorly); pleonites 1 – 4 width 1.18 – 1.21 composite length in dorsal view. Pleonites 1 – 5 dorsal length:maximum width of pleonites 1 – 5 respectively 0.16, 0.19, 0.22, 0.27 and 0.58. Pleonites 1 – 5 depth:pereonite 7 depth respectively 1.47, 1.94, 2.09, 2.06 and 1.85.

Pleotelson dorsal surface in lateral view inflected ventrally, covered with abundant elongate setae, length 0.99 – 1.02 width; median ridge absent; lateral length approximately 0.13 body length, less (0.75 – 0.84) than depth; depth 1.35 – 1.68 pereonite 7 depth; ventral margin anterior to uropods with single row of 4 simple robust setae; lateral uropodal ridge terminating at pleotelson margin above uropods, lacking setae. Posterior apex with one pair of robust setae; additional pair occurs more laterally.

Antennula (Fig. 4.17A) length 0.15 body length, with 7 – 8 articles. Antepenultimate article may be divisible into one large or two small articles. Article 5 length:width 1.33 – 1.50. Article 6 length:width 1.21. Three fine aesthetascs, 5 simple setae on terminal article, single aesthetasc and 2 simple setae on subterminal article distal margin. Terminal article length:width 0.36; length:antennular length 0.01 – 0.02. Penultimate article distinctly longer than any other article. Distal articles in cross-section circular to oval.

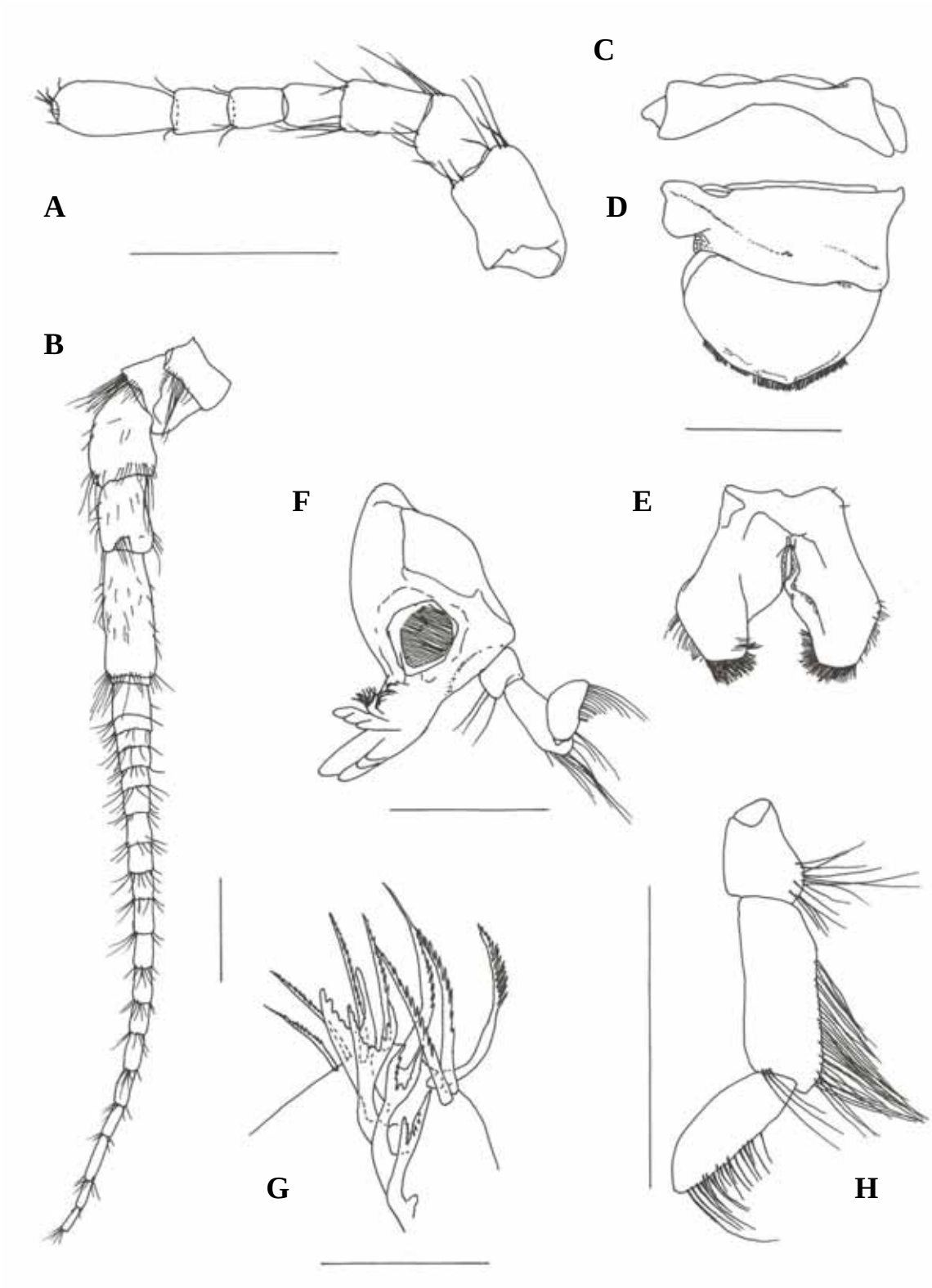


Figure 4.17: *Mesamphisopus kensleyi* n. sp., dissected male (SAM A44940). A, antennule; B, antenna; C, clypeus; D, labrum; E, paragnath; F, left mandible; G, left mandible spine row; H, left mandibular palp. Scale lines 0.5 mm, except G (0.1 mm).

Antenna (Fig. 4.17B) length 0.51 body length. Flagellum length 0.61 total antenna length, with 16 – 20 articles. Article 5 length subequal to article 4; article 6 shorter than articles 4 and 5 combined.

Mouthfield. Clypeus (Fig. 4.17C) greatly expanded and broadly triangular laterally; width 0.61 head width. Labrum (Fig. 4.17D) ventrally semicircular in anterior view, with fine fringe of setae ventrally; asymmetrical, with invagination along right margin; dorsal margin approximately same width as clypeus. Paragnath (Fig. 4.17E) lobes distolaterally rounded to angular, distally more truncate; distomedial margin with dense rows of very fine setae projecting inwards; elongate simple setae scattered distolaterally.

Mandible (Figs 4.17F,G,H, 4.18A,B,C) palp length 1.01 mandible length; 3rd article setae with 19 – 21 finely setulate setae on medial-distal margins, additional medial surface setae absent; 2nd article with numerous elongate simple setae in longitudinal row along ventral margin or concentrated along anterior-medial margin, separate distal row of 3 setae dorsolaterally; article 1 with elongate simple setae distoventrally; articles 1 – 2 setae longer than half respective segment lengths. Left spine row with 13 spines, 4 – 5 of which bifurcate. Right spine row with 11 spines, 4 of which bifurcate. Molar process length subequal to width or longer than wide; fine simple spines forming posterior row.

Maxillula (Figs 4.18D,E) medial lobe length 0.70 lateral lobe length; width 0.54 lateral lobe width; with 2 'accessory' setae, one on distolateral margin and one between distomedial pappose setae, lateral 'accessory' seta simple, medial 'accessory' seta distally denticulate; short weakly setulate seta on distal tip absent. Lateral lobe distal margin with 7 denticulate robust setae, 5 smooth robust setae, distal setal row with 3 robust setae; ventral face with 1 plumose, 2 pectinate-plumose setae, setae widely spaced; additional plumose seta absent.

Maxilla (Fig. 4.18F) medial lobe width 0.90 outer lateral lobe width; proximal portion distinctly angled to distal portion; proximal and distal setal rows separated by gap; 13 broad based, elongate simple setae in single ventral basal row; 35 – 45 closely set, elongate setae with distinct base, sparsely plumose shaft in dorsal basal row; setae in multiple distal rows includes large number of simple, pectinate/setulate and plumose setae. Outer lateral lobe length subequal to inner lateral lobe, wider than inner lateral lobe; distal margin with 17 long bidenticulate setae. Inner lateral lobe with 13 long bidenticulate setae. Lateral lobes with bidenticulate setae only on distal tips.

Maxilliped (Fig. 4.19A) epipod length:width 1.13 – 1.18; distal tip truncate; distal margin setae simple and scattered, finer fringe along medial margin. Endite length:total basis length 0.42; medial margin with 3 – 4 coupling hooks on left side, 3 on right side; dorsal ridge with 18 large distally denticulate plumose setae. Palp insertion on basis lateral margin without plumose setae; medial margin with 3 simple setae distally along ventral surface; ventral surface with 3 – 4 subdistal smooth setae; 2 additional elongate simple setae ventral subdistal/distolateral at palp insertion onto basis; palp length:basis length 0.96; width across articles 2 – 3:endite width 1.79; article 4 subcircular, length:width 1.00; article 5 length:width 1.23, article 5 length:article 4 length 0.67.

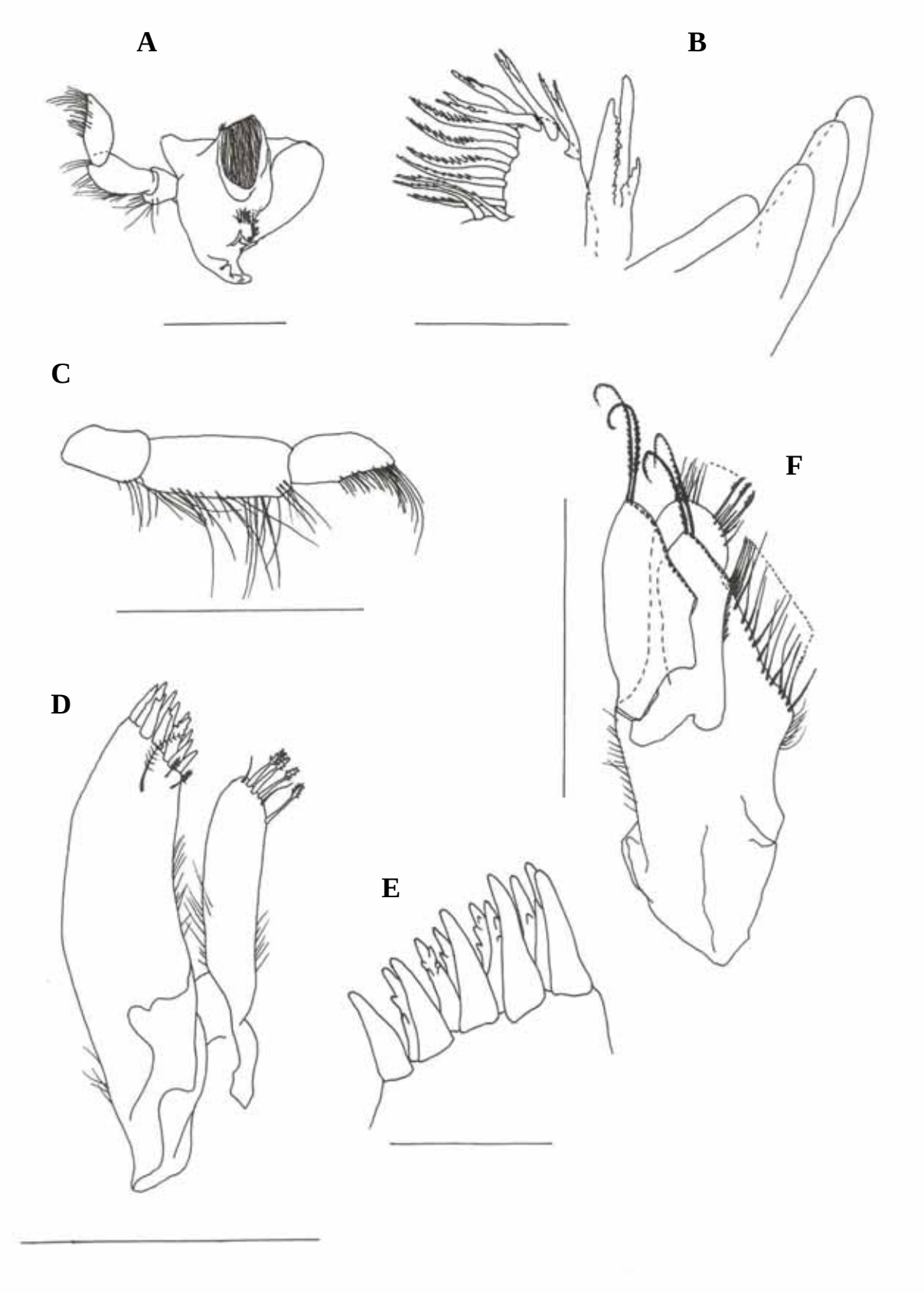


Figure 4.18: *Mesamphisopus kensleyi* n. sp., dissected male (SAM A44940). A, right mandible; B, right mandible incisor process and spine row; C, right mandibular palp; D, maxillula; E, maxillula lateral lobe distal margin; F, maxilla. Scale lines 0.5 mm, except for B and E, where they represent 0.1 mm.

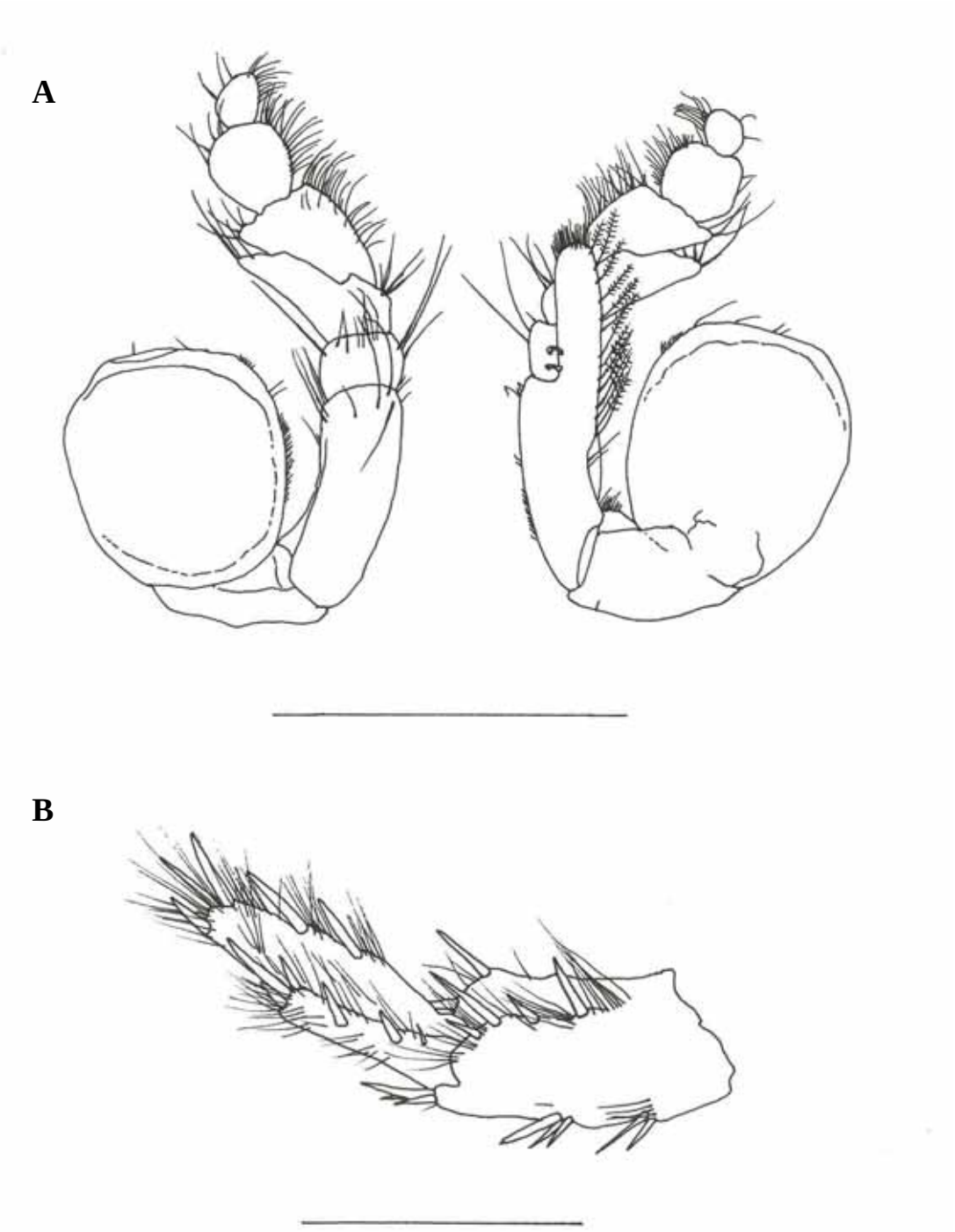


Figure 4.19: *Mesamphisopus kensleyi* n. sp., dissected male (SAM A44940). A, right maxilliped ventral view (left) and dorsal view (right); B, uropod. Scale lines 1 mm.

Pereopod I (Figs 4.20A,B) length:body length 0.49. Dactylus length subequal to palm or longer than palm, length:palm length 1.33 – 1.42; ventrodistal margin smooth; claw length:dactylus length 0.14; distal accessory claw ventrolateral to primary claw, 0.30 length of primary claw. Propodus length:pereopod length 0.25; length:width 1.16; dorsal margin setae in several groups between proximal and distal margin, 14 setae altogether, including 9 in distal group. Propodal palm cuticular fringe weakly developed; stout denticulate setae bifid, 4 altogether; 3 basally inflated stout robust simple setae altogether; 2 elongate broad based setae present. Merus distodorsal margin with numerous elongate simple setae, one more robust. Ischium dorsal margin with 1 simple seta and row of 7 setae lateral to margin, including 1 robust. Basis length:width 1.89; single dorsal seta positioned proximally; ventrodistal margin with 5 elongate setae.

Pereopods II – III (Figs 4.20C,D). Pereopod II length:body length approximately 0.41. Dactylus length:propodus length approximately 0.73. Propodus length:pereopod length 0.14; length:width 1.77. Carpus length:pereopod length 0.12; length:width 1.23. Basis length:pereopod length 0.27; length:width 2.00. Pereopod III length:body length 0.40. Dactylus length:propodus length 0.82; primary claw length:dactylar length 0.29. Propodus length:pereopod length 0.13; length:width 1.72. Carpus length:pereopod length 0.12; length:width 1.21. Basis length:pereopod length 0.27; length:width 2.04. Pereopods II – III with singular penicillate seta present on dorsal ridge of pereopod III basis. Dactylus distal accessory claw ventrolateral to primary claw, 0.33 – 0.38 primary claw length. Propodus broad based setae present, respectively 5, 4 on pereopods II and III; on pereopod II increasing in length from proximal seta (0.16 propodus length) to fourth seta (0.40 propodus length), most distal seta 0.25 propodus length, evenly spaced along margin; on pereopod III increasing in length from proximal seta (0.26 propodus length) to third seta (0.40 propodus length), most distal seta 0.24 propodus length, evenly spaced along margin. Carpus broad based setae present, respectively 7, 7 on pereopods II and III; on pereopod II increasing in length from proximal seta (0.10 carpus length) to fourth seta (0.54 carpus length), and from fifth seta (0.44 carpus length) to distal seta (0.65 carpus length), evenly spaced along margin; on pereopod III progressively increasing in length from proximal seta (0.15 carpus length) to distal seta (0.61 carpus length), with sixth seta shorter (0.33 carpus length), evenly spaced along margin. Basis dorsal ridge in cross-section rounded to angular and produced without forming distinct plate, with 11 – 18 elongate simple setae along margin length or just laterally/medially. Pereopods II – IV ischium dorsal margin with 13 – 15 simple setae, including 1 robust seta on pereopod II.

Pereopod IV (Fig. 4.20E) length:body length 0.35. Penicillate setae present on dorsal margin of basis. Dactylus longer than or subequal to propodal palm; distal accessory claw approximately 0.25 length of primary claw. Propodus length:pereopod length 0.12, length:width 1.36; distal width:palm width 0.82; with 2 broad based setae on ventral margin, none distinctly larger than others; articular plate subequal in length to dactylar claw. Carpus length:pereopod length 0.13; with 6 broad based setae on ventral margin, some distinctly larger than others. Ischium posterodistal margin 7 setae. Basis length:width

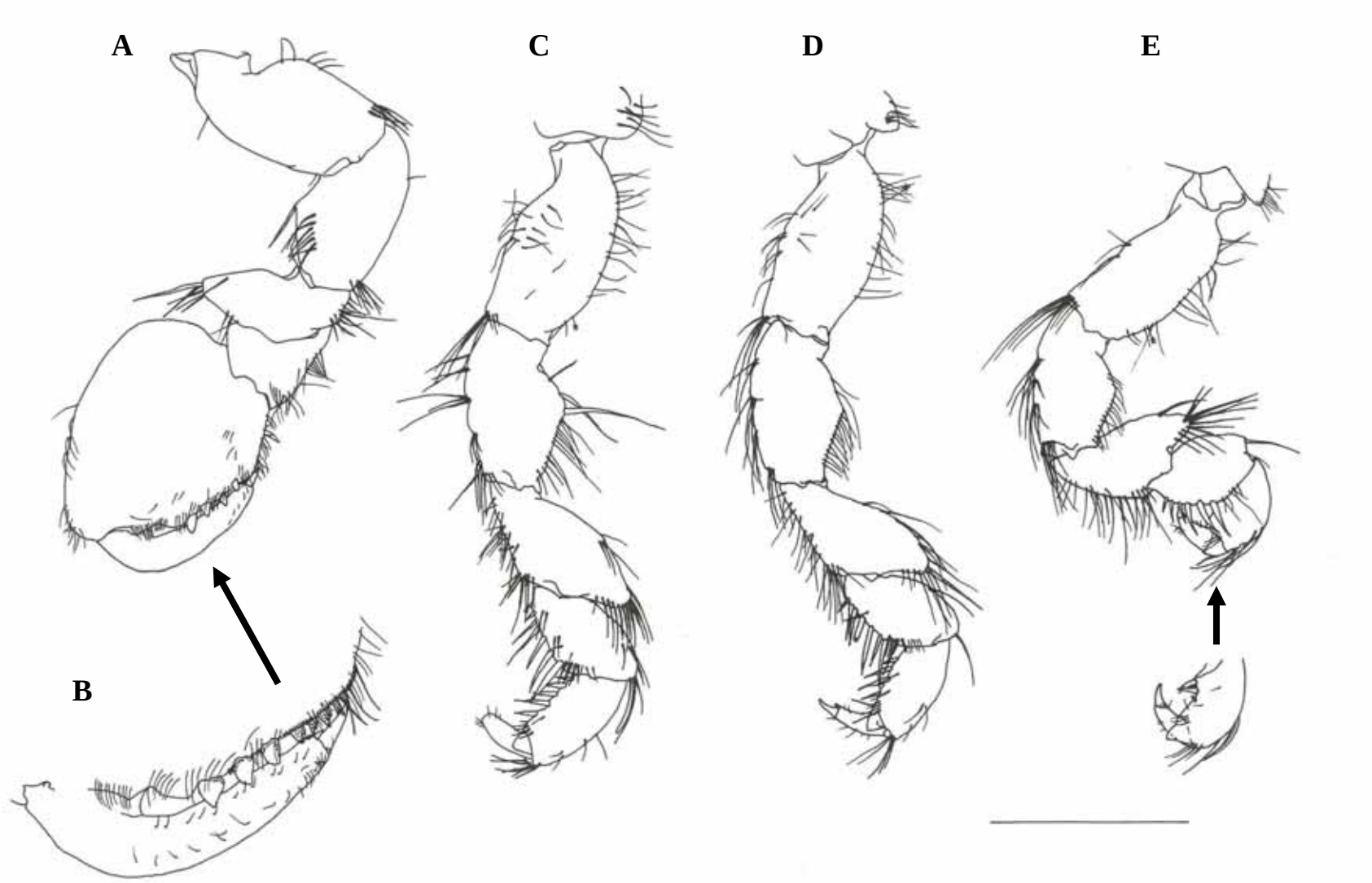


Figure 4.20: *Mesamphisopus kensleyi* n. sp., dissected male (SAM A44940). A, pereopod I (left); B, pereopod I propodal palm; C, pereopod II (right); D, pereopod III (right); E, pereopod IV (right). Scale line 1 mm.

2.12; dorsal ridge in cross-section rounded to angular and produced but not forming distinct plate, with approximately 18 setae.

Pereopods V – VII (Fig. 4.21). Pereopod V length:body length 0.36. Dactylus claw length:dactylar length 0.24. Propodus length:pereopod length 0.16. Carpus length:pereopod length 0.14. Basis length:width 1.88. Pereopod VI length:body length approximately 0.45. Propodus length:pereopod length approximately 0.16. Carpus length:pereopod length approximately 0.15. Basis length:width 1.44. Pereopod VII length:body length 0.47. Dactylus claw length:dactylar length 0.32. Propodus length:pereopod length 0.15. Carpus length:pereopod length 0.13. Basis length:width 1.51. Pereopods V – VII penicillate setae on dorsal ridge of basis. Dactylus distal accessory claw ventrolateral to primary claw, 0.33 – 0.43 length of primary claw. Propodus distal margins with 5 – 7 elongate robust setae, 3 – 5 more elongate than others. Pereopods V – VII ischium dorsal margin with approximately 8 – 10 simple setae, including 2 – 5 robust setae. Basis dorsal ridge not distinctly separated from basis shaft, in cross-section angular on V, produced and forming distinct plate on VI – VII, with elongate fine setae positioned along entire margin; lateral face central ridge present on pereopods VI – VII; lateral face ventral ridge absent. Pereopod VII ischium dorsal ridge flange absent.

Penes length 0.47 body width at pereonite 7; with seta on tip; distally tubular, tapering slightly; distal tip rounded.

Pleopods (Figs 4.22, 4.23). Pleopod I length:body length 0.16. Exopod length:width 2.59. Endopod length:width 2.42; endopod length:exopod length 0.89. Pleopod II length:body length 0.18. Exopod length:width 1.93; length of distal article:exopod length 0.37. Endopod length:width 1.92; endopod length:exopod length 0.77. Pleopod III length:body length 0.18. Exopod length:width 1.50; length of distal article:exopod length 0.32. Endopod length:width 1.55; endopod length:exopod length 0.88. Pleopod IV length:body length 0.16. Exopod length:width 1.45 – 1.53; length of distal article:exopod length 0.36. Endopod length:width 1.29 – 1.40; endopod length:exopod length 0.63 – 0.71. Pleopod V length:body length 0.15. Exopod length:width 1.21; length of distal article:exopod length 0.39. Endopod length:width 1.24; endopod length:exopod length 0.62. Endopods unilobed, III – V with invagination or shallow cleft distomedially; I – V with setae on margins, setae plumose on I – IV, simple on V. Protopods medial margins/epipods I – IV with coupling hooks, respective counts 4, 2, 1, 1; with 4, 3, 4, 5 and 4 elongate inflexible simple setae on I, II, III, IV and V respectively; lateral epipod III length 1.75 width, lateral epipod V length 1.37 width. Protopods with 19 (8 lateral, 11 medial to apex), 21 (10 lateral, 11 medial to apex) and 20 (8 medial to apical, 12 lateral) elongate inflexible simple setae on margins of lateral epipods of pleopods III, IV and V respectively. Pleopod I exopod broadest proximally, medial margin straight to slightly convex — divergent from lateral margin proximally, dorsal surface with setae; protopod length subequal to that of other pleopods, width subequal length or longer than wide. Pleopod II endopod appendix masculina basal musculature

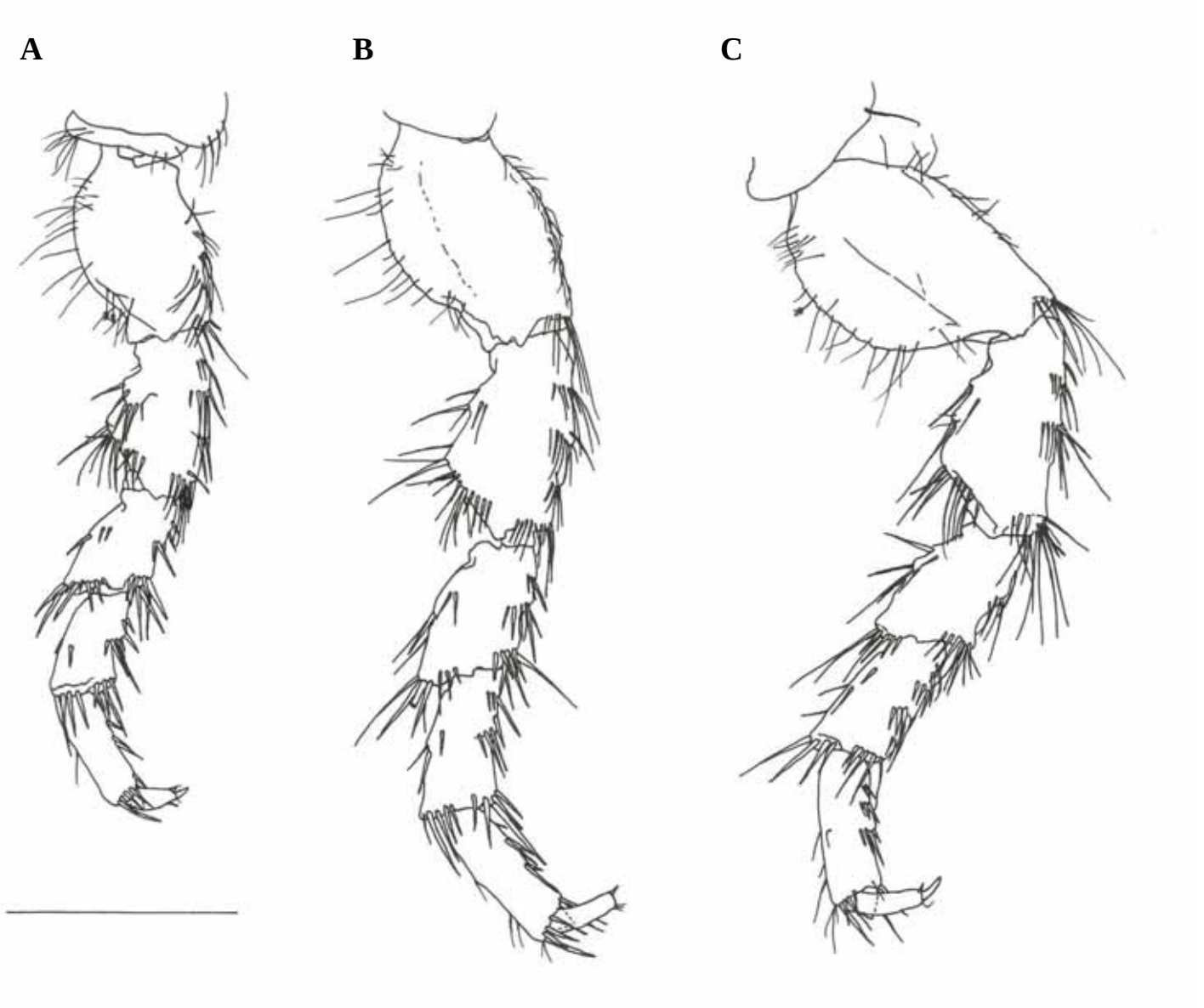


Figure 4.21: *Mesamphisopus kensleyi* n. sp., dissected male (SAM A44940). A, pereopod V; B, pereopod VI; C, pereopod VII. Scale line represents 1 mm.

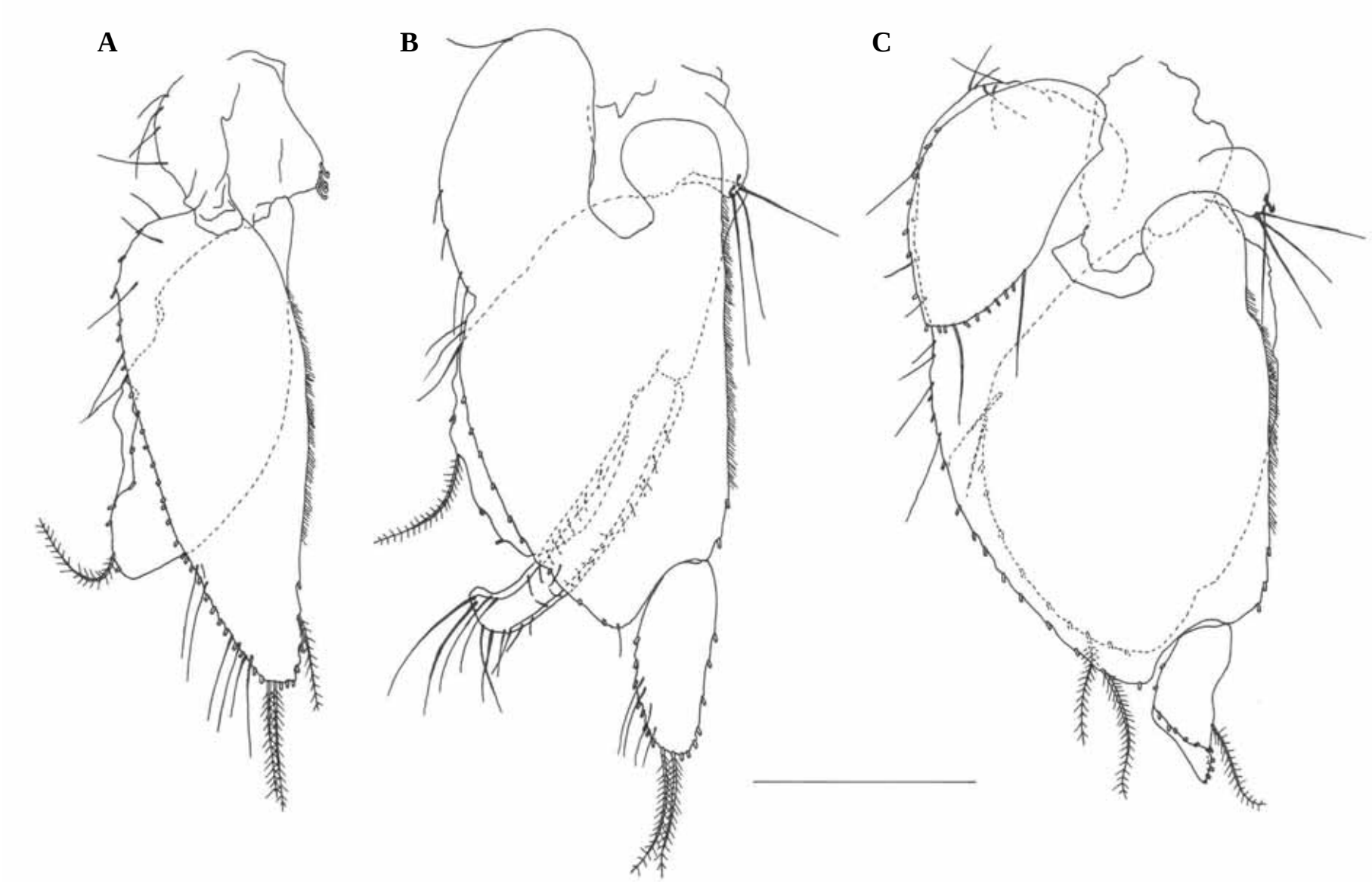


Figure 4.22: *Mesamphisopus kensleyi* n. sp., dissected male (SAM A44940). A, pleopod I; B, pleopod II; C, pleopod III. Scale line represents 0.5 mm.

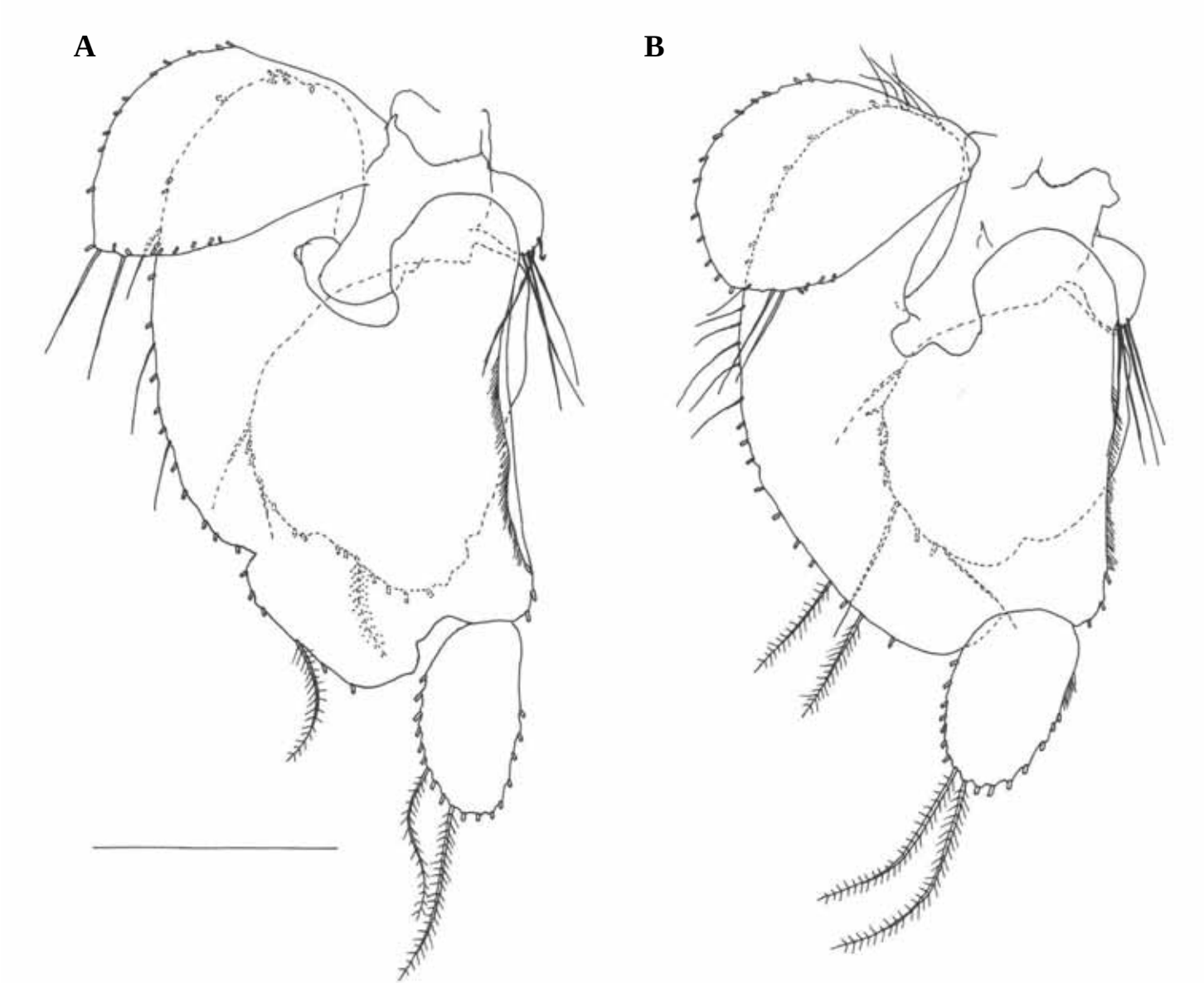


Figure 4.23: *Mesamphisopus kensleyi* n. sp., dissected male (SAM A44940). A, pleopod IV; B, pleopod V. Scale line 0.5 mm.

not pronounced; with 32 setae on margin, 13 laterally, 19 medially; length 0.43 pleopod length; distal tip extending beyond distal margin of endopod.

Uropod (Fig. 4.19B) total length 1.79 pleotelson length. Protopod length:width 3.22; length 0.43 uropod total length; extending posteriorly subequal to pleotelson apex; dorsomedial ridge produced, plate-like, margin smooth, in lateral view approximately straight, ridge length:endopod length approximately 0.56; ventral ridge without rows of long laterally projecting setae. Rami cross-sectional shape flattened on dorsal surface only. Endopod dorsal margin robust setae along length, with 5 robust setae, 2 lateral, 3 medial, excluding apical seta. Exopod length 0.82 endopod length; dorsal margin with 3 robust setae, excluding apical seta.

Sexual dimorphism, female differences from male. *Pereon.* Pereonite 1 dorsal margin in lateral view shorter than on pereonite 2; length:width in dorsal view 0.31. Pereonite 2 length:width in dorsal view 0.40 – 0.43. Pereonite 3 length:width 0.42 – 0.44. Pereonite 4 length:width 0.38 – 0.40. Pereonite 5 length:width 0.34 – 0.37. Pereonite 6 length:width 0.39. Pereonite 7 length:width 0.21.

Antennula length 0.14 body length, with 7 articles. Article 6 (subterminal article) length:width 1.64. Four fine aesthetascs on terminal article or terminal article periphery, 2 along subterminal article distal margins; 5 simple setae on terminal article and subterminal article distal margins.

Antenna length approximately 0.47 body length. Flagellum length approximately 0.61 total antenna length, with 19 articles.

Pereopod I length:body length 0.38. Dactylus projecting beyond palm, length:palm length 1.24; ventrodistal margin with row of thin scale-like spines, along 0.26 total length; claw length:dactylus length 0.17 – 0.21. Propodus length:pereopod length 0.21; length:width 1.29. Propodal palm concave; cuticular fringe weakly developed; serrate and bifid stout denticulate setae present, 7 altogether; stout robust simple setae absent; 3 elongate broad based setae present. Ischium dorsal margin with 7 simple setae, none robust. Basis length:width 1.78; dorsal setae positioned proximally, 4 – 5 altogether; ventrodistal margin with 3 – 4 elongate setae.

Pereopods II – III. Pereopod II length:body length 0.36. Dactylus length:propodus length 0.85; primary claw length:dactylar length 0.39 – 0.42. Propodus length:pereopod length 0.13; length:width 1.86. Carpus length:pereopod length 0.12; length:width 1.25. Basis length:pereopod length 0.26; length:width 1.71. Pereopod III length:body length 0.35. Dactylus length:propodus length 0.74; primary claw length:dactylar length 0.36. Propodus length:pereopod length 0.13; length:width 1.81 – 1.90. Carpus length:pereopod length 0.11; length:width 1.25. Basis length:pereopod length 0.30; length:width 2.05. Propodus broad based setae present, respectively 3, 3 on pereopods II and III; on pereopod II proximal two setae 0.31 propodus length, most distal seta 0.23 propodus length, proximal two setae closely set, distal seta placed at midpoint of margin; on pereopod III proximal seta 0.16 propodus length, median seta 0.26 propodus length, distal seta 0.13 propodus length, evenly spaced from midpoint of margin to distal margin. Carpus broad based setae present, respectively 6, 5 on

pereopods II and III; on pereopod II proximal seta 0.20 carpus length, second seta 0.29 carpus length, increasing in length from third seta (0.26 carpus length) to distal seta (0.46 carpus length), evenly spaced along length of margin; on pereopod III increasing in length from proximal seta (0.30 carpus length) to fourth seta (0.87 carpus length), distal seta 0.77 carpus length, evenly spaced along margin.

Pereopod IV simple, approaching prehensility. Length:body length 0.32. Penicillate setae absent. Dactylus distal accessory claw approximately 0.33 length of primary claw. Propodus length:pereopod length 0.12; length:width 1.60. Propodus with 3 broad based setae on ventral margin. Carpus length:pereopod length 0.12; with 5 broad based setae on ventral margin, 4 broad based setae distolaterally. Ischium posterodistal margin with 8 setae. Basis length:width 1.89; dorsal ridge with approximately 12 setae.

Pereopods V – VII. Pereopod V length:body length 0.30. Dactylus claw length:dactylar length 0.31. Propodus length:pereopod length 0.15. Carpus length:pereopod length 0.14. Basis length:width 1.34. Pereopod VI length:body length approximately 0.39. Propodus length:pereopod length approximately 0.15. Carpus length:pereopod length approximately 0.14. Basis length:width 1.50. Pereopod VII length:body length 0.41. Dactylus claw length:dactylar length 0.35. Propodus length:pereopod length 0.15. Carpus length:pereopod length 0.14. Basis length:width 1.35.

Pleopods. Pleopod I length:body length 0.14. Exopod length:width 2.64. Endopod length:width 3.04; endopod length:exopod length 1.15. Pleopod II length:body length 0.16. Exopod length:width 2.13; length of distal article:exopod length 0.31. Endopod length:width 2.00; endopod length:exopod length 0.80. Pleopod III length:body length 0.17. Exopod length:width 1.64; length of distal article:exopod length 0.32. Endopod length:width 1.22; endopod length:exopod length 0.66. Pleopod IV length:body length 0.17. Exopod length:width 1.46; length of distal article:exopod length 0.35. Endopod length:width 1.39; endopod length:exopod length 0.81. Pleopod V length:body length 0.13. Exopod length:width 1.14; length of distal article:exopod length 0.32. Endopod length:width 1.13; endopod length:exopod length 0.68. Protopods medial margins/epipods I – IV with coupling hooks, respective counts 2, 1, 1, 1; with 2, 3, 3, 3 and 5 elongate inflexible simple setae on pleopods I, II, III, IV and V respectively; lateral epipod III length 2.50 width, lateral epipod V length 1.56 width. Protopods with 19 (7 lateral, 12 medial to apical), 22 (14 medial, 8 lateral and apical) and 15 elongate inflexible simple setae on margins of lateral epipods of pleopods III, IV and V respectively.

Uropod total length 1.55 pleotelson length. Protopod length:width 2.83, length 0.39 uropod total length; dorsomedial ridge length:endopod length 0.49. Endopod with 8 robust setae. Exopod length 0.77 endopod length; with 4 robust setae.

General Distribution. Known only from the type locality.

Remarks. In terms of the setation of the head, pereon and pleotelson, ***M. kensleyi*** n. sp. is most similar to ***M. baccatus*** n. sp., and perhaps ***M. depressus*** and ***M. abbreviatus***, while being more setose

than *M. capensis*, *M. paludosus* n. sp. and *M. penicillatus*. While the degree of setation of the pereopods is similar between this species and *M. setosus* n. sp., the setae of the latter species appear to be characteristically more robust, while the body, particularly the head, is less setose. Although the setation of the endopods of pleopods I – V is again more typical of *Mesamphisopus*, the endopods of pleopods III – V (of both the males and females) of this species are unique in being weakly cleft (having a dorsomedial invagination). The endopod of pleopod V in *M. setosus* n. sp. is similarly cleft, albeit more slightly, while those of the remaining pleopods have the margin entire. The absence of a fringe of small cuticular spines on the ventrodistal margin of the dactylus of pereopod I of the examined male individuals is noteworthy, as this cuticular fringe is regarded as common to all species within the genus (Nicholls, 1943).

***Mesamphisopus paludosus* n. sp.**

Figures 4.24 – 4.32

Type locality. Temporary wetland along Elim – Struisbaai road, opposite "Crane's Nest" guest farm, Agulhas Plain, South Africa (34°38'27"S 19°52'05"E).

Material examined. Syntypes: SAM A45157, one dissected adult male (bl 11.6 mm) and one dissected brooding female (bl 12.0 mm) parts slide mounted and in microvials, additional six males, six females, temporary wetland along Elim – Struisbaai road, opposite "Crane's Nest" guest farm, Agulhas Plain, South Africa (34°38'27"S 19°52'05"E), collected on 05/XII/2001 by S. R. Daniels and G. Gouws. SAM A45158, one male, two females, collection locality as for syntypes, collected on 24/XI/2001 by G. Gouws and H. Endemann.

Other material. SAM A45159, temporary wetland to the east of vlei, in kraal of "Ratel's River" farm, Agulhas Plain, South Africa (34°44'30"S 19°40'48"E) collected on 05/XII/2001 by S. R. Daniels and G. Gouws. University of Cape Town, Freshwater Research Unit WCW B13, Rattelrivier, on Agulhas Plain, South Africa (34°44'28"S 19°40'42"E) (further collection details unavailable).

Etymology. The species epitheton is the Latin adjective "paludosus" meaning "marshy" or "swampy", in reference to the temporary wetlands of the Agulhas Plain in which this species is found.

Diagnosis. Mandibular groove with acute indentation. Pereonites with fine short setae, setae 0.05 – 0.06 body depth. Pleonites 1 – 4 individual depths:pereonite 7 depth 1.35 – 2.15. Pleotelson dorsal surface in lateral view evenly curving, sparsely covered with fine setae; lateral length subequal to depth, 0.95 – 1.00 depth; depth 1.65 – 1.70 pereonite 7 depth; ventral margin anterior to uropods with

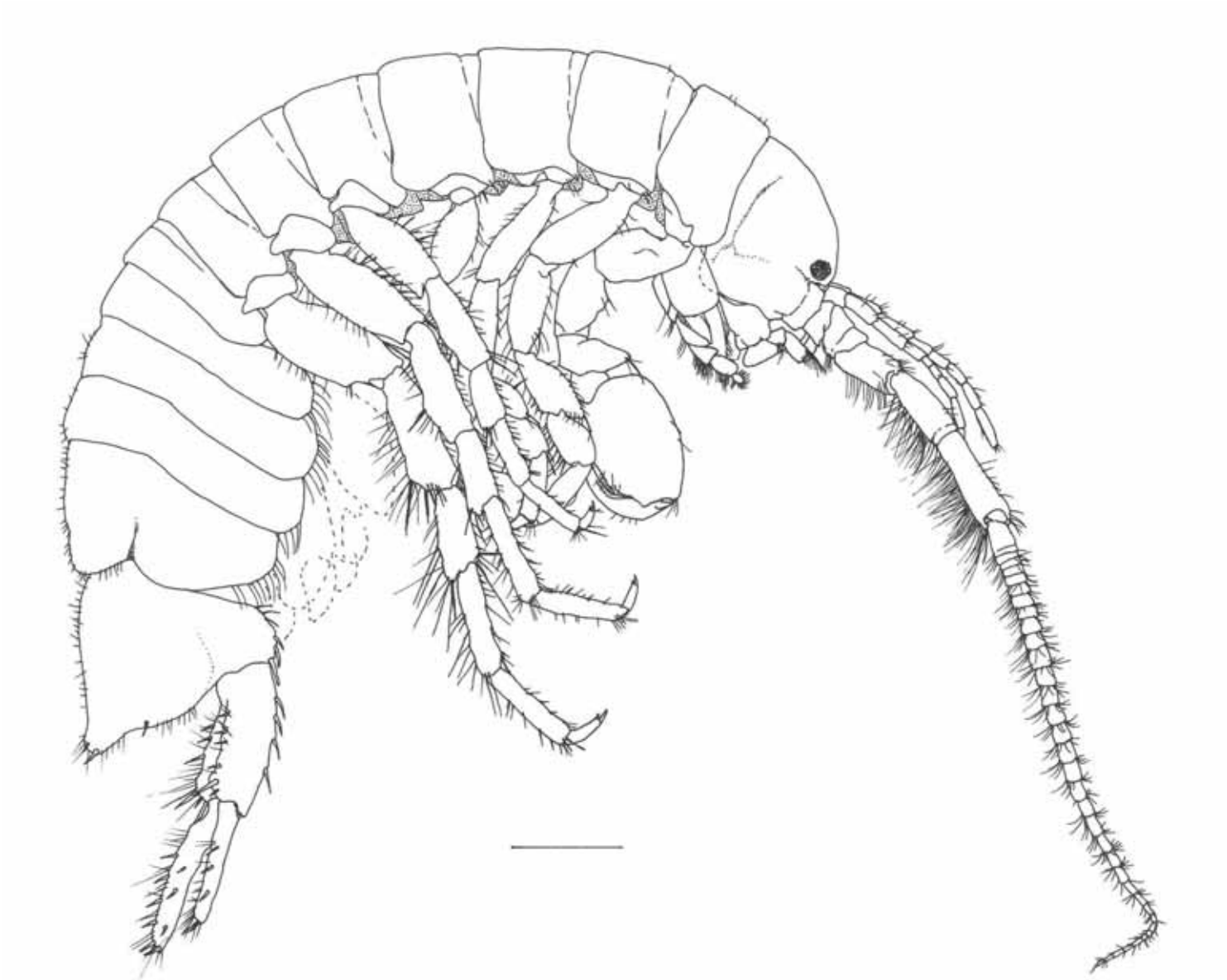


Figure 4.24: *Mesamphisopus paludosus* n. sp., dissected male syntype (SAM A45157), lateral view. Scale line represents 1 mm.

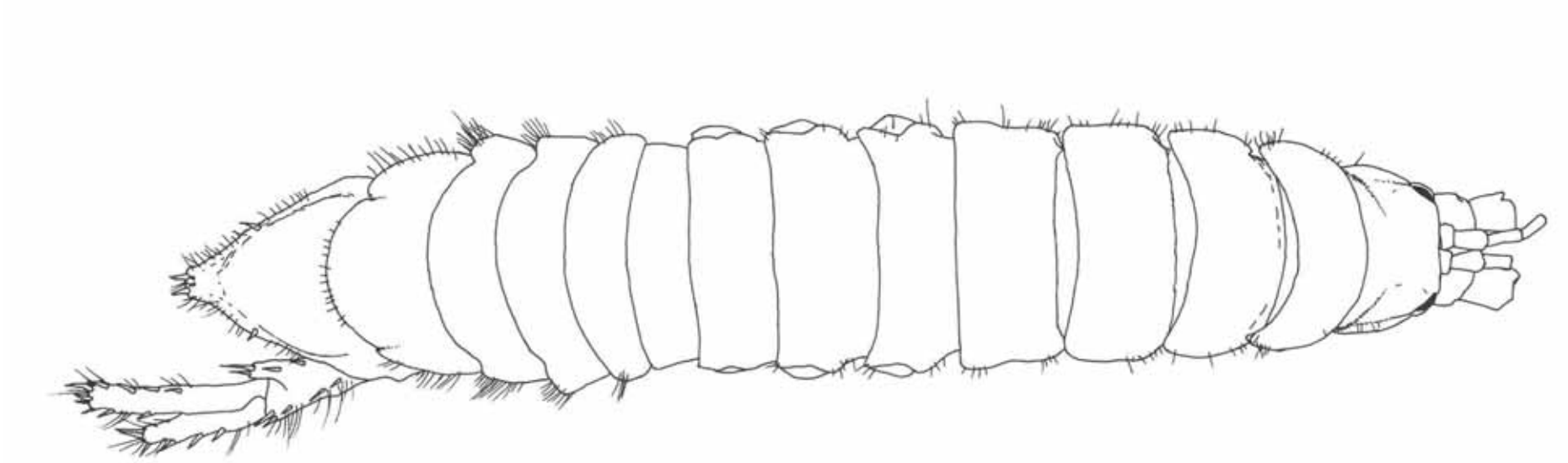


Figure 4.25: *Mesamphisopus paludosus* n. sp., dissected male syntype (SAM A45157), dorsal view. Only one uropod is figured. Antennules and antennae are incompletely illustrated.

single row of simple robust setae grading anteriorly to fine setae; lateral uropodal ridge absent; posterior apex not reflexed, with two pairs of robust setae and subapical pair of robust setae dorsally. Antennula long, length 0.23 body length, with 10 long slender articles; penultimate article subequal to length of other articles; distal articles in cross-section circular. Antenna long, length 0.78 body length; article 5 longer than article 4; article 6 length subequal to articles 4 and 5 combined. Mandibular palp article 3 with > 32 setae. Maxillula medial lobe width 0.61 lateral lobe width; lateral lobe distal margin with 3 smooth robust setae. Maxilliped palp article 5 length:width > 1.80. Pereopod I dactylus ventrodistal margin with row of thin scale-like spines, along 0.08 total length; propodal palm stout denticulate setae serrate; basis dorsal margin setae positioned along ridge, > 10 altogether. Pereopod II propodus length:width > 2.50, with 8 broad based setae; carpus with 10 – 11 broad based setae; basis length:width approximately 2.40. Pereopod III propodus length:width approximately 2.50, with 6 broad based setae; carpus length:width approximately 2.50, with 18 broad based setae. Pereopod IV dactylus length subequal to palm; propodus length:width > 1.80; carpus ventral margin with 7 broad based setae; basis length:width > 2.60. Pereopods V – VII basis dorsal ridge in cross-section angular on V – VI, produced and forming distinct plate on VII, lateral face ventral ridge absent. Pereopod VI basis length:width > 2.10; pereopod VII basis length:width > 1.70. Pleopodal endopods setae simple and plumose on I, plumose on II. Pleopod I protopod longer than wide. Pleopod II appendix masculina short, distal tip not reaching distal margin of endopod. Uropod total length 1.80 pleotelson length; endopod dorsal margin with 10 robust setae, along length; exopod with 4 robust setae.

Description based on male. *Coloration.* Body strongly pigmented, dark-brown to black-brown dorsally and laterally; off-white (living specimens) to yellowish-white (preserved specimens) where not pigmented; more heavily pigmented patches or spots formed laterally on pleonites, pigmentation forms mottlings on pereopods and dendritic patterns laterally on cephalon and on pereopods.

Head width 0.79 – 0.89 pereonite 1 width; setae sparse, fine. Eyes projecting anteriorly; maximum diameter 0.15 – 0.18 head depth; approximately round. Cervical groove extending nearly to dorsal margin of head. Mandibular (genal or cheek) groove with acute indentation. Antennal notch posterior extension reaches just below anterior portion of eye. Maxillipeds insertion from posterior margin of head approximately 0.10 head length.

Pereon width exceeding head width; setae on dorsal surface sparse, length of setae 0.05 – 0.06 body depth. Pereonite 1 dorsal margin in lateral view shorter than on pereonite 2; length:width in dorsal view 0.30 – 0.39. Pereonite 2 length:width in dorsal view 0.39 – 0.41. Pereonite 3 length:width 0.43 – 0.50. Pereonite 4 length:width 0.42 – 0.50. Pereonite 5 length:width 0.47. Pereonite 6 length:width 0.43. Pereonite 7 length:width 0.36.

Pleonites in dorsal view 2 – 4 respective lengths more than half the length of pleonite 5, 1 – 4 relative lengths unequal, pleonite 4 length greater than or subequal to pleonites 1 – 3; pleonites 1 – 4 width 0.95 – 0.98 composite length in dorsal view. Pleonites 1 – 5 dorsal length:maximum width of

pleonites 1 – 5 respectively 0.25, 0.25, 0.25, 0.28 and 0.41. Pleonites 1 – 5 depth:pereonite 7 depth respectively 1.37, 1.99, 2.13, 2.16 and 1.91.

Pleotelson dorsal surface in lateral view evenly curving, sparsely covered with fine setae, length 1.04 – 1.08 width; median ridge absent; lateral length 0.14 – 0.15 body length, subequal (0.94 – 0.98) to depth; depth 1.64 – 1.69 pereonite 7 depth; ventral margin anterior to uropods with single row of 3 – 5 simple robust setae grading anteriorly to fine setae; lateral uropodal ridge absent. Posterior apex not reflexed, with one pair of robust setae; additional pair towards ventrolateral margin.

Antennula (Fig. 4.26A) length 0.23 body length, with 9 – 10 articles. Article 5 divisible into one large or two small articles. Four aesthetascs on terminal article; 3 aesthetascs and one simple seta peripherally on distal margin of subterminal article. Terminal article length:width 0.73 – 0.80; length:antennular length 0.01. Penultimate article length approximately subequal to length of other articles. Distal articles in cross-section circular.

Antenna (Fig. 4.26B) length 0.78 body length. Flagellum length 0.69 total antenna length, with 28 – 36 articles, with abundant fine setae along distal margins. Article 5 longer than article 4; article 6 length shorter-subequal than articles 4 and 5 combined.

Mouthfield. Clypeus not widening laterally; width approximately 0.89 head width. Labrum (Fig. 4.26C) ventrally semicircular in anterior view, with fringe of fine setae along ventral margin; asymmetrical, with invagination along right margin; dorsal margin wider than clypeus. Paragnaths (Fig. 4.26D) with dense mats of fine setae distomedially along lobes, becoming shorter proximally; longer simple setae scattered apically and distolaterally.

Mandible (Figs 4.26E,F,G, 4.27A) palp length 1.07 mandible length; 3rd article medial-distal margins with 32 – 38 finely setulate setae, additional medial surface setae absent; 2nd article longitudinal row of elongate simple setae along ventral margin, separate row of 6 elongate simple setae along distolateral surface near dorsal margin; article 1 with elongate simple setae at distoventral margin; articles 1 – 2 setae more than half respective article lengths. Left spine row with 13 spines, 3 of which bifurcate. Right spine row with 11 spines, 2 of which bifurcate. Molar process wider than long; spines absent.

Maxillula (Figs 4.27B,C) medial lobe length 0.49 lateral lobe length; width 0.61 lateral lobe width; with 2 ‘accessory’ setae, one on distolateral margin and one at base of medial of two central pappose setae, one ‘accessory’ seta distally denticulate, one simple; short weakly setulate seta on distal tip absent. Lateral lobe distal margin with 9 denticulate robust setae, 3 smooth robust setae, distal setal row with 4 robust setae; ventral face with 2 plumose setae, setae widely spaced; additional plumose setae absent.

Maxilla (Fig. 4.27D) medial lobe width 0.71 outer lateral lobe width; proximal portion smoothly continuous with distal portion; proximal and distal setal rows separated by gap; 8 fairly robust serrate/pectinate setae in single ventral basal row; 36 closely-set elongate, weakly plumose setae in dorsal basal row; approximately 18 simple and plumose setae and 3 pectinate setae in multiple distal

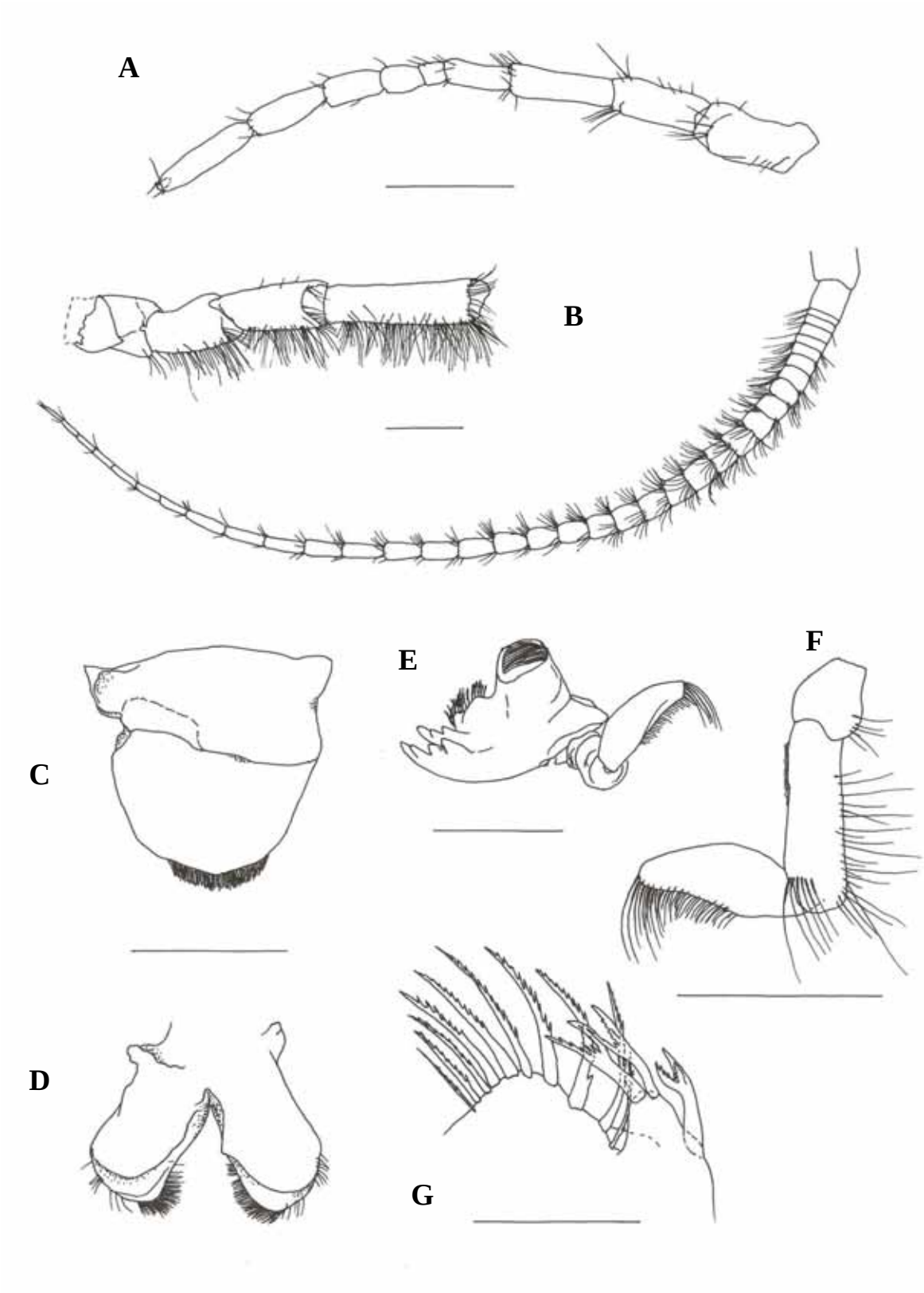


Figure 4.26: *Mesamphisopus paludosus* n. sp., dissected male syntype (SAM A45157). A, antennula; B, antennal peduncle and flagellum; C, labrum; D, paragnaths; E, left mandible; F, left mandibular palp; G, left mandible spine row. Scale lines represent 0.5 mm, except for G, where the scale line represents 0.1 mm.

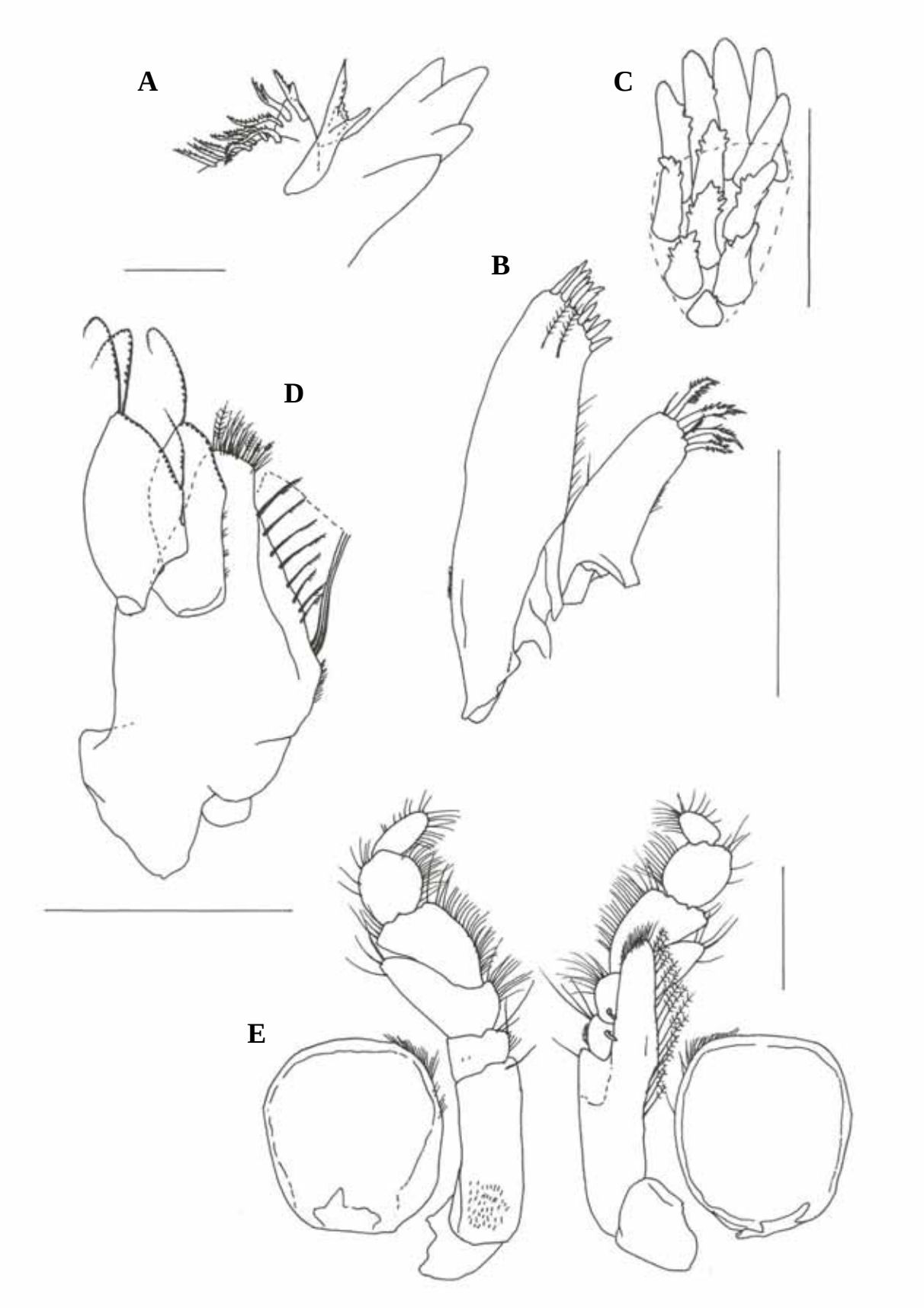


Figure 4.27: *Mesamphisopus paludosus* n. sp., dissected male syntype (SAM A45157). A, right mandible spine row and incisor process; B, maxillula; C, maxillula lateral lobe distal margin; D, maxilla; E, right maxilliped, ventral (left) and dorsal view (right). Scale lines 0.5 mm, except for A and C, where they represent 0.1 mm.

rows. Outer lateral lobe length subequal to inner lateral lobe, wider than inner lateral lobe; distal margin with 18 long bidenticulate setae. Inner lateral lobe with 13 long bidenticulate setae. Lateral lobes with bidenticulate setae only on distal tips.

Maxilliped (Fig. 4.27E) epipod length:width 1.14; distal tip truncate; distal margin setae fine and in fringe. Endite length:total basis length 0.41; medial margin with 2 coupling hooks on left side, 2 on right side; dorsal ridge with 15 large distally denticulate plumose setae. Palp insertion on basis lateral margin without plumose setae; medial margin with 1 simple seta; ventral surface without subdistal smooth setae; length:basis length 1.02; width across articles 2 – 3:endite width 1.33 – 1.40; article 4 subcircular, length:width 0.96; article 5 length:width 1.85, article 5 length:article 4 length 0.92.

Pereopod I (Figs 4.28A,B) length:body length 0.44. Dactylus length subequal to palm or slightly longer, length:palm length 1.36; ventrodistal margin with row of thin scale-like spines, along 0.08 total length; claw length:dactylus length 0.10; distal accessory claw ventral to primary claw, 0.27 primary claw length. Propodus length:pereopod length 0.26; length:width 1.23; dorsal margin setae distributed singularly between proximal and distal margin, forms group distally, 10 – 11 setae altogether, including 5 in distal group. Propodal palm cuticular fringe well developed, continuous along proximal third of palm, intermittent towards distal portion; stout denticulate setae serrate, 4 altogether; 3 basally inflated stout robust simple setae altogether; 4 elongate broad based setae present. Ischium dorsal margin with 3 – 5 simple setae, none robust. Basis length:width 1.93; dorsal setae positioned along ridge or just lateral to ridge, 12 altogether; ventrodistal margin with 3 elongate setae.

Pereopods II – III (Figs 4.28C,D). Pereopod II length:body length 0.42. Dactylus length:propodus length 0.60; primary claw length:dactylar length 0.23. Propodus length:pereopod length 0.16; length:width 2.58. Carpus length:pereopod length 0.14; length:width 1.71. Basis length:pereopod length 0.25; length:width 2.45. Pereopod III length:body length 0.39. Dactylus length:propodus length 0.64; primary claw length:dactylar length 0.32. Propodus length:pereopod length 0.13; length:width 2.46. Carpus length:pereopod length 0.13; length:width 1.65. Basis length:pereopod length 0.26; length:width 2.48. Pereopods II – III penicillate setae present, scattered along dorsal margin of basis. Dactylus distal accessory claw ventral to primary claw, 0.35 – 0.50 primary claw length. Propodus broad based setae present, respectively 8, 6 on pereopods II and III; on pereopod II all of approximate equal length (0.14 – 0.18 propodus length), third seta shortest (0.10 propodus length), evenly spaced along margin; on pereopod III all of approximate equal length (0.11 – 0.17 propodus length), distal three setae largest, evenly spaced along margin. Carpus broad based setae present, respectively 10 – 11, 18 on pereopods II and III; on pereopod II long broad based setae (0.19 – 0.34 carpus length) interspersed with short robust broad based setae (0.09 – 0.13 carpus length), evenly spaced along margin, shorter setae lateral to margin; on pereopod III long broad based setae (0.10 – 0.16 carpus length) interspersed with shorter robust broad based setae, evenly spaced along margin, with series of 4 broad based robust setae (0.10 – 0.31 carpus length) near distolateral margin. Basis dorsal ridge in cross-section rounded to angular and produced without forming distinct plate,

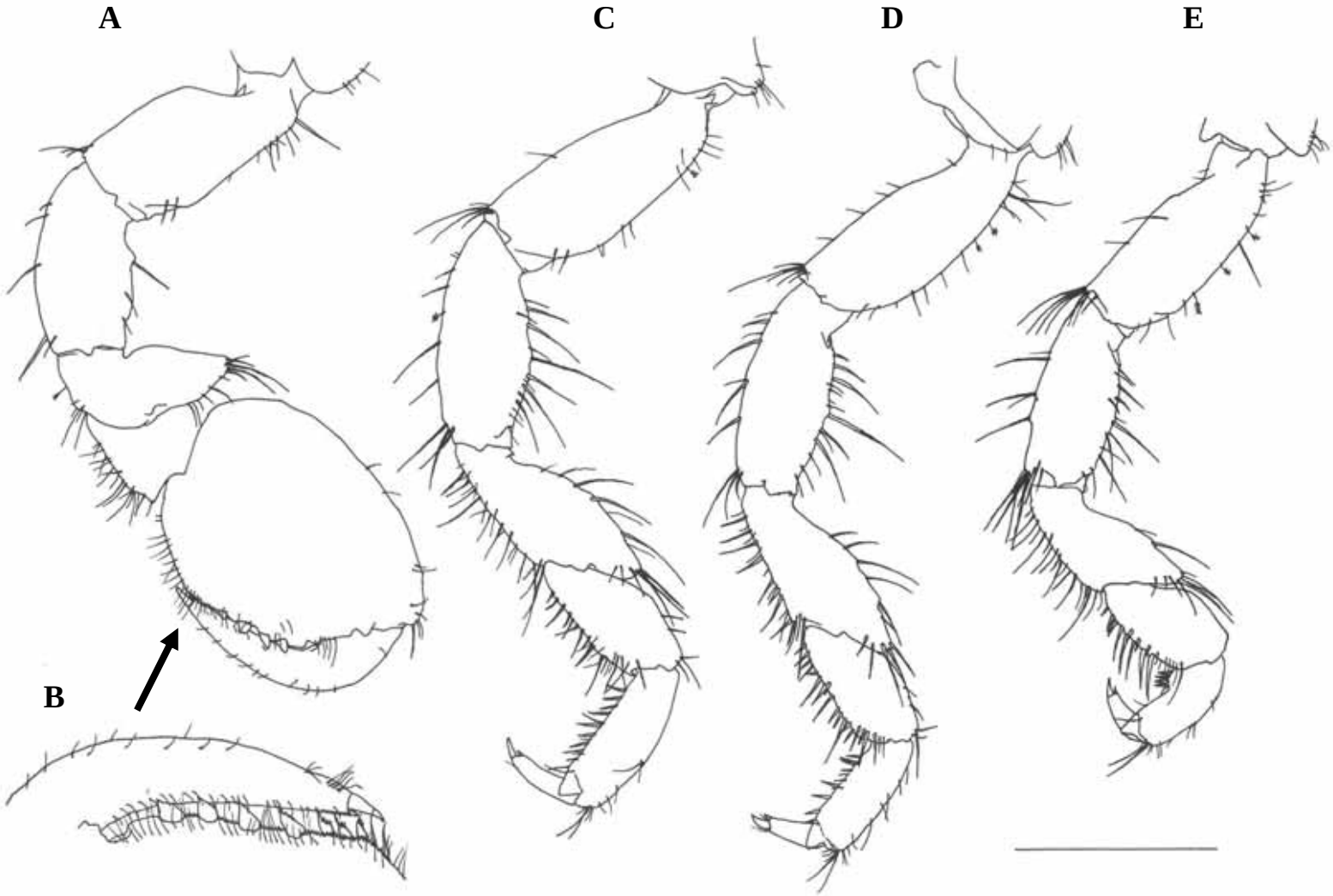


Figure 4.28: *Mesamphisopus paludosus* n. sp., dissected male syntype (SAM A45157). A, pereopod I; B, pereopod I propodal palm; C, pereopod II; D, pereopod III; E, pereopod IV. Scale line represents 1 mm.

with 10 – 13 elongate simple setae along dorsal ridge, 1 – 2 more robust than others. Pereopods II – IV ischium dorsal margin with 11 – 12 simple setae, including 2 robust setae.

Pereopod IV (Fig. 4.28E) length:body length 0.35. Penicillate setae present on dorsal margin of basis. Dactylus length subequal to propodal palm; distal accessory claw approximately 0.25 length of primary claw. Propodus length:pereopod length 0.12, length:width 1.89; distal width:palm width 0.82; with 4 broad based setae on ventral margin, none distinctly larger than others; articular plate subequal in length to dactylar claw. Carpus length:pereopod length 0.14; with 7 broad based setae on ventral margin, 5 distinctly larger than others. Ischium posterodistal margin with 5 – 6 setae. Basis length:width 2.63; dorsal ridge in cross-section rounded or angular and produced but not forming distinct plate, with 9 setae.

Pereopods V – VII (Fig. 4.29). Pereopod V length:body length 0.33. Dactylus claw length:dactylar length 0.29. Propodus length:pereopod length 0.16. Carpus length:pereopod length 0.16. Basis length:width 1.96. Pereopod VI length:body length 0.44. Dactylus claw length:dactylar length 0.26. Propodus length:pereopod length 0.15. Carpus length:pereopod length 0.17. Basis length:width 2.16. Pereopod VII length:body length 0.45. Dactylus claw length:dactylar length 0.28. Propodus length:pereopod length 0.16. Carpus length:pereopod length 0.18. Basis length:width 1.77. Pereopods V – VII penicillate setae on dorsal ridge of basis. Dactylus distal accessory claw ventral to and separated from primary claw, approximately 0.33 length of primary claw. Propodus distal margins with 3 – 5 elongate robust setae. Pereopods V – VII ischium dorsal margin with 5 – 19 simple setae, including 1 – 3 robust setae. Basis dorsal ridge not distinctly separated from basis shaft, in cross-section angular on V – VI, produced and forming distinct plate on VII, with elongate fine setae positioned along entire margin; lateral face central ridge or groove present; lateral face ventral ridge absent. Pereopod VII ischium dorsal ridge flange absent.

Penes length 0.44 body width at pereonite 7; with setae on shaft; distal tip rounded to truncate.

Pleopods (Figs 4.30, 4.31). Pleopod I length:body length 0.19 – 0.21. Exopod length:width 2.90 – 2.91. Endopod length:width 2.51 – 2.94; endopod length:exopod length 0.92 – 0.98. Pleopod II length:body length 0.19 – 0.24. Exopod length:width 2.18 – 2.25; length of distal article:exopod length 0.28 – 0.29. Endopod length:width 2.53 – 2.61; endopod length:exopod length 0.86. Pleopod III length:body length 0.2 – 0.24. Exopod length:width 1.68 – 1.91; length of distal article:exopod length 0.28 – 0.30. Endopod length:width 2.13 – 2.18; endopod length:exopod length 0.83 – 0.99. Pleopod IV length:body length 0.18 – 0.22. Exopod length:width 1.58 – 1.86; length of distal article:exopod length 0.33 – 0.34. Endopod length:width 1.87 – 1.93; endopod length:exopod length 0.79 – 0.87. Pleopod V length:body length 0.18. Exopod length:width 1.15 – 1.29; length of distal article:exopod length 0.34 – 0.35. Endopod length:width 1.51 – 1.70; endopod length:exopod length 0.72 – 0.76. Endopods I – II with setae on margins, plumose and simple on I, singular plumose seta on II. Protopods medial margins/epipods I – IV with coupling hooks, respective counts 6, 3, 3, 2; with 4, 7, 8 and 9 elongate inflexible simple setae on II, III, IV and V respectively; lateral epipod III length

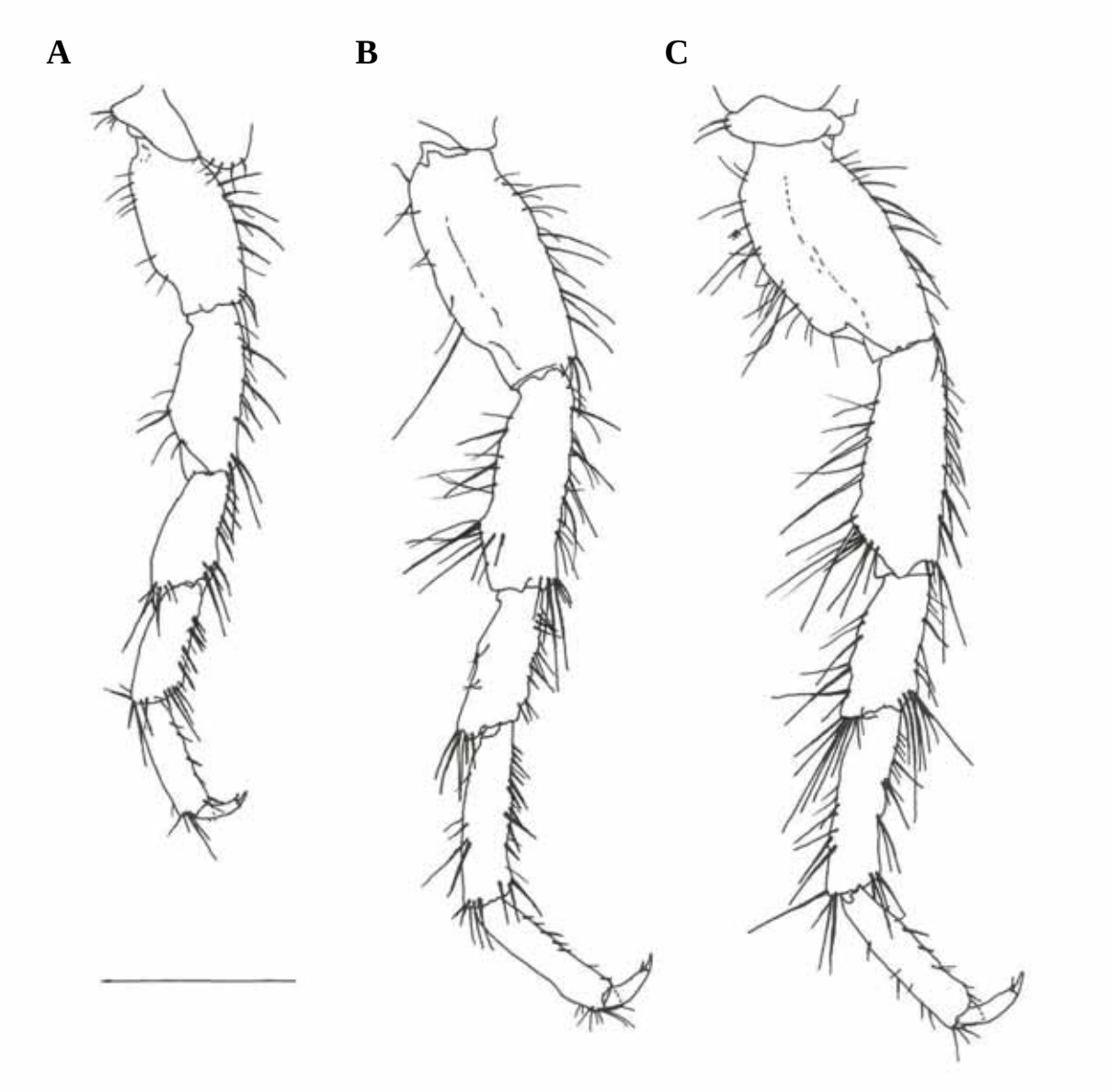


Figure 4.29: *Mesamphisopus paludosus* n. sp., dissected male syntype (SAM A45157). A, pereopod V; B, pereopod VI; C, pereopod VII. Scale line 1 mm.

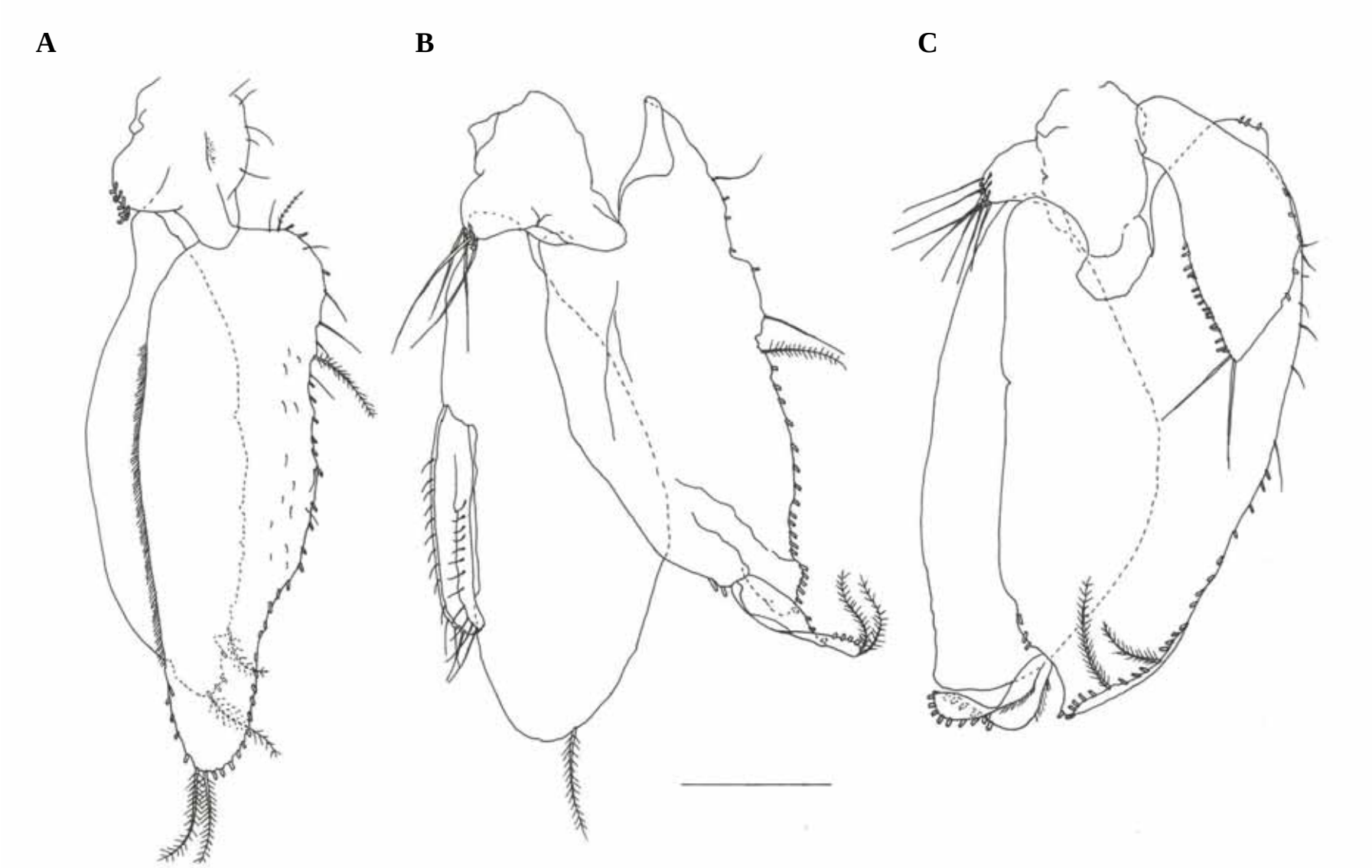


Figure 4.30: *Mesamphisopus paludosus* n. sp., dissected male syntype (SAM A45157). A, pleopod I; B, pleopod II; C, pleopod III. Scale line represents 0.5 mm.

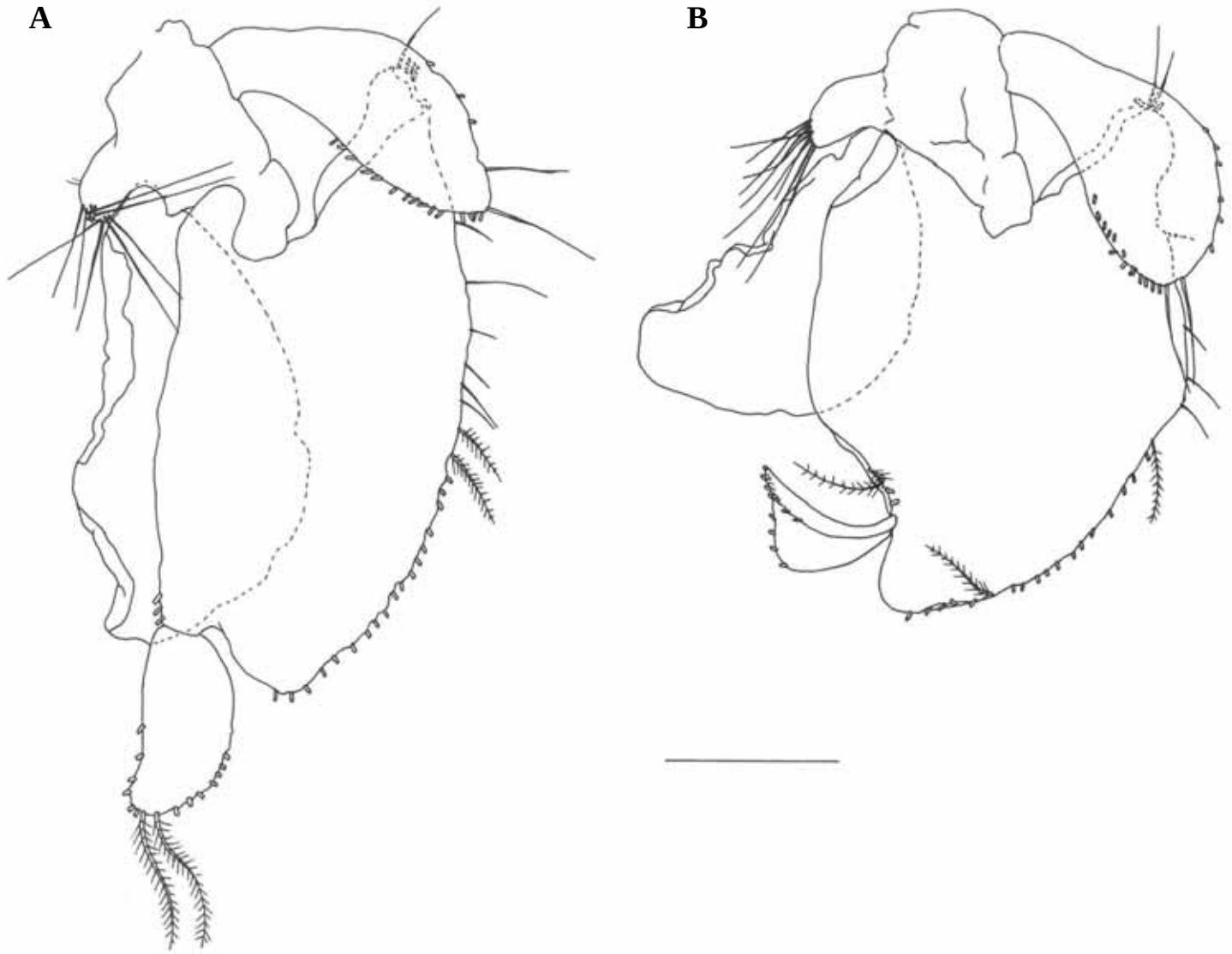


Figure 4.31: *Mesamphisopus paludosus* n. sp., dissected male syntype (SAM A45157). A, pleopod IV; B, pleopod V. Scale line 0.5 mm.

2.04 – 2.17 width, lateral epipod V length 1.88 – 1.94 width. Protopods with 4 fine elongate setae on lateral margin on pleopod I; 23 (5 lateral, 18 medial to apical), 22 (5 lateral, 17 medial to apical) and 22 (6 lateral, 16 medial to apical) elongate inflexible simple setae on margins of lateral epipods of pleopods III, IV and V respectively. Pleopod I exopod broadest proximally, medial margin straight — divergent from lateral margin proximally, dorsal surface with setae; protopod length subequal to that of other pleopods, longer than wide (1.29 length:width). Pleopod II endopod appendix masculina weakly curved; basal musculature not pronounced; with 25 setae on margin, 13 laterally, 12 medially; length 0.29 – 0.34 pleopod length; distal tip not reaching to distal margin of endopod, less than subequal endopod length.

Uropod (Fig. 4.32) total length 1.82 pleotelson length. Protopod length:width 3.90; length 0.45 uropod total length; extending posteriorly subequal to pleotelson apex; dorsomedial ridge produced, plate-like, margin smooth, in lateral view approximately straight, ridge length:endopod length 0.52; ventral ridge without rows of long laterally projecting setae. Rami cross-sectional shape flattened on dorsal surface only. Endopod dorsal margin robust setae along length, with 10 robust setae, 6 medial, 4 lateral, excluding apical seta. Exopod length 0.80 endopod length; dorsal margin with 4 robust setae, excluding apical seta.

Sexual dimorphism, female differences from male. *Head.* Cervical groove smoothly curved to straight.

Pereon. Pereonite 1 length:width in dorsal view 0.38. Pereonite 2 length:width 0.44 – 0.52. Pereonite 3 length:width 0.48. Pereonite 4 length:width 0.45 – 0.51. Pereonite 5 length:width 0.43. Pereonite 6 length:width 0.37. Pereonite 7 length:width 0.29.

Antennula length 0.21 body length, with 9 articles. Article 5 length:width 1.50. Article 6 length:width 2.00. Terminal article with 4 tiny aesthetascs and 1 simple seta; additional 4 aesthetascs and 1 simple seta on subterminal article distal margin, peripheral to terminal article.

Antenna length 0.76 body length. Flagellum length 0.72 total antenna length, with approximately 35 articles.

Pereopod I length:body length 0.38. Dactylus length:palm length 1.19 – 1.31; ventrodistal margin with row of thin scale-like spines, along 0.17 total length; claw length:dactylus length 0.13; distal accessory claw ventrolateral to primary claw, 0.41 primary claw length. Propodus length:pereopod length 0.21; length:width 1.31. Propodal palm straight; cuticular fringe well developed, shorter than half palm length, but more intermittently distally; serrate and bifid stout denticulate setae present, 10 altogether; stout robust simple seta basally inflated; 3 – 4 elongate broad based setae present. Ischium dorsal margin with 4 simple setae, none robust. Basis length:width 1.93; dorsal setae positioned along ridge, 7 – 8 altogether; ventrodistal margin with 4 elongate setae, 2 shorter than remainder.

Pereopods II – III. Pereopod II length:body length 0.38. Dactylus length:propodus length 0.61; primary claw length:dactylar length 0.23. Propodus length:pereopod length 0.14; length:width 2.36.

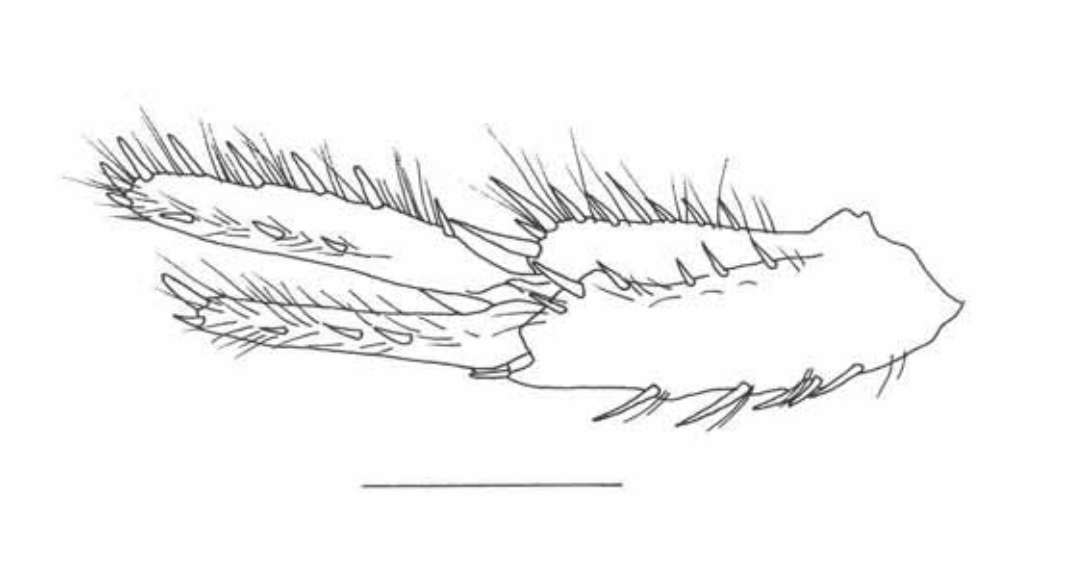


Figure 4.32: *Mesamphisopus paludosus* n. sp., dissected male syntype (SAM A45147). Uropod. Scale line 1 mm.

Carpus length:pereopod length 0.15; length:width 1.84. Basis length:pereopod length 0.27; length:width 2.32. Pereopod III length:body length 0.36. Dactylus length:propodus length 0.60; primary claw length:dactylar length 0.36. Propodus length:pereopod length 0.14; length:width 2.60. Carpus length:pereopod length 0.15; length:width 1.94. Basis length:pereopod length 0.27; length:width 2.26. Propodus broad based setae present, respectively 6, 9 (8 along ventral margin, 1 lateral) on pereopods II and III; on pereopod II proximal two setae 0.20 propodus length, increasing in length to fourth seta (0.24 propodus length), fifth as long as proximal seta, distal seta longest (0.33 propodus length), evenly spaced along margin; on pereopod III increasing in length from proximal (0.09 propodus length) to fourth seta (0.22 propodus length), fifth seta 0.13 propodus length, sixth seta 0.21 propodus length, seventh 0.12 propodus length, distal seta 0.22 propodus length, evenly spaced along margin, seta on lateral surface 0.10 propodus length. Carpus broad based setae present, respectively 6, 8 on pereopods II and III; on pereopod II progressively increasing in length from proximal seta (0.13 carpus length) to distal seta (0.43 carpus length) with fifth setae shorter, as long as second seta (0.20 carpus length), evenly spaced along margin; on pereopod III proximal seta 0.12 carpus length, second 0.15 carpus length, increasing in length from third (0.11 carpus length) to sixth (0.27 carpus length), seventh seta 0.18 carpus length, most distal longest (0.44 carpus length), generally evenly spaced along margin, with second to fourth setae more closely set.

Pereopod IV simple. Length:body length 0.33. Penicillate setae lacking. Dactylus distal accessory claw approximately 0.33 length of primary claw. Propodus length:pereopod length 0.14; length:width 2.43. Propodus with 4 broad based setae on ventral margin. Carpus length:pereopod length 0.13; with 7 broad based setae on ventral margin. Ischium posterodistal margin with 6 – 7 setae. Basis length:width 2.51; dorsal ridge with 9 setae.

Pereopods V – VII. Pereopod V length:body length 0.28. Dactylus claw length:dactylar length 0.31. Propodus length:pereopod length 0.16. Carpus length:pereopod length 0.17. Basis length:width 1.69. Pereopod VI length:body length 0.39. Dactylus claw length:dactylar length 0.26. Propodus length:pereopod length 0.16. Carpus length:pereopod length 0.16. Basis length:width 1.84. Pereopod VII length:body length 0.39. Dactylus claw length:dactylar length 0.27. Propodus length:pereopod length 0.14. Carpus length:pereopod length 0.18. Basis length:width 1.79.

Pleopods. Pleopod I length:body length 0.20. Exopod length:width approximately 2.51. Endopod length:width 2.73; endopod length:exopod length 1.03. Pleopod II length:body length 0.23. Exopod length:width 2.02; length of distal article:exopod length 0.23. Endopod length:width 2.15; endopod length:exopod length 0.85. Pleopod III length:body length 0.23. Exopod length:width 1.62; length of distal article:exopod length 0.25. Endopod length:width 2.00; endopod length:exopod length 0.90. Pleopod IV length:body length 0.21. Exopod length:width 1.41; length of distal article:exopod length 0.30. Endopod length:width 1.83; endopod length:exopod length 0.83. Pleopod V length:body length 0.18. Exopod length:width 1.21; length of distal article:exopod length 0.29. Endopod length:width 1.46; endopod length:exopod length 0.71. Endopod I only with seta on margins, seta plumose.

Protopods medial margin/epipods I – IV with coupling hooks, respective counts 6, 3, 3, 4; with 5, 8, 8 and 7 elongate inflexible simple setae on II, III, IV and V respectively. Protopods with 1 fine elongate seta on lateral margin of pleopod I; lateral epipod margins with 21 (16 medial to apical, 5 lateral), 26 (6 lateral, 20 medial to apical) and 26 (6 lateral, 20 medial to apical) elongate inflexible simple setae on pleopods III, IV and V respectively.

Uropod total length 1.69 pleotelson length. Protopod length:width 3.89, length 0.45 uropod total length; dorsomedial ridge length:endopod length 0.61. Endopod with 11 robust setae. Exopod length 0.79 endopod length; with 6 robust setae.

General Distribution. Known only from the above localities, but perhaps with wider distribution across the temporary wetlands of the Agulhas Plain.

Remarks. *Mesamphisopus paludosus* n. sp. appears to show its closest resemblance to *M. penicillatus*, sharing with this species the sparse, short, fine setation of the head, pereon and pleotelson, the abundant and diagnostic fine setation of the ventral margin of the antennal peduncles, and the presence of a pair of sub-apical robust setae dorsally on the pleotelson (Barnard, 1940; Kensley, 2001). However, *M. paludosus* n. sp. differs from this species and all others within the genus, by having an elongate, shallow pleotelson, that curves smoothly or extends almost linearly, rather than being sharply ventrally inflected, along the dorsal margin (see Barnard, 1927; Nicholls, 1943; Kensley, 2001). The posterior apex of the pleotelson also not reflexed or upturned. Although the apex in *M. abbreviatus* has also been reported as not being upturned (Nicholls, 1943), the ventral inflection along the dorsal margin of the relatively elongate pleotelson appears to be extreme within this species (Nicholls, 1943: Fig. 10.3s; Kensley, 2001: Fig. 3.8a), giving the pleotelson an abrupt appearance (Nicholls, 1943). While the pleopods of *M. penicillatus* remain unexamined, the setation of the pleopodal endopods of *M. paludosus* n. sp. appear to be unique within *Mesamphisopus*, with setae found only on pleopod I – II (plumose on both) in the male and pleopod I (also plumose) in the female. The occurrence of setae on all five pleopodal endopods is regarded as a key diagnostic feature of the genus. No further evidence, at this stage, suggests that *M. paludosus* n. sp. be excluded from the genus. This does however argue for a re-examination of the characters believed to be of systematic importance within the genus. Further distinct features of *M. paludosus* n. sp. include an antennule reaching one-quarter the body length, with ten articles, and an antenna reaching four-fifths the body length, making them the longest observed within *Mesamphisopus*. The appendix masculina is, relatively, the shortest within *Mesamphisopus*, not extending to the distal margin of the endopod. In other species the appendix maculina is described or figured as reaching to the distal margin of the endopod (*M. abbreviatus*, *M. baccatus* n. sp., *M. depressus*, *M. tsitsikamma* n. sp.) or extending beyond it (*M. albidus* n. sp., *M. capensis*, *M. kensleyi* n. sp., *M. setosus* n. sp.). More broad based setae are also encountered on the carpus and propodus of pereopods II – IV than in other species; these

are however not as stout as those in other species, and are scarcely more stout than other setae on the particular limbs. A completely developed appendix masculina was observed arising from the medial margin of the endopod of the right pleopod I in one dissected male — a developmental abnormality rather than a feature of the species.

Mesamphisopus setosus n. sp.

Figures 4.33 – 4.41

Type locality. Small pool in river, below Guardian Peak, Jonkershoek Nature Reserve, Western Cape, South Africa (34°00'48"S 19°00'04"E).

Material examined. Holotype: SAM A45155, one adult male (bl 10.8 mm), small pool in river, below Guardian Peak, Jonkershoek Nature Reserve, South Africa (34°00'48"S 19°00'04"E), collected on 18/IV/2003 by G. Gouws, S. R. Daniels, A. Pardini and S. Willows-Munro. SAM A45156, one dissected adult male (bl 11.4 mm) and one dissected brooding female (bl 10.5 mm) parts slide mounted and in microvials, additional three males, three females, collection details as for holotype. SAM A44939, one male, one female, collection locality as for holotype, collected on 12/III/2001 by G. Gouws.

Etymology. The species is given the derived Latin epitheton 'setosus', in reference to the relatively abundant robust setation of the pereopods.

Diagnosis. Head setae absent. Cervical groove straight to smoothly curved. Mandibular groove smoothly indented. Pereon setae short and fine, 0.05 – 0.08 body depth. Pleonites 1 – 4 individual depths:pereonite 7 depth 1.35 – 2.25. Pleotelson dorsal surface sparsely covered with fine setae; median ridge present posteriorly; lateral length less than depth; depth 1.75 – 1.95 pereonite 7 depth; ventral margin anterior to uropods with single row of simple robust setae; lateral uropodal ridge terminating at pleotelson margin above uropods; posterior apex with one pair of robust setae. Antennula short, 0.13 body length, with 7 articles; penultimate article distinctly longer than any other article; distal articles in cross-section circular. Antenna long, 0.65 body length; article 5 length subequal to article 4. Mandibular palp article 3 with 27 – 32 setae. Maxillula medial lobe width 0.54 lateral lobe width; medial lobe with 4 'accessory' setae; lateral lobe distal margin with 4 smooth robust setae. Maxilla medial lobe proximal and distal setal rows continuous; ventral basal setal row consisting of a double row of setae. Maxilliped palp article 4 shape elongate-oval. Pereopod I dactylus ventrodistal margin with row of thin scale-like spines, along 0.16 total length; propodus dorsal margin setae confined to single group at distal margin; propodal palm with 9 serrate stout

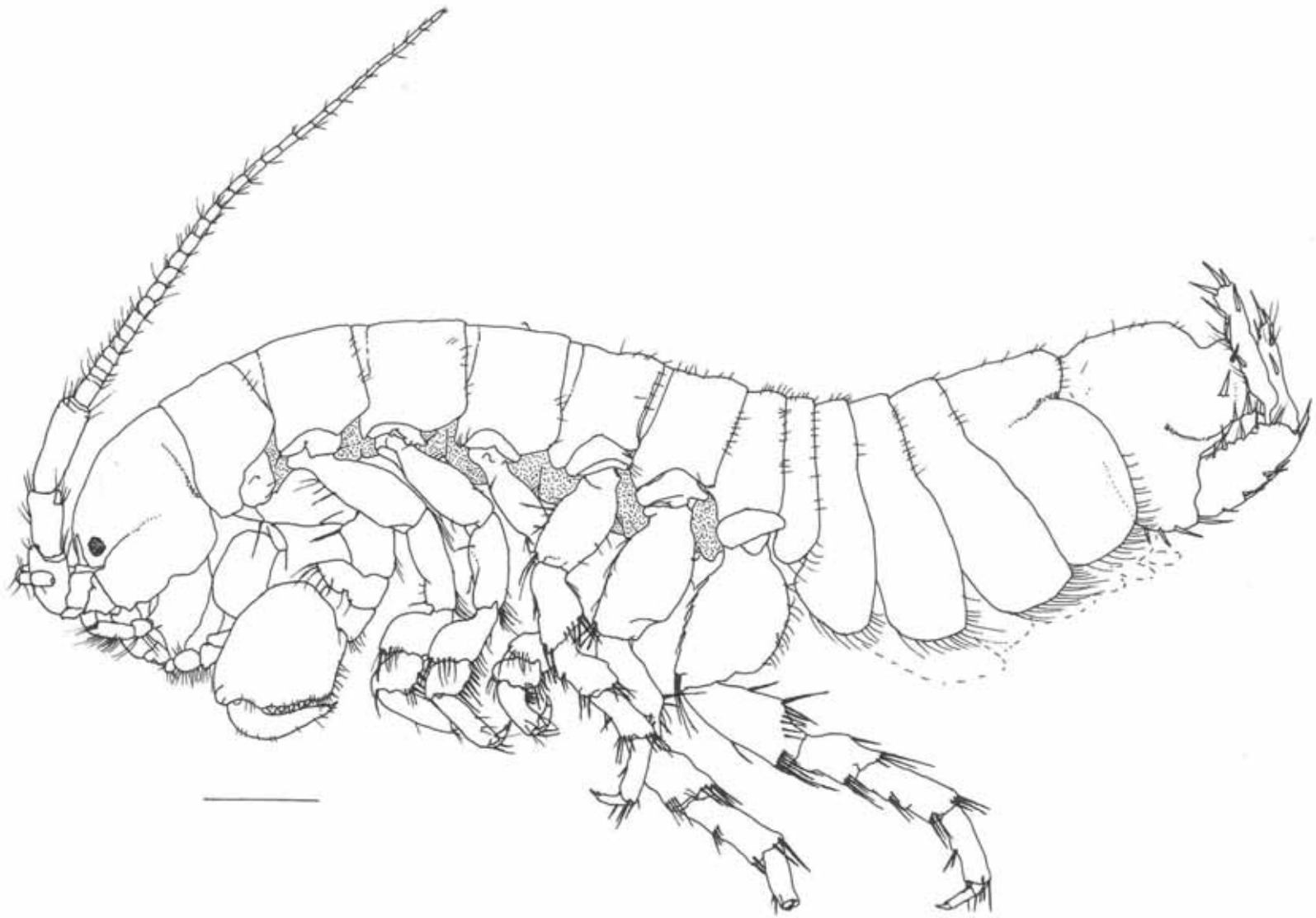


Figure 4.33: *Mesamphisopus setosus* n. sp., male holotype (SAM A45155), lateral view. Scale line 1 mm.

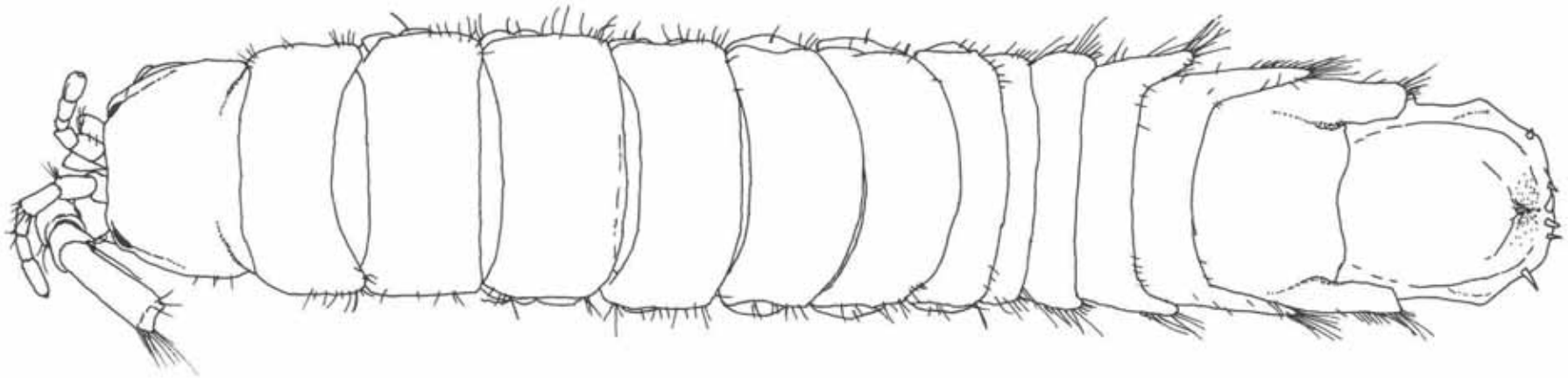


Figure 4.34: *Mesamphisopus setosus* n. sp., male holotype (SAM A45155), dorsal view. Uropods not illustrated, antennae incompletely illustrated.

denticulate setae, 2 basally inflated stout simple setae; ischium dorsal margin with single robust seta; basis dorsal setae positioned proximally. Pereopod II propodus length:width < 2.00. Pereopod III propodus with 7 broad based setae. Pereopod IV dactylus longer than propodal palm; propodus length:width approximately 1.60; basis length:width < 2.20. Pereopods V – VII basis lateral face ventral ridge present. Pereopods VI – VII length:body length > 0.50. Pleopodal endopods setae plumose on I – IV, simple on V; endopod V shallowly cleft dorsomedially. Pleopod II appendix masculina distal tip extending beyond distal margin of endopod. Uropod total length 2.15 pleotelson length; protopod extending posterior to pleotelson apex; endopod dorsal margin with 9 robust setae, along length; exopod dorsal margin with 10 robust setae.

Description based on male. *Coloration.* Bodies lightly pigmented and light brown to orange-brown; less pigmented along pleotelson, lateral portions of pereonites, ventral parts of pleura of pleonites; darker pigmentation along dorsal longitudinal band, towards posterior margins of pereonites and pleonites; unpigmented patches along lateral portions of pereonites give body light mottled appearance; pereopods generally unpigmented and white, may have slight pigmentation on bases; some individuals wholly lacking pigmentation, white to off-white in colour; pigmentation fades to off-white to cream upon preservation, eyes remain black.

Head width 0.84 – 0.85 pereonite 1 width; setae absent. Eyes projecting anteriorly; maximum diameter 0.10 – 0.12 head depth; approximately round. Cervical groove straight to smoothly curved, extending nearly to dorsal margin of head. Mandibular (genal or cheek) groove smoothly indented, more acutely indented anteriorly. Maxillipeds insertion from posterior margin of head approximately 0.08 head length.

Pereon width exceeding head width; setae on dorsal surface concentrated along posterior pereonite margins, length of setae 0.05 – 0.08 body depth. Pereonite 1 dorsal margin in lateral view shorter than on pereonite 2; length:width in dorsal view 0.41 – 0.45. Pereonite 2 length:width in dorsal view 0.42 – 0.54. Pereonite 3 length:width 0.49 – 0.59. Pereonite 4 length:width 0.48. Pereonite 5 length:width 0.41 – 0.45. Pereonite 6 length:width 0.39 – 0.43. Pereonite 7 length:width 0.21.

Pleonites in dorsal view 2 – 4 respective lengths less than half the length of pleonite 5, 1 – 4 relative lengths unequal, increasing in length from anterior to posterior; pleonites 1 – 4 width 1.21 – 1.24 composite length in dorsal view. Pleonites 1 – 5 dorsal length:maximum width of pleonites 1 – 5 respectively 0.16, 0.19, 0.20, 0.25 and 0.58. Pleonites 1 – 5 depth:pereonite 7 depth respectively 1.35, 1.92, 2.16, 2.23 and 2.00.

Pleotelson dorsal surface in lateral view inflected ventrally, sparsely covered with fine setae, length 1.04 – 1.10 width; median ridge present posteriorly between vaulted telson and apex; lateral length 0.13 – 0.14 body length, less (0.78 – 0.79) than depth; depth 1.77 – 1.92 pereonite 7 depth; ventral margin anterior to uropods with single row of 4 – 5 simple robust setae, interspersed with elongate,

fine setae; lateral uropodal ridge terminating at pleotelson margin above uropods, lacking setae. Posterior apex with one pair of robust setae.

Antennula (Fig. 4.35A) length 0.13 body length, with 7 articles. No articles divisible into one large or two small articles. Article 3 with antennal scale. Article 5 length:width 1.55 – 1.78. Article 6 length:width 1.93 – 2.55. 6 – 7 Tiny aesthetascs, approximately 4 sensory setae on terminal article and along distal margin of subterminal article. Terminal article length:width 0.50 – 0.58; length:antennular length 0.02. Penultimate article distinctly longer than any other article. Distal articles in cross-section circular.

Antenna (Fig. 4.35B) length 0.65 body length. Flagellum length 0.68 total antenna length, with 29 – 35 articles. Article 5 length longer than or subequal to article 4; article 6 shorter than articles 4 and 5 combined.

Mouthfield. Clypeus widening and broadly triangular laterally; width 0.72 head width. Labrum (Fig. 4.35C) ventrally broad and truncate, with slight median point and margin of fine setae; asymmetrical, with invagination along right margin; dorsal margin approximately same width as clypeus. Paragnaths (Fig. 4.35D) with dense mat of fine setae from distal extent of lobes inwards along medial margins; simple setae scattered along proximal medial margins; lateral margin with dense mat of fine setae, discontinuous with apical setal rows.

Mandible (Figs 4.35E,F,G, 4.36A,B) palp length 1.08 – 1.25 mandible length; 3rd article with 27 – 32 finely setulate setae on medial-distal margins, additional medial surface additional setae present; 2nd article with elongate simple setae along length ventrolaterally and ventromedially, separate distal groups of elongate setae laterally and ventrally; article 1 with elongate simple setae distoventrally; articles 1 – 2 elongate setae greater than half respective article length. Left spine row with 12 spines, 6 of which bifurcate. Right spine row with 8 spines, 3 of which bifurcate. Molar process length subequal to width or longer than wide; spines absent.

Maxillula (Figs 4.36C,D) medial lobe length 0.50 lateral lobe length; width 0.54 lateral lobe width; with 4 ‘accessory setae’, one on distolateral margin, one between central pappose setae and two between distomedial setae, lateral ‘accessory’ seta simple, remainder distally denticulate; with 1 short weakly setulate seta on distal tip. Lateral lobe distal margin with 8 denticulate robust setae, 4 smooth robust setae, distal setal row with 3 robust setae; ventral face with 2 plumose setae, setae widely spaced; additional plumose seta absent.

Maxilla (Fig. 4.37A) medial lobe width 0.74 outer lateral lobe width; proximal portion distinctly angled to distal portion; proximal and distal setal rows continuous; 24 elongate, finely serrate setae in two ventral basal rows; approximately 36 closely-set elongate setae with distinct bases and thick shafts in dorsal basal row; approximately 30 simple, plumose and pectinate setae, of which 4 are more robust pectinate setae, in multiple distal rows. Outer lateral lobe longer than inner lateral lobe, wider than inner lateral lobe; distal margin with 19 long bidenticulate setae. Inner lateral lobe with 14 long bidenticulate setae. Lateral lobes with bidenticulate setae only on distal tips.

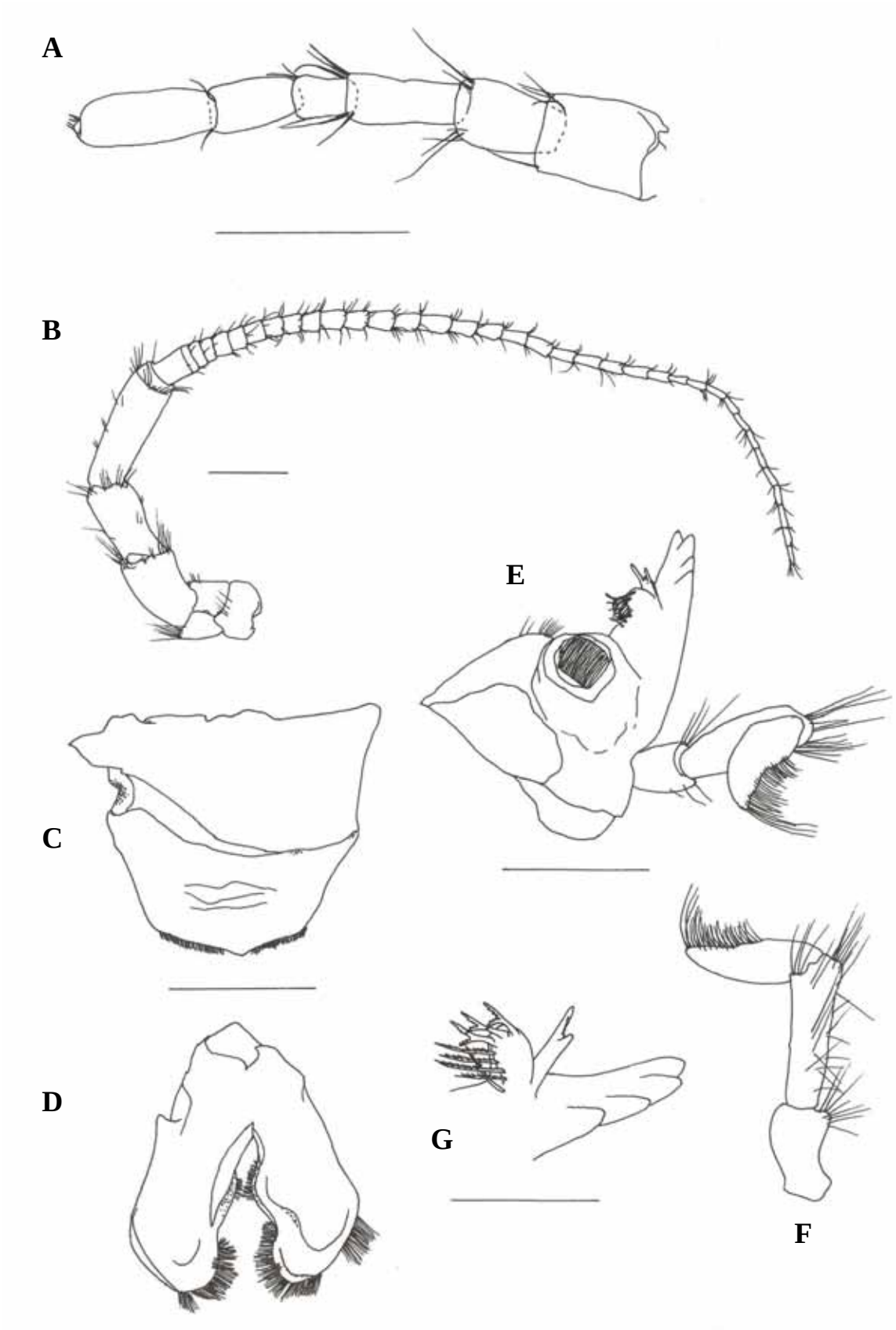


Figure 4.35: *Mesamphisopus setosus* n. sp., dissected male (SAM A45156). A, antennule; B, antenna; C, labrum; D, paragnaths; E, right mandible; F, right mandibular palp; G, right mandible incisor process and spine row. Scale lines represent 0.5 mm, except for G, where it represents 0.1 mm.

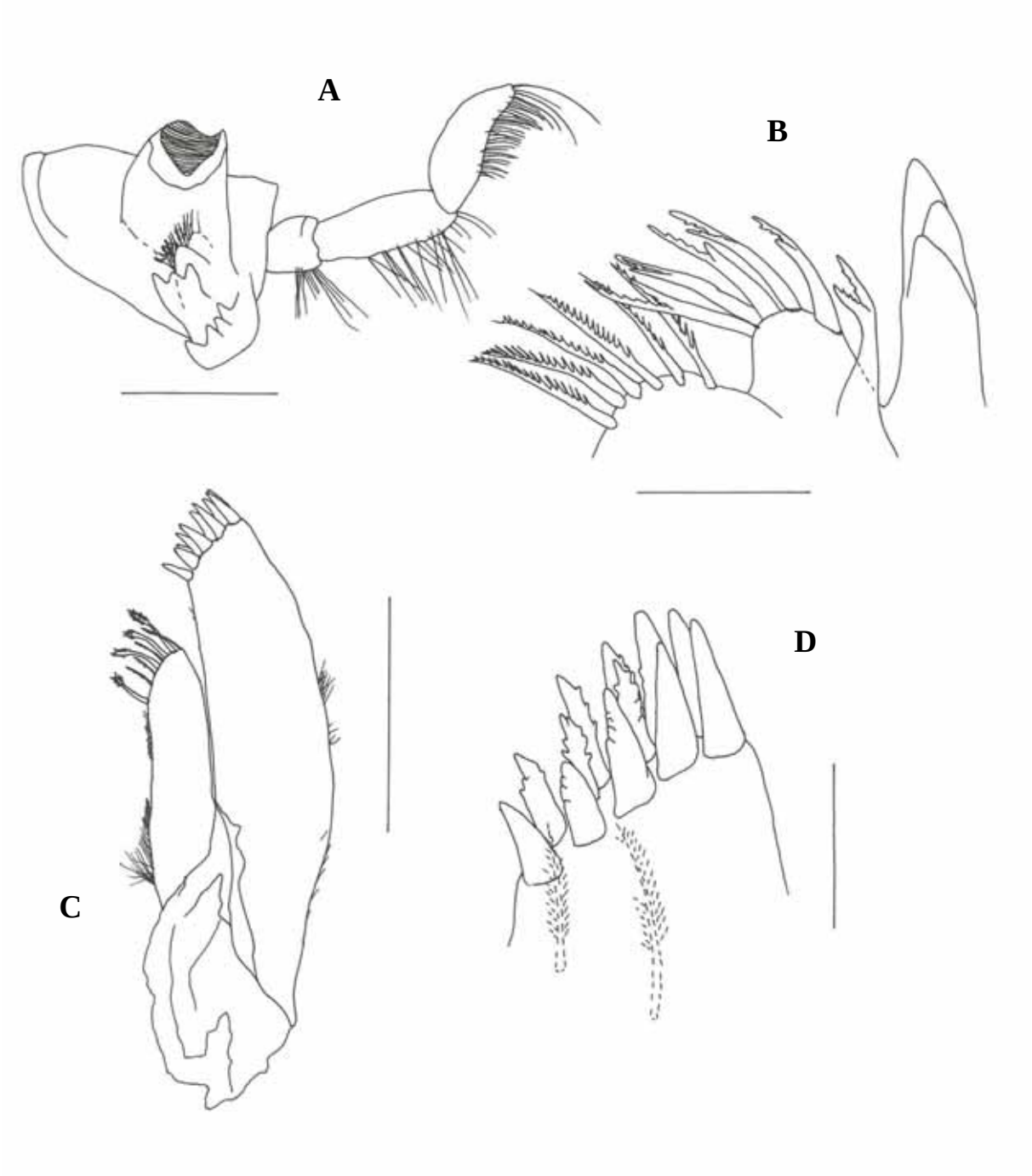


Figure 4.36: *Mesamphisopus setosus* n. sp., dissected male (SAM A45156). A, left mandible; B, left mandible spine row and lacinia mobilis; C, maxillula; D, maxillula lateral lobe distal margin. Scale lines represent 0.5 mm (A and C) or 0.1 mm (B and D).

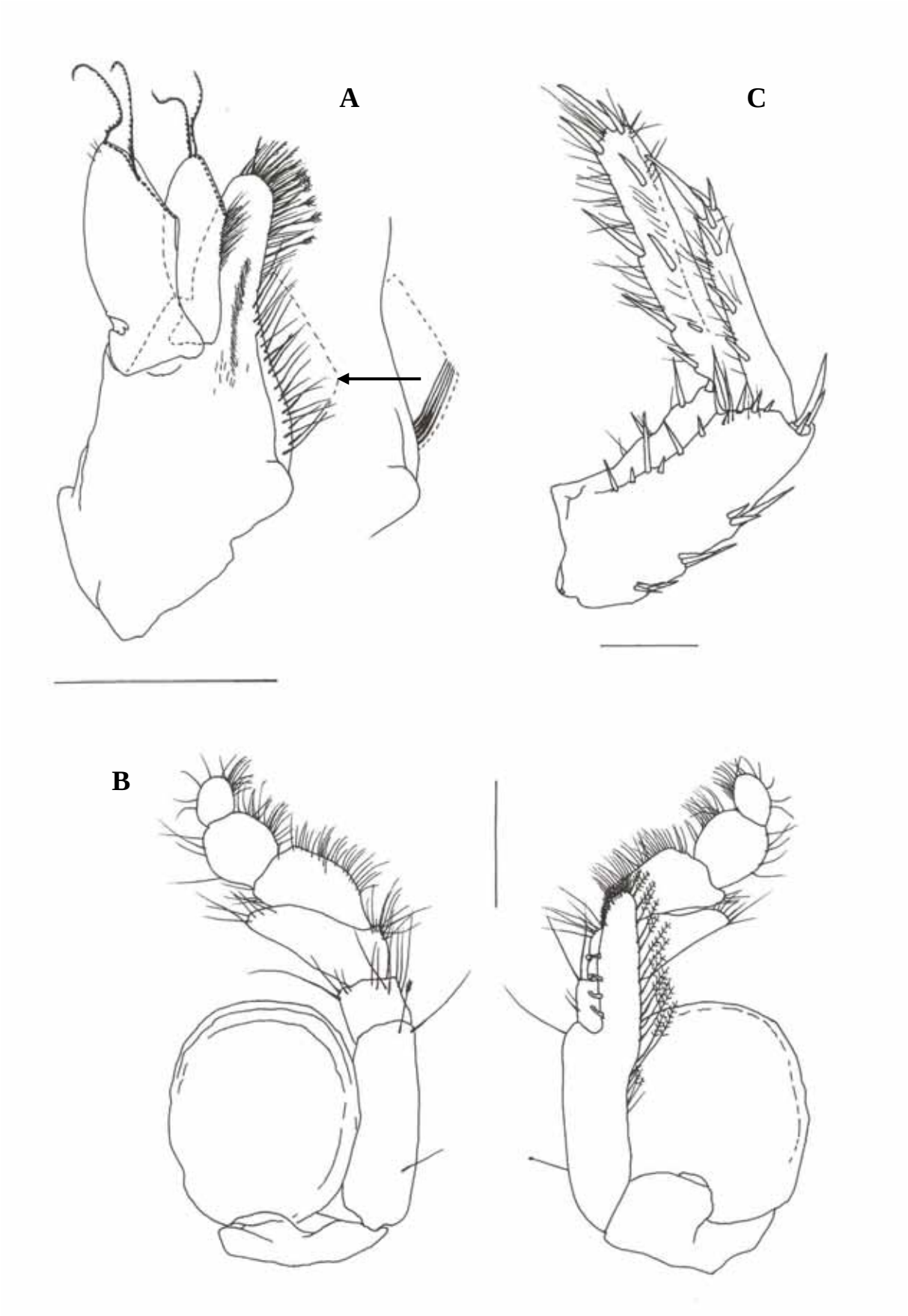


Figure 4.37: *Mesamphisopus setosus* n. sp., dissected male (SAM A45156). A, maxilla; B, right maxilliped, ventral (left) and dorsal (right) views; C, uropod. Scale lines 0.5 mm.

Maxilliped (Fig. 4.37B) epipod length:width 1.15; distal tip truncate to rounded; distal margin setae absent. Endite length:total basis length 0.37 – 0.41; medial margin with 2 coupling hooks on left side, 4 on right side; dorsal ridge with 20 large distally denticulate plumose setae. Palp insertion on basis lateral margin without plumose setae; medial margin with 1 simple seta; ventral surface without subdistal smooth setae, one elongate distally plumose/pectinate seta occurs subdistally towards medial margin; palp length:basis length 0.90; width across articles 2 – 3:endite width 1.81; article 4 elongate-oval, length:width 1.24; article 5 length:width 1.57, article 5 length:article 4 length 0.71.

Pereopod I (Figs 4.38A,B) length:body length 0.48. Dactylus length subequal to or longer than palm, length:palm length 1.57; ventrodistal margin with row of thin scale-like spines, along 0.16 total length; claw length:dactylus length 0.11; distal accessory claw ventrolateral to primary claw, 0.25 length of primary claw. Propodus length:pereopod length 0.27; length:width 1.18 – 1.22; dorsal margin setae confined to single group of 6 setae at distal margin. Propodal palm cuticular fringe well developed; with low stout cuticular projection distally; stout denticulate setae serrate, 9 altogether; 2 basally inflated stout robust simple setae altogether; 6 elongate broad based setae present. Merus distodorsal margin with numerous elongate simple setae, one robust. Ischium dorsal margin with 1 robust simple seta. Basis length:width 2.05; dorsal setae positioned proximally, few positioned further along length, 4 altogether; ventrodistal margin with 2 – 3 elongate setae.

Pereopods II – III (Figs 4.38C,D). Pereopod II length:body length 0.43. Dactylus length:propodus length 0.82; primary claw length:dactylar length 0.28. Propodus length:pereopod length 0.13; length:width 1.89. Carpus length:pereopod length 0.14; length:width 1.81. Basis length:pereopod length 0.27; length:width 2.15. Pereopod III length:body length 0.42. Dactylus length:propodus length 0.75; primary claw length:dactylar length 0.28. Propodus length:pereopod length 0.14; length:width 2.25. Carpus length:pereopod length 0.13; length:width 1.67. Basis length:pereopod length 0.26; length:width 2.11. Pereopods II – III penicillate setae present, (single seta) on dorsal ridge of basis of pereopod III. Dactylus with few fine setae; distal accessory claw ventral to ventrolateral of primary claw, 0.30 – 0.50 primary claw length. Propodus broad based setae present, respectively 5, 7 on pereopods II and III; on pereopod II increasing in length from proximal seta (0.18 propodus length) to median seta (0.32 propodus length), decreasing in length to distal seta (0.09 propodus length), evenly spaced along margin; on pereopod III increasing in length from proximal seta (0.21 propodus length) to third seta (0.28 propodus length), most distal seta shorter (0.15 propodus length), proximal three setae evenly spaced along margin, with three short setae (0.06 propodus length) occurring lateral to basal insertion of each, larger gap present between third and distal setae. Carpus broad based setae present, respectively 5, 6 on pereopods II and III; on pereopod II increasing in length from proximal seta (0.08 carpus length) to distal seta (0.36 carpus length), evenly spaced along margin; on pereopod III progressively increasing in length from proximal seta (0.14 carpus length) to distal seta (0.36 carpus length), with fifth seta shorter (0.23 carpus length), generally evenly spaced along margin, fifth and distal setae more closely set. Basis dorsal ridge in

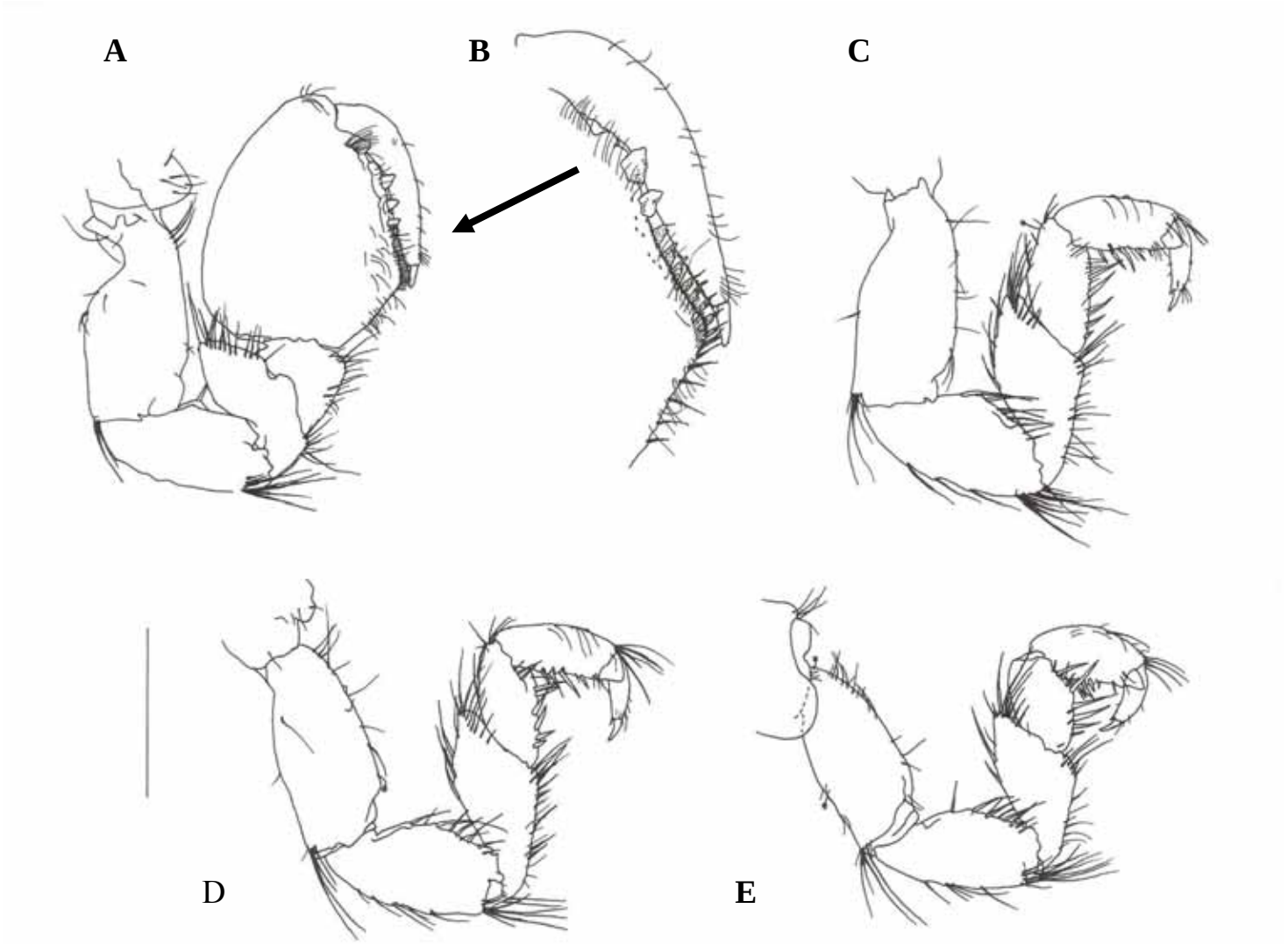


Figure 4.38: *Mesamphisopus setosus* n. sp., dissected male (SAM A45156). A, pereopod I; B, pereopod I propodal palm; C, pereopod II; D, pereopod III; E, pereopod IV. Scale line 1 mm.

cross-section angular and produced but not forming distinct plate, with 8 – 12 elongate and fine simple setae positioned along margin. Pereopods II – IV ischium dorsal margin with 9 simple setae, including 2 – 3 robust setae.

Pereopod IV (Fig. 4.38E) length:body length 0.36. Penicillate setae present on dorsal and ventral margin of basis. Dactylus longer than propodal palm; distal accessory claw approximately 0.25 length of primary claw. Propodus length:pereopod length 0.15, length:width 1.59; distal width:palm width 0.69; with 6 broad based setae on ventral margin, 3 distinctly larger than remainder; articular plate subequal in length to dactylar claw. Carpus length:pereopod length 0.11; with 5 broad based setae on ventral margin, 3 distinctly larger than others. Ischium posterodistal margin with 7 – 9 setae. Basis length:width 2.11; dorsal ridge in cross-section angular and produced but not forming distinct plate, with 15 – 16 setae.

Pereopods V – VII (Fig. 4.39). Pereopod V length:body length 0.38. Dactylus claw length:dactylar length 0.33. Propodus length:pereopod length 0.14. Carpus length:pereopod length 0.14. Basis length:width 1.72. Pereopod VI length:body length 0.50. Dactylus claw length:dactylar length 0.28. Propodus length:pereopod length 0.15. Carpus length:pereopod length 0.16. Basis length:width 1.56. Pereopod VII length:body length 0.53. Dactylus claw length:dactylar length 0.30. Propodus length:pereopod length 0.15. Carpus length:pereopod length 0.16. Basis length:width 1.59. Pereopods V – VII penicillate setae on dorsal ridge of basis of pereopod VII, dorsodistally on propodus of pereopods V – VII. Dactylus distal accessory claw ventral to ventrolateral to primary claw, 0.20 – 0.42 length of primary claw. Propodus distal margins with 6 – 7 robust setae, including 3 – 4 elongate robust setae. Pereopods V – VII ischium dorsal margin with 2 – 4 simple setae, including 2 – 3 robust setae. Basis dorsal ridge not distinctly separated from basis shaft, in cross-section angular on V, produced and forming distinct plate on VI – VII, with elongate fine setae positioned along entire margin and lateral to margin; lateral face central ridge present; lateral face ventral ridge present, setae absent. Pereopod VII ischium dorsal ridge flange absent.

Penes length 0.39 body width at pereonite 7; with setae on shaft; distal tip rounded.

Pleopods (Figs 4.40, 4.41). Pleopod I length:body length 0.18. Exopod length:width 2.65. Endopod length:width 2.71; endopod length:exopod length 1.06. Pleopod II length:body length 0.20. Exopod length:width 2.14; length of distal article:exopod length 0.30. Endopod length:width 1.90 – 2.71; endopod length:exopod length 0.78. Pleopod III length:body length 0.19. Exopod length:width 1.65; length of distal article:exopod length 0.21. Endopod length:width approximately 2.23; endopod length:exopod length approximately 0.75. Pleopod IV length:body length 0.18. Exopod length:width 1.44; length of distal article:exopod length 0.27. Endopod length:width 1.72; endopod length:exopod length 0.91. Pleopod V length:body length 0.16. Exopod length:width 1.26 – 1.31; length of distal article:exopod length 0.33 – 0.35. Endopod length:width 1.29 – 1.36; endopod length:exopod length 0.64 – 0.76. Endopods unilobed; V slightly cleft, with invagination in distomedial margin; I – V with setae on margins, setae plumose and simple on I – IV, simple on V. Protopods medial margin I – IV

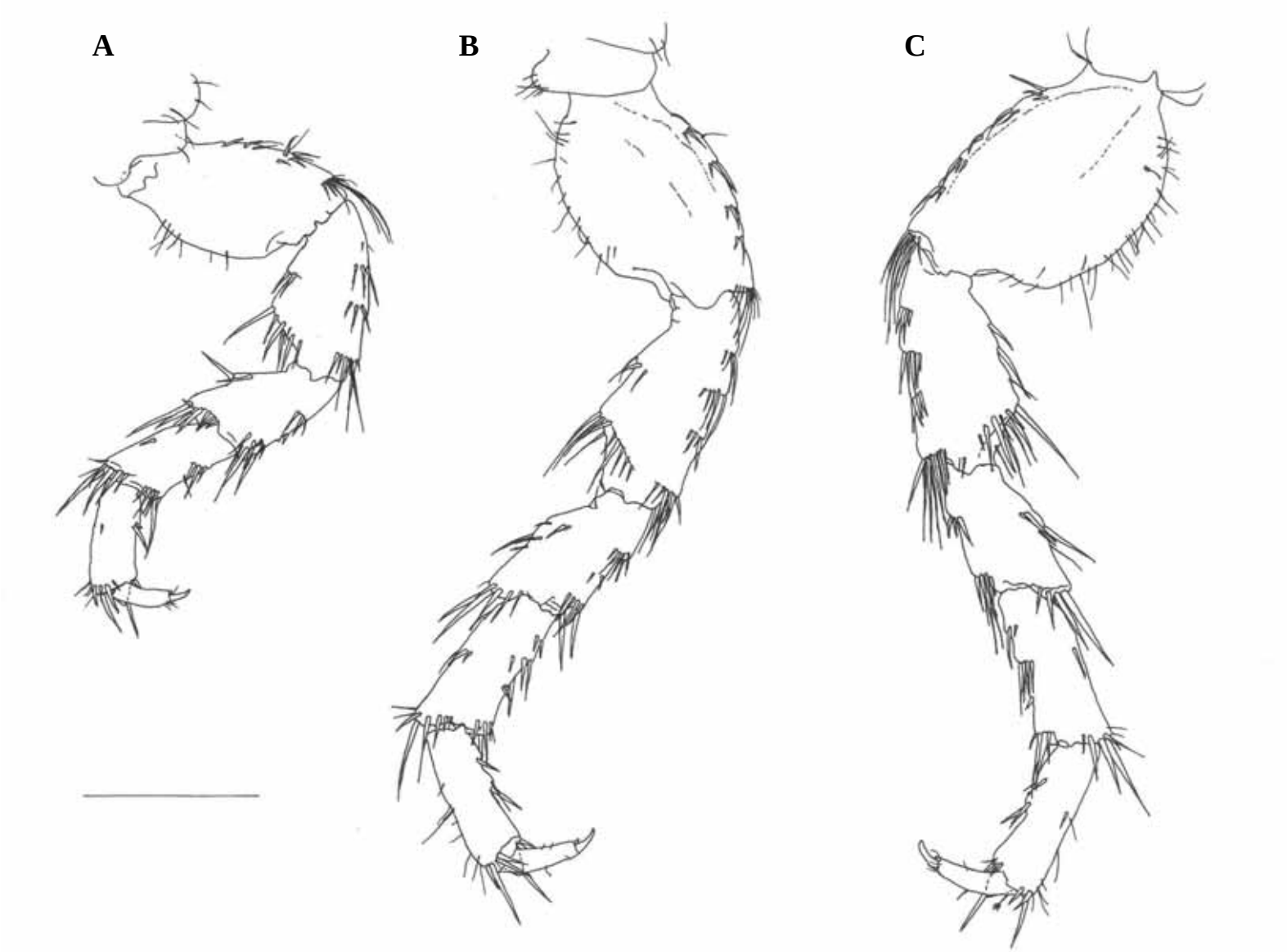


Figure 4.39: *Mesamphisopus setosus* n. sp., dissected male (SAM A45146). A, pereopod V; B, pereopod VI; C, pereopod VII (left). Scale line 1 mm.

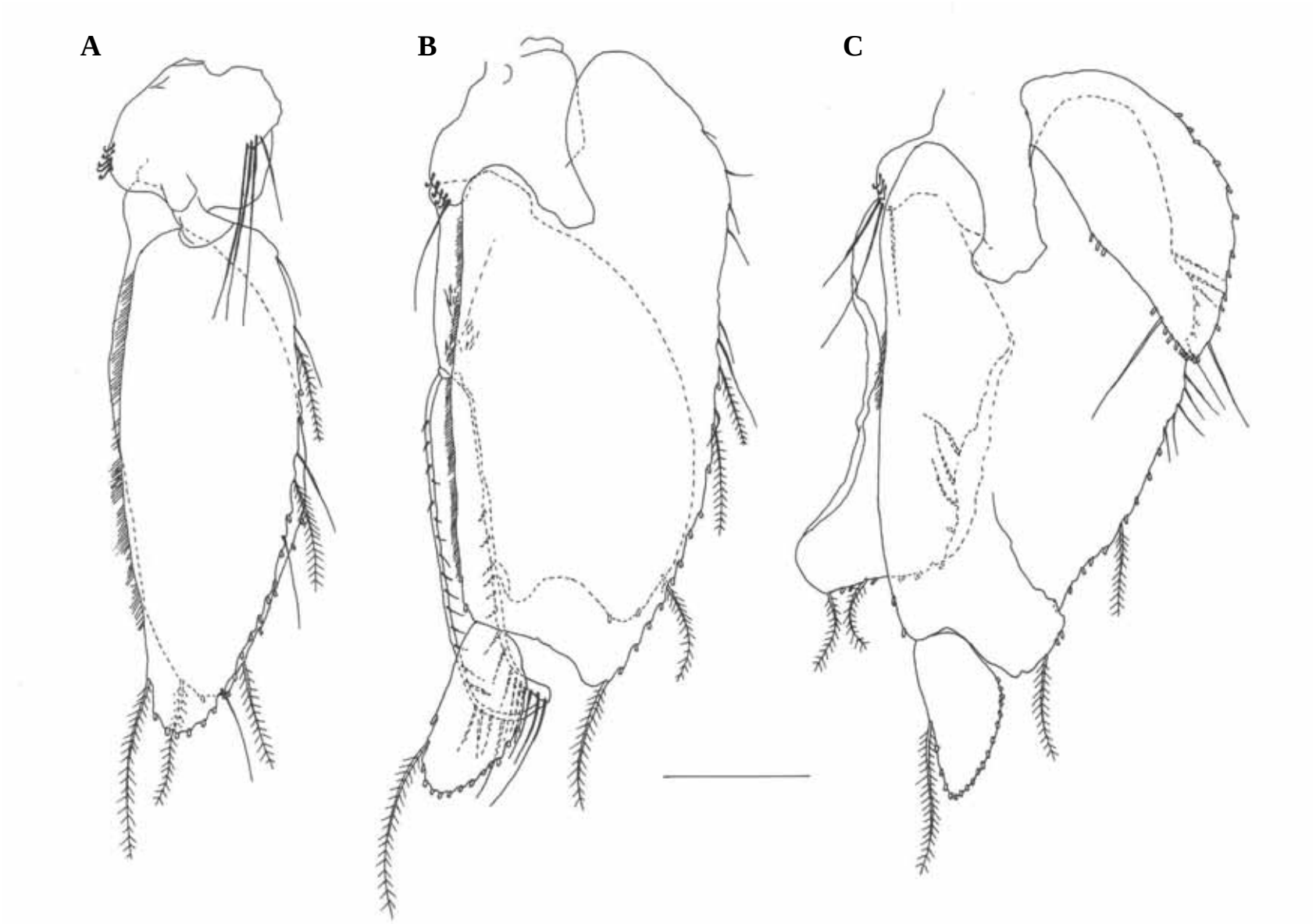


Figure 4.40: *Mesamphisopus setosus* n. sp., dissected male (SAM A45156). A, pleopod I; B, pleopod II; C, pleopod III. Scale line 0.5 mm.

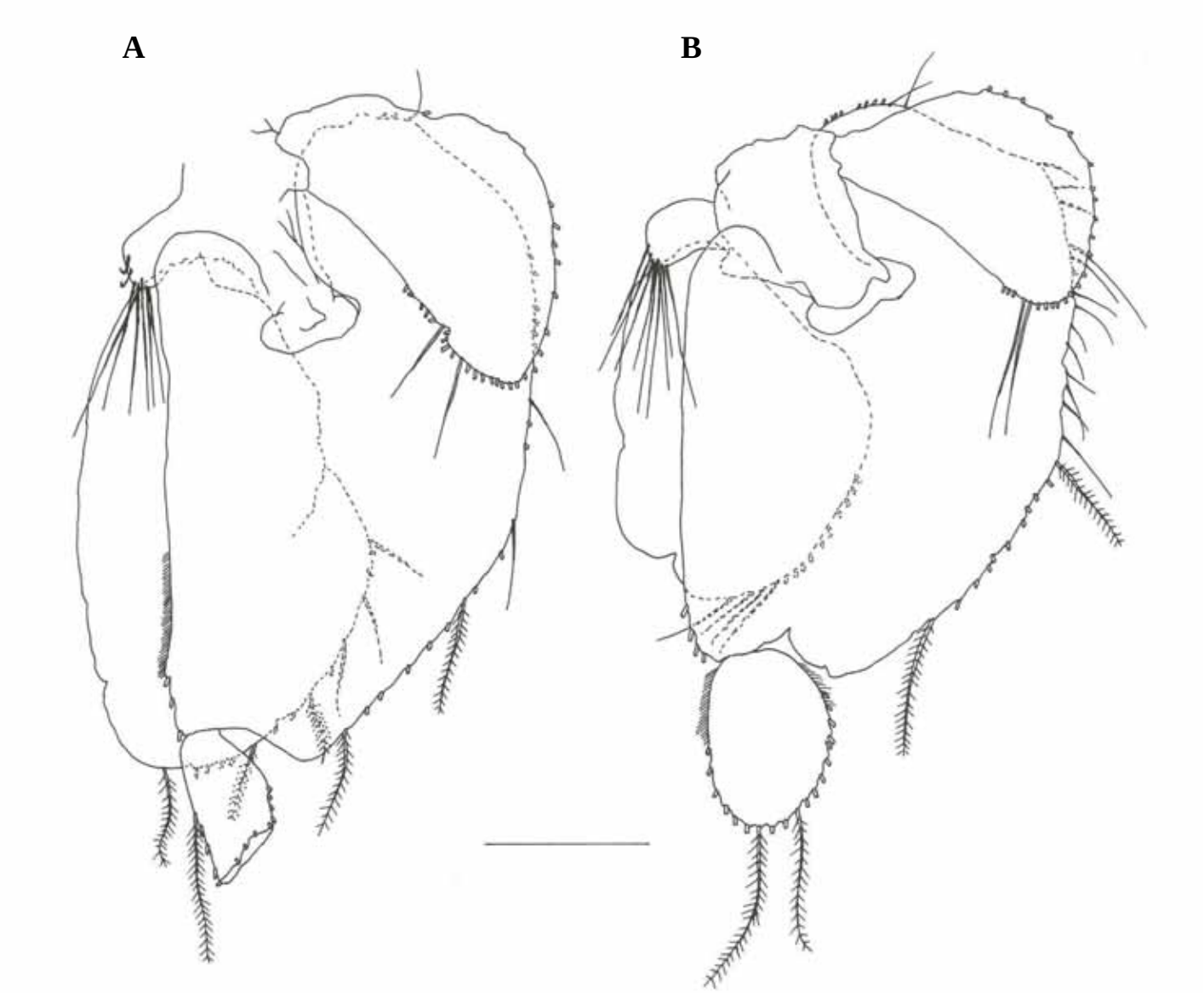


Figure 4.41: *Mesamphisopus setosus* n. sp., dissected male (SAM A45156). A, pleopod IV; B, pleopod V. Scale line 0.5 mm.

with coupling hooks, respective counts 5, 4, 3, 2; with 1, 5, 6 and 9 – 10 elongate inflexible simple setae on II, III, IV and V respectively; lateral epipod III length 1.93 – 2.13 width, lateral epipod V length 1.73 – 1.81 width. Protopods with 3 – 4 elongate inflexible simple setae on lateral margin on pleopod I; 25 (13 medial to apical, 12 lateral), 31 (19 medial to apical, 12 lateral) and 30 – 31 elongate inflexible simple setae on margins of lateral epipods of pleopods III, IV and V respectively. Pleopod I exopod broadest proximally, medial margin straight — divergent from lateral margin proximally, dorsal surface with setae; protopod length subequal to that of other pleopods, width subequal length. Pleopod II endopod appendix masculina basal musculature pronounced; with 34 setae on margin; length 0.47 pleopod length; distal tip extending beyond distal margin of endopod.

Uropod (Fig. 4.37C) total length 2.15 pleotelson length. Protopod length:width 3.18; length 0.40 uropod total length; extending posteriorly subequal to or extending posterior to pleotelson apex; dorsomedial ridge weakly produced, plate-like, margin smooth, in lateral view approximately straight, ridge length:endopod length 0.50; ventral ridge without rows of long laterally projecting setae. Rami cross-sectional shape flattened on dorsal surface only. Endopod dorsal margin robust setae along length, with 9 robust setae, 4 medial, 5 lateral, excluding apical seta. Exopod length 0.82 endopod length; dorsal margin with 10 robust setae, excluding apical seta.

Sexual dimorphism, female differences from male. *Head.* Cervical groove smoothly curved to nearly sigmoidal.

Pereon. Pereonite 1 length:width in dorsal view 0.35 – 0.38. Pereonite 2 length:width 0.51 – 0.54. Pereonite 3 length:width 0.56. Pereonite 4 length:width 0.43. Pereonite 5 length:width 0.33. Pereonite 6 length:width 0.35. Pereonite 7 length:width 0.19.

Pleonites in dorsal view 2 – 3 respective lengths less than half the length of pleonite 5, pleonite 4 equal to or more than half the length of pleonite 5.

Pleotelson ventral margin anterior to uropods with single row of 5 – 6 simple robust setae, interspersed with elongate, fine setae, posterior seta generally longer than anterior adjacent setae with shorter robust seta placed between two most posterior setae.

Antennula length 0.13 body length, with 7 articles. Terminal article with 4 aesthetascs. Penultimate article length subequal to or less than length of other articles.

Antenna length 0.66 body length. Flagellum length 0.69 total antenna length, with 30 articles. Article 5 length subequal to or shorter than article 4.

Pereopod I length:body length 0.39. Dactylus length projecting beyond palm, length:palm length 1.53; ventrodistal margin with row of thin scale-like spines, along 0.38 total length; claw length:dactylus length 0.15. Propodus length:pereopod length 0.21; length:width 1.25 – 1.27; dorsal margin setae along entire margin. Propodal palm concave; cuticular fringe well developed; serrate and bifid stout denticulate setae present, 10 altogether; stout robust simple setae absent; 5 elongate broad based setae present. Merus distodorsal margin with numerous elongate simple setae, 1 – 3 more

robust than remainder. Ischium dorsal margin setae absent. Basis length:width 2.07; dorsal setae positioned along ridge, 3 altogether; ventrodistal margin with 4 elongate setae, 2 more elongate than others.

Pereopods II – III. Pereopod II length:body length 0.39. Dactylus length:propodus length 0.70; primary claw length:dactylar length 0.31. Propodus length:pereopod length 0.14; length:width 2.22. Carpus length:pereopod length 0.12; length:width 1.69. Basis length:pereopod length 0.28; length:width 2.26. Pereopod III length:body length 0.39. Dactylus length:propodus length 0.78; primary claw length:dactylar length 0.26. Propodus length:pereopod length 0.13; length:width 2.20. Carpus length:pereopod length 0.12; length:width 1.67. Basis length:pereopod length 0.29; length:width 2.40. Single penicillate seta present on ventral margin of basis of pereopod II. Propodus broad based setae present, respectively 4, 3 on pereopods II and III; on pereopod II increasing in length from short proximal seta (0.07 propodus length) to third seta (0.27 propodus length), distal seta 0.13 propodus length, evenly spaced along margin; on pereopod III increasing in length from proximal seta (0.09 propodus length) to distal seta (0.25 propodus length), second and most distal setae closely set near midlength of margin, proximal seta midway between these and proximal margin. Carpus broad based setae present, respectively 5, 7 (4 along margin, 3 distolaterally) on pereopods II and III; on pereopod II progressively increasing in length from proximal seta (0.07 carpus length) to distal seta (0.41 carpus length), evenly spaced along margin; on pereopod III increasing in length along margin from proximal seta (0.18 carpus length) to distal seta (0.43 carpus length), evenly spaced along margin, seta along distolateral margin 0.12 – 0.41 carpus length, placed towards ventral margin and closely set.

Pereopod IV simple to slightly prehensile. Length:body length 0.35. Penicillate setae occurring on dorsal margin of basis and anterodorsal margin of carpus. Dactylus distal accessory claw approximately 0.33 length of primary claw. Propodus length:pereopod length 0.11; length:width 1.83. Propodus with 1 broad based seta on ventral margin. Carpus length:pereopod length 0.12; with 5 broad based setae on ventral margin. Ischium posterodistal margin with 4 setae ventrolaterally. Basis length:width 2.37; dorsal ridge with approximately 11 setae.

Pereopods V – VII. Pereopod V length:body length 0.34. Dactylus claw length:dactylar length 0.34. Propodus length:pereopod length 0.15. Carpus length:pereopod length 0.15. Basis length:width 1.63. Pereopod VI length:body length 0.49. Dactylus claw length:dactylar length 0.28. Propodus length:pereopod length 0.15. Carpus length:pereopod length 0.15. Basis length:width 1.72. Pereopod VII length:body length 0.49. Dactylus claw length:dactylar length 0.39. Propodus length:pereopod length 0.13. Carpus length:pereopod length 0.17. Basis length:width 1.84.

Pleopods. Pleopod I length:body length 0.16. Exopod length:width approximately 2.89. Endopod length:width 2.38; endopod length:exopod length approximately 1.09. Pleopod II length:body length 0.17. Exopod length:width 2.01; length of distal article:exopod length 0.27. Endopod length:width 2.31; endopod length:exopod length 0.95. Pleopod III length:body length 0.18. Exopod length:width

1.44; length of distal article:exopod length 0.25. Endopod length:width 1.87; endopod length:exopod length 0.97. Pleopod IV length:body length 0.16. Exopod length:width 1.25; length of distal article:exopod length 0.25. Endopod length:width approximately 1.87; endopod length:exopod length 0.82. Pleopod V length:body length 0.15. Exopod length:width 1.23; length of distal article:exopod length 0.32. Endopod length:width approximately 1.16; endopod length:exopod length 0.54. Protopods medial margins/epipods I – IV with coupling hooks, respective counts 2, 1, 1, 1; with 3, 6, 6 and 9 elongate inflexible simple setae on pleopods II, III, IV and V respectively. Protopods with 3 elongate inflexible simple setae on lateral margin on pleopod I; 21 (7 lateral, 14 medial and apical), 29 (18 medial, 11 lateral) and 23 (12 medial and apical, 11 lateral) elongate inflexible simple setae on margins of lateral epipods of pleopods III, IV and V respectively.

Uropod total length 2.00 pleotelson length. Protopod length:width 3.54, length 0.41 uropod total length; dorsomedial ridge length:endopod length 0.46. Endopod with 11 robust setae. Exopod length 0.89 endopod length; with 10 robust setae.

General Distribution. Known only from the type locality.

Remarks. *Mesamphisopus setosus* n. sp. shows its closest morphological affinity to *M. albidus* n. sp. Both species are lightly pigmented (*M. setosus* n. sp. individuals are occasionally depigmented), both are similarly setose along the pereon and pleotelson, both have an indication of a median ridge posteriorly on the pleotelson (just anterior to the apex) and apparently lack the dorsal sub-apical robust setal pair, both have a well developed, low, stout cuticular projection distally along the propodal palm of pereopod I (a feature seen too in *M. tsitsikamma* n. sp.), and both have a similar extension of the appendix masculina. *Mesamphisopus setosus* n. sp. is superficially distinguished from *M. albidus* n. sp. by the larger eyes, the sparser setation of the head, the longer antenna, the heavier setation of the limbs, and the greater relative length of the posterior pereopod (V – VII) series. Further characteristic features of *M. setosus* n. sp. are found amongst the mouthparts. Four 'accessory' setae are found amongst the four pappose setae on the distal (and medial) margin of the medial lobe of the maxillula. In the remaining five species described herein only two 'accessory' setae are found. Two 'accessory' setae have been documented for *M. abbreviatus* and *M. depressus*, and between two and three (among four to five pappose setae) for *M. capensis* (Barnard, 1914; Nicholls, 1943). The maxilla medial lobe ventral basal setal row is represented by a double row of setae in *M. setosus* n. sp., a condition documented in *M. depressus* (Nicholls, 1943), but not seen in other species of the genus, where a single row is present. The number of setae (24) forming the double rows is greater than observed in the other species. The proximal and distal setal rows of the medial margin are also continuous, being separated by a gap in other species. The uropod of *M. setosus* n. sp. appears to be the longest, relatively, within *Mesamphisopus*, being twice as long as the pleotelson. Even though the setation of the uropodal rami is known to vary and be inconsistent as a character (Nicholls, 1943), this too

appears to distinguish *M. setosus* n. sp., with up to ten robust setae occurring on the dorsal margins of the endopod and exopod, respectively. This degree of setation is perhaps only approached in *M. albidus* n. sp. (with a relatively shorter uropod) and *M. paludosus* n. sp., where a similar number of robust setae are counted on the endopod only. Although the distal margin of the figured endopod of pleopod II (Fig. 4.40B) was truncate and concave, the distal margin of the right pleopod II endopod was rounded, approaching the "normal" condition seen in the remaining endopods of both the male and female.

***Mesamphisopus tsitsikamma* n. sp.**

Figures 4.42 – 4.49

Type locality. Stream near "Big Tree", Tsitsikamma forest, Eastern Cape, South Africa (33°57'57"S 23°53'48"E).

Material examined. Holotype: SAM A45154, one adult male (bl 10.2 mm), stream near "Big Tree", Tsitsikamma forest, Eastern Cape, South Africa (33°57'57"S 23°53'48"E), collected on 21/II/2000 by S. R. Daniels and G. Gouws. SAM A44935, one dissected adult male (bl 11.1 mm) and one dissected preparatory female (bl 8.9 mm) parts slide mounted and in microvials, additional three males, collection details as for holotype.

Other material. SAM A40957, near "Big Tree" on N2 freeway, Storms River Forest, collected XII/1992 by C. L. Griffiths.

Etymology. The species epitheton is the Khoi-San name, "Tsitsikamma", for the area of temperate forest along the South African south coast in which the type locality is situated. The name is translated as "place of many waters" and is a noun in apposition.

Diagnosis. Mandibular groove smoothly indented. Pereon width in dorsal view near head width. Pleonites in dorsal view 2 – 3 respective lengths less than half the length of pleonite 5, pleonite 4 more than half the length of pleonite 5; 1 – 4 width 0.90 composite length in dorsal view; 1 – 4 individual dorsal lengths:maximum width of pleonites 1 – 5 0.30 – 0.35; pleonite 5 dorsal length:maximum width of pleonites 1 – 5 0.30; individual pleonite 1 – 4 depths:pereonite 7 depth 1.60 – 2.80. Pleotelson dorsal surface sparsely covered with fine setae, length 1.15 – 1.35 width; lateral length less than depth; depth 2.00 pereonite 7 depth; ventral margin anterior to uropods with single row of simple robust setae; lateral uropodal ridge curving strongly and extending posteriorly from uropods on pleotelson margin; posterior apex with two pairs of robust setae. Antennula penultimate article distinctly longer

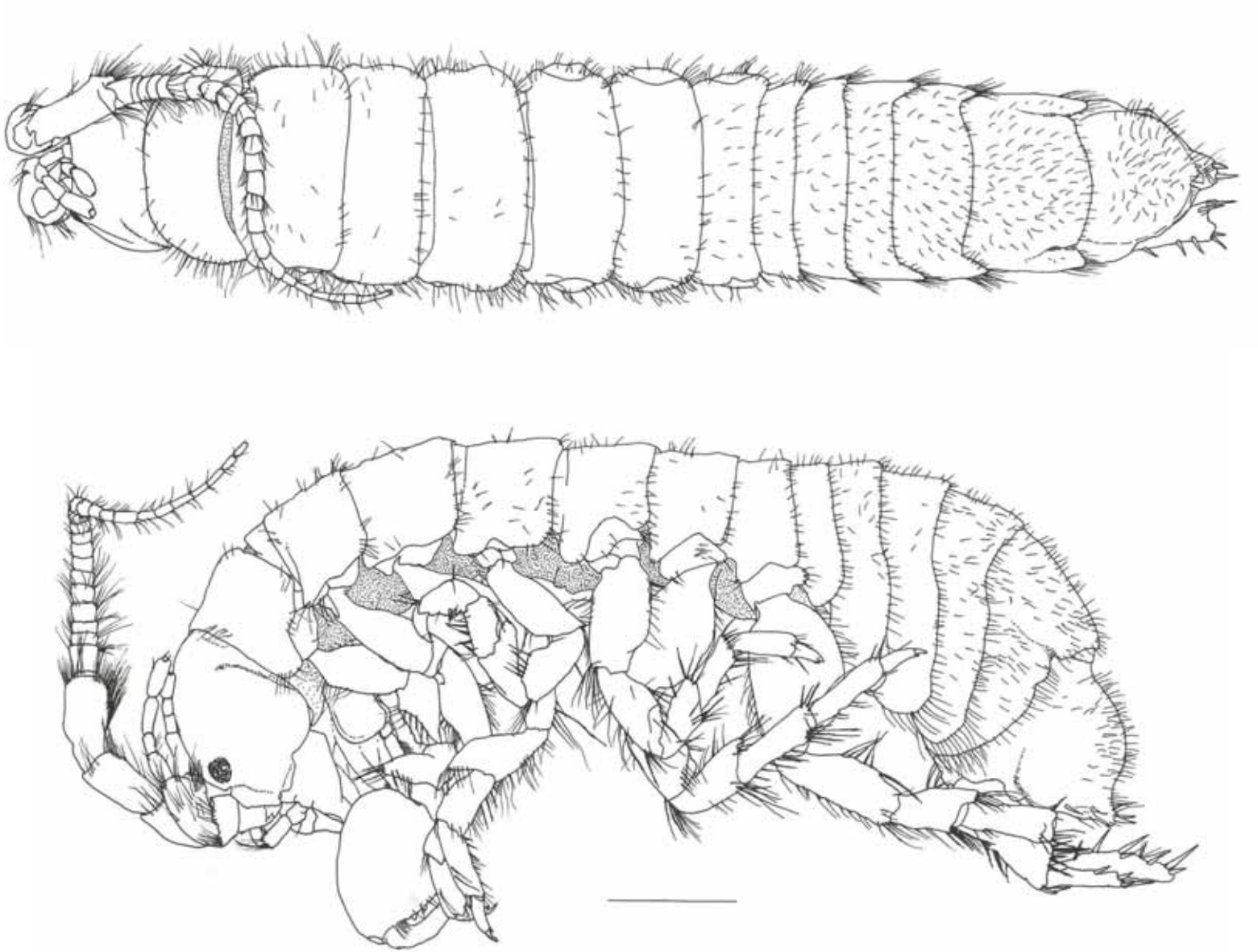


Figure 4.42: *Mesamphisopus tsitsikamma* n. sp., male holotype (SAM A45154), dorsal view (above) and lateral view (below). Scale line 1 mm. Uropods incompletely illustrated in dorsal view.

than any other article; distal articles in cross-section oval. Antenna article 5 longer than article 4. Mandibular palp article 3 with 9 – 11 smooth setae. Maxillula medial lobe width 0.64 – 0.80 lateral lobe width; lateral lobe distal margin with 5 smooth robust setae, ventral face setae absent. Maxilla medial lobe wider than outer lateral lobe; outer lateral lobe width subequal to inner lateral lobe; few long bidenticulate setae present on inner (9 – 11) and outer (9 – 13) lateral lobes. Pereopod I dactylus ventrodistal margin with row of thin scale-like spines, along 0.10 total length; propodal palm with 5 bifid stout denticulate setae, 5 basally inflated stout robust simple setae; basis dorsal margin setae positioned along ridge, > 10 altogether. Pereopod II propodus length:width approximately 2.50; with 6 broad based setae. Pereopod III propodus length:width > 2.50; carpus length:width > 1.75, with 5 broad based setae. Pereopod IV dactylus longer than propodal palm; propodus length:width approximately 1.60; basis length:width approximately 2.40. Pereopods V – VII penicillate setae absent; basis lateral face ventral ridge present. Pereopod VII ischium dorsal ridge forming flange subequal to shaft width. Pereopod V basis length:width > 2.00. Pereopod VII without articular plate. Pleopodal endopods with setae plumose on I – V. Pleopod I exopod broadest at midlength; medial margin convex; protopod longer than wide, significantly longer than other protopods. Pleopod II appendix masculina distal tip extending near to distal margin of endopod. Uropod total length 1.60 pleotelson length; protopod dorsomedial ridge not produced; endopod dorsal margin with 5 robust setae; exopod dorsal margin with 5 robust setae.

Description based on male. *Coloration.* Darkly coloured from dark brown to dark slate-grey, fading to much lighter brown upon preservation, with eyes remaining black. Where unpigmented, white to off-white, turning darker yellowish-white upon preservation. Unpigmented patches give body slight mottled appearance. Pigmentation darkest in longitudinal dorsal band; lateral cephalon, pereon, pleon and pleotelson more lightly pigmented. Pereopods lightly coloured, mottled; pigmentation concentrated along dorsal portions of limbs. Infrequently, individuals may lack pigmentation.

Head width 0.88 pereonite 1 width; surface generally smooth, more granular than shiny. Eyes bulging dorsolaterally to projecting anteriorly; maximum diameter 0.17 – 0.22 head depth; approximately round. Cervical groove extending nearly to dorsal margin of head. Mandibular (genal or cheek) groove smoothly indented. Maxillipeds insertion from posterior margin of head approximately 0.06 head length.

Pereon width near head width; length of setae on dorsal surface approximately 0.16 body depth. Pereonite 1 dorsal margin in lateral view shorter than on pereonite 2; length:width in dorsal view 0.39. Pereonite 2 length:width in dorsal view 0.37 – 0.56. Pereonite 3 length:width 0.41 – 0.59. Pereonite 4 length:width 0.43 – 0.45. Pereonite 5 length:width 0.42 – 0.52. Pereonite 6 length:width 0.36. Pereonite 7 length:width 0.25.

Pleonites in dorsal view 2 – 3 respective lengths less than half the length of pleonite 5, pleonite 4 length more than half the length of pleonite 5, 1 – 4 relative lengths unequal, pleonite 4 length greater

than pleonites 1 – 3; pleonites 1 – 4 width 0.89 composite length in dorsal view. Pleonites 1 – 5 dorsal length:maximum width of pleonites 1 – 5 respectively (approximately) 0.32, 0.28, 0.34, 0.32 and 0.31. Pleonites 1 – 5 depth:pereonite 7 depth respectively 1.60, 2.35, 2.73, 2.79 and 2.40.

Pleotelson dorsal surface in lateral view inflected ventrally, sparsely covered with fine setae, length 1.17 – 1.35 width; median ridge absent; lateral length 0.13 body length, less (0.79) than depth; depth 2.02 pereonite 7 depth; ventral margin anterior to uropods with single row of 4 simple robust setae; lateral uropodal ridge curving strongly and extending posteriorly from uropods on pleotelson margin, lacking setae. Posterior apex with two pairs of robust setae; additional pair of subapical robust setae can occur dorsally.

Antennula (Fig. 4.43A) length 0.16 – 0.18 body length, with 7 – 8 articles. No articles divisible into one large or two small articles. Single tiny aesthetascs, 4 – 5, on terminal and penultimate articles. Terminal article length:width 0.55; length:antennular length 0.02. Penultimate article distinctly longer than any other article. Distal articles in cross-section oval.

Antenna (Fig. 4.43B) length 0.62 body length. Flagellum length 0.63 total antenna length, with 28 – 31 articles. Article 5 longer than article 4; article 6 shorter than articles 4 and 5 combined.

Mouthfield. Clypeus slightly rounded to truncate at mandibular fossae; width 0.78 head width. Labrum (Fig. 4.43C) ventrally semi-circular in anterior view; slightly asymmetrical; dorsal margin narrower than clypeus. Paragnaths (Fig. 4.43D) medial margins with multiple setal rows, forming dense mat of fine elongate setae; lateral margins of lobes with sparse elongate simple setae and more robust setae; dorsal and ventral surfaces free of setation.

Mandible (Figs 4.43E,F,G, 4.44A,B,C) palp length 0.87 – 0.94 mandible length; 3rd article with 9 – 11 smooth setae on medial-distal margins, additional medial surface setae absent; 2nd article longitudinal row of setae absent, separate distal group of setae absent; articles 1 – 2 with elongate simple setae around entire distal margins, setae longer than respective articles. Left spine row with 11 spines, 3 of which bifurcate. Right spine row with 9 spines, 4 of which bifurcate. Molar process longer than wide; spines absent.

Maxillula (Figs 4.44D,E) medial lobe length 0.64 – 0.88 lateral lobe length; width 0.64 – 0.80 lateral lobe width; with 2 ‘accessory’ setae, one on distolateral margin and one between central pappose setae, ‘accessory’ setae distally denticulate; short weakly setulate seta on distal tip absent. Lateral lobe distal margin with 7 denticulate robust setae, 5 smooth robust setae, distal setal row with 4 robust setae; ventral face setae absent; additional plumose seta absent.

Maxilla (Fig. 4.44F) medial lobe width 1.08 – 1.11 outer lateral lobe width; proximal portion smoothly continuous with distal portion; proximal and distal setal rows separated by gap; 9 thickly set elongate setae in single ventral basal row; 29 – 30 closely-set setae with distinct base and long smooth shaft in dorsal basal row; 27 – 37 elongate, simple or plumose setae and few strongly pectinate (in distal third) setae in distal row. Outer lateral lobe length subequal to inner lateral lobe, width subequal to inner lateral lobe; distal margin setal row curving and extending proximally along medial margin, with 9 –

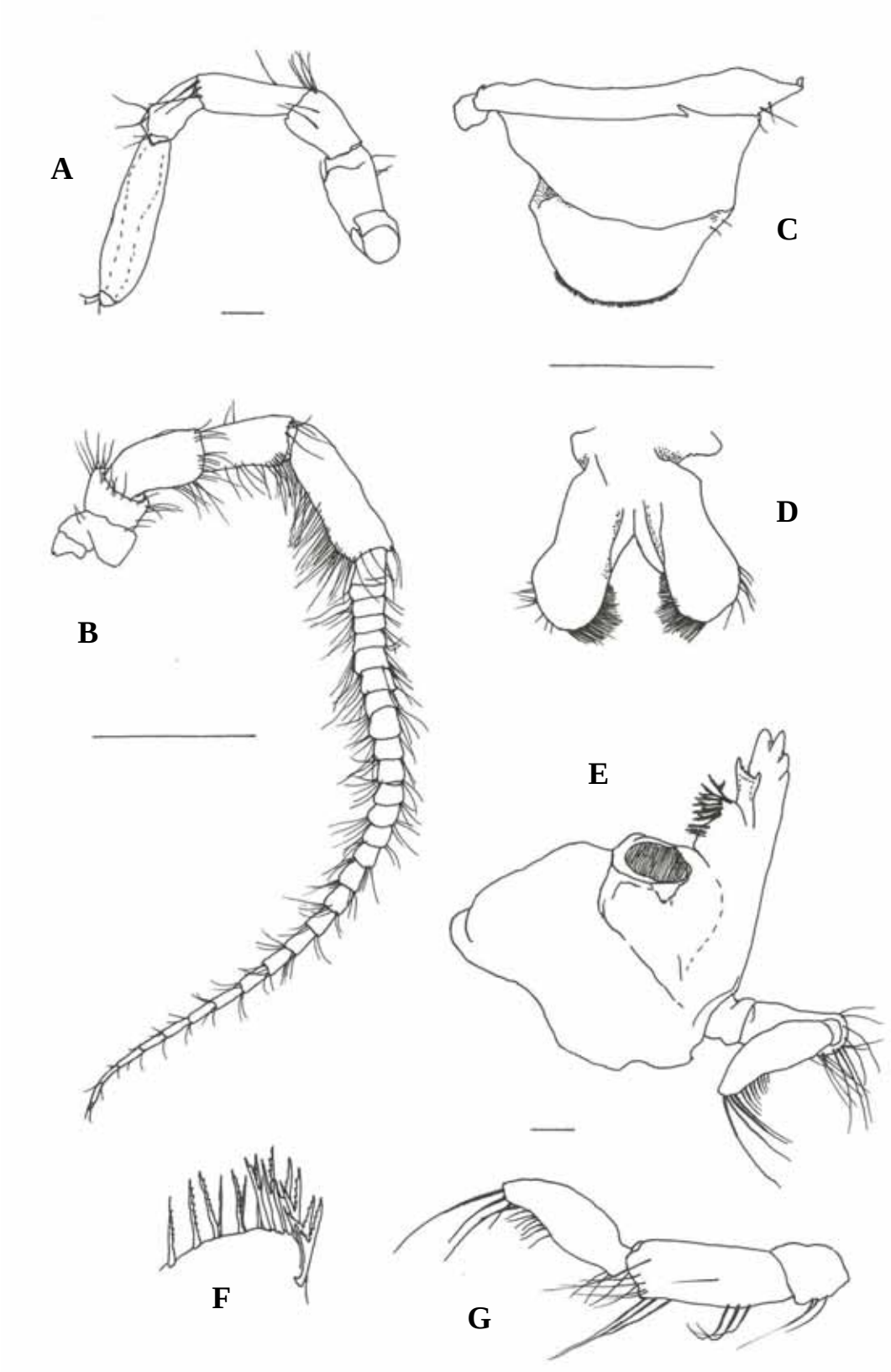


Figure 4.43: *Mesamphisopus tsitsikamma* n. sp., dissected female and male (SAM A44935). A, antennule (female; scale line 0.1 mm); B, antenna (male; scale line 1 mm); C, labrum, (female), anterior view; D, paragnaths (female; scale line 0.5 mm); E, right mandible (female; scale line 0.1 mm); F right mandible spine row; G, right mandibular palp.

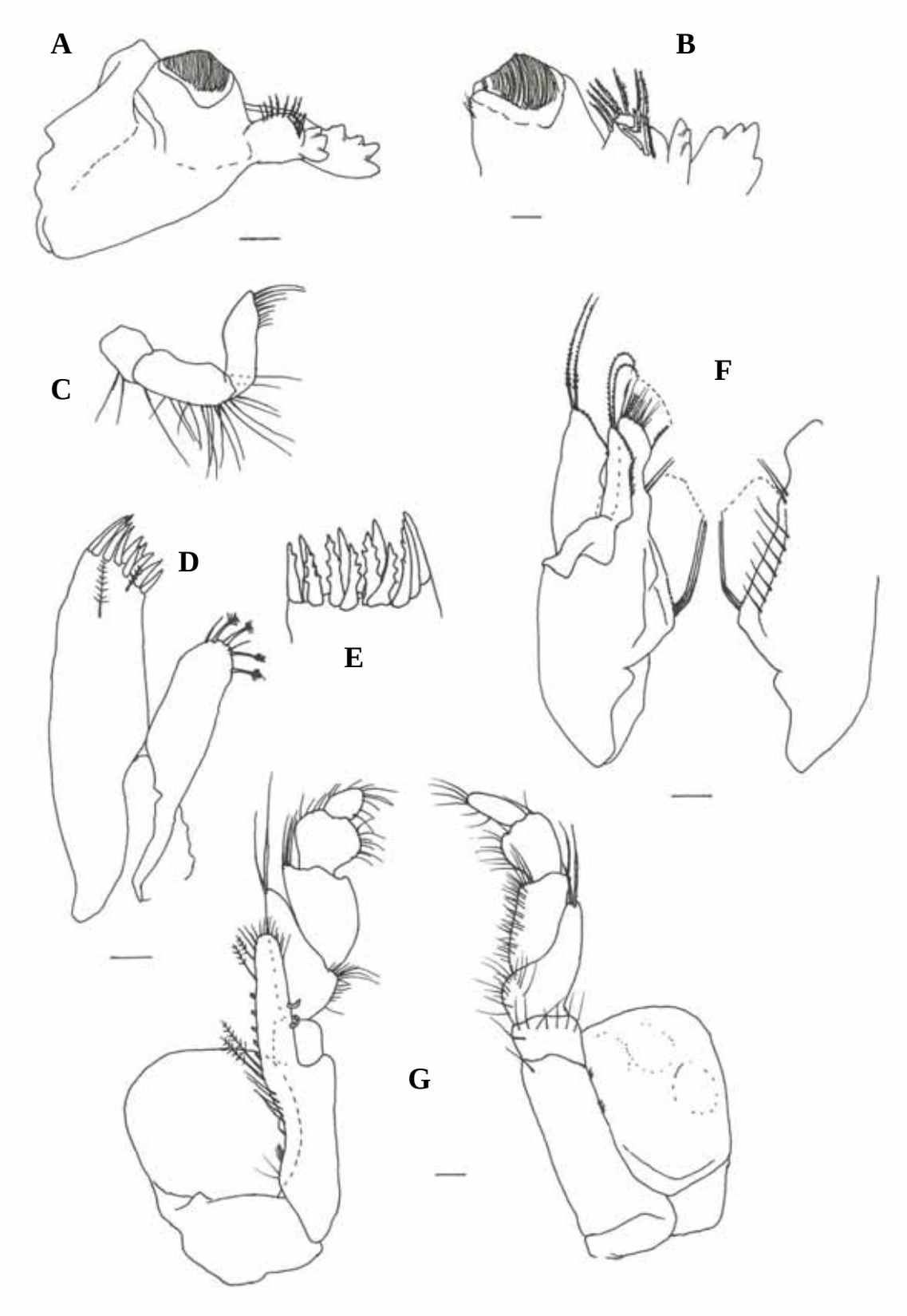


Figure 4.44: *Mesamphisopus tsitsikamma* n. sp., dissected male and female (SAM A44935). A, left mandible (female); B, left mandible molar process, spine row and incisor process; C, left mandibular palp; D, maxillula (female); E, maxillula lateral lobe distal margin; F, maxilla (male); G, left maxilliped (female), dorsal view (left) and ventral view (right). Scale lines 0.1 mm.

13 long bidenticulate setae. Inner lateral lobe with 9 – 10 long bidenticulate setae. Lateral lobes with bidenticulate setae only on distal tips.

Maxilliped (Fig. 4.44G) epipod length:width 1.09; distal tip truncate to broadly rounded; distal margin setae absent. Endite length:total basis length 0.44 – 0.56; medial margin with 2 coupling hooks on left side, 3 on right side. Palp insertion on basis lateral margin without plumose setae; medial margin with 1 simple seta; ventral surface with 7 subdistal elongate smooth setae; palp length:basis length 0.88 – 1.03; width across articles 2 – 3:endite width 1.36 – 1.79; article 4 subcircular, length:width 0.92 – 1.14; article 5 length:width 1.54, article 5 length:article 4 length 0.68 – 0.83.

Pereopod I (Fig. 4.45A) length:body length 0.42. Dactylus length subequal to palm or slightly shorter, length:palm length 0.93; ventrodistal margin with row of thin scale-like spines, along 0.10 total length; claw length:dactylus length 0.09; distal accessory claw small, triangular, ventrolateral to primary claw, 0.33 – 0.50 primary claw length. Propodus length:pereopod length 0.25; length:width 1.05; dorsal margin setae in several groups between proximal and distal margin, 10 setae altogether, excluding distal group. Propodal palm cuticular fringe weakly developed; with low stout cuticular projection distally; stout denticulate setae bifid, 5 altogether; 5 basally inflated stout robust simple setae altogether; approximately 12 elongate broad based setae present. Merus distodorsal margin with numerous elongate simple setae or 1 – 2 robust simple setae. Ischium dorsal margin with 6 simple setae, none robust. Basis length:width 2.15; dorsal setae positioned along ridge, 11 – 12 altogether; ventrodistal margin with 6 – 7 elongate setae.

Pereopods II – III (Figs 4.45B,C). Pereopod II length:body length 0.44. Dactylus length:propodus length 0.62; primary claw length:dactylar length 0.21. Propodus length:pereopod length 0.15; length:width 2.52. Carpus length:pereopod length 0.15; length:width 1.90. Basis length:pereopod length 0.25; length:width 2.29. Pereopod III length:body length 0.41. Dactylus length:propodus length 0.68; primary claw length:dactylar length 0.28. Propodus length:pereopod length 0.15; length:width 2.64. Carpus length:pereopod length 0.14; length:width 1.84. Basis length:pereopod length 0.25; length:width 2.30. Pereopods II – III distal accessory claw ventrolateral to primary claw, 0.30 – 0.50 length of primary claw. Propodus broad based setae present, respectively 6, 5 on pereopods II and III; on pereopod II increasing in length from first to fourth (0.20 propodus length) setae, fifth shorter, sixth as long as fourth, series of five evenly spaced from one-third propodus length to two-thirds length, sixth occurs more distally; on pereopod III increase in size from first to third (0.20 propodus length) setae, fourth shorter, fifth as long as third, series of four evenly spaced along ventral margin from one-third propodus length to two-thirds length, fifth occurs more distally. Carpus broad based setae present, respectively 5, 5 on pereopods II and III; on pereopod II between 0.06 and 0.20 carpus length, increasing in length distally, series of 4 evenly spaced along margin, proximal to half-length of margin, fifth more distal at two-thirds length of margin; on pereopod III increasing in length from 0.20 to 0.48 carpus length, series of 4 evenly spaced from proximal to half-length along margin, fifth more distal at three-quarter margin length. Basis dorsal ridge in cross-section angular

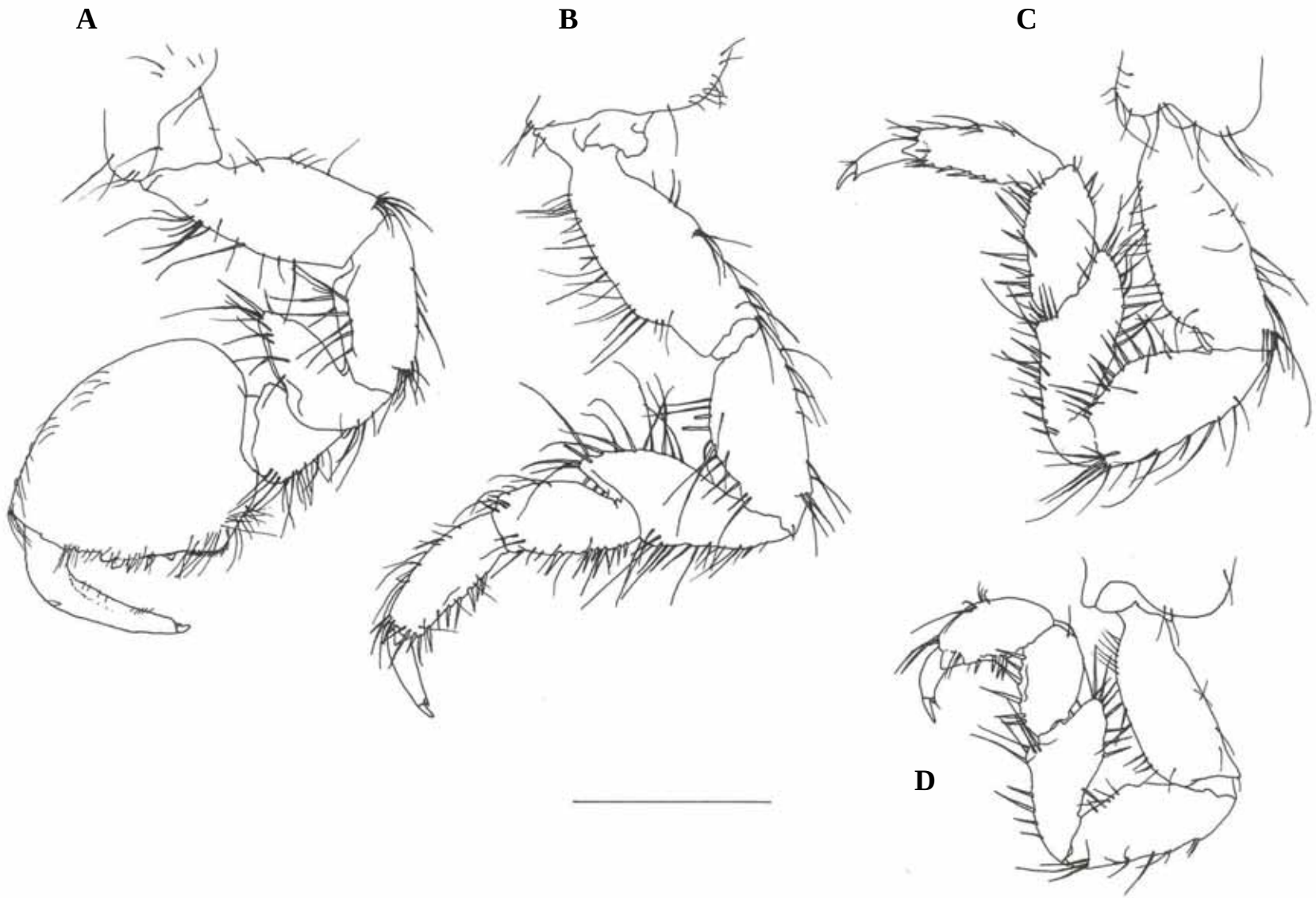


Figure 4.45: *Mesamphisopus tsitsikamma* n. sp., dissected male (SAM A44935). A, pereopod I; B, pereopod II; C, pereopod III; D, pereopod IV. Scale line represents 1 mm.

and produced but not forming distinct plate, with 9 – 18 elongate simple setae distributed along ridge or medially and laterally to margin, densest proximally. Pereopods II – IV ischium dorsal margin with 7 – 12 simple setae, including 3 robust setae.

Pereopod IV (Fig. 4.45D) length:body length 0.33. Penicillate setae absent. Dactylus longer than propodal palm; distal accessory claw approximately 0.33 length of primary claw. Propodus length:pereopod length 0.14, length:width 1.59; distal width:palm width 0.74; with 6 broad based setae on ventral margin, 2 – 3 distinctly larger than remainder; articular plate on posterior side of limb subequal in length to dactylar claw. Carpus length:pereopod length 0.13; with 6 broad based setae on ventral margin, 3 distinctly larger than others. Ischium posterodistal margin with 5 setae, 3 on margin, with series continuing round to anterodistal margin. Basis length:width 2.45; dorsal ridge in cross-section angular and produced but not forming distinct plate, with 12 setae positioned along ridge and in dense cluster proximally.

Pereopods V – VII (Fig. 4.46). Pereopod V length:body length 0.32. Dactylus claw length:dactylar length 0.29. Propodus length:pereopod length 0.13. Carpus length:pereopod length 0.16. Basis length:width 2.07. Pereopod VI length:body length 0.48. Dactylus claw length:dactylar length 0.33. Propodus length:pereopod length 0.13. Carpus length:pereopod length 0.17. Basis length:width 1.73. Pereopod VII length:body length 0.42 – 0.43. Dactylus claw length:dactylar length 0.33. Propodus length:pereopod length 0.14. Carpus length:pereopod length 0.15 – 0.16. Basis length:width 1.61. Pereopods V – VII penicillate setae absent. Dactylus distal accessory claw ventrolateral to primary claw, 0.25 – 0.66 primary claw length. Propodus distal margins with 3 – 5 elongate robust setae. Pereopods V – VII ischium dorsal margin with 1 – 11 simple setae, including 1 – 6 robust setae. Basis dorsal ridge not distinctly separated from basis shaft, in cross-section angular on V, produced and forming distinct plate on VI – VII, with elongate fine setae positioned along entire margin; lateral face central ridge present; lateral face ventral ridge present, setae absent. Pereopod VII ischium dorsal ridge forming flange subequal to shaft width.

Penes length 0.41 body width at pereonite 7; with setae on shaft; distal tip broadly rounded to truncate.

Pleopods (Figs 4.47, 4.48). Pleopod I length:body length 0.16. Exopod length:width 2.73. Endopod length:width 2.46; endopod length:exopod length 1.02. Pleopod II length:body length 0.18. Exopod length:width 2.49; length of distal article:exopod length 0.32. Endopod length:width 2.52; endopod length:exopod length 0.74. Pleopod III length:body length 0.14. Exopod length:width 1.85; length of distal article:exopod length 0.32. Endopod length:width 1.72; endopod length:exopod length 0.73. Pleopod IV length:body length 0.18. Exopod length:width 1.37; length of distal article:exopod length 0.33. Endopod length:width 1.78; endopod length:exopod length 0.73. Pleopod V length:body length 0.11. Exopod length:width 1.63; length of distal article:exopod length 0.34. Endopod length:width 1.67; endopod length:exopod length 0.91. Endopods I – V with plumose setae on margins. Protopods medial margins/epipods I – IV with coupling hooks, respective counts 4, 2, 2, 2; with 2, 3, 6 and 8 elongate simple setae on II, III, IV and V respectively; lateral epipod III length 1.76 – 2.26 width,

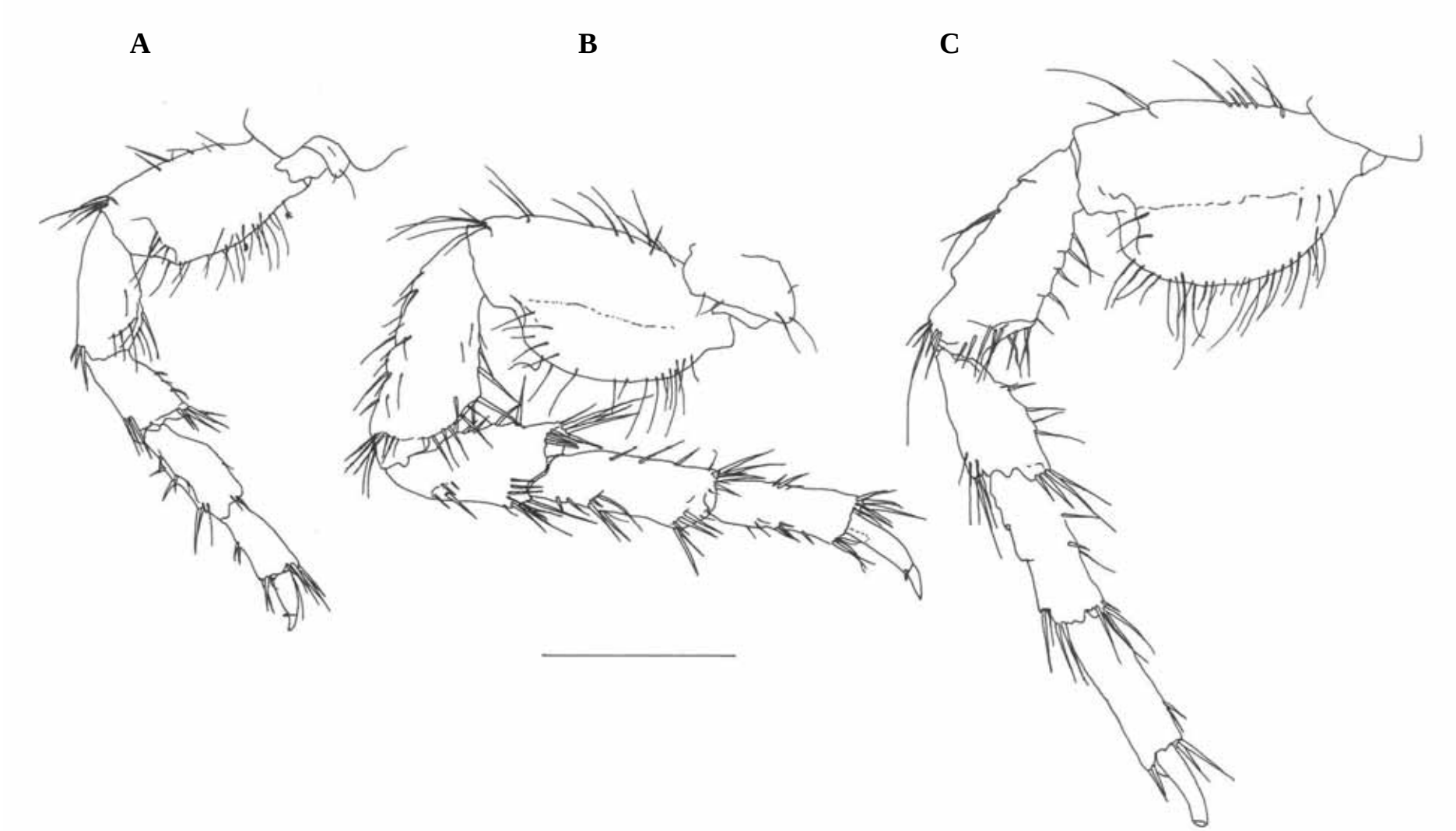


Figure 4.46: *Mesamphisopus tsitsikamma* n. sp., dissected male (SAM A44935). A, pereopod V; B, pereopod VI; C, pereopod VII. Scale line 1 mm.

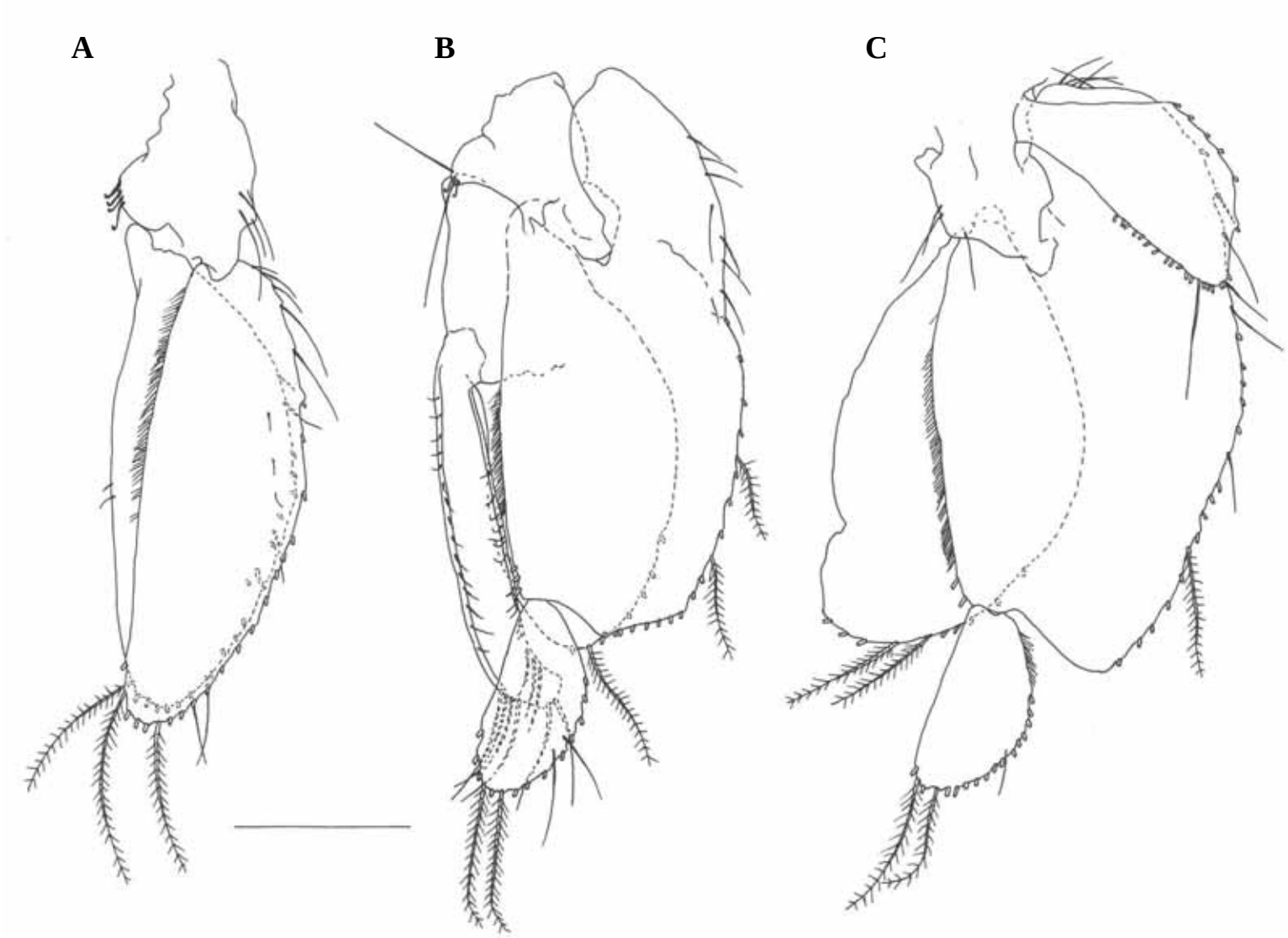


Figure 4.47: *Mesamphisopus tsitsikamma* n. sp., dissected male (SAM A44935). A, pleopod I; B, pleopod II; C, pleopod III. Scale line represents 0.5 mm.

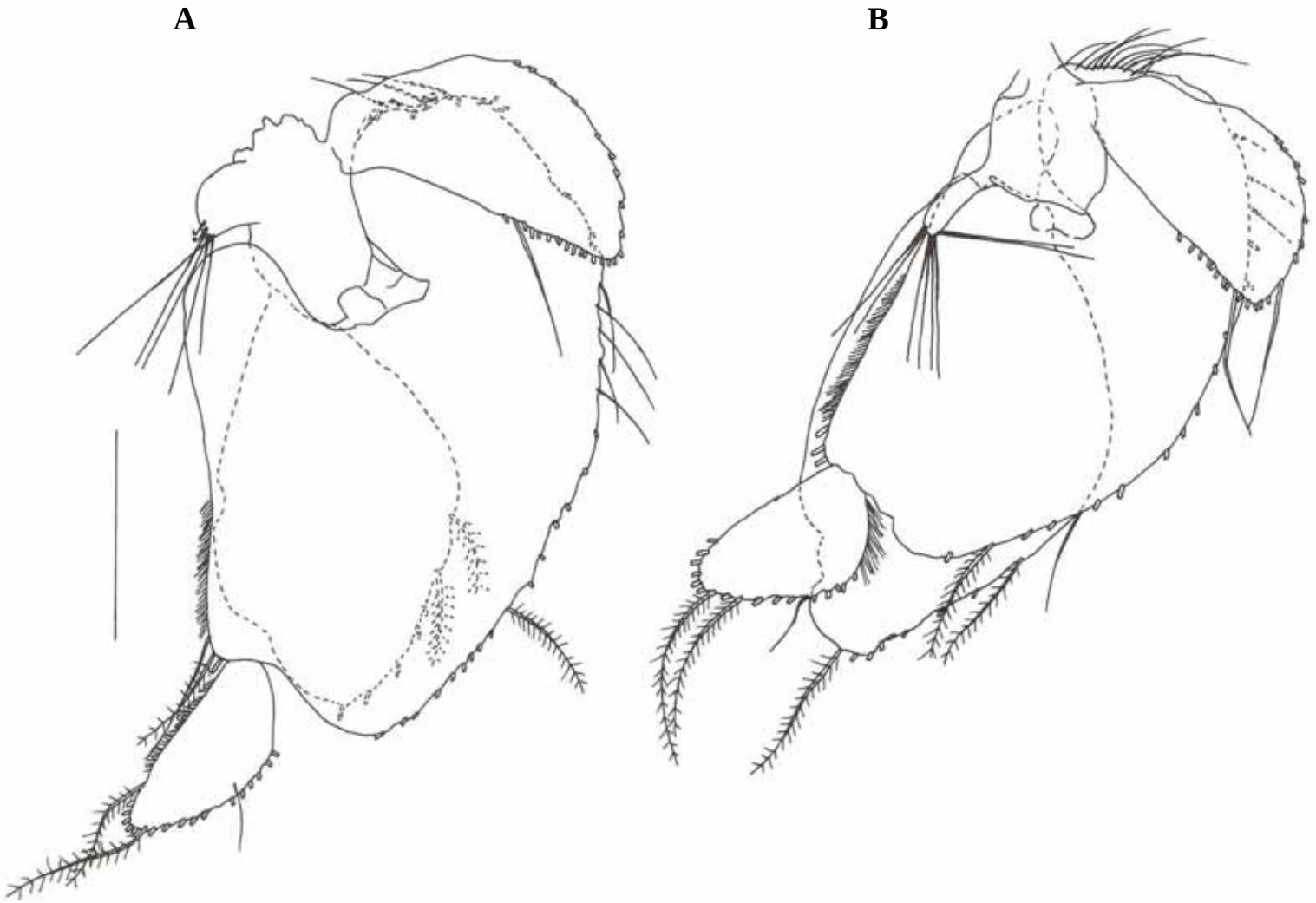


Figure 4.48: *Mesamphisopus tsitsikamma* n. sp., dissected male (SAM A44935). A, pleopod IV; B, pleopod V. Scale line represents 0.5 mm.

lateral epipod V length 1.67 – 1.97. Pleopod I exopod broadest at midlength, medial margin convex — divergent from lateral margin proximally, dorsal surface with setae; protopod significantly longer than other pleopods, longer than wide. Pleopod II endopod appendix masculina basal musculature pronounced; with 27 – 34 setae on margin; length 0.53 pleopod length; distal tip extending near to distal margin of endopod.

Uropod (Fig. 4.49) total length 1.62 pleotelson length. Protopod length:width 4.07; length 0.46 uropod total length; extending posteriorly subequal to pleotelson apex; dorsomedial ridge not produced, ridge length:endopod length 0.64; ventral ridge without rows of long laterally projecting setae. Rami cross-sectional shape flattened on dorsal surface only. Endopod dorsal margin robust setae along length, with 5 robust setae, excluding apical seta. Exopod length 0.86 endopod length; dorsal margin with 5 robust setae, excluding apical seta.

Sexually dimorphic, female differences from male. *Pereon.* Pereonite 1 length:width in dorsal view 0.42. Pereonite 2 length:width in dorsal view 0.41 – 0.49. Pereonite 3 length:width 0.50 – 0.54. Pereonite 4 length:width 0.48. Pereonite 5 length:width 0.45 – 0.52. Pereonite 6 length:width 0.42. Pereonite 7 length:width 0.36.

Antennula length 0.15 body length, with 6 articles.

Antenna length 0.57 body length. Flagellum length 0.69 total antenna length, with 31 articles.

Pereopod I length:body length 0.36. Dactylus length subequal to palm, length:palm length 1.04; ventrodistal margin with row of thin scale-like spines, along 0.49 total length; claw length:dactylus length 0.15 – 0.19. Propodus length:pereopod length 0.20; length:width 1.24. Propodal palm straight; stout denticulate setae serrate, 9 altogether; stout robust simple setae absent; 3 – 4 elongate broad based setae present. Ischium dorsal margin with at least 1 simple seta, seta robust. Basis length:width 2.36; dorsal setae positioned along ridge, approximately 9 altogether; ventrodistal margin with 2 – 3 elongate setae.

Pereopods II – III. Pereopod II length:body length 0.41. Dactylus length:propodus length 0.74; primary claw length:dactylar length 0.45. Propodus length:pereopod length 0.14; length:width 2.70. Carpus length:pereopod length 0.14; length:width 2.00. Basis length:pereopod length 0.26; length:width 2.45. Pereopod III length:body length 0.39. Dactylus length:propodus length 0.67; primary claw length:dactylar length 0.33. Propodus length:pereopod length 0.15; length:width 2.84. Carpus length:pereopod length 0.13; length:width 1.92. Basis length:pereopod length 0.26; length:width 2.67. Propodus broad based setae present, respectively 4, 3 on pereopods II and III; on pereopod II increasing in length from proximal seta (0.10 propodus length) to third seta (0.26 propodus length), distal shorter, evenly spaced from quarter-length to three-quarter length of propodus margin; on pereopod III proximal seta equal in length to distal seta (0.15 propodus length), second longest (0.22 propodus length), evenly spaced from one-third to two-thirds length along margin. Carpus broad based setae present, respectively 4, 4 on pereopods II and III; on pereopod II increasing

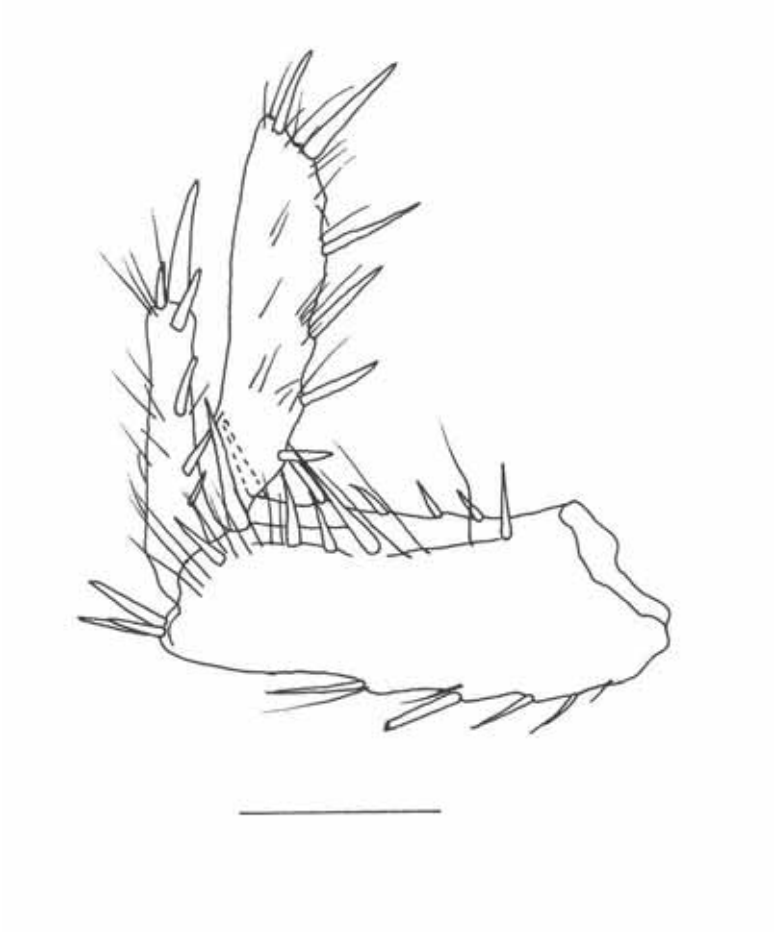


Figure 4.49: *Mesamphisopus tsitsikamma* n. sp., dissected male (SAM A44935). Uropod. Scale line 0.5 mm.

in length from proximal to distal setae, from 0.15 to 0.46 carpus length, evenly spaced proximally to two-thirds along ventral margin length; on pereopod III increasing in length from proximal to distal setae, from 0.18 to 0.65 carpus length, evenly spaced along ventral margin from proximally to two-thirds margin length.

Pereopod IV simple. Length:body length 0.35. Dactylus distal accessory claw approximately 0.50 length of primary claw. Propodus length:pereopod length 0.14; length:width 1.21; with 2 – 3 broad based setae on ventral margin. Carpus length:pereopod length 0.11; with 3 broad based setae on ventral margin. Ischium posterodistal margin with 3 setae, fourth anteriorly. Basis length:width 2.33; dorsal ridge with 9 – 11 setae.

Pereopods V – VII. Pereopod V length:body length 0.34. Dactylus claw length:dactylar length 0.47. Propodus length:pereopod length 0.14. Carpus length:pereopod length 0.14. Basis length:width 1.95. Pereopod VI length:body length 0.43. Dactylus claw length:dactylar length 0.32. Propodus length:pereopod length 0.14. Carpus length:pereopod length 0.15. Basis length:width 1.90. Pereopod VII length:body length 0.44. Dactylus claw length:dactylar length 0.37. Propodus length:pereopod length 0.15. Carpus length:pereopod length 0.14. Basis length:width 1.76.

Pleopods. Pleopod I length:body length 0.14. Exopod length:width 2.49. Endopod length:width 2.11; endopod length:exopod length 0.98. Pleopod II length:body length 0.16. Exopod length:width 1.97; length of distal article:exopod length 0.29. Endopod length:width 1.82; endopod length:exopod length 0.77. Pleopod III length:body length 0.17. Exopod length:width 1.72; length of distal article:exopod length 0.29. Endopod length:width 1.79; endopod length:exopod length 0.79. Pleopod IV length:body length 0.16. Exopod length:width 1.40; length of distal article:exopod length 0.30. Endopod length:width 1.64; endopod length:exopod length 0.88. Pleopod V length:body length 0.13. Exopod length:width 1.19; length of distal article:exopod length 0.51. Endopod length:width 1.27 – 1.44; endopod length:exopod length 0.56 – 0.64. Endopods I – V with setae on margins, setae plumose on I – IV, simple on V. Protopods medial margins/epipods with coupling hooks on I – IV, respective counts 4, 2, 2 and 2; with 2, 3, 4 and 4 – 5 elongate simple setae on pleopods II, III, IV and V respectively.

Uropod total length 1.72 pleotelson length. Protopod length:width 4.08, length 0.45 uropod total length; dorsomedial ridge length:endopod length 0.45. Endopod with 6 robust setae. Exopod length 0.78 endopod length; with 4 robust setae.

General Distribution. Known from the type locality only.

Remarks. The most distinguishing feature of *M. tsitsikamma* n. sp. is the dorsomedial margin of the peduncle of the uropod being scarcely produced, and relatively linear. The dorsomedial margin forms a ridge and is produced distally, forming a plate-like projection, in all of the species within the genus. While described as being weakly produced in *M. depressus* (Nicholls, 1943), the extent of the

projection figured for *M. depressus* (Nicholls, 1943: Figs 8.3 and 9.15) approaches the condition seen in, but still may be more produced than in *M. tsitsikamma* n. sp. An additional peculiarity of the species is the presence of plumose setae on all five pleopodal endopods, known otherwise only in *M. baccatus* n. sp., distinguishing these species from the remainder. As in *M. setosus* n. sp., a number of unique features are found among the mouthparts of *M. tsitsikamma* n. sp. The medial-distal setae of third article of the mandibular palp appear to be smooth in *M. tsitsikamma* n. sp., while being finely setulate in the remaining species described here. They are also few, with approximately ten present in *M. tsitsikamma* n. sp.; fewer have been documented in *M. depressus* (Nicholls, 1943), but 20 or more have been recorded in the remaining five species described above, with the greatest numbers found in *M. setosus* n. sp. and *M. paludosus* n. sp. Fewer setae are also encountered on the maxilla, than in the above species, particularly distally on the inner and outer lateral lobes, and in the ventral basal row of the medial lobe (although a similar number are found in this row in *M. paludosus* n. sp.). The pair of sub-apical dorsal robust setae on the pleotelson, recorded for *M. abbreviatus*, *M. depressus* and *M. penicillatus* (see Barnard, 1927; Kensley, 2001) and observed in *M. paludosus* n. sp., was not observed in all examined individuals (see Chapter 3).

4.4) Discussion

The description of these six species brings the number now known from South Africa to ten. This represents a substantial increase in the recognised diversity of the suborder within South Africa, ranking *Mesamphisopus* among the more speciose genera (e.g. *Colubotelson* Nicholls, 1944 (see Nicholls, 1944) and *Crenoicus* Nicholls, 1944 (see Wilson and Keable, 2001)) within the suborder, and results from minimal collection effort — the six described species being represented in only seven localities. Given the large areas remaining unsampled, the possibility of many species existing as cryptic species or closely related species complexes (e.g. Chapter 2, Chapter 3), and the additional fact that most *Mesamphisopus* species are known from their type localities only (see Barnard, 1927; Nicholls, 1943; above descriptions), it appears that the diversity of the group in South Africa is greatly underestimated. As suggested by Kensley (2001), potentially many more species remain to be examined and

described. Similar intensive collection, systematic and taxonomic studies have too increased the recognized diversity within Australia, where, until recently, fewer than 50 species were known (Wilson and Keable, 1999, 2001). Recent work, however, has led to the description of numerous new genera and species (Wilson and Ho, 1996; Knott and Halse, 1999; Wilson and Keable, 1999, 2002a, 2002b, 2004), with many new species being identified and awaiting description (see Wilson and Ho, 1996; Wilson and Johnson, 1999; Wilson and Keable, 2001, 2002a). Present extrapolations place the Australian diversity in excess of 200 species (Wilson and Keable, 2001).

Through the examination and comparison of the existing literature, it becomes apparent that a revision of the genus is required. This is not only necessary to provide detailed descriptions of the species, but to give some clarity on the importance of certain characters within the genus. The existing descriptions of the taxa, with the exception of that of *M. capensis*, are brief and inadequate. This criticism was raised by Nicholls (1943) in his revision, and while he improved upon the brief (paragraph) descriptions provided by Barnard (1927), the descriptions of *M. abbreviatus* and *M. depressus* were not as detailed as that provided for *M. capensis*, and offer relatively little to discriminate these species. Additionally, *M. penicillatus* remained unexamined. Since this revision, Kensley (2001) provided a key, and only brief diagnoses for the species of *Mesamphisopus*, including *M. penicillatus*. The diagnosis provided for *M. penicillatus* (and the others) included only the description of "external" features, i.e. setation, pleotelson shape, and pereopod I shape and setation. The pereopods, mouthparts, pleopods and uropods of *M. penicillatus* remain largely unexamined and their features unknown. In mitigation, however, Kensley's (2001) contribution was not intended to be a systematic or taxonomic account. The examination and comparison of the species described above calls into question the importance of certain features within *Mesamphisopus*.

For example, the presence of setae on the margins of the endopods of all five pleopods was regarded as a diagnostic (although not synapomorphic) character for *Mesamphisopus* (Nicholls, 1926, 1943). *Mesamphisopus paludosus* n. sp., described above, bears setae on the margins of the endopods of only the first two pleopods. The importance of certain variable characters (e.g. the presence of a cuticular fringe on the ventrodistal dactylus margin in *M. kensleyi* n. sp.), particularly of those on which taxonomic delineations have been based (e.g. the presence of subapical robust setae dorsally on the pleotelson), also needs to be assessed. The re-examination of the known species and the description of additional new species within *Mesamphisopus* will shed new light on the importance of the diagnostic characters mentioned earlier, and may highlight more diagnostic, potentially synapomorphic, characters of the genus. The resolution of the phylogenetic placement of *Mesamphisopus* within the Phreatoicidea (see Wilson and Johnson, 1999; Wilson and Keable, 1999, 2001, 2002b; Wilson and Edgecombe, 2003) will also be instructive in this regard.

The species described above, initially identified genetically, were able to be delineated morphologically, and can be identified, using only a combination of characters, including features of the mouthparts, pereopod I, pleopods, pleotelson and uropods, and coloration. The examination of additional material may possibly highlight a smaller suite of features useful for the diagnosis of species within the genus. There does not, however, appear to be a particular set of characters or features that are best suited for species delimitation within the Phreatoicidea, as different characters prove to be discriminatory in different genera. For example, among the recently examined genera, species have been distinguished on the basis of: features of the maxillipeds, pleopods and appendix masculina (*Crenoicus*: see Wilson and Ho, 1996); features of the maxillula, mandible and penes (*Phreatoicus*: see Wilson and Fenwick, 1999); spination of the propodal palm of pereopod I, setation of the body, appendix

masculina and uropodal protopod (*Synamphisopus*: see Wilson and Keable, 2002b); the shape of the uropodal protopod and setation of pereopod VII (*Phreatoicopsis*: see Wilson and Keable, 2002b); and, the relative sizes of the propodus of pereopod I and antennula articles (*Gariwerdeus* Wilson & Keable, 2002: see Wilson and Keable, 2002b). The features, shape and setation of the pleotelson and its medial and lateral lobes are used more extensively to delineate species in these genera (Wilson and Ho, 1996; Wilson and Fenwick, 1999; Wilson and Keable, 2002b), and may prove useful within *Mesamphisopus*.

The completion of a revision for this genus, deferred for the time being, will, however, be impeded by the poor condition of some of Barnard's syntypic series, particularly that of *M. abbreviatus*. The success of such an endeavour, alternatively hinges upon the acquisition of additional topotypic material. While Barnard's (1914) description (see too Sheppard, 1927; Nicholls, 1943) of the type locality of *M. capensis* is accurate, and abundant material has been recollected from this locality, the descriptions of the type localities of *M. abbreviatus* and *M. depressus* (Barnard, 1927, 1940; Nicholls, 1943) are more broad and equivocal. This is likely to be problematic given the apparently narrow distributions of certain species. The description of the locality of *M. penicillatus* provided by Barnard (1940) is accurate, but the locality is so influenced by human activity (now bordering on a residential area and popular coastal picnic site) that collection attempts have proved futile.

Chapter 5: Towards a multiple data set phylogeny for the known species of the endemic South African freshwater isopod genus *Mesamphisopus*: taxonomic and biogeographic implications.

5.1) Introduction

The ancient, and most basal (Wägele, 1989; Brusca and Wilson, 1991), isopodan suborder Phreatoicidea is represented in South Africa by ten known species belonging to the endemic genus *Mesamphisopus*. While four species (*M. abbreviatus, M. capensis, M. depressus* and *M. penicillatus*) of this genus have long been known to occur within isolated, predominantly high-altitude, freshwater habitats of the south-western Cape (Barnard, 1914, 1927, 1940; Nicholls, 1943; Kensley, 2001), recent interest in the group has led to the recognition (Chapter 2; Chapter 3) and description (Chapter 4) of six new species. These, mostly cryptic, species have primarily been delineated using a combination of allozyme and mtDNA sequence data, coupled with morphometric data.

The evolutionary relationships of the species within the genus are, however, largely unknown. Probably with so few species being recognized earlier, no systematists examining species of *Mesamphisopus* (Barnard, 1927; Nicholls, 1943) ventured to discuss the evolutionary relationships among the species of the genus. In addition, with morphological differentiation among species being subtle, few relationships can be readily and unambiguously proposed using morphological characters (see Chapter 4). For example, only close relationships between *M. albidus* and *M. setosus,* and *M. penicillatus* and *M. paludosus* were suggested in the description of new species (Chapter 4), while published descriptions would indicate a

close morphological affinity between *M. abbreviatus* and *M. depressus*, and perhaps *M. penicillatus* (Nicholls, 1943; Kensley, 2001).

Notwithstanding the interest in the flora of the southern and south-western Cape, many aspects of the biogeography and ecosystem evolution of the region remain poorly understood (Deacon, 1983). With the exception of Barnard's (1927) discussion of the probable factors influencing the distribution of *Mesamphisopus*, biogeographic patterns were also not discussed in earlier work, perhaps also due to a paucity of material. With the recognition of more taxa, however, a well-resolved phylogeny can provide the framework with which to examine these patterns and can contribute significantly to the understanding of the biogeography and evolutionary processes within the region. It is therefore aimed, through this study, to present a phylogeny for the genus *Mesamphisopus*, based on the independent and combined analyses of sequence data derived from two mitochondrial DNA gene regions and allele frequency data derived from the electrophoresis of 12 allozyme loci.

Freshwater organisms are generally restricted to drainages and associated water bodies and their dispersal, distributions and evolutionary relationships are determined by geology and hydrographic processes, such as river captures (Jubb, 1964; Tsigenopoulos, Karakousis and Berrebi, 1999; Wong, Keogh and McGlashan, 2004). Studying the evolutionary relationships and biogeography of freshwater organisms can provide novel insights and an independent assessment of geological patterns or drainage basin evolution (Waters *et al.*, 2001). In this regard, ancient freshwater groups, such as the phreatoicidean isopods and paramelitid amphipods (see Stewart, 1992), may be instructive in providing an organismal assessment of the hydrogeographic evolution of the southern and south-western Cape and may be representative of, or produce comparable biogeographic patterns to that of many freshwater

taxa of the region. Fossil evidence has indicated a freshwater existence for phreatoicideans since the Middle Triassic (*ca* 236 Myr) (Chilton, 1918; Wilson and Edgecombe, 2003), while the distribution of taxa (particularly belonging to the sub-family Phreatoicopsinae) in Australia suggests an exclusive occupation of freshwater habitats since Cretaceous, Jurassic or even earlier times (Nicholls, 1944). With major cladogenic events (e.g. the divergence of Nicholls' (1943, 1944) families Amphisopodidae and Phreatoicidae) occurring prior to the fragmentation of Gondwana (Wilson and Johnson, 1999; Wilson and Edgecombe, 2003), it is likely that phreatoicideans were represented within their present South African distribution since similar early Mesozoic times. If so, their occurrence within southern Africa may have followed shortly upon the orogenic episodes (278 – 215 Myr) resulting in the formation of the Cape Fold Mountains (Deacon, 1983; Linder, 2003), and coincided temporally with the subsequent major erosion and deposition cycles, uplift and denudation, and the later drastic Cenozoic climate and sea-level changes (see Hendey 1983a, b; Deacon, 1983; Linder, 2003). These would have, in sculpting the present landscape, influenced evolutionary patterns within the genus and the contemporary distribution of its constituent species.

Previous work (Chapter 3), employing independent analyses of allozyme data and sequence data from the mitochondrial protein-coding cytochrome *c* oxidase subunit I (COI) gene region, had largely failed to resolve relationships or extricate species boundaries among populations initially identified as *M. abbreviatus* or *M. depressus*. Here, representatives of each of these populations are included, along with representatives of all recognized southern African taxa, to assess whether the sequencing of a fragment of the 12S rRNA gene, as well as the combined analyses of allozyme and sequence data, would additionally resolve relationships among these populations and shed new light on their taxonomic status.

5.2) Materials and methods

5.2.1) Taxonomic sampling

5.2.1.1) Specimens:

Twenty-three ingroup taxa were included in the phylogenetic analyses of sequence data. Multiple representatives of each taxon were included, where possible, from geographically distant localities. As the relationships among, and the specific status of, representative populations belonging to the *M. abbreviatus – depressus* group were largely unresolved (see Chapter 3), a representative from each of the sampled populations was included in the analyses. These taxa are identified by their collection localities (Table 5.1).

5.2.1.2) Outgroup selection:

Sequences of *Paramphisopus palustris* (12S rRNA: AF259523; COI: AF255777) and *Colubotelson thomsoni* Nicholls, 1944 (12S rRNA: AF259525; COI: AF255775) were retrieved from GenBank, while the two gene fragments were sequenced from a single *Amphisopus* individual, to be used as outgroups. Cladistic analyses of morphological characters (Wilson and Johnson, 1999; Wilson and Keable, 1999, 2001, 2002b; Wilson and Edgecombe, 2003) have generally failed to consistently resolve the phylogenetic placement of, and relationships among, several phreatoicidean genera. *Mesamphisopus* is, albeit inconsistently, regarded as the most basal phreatoicidean genus in these analyses, and its sister taxa are not clear, complicating the choice of outgroup. Nonetheless, *Mesamphisopus* (presently in the family Mesamphisopodidae) was previously included in the same family (Amphisopodidae), albeit in a different subfamily, as *Paramphisopus* and *Amphisopus* (Nicholls, 1943). Knott and Halse (1999) have further hinted at a possible sister-group

Table 5.1: Taxa (23 ingroup taxa and three outgroups) included in the analyses of sequence data. Where possible, multiple representatives of taxa were included from geographically distant localities. Taxa belonging to the *Mesamphisopus abbreviatus – depressus* complex are identified, and subsequently referred to in the text, by collection locality. Accession numbers of sequences of both mitochondrial gene fragments (12S rRNA and COI) obtained from GenBank are provided for the outgroup specimens and, where available, for sequences generated in Chapter 2. Sequences of both gene fragments were derived from the same representative individual, with the exception of two cases (indicated by asterices), where sequences of each fragment were derived from different individuals from the same collection lot.

Taxa		Collection locality	GenBank accession numbers	
			12S rRNA	COI
Ingroup				
Mesamphisopus abbreviatus-depressus	Barrydale	Melmoth Nature Reserve, Langeberg Mountains, Western Cape	This study	Chapter 3
	Betty's Bay	Harold Porter Botanical Gardens, Betty's Bay, Western Cape	This study	Chapter 3
	Grabouw	Grabouw, Hottentots Holland Mountains, Western Cape	This study	Chapter 3
	Greyton	Greyton, Riviersonderend Mountains, Western Cape	This study	Chapter 3
	Grootvadersbos	Grootvadersbos Nature Reserve, Langeberg Mountains, Western Cape	This study	Chapter 3
	Kogelberg	Kogelberg, Hottentots Holland Mountains, Western Cape	This study	Chapter 3
	Protea Valley	Protea Valley, Melmoth Nature Reserve, Langeberg Mountains, Western Cape	This study	Chapter 3
	Riversdale	Riversdale, Garcia's Pass, Riversdale Mountains, Western Cape	This study	Chapter 3
	Steenbras 1	Grabouw plantation, Hottentots Holland Mountains, Western Cape	This study	Chapter 3
	Steenbras 2	Steenbras Dam, Hottentots Holland Mountains, Western Cape	This study	Chapter 3
	Steenbras 3	Boskloof Peak, Hottentots Holland Mountains, Western Cape	This study	Chapter 3
	Tradouw Pass	Tradouw's Pass, Langeberg Mountains, Western Cape	This study	Chapter 3
	Wemmershoek	Wemmershoek dam, Klein Drakenstein Mountains, Western Cape	This study	Chapter 3
Mesamphisopus albidus		Franschhoek Pass, Hottentots Holland Mountains, Western Cape	AY322180	This study
Mesamphisopus baccatus		Silvermine, Cape Peninsula, Western Cape	AY322176	This study
*Mesamphisopus kensleyi**		Gordon's Bay, Hottentots Holland Mountains, Western Cape	AY322182	This study
Mesamphisopus capensis 1*		Echo Valley, Table Mountain, Cape Peninsula, Western Cape	AY322172	This study
Mesamphisopus capensis 2		Schuster's River, southern Peninsula, Western Cape	AY322179	This study
Mesamphisopus paludosus 1		"Crane's Nest", Agulhas Plain, Western Cape	This study	This study
Mesamphisopus paludosus 2		"Ratels River", Agulhas Plain, Western Cape	This study	This study
Mesamphisopus penicillatus		Stanford, Western Cape	AY322183	This study
Mesamphisopus setosus		Jonkershoek Nature Reserve, Hottentots Holland Mountains, Western Cape	AY322181	This study
Mesamphisopus tsitsikamma		Storms River, Tsitsikamma forest, Eastern Cape	This study	This study
Outgroups				
Amphisopus sp.		King River, Albany, Western Australia	This study	This study
Colubotelson thomsoni		Collection details unavailable (Wetzer, 2001)	AF259525	AF255775
Paramphisopus palustris		Collection details unavailable (Wetzer, 2001)	AF259523	AF255777

relationship among these respective former subfamilies. *Paramphisopus* and *Amphisopus*, thus, appear to be taxonomically the most closely related to *Mesamphisopus* of the available outgroup specimens (see Nicholls, 1943). *Colubotelson*, included as a more distantly related outgroup, belongs to the family Phreatoicidae and appears more derived than *Mesamphisopus* in the morphological phylogenies (Wilson and Keable, 2001, 2002b; Wilson and Edgecombe, 2003). As no suitable material was available to perform allozyme electrophoresis on any outgroup population, trees derived in the cladistic analysis of the allozyme data were rooted using the most basal ingroup taxa, as revealed by the sequence data analyses. Allozyme data for the outgroup taxa were coded as missing in the total data analysis.

5.2.2) MtDNA sequencing and sequence data analyses

Sequence data for the 12S rRNA and COI gene fragments had been collected earlier for certain representative taxa or populations (Table 5.1; Chapters 2; Chapter 3). This data set was augmented here to include sequences of the two gene fragments generated from the same representative individual, where possible. In the case of the *M. capensis* 1 (Echo Valley) and *M. kensleyi* representatives, each of the fragments was sequenced from a different individual from the same collection lot.

For the *Amphisopus* individual and a number of representatives for which sequence data had not been collected earlier (*M. paludosus* and *M. penicillatus*), total genomic DNA was extracted from representative individuals using commercial extraction kits and protocols as discussed earlier (Chapter 2; Chapter 3). Polymerase chain reactions (PCRs) were set up, using the 12SCRF and 12SCRR (Wetzer, 2001) primer pair to amplify the 12S rRNA gene fragment, and the LCO1490 and HCO2198 (Folmer *et al.*, 1994) primer pair to amplify the

COI gene fragment, respectively. As amplification of certain individuals was problematic, due to degraded DNA, internal primers (COI-intF and COI-intR; Chapter 3) were used in combination with the Folmer *et al.* (1994) primer pair to amplify the latter fragment in these individuals (see Chapter 3). PCR protocols and thermo-cycling regimes have been reported earlier (Chapters 2; Chapter 3). Following purification of PCR products, using commercial kits, and standard Big-Dye (ABI Prism, Perkin-Elmer) chemistry cycle-sequencing, samples were analysed using an AB 3100 automated sequencer.

Upon inspection of chromatograms, sequences of the 12S rRNA data partition were aligned using Clustal X 1.81 (Thompson *et al.*, 1997). As the default gap opening and gap extension penalties produced alignments determined to be spurious by visual inspection, a gap penalty of 9.00 and gap extension penalty of 6.66 were implemented for pair-wise and multiple sequence alignment. The default settings of all other parameters were maintained. Other gap penalties investigated produced alignments of equal length to that obtained with the above parameters and with comparable numbers of parsimony informative characters (118 – 119 characters), but provided trees that were substantially less parsimonious in preliminary analyses (results not shown). Sequences of the protein-coding COI partition were aligned manually. The accuracy of the sequences and the functionality of this fragment were examined by translation to amino acid residues based on the *Drosophila* mitochondrial code in MacClade 4.05 (Maddison and Maddison, 2000). In both alignments, sequences were trimmed to equal length by removing gaps at the ends.

Gene fragments were analysed independently, in combination (the combined mtDNA data set), and in combination with the recoded allele frequency data (see below) from the allozyme analysis (the total data set), using PAUP*4b10 (Swofford, 2001). Phylogenies were

reconstructed using three approaches (parsimony, maximum likelihood and Bayesian inference), discussed below. For the combined analyses, data partitions were concatenated following the determination of combinability, using the Incongruence Length Difference test (ILD; Farris *et al.*, 1994, 1995) — the partition homogeneity test as implemented in PAUP*. Following Wetzer (2002), the ILD test was performed including variable characters only, in order to negate unequal informative: uninformative character ratios among the partitions in the resampling of characters.

5.2.2.1) Parsimony analyses:

All parsimony analyses (including the independent analysis of the recoded allele frequency data) were conducted using only parsimony informative characters. Heuristic searches were employed using the Tree-Bisection-Reconnection (TBR) algorithm, accelerated (ACCTRAN) character optimisation and a random addition of taxa (1000 replicates) to find the most parsimonious tree. Gaps/indels (restricted to the 12S rRNA partition) were regarded as missing data (but see below). Missing data, generally restricted to only one of the outgroup representatives (*Paramphisopus palustris*) in the COI data partition, were not excluded from the analyses (resulting in the exclusion of alignment positions where the missing data occur). In all parsimony analyses, phylogenetic confidence in the relationships was determined by nonparametric bootstrapping (Felsenstein, 1985), using 1000 pseudo-replicates, each with 100 random additions of taxa. As weighting schemes are often arbitrary and rarely justified, and do not always provide a more resolved phylogenetic hypothesis (Baker, Wilkinson and DeSalle, 2001; Creer, Malhotra and Thorpe, 2003), characters were unweighted in all present analyses.

While treating gaps (indels) introduced into an alignment as fifth character states has been shown to be phylogenetically inappropriate and untenable (Simmons and Ochoterena, 2000), the omission of gaps (or their treatment as missing data) is equally undesirable, as potentially informative, historically significant events are ignored (Giribet and Wheeler, 1999). The inclusion of coded gaps, for which various coding methodologies have been proposed, in analyses has been shown to introduce less homoplasy than nucleotide characters, to improve topology and resolution, and to increase branch support (Simmons and Ochotorena, 2000; Simmons, Ochoterena and Carr, 2001). The effect of coding gaps introduced into the 12S rRNA sequence alignment was also explored using parsimony analysis, with gaps coded as present or absent according to the "simple indel coding" procedure of Simmons and Ochoterena (2000).

5.2.2.2) Maximum likelihood analyses:

Prior to the independent analysis of each of the mtDNA data partitions, MODELTEST 3.06 (Posada and Crandall, 1998) was used to determine the optimal model of nucleotide substitution for each partition. The parameters of the most appropriate model were then employed in the ML tree search. Heuristic searches were employed to find the most likely topology. Confidence in the nodes was determined by bootstrapping (Felsenstein, 1985), using 100 pseudo-replicates. Phylogenies were not inferred for the combined mtDNA data set and total data set using ML. This was primarily motivated by cumbersome computational times.

5.2.2.3) Bayesian inferences of phylogeny:

Bayesian inference is a likelihood-based approach that aims for the incorporation of prior knowledge (e.g. a prior probability distribution of trees), and provides a logical representation

of uncertainty in phylogenetic reconstructions (Lewis, 2001a; Huelsenbeck *et al.*, 2002; Archibald, Mort and Crawford, 2003). The approach is, moreover, computationally efficient (Huelsenbeck *et al.*, 2002), particularly as topological hypotheses and nodal support are evaluated simultaneously (Lewis, 2001a). For full overviews of the procedure, the Bayesian-statistical underpinnings, applications and considerations (as well as references to the key technical literature) consult Lewis (2001a), Huelsenbeck *et al.* (2002), Archibald *et al.* (2003) and Nylander *et al.* (2004).

Phylogenies were inferred using Bayesian methods for the independent data partitions (12S rRNA and COI) and, as MRBAYES (unlike PAUP*) can independently estimate model parameters for each of the partitions in a combined analysis, for the combined mtDNA data set. While stochastic evolutionary models for discrete morphological data have recently been proposed (Lewis, 2001b), incorporated in MRBAYES (see Hipp, Hall and Sytsma, 2004) and used in combined analyses (Nylander *et al.*, 2004), the application of these or similar models to the binary-coded, allele frequency data is, as far as is known, unprecedented. Thus, Bayesian inferences of phylogeny and ML (above) were not considered for the total data set. Four Markov chains (three heated and one cold) were started from a random tree and run simultaneously for 1 000 000 generations in each analysis. Trees, likelihood scores and estimates of substitution parameters were sampled from the posterior probability distribution every fifty generations. Stationarity (convergence) was determined by using the *sump*-command in MRBAYES. The generations (and hence trees) sampled prior to stationarity being attained were discarded as “burn-in”. Majority-rule consensus trees were constructed from the remaining trees sampled, these approximating the posterior probability distribution of trees, with the frequency of a clade being retrieved representing the posterior probability of that clade being true given the priors, data and model (but see Simmons, Pickett and Miya,

2004). To confirm that the Markov chains converged upon and sampled similar regions of the posterior distribution, rather than trees with similar likelihood scores from different regions of the distribution, four independent MRBAYES (version 3.0b3; Huelsenbeck and Ronquist, 2001) runs were performed each time. For the independent analysis of each partition, the General Time Reversible (GTR) model (Rodríguez *et al.*, 1990) of sequence evolution, with a proportion of invariant sites and a Γ-distribution of variable sites was implemented. Individual parameters were estimated by MRBAYES. In the combined mtDNA analysis, the parameters of the GTR model were estimated for each partition, independently.

5.2.3) Application of a molecular clock

The time of divergence of clades was determined using the relaxed Bayesian molecular clock of Thorne, Kishino and Painter (1998), and Thorne and Kishino (2002). This approach relaxes the requirements of the molecular clock, i.e. uniform evolutionary rates among lineages or among molecular markers (Rambaut and Bromham, 1998), and accommodates variable rates among genes or lineages through continuous autocorrelation of rates along branches, enabling multiple data partitions, with differing evolutionary models, to be used to date divergences (Thorne and Kishino, 2002; Yang and Yoder, 2003; Hassanin and Douzery, 2003). The method also allows multiple independent calibration points and the inclusion of lower and upper bounds on the divergence time of nodes (Thorne and Kishino, 2002; Yang and Yoder, 2003; Hassanin and Douzery, 2003; Schrago and Russo, 2003). A Bayesian approach, using a computationally efficient Markov chain Monte Carlo algorithm, is adopted to derive a posterior distribution of rates and divergence times, with the prior distribution of rates provided by a stochastic model of evolutionary change (Yang and Yoder, 2003).

Here, both the 12S rRNA and COI partitions were used to date divergences. First, given the respective ML topologies for each of the partitions, base frequencies and substitution parameters (assuming eight discrete rate categories) of the F84 model (Felsenstein, 2002) were determined for each partition using the BASEML program of the PAML (Version 3.14; Yang, 1997) package. The ESTBRANCHES program of the MULTIDIVERGENCE (Thorne and Kishino, 2002) package was then used to estimate, for each of the data partitions, the ML branch lengths of the outgroup-rooted topology on which the divergences are dated, and their variance-covariance matrices. Finally, the MULTIDIVTIME program of the latter package was used to estimate the prior and posterior distributions of substitution rates and the ages of the divergence of clades, together with their respective 95% credibility intervals. Although many major cladogenic events within the Phreatoicidea are thought to predate the fragmentation of Gondwana (Wilson and Johnson, 1999; Wilson and Edgecombe, 2003), the maximum time between the root and tip was set to be 140 Myr (with a standard deviation of 70 Myr), reflecting Gondwanan fragmentation (see below). The rate of evolution at the root node, determined from the median of the individual root to tip lengths for both data partitions, was set at 0.006 substitutions per site per Mya (SD = 0.006). Two prior time constraints were placed on nodes. As fragmentation of Gondwana was initiated some 140 Myr ago and completed about 100 Myr ago (Hendey, 1983b), the divergence between the western Australian *Amphisopus* – *Paramphisopus* clade and the southern African ingroup (*Mesamphisopus*) was liberally constrained to be no younger than 100 Mya. Earlier analyses (Chapter 2) had suggested that divergence between the taxa of the Cape Peninsula and those from Hottentot's Holland Mountains had been brought about by transgression-regression events relating to Cenozoic climate change. Although it cannot be determined with confidence which of these events led to the divergence of these populations or taxa, and the dating of these events is somewhat speculative (Hendey, 1983b), the divergence of the Cape

Peninsula clade (*M. capensis* - *M. baccatus*) from those remaining ingroup taxa occurring on the Hottentot's Holland Mountains and eastwards (see below) was constrained to be no older than 20 Myr. This corresponds to the onset of the first major Miocene transgression episode (Hendey, 1983b). After an initial "burn-in" of 10 000 generations, the Markov chain was run for 10 000 generations, sampling every 100th generation. Four independent runs were conducted to monitor convergence of the Markov chains, while one approximation of the prior distribution was obtained for examination, following Yoder *et al.* (2003).

5.2.4) Allozyme electrophoresis and data analyses

Twenty-three populations from identical sampling localities as the ingroup representatives sequenced in the mtDNA study were included in the allozyme study. Allele frequency data, derived from at least 20 individuals, for certain included populations have been reported earlier (Chapter 2; Chapter 3). For newly included populations, allele frequency data were collected by starch gel electrophoresis using identical buffer systems, running conditions, staining recipes and scoring approach (Chapter 2; Chapter 3). Allozyme differentiation was assayed at ten enzyme systems, encoded by 12 loci (see Table 2.1). The scoring of alleles and the determination of mobilities were standardized across all populations by the inclusion of a reference population in sequential runs (see Chapter 3), or by the direct side-by-side running of representatives of all alleles.

Thus, following calculation of allele frequencies at the twelve examined loci, Cavalli-Sforza and Edwards (1967) chord distances (CSE) among these populations were calculated using BIOSYS-1 (Swofford and Selander, 1981). This distance measure, in combination with the Neighbour-Joining (Saitou and Nei, 1987) tree reconstruction, provides better estimates of

topology than commonly used Nei (1978) distances or other distance measures (Wiens, 2000; Monsen and Blouin, 2003). Using MEGA2.1 (Kumar *et al.*, 2001), a midpoint-rooted neighbour-joining tree was then constructed based upon these distances.

The use of allozyme data, and in particular allele frequency data, in phylogenetic analyses has been widely criticized in the past, principally on the grounds that allele frequencies are not temporally stable (Crother, 1990). However, proponents favouring phylogenetic/cladistic approaches over phenetic approaches and other authors (e.g. Mickevich and Johnson, 1976; Farris, 1981; Mickevich and Mitter, 1983; Buth, 1984; Lessios and Weinberg, 1994; King and Hanner, 1998) have suggested that it is qualitative differences (i.e. composition of allelic arrays), rather than quantitative differences (i.e. allele frequencies), that are evolutionarily most significant and perhaps of greater utility in determining the systematic relationships among populations. Additionally, recent isopod studies have demonstrated the temporal stability of allele frequencies (Lessios *et al.*, 1994) or allele frequency differences (Piertney and Carvalho, 1995a), in the face of presumed drastic demographic changes, suggesting that contemporary allele frequency "snap shots" may be instructive of the evolutionary history of populations or taxa.

The cladistic analysis of allozyme data thus proceeded with alleles being coded as present or absent in populations (OTUs) following the procedure of Mickevich and Johnson (1976), termed the "independent allele" model by Mickevich and Mitter (1981). Following Michevich and Johnson (1976), alleles were coded as present if they occurred at a frequency ≥ 0.05 in a particular population. After coding, parsimony analysis was performed in PAUP, with statistical support for nodes assessed by bootstrapping, as above.

The use of presence/absence coding of alleles has been criticized and regarded as phylogenetically unsuitable (for detailed criticism see Murphy, 1993). The use of loci as characters has been suggested as a viable and phylogenetically defensible alternative to using alleles as characters, and various methodologies for coding, ordering and polarizing allelic arrays (character states) have been proposed (Mickevich and Mitter, 1981, 1983; Buth, 1984; Murphy, 1993; Hillis, 1998; Wiens, 2000). These approaches were not considered here, however, as the number of loci would have yielded fewer characters (12 loci) than the number of included taxa (23 OTUs). An additional concern with the coding methodology employed here, not resolved without allele frequency data from more basal outgroups, is that persistent ancestral (plesiomorphic) alleles shared among populations, often at low frequency (Murphy, 1993), are incorrectly interpreted as synapomorphies (Avise, 1983), violating the Hennigian principles on which cladistic analyses are based. Given these concerns, the allele-based cladistic analysis presented here serves mostly as a point of comparison with topologies derived from the distance-based (phenetic) examination of relationships among populations and phylogenetic analyses of the sequence data.

5.3) Results

5.3.1) 12S rRNA mtDNA

Individual sequences were aligned and, following the trimming of the ingroup sequences and the removal of an ambiguously aligned eight nucleotide region at the 5'-end of the alignment, provided 328 nucleotide characters (Appendix 9). Base frequencies showed an AT-bias (A = 0.403, C = 0.121, G = 0.122, T = 0.354), but were homogenous (χ^2 = 44.728, df = 75, *P* =

0.998) across all the included taxa. The alignment included 147 variable characters (excluding gaps), of which 107 were parsimony informative. Considering the ingroup only, 89 characters were variable. Parsimony analysis of this alignment yielded 155 trees of 254 steps (CI = 0.610, RI = 0.713; Rescaled CI = 0.435).

A region of particular alignment ambiguity, corresponding to positions 153 to 178, inclusive, of the above trimmed alignment, was identified. This region corresponds to loop region designated as helices 39 and 40 of Van Raay and Crease's (1994) inferred secondary structure of the 12S rRNA molecule in *Daphnia pulex*. In an attempt to improve resolution, this region was omitted in a preliminary parsimony analysis, resulting in the loss of eight parsimony informative characters. Parsimony analysis of this reduced alignment retrieved 156 equally parsimonious trees of 225 steps (CI = 0.622, RI = 0.728, Rescaled CI = 0.453).

In the investigation of the effect of coding gaps, thirty-three unique gaps (having different 5' and 3' termini) were recognized and coded as present or absent. With the inclusion of these recoded gaps, and exclusion of alignment positions where gaps were present, the 132 parsimony informative characters provided 90 equally parsimonious trees of 292 steps (CI = 0.616, RI = 0.733, Rescaled CI = 0.452). However, neither the omission of ambiguous alignment regions, nor the coding of gaps provided a substantially improved phylogeny. Indeed, fewer relationships were resolved in these analyses (strict consensus trees not shown) than in the analysis of the initial 328 nucleotide character matrix. Subsequent analyses of the combined data partitions proceeded with this unaltered data set, while discussion and comparison of topologies concerns the strict consensus (Fig. 5.1a) of the 155 trees obtained from its analysis.

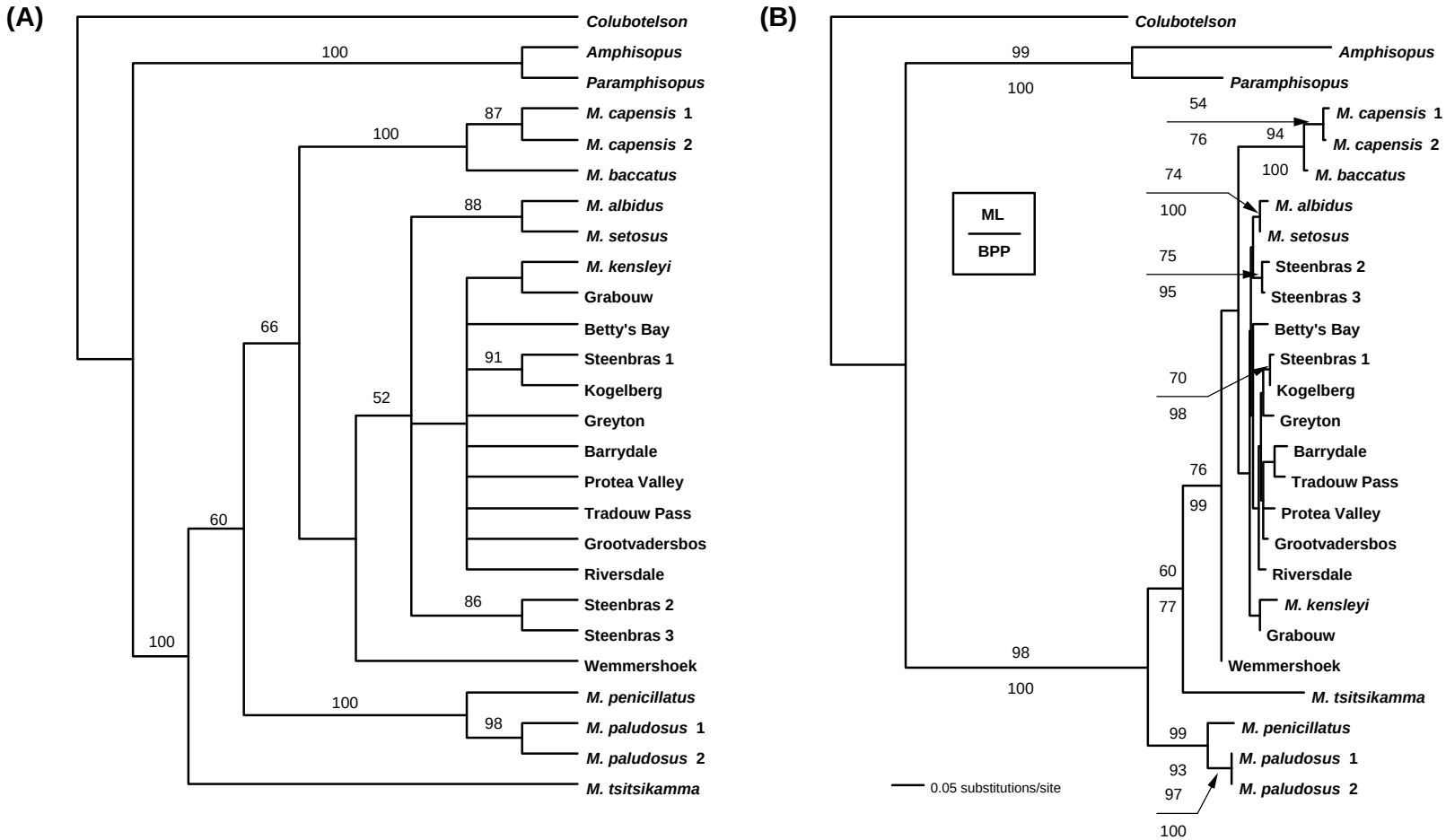


Figure 5.1: (A) Strict consensus of 155 trees obtained in the parsimony analysis of 328 nucleotides of the 12S rRNA mtDNA fragment, in 23 *Mesamphisopus* and three outgroup (*Colubotelson*, *Amphisopus* and *Paramphisopus*) representatives. Numbers above the branches indicate bootstrap (Felsenstein, 1985) support calculated from 1000 replicates (with 100 random taxon addition iterations). Only bootstrap support > 50% is indicated. (B) Maximum likelihood tree (-lnL = 1970.852) from analysis of the same gene fragment with the implementation of a GTR + Γ model of nucleotide evolution (consult Table 5.2). Numbers above the branches indicate bootstrap support (100 pseudo-replicates). Numbers below the branches represent the lowest of the Bayesian Posterior Probabilities (BPPs), presented as percentages for ease of comparison, obtained in the four independent Bayesian inferences of phylogeny. Only bootstrap support > 50% and BPPs > 75% are indicated.

The hierarchical likelihood ratio test (hLRT; Huelsenbeck and Crandall, 1997) and the Akaike Information Criteria (AIC; Akaike, 1974) employed in MODELTEST each suggested a different substitution model. These were, respectively, the GTR (hLRT) and TIM models (AIC), both with a gamma-distribution of variable sites. Substitution parameters for each of the models are presented in Table 5.2. Topologies obtained with the implementation of each of the models were identical (Fig. 5.1b; GTR + Γ: -lnL = 1970.862; TIM + Γ: -lnL = 1971.511). The bootstrap analysis proceeded using the parameters of the GTR + Γ model.

In the Bayesian inference, stationarity was achieved after the first 20 000 generations, resulting in the discarding of 401 trees and data sampled from the "burn-in" in each of the four runs. Similar clade probabilities were obtained and model parameters estimated in each of the four runs, indicating convergence upon the similar regions of the posterior distribution of trees. The mean base frequencies and substitution parameters estimated at each of the sampled post-"burn-in" generations are presented in Table 5.2 for each of the four runs. Identical majority-rule consensus trees were obtained from the remaining 19 600 trees in each of the four independent runs. The Bayesian inference topologies were congruent with the ML phylogram, and the Bayesian posterior clade probabilities (BPPs) are indicated on Figure 5.1b.

The topologies derived from the independent analyses of the 12S rRNA data partition (above) and the COI partition (below) are discussed together with, and in reference to, the topologies derived from the analyses of the combined mtDNA partitions (below).

Table 5.2: Likelihood scores, base frequencies, and substitution parameters (including the proportion of invariant sites (I), and the α-shape parameter of the Γ-distribution of variable sites) for implementation in the maximum-likelihood analyses of the 12S rRNA and COI mtDNA sequence data partitions, determined using MODELTEST (Posada and Crandall, 1998), implementing hierarchical likelihood ratio tests (hLRT: Huelsenbeck and Crandall, 1997) and the Akaike Information Criteria (AIC: Akaike, 1974). These parameters, sampled from the posterior probability distribution by the four Markov chains in the Bayesian inference of phylogeny are also presented for each of the data partitions. Means for each parameter and standard deviations (presented below) were calculated from the sampled post-"burn-in" generations for each of the four independent MRBAYES runs performed on each partition.

			-lnL	Base frequencies				Substitution rate matrix (G ↔ T = 1.000)					I	α
				A	C	G	T	A ↔ C	A ↔ G	A ↔ T	C ↔ G	C ↔ T		
12S rRNA	ModelTest	hLRT	1979.749	0.409	0.094	0.137	0.360	1.704	3.755	1.919	0.971	16.375	-	0.351
		AIC	1980.497	0.415	0.092	0.129	0.364	1.000	3.149	1.430	1.430	13.002	-	0.344
	MRBAYES	Run 1	2073.088	0.393	0.092	0.110	0.405	2.688	6.336	1.171	3.381	33.466	0.060	0.159
			±9.843	±0.021	±0.010	±0.012	±0.022	±1.345	±2.632	±0.535	±2.498	±10.794	±0.043	±0.019
		Run 2	2071.972	0.392	0.092	0.110	0.406	3.162	7.200	1.304	4.692	37.638	0.061	0.160
			±9.578	±0.020	±0.010	±0.012	±0.021	±1.544	±2.771	±0.575	±3.516	±10.369	±0.046	±0.021
		Run 3	2073.098	0.391	0.094	0.109	0.405	2.522	7.135	1.279	3.278	31.231	0.067	0.162
			±9.582	±0.020	±0.010	±0.012	±0.021	±1.405	±2.953	±0.627	±1.937	±11.662	±0.046	±0.020
		Run 4	2071.840	0.394	0.093	0.111	0.402	2.370	5.448	1.096	2.634	30.196	0.063	0.161
			±9.759	±0.021	±0.010	±0.012	±0.021	±1.281	±1.934	±0.475	±1.621	±10.821	±0.042	±0.017
COI	ModelTest	hLRT	3921.578	0.317	0.107	0.129	0.447	0.148	11.084	0.907	1.372	4.771	0.315	0.443
		AIC	3921.578	0.317	0.107	0.129	0.447	0.148	11.084	0.907	1.372	4.771	0.315	0.443
	MRBAYES	Run 1	3985.125	0.322	0.089	0.125	0.464	0.376	26.306	0.879	3.083	9.074	0.349	0.386
			±13.733	±0.014	±0.009	±0.009	±0.016	±0.339	±5.899	±0.388	±1.373	±3.421	±0.057	±0.075
		Run 2	3987.518	0.321	0.091	0.124	0.465	0.339	30.178	0.907	3.159	8.700	0.351	0.382
			±15.511	±0.014	±0.009	±0.009	±0.017	±0.311	±9.240	±0.441	±1.617	±3.593	±0.053	±0.070
		Run 3	3980.342	0.321	0.090	0.126	0.463	0.463	26.838	1.037	3.333	9.855	0.337	0.389
			±13.693	±0.014	±0.009	±0.009	±0.017	±0.505	±8.793	±0.518	±1.635	±4.826	±0.066	±0.079
		Run 4	3987.218	0.321	0.091	0.124	0.464	0.342	28.670	0.897	3.183	8.928	0.352	0.386
			±14.214	±0.014	±0.010	±0.009	±0.017	±0.332	±8.357	±0.486	±1.933	±4.487	±0.055	±0.073

5.3.2) Cytochrome oxidase c *subunit I (COI)*

After aligned sequences were trimmed to equal length, and two uninformative nucleotide positions were removed from the end of the alignment (to allow the alignment to contain only complete codons), 585 nucleotide characters were available for analysis (Appendix 10). These included 272 variable characters, of which 218 were parsimony informative. Of the variable characters, 66 (24.3%), 30 (11.0%) and 176 (64.7%) were found in first, second and third codon positions, respectively. Significant heterogeneity (χ^2 = 123.418, df = 75, P < 0.001) in base frequencies was observed among the included taxa. However, upon the omission of the *Paramphisopus* outgroup representative, possessing much missing data for this partition, base frequencies among the remaining taxa were not significantly different (χ^2 = 69.877, df = 72, P = 0.549) and were again AT-rich (A = 0.232, C = 0.130, G = 0.186, T = 0.453). Parsimony analysis of the total 218 parsimony informative characters provided three equally parsimonious trees of 642 steps (CI = 0.525, RI = 0.639, Rescaled CI = 0.336). The strict consensus of these trees (Fig. 5.2a) appeared to have more internal relationships resolved than in the analyses of the 12S rRNA partition.

MODELTEST, using both the hLRT and AIC criteria, suggested the use of a General Time Reversible model, with a proportion of invariant sites and a gamma-distribution of variable sites (GTR + I + Γ) to be the most appropriate for the data set. The substitution parameters of the model are presented in Table 5.2. The ML tree (-lnL = 3918.843) is presented in Figure 5.2b.

In the Bayesian inference, the first 10 000 generations were determined to represent the "burn-in" period. As a result, 201 trees were discarded and the majority-rule consensus trees

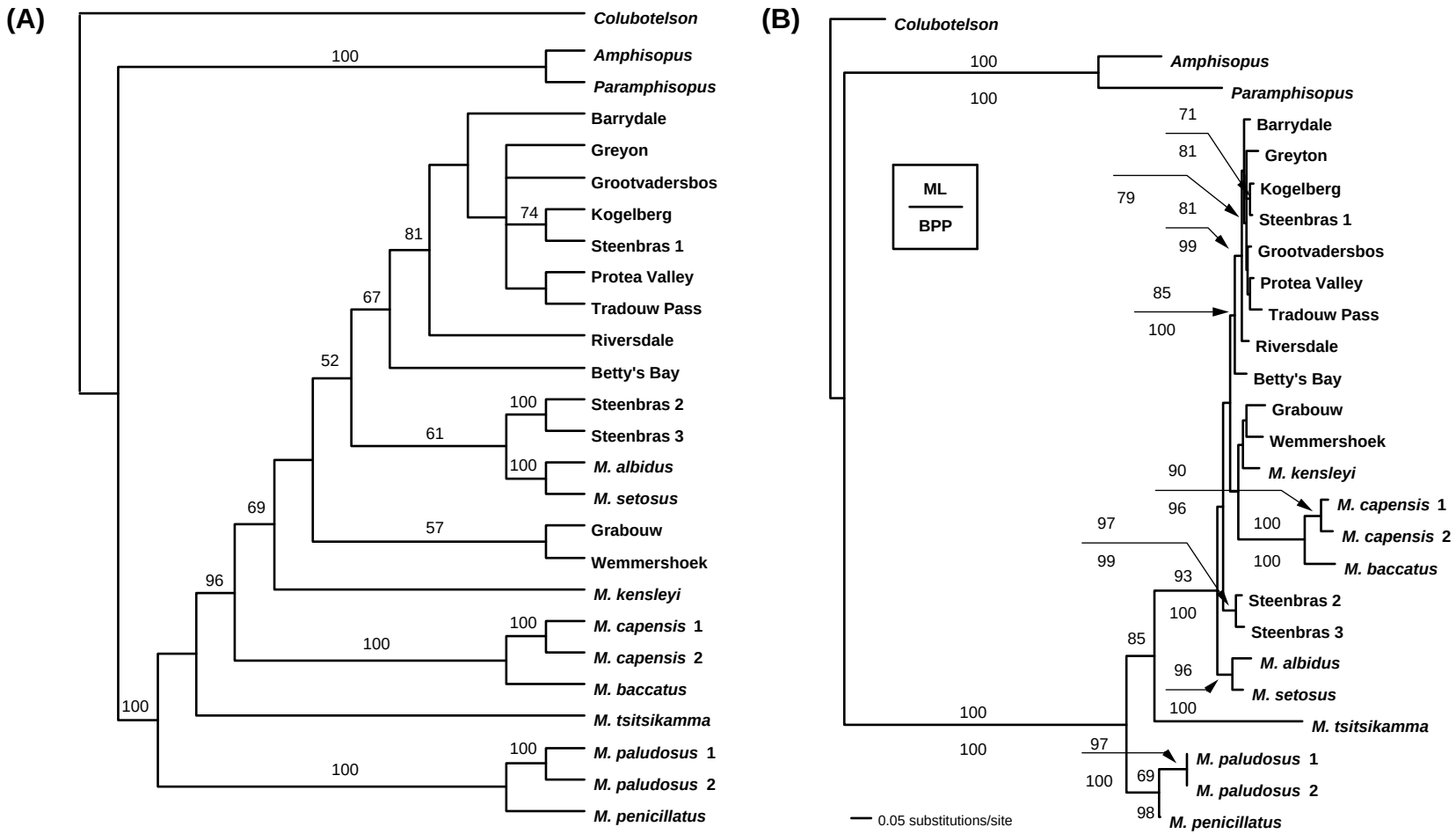


Figure 5.2: (A) Strict consensus of three equally parsimonious trees obtained in the parsimony analysis of 585 nucleotide characters from the COI mtDNA gene fragment in 23 *Mesamphisopus* representatives and three outgroup taxa (*Colubotelson*, *Amphisopus* and *Paramphisopus*). Numbers above branches indicate bootstrap support from 1000 pseudo-replicates (with 100 random taxon addition iterations). Only bootstrap support > 50% is indicated. (B) Maximum likelihood tree (-lnL = 3918.843) from the analysis of the same gene fragment with the implementation of a GTR + I + Γ model of nucleotide evolution (consult Table 5.2 for substitution parameters). Numbers above the branches indicate bootstrap support (100 pseudo-replicates). Numbers below the branches represent the lowest of the Bayesian Posterior Probabilities (BPP), presented as percentages for ease of comparison, obtained in the four independent Bayesian inferences of phylogeny. Only bootstrap support > 50% and BPPs > 75% are indicated.

constructed, and mean likelihood scores and substitution parameters (Table 5.2) calculated, from 19 800 sampled generations in each of the four runs. Identical tree topologies, and comparable likelihood scores and substitution parameter estimates were obtained in each of the runs. These topologies were generally congruent to the ML tree.

5.3.3) Combined mtDNA data set

The Incongruence Length Difference test indicated that the two respective genes (12S rRNA and COI) exhibited no greater intergenic incongruence than two partitions drawn randomly from a homogenous data set, considering only variable ($P = 0.563$) or parsimony informative characters ($P = 0.542$) in both partitions.

The concatenated 12S rRNA + COI data set (923 bp) included 308 parsimony informative characters. The parsimony analysis recovered four equally parsimonious trees of 904 steps. More relationships were resolved in the strict consensus (Fig. 5.3) of these trees than in each of the strict consensus trees from the independent analyses of these partitions.

In the Bayesian inference, the first 20 000 generations were discarded as "burn-in". Consequently, majority rule consensus trees were constructed from the remaining 19 600 sampled trees for each of the four runs. These were largely congruent with the strict consensus tree presented in Figure 5.3. The reduction of the number of trees in a given credibility interval gives an indication of the increased information content and resolution of the combined data set (Buckley *et al.*, 2002). While between 13 759 and 13 867, and 7 064 and 8 468 trees fell within the 99% credible set in the four runs in independent analyses of the

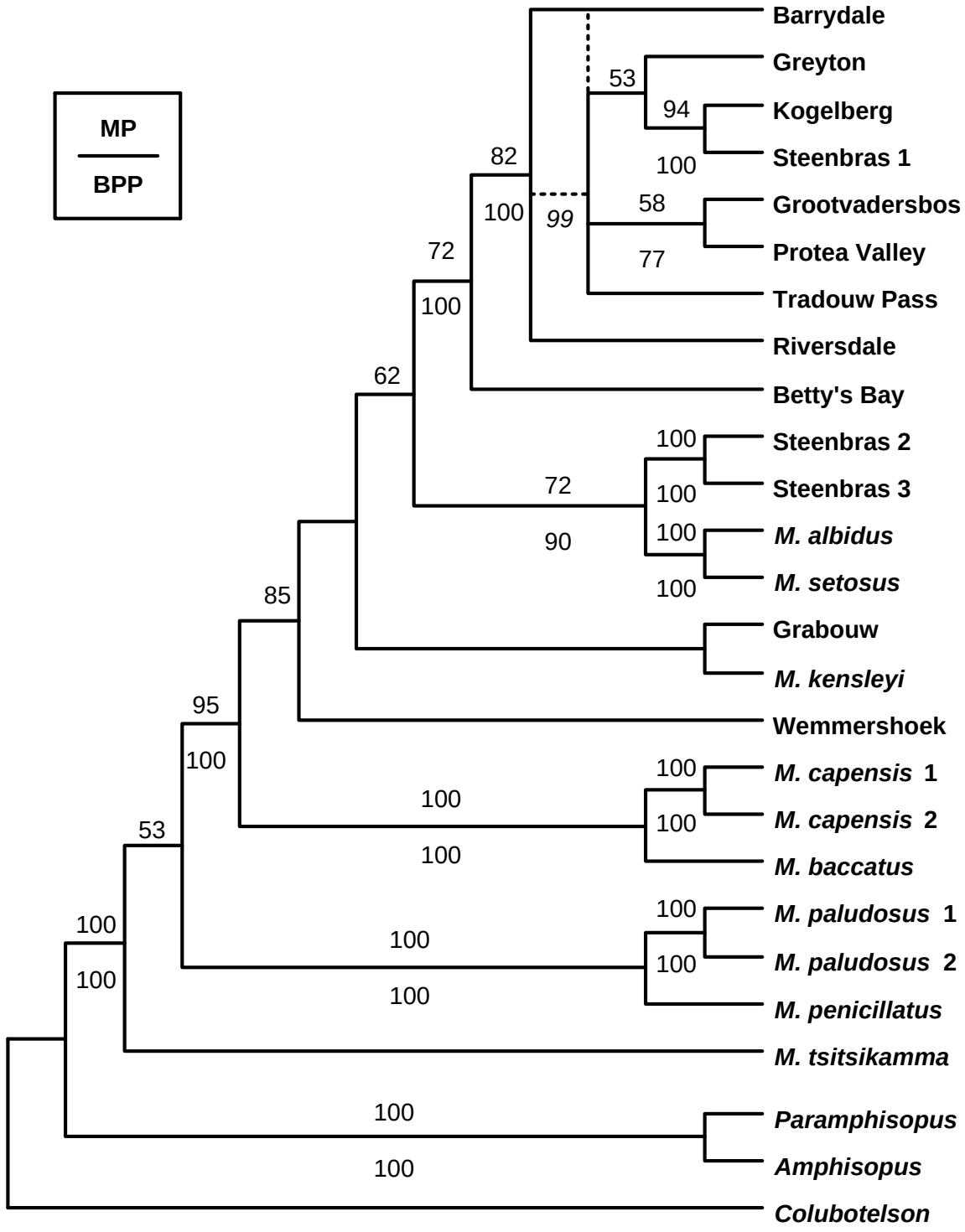


Figure 5.3: Strict consensus of four equally parsimonious trees obtained in the parsimony analysis of the combined mtDNA (12S rRNA + COI) data set. Numbers above branches indicate bootstrap support (Felsenstein, 1985) from 1000 pseudo-replicates (each using 100 random taxon addition iterations). Numbers below the branches represent the lowest of the posterior clade probabilities (presented as percentages for ease of comparison) obtained in the four independent Bayesian inferences of phylogeny. Only posterior probabilities > 75% and bootstrap support > 50% are indicated. Dashed lines indicate relationships supported, with high support, in the Bayesian inferences, but not in the parsimony analysis.

12S rRNA and COI partitions, respectively, only 3 849 – 4 076 trees were found in this set in the combined analysis.

Parsimony analysis of the combined data set retrieved *M. tsitsikamma* as the basal sister taxon to the remaining ingroup. A strongly-supported (100% bootstrap, 1.00 BPP) clade, comprising *M. penicillatus* and *M. paludosus*, was next basal. This clade was well-supported in analyses of the individual partitions (12S rRNA and COI), although support was weaker in certain ML (COI: 69% bootstrap) and Bayesian (12S rRNA: 0.93 BPP – non-significant support) analyses. The basal relationship among the *M. penicillatus – M. paludosus* clade and *M. tsitsikamma* was, however, not well resolved, with the *M. penicillatus – M. paludosus* clade appearing basally in the Bayesian analyses of the combined mtDNA data set (not shown on Figure 5.3). With the exception of the parsimony analysis of the 12S rRNA partition, this clade was also recovered basally in all analyses of the independent data partitions. The remaining ingroup received fair to high support (≥ 66% bootstrap, ≥ 0.99 BPP), to the exclusion of *M. paludosus*, *M. penicillatus* and *M. tsitsikamma*, in all analyses of all partitions. However, the placement of *M. tsitsikamma* as a sister taxon to the remaining ingroup to the exclusion of the *M. penicillatus – M. paludosus* clade, or vice versa, was not supported in most analyses of the 12S rRNA (60% bootstrap, no significant BPP), COI (no bootstrap support from the parsimony analysis, no significant BPP) and combined (53% bootstrap, 0.84 – a non-significant BPP) partitions. The only exception was the ML analysis of the COI data set, where the position of *M. tsitsikamma* as a sister taxon to the remaining ingroup (to the exclusion of the *M. penicillatus – M. paludosus* clade) was well-supported (85% bootstrap).

A strongly supported clade (100% bootstrap, 1.00 BPP) comprising *M. capensis* and *M. baccatus* clade was recovered by parsimony and Bayesian analyses of the combined data set, as well as by all analyses of the individual partitions (≥ 94% bootstrap, 1.00 BPP). Its placement as a sister clade (with 85% bootstrap support in the parsimony analysis of the combined partitions) to the larger ingroup clade, containing *M. albidus*, *M. kensleyi*, *M. setosus* and representatives of the *M. abbreviatus – M. depressus* group, was not supported in the Bayesian analyses of the combined data set (no significant posterior probability). The basal relationships within this larger clade, and the position of the *M. capensis – M. baccatus* clade, were poorly resolved and unsupported in independent analyses of the individual partitions, with this latter clade being nested within the larger clade in the ML analyses.

While basal relationships within this remaining ingroup clade were unresolved in the combined data analyses, and conflict was observed in some of the more terminal relationships, a number of relationships were well supported. Sister taxon relationships between Steenbras 2 and Steenbras 3 (100% bootstrap, 1.00 BPP), *M. albidus* and *M. setosus* (100% bootstrap, 1.00 BPP), and Kogelberg and Steenbras 1 (94% bootstrap, 1.00 BPP) were well-supported in the parsimony and Bayesian analyses. These sister taxon relationships were, likewise, retrieved in all analyses of the individual partitions. Aside from these relationships, relationships within this remaining ingroup clade were wholly unresolved or poorly supported in analyses of the 12S rRNA partition. The sister group relationship (72% bootstrap) between *M. albidus – M. setosus* and Steenbras 2 – Steenbras 3 was further supported in the parsimony analysis of the combined data. Both parsimony and Bayesian analysis of the combined data partition supported (82% bootstrap, 1.00 BPP) a 'derived' clade, consisting of the Barrydale, Greyton, Grootvadersbos, Kogelberg, Protea Valley, Riversdale, Steenbras 1 and Tradouw Pass representatives. The Betty's Bay representative

was placed as a sister taxon to this clade with high support (72% bootstrap, 1.00 BPP). Within the 'derived' clade, the Bayesian analyses supported, with 0.99 BPP, the Riversdale representative as a basal sister taxon to the remaining representatives; this relationship not supported by the parsimony analysis. The 'derived' clade was also retrieved with significant support (≥ 81% bootstrap, 0.99 BPP) in analyses of the COI partition, although the placement of the Betty's Bay individual as its sister taxon was only supported in the ML (81% bootstrap) and Bayesian (1.00 BPP) analyses.

5.3.4) Allozyme data

The among-population CSE-chord distances calculated from the allele frequencies at 12 loci ranged from 0.112 to 0.868 (matrix not shown). While low values were observed between representative populations of the same species (*M. paludosus* 1 – *M. paludosus* 2: 0.112; *M. capensis* 1 – *M. capensis* 2: 0.290), similarly low values were observed in comparisons within the *M. abbreviatus* – *depressus* complex (e.g. Steenbras 2 – Steenbras 3: 0.192; Grootvadersbos – Tradouw Pass: 0.209) and in certain interspecific comparisons (e.g. *M. penicillatus* – *M. paludosus* 1: 0.289). At the other end of the spectrum, the highest values were obtained in comparisons involving *M. tsitsikamma* (e.g. *M. tsitsikamma* – Wemmershoek: 0.868; *M. tsitsikamma* – Grabouw: 0.855). Similarly high values were obtained in other interspecific comparisons (e.g. *M. capensis* 1 – *M. penicillatus*: 0.794; *M. baccatus* – *M. paludosus* 2: 0.778), while certain comparisons within the *M. abbreviatus* – *depressus* group approached these values (e.g. Grabouw – Steenbras 3: 0.647; Wemmershoek – Steenbras 2: 0.633).

The midpoint-rooted neighbour-joining tree (Fig. 5.4) revealed four main clusters: a cluster was formed by the *M. tsitsikamma*, *M. penicillatus* and *M. paludosus* populations; a second cluster was formed by the two *M. capensis* populations and the *M. baccatus* population; the third cluster contained the *M. albidus* and *M. setosus* populations, as well as the Steenbras 2 and Steenbras 3 populations of the *M. abbreviatus – depressus* group. Finally, the remaining populations of the *M. abbreviatus – depressus* group formed a cluster, with the *M. kensleyi* population nested within. This topology differed from those obtained in the analyses, both independent and combined, of the sequence data partitions only in the placement of the Wemmershoek, *M. kensleyi* and Grabouw populations within the 'derived' *M. abbreviatus – depressus* clade; representatives of these populations mostly being placed basal to the *M. albidus – M. setosus* – Steenbras 2 – Steenbras 3 clade elsewhere.

Sixty-seven alleles were detected at the 12 examined loci in the 23 ingroup taxa, with two additional null alleles being fixed at each of the *Ldh*- and *Lt-2*-loci in certain populations. Of these, 54 occurred at a frequency of 0.05 or greater in at least one taxon, and were scored as present or absent in each population (Appendix 11). The null alleles were not scored, as the two scoring methodologies proposed for loci possessing a fixed null allele (Berrebi *et al.*, 1990) would either present the null allele as a synapomorphy uniting all taxa in which it occurs (the "minimizing" criterion), or as an autapomorphy for each of the taxa (the "maximizing" criterion) in which it is present. These approaches, respectively, could introduce additional homoplasy into the data set, or would not be informative regarding phylogenetic relationships within the ingroup. Rather than make such assumptions *a priori*, the presence of fixed null alleles was mapped onto cladograms derived from the total data analysis to determine the likely patterns of loss of expression of the *Ldh*- and *Lt-2*-loci.

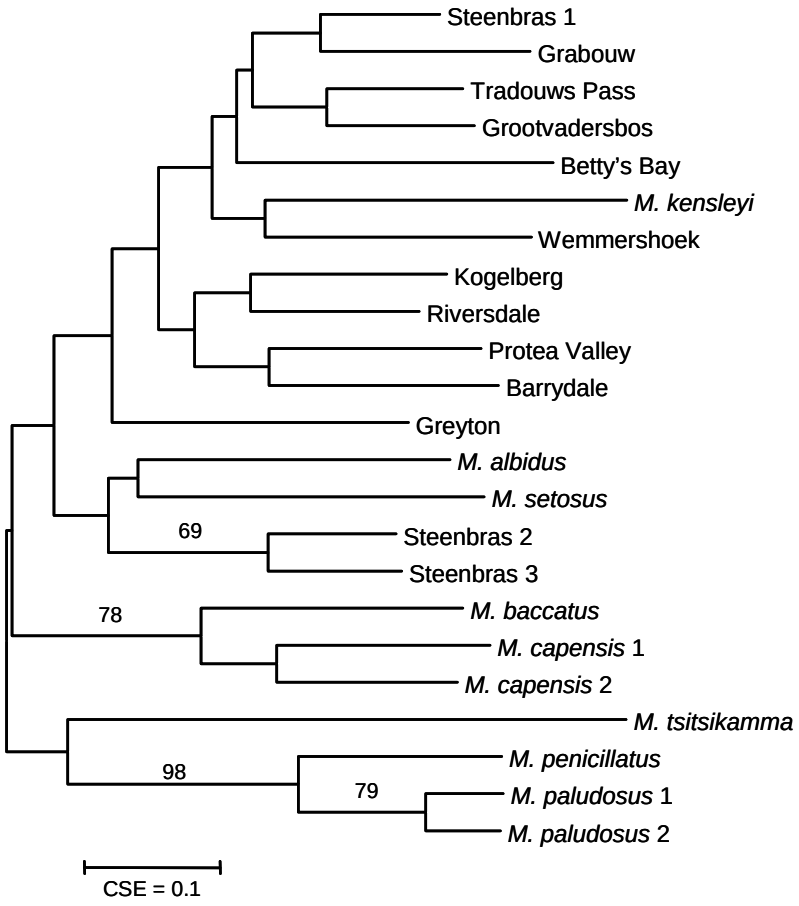


Figure 5.4: Midpoint-rooted neighbour-joining (Saitou and Nei, 1987) tree constructed using Cavalli-Sforza and Edwards (1967) chord-distances (CSE) calculated among 23 representative *Mesamphisopus* populations using allele frequency data from the electrophoresis of 12 allozyme loci. Numbers above the branches indicate nodal support (> 50%) for relationships determined by 1000 bootstrapping (Felsenstein, 1985) replicates, with 100 random taxon addition iterations, in the parsimony analysis of 54 alleles, coded as present or absent in each of the representative populations. The strict consensus of the 56 equally parsimonious trees (95 steps) obtained in the cladistic analysis is largely congruent (see text) to the neighbour-joining tree presented here and is not shown.

Of these 54 alleles, 39 were parsimony informative. Parsimony analysis resulted 56 equally parsimonious trees of 95 steps (CI = 0.411, RI = 0.636, rescaled CI = 0.261). The strict consensus tree, rooted using the *M. paludosus, M. penicillatus* and *M. tsitsikamma* populations as outgroups, is topologically largely congruent with the neighbour-joining tree and is not presented. Within the *M. abbreviatus – depressus* cluster identified in the neighbour-joining tree, the Greyton population formed a sister taxon to an unresolved polytomy, with only the sister-relationships between the Wemmershoek and *M. kensleyi*, Grabouw and Steenbras 1, and Barrydale and Protea Valley populations being retrieved within this polytomy. The two *M. capensis* population and the *M. baccatus* population also formed a three-way polytomy. Further relationships were identical to those revealed by the neighbour-joining tree. Few relationships were supported, with only the association of the *M. baccatus* and *M. capensis* populations, and the sister taxon relationship between *M. penicillatus* and *M. paludosus*, and between Steenbras 1 and Steenbras 2 receiving bootstrap support (greater than 50%).

5.3.5) Total evidence

The Incongruence Length Difference test indicated significant heterogeneity among the three (two mtDNA and the nuclear/allozyme) data partitions (variable characters only $P = 0.025$; parsimony informative characters only $P = 0.028$). As earlier ILD tests had detected no significant heterogeneity among the two mtDNA partitions (see above), non-compatibility among the data sets was introduced into this concatenated data set with the inclusion of the recoded allozyme data partition, a possible artefact of the coding methodology as discussed by Buth (1984) and Murphy (1993). Among wider criticism of the efficacy of the ILD test as a indicator of topological congruence, partition homogeneity and partition combinability

(Barker and Lutzoni, 2002), several authors have highlighted the propensity of the ILD test to Type I errors, i.e. the rejection of combinability of data partitions, when the combination of such partitions would lead to more accurate estimates of phylogeny (Huelsenbeck, Bull and Cunningham, 1996; Yoder, Irwin and Pasteur, 2001; Hipp *et al.*, 2004). Indeed, better estimates of phylogeny have been obtained through the combined analysis of data partitions than provided by individual partitions, despite the rejection of combinability by the ILD test (Sullivan, 1996; Creer *et al.*, 2003; Yoder *et al.*, 2001; but see Hipp *et al.*, 2004). Consequently, several authors have conceded that the ILD test is too conservative and have suggested that a critical value (α) of 0.01 or even 0.001 would be more appropriate for determining combinability than the critical value of 0.05 generally used (see Yoder *et al.*, 2001; Barker and Lutzoni, 2002). Considering this, parsimony analysis proceeded with the three partitions combined.

The total data set included 977 characters, of which 347 were parsimony informative. Seven equally parsimonious trees of 1013 steps were retrieved (CI = 0.524, RI = 0.641, Rescaled CI = 0.336) in the MP analysis. This total evidence topology (Fig. 5.5) was congruent in most respects to other topologies. The *M. tsitsikamma* and the *M. paludosus – M. penicillatus* lineages were again retrieved basally; the consistent most-basal placement of one lineage to the exclusion of the other was again not supported. The next basal *M. capensis – M. baccatus* clade was strongly supported, as was the 'derived' *M. abbreviatus – depressus* clade, and the Steenbras 1 – Steenbras 2 – *M. albidus – M. setosus* association. A weakly supported (51% bootstrap) relationship was recovered between *M. kensleyi* and the Grabouw – Wemmershoek clade in the bootstrap analysis (indicated by dashed branches in Fig. 5.5).

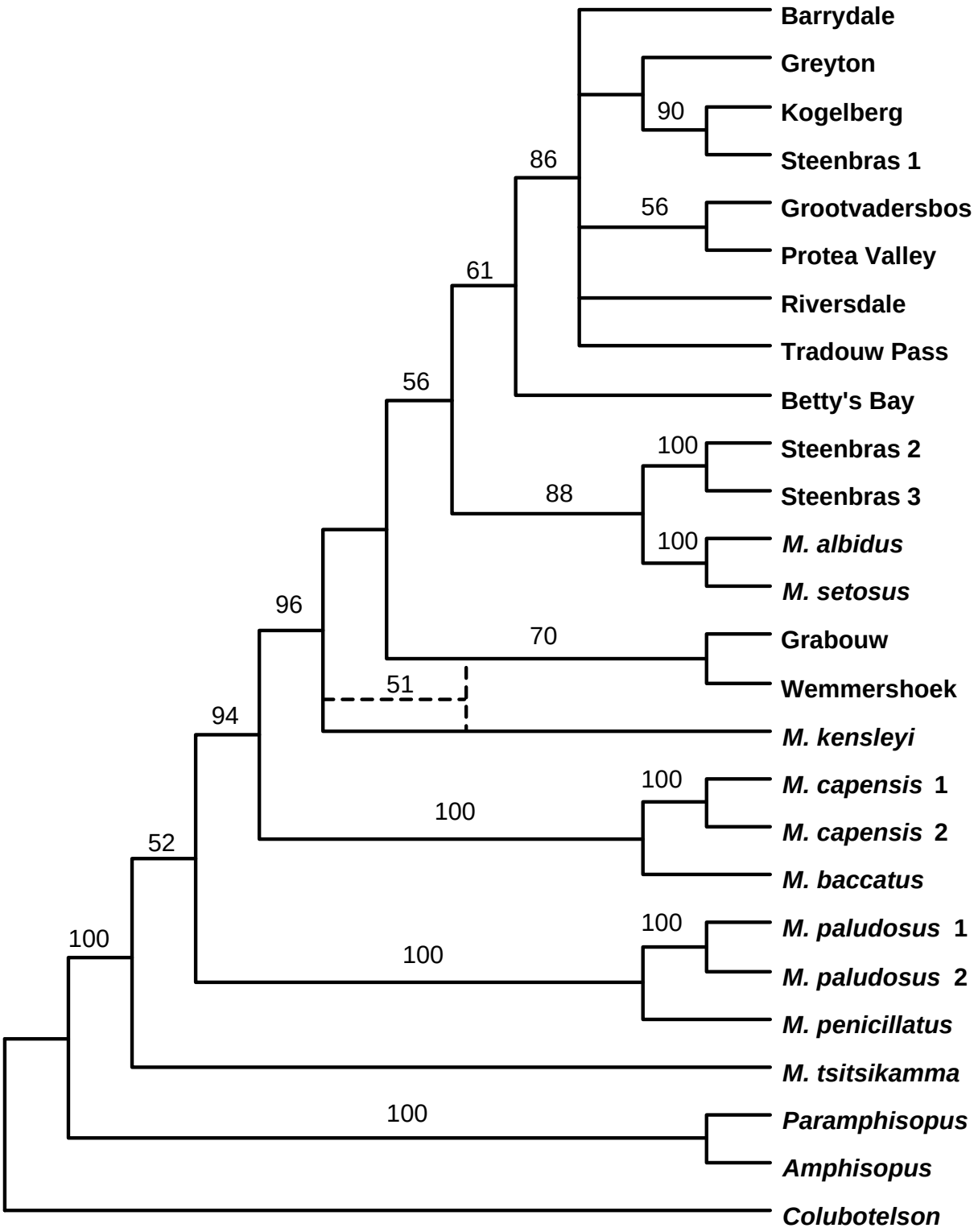


Figure 5.5: Strict consensus of the seven equally-parsimonious trees obtained in the parsimony analysis of the total data set, including the two mitochondrial DNA partitions (12S rRNA + COI) and the nuclear data partition (presence/absence coded matrix of 54 alleles from the allozyme data set). Numbers above the branches indicate bootstrap support (Felsenstein, 1985) from 1000 replicates, employing 100 random taxon addition iterations. Bootstrap support < 50% is not shown. Dashed lines indicate relationships weakly supported by the bootstrap analysis of the data set, but not unambiguously supported by the strict consensus of the most parsimonious trees.

The strict consensus topology from the total evidence analysis was used to map and evaluate character distributions, particularly the duplication or the inactivation (or reduced activity) of loci observed as fixed null alleles in certain populations during the electropheretic procedure. Mapping the allozyme data partition, including the null alleles, coded as being identical in all populations (i.e. using the "minimizing" criterion), to this topology indicated a tree length of 128 steps. A single duplication of the *Lt-2*-locus was proposed (Fig. 5.6) after the derivation of *M. tsitsikamma* and the *M. paludosus – M. penicillatus* clade. This topology also postulated a single deactivation (see Fig. 5.6) of the *Ldh*-locus (ancestral to the 'derived' *M. abbreviatus – depressus* clade) and three reversals (along the terminal branches leading to the Grootvadersbos, Steenbras 1 and Tradouw Pass representatives). The "maximizing" coding procedure for the null alleles proposed less parsimonious solutions: five and four steps were required to explain the character distributions of the null alleles at the *Ldh*- and *Lt-2*-loci, respectively. The independent emergence of the identical alleles or, in this case, the independent reversal and expression of the same loci in different populations is less likely than the independent loss of expression of alleles or loci (Tsigenopoulos *et al.*, 1999) – although the coding methodology employed here for fixed null alleles proposes a common ancestral inactivation of expression. For the aforementioned reason, the strict consensus topology was constrained to allow a single inactivation of the *Ldh*-locus without reversals (i.e. Barrydale – Greyton – Kogelberg – Protea Valley – Riversdale, and Grootvadersbos – Steenbras 1 – Tradouw Pass forming respective polytomies) and was shorter (125 steps) than the unconstrained tree, although not significantly so (Templeton (1983) test/Wilcoxon signed ranks test: N = 5, T = 6, Z = -0.414, P = 0.679). Thus, a more parsimonious single inactivation of the *Ldh*-locus, ancestral to the Barrydale – Greyton – Kogelberg – Protea Valley – Riversdale populations, cannot be excluded.

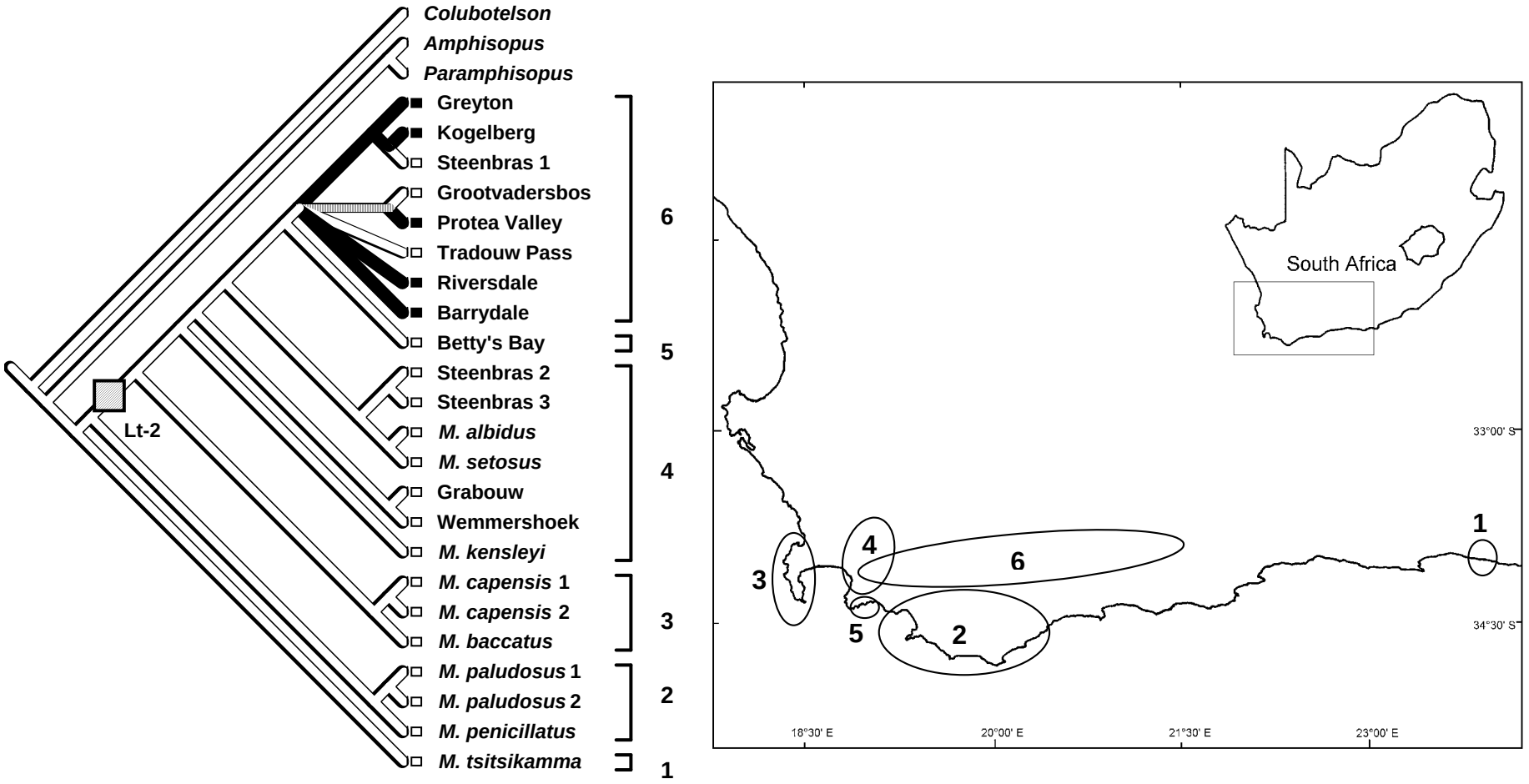


Figure 5.6: Character distribution of the presence/absence coded null allele at the *Ldh*-locus, mapped onto the strict consensus (see Fig. 5.5) of the the most parsimonious trees obtained in the analysis of the total data set (12S rRNA + COI + allozymes). Null-alleles were identically coded in all terminals, following the "minimizing" procedure of Berrebi *et al.* (1990). Filled boxes (left of terminals) indicate the presence of a null allele (absence of other alleles and the inactivation of the locus), while empty boxes indicate the absence of the null allele (and the presence of alternative alleles). The hatched branch represents equivocal character states. The hatched block indicates the duplication of the *Lt-2*-locus, this character change representing the most parsimonious explanation for the distribution of null alleles at that locus. The broad geographic distributions of identified clades or lineages (numbered to the right of terminals) are indicated on the map of the southern and south-western Cape, South Africa (right).

5.3.6) Dating of divergences

The divergence times of the clades revealed by the strict consensus topology from the analysis of the total data set were estimated using the relaxed Bayesian molecular clock (Thorne and Kishino, 2002), with prior constraints on divergence time placed on two nodes, as described earlier. The prior and posterior estimates of divergence times and their respective 95% confidence intervals are presented in Table 5.3. Prior and posterior estimates of divergence time and their confidence intervals determined in each of four MULTIDIVTIME runs were similar. Thus, only the divergence estimates and confidence intervals results of the first run are presented or discussed. The large differences in posterior and prior estimates of divergence time, as well as a narrowing of the posterior 95% confidence intervals (Table 5.3), indicate that the priors did not have an undue influence and that the dating information is derived from the actual data (Hassanin and Douzery, 2003).

5.4) Discussion

Largely congruent topologies were obtained in analyses of all individual sequence data partitions, and the combination of these partitions. The phenetic analysis of the allele frequency data provided a topology congruent, to a large extent, to those obtained in the sequence data (and combined data) analyses, whereas the cladistic analysis of these data provided a similarly congruent, but poorly supported, topology. In summary: (1) all analyses supported the monophyly of *Mesamphisopus* with respect to the included outgroup taxa; (2) all analyses supported the sister taxa relationship between *Paramphisopus* and *Amphisopus*; (3) *Mesamphisopus tsitsikamma* and the *M. paludosus – M. penicillatus* clade were

Table 5.3: Molecular dating of the divergences within *Mesamphisopus* and included outgroup taxa, as revealed by the strict consensus topology from the analysis of the total data set and determined using the relaxed Bayesian clock of Thorne and Kishino (1992). Maximum likelihood branch lengths and variance-covariance matrices for implementation in the MULTIDIVTIME program were determined for each of the 12S rRNA and COI data partitions. The root node was assumed to be 140 Mya old, with a rate of evolution of 0.006 (±0.006) substitutions per site per Mya. Specific prior constraints on nodes are indicated in parenthesis. The estimated prior and posterior divergence times (in Mya before present) are presented, along with their 95% confidence intervals (95% CI). "Rest" refers to the remaining, more-derived representatives of the ingroup; the sister group of the lineage in question. Divergences are arranged from oldest to youngest, according to the posterior divergence times.

Split/Divergence	Divergence times (x 10^6 years before present)			
	Prior		Posterior	
	Divergence time	95% CI	Divergence time	95% CI
Amphisopus/*Paramphisopus* – *Mesamphisopus* (no younger than 100 Myr)	116.097	100.392 – 164.149	112.135	100.288 – 144.326
Paramphisopus – *Amphisopus*	60.497	3.547 – 127.578	49.702	23.380 – 88.417
M. tsitsikamma – rest	77.184	28.411 – 126.419	44.643	28.435 – 68.691
M. penicillatus/*M. paludosus* – rest	47.890	18.197 – 97.169	36.475	22.945 – 54.939
M. capensis/*M. baccatus* – rest (no older than 20 Myr)	17.671	12.303 – 19.930	17.538	12.429 – 19.919
M. kensleyi – rest	15.516	9.404 – 19.357	11.744	7.043 – 17.143
M. penicillatus – *M. paludosus* 1/*M. paludosus* 2	31.589	5.776 – 77.167	10.861	3.489 – 24.393
Grabouw/Wemmershoek – rest	13.355	7.189 – 18.258	10.838	6.466 – 15.942
M. albidus/*M. setosus*/Steenbras 2/Steenbras 3 – rest	11.138	5.012 – 16.791	8.986	5.102 – 13.862
Grabouw – Wemmershoek	6.773	0.374 – 15.158	8.527	4.513 – 13.565
M. baccatus – *M. capensis* 1/*M. capensis* 2	11.583	2.643 – 18.723	7.524	3.255 – 14.130
M. albidus/*M. setosus* – Steenbras 2/Steenbras 3	7.360	1.471 – 14.272	7.113	3.660 – 11.681
Betty's Bay – 'derived' clade	8.890	3.165 – 14.924	6.978	3.606 – 11.563
Lineages of the polytomic 'derived' clade	6.642	1.704 – 12.913	4.413	2.133 – 7.945
Greyton – Kogelberg/Steenbras 1	4.404	0.655 – 10.398	3.540	1.535 – 6.686
M. albidus – *M. setosus*	3.670	0.107 – 10.650	3.196	0.994 – 6.665
Steenbras 2 – Steenbras 3	3.765	0.120 – 10.999	2.954	0.942 – 6.060
M. capensis 1 – *M. capensis* 2	5.839	0.215 – 15.410	2.868	0.879 – 6.311
Grootvadersbos – Protea Valley	3.297	0.108 – 9.443	2.549	0.842 – 5.347
Kogelberg – Steenbras	2.211	0.063 – 7.252	1.622	0.333 – 3.713
M. paludosus 1 – *M. paludosus* 2	16.051	0.490 – 54.162	0.597	0.015 – 2.381

consistently retrieved basally, but the relationship among these lineages was not consistently resolved; (4) the well-supported *M. capensis – M. baccatus* clade was retrieved as the next basal clade; and (5) relationships within the clade of 'derived' populations (Barrydale + Greyton + Grootvadersbos + Kogelberg + Protea Valley + Riversdale + Steenbras 1 + Tradouw Pass) were less well resolved, but the monophyly of this clade was consistently retrieved.

5.4.1) Some methodological considerations

The use of Bayesian approaches to infer phylogeny is still in its infancy, and many theoretical, practical and interpretative aspects are still poorly understood (see Huelsenbeck *et al.*, 2002; Archibald *et al.*, 2003; Simmons *et al.*, 2004). For example, while there is wide and general acceptance of the levels of support indicated by nonparametric bootstrapping (Hillis and Bull, 1993), the interpretation of Bayesian Posterior Probabilities (BPPs) is less well understood. A discrepancy among bootstrap and BPP nodal support has been well noted (Buckley *et al.*, 2002; Wilcox *et al.*, 2002; Archibald *et al.*, 2003; Simmons *et al.*, 2004), with BPPs being higher than bootstrap support. This discrepancy is thought to result from fundamental statistical and methodological differences in the calculation of each (Buckley *et al.*, 2002; Huelsenbeck *et al.*, 2002; Suzuki, Glazko and Nei, 2002). As a result, BPPs have been variably regarded as excessively liberal, with a BPP of 100% representing 60 – 70% bootstrap support (Suzuki *et al.*, 2002), or as being a conservative, accurate representation of nodal support, with the bootstrap being overly conservative (Wilcox *et al.*, 2002). Several authors (Whittingham *et al.*, 2002; Weekers *et al.*, 2002; Douady *et al.*, 2003) have suggested that a moderate correlation exists between BPP and bootstrap support, although the measures are not directly comparable (Archibald *et al.*, 2003). It has, thus, been suggested that nodes

with BPP above 80% (corresponding to a bootstrap of above 70%) (Whittingham *et al.*, 2002; Weekers *et al.*, 2002), or above 95% (Wilcox *et al.*, 2002) be considered as well-supported. With broadly congruent topologies obtained in all analyses, support of certain nodes in the Bayesian inference, as measured by BPPs, was slightly higher than support values obtained by nonparametric bootstrapping in the MP and ML analyses. This discrepancy did not always hold and the broad correlation is questioned in this case, as several nodes were significantly supported by bootstrap values (≥ 70%), but not by significant BPPs (≥ 95%), while only one node was supported by a significant BPP and a non-significant bootstrap. An additional concern with Bayesian inference, only recently being addressed (see Nylander *et al.*, 2004), is the unknown sensitivity of the posterior probability distribution to the proposed prior distribution and model choice (Buckley *et al.*, 2002; Archibald *et al.*, 2003). Although most software presently used for Bayesian inference cannot implement any priors other than a uniform ("flat") prior probability distribution (Archibald *et al.*, 2003), particularly with respect to topology, posterior probability distributions are thought to be relatively insensitive to the prior, especially given large data sets (Lewis, 2001a; Huelsenbeck *et al.*, 2002; but see Nylander *et al.*, 2004). With the recovery of topologies in the Bayesian inference largely congruent with the topologies derived from the MP and ML analyses, the similarity in degree of support of most nodes (at least categorically – accepting the above criteria), and the approximation of the model/substitution parameters sampled from the posterior probability to those determined as being most appropriate for the data partitions using MODELTEST, there is no reason to suspect spurious hypotheses of phylogeny resulting from inappropriate models or priors in this study. This is heartening, especially considering combined analyses where cumbersome computational times required by ML analyses were, and are often, prohibitive. The use of Bayesian approaches is again vindicated and provides additional support for the evolutionary hypotheses proposed by parsimony and likelihood analyses.

5.4.2) Taxonomic implications

Two independent lines of evidence exist to suggest that the populations included in the *M. abbreviatus – M. depressus* group represent a complex of closely related species, rather than genetically differentiated conspecific populations, and that previous caution with regard to the delineation of species within this group was unwarranted (see Chapter 3). In the first instance, a great overlap of CSE-distances was observed among recognized species and between populations belonging to the previously identified *M. abbreviatus – M. depressus* group. As Nei's (1978) genetic distances and identities are more commonly used in allozyme studies of Crustacea, certain standard or threshold values have been proposed and are routinely applied as guidelines for species delineation (Chapter 2 and references therein). CSE-distances are less frequently used and such standards have not been proposed, prohibiting a comparison or application here. Nonetheless, certain comparisons involving representatives of this group showed greater CSE-distances than those obtained in certain interspecific comparisons of recognized taxa. The second line of evidence is the fact that valid species (*M. albidus*, *M. kensleyi* and *M. setosus*), recognised morphologically, are nested within the larger *M. abbreviatus – M. depressus* clade. Even conceding that the Steenbras 2 and Steenbras 3 populations, collected near the type locality of *M. depressus* and morphometrically similar to the syntypes of the species (Chapter 3), may be the only representatives of this species, the remaining populations of the group (reasonably regarded as *M. abbreviatus* on the basis of published descriptions, the single key and morphometry (Barnard, 1927; Nicholls, 1943; Kensley, 2001; Chapter 3)) do not form a monophyletic assemblage in any of the analyses. This assemblage remains paraphyletic with *M. albidus*, *M. setosus* and these two populations nested within. Given that relationships within this clade were not consistently well-resolved or well-supported, precluding a tree based approach to

species delimitation *sensu* Wiens (1999), and given that cryptic species, morphologically diagnosable upon closer examination (e.g. *M. setosus* and *M. albidus*), may be separated by low sequence divergences (Chapter 2), detailed morphological examination of representatives of each population within this group will be required to determine which represent distinct species.

The additional analysis of the 12S rRNA gene fragment, cladistic analysis of allele frequency data and the combined analyses of all partitions failed to significantly resolve relationships among the members of the *M. abbreviatus – M. depressus* group. Resolution provided by the 12S rRNA partition was generally restricted to deeper divergences, while resolution provided by the COI partition was restricted to deeper and intermediate relationships, leaving many of the terminal relationships unresolved. As reported earlier (Chapter 3), relationships within this group were largely unrelated to geographic locality, mountain range, drainage system, or altitude. The poor resolution may result from the rapid radiation of the group, reflected by the short branch lengths on the phylograms, with few synapomorphies defining relationships (Remigio *et al.*, 2001). Alternatively, the lack of structure may result from (transitional) saturation of the genes examined. However, examination of saturation plots (not shown) only revealed evidence of saturation in the third codon positions of the COI gene, and only with regard to comparisons involving outgroup sequences. Additionally, retention indices were relatively high (≥ 0.636) in analyses of all individual and combined partitions.

5.4.3) Biogeographic patterns and evolutionary implications

A number of broad biogeographic regions can be identified, corresponding to major clades or lineages revealed by the above analyses (Fig. 5.6). *Mesamphisopus tsitsikamma* occurs on an elevated coastal shelf (see Lambrechts, 1979; Hendey, 1983b) at a locality adjacent to the coast in the Eastern Cape (Fig. 5.6: 1). Representatives of *M. penicillatus – M. paludosus* clade have an essentially low-lying, coastal plain (see Lambrechts, 1979; Hendey, 1983b; Linder, 2003) distribution (2), occurring adjacent to the coast in the Western Cape (*M. penicillatus*) (see Barnard, 1940), or slightly further inland (*M. paludosus*) across the low-lying Agulhas Plain (see Cowling *et al.*, 1992). Large parts of the Agulhas Plain formed the Bredasdorp Embayment, a former coastal deposition platform of Tertiary (Neogene) age (Hendey, 1983b: Fig. 1). Members of the *M. capensis – M. baccatus* clade are restricted to the Cape Peninsula (3). Representatives of the large remaining clade are restricted to the Cape Fold Mountains, extending from the Hottentot's Holland Mountains eastwards, along the Riviersonderend, Langeberg and Riversdale Mountains. Within this region, further biogeographic patterns can be identified. These are, however, less discrete. The weakly supported clade (by bootstrap analysis) containing *M. kensleyi* and the Grabouw and Wemmershoek representatives, as well as the Steenbras 2 – Steenbras 3 – *M. albidus – M. setosus* clade, are confined to the south-western portion of the Cape Fold Mountains (4), from the southern Hottentot's Holland Mountains to the Franschhoek and Drakenstein Mountains in the north. This region, with folding in various directions, represents the syntaxis of the north-south and east-west axes of the Cape Fold Mountains (Lambrechts, 1979). The Betty's Bay representative lies at the southern foot of the Hottentot's Holland Mountains (5), near sea level on the coastal plain, with individuals probably being washed down from high-altitude areas and populations established in the small lakes of the area. The 'derived' *M. abbreviatus*

– *M. depressus* clade is primarily restricted to the eastern zone of the Cape Fold Mountains (6), which runs west to east, roughly parallel to the south coast (Lambrechts, 1979). With the exception of the Steenbras 1 and Kogelberg representatives that occur within the Hottentot's Holland Mountains, causing some overlap of the identified biogeographic areas (4 and 6), these taxa occur exclusively along this eastern zone, in the Riviersonderend, Langeberg and Riversdale Mountains.

The most immediately striking biogeographic pattern revealed by the phylogenetic analyses is the consistent basal placement of *M. tsitsikamma* and the *M. paludosus – M. penicillatus* clade, although the relationships among these are not consistently resolved. Early work regarding the evolutionary relationships of the phreatoicidean facies, although strictly more applicable at higher taxonomic levels, had suggested that extant taxa with low-lying distributions were likely to be derived from taxa that had persisted and speciated in refugial high-altitude or sub-alpine habitats, following the extirpation of the ancestral low-lying surface-water fauna (Nicholls, 1924, 1926). Moreover, this pattern is also expected when viewed against the paleogeographic history of the region. Large portions of the coastal belt, including the Agulhas Plain, were exposed, as were the Cape Flats (see Chapter 2), to periodic transgression and regression events. For most of the Miocene and Pliocene, the Agulhas Plain would have been under marine transgressions (Linder, 2003). Under these conditions, it would be expected that mountainous areas, with their broad, ancient mature valleys (Barnard, 1927), would act as refugia and, following recession, populations would be established from these high-altitude areas. Taxa inhabiting mountainous regions would be expected to be most basal, with the low-lying taxa derived. Indeed, speciation in many floristic components of the Agulhas Plain has been recent (3 – 4 Myr) and, with many regional endemics restricted to geologically young strata, an abundance of phylogenetically derived taxa is expected

(Cowling *et al.*, 1992). In the case of *Mesamphisopus*, however, the pattern appears to be reversed. It is, however, entirely probable that not all taxa inhabiting the coastal foreland had been extirpated during these transgression events, as discontinuous fold ridge outliers lying on the Agulhas Plain (Lambrechts, 1979) could have acted as refugia. These events could have also induced allopatric divergence of a more widespread taxon as these refugial habitats were occupied. Calculated divergence times between these coastal belt lineages and those inhabiting the Cape Peninsula and Cape Fold Mountains, however, predate these Cenozoic sea level increases. For example, *M. tsitsikamma* is shown to have diverged ~ 44.5 Myr ago, with the *M. penicillatus – M. paludosus* clade diverging from the Cape Peninsula and Cape Fold Mountains taxa ~ 36.5 Myr ago. These dates rather correspond to, and suggest that divergence among these coastal taxa and the montane taxa may result from, a tectonically induced transgression proposed to have occurred in the late Eocene (Hendey, 1983b). While the sea levels rose by 150 m during the major Miocene transgression (Hendey, 1983b), incompletely inundating the coastal foreland and Agulhas Plain (Linder, 2003), Eocene transgressions were greater in magnitude (200 m) and would have inundated these area almost entirely (Linder, 2003) – making the persistence of these taxa in refugial areas remarkable. A pattern similar to that proposed by Nicholls (1926, 1943) as an alternative to the above scenario, where sub-alpine species are specifically derived from robust, widespread lowland species, is thus not entirely inconsistent with that seen in *Mesamphisopus*. Here, the lowland taxa have persisted and underwent speciation *in situ*; e.g. the *M. penicillatus* and *M. paludosus* having diverged ~ 11 Mya ago, perhaps as a consequence of the Miocene transgression. The apparent taxonomic and phylogenetic isolation of *M. tsitsikamma* and the *M. paludosus – M. penicillatus* clade, with long branches (relative to others within the single gene phylogenies) leading to each, may result from the extirpation of closely related taxa by the subsequent major transgression of the Miocene and lesser trangressions of the Pliocene

and Pleistocene (see Hendey, 1983b), although sampling artifacts cannot be discounted here. Still, no phreatoicideans have yet been recorded from the low-lying coastal regions between the temporary wetlands of the Agulhas Plain and the collection locality of *M. tsitsikamma* in the Eastern Cape. Populations from longitudinally intermediate localities have been sampled only from high-altitude localities in the Cape Fold Mountains. Phreatoicidean populations are also not expected throughout much of this intermediate area, as suitable habitat is presently lacking. Although the broad, lower reaches of two major river systems cut through this coastal belt, temporary wetlands and slow-flowing streams are largely absent. Towards the coastal mountains in the east, some freshwater lakes occur (from which phreatoicideans have yet to be recorded), but most drainages are precipitous and fast-flowing. Interestingly, the biogeographic and phylogenetic distinction of *M. tsitsikamma* and the *M. penicillatus – M. paludosus* clade is reflected within the paramelitid amphipods (Stewart, 1992), where a large phenetic divergence was evident among species collected from largely the same localities in the Eastern Cape and Agulhas Plain, and those from elsewhere (Stewart, 1992: Fig. 5). In correspondence with the basal position of *M. tsitsikamma* (and the *M. penicillatus – M. paludosus* clade), it is also interesting to note that many groups within the Cape flora have clades or lineages presently centred within the southern Cape occurring basally to the more species-rich Western Cape clades (Linder, 2003).

While vicariance brought about by transgressions is provided as explanation for many of the patterns observed, it is important to point out that regressional events throughout the Cenozoic may also have played a critical role in isolating taxa or populations. Under typically xeric, regressional conditions (Hendley, 1983b), distances between mountains and the coast also increased (Linder, 2003), increasing the length of river courses and altering them substantially. Additionally, the lack of rainfall, particularly in mountainous regions (Linder,

2003), under these conditions would also have diminished the probability of both active and passive migration among populations in different watersheds and separate drainages, contributing to their isolation.

Mesamphisopus capensis and *M. baccatus* are restricted to outlying massifs of the Cape Fold Mountains (Lambrechts, 1979; Hendey, 1983b) on the Cape Peninsula (Harrison and Barnard, 1972; Chapter 2). The divergence of this clade from taxa present on the Hottentot's Holland Mountains and mountains eastwards was dated as occurring ~17.5 Myr ago. This is indicative of separation coinciding with the major Miocene transgression (Hendey, 1983b) discussed above. The geological history of the formation of the Cape Flats, the separation of the Cape Peninsula and the Cape Fold Mountains, and the Cenozoic history of transgressions, regressions and climate change in the region have been discussed earlier (Chapter 2; see too Hendey, 1983a, b). Dating using ML-corrected sequence divergences from the above COI data set and applying an intermediate divergence rate (2.3% per Mya) derived from molecular clocks calibrated for this gene fragment in Crustacea (Knowlton *et al.*, 1993; Knowlton and Weigt, 1998; Schubart, Diesel and Hedges, 1998; Baldwin *et al.*, 1998; Wares, 2001b) places this divergence 11 Mya ago. Earlier dating (Chapter 2) based on 12S rRNA sequence divergences and using molecular clocks calibrated for non-homologous gene regions indicated a later divergence (~ 7 – 8 Myr) than that presented here, while dating based on allozyme divergence, implementing a protein clock specifically calibrated for isopods (see Chapter 2), was more comparable to estimates derived from the relaxed Bayesian clock. Few taxa from the Hottentot's Holland Mountains were included in this latter study, however. Included taxa were also presumably closely related, at least in terms of morphological similarity, to *M. capensis* (the fact that these are found nested within the *M. depressus – M. abbreviatus* clade is indicative of the unreliability of certain characters deemed to be of

systematic importance (see Kensley, 2001; Chapter 2), and of the taxonomic difficulty of the group). Interestingly, Wishart and Hughes (2003) dated the divergence of blephariceridid dipterans (midges) across the Cape Flats, using the COI gene fragment and Brower's (1994) identical rate of sequence divergence, at ~ 2 – 3.5 Myr. These discrepancies illustrate the difficulties in the application of molecular clocks, particularly with regards to different taxa (with different life-histories, generation times and dispersal capacities) and non-homologous gene fragments, and the need for accurate and taxon-specific calibration. These discrepancies further indicate that many studies may benefit from the re-examination of estimates of divergence times, through the application of the relaxed Bayesian clock. This is particularly pertinent in situations where conclusions are drawn from, or explanations for phylogeographic and phylogenetic patterns are sought in, complex and recurring paleogeological and paleoclimatic histories.

The distribution of *M. capensis* on the Cape Peninsula (see Chapter 2) is peculiar, occurring on Table Mountain in the north to the low-lying southern parts of the peninsula, with the type locality of *M. baccatus* apparently nested within this distribution (Chapter 4). As the low-lying parts (including much of the southern extent) of the peninsula were also affected by Cenozoic trangression-regression events (Chapter 2), it is probable that separation of *M. capensis* and *M. baccatus* on isolated mountain massifs (Table Mountain and Silvermine/Constantiaberg, respectively) led to their allopatric divergence, approximately ~7.5 Myr ago. Chance dispersal or establishment events, following the last regression may have led to the curious distribution of *M. capensis* – with populations on the southern peninsula (of which *M. capensis* 2 was representative) perhaps being established from higher-altitude Table Mountain populations (*M. capensis* 1) about 2.9 Mya ago.

Relationships within the remaining clade are less resolved and the explanation of biogeographic patterns is difficult, primarily due to a paucity of paleogeological or palaeoclimatic data for these mountainous regions or comparative patterns from other fauna. The divergence of the 'derived' *M. abbreviatus – M. depressus* clade (together with the Betty's Bay representative) from the more western Hottentot's Holland clades and lineages (4) is minimally dated at ~ 9 Myr. While lineages of the western zone were derived earlier (~ 9 – 11 Myr ago), the Steenbras 1 – Steenbras 2 (southern Hottentot's Holland Mountains) and *M. albidus – M. setosus* (northern Hottentot's Holland Mountains) sister clades were shown to diverge from each other around the same period (~ 7 Myr). These divergences all coincide with period during the late Miocene, during which the major transgression was subsiding (Hendey, 1983b). Climatically, conditions would have still been warm and mesic (Hendey, 1983b). The mechanisms responsible for divergence are not known, but may relate to increased isolation of habitats and drainage system alterations as conditions became more xeric (Hendey, 1983a, b).

Following the derivation of the Betty's Bay lineage (~ 7 Myr ago), radiation within the 'derived' clade was rapid, evident from the short branches within this clade, and recent. The divergence of lineages (forming a polytomy) arising basally in this clade occurred ~ 4.4 Myr ago, while more recent splits supported in this clade were between ~ 3.5 and 1.5 Myr old. As found previously (Chapter 3), relationships were poorly resolved within this clade and bore no apparent relation to altitude, mountain range, or drainage system. This is despite the Cape Fold Mountains being largely unchanged, and the southern Cape river systems, draining this eastern Cape Fold zone, being independent throughout the Cenozoic (Hendey, 1983b). The above patterns cast serious doubt on the utility of *Mesamphisopus* as a biogeographic indicator of hydrogeographic change. In contrast, biogeographic, phylogenetic and

phylogeographic patterns within other freshwater fauna of the region have revealed evidence of the ancient connectivity of drainages (Farquharson, 1962; Jubb, 1964; Waters and Cambray, 1997; Bloomer and Impson, 2000; Daniels, 2003) or their hydrogeographic independence (Daniels, 2003) within the region. Interestingly, the paramelitid ampipods, a similarly ancient relictual Gondwanan group, too reveal surprisingly little concordance with drainage systems and appear to be of little use as biogeographic indicators in this regard. A morphological phylogeny of the family Paramelitidae (Stewart and Griffiths, 1995) shows also surprisingly little concordance with the phylogenies above. However, fine-scale patterns are possibly obscured by the presence of a number of taxa with fairly wide distributions (Stewart and Griffiths, 1995).

This recent radiation and lack of resolution of this clade, and the apparent lack of macrogeographic structure (geographic-phylogenetic discordance) perhaps argue for random long-distance dispersal events leading to the establishment and subsequent isolation of populations/taxa within this region (and to a lesser extent within the western Hottentot's Holland region). The distribution of null alleles at the *Ldh*-locus provides some evidence in this regard, if a common ancestral inactivation of the *Ldh*-locus is assumed, as the "minimizing" coding methodology does (Berrebi *et al.*, 1990). Populations fixed for these null alleles are not necessarily geographically proximate (see Chapter 3), although most are found within this eastern zone, and their presence at their respective localities could be explained by long-distance dispersal from an ancestral population, lacking expression of the locus, to different localities within this eastern zone and to localities within the Hottentot's Holland Mountains (e.g. Kogelberg). The presence of populations (i.e. the geographically proximate Tradouw Pass and Grootvadersbos populations) in which the *Ldh*-locus is expressed in this eastern zone, may too reflect such long-distance dispersal events and could

perhaps be derived from an ancestor originating from the western/Hottentot's Holland clade. Plausibly, this eastern ancestor could give rise to the ancestral population in which the locus is not expressed, as indicated above. Similar long-distance dispersal event have often been invoked to explain biogeographic, phylogenetic or phylogeographic patterns within Crustacea (Taylor *et al.*, 1996; Taylor *et al.*, 1998; Pálsson, 2000; Cox and Hebert, 2001; Remigio *et al.*, 2001; Adamowicz *et al.*, 2002; Michels *et al.*, 2003). However, most of these studies investigated micro-crustaceans (including anostracans and cladocerans), with passive dispersal of adhesive, digestion-resistant diapausing eggs (ephippia) or resting stages being facilitated by abiotic (wind or water) agents and biotic vectors. Primarily, these were thought to be water birds, distributing the micro-crustacea along their migration routes. As these isopods are presently largely restricted to seepage areas, isolated runnels and small first-order streams in broad, ancient valleys, more mesic periods during the Pliocene and Pleistocene may have been more conducive to dispersal, providing more opportunity for contact with animal (bird) vectors. With peracarid young being brooded in the female marsupium, it is however difficult to envisage such passive transport of any individuals other than the smallest, recently released *Mesamphisopus* mancas, and in large enough numbers to establish viable populations. The mechanisms facilitating such dispersal events, should they be responsible for the patterns observed, remain uncertain here. Alternatively, patterns in these two regions can perhaps be explained by random lineage sorting, and divergence through repeated bottlenecks, as has been proposed for the differentiation of populations within the *M. abbreviatus – depressus* group (Chapter 3). Here, the populations of the eastern zone may represent relicts of a previously widespread taxon, originating from the western zone. Long-distance dispersal events may still need to be invoked to account for the distributions of the Steenbras 1 and Kogelberg populations.

5.4.4) Shortcomings and future directions

The above estimation of divergences is hampered by the unavailability of well-dated, independent, external and internal calibration points. The wide confidence intervals of the estimated times of divergence suggest inherent inaccuracy, and together with the poor resolution of, or support for, certain relationships, argue for cautious interpretation of the patterns and only tentative acceptance of the explanations provided. Including all *Mesamphisopus* species within a broader molecular phylogeny of the Phreatoicidea, once more data are available, may provide better estimates of divergence times, as certain nodes can be constrained, for example by additional geological events (e.g. Gondwanan fragmentation of Australia, New Zealand and India) (Wilson and Johnson, 1999; Wilson and Edgecombe, 2003) and by the age and placement of a fossil phreatoicidean (*Protamphisopus wianamattensis*) among extant taxa in a morphological phylogeny (Wilson and Edgecombe, 2003).

A morphological phylogeny will too be more instructive, and will be completed following the examination and identification of possible species within the *M. abbreviatus – M. depressus* complex. Morphological and morphometric analyses (morphometric data have been collected for representatives of all included taxa/populations) were not attempted here. This was primarily due to the concern that, given the morphological conservatism of the genus (Barnard, 1927), differentiation within taxa may be greater than differentiation among taxa, resulting in discordance or poorly resolved phylogenies (Wiens and Penkrot, 2002). With increased taxonomic work, the systematic importance of certain characters will be revealed and these concerns perhaps negated. The consistently retrieved sister relationships of *M. penicillatus* and *M. paludosus*, and *M. albidus* and *M. setosus*, respectively, suggested by

morphology, already provides some indication that, despite the morphological conservatism, parsimony informative morphological characters may be identified upon closer examination.

This study provides a first approximation of evolutionary and biogeographic patterns within an obligate freshwater southern and south-western Cape endemic. Unfortunately, save for broad patterns, fine-scale patterns and the processes responsible for them do not intuitively follow from these analyses. The genus examined is apparently only of limited use for examining hydrogeographic patterns. Comparable data also remain scarce. For instance, recent phylogenetic and phylogeographic investigations of freshwater taxa of the region have been relevant at a more local geographic scale (e.g. Wishart and Hughes, 2003), precluding an examination of wider biogeographic patterns, or have had a substantially different biogeographic focus, and sampling design (Daniels *et al.*, 2002b). Further work, focussing on similarly endemic, narrowly distributed freshwater endemics should contribute greatly to an understanding of biogeographic and evolutionary patterns. In this regard, the study of the many paleoendemic and enigmatic groups of the region should be fruitful. Stuckenberg (1962) and Harrison and Barnard (1972) had listed many additional paleoendemic freshwater groups occurring on the Cape Peninsula (Table Mountain), and having a wider distribution across the Cape Fold Mountains of the "mainland". These included corydalid Megaloptera Latreille, 1802, notonemourine Plecoptera Burmeister, 1838, leptophlebiid and ephemerellid Ephemeroptera Hyatt & Arms, 1890, the Synlestidae Tillyard, 1917 (Zygoptera Selys, 1854), sericostomatid and molannid Trichoptera Kirby, 1815, and helminthid Coleoptera Linnaeus, 1758. The hydraenid Coleoptera, being incapable of flight, may be of particular interest in this regard (Harrison and Barnard, 1972). While a morphological phylogeny of the paramelitid amphipods has been presented (Stewart and Griffiths, 1995), a molecular phylogeny may reveal undetected, corresponding biogeographic patterns and will enable

tentative dating of the divergence of the major lineages and clades. This should contribute greatly, in substantiating or refuting certain aspects of the patterns described above, to an understanding of the biogeography and evolution of freshwater invertebrates of the region.

Chapter 6: Summary and general conclusion

6.1) Diversity

The present study made a significant contribution to the taxonomy and systematics of the southern African phreatoicidean genus *Mesamphisopus*. Six new species were identified and described (Chapter 4), bringing the total number of known species in the genus to ten (Kensley, 2001). Although the relationships among populations initially identified as *Mesamphisopus abbreviatus* or *M. depressus* remained unresolved through the analyses of molecular data (Chapter 3; Chapter 5), the phylogenetic relationships among these and the recognized species, distance criteria and limited morphometric data suggested that this group (the Hottentot's Holland Mountains clade and, particularly, the eastern clade identified in Chapter 5) contains undescribed species-level diversity. Such geographically defined clusters of morphologically similar species are typical within the Phreatoicidea (Wilson and Keable, 2002a). Clearly, more work is required to fully resolve relationships within these problematic groups and to accurately delineate species within them. However, a casual estimate, based only on data from populations included in this study, suggests that as many as 20 species could eventually be recognized within the genus.

However, caution does need to be expressed. These casual estimates of species diversity are based primarily on genetic distances or on distance-based topologies, as were the initial delimitations of the newly described species. In the latter case, however, additional evidence corroborated the designations, with the species being morphologically distinguishable. It has been suggested that genetic distances are ideally suited to, and perhaps preferred over

phylogenetic approaches to, species delineation (see Ferguson, 2002; and references therein). This is primarily due to the fact that these estimates are objective measures of the extent of genetic divergence among populations or species and are not explicitly tied to any species concept or theory concerning the speciation process (Ferguson, 2002). Nonetheless, there are limited practical difficulties (see Chapter 2) and more serious conceptual problems (see Ferguson, 2002) in using genetic distances in this regard. A further potentially problematic aspect in the present study is the fact that genetic distances (and hence allozyme topologies) were determined by a limited sample of loci. Although many enzyme systems were initially assayed, only 12 loci provided reliably interpretable banding patterns and were included in the analyses. Small sample sizes, both in terms of the number of individuals and the number of loci studied, are known to bias distance estimates (Mueller and Ayala, 1982). Many authors have further highlighted the need for assaying a large number of loci for accurate determination of genetic distances (Nei and Roychoudhury, 1974; Nei, 1978; Gorman and Renzi, 1979; Hillis, 1987). Although small sample sizes do not necessarily bias distance estimates in a particular direction, it needs to be considered that increased sampling of loci reduces the substantial standard errors involved in calculation of distance (and heterozygosities) (Nei, 1978). While some proposed sample sizes (see Nei, 1978; Mueller and Ayala, 1982) are practically unfeasible, the study of a large number of individuals may offset the effect of a small sample of loci on genetic distances (Nei, 1978). A large number of individuals were assayed for many populations included in the study, but it is also worth pointing out that varying sample sizes (in terms of the number of individuals and loci) may influence distance estimates and resultant dendrograms (Archie *et al.*, 1989). The above considerations may, indeed, alter the conclusions drawn here. Genetic distances are also likely to be biased by which particular loci are examined. For example, Gillespie and Langley (1974), Sarich (1977), and Nelson and Hedgecock (1980) have suggested the

existence of two protein classes, differing in their rates of evolution: rapidly evolving proteins (e. g. non-specific phosphotases, esterases, plasma proteins, and alcohol and aldehyde dehydrogenases, all with general functions and multiple substrates) and conserved proteins (e.g. structural, regulatory, ribosomal proteins and proteins involved in the glycolysis and citric acid cycles, all substrate-specific and acting intracellularly). While the choice of loci examined was not explicitly biased towards any particular class of enzymes, the effects of locus choice and the proportional representation of variable and less-variable loci on genetic distance cannot be ignored.

The sampling program embarked upon in the present study has been the most extensive yet for the group. Despite this, time limitations and the inaccessibility of many localities, where phreatoicideans are likely to occur, means that sampling was by no means exhaustive. Indeed, the reliance on fresh tissue necessary for the allozyme analyses further hindered collection effort. Considering this, and the apparently restricted distributions of individual taxa (see below), potentially many more species still remain to be discovered within South Africa. Many areas with suitable habitat still remain to be sampled, even in close geographic proximity to where collections were made and it is not improbable that populations or species will be found geographically intermediate to the localities included in this study. These populations could potentially resolve some of the relationships discussed earlier. Fervent recent interest in the Phreatoicidea in Australia has led to the description of many new genera and species (e.g. Wilson and Ho, 1996; Knott and Halse, 1999; Wilson and Keable, 1999, 2002a, b) and the discovery of new taxa shows no signs of abating. As many as one new genus and 20 new species (including 14 in a single existing genus) have been identified and await description in Australia (Wilson and Johnson, 1999; Wilson and Keable, 2002a; Wilson and Edgecombe, 2003). The belief that much undescribed diversity remains to be discovered

with increased sampling and study, both in South Africa (Kensley, 2001) and elsewhere (Wilson and Ponder, 1992; Wilson and Ho, 1996; Wilson and Keable, 2001, 2002a), thus remains. Although the South African fauna is likely to be less diverse than the Australian fauna, which shows high levels of generic and specific diversity and endemism (Wilson and Johnson, 1999) and where linear extrapolations have suggested the presence of at least 200 species (Wilson and Keable, 2001), the present understanding of the levels of diversity and endemism is far from complete. Nonetheless, the phreatoicidean isopods clearly present another example of a freshwater invertebrate with high diversity and endemism in South Africa (Wishart and Day, 2002).

With the geographic extent of sampling in the present study overlapping that of earlier amphipodan studies (see Stewart and Griffiths, 1995), the taxonomic diversity of the phreatoicidean isopods in South Africa is likely to be similar to that of the paramelitid amphipods. This family, represented in South Africa by some 25 species in three genera (Stewart and Griffiths, 1995), is the only other peracarid crustacean group represented widely and abundantly throughout freshwater habitats of the southern and south-western parts. These amphipods are similarly ancient, taxonomically isolated, Gondwanan relicts and are thought to have a similar evolutionary history within southern Africa (Barnard, 1927).

At the generic level, still only a single phreatoicidean genus is recognized within South Africa, although morphological evidence suggests that this genus needs to be redefined (Chapter 4). Fossil evidence (Chilton, 1918; Wilson and Edgecombe, 2003) indicates a long occupancy of Australia by the Phreatoicidea. This is substantiated by the high generic and species diversity and endemism of the suborder (Wilson and Johnson, 1999), and the taxonomic isolation of many lineages, now represented by monotypic genera (e.g. Knott and

Halse, 1999; Wilson and Keable, 2002a). The presence of only one genus in South Africa indicates that this area was perhaps only on the periphery of the phreatoicidean distribution, centred on western and "Antarctic" Gondwana, during pre-fragmentation times (Bănărescu, 1995; Wilson and Edgecombe, 2003) or had perhaps only been occupied more recently, just prior to the Gondwanan fragmentation. Both cases find some support in the fact that no phreatoicideans have yet been documented from South America (Wilson and Keable, 1999; Kensley, 2001). The further absence of true hypogean species from (South) Africa can also be taken as evidence of the region's peripheral status or recent occupancy (Kensley, 2001).

6.2) Distribution

A pattern of highly restricted, refugial distributions appears to be typical of taxa (both genera and species) within the Phreatoicidea (Wilson and Johnson, 1999; Knott and Halse, 1999; Wilson and Keable, 2001, 2002a). Many species are known from only a few geographically proximate localities, or are known from their respective type localities only (e.g. Nicholls, 1943, 1944; Wilson and Ho, 1996; Knott and Halse, 1999; Wilson and Keable, 2002a, b). Even the distribution of potential conspecifics appears to be highly restricted and isolated (Barnard, 1927), as has been demonstrated, for example, for *Crenoicus*, where morphologically differentiated populations were isolated in swamps over very short geographic (< 1 km) distances (Wilson and Ho, 1996). Barnard's (1927) description of varieties and eventual species (Nicholls, 1943) from only a few localities suggested that this might too be the case in *Mesamphisopus*. The distribution of populations and taxa in the present study substantiates this view. Most of the newly described species (*M. albidus*, *M. baccatus*, *M. kensleyi*, *M. setosus* and *M. tsitsikamma*), as well as *M. abbreviatus* and *M.*

depressus (accepting the species assignment of museum specimens, but see Chapter 3 and 5) are known from the type localities only. The restricted distributions of these taxa substantiate, incidentally, the belief that many individual populations of the unresolved "*M. abbreviatus* and *M. depressus*" group may indeed represent unique species. *Mesamphisopus capensis*, *M. paludosus* and *M. penicillatus* are known from more localities, but are still restricted in distribution to relatively small geographic areas. *Mesamphisopus capensis* occurs on the Cape Peninsula, from Table Mountain at the northern extent of the peninsula to the south (Chapter 2). *Mesamphisopus penicillatus* is known from Barnard's (1940) type locality, from which subsequent collection attempts have proved futile (see Chapter 4), and one other proximate locality. *Mesamphisopus paludosus* is perhaps the most widespread of the taxa included or identified in this study; collected from two localities on the Agulhas Plain. The species probably has a wider distribution across the temporary wetlands of the region.

Although collection was not extensive and ecological or physical data pertaining to habitat was not always collected, certain broad generalizations can be made regarding the distribution of the genus. Barnard (1927) had suggested that the distribution of *Mesamphisopus* was determined primarily by the extent of the mist belt, rather than rainfall *per se*. Mist carried by the south-eastern trade winds would provide sufficient moisture during dry summer months to ensure population survival. As a result, *Mesamphisopus* occurred only on the mountains of the Cape Peninsula, the Franschhoek and Hottentot's Holland Mountains, and, to the east, along the southernmost ranges of the east-west lying Cape Fold Mountains (Barnard, 1927) – a distribution wholly substantiated by the present collections. Mountainous areas, some with suitable habitat, occurring to the north of an east-west lying "mist belt line" (Barnard, 1927) were uninhabited. These included the Witteberg Mountains in the vicinity of Tulbagh and Ceres, the Langeberg Mountains in the vicinity of Montagu, and the Cedarberg Mountains –

the western extent of the Cape Fold Mountains with a north-south axis (Lambrechts, 1979). Further, phreatoicideans were not found in areas of the Cape Fold Mountains subject to the most intense folding (Barnard, 1927; this study), as physically suitable habitat was absent. Geologically, as noted by Barnard (1927), most of the present collections (with the exception of the taxa/populations collected from the lakes and temporary wetlands of the coastal belt) were taken from localities situated on Table Mountain Sandstone. No populations were sampled from localities situated on the overlying Bokkeveld Shale beds. These habitats, as stated by Barnard (1927), are markedly dry and have substantially different water chemistry – *Mesamphisopus* is believed to be confined, presently, to highly acidic water bodies (Harrison and Barnard, 1972).

Mesamphisopus was predominantly encountered in high-altitude, broad, flat, mature valleys, where streams were not more than slow-flowing seepages, springs or runnels. Where these streams were larger, they were slow flowing and often formed disconnected stagnant pools. With limited exceptions, specimens were not found in the typically rocky, fast-flowing, narrow and geologically young, mountain streams. Their occasional presence in these streams (such as the *M. penicillatus* population sampled) probably results from individuals being washed down from their high-altitude habitats. Although *M. penicillatus* was described from a locality that was formerly a coastal lagoon, this study is the first to record the presence of *Mesamphisopus* in the larger water bodies (lakes and temporary wetlands) of the coastal belt. Interestingly, measured water temperature in these water bodies, where *Mesamphisopus* was abundant, occasionally exceeded 20 °C. This casts some doubt on Barnard's (1927) belief that high water temperatures also limit phreatoicidean distribution.

While Barnard (1927) had suggested that altitude was not a factor influencing phreatoicidean distribution, Kensley (2001) believed that the altitudinal distribution of the South African phreatoicideans warranted investigation. However, both earlier (Barnard, 1940) and subsequent (this study) collections, with isopods occurring at high altitude and at sea level, substantiate Barnard's (1927) view. Altitude does broadly appear to influence phreatoicidean distributions (more populations were sampled from mountainous regions than at lower altitude), but only insofar as the occurrence of suitable habitat (i.e. broad, low valleys with slowly-moving water) is related to altitude.

6.3) Differentiation

This study has demonstrated marked differentiation among putative taxa, in terms of allozymes, mtDNA and morphometrics (Chapter 2; Chapter 3). Further differentiation, particularly at allozyme loci, has been documented among populations of individual taxa (e.g. Chapter 2; Chapter 3). Differentiation within *Mesamphisopus* appears to reflect a general pattern within aquatic invertebrates, i.e. taxa morphologically identified as a single species actually represent a multitude of genetically differentiated populations (or taxa) subdivided by watershed boundaries (Perry, Lodge and Feder, 2002). This pattern has been documented in certain Australian phreatoicideans, where multiple, (presumably genetically) differentiated species occur in individual watershed of drainages (Wilson and Ho, 1996) and in certain instances in the present study (Chapter 3). The marked differentiation among populations in the present study has been interpreted as resulting from population genetic processes such as repeated population bottlenecks (brought about by extreme variations in seasonal aridity), or multiple extinction and recolonization events, followed by stochastic processes of mutation,

genetic drift and lineage sorting (Chapter 3). Further towards the phylogenetic end of the continuum (Chapter 5), patterns of differentiation among taxa are explained within a paradigm of allopatric or vicariant divergence, and speciation primarily brought about by Miocene climate and sea-level changes. Consequently, biogeographic patterns reflect these events, with patterns of chance long distance dispersal invoked to account for the few unusual patterns observed. At both levels, patterns of differentiation may be explained by the stochastic differentiation of a formerly widespread taxon and its subsequent extinction from many habitats.

While surveys of the allozyme literature have been conducted for invertebrates (Thorpe, 1982; Thorpe and Solé-Cava, 1994), Crustacea in general (Hedgecock, Tracey and Nelson, 1982), and specific peracarid Crustacea (e. g. Amphipoda; Stewart, 1993), similar surveys are lacking for the Isopoda. As a result, it becomes more difficult to place the extent of genetic differentiation among taxa/populations in context. For sequence-level divergence determined from mtDNA gene fragments, Wetzer (2001) has provided a hierarchical examination of the systematic utility of these data. Nonetheless, the comparison of the above data (Chapter 2; Chapter 3) with widely disparate isopod literature (references in individual chapters) reveals a large correspondence in most cases (but see Chapter 3), and has provided a systematic framework in which to interpret these data.

Where examined, taxa or individual populations were determined to be morphometrically distinct. Similar morphological and morphometric differentiation has been documented over very short geographic distances in the Australian fauna (Wilson and Ho, 1996). This differentiation belies the often-documented morphological conservatism of the group (Barnard, 1927; Nicholls, 1943; Wilson and Ho, 1996), as morphometrically distinct

populations have been shown to be valid species, diagnosable morphologically upon closer investigation (see Chapter2; Chapter 4).

Upon closer examination, taxa described in this study, and the four known species, were distinguishable morphologically, mostly through the examination of a combination of features. These included features of the mouthparts, pereopods, uropods, and, more specifically, pleopodal and setation features. Few taxa could be reliably identified using single (autapomorphic) features, and certain features believed to diagnose species (see Kensley, 2001; Chapter 2; Chapter 3) were shown to be of little systematic importance. Other peracarid crustacean groups are often plagued by similar morphological conservatism and intraspecific variability (e.g. Meyran *et al.*, 1997). Consequently, a similar lack of reliable diagnostic or apomorphic characters for both species identification and phylogenetic analysis has been documented in Amphipoda (e.g. Englisch and Koenemann, 2001; Englisch, Coleman and Wägele, 2003), impeding an understanding of the diversity and relationships in these groups. Nonetheless, the systematic importance of certain features has been noted in certain morphologically conservative groups, e.g. the second antenna and pereopod III in *Paramelita* Schellenberg, 1926 (see Stewart and Griffiths, 1995). While *Mesamphisopus* appears to be more conserved, at least superficially, the examination of more representatives and more characters will undoubtedly highlight more variable, and systematically relevant, features.

6.4) Conservation

In the most comprehensive treatise on the distribution and ecology of *Mesamphisopus* to date, Barnard (1927) raised concerns over the conservation of the Phreatoicidea, and freshwater peracarid Crustacea in general, highlighting the threats of, particularly, afforestation and veld-burning (the rotational burning of fynbos – the sclerophyllous, low-growing vegetation of the Western Cape – to maintain a mosaic of differing vegetation age classes). Poorly managed or uncontrolled veld-burning regimes and aseasonal fires are expected to impact negatively on invertebrate taxa, particularly those with poor dispersal ability and isolated, restricted distributions (Bigalke, 1979; Panzer, 2002). Additional threats to both floral and faunal components of the region identified subsequently include encroachment by alien vegetation (and concomitant water loss), water abstraction, and habitat modification through urban expansion and agricultural development (Rebelo, 1992; Cowling *et al.*, 1996; Picker and Samways, 1996). Similar concerns have been raised over the Australian isopod fauna, where afforestation (causing exaggerated wet-dry cycles and increased run-off), agricultural and mining practices, and impoundments have led to habitat degradation (Wilson and Johnson, 1999; Wilson and Keable, 1999, 2001, 2002b; Wilson, 2003). While groundwater abstraction and contamination (Wilson and Fenwick, 1999) are unlikely to be a threat to the South African fauna – no true hypogean phreatoicidean species have yet been discovered in South Africa (Kensley, 2001; this study), the other, more universal, threats remain.

While large portions of the Cape Fynbos region, including more than half of the Cape Peninsula itself and mountain fynbos regions, are protected in national or provincial reserves (Rebelo, 1992; Cowling *et al.*, 1996), management practices in the Western Cape are directed towards the floral assemblages, or are driven by aesthetic considerations, with the faunal

component largely disregarded (Picker and Samways, 1996). Given the natural paucity of water in the region (New, 2002), conservation efforts are also directed towards catchment management rather than the preservation of flora and fauna (Bigalke, 1979; Rebelo, 1992), these benefiting only as surrogates of these catchments (Wishart, 2000). Studies such as the above are thus important in highlighting the diversity of, and threats to, taxa that are often overlooked. In so doing, conservation efforts can perhaps be more directed towards the taxa in question or can aim to be more inclusive.

The sampling strategy employed in the molecular analyses in this study did not always facilitate the designation of evolutionarily significant units and management units for the purposes of conservation. Additionally, certain criteria for defining such units are not easily ascertained, operationally or empirically. For example, the ESU concepts of Ryder (1986) and Waples (1991) hinge upon on the demonstration of adaptively significant variation, or the evolutionary potential of the putative ESUs (Bowen, 1998): these cannot be assessed empirically with the data at hand, at least not on a contemporary timescale. Nonetheless, most populations were distinguished by significant frequency differences at nuclear (allozyme) loci (Chapter 2; Chapter 3) and, when multiple individuals were sequenced from populations (Chapter 3), these geographically isolated populations were monophyletic for mtDNA. This suggests that, given more extensive sampling, sequencing and analyses, most populations would indeed be recognized as ESUs or MUs under the criteria of Moritz (1994). The fact that these concepts have yet to gain a legislative foothold in South African conservation is of great concern (note, for example, their absence from provincial conservation ordinances included by Bürgener *et al.* (2001)), and negates their designation. Nevertheless, the demonstration of increased diversity within the group immediately increases their importance for conservation, as the conservation of groups that provide the highest

phylogenetic diversity for an area should be prioritised (Faith, 1992). Further, as is argued for the Australian fauna (Wilson and Keable, 2002a), the narrow endemic distributions, the phylogenetic distinctiveness of individual taxa and their vulnerability to extinction warrants their conservation. The relative conservation priorities of individual taxa or populations need to be considered on a case-by-case basis, based on perceived threats.

Many of the taxa and populations included in the present study are represented within conservation areas, and are thus not immediately threatened. For example, populations sampled (during this study and earlier) from the eastern mountains (Langeberg, Riviersonderend and Riversdale Mountains) of the Cape Fold Belt (see Chapter 5), initially identified as *M. abbreviatus* or *M. depressus* (Chapter 3), mostly occur within nature reserves falling under the jurisdiction of the Western Cape Nature Conservation Board. The high-altitude, relatively inaccessible nature of these habitats also affords these populations a degree of protection from anthropogenic influences, although aseasonal fires or uncontrolled fire-regimes may have a negative influence here. *Mesamphisopus tsitsikamma* was collected from a coastal temperate forest, bounded by a northern mountain range, within the Tsitsikamma National Park, and is protected. Increased tourist visitation to the only known locality from which this species has been collected, may place pressure on the habitat. While *M. albidus*, *M. setosus* and the Kogelberg population were collected from relatively inaccessible, high-altitude localities within nature reserves, the remaining populations from the western Hottentot's Holland Mountains may be more threatened. For example, *M. kensleyi*, collected from a single locality on the lower western slopes of the Hottentot's Holland Mountains above Gordon's Bay, may be threatened by future property development. The three Steenbras populations were collected from the periphery of a large dam, with one of the populations (as is the Grabouw population) situated within a commercially forested region. Interestingly, the

presence of this impoundment has not facilitated movement of isopods, as is evident from the lack of gene flow or apparent migration among populations in different streams entering the dam (Chapter 3). This western Hottentot's Holland area is also under intensive agriculture, particularly in the region around Grabouw. Forestry and agricultural practices, and growing informal settlements in the area may have already significantly influenced freshwater habitats in which isopods are expected to occur. On the Cape Peninsula, *M. capensis* and *M. baccatus* are found on in areas incorporated within the Cape Peninsula National Park (South African National Parks), and are thus afforded a degree of protection. However, increased tourist pressure may impact upon the *M. capensis* population on Table Mountain (Chapter 2) and *M. baccatus*, known only from the type locality in the Silvermine Nature Reserve. The southern Peninsula populations of *M. capensis*, collected from less accessible and less frequently visited parts of the Cape Peninsula National Park, seem to be less threatened in this regard. Appearing the most threatened, however, are *M. penicillatus*, *M. paludosus* and the Betty's Bay populations, occurring on the low-lying coastal foreland. The apparent destruction of the type locality of *M. penicillatus* by human activity has already been noted (Chapter 4). The other locality at which *M. penicillatus* was collected may, however, be threatened, as are the Betty's Bay populations, by urban expansion along this narrow coastal belt and increased human pressure. The *M. paludosus* populations occur in temporary wetlands (already sensitive environments) across the Agulhas Plain, an area under relatively intense livestock (and crop) agriculture, and under threat from alien vegetation.

A great conservation concern, over-riding the speculative threats highlighted above, is the effect of anthropogenically-induced climate change. The reality and threat of global climate change is becoming increasingly recognized, and climate change has been demonstrated to be threatening, or has been implicated in, the extinction of populations or taxa (e.g. McLaughlin

et al., 2002; Erasmus *et al.*, 2002; Thomas *et al.*, 2004). Given the narrow, highly relictual distributions of taxa, and their poor dispersal capabilities, rapid climate change could have catastrophic consequences for the survival of phreatoicidean populations or taxa. With a distribution chiefly determined by the presence of sufficient moisture, gathered from south-eastern mist belt, and affected by water temperature and chemistry (Barnard, 1927; Harrison and Barnard, 1972) increases in temperature and increased aridification could have serious repercussions. Although repeated population bottlenecks (or local extinctions and recolonizations) resulting from extreme seasonal aridity have been implicated in hastening the differentiation and contributing to allopatric speciation of populations (Chapter 3), the effects of climatic change, perhaps bringing about similar phenomena of longer duration, are unknown.

6.5) Importance of this study

While recent fervent interest in the Phreatoicidea has substantially increased our knowledge of the diversity, biogeography and evolution of the group (Wilson and Keable, 2001), most research thus far has concerned the generic level and higher. Taxonomic descriptions aside, most research has focused on resolving generic relationships within the suborder (Wilson and Johnson, 1999; Wilson and Keable, 1999, 2001, 2002b; Wilson and Edgecombe, 2003; ongoing research: R. Wetzer, Los Angeles County Natural History Museum; G. D. F. Wilson, Australian Museum; S. J. Keable, Australian Museum) or the phylogenetic placement of the suborder within the Isopoda (e.g. Brusca and Wilson, 1991; Wetzer, 2002; Dreyer and Wägele, 2003).

This study represents the first to specifically focus on a genus and to address questions pertaining to diversity, phylogeny and biogeography at the species-level within the Phreatoicidea. Specifically, this study is the first to implement genetic data (allozyme and mitochondrial DNA sequence data) and morphometric data to elucidate patterns of differentiation and examine species boundaries within a phreatoicidean genus. As such, it entrenches methodologies for examining differentiation at the species level within the Phreatoicidea, and establishes a framework for the identification of diversity and the delineation of species within the group. As this study represents the first such study on any isopod group within South Africa, and the only molecular investigation on South African peracarid Crustacea other than Stewart's (1992) and Stewart *et al.*'s (1994) investigations of the paramelitid amphipods, the procedures and approach adopted here may find wider application in much needed crustacean and invertebrate systematic studies.

This study also contains the first detailed taxonomic accounts of South African phreatoicideans since Nicholls' (1943, 1944) seminal revision. As such it is hoped that this study will stimulate interest in this group, and not only locally. Given the apparent diversity of the group (discussed above), similar species investigations will be fruitful and are much needed.

Although biogeographic patterns were only broadly resolved and tentatively explained, the evolutionary scenarios presented here provide a useful null-hypothesis that needs to be tested with data from various taxa. Such work is sorely needed to address the paucity of knowledge concerning the biogeography (particularly of invertebrates) of the Western Cape.

6.6) Future directions

The problems of time and the accessibility of sampling localities have been highlighted previously. Additional field work will, without a doubt, highlight additional taxa and enable more accurate conclusions to be drawn regarding distribution and biogeographic patterns and the relationships among taxa.

Morphological examinations of representatives of many populations have not yet been completed. These (ongoing) examinations should reveal the importance of various characters for species delineation within the genus, and in reconstructing evolutionary relationships. Many of the problematic or unresolved relationships proposed by molecular data in the current study may well be resolved through morphological examinations.

The need for morphological and, more particularly, molecular phylogenies of the Phreatoicidea, incorporating all genera and all known species, has been highlighted in the individual chapters. These may determine the correct placement of *Mesamphisopus* within the suborder, highlight the diagnostic morphological characters of this genus, and enable more accurate dating of cladogenic events within the genus.

Barnard (1927) had made ecological observations on *Mesamphisopus* over many years, noting aspects of population density and composition (in terms of sex and size), and reproductive biology. Despite his apparent lack of confidence in his data, these remain the only ecological data on *Mesamphisopus* yet presented. Such, albeit rudimentary, ecological observations may prove instructive, given the apparent species diversity of the group, as differences in these patterns may provide additional evidence of taxonomic status, or may reflect the influence of

local environments. The latter may be pertinent, given the perceived importance of demographic processes (e.g. population bottlenecks) in the differentiation, speciation and evolution of the genus.

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Appendix 1: Presumed key synapomorphies, and characters previously considered diagnostic (in combination), for the suborder Phreatoicidea. References, presented below the table, are numbered chronologically.

	Characteristic features	References[1]
Body	Fusiform/elongate, (sub)cylindrical, appears laterally compressed[2]	1 – 4, 6 – 9, 11
Head capsule	Deeper than broad	13
Pereon	First thoracic segment (and occasionally the second) fused to head	6, 7
	Six to seven free pereonites	7
Pleon	Long, six pleonites, first five distinct and movable, last fused to telson	1, 2 – 4, 6, 11
	Pleonite 5 longer than others[3]	7, 9, 10, 13
	Suture between pleonite 6 and telson may be strongly developed	7
	Pleura may be developed, projecting ventrally, or not	6, 13
Pleotelson	Large, subconical	1
	Vaulted, higher than broad, flexed ventrally with dorsally recurved distal tip[3]	9, 10, 12, 13
Eyes	Large, small or lacking; sessile, compound; widely separated, laterally placed or closely set; near anterior margin	6, 7, 11, 13
Labrum	Asymmetrical, freely movable from stout epistome	7
Antennula	Short, with peduncle of three articles	1, 6
	Uniramous, lacks rudiment of second flagellum	10, 11
Antenna	Long, with flagellum (equal to, or exceeding peduncle length)	1, 6
	Well defined peduncle of five articles	6, 7, 9
	Uniramous, lacking exopodite	6, 7, 11
	Article 3 without scale	13
	Basal article (article 1) of protopod reduced or absent[3]	10, 13
Mandibles	With well developed, three-jointed mandibular palp	1, 2, 4, 6, 7, 11
	Lacinia mobilis present on both mandibles, or on left mandible only (right lacinia mobilis variably reduced in many species)	6, 7, 9, 13
	Molar process (broad, flat, truncate, grinding) separated from incisor process by spine row	6, 7, 9
	Row of free setae separate spine row from molar	7
	Spine row on distinct medially projecting ridge/process[3]	6, 7, 10, 12
	Bifurcate spines present in spine row[3], adjacent to lacinia mobilis	12, 13
Maxillula	Proximal endite with many or few terminal setospines	7
Maxilla	Medial margin bears row of filter setae[4]	9
Maxilliped	Well developed, incorporated into mouthfield	7, 11
	Palp long, five-jointed, with robust plumose seta distolaterally on basis	7
	Coxa with epipodite, and vestigial oostegite in mature females	7

Pereopods	Anterior series of four directed foward, posterior series of three directed backwards	1, 2, 4 – 7, 11
	Pereopod I subchelate, prehensile, with inflated propodus	1, 6 – 8, 11
	Pereopods II – VII simple, II – IV articulate towards anterior of pereonites, and V – VII towards posterior	1, 6
	Pereopods II – IV ambulatory, rarely prehensile, IV generally sexually dimorphic, V – VII ambulatory	7
	Coxa small, or expanded, with well defined articulations with pereonites (at least last six)	3, 6, 9
	Coxae not developed into lateral plates (obscuring coxa-basis articulation)	9, 11
Pleopods	Broad, foliaceous, not protected by operculum	2, 4, 6
	Natatory and respiratory in function[2]	2, 4, 7, 8, 11
	Exopods of pleopod I uniarticulate, pleopods II – V biarticulate	7, 9, 13
	Narrow articulation between proximal and distal segments of biarticulate exopods[3]	10
	Lateral and medial epipods present, may be reduced on anterior pleopods (epipodites appear present on pleopods III – V)	6, 7, 13
	Pleopod II in male with appendix masculine arising from mesial border of endopodite	7, 11
Uropoda	Single pair; robust, biramous and styliform[3]	1 – 4, 7, 11, 13
	Lateral (subterminal), ambulatory[2]	6 – 8
	Protopod may be produced into distomesial process	7
	Rotated ventromedially[3]; projecting ventrally and posteriorly	10, 13
Genital pores	Both male and female genital pores on coxa of pereopods	9
Penes	Long, arising from coxa of pereopod VII[4]	7, 9, 11
Oostegites	Thoracic oostegites, four pairs on pereopods I – IV	7, 9, 11
	Two additional vestigial pairs (on maxilliped and pereopod V) may be present	7, 9

[1]References: (1) Chilton, 1883; (2) Chilton, 1891; (3) Calman, 1918; (4) Glauert, 1924; (5) Barnard, 1927; (6) Sheppard, 1927; (7) Nicholls, 1943; (8) Williams, 1966; (9) Brusca and Wilson, 1991; (10) Wilson and Ponder, 1992; (11) Kensley, 2001; (12) Wilson and Keable, 2001; and (13) Poore *et al.*, 2002.

[2]Characteristics used by Nicholls (1943) to distinguish the Phreatoicidea in his dichotomous key to the isopod suborders.

[3]Key synapormorphies of the Phreatoicidea, recently identified by Brusca and Wilson (1991), Wilson and Ponder (1992), Wilson and Keable (2001), and Poore *et al.* (2002).

[4]Symplesiomorphic characters of the Phreatoicidea, lost in other isopod suborders, but primitive within the Peracarida.

Appendix 2: Allele frequencies at the 11 polymorphic loci for the 11 populations of *Mesamphisopus* studied in Chapter 2. *N* = sample size. Allele frequencies in bold typeface indicate cases where genotype frequencies were found not to conform to Hardy-Weinberg expectations (all at $P < 0.05$). Refer to Figure 2.1 for full population names.

		Population										
Locus		EV	VRG	Kas	Nurs	Silv	Smit	KR	Sch	Fran	Jonk	GB
Ao	*N*	62	45	44	30	38	19	35	26	70	19	25
100		0.992	1.000	1.000	1.000	1.000	1.000	1.000	1.000	**0.764**	0.605	1.000
95		0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.236**	0.395	0.000
Ark	*N*	57	39	40	30	30	19	35	29	58	20	30
130		0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
115		0.991	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000
100		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000
Gpi	*N*	60	42	43	30	33	19	35	29	64	20	30
170		0.017	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000
145		0.000	0.000	0.000	0.000	0.682	0.000	0.000	0.000	0.000	0.000	0.000
140		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.033
125		0.983	1.000	1.000	1.000	0.212	0.395	0.043	0.707	0.000	0.000	0.000
115		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000
105		0.000	0.000	0.000	0.000	0.061	0.000	0.000	0.000	0.000	0.000	0.000
100		0.000	0.000	0.000	0.000	0.000	0.447	0.957	0.293	1.000	0.000	0.967
95		0.000	0.000	0.000	0.000	0.030	0.000	0.000	0.000	0.000	0.000	0.000
70		0.000	0.000	0.000	0.000	0.000	0.132	0.000	0.000	0.000	0.000	0.000
40		0.000	0.000	0.000	0.000	0.000	0.026	0.000	0.000	0.000	0.000	0.000
Hk	*N*	61	45	49	30	36	17	35	28	63	20	29
125		0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.000**	0.008	0.000	0.000
100		0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.036**	0.992	0.400	0.931
95		0.000	0.011	0.010	0.000	0.708	0.412	0.800	**0.607**	0.000	0.450	0.069
85		0.992	0.989	0.990	1.000	0.292	0.588	0.200	**0.357**	0.000	0.150	0.000
75		0.008	0.000	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	0.000
Idh	*N*	59	48	40	30	39	19	32	29	41	20	30
170		1.000	1.000	1.000	1.000	0.000	1.000	0.984	1.000	0.000	0.000	0.000
125		0.000	0.000	0.000	0.000	0.987	0.000	0.000	0.000	0.000	0.000	0.000

120		0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	1.000	0.000
100		0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	1.000	0.000	0.000
90		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
Ldh	*N*	62	43	49	30	39	15	32	28	67	20	30
100		**0.024**	0.000	0.000	0.017	0.000	0.000	0.422	**0.964**	1.000	0.975	0.000
80		**0.976**	1.000	1.000	0.983	1.000	1.000	0.578	**0.036**	0.000	0.025	0.000
70		**0.000**	0.000	0.000	0.000	0.000	0.000	0.000	**0.000**	0.000	0.000	1.000
Lt-1	*N*	56	34	41	10	35	19	15	29	65	20	30
100		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	1.000
95		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000
Mdh-1	*N*	64	48	48	30	39	19	35	29	69	20	30
100		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000
80		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
Mdh-2	*N*	64	48	49	30	39	19	35	29	69	20	30
190		0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000
100		1.000	1.000	1.000	1.000	0.987	1.000	1.000	1.000	1.000	1.000	1.000
Me	*N*	60	45	46	30	36	19	35	29	67	20	30
115		0.000	0.056	0.054	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
100		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000
75		1.000	0.944	0.946	1.000	1.000	1.000	1.000	1.000	0.000	0.000	1.000
Pgm	*N*	58	42	43	30	33	19	35	29	67	20	30
120		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	**0.000**	0.000	0.000
105		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.015**	0.000	0.000
100		1.000	1.000	1.000	1.000	0.894	1.000	1.000	0.983	**0.963**	0.900	0.000
90		0.000	0.000	0.000	0.000	0.106	0.000	0.000	0.000	**0.022**	0.000	0.000
80		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.000**	0.100	1.000

Appendix 3: Clustal X sequence alignment (338bp) of the 12S rRNA mtDNA gene fragment used to examine relationships among individuals tentatively identified as *M. capensis* (Chapter 2). Missing data are represented by '?', with indels (gaps) represented by hyphens.

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M. penicillatus           TTAGATTAATATTCTTCAAACCCAAAGAATATGGCGGTGTTTTTTCATAATTAGAGGAACCTGTCTATTA
Echo Valley               CTAAAT--ATGATTTTCAAACCTAAAGAATATGGCGGTGTTTTATTATAATTAGAGGAACCTGTTTATTA
Valley of the Red Gods    CTAAAT--ATGATTTTCAAACCTAAAGAATATGGCGGTGTTTTATTATAATTAGAGGAACCTGTTTATTA
Kasteelspoort             CTAAAT--ATGATTTTCAAACCTAAAGAATATGGCGGTGTTTTATTATAATTAGAGGAACCTGTTTATTA
Nursery Ravine            CTAAAT--ATGATTTTCAAACCTAAAGAATATGGCGGTGTTTTATTATAATTAGAGGAACCTGTTTATTT
Silvermine                CTAAAT--ATGATTTTCAAACCTAAAGAATATGGCGGTGTTTAACTATAATTAGAGGAACCTGTTTATTA
Smitswinkelbaai           CTAAAT--ATGATTTTCAAACCTAAAGAATATGGCGGTGTTTTATTATAATTAGAGGAACCTGTTTATTA
Krom River                CTAAAT--ATGATTTTCAAACCTAAAGAATATGGCGGTGTTTTATTATAATTAAAGGAACCTGTTTATTA
Schusters River           CTAAAT--ATGATTTTCAAACCTAAAGAATATGGCGGTGTTTTATTATAATTAGAGGAACCTGTTTATTA
Franschhoek               TTAGATTTATGTTCTTCAAACCTAAAGAATATGGCGGTGTTTAATTATAATTAGAGGAACCTGTTTATTA
Jonkershoek               TTAGATTTATGTTCTTCAAACCTAAAGAATATGGCGGTGTTTAATTATAATTAGAGGAACCTGTTTATTA
Gordon's Bay              TTA?ATTTATGTTCTTCAAACCTAAAGAATATGGCGGTGTTTAATTATAATTAGAGGAACCTGTTTACTA
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M. penicillatus           ATTCGATAATCCACGAAAATCTTACTTAAATTTAAAAAAATTAAAAGCTTGCATACCGTCGTTTGAAATA
Echo Valley               AT-CGATAATCCACGAAAATCTCACTTAAATTT----------CAAAGTTTGTATACCGTCGTCTAAAATA
Valley of the Red Gods    AT-CGATAATCCACGAAAATCTCACTTAAATTT----------CAAAGTTTGTATACCGTCGTCTAAAATA
Kasteelspoort             AT-CGATAATCCACGAAAATCTCACTTAAATTT----------CAAAGTTTGTATACCGTCGTCTAAAATA
Nursery Ravine            AT-CGATAATCCACGAAAATCTCACTTAAATTT----------CAAAGTTTGTATACCGTCGTCTAAAATA
Silvermine                AT-CGATAATCCACGAAAATCTCACTTAAATTT----------AAAAGTTTGTATACCGTCGTCTAAAATA
Smitswinkelbaai           AT-CGATAATCCACGAAAATCTCACTTAAATTT----------CAAAGTTTGTATACCGTCGTCTAAAATA
Krom River                AT-CGATAATCCACGAAAATCTCACTTAAATTT----------CAAAGTTTGTATACCGTCGTCTAAAATA
Schusters River           AT-CGATAATCCACGAAAATCTCACTTAAATTT----------AAAAGTTTGTATACCATCGTCTAAAATA
Franschhoek               AT-CGATAATCCACGAAAATCTTACTTAAATT-----------AAAAGTTTGTATACCATCGTTTGAAATA
Jonkershoek               AT-CGATAATCCACGAAAATCTTACTTAAATT-----------AAAAGTTTGTATACCATCGTTTGAAATA
Gordon's Bay              AT-CGATAATCCACGAAAATCTTACTTAAATT-----------AAAAGTTTGTATACCATCGTTTGAAATA
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M. penicillatus	ATATTCGAAAATCTTATTATCACATACCAATATAAATTTAAGGTCAGATCATGGCGCAGCTATATTTAAG
Echo Valley	ATATCTAAAAATTTTATTACCAAATAT--ACAAAA-TAAAATGTCAGATCATGGTGCAGCAATATTTAAG
Valley of the Red Gods	ATATCTAAAAATTTTATTACTAAATAT--ACAAAA-TAAAATGTCAGATCATGGTGCAGCAATATTTAAG
Kasteelspoort	ATATCTAAAAATTTTATTACTAAATAT--ACAAAA-TAAAATGTCAGATCATGGTGCAGCAATATTTAAG
Nursery Ravine	ATATCTAAAAATTTTATTACCAAATAT--ACAAAA-TAAAATGTCAGATCATGGTGCAGCAATATTTAAG
Silvermine	ATATCTAAAAATTTTATTGCCAAATAC--ACAAAA-TAAAATGTCAGATCATGGTGCAGCAATATTTAAG
Smitswinkelbaai	ATATCTAAAAATTTTATTACCAAATAT--ACAAAA-TAAAATGTCAGATCATGGTGCAGCAATATTTAAG
Krom River	ATATCTAAAAATTTTATTACCAAATAT--ACAAAA-TAAAATGTCAGATCATGGTGCAGCAATATTTAAG
Schusters River	ATATCTAAAAATTTTATTACCAAATAT--ACAAAA-TAAAATGTCAGATCATGGTGCAGCAATATTTAAG
Franschhoek	ATATTTAAAAATTCTATTTGTACATAT--ACATAAATAAAATGTCAGATCATGGTGCAGCAATATTTAAG
Jonkershoek	ATATTTAAAAATTCTATTTCTACATAT--ACATAAATAAAATGTCAGATCATGGTGCAGCAATATTTAAG
Gordon's Bay	ATATTAAAAGATTCTATTTTCACATAT--ACATAAATAAAATGTCAGATCATGGTGCAGCAATATTTAAG

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M. penicillatus	GTTAAATTGGTTACATTCTAAAATCTATTGACAGAAAATAAAATGAAAAATTATTTTAAGCCGAATCTAA
Echo Valley	ATTAAATTGGTTACATTCTATAATCTATCGACATTATTTGAATTTTAAACTCATTA-AAGTAGGATTTAA
Valley of the Red Gods	ATTAAATTGGTTACATTCTATAATCTATCGACATTATTTGAATTTTAAACTCATTA-AAGTAGGATTTAA
Kasteelspoort	ATTAAATTGGTTACATTCTATAATCTATCGACATTATTTGAATTTTAAACTCATTA-AAGTAGGATTTAA
Nursery Ravine	ATTAAATTGGTTACATTCTATAATCTATCGACATTATTTGAATTTTAAACTCATTA-AAGTAGGATTTAA
Silvermine	ATTAAATTGGTTACATTCTACAATCTATTGACATTATTTGAATTTAAAATTCATTA-AAGTAGGATTTAA
Smitswinkelbaai	ATTAAATTGGTTACATTCTATAATCTATCGACATTATTTGAATTTAAAACTCATTA-AAGTAGGATTTAA
Krom River	ATTAAATTGGTTACATTCTATAATCTATCGACATTGTTTGAATTTAAAACTCATTA-AAGTAGGATTTAA
Schusters River	ATTAAATTGGTTACATTCTATAATCTATCGACATTATTTGAATTTAAAACTCATTA-AAGTAGGATTTAA
Franschhoek	ATTAAATTGGTTACATTCTGTAATCTATCGACGTTATCTGAATTTAAAAATCACAA-AAGCAGAATTTAA
Jonkershoek	ATTAAATTGGTTACATTCTATAATCTATCGACGTTATCTGAATTTAAAAATCACAA-AAGCAGAATTTAA
Gordon's Bay	ATTAAATTGGTTACATTCTACAATCTATTGACGTTACATGAATTTAAAAATCAATA-AAGCAGAATTTAA

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M. penicillatus	A C G T A A T T T A A C A A G T T A T A A A C T T T T A A T G A A T A C T - A C A A A A C A T G C A C A T A T C G C
Echo Valley	A T G T A A T T - - A A A A A C T A T A A A T T T A T A A T G A A T A T T T C C A A A - C A T G T A C A C A T C G C
Valley of the Red Gods	A T G T A A T T - - A A A A A C T A T A A A T T T A T A A T G A A T A T T T C C A A A - C A T G T A C A T A T C G C
Kasteelspoort	A T G T A A T T - - A A A A A C T A T A A A T T T A T A A T G A A T A T T T C C A A A - C A T G T A C A T A T C G C
Nursery Ravine	A T G T A A T T - - A A A A A C T A T A A A T T T A T A A T G A A T A T T T C C A A A - C A T G T A C A T A T C G C
Silvermine	A T G T A A T T - - A A A A A C T A T A A A T T T A T A A T G A A T A T T T T C A A A A C A T G T A C A T A T C G C
Smitswinkelbaai	A T G T A A T T - - A A A A A C T A T A A A T T T A T A A T G A A T A T T T C C A A A - C A T G T A C A T A T C G C
Krom River	A T G T A A T T - - A A A G A C T A T A A A T T T A T A A T G A A T A T T T C C A A A - C A T G T A C A T A T C G C
Schusters River	A T G T A A T T - - A A A A A C T A T A A A T T T A T A A T G A A T A T T T C C A A A - C A T G T A C A C A T C G C
Franschhoek	A T G T A A T A - - A T A A A C T A T A A T T T T A T A A T G A A T A T T T A C A A A A C A T G C A C A C A T C G C
Jonkershoek	A T G T A A T A - - A T A A A C T A T A A T T T T A T A A T G A A T A T T T A C A A A A C A T G C A C A T A T C G C
Gordon's Bay	A T G T A A T T - - A C A A A C T A T A A T T T T T T A A T G A A T A T T T A C A A A A C A T G C A C A T A T C G C

Appendix 4: Means, sample sizes (in parentheses) and standard deviations of the 47 variables for the 11 *Mesamphisopus* populations included in the morphometric analyses in Chapter 2. Consult Table 2.6 for full variable details.

Population	Variables												
	BL	HW	HL	HD	P1W	P1L	P1D	P3W	P3L	P3D	P5W	P5L	P5D
Echo Valley	12.500 (5) ±1.027	1.520 (5) ±0.192	1.280 (5) ±0.130	1.480 (5) ±0.148	1.860 (5) ±0.152	0.920 (5) ±0.110	0.840 (5) ±0.230	2.100 (5) ±0.224	1.180 (5) ±0.084	0.860 (5) ±0.230	2.240 (5) ±0.207	0.900 (5) ±0.071	0.780 (5) ±0.192
Red Gods Valley	9.800 (2) ±0.990	1.200 (2) ±0.141	1.050 (2) ±0.071	1.200 (2) ±0.000	1.550 (2) ±0.212	0.700 (2) ±0.141	0.750 (2) ±0.071	1.650 (2) ±0.071	0.800 (2) ±0.000	0.700 (2) ±0.000	1.700 (2) ±0.000	0.700 (2) ±0.141	0.600 (2) ±0.000
Kasteelspoort	13.800 (5) ±0.834	1.520 (5) ±0.130	1.360 (5) ±0.152	1.520 (5) ±0.217	1.860(5) ±0.152	0.900 (5) ±0.071	0.900 (5) ±0.200	2.060 (5) ±0.152	1.200 (5) ±0.158	0.900 (5) ±0.187	2.040 (5) ±0.167	0.920 (5) ±0.045	0.740 (5) ±0.152
Nursery Ravine	9.200 (5) ±0.992	1.200 (5) ±0.173	1.080 (5) ±0.130	1.100 (5) ±0.100	1.400 (5) ±0.200	0.640 (5) ±0.114	0.700 (5) ±0.122	1.560 (5) ±0.167	0.800 (5) ±0.122	0.680 (5) ±0.110	1.600 (5) ±0.100	0.680 (5) ±0.084	0.580 (5) ±0.130
Silvermine	10.340 (5) ±0.508	1.320 (5) ±0.084	1.120 (5) ±0.045	1.280 (5) ±0.130	1.580 (5) ±0.045	0.700 (5) ±0.000	0.900 (5) ±0.141	1.680 (5) ±0.045	0.960 (5) ±0.055	0.900 (5) ±0.200	1.760 (5) ±0.055	0.840 (5) ±0.114	0.800 (5) ±0.200
Smitswinkelbaai	8.820 (5) ±0.522	1.140 (5) ±0.055	1.060 (5) ±0.089	1.080 (5) ±0.045	1.340 (5) ±0.055	0.640 (5) ±0.055	0.720 (5) ±0.084	1.560 (5) ±0.055	0.860 (5) ±0.114	0.700 (5) ±0.071	1.580 (5) ±0.084	0.700(5) ±0.071	0.580 (5) ±0.045
Krom River	11.560 (5) ±0.493	1.440 (5) ±0.134	1.200 (5) ±0.071	1.480 (5) ±0.130	1.800 (5) ±0.141	0.820 (5) ±0.084	1.000 (5) ±0.187	2.140 (5) ±0.089	1.040 (5) ±0.089	0.960 (5) ±0.152	2.180 (5) ±0.084	0.900 (5) ±0.071	0.900 (5) ±0.122
Schusters River	12.820 (5) ±0.622	1.580 (5) ±0.045	1.340 (5) ±0.055	1.480 (5) ±0.164	1.960 (5) ±0.055	0.860 (5) ±0.089	1.020 (5) ±0.148	2.220 (5) ±0.084	1.120 (5) ±0.084	0.980 (5) ±0.179	2.320 (5) ±0.084	0.960 (5) ±0.055	0.880 (5) ±0.110
Franschhoek	10.740 (5) ±1.328	1.360 (5) ±0.089	1.160 (5) ±0.114	1.280 (5) ±0.045	1.700 (5) ±0.187	0.800 (5) ±0.071	1.040 (5) ±0.055	1.840 (5) ±0.241	0.980 (5) ±0.110	0.920 (5) ±0.045	1.840 (5) ±0.195	0.820 (5) ±0.084	0.860 (5) ±0.152
Jonkershoek	12.240 (5) ±0.802	1.540 (5) ±0.134	1.360 (5) ±0.055	1.460 (5) ±0.182	1.840 (5) ±0.114	0.880 (5) ±0.045	0.920 (5) ±0.192	2.000 (5) ±0.071	1.120 (5) ±0.084	1.000 (5) ±0.235	2.020 (5) ±0.110	0.960 (5) ±0.114	0.880 (5) ±0.259
Gordon's Bay	8.160 (5) ±0.767	1.140 (5) ±0.089	0.922 (5) ±0.047	0.858 (5) ±0.081	1.340 (5) ±0.114	0.559 (5) ±0.068	0.586 (5) ±0.119	1.460 (5) ±0.134	0.647 (5) ±0.062	0.566 (5) ±0.057	1.460 (5) ±0.207	0.577 (5) ±0.048	0.507 (5) ±0.076

Population	Variables												
	P7W	P7L	P7D	PL4W	PL4L	PL4D	TW	TL	TD	Pe1L	Pe1BL	Pe1BW	Pe1PL
Echo Valley	2.140 (5) ±0.219	0.720 (5) ±0.084	1.060 (5) ±0.219	2.060 (5) ±0.167	0.760 (5) ±0.089	2.600 (5) ±0.158	1.340 (5) ±0.207	1.600 (5) ±0.122	1.580 (5) ±0.205	5.359 (1) ---	1.108 (4) ±0.142	0.558 (5) ±0.082	1.229 (4) ±0.134
Red Gods Valley	1.650 (2) ±0.071	0.600 (2) ±0.000	0.900 (2) ±0.000	1.550 (2) ±0.071	0.550 (2) ±0.071	1.950 (2) ±0.071	1.050 (2) ±0.071	1.100 (2) ±0.424	1.200 (2) ±0.141	3.373 (1) ---	0.844 (1) ---	0.428 (2) ±0.016	0.904 (2) ±0.096
Kasteelspoort	2.060 (5) ±0.152	0.700 (5) ±0.071	1.080 (5) ±0.192	2.060 (5) ±0.152	0.820 (5) ±0.084	2.800 (5) ±0.245	1.380 (5) ±0.045	1.633 (3) ±0.153	1.600 (3) ±0.100	4.635 (2) ±0.491	1.170 (3) ±0.227	0.563 (3) ±0.053	1.238 (4) ±0.131
Nursery Ravine	1.520 (5) ±0.148	0.500 (5) ±0.071	0.820 (5) ±0.130	1.460 (5) ±0.089	0.640 (5) ±0.114	2.000 (5) ±0.158	1.200 (5) ±0.122	1.060 (5) ±0.055	1.140 (5) ±0.055	3.417 (2) ±0.582	0.737 (2) ±0.098	0.420 (3) ±0.050	0.868 (3) ±0.107
Silvermine	1.740 (5) ±0.055	0.600 (5) ±0.000	1.000 (5) ±0.200	1.560 (5) ±0.089	0.700 (5) ±0.071	2.160 (5) ±0.134	1.180 (5) ±0.084	1.080 (5) ±0.110	1.380 (5) ±0.110	4.275 (3) ±0.064	0.902 (3) ±0.075	0.456 (5) ±0.020	1.239 (5) ±0.087
Smitswinkelbaai	1.540 (5) ±0.089	0.520 (5) ±0.045	0.880 (5) ±0.045	1.360 (5) ±0.089	0.580 (5) ±0.045	1.760 (5) ±0.114	1.060 (5) ±0.089	1.000 (5) ±0.173	1.180 (5) ±0.110	3.695 (5) ±0.240	0.813 (5) ±0.069	0.398 (5) ±0.068	1.045 (5) ±0.114
Krom River	2.120 (5) ±0.084	0.700 (5) ±0.071	1.240 (5) ±0.152	1.860 (5) ±0.114	0.775 (4) ±0.050	2.550 (4) ±0.238	1.520 (5) ±0.179	1.380 (5) ±0.130	1.640 (5) ±0.167	4.898 (4) ±0.452	1.047 (4) ±0.074	0.517 (4) ±0.036	1.384 (4) ±0.156
Schusters River	2.280 (5) ±0.110	0.720 (5) ±0.084	1.220 (5) ±0.179	1.940 (5) ±0.134	0.840 (5) ±0.055	2.540 (5) ±0.219	1.540 (5) ±0.114	1.320 (5) ±0.084	1.700 (5) ±0.122	5.196 (2) ±0.296	1.078 (2) ±0.086	0.544 (4) ±0.046	1.438 (4) ±0.106
Franschhoek	1.800 (5) ±0.200	0.660 (5) ±0.089	1.120 (5) ±0.045	1.680 (5) ±0.228	0.700 (5) ±0.071	2.160 (5) ±0.182	1.320 (5) ±0.110	1.040 (5) ±0.055	1.440 (5) ±0.114	4.393 (5) ±0.632	0.918 (5) ±0.117	0.499 (5) ±0.066	1.284 (5) ±0.246
Jonkershoek	1.980 (5) ±0.148	0.720 (5) ±0.045	1.140 (5) ±0.114	1.820 (5) ±0.084	0.900 (5) ±0.000	2.620 (5) ±0.179	1.460 (5) ±0.089	1.280 (5) ±0.148	1.760 (5) ±0.167	4.597 (4) ±0.203	0.998 (4) ±0.030	0.523 (4) ±0.007	1.204 (4) ±0.069
Gordon’s Bay	1.400 (5) ±0.158	0.493 (5) ±0.075	0.731 (5) ±0.083	1.280 (5) ±0.130	0.558 (5) ±0.062	1.548 (5) ±0.093	0.940 (5) ±0.055	1.149 (5) ±0.091	1.183 (5) ±0.145	2.725 (5) ±0.210	0.632 (5) ±0.052	0.369 (5) ±0.031	0.663 (5) ±0.060

Population	Variables												
	Pe1PW	Pe3L	Pe3BL	Pe3BW	Pe3PL	Pe3PW	Pe4L	Pe4BL	Pe4BW	Pe4PL	Pe4PW	Pe5L	Pe5BL
Echo Valley	0.852 (4) ±0.091	4.710 (4) ±0.460	1.242 (4) ±0.109	0.470 (5) ±0.027	0.720 (5) ±0.055	0.221 (5) ±0.024	3.875 (5) ±0.319	1.080 (5) ±0.078	0.420 (5) ±0.029	0.524 (5) ±0.038	0.274 (5) ±0.022	4.496 (3) ±0.119	1.003 (4) ±0.058
Red Gods Valley	0.621 (2) ±0.047	3.198 (1) ---	0.770 (1) ---	0.324 (1) ---	0.481 (1) ---	0.193 (1) ---	3.062 (1) ---	0.843 (1) ---	0.308 (1) ---	0.391 (1) ---	0.232 (1) ---	4.570 (1) ---	0.824 (2) ±0.170
Kasteelspoort	0.833 (4) ±0.039	4.388 (4) ±0.208	1.138 (4) ±0.047	0.462 (4) ±0.021	0.693 (4) ±0.094	0.247 (4) ±0.017	3.893 (3) ±0.227	1.090 (4) ±0.061	0.423 (4) ±0.043	0.511 (3) ±0.028	0.320 (3) ±0.017	4.840 (5) ±0.333	1.127 (5) ±0.118
Nursery Ravine	0.591 (3) ±0.106	3.287 (5) ±0.311	0.835 (5) ±0.077	0.322 (5) ±0.043	0.489 (5) ±0.065	0.174 (5) ±0.028	3.110 (3) ±0.281	0.854 (4) ±0.091	0.323 (4) ±0.022	0.419 (3) ±0.064	0.227 (3) ±0.009	3.449 (3) ±0.371	0.802 (5) ±0.139
Silvermine	0.973 (5) ±0.072	3.318 (5) ±0.269	0.917 (5) ±0.102	0.398 (5) ±0.019	0.498 (5) ±0.056	0.241 (5) ±0.023	3.022 (3) ±0.171	0.831 (3) ±0.075	0.371 (3) ±0.007	0.412 (3) ±0.024	0.275 (3) ±0.008	2.858 (2) ±0.383	0.811 (4) ±0.087
Smitswinkelbaai	0.811 (5) ±0.092	2.978 (5) ±0.353	0.850 (5) ±0.070	0.387 (5) ±0.041	0.461 (5) ±0.061	0.248 (5) ±0.029	2.803 (5) ±0.124	0.813 (5) ±0.036	0.344 (5) ±0.015	0.373 (5) ±0.021	0.244 (5) ±0.017	2.906 (4) ±0.276	0.774 (4) ±0.088
Krom River	1.031 (4) ±0.115	3.981 (5) ±0.235	1.035 (5) ±0.065	0.478 (5) ±0.026	0.560 (5) ±0.062	0.291 (5) ±0.014	3.644 (5) ±0.178	1.011 (5) ±0.045	0.436 (5) ±0.013	0.498 (5) ±0.020	0.321 (5) ±0.019	3.788 (5) ±0.312	0.908 (5) ±0.070
Schusters River	1.032 (4) ±0.129	4.464 (5) ±0.200	1.130 (5) ±0.138	0.524 (5) ±0.025	0.691 (5) ±0.042	0.290 (5) ±0.016	4.006 (5) ±0.233	1.153 (5) ±0.098	0.480 (5) ±0.019	0.540 (5) ±0.046	0.331 (5) ±0.018	4.388 (4) ±0.333	1.043 (4) ±0.148
Franschhoek	1.039 (5) ±0.191	3.703 (5) ±0.395	0.985 (5) ±0.086	0.489 (5) ±0.063	0.531 (5) ±0.087	0.275 (5) ±0.039	3.331 (4) ±0.312	0.878 (5) ±0.137	0.400 (5) ±0.063	0.481 (4) ±0.054	0.295 (4) ±0.038	3.291 (4) ±0.464	0.891 (4) ±0.132
Jonkershoek	0.929 (4) ±0.091	4.159 (3) ±0.103	1.031 (4) ±0.011	0.501 (5) ±0.038	0.605 (4) ±0.056	0.269 (4) ±0.015	3.897 (2) ±0.307	1.058 (3) ±0.063	0.495 (3) ±0.029	0.547 (2) ±0.026	0.319 (2) ±0.044	4.023 (5) ±0.312	1.072 (5) ±0.074
Gordon's Bay	0.497 (5) ±0.061	2.106 (5) ±0.426	0.597 (5) ±0.094	0.309 (5) ±0.064	0.301 (5) ±0.066	0.176 (5) ±0.037	2.190 (3) ±0.168	0.558 (3) ±0.064	0.303 (4) ±0.016	0.323 (5) ±0.024	0.209 (5) ±0.013	2.244 (4) ±0.207	0.577 (5) ±0.043

Population	Variables							
	Pe5BW	Pe5PL	Pe5PW	Pe7L	Pe7BL	Pe7BW	Pe7PL	Pe7PW
Echo Valley	0.550 (5) ±0.140	0.971 (5) ±0.298	0.197 (5) ±0.059	5.746 (1) ---	1.468 (3) ±0.236	0.765 (3) ±0.156	1.062 (2) ±0.072	0.184 (2) ±0.006
Red Gods Valley	0.368 (2) ±0.025	0.739 (1) ---	0.156 (1) ---	--- ---	1.104 (2) ±0.128	0.590 (2) ±0.057	--- ---	--- ---
Kasteelspoort	0.534 (5) ±0.034	0.908 (5) ±0.107	0.192 (5) ±0.022	6.705 (5) ±0.661	1.600 (5) ±0.170	0.793 (5) ±0.076	1.249 (5) ±0.060	0.206 (5) ±0.009
Nursery Ravine	0.386 (5) ±0.017	0.601 (3) ±0.104	0.141 (3) ±0.015	4.603 (3) ±0.424	1.043 (5) ±0.128	0.523 (5) ±0.062	0.806 (3) ±0.045	0.154 (3) ±0.017
Silvermine	0.439 (4) ±0.033	0.393 (3) ±0.096	0.168 (3) ±0.041	4.227 (3) ±0.124	1.093 (3) ±0.043	0.641 (5) ±0.021	0.634 (5) ±0.053	0.221 (5) ±0.010
Smitswinkelbaai	0.445 (5) ±0.022	0.501 (5) ±0.049	0.457 (5) ±0.572	3.709 (5) ±0.300	1.021 (5) ±0.073	0.613 (5) ±0.049	0.560 (5) ±0.078	0.215 (5) ±0.022
Krom River	0.542 (5) ±0.034	0.593 (5) ±0.068	0.236 (5) ±0.013	5.140 (5) ±0.538	1.375 (5) ±0.163	0.793 (5) ±0.072	0.835 (5) ±0.097	0.279 (5) ±0.029
Schusters River	0.549 (5) ±0.054	0.740 (5) ±0.049	0.207 (5) ±0.022	5.656 (5) ±0.398	1.487 (5) ±0.087	0.724 (5) ±0.092	0.980 (5) ±0.086	0.229 (5) ±0.041
Franschhoek	0.552 (4) ±0.068	0.469 (4) ±0.110	0.215 (4) ±0.032	4.534 (5) ±0.511	1.225 (5) ±0.153	0.828 (5) ±0.099	0.684 (5) ±0.059	0.257 (5) ±0.025
Jonkershoek	0.639 (5) ±0.125	0.596 (5) ±0.057	0.210 (5) ±0.027	5.733 (3) ±0.231	1.559 (4) ±0.116	0.915 (4) ±0.059	0.933 (3) ±0.058	0.300 (3) ±0.000
Gordon’s Bay	0.376 (5) ±0.043	0.368 (4) ±0.026	0.151 (4) ±0.009	2.844 (5) ±0.248	0.759 (5) ±0.086	0.524 (5) ±0.052	0.453 (5) ±0.031	0.186 (5) ±0.022

Appendix 5: Sample sizes (*N*), means and standard deviations of 22 variables for the *Mesamphisopus abbreviatus* and *M. depressus* syntypes, and the 14 *Mesamphisopus* populations included in the morphometric analyses in Chapter 3. Refer to Table 2.6 for full variable details.

Population	*N*	Variables										
		BL	HW	HL	HD	P1W	P1L	P1D	P3W	P3L	P3D	P5W
M. abbreviatus	21	8.205 ±1.563	1.067 ±0.132	1.014 ±0.190	1.024 ±0.155	1.219 ±0.144	0.548 ±0.103	0.762 ±0.107	1.319 ±0.166	0.748 ±0.144	0.714 ±0.135	1.314 ±0.149
M. depressus	21	8.452 ±0.818	1.362 ±0.160	0.986 ±0.079	1.100 ±0.126	1.686 ±0.188	0.624 ±0.077	0.776 ±0.104	1.948 ±0.236	0.695 ±0.097	0.890 ±0.089	2.019 ±0.256
Betty's Bay A	5	16.600 ±1.049	1.980 ±0.192	1.600 ±0.100	1.820 ±0.130	2.520 ±0.268	1.200 ±0.071	1.280 ±0.228	2.820 ±0.268	1.340 ±0.055	1.500 ±0.265	3.020 ±0.295
Wemmershoek	5	9.500 ±0.354	1.260 ±0.152	1.040 ±0.089	1.140 ±0.134	1.520 ±0.084	0.740 ±0.055	0.880 ±0.110	1.620 ±0.084	0.880 ±0.045	0.800 ±0.158	1.600 ±0.071
Steenbras A	2	16.500 ±0.990	2.000 ±0.283	1.600 ±0.000	1.800 ±0.283	2.500 ±0.283	1.450 ±0.212	1.150 ±0.212	2.750 ±0.212	1.400 ±0.141	1.250 ±0.354	2.800 ±0.283
Steenbras B	5	9.540 ±1.457	1.540 ±0.152	1.080 ±0.130	1.240 ±0.251	1.920 ±0.228	0.700 ±0.158	0.860 ±0.195	2.220 ±0.311	0.900 ±0.158	0.940 ±0.344	2.300 ±0.339
Steenbras C	5	13.000 ±0.927	1.840 ±0.114	1.480 ±0.179	1.600 ±0.367	2.400 ±0.141	0.860 ±0.055	1.120 ±0.277	2.740 ±0.182	1.080 ±0.084	1.320 ±0.370	2.860 ±0.207
Kogelberg	5	17.000 ±2.636	2.140 ±0.230	1.700 ±0.173	1.960 ±0.270	2.660 ±0.365	1.340 ±0.207	1.280 ±0.130	2.860 ±0.439	1.400 ±0.200	1.220 ±0.130	2.960 ±0.518
Grabouw	5	7.760 ±0.488	1.040 ±0.055	0.847 ±0.064	0.928 ±0.099	1.180 ±0.084	0.551 ±0.032	0.607 ±0.109	1.280 ±0.045	0.681 ±0.033	0.538 ±0.110	1.240 ±0.055
Greyton	5	12.900 ±1.387	1.580 ±0.110	1.300 ±0.187	1.420 ±0.259	1.940 ±0.114	0.940 ±0.152	0.900 ±0.122	2.100 ±0.141	1.120 ±0.084	1.000 ±0.071	2.120 ±0.148
Protea Valley	5	8.740 ±0.654	1.240 ±0.089	1.008 ±0.105	1.130 ±0.062	1.420 ±0.110	0.632 ±0.060	0.634 ±0.092	1.540 ±0.152	0.766 ±0.082	0.702 ±0.131	1.540 ±0.152
Barrydale	5	7.620 ±0.622	1.120 ±0.045	0.776 ±0.042	0.913 ±0.178	1.220 ±0.084	0.548 ±0.037	0.635 ±0.174	1.380 ±0.110	0.655 ±0.061	0.540 ±0.124	1.340 ±0.055
Tradouw Pass	5	13.260 ±1.036	1.660 ±0.207	1.300 ±0.158	1.480 ±0.179	2.060 ±0.114	1.120 ±0.130	0.920 ±0.110	2.280 ±0.179	1.180 ±0.084	1.000 ±0.071	2.340 ±0.230
Grootvadersbos	5	11.120 ±1.080	1.440 ±0.089	1.180 ±0.164	1.380 ±0.130	1.640 ±0.089	0.880 ±0.130	1.000 ±0.100	1.820 ±0.148	0.980 ±0.045	0.940 ±0.114	1.880 ±0.164
Riversdale	5	7.360 ±0.261	1.060 ±0.055	0.795 ±0.048	1.029 ±0.064	1.240 ±0.055	0.522 ±0.046	0.652 ±0.073	1.300 ±0.071	0.604 ±0.035	0.657 ±0.085	1.340 ±0.055
Tsitsikamma	5	10.580 ±0.785	1.360 ±0.167	1.100 ±0.071	1.260 ±0.167	1.560 ±0.089	0.740 ±0.089	0.900 ±0.122	1.740 ±0.152	0.980 ±0.130	0.960 ±0.114	1.760 ±0.182

Population	*N*	Variables										
		P5L	P5D	P7W	P7L	P7D	PL4W	PL4L	PL4D	TW	TL	TD
M. abbreviatus	21	0.652 ±0.136	0.671 ±0.101	1.271 ±0.155	0.481 ±0.098	0.795 ±0.116	1.081 ±0.204	0.490 ±0.104	1.529 ±0.159	0.795 ±0.132	1.295 ±0.166	1.300 ±0.182
M. depressus	21	0.690 ±0.089	0.724 ±0.109	1.852 ±0.242	0.567 ±0.080	0.876 ±0.114	1.448 ±0.147	0.619 ±0.081	1.919 ±0.225	1.181 ±0.121	1.267 ±0.180	1.500 ±0.197
Betty's Bay A	5	1.200 ±0.122	1.260 ±0.182	2.940 ±0.336	0.900 ±0.122	1.640 ±0.251	3.200 ±0.406	1.180 ±0.084	3.520 ±0.390	2.620 ±0.342	3.220 ±0.303	2.840 ±0.391
Wemmershoek	5	0.800 ±0.000	0.800 ±0.173	1.600 ±0.071	0.600 ±0.000	1.020 ±0.148	1.440 ±0.089	0.800 ±0.100	1.980 ±0.130	1.240 ±0.089	1.480 ±0.084	1.500 ±0.122
Steenbras A	2	1.300 ±0.141	0.950 ±0.212	2.600 ±0.283	0.900 ±0.141	1.300 ±0.283	2.900 ±0.141	1.000 ±0.141	2.950 ±0.495	2.150 ±0.212	2.850 ±0.212	2.500 ±0.283
Steenbras B	5	0.780 ±0.164	0.820 ±0.259	2.220 ±0.268	0.600 ±0.100	1.060 ±0.329	1.800 ±0.245	0.600 ±0.071	2.100 ±0.374	1.400 ±0.158	1.560 ±0.288	1.620 ±0.327
Steenbras C	5	1.080 ±0.084	0.980 ±0.383	2.680 ±0.277	0.860 ±0.089	1.380 ±0.356	2.340 ±0.270	0.820 ±0.084	2.780 ±0.432	1.720 ±0.217	2.060 ±0.134	2.120 ±0.277
Kogelberg	5	1.340 ±0.207	0.960 ±0.089	3.020 ±0.497	0.960 ±0.114	1.340 ±0.055	2.860 ±0.586	0.880 ±0.130	3.280 ±0.286	1.960 ±0.251	3.100 ±0.374	2.780 ±0.319
Grabouw	5	0.586 ±0.019	0.449 ±0.055	1.200 ±0.000	0.529 ±0.116	0.636 ±0.134	1.140 ±0.055	0.524 ±0.080	1.457 ±0.097	0.900 ±0.100	1.209 ±0.143	1.157 ±0.130
Greyton	5	0.940 ±0.114	0.860 ±0.089	2.120 ±0.148	0.720 ±0.084	1.180 ±0.084	2.060 ±0.230	0.760 ±0.055	2.600 ±0.122	1.620 ±0.164	2.180 ±0.239	2.040 ±0.114
Protea Valley	5	0.652 ±0.031	0.584 ±0.124	1.520 ±0.164	0.586 ±0.060	0.732 ±0.203	1.380 ±0.192	0.568 ±0.086	1.758 ±0.237	1.060 ±0.152	1.316 ±0.068	1.478 ±0.190
Barrydale	5	0.543 ±0.030	0.452 ±0.117	1.300 ±0.071	0.445 ±0.049	0.651 ±0.146	1.160 ±0.055	0.539 ±0.067	1.353 ±0.250	0.880 ±0.110	1.088 ±0.116	1.154 ±0.097
Tradouw Pass	5	1.060 ±0.055	0.760 ±0.055	2.320 ±0.164	0.800 ±0.071	1.200 ±0.100	2.200 ±0.158	0.880 ±0.045	2.480 ±0.239	1.660 ±0.219	2.240 ±0.152	2.060 ±0.207
Grootvadersbos	5	0.900 ±0.071	0.800 ±0.200	1.800 ±0.122	0.760 ±0.089	1.000 ±0.071	1.760 ±0.152	0.760 ±0.114	2.080 ±0.217	1.360 ±0.055	1.820 ±0.192	1.740 ±0.167
Riversdale	5	0.546 ±0.030	0.553 ±0.069	1.300 ±0.071	0.420 ±0.040	0.714 ±0.080	1.200 ±0.071	0.506 ±0.062	1.474 ±0.109	1.000 ±0.000	1.114 ±0.055	1.112 ±0.094
Tsitsikamma	5	0.820 ±0.045	0.740 ±0.114	1.700 ±0.158	0.600 ±0.100	0.960 ±0.114	1.600 ±0.141	0.700 ±0.071	2.240 ±0.152	1.260 ±0.152	1.620 ±0.164	1.660 ±0.182

Appendix 6: Allele frequencies at the 12 polymorphic loci in the 15 populations of *Mesamphisopus* studied in Chapter 3. *N* denotes the sample size for each of the populations at the respective locus. Alleles are numbered following their mobility relative to an allele present in a reference population (consult Chapter 3: Materials and Methods). Refer to Figure 3.1 for full population names.

Locus		Population														
		BetA	BetB	Wem	StA	StB	StC	Kog	Grab	Grey	PV	Bar	Trad	Gvb	Riv	Tsi
Ao	*N*	5	20	21	23	21	30	20	21	19	25	25	30	30	28	36
100		1.000	1.000	0.000	0.848	1.000	1.000	1.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
90		0.000	0.000	1.000	0.152	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ark	*N*	35	33	28	23	25	30	20	48	20	14	11	30	29	28	40
115		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
105		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.086	0.000	0.000
100		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.914	1.000	0.000
Gpi	*N*	35	33	28	23	30	30	20	48	20	30	30	27	28	30	40
160		0.000	0.061	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
155		0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
145		0.986	0.939	0.000	0.022	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000
140		0.000	0.000	0.000	0.000	0.383	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
120		0.000	0.000	0.000	0.391	0.617	0.000	1.000	0.188	0.000	0.967	0.000	0.019	0.107	1.000	0.000
110		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000
105		0.000	0.000	0.000	0.348	0.000	0.000	0.000	0.490	0.000	0.000	0.000	0.000	0.000	0.000	0.000
100		0.014	0.000	0.982	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
95		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000

90		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.444	0.893	0.000	0.000
80		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000
70		0.000	0.000	0.018	0.152	0.000	0.000	0.000	0.323	0.000	0.000	0.000	0.000	0.000	0.000	0.000
60		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.537	0.000	0.000	0.000
40		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.775
10		0.000	0.000	0.000	0.043	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
5		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.225
Hk	*N*	28	27	28	23	26	30	20	35	18	27	18	30	27	28	40
125		0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
100		0.964	0.981	0.661	0.935	0.192	0.600	0.975	0.986	0.556	0.000	0.000	0.717	0.963	1.000	1.000
95		0.036	0.000	0.304	0.065	0.731	0.400	0.000	0.014	0.417	1.000	1.000	0.283	0.037	0.000	0.000
85		0.000	0.000	0.036	0.000	0.077	0.000	0.025	0.000	0.028	0.000	0.000	0.000	0.000	0.000	0.000
Idh	*N*	7	26	28	7	29	20	20	14	20	29	18	30	26	27	24
170		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.792
135		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.000
120		1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.929	1.000	0.293	0.806	1.000	0.635	1.000	0.000
100		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.707	0.194	0.000	0.365	0.000	0.208
Ldh	*N*	23	33	28	23	30	30	20	34	20	30	30	30	30	30	40
100		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
80		0.000	0.152	0.000	0.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
70		1.000	0.848	1.000	0.978	0.000	0.000	0.000	1.000	0.000	0.000	0.000	1.000	1.000	0.000	0.000
50		0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
null		0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	1.000	1.000	1.000	0.000	0.000	1.000	0.000

Lt-1	*N*	25	28	25	23	26	30	20	36	20	30	27	30	30	22	37
100		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000
90		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
Lt-2	*N*	25	18	25	23	19	30	20	24	17	30	21	30	30	13	35
100		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000
null		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
Mdh-1	*N*	33	31	28	23	30	30	20	37	19	30	30	30	27	30	40
130		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
100		0.000	0.000	0.000	0.000	1.000	1.000	0.000	0.000	0.658	0.000	0.000	0.000	0.000	0.000	0.000
80		1.000	1.000	1.000	0.957	0.000	0.000	1.000	1.000	0.342	1.000	1.000	1.000	1.000	1.000	0.000
70		0.000	0.000	0.000	0.043	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh-2	*N*	33	33	28	23	30	30	20	40	20	30	30	30	30	30	37
100		1.000	1.000	1.000	0.978	1.000	1.000	0.900	1.000	1.000	0.983	1.000	1.000	0.983	1.000	0.000
40		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
15		0.000	0.000	0.000	0.022	0.000	0.000	0.100	0.000	0.000	0.017	0.000	0.000	0.017	0.000	0.000
Me	*N*	34	33	28	23	30	30	20	27	20	30	30	30	30	30	23
115		0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
100		1.000	0.985	0.000	0.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
90		0.000	0.000	1.000	1.000	0.000	0.000	1.000	0.963	1.000	1.000	1.000	1.000	1.000	1.000	0.000
75		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.037	0.000	0.000	0.000	0.000	0.000	0.000	1.000

Pgm	*N*	30	27	28	23	30	30	20	36	20	30	30	30	30	30	40
110		0.000	0.000	0.000	0.000	0.083	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.962
100		0.000	0.000	0.036	0.087	0.917	1.000	0.000	0.000	1.000	0.167	0.000	0.000	0.000	0.000	0.038
90		1.000	1.000	0.018	0.826	0.000	0.000	0.000	0.972	0.000	0.350	1.000	0.967	0.967	0.700	0.000
80		0.000	0.000	0.875	0.087	0.000	0.000	1.000	0.028	0.000	0.467	0.000	0.033	0.033	0.000	0.000
70		0.000	0.000	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.300	0.000

Appendix 7: The "reduced" data set (see Chapter 3) sequence alignment of 600 bp of the COI mtDNA gene region used to investigate relationships among representative individuals, identified as *Mesamphisopus abbreviatus* or *M. depressus*, collected from 15 localities. *Mesamphisopus* sp. nov. and *M. penicillatus* were included as outgroups.

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                                1 1 1 1 1 1 1 1 1 1 2 2 2 2 2 2 2 2 2 2 3 3 3 3 3 3 3 3 3 3 4 4 4 4 4 4 4 4 4 4 5 5 5 5 5 5 5 5 5 5 6
                    1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0

Betty's Bay A       G G A A C T G G T C T T A G T A T A C T T A T T C G A A T T G A A T T A G G T C A A C C T G G T G G T T T A A T T T G T
Betty's Bay B       G G A A C T G G T C T T A G T A T A C T T A T T C G A A T T G A A T T A G G T C A A C C T G G T G G T T T A A T T T G T
Wemmershoek         G G A A C T G G T C T T A G T A T A C T T A T T C G A A T T G A G T T A G G T C A A C C T G G T G G T T T A A T T T G T
Steenbras A         G G A A C T G G T C T T A G T A T G C T T A T T C G A A T T G A A T T A G G T C A A C C T G G T G G T T T A A T T T G T
Steenbras B         G G A A C T G G T C T T A G T A T A C T T A T T C G A A T T G A A T T G G G T C A A C C T G G T G G C T T A A T T T G T
Steenbras C         G G A A C T G G T C T T A G T A T G C T T A T T C G A A T T G A A T T G G G T C A A C C T G G C G G T T T A A T T T G T
Kogelberg           G G A A C T G G G C T T A G T A T G C T T A T T C G A A T T G A A T T A G G T C A A C C T G G T G G T T T A A T T T G T
Grabouw             G G A A C T G G T C T C A G T A T A C T T A T C C G A A T T G A G T T A G G T C A A C C T G G T G G T T T A A T T T G T
Greyton             G G A A C T G G T C T T A G T A T A C T T A T T C G A A T T G A A T T A G G T C A A C C T G G T G G C T T A A T T T G T
Protea Valley 1     G G A A C T G G T C T T A G T A T A C T T A T T C G A A T T G A A T T A G G T C A A C C T G G T G G T T T A A T T T G T
Protea Valley 2     G G A A C T G G T C T T A G T A T A C T T A T T C G A A T T G A A T T A G G T C A A C C T G G T G G T T T A A T T T G T
Barrydale           G G A A C T G G T C T T A G T A T A C T T A T T C G A A T T G A A T T A G G T C A A C C T G G T G G T T T A A T T T G T
Tradouw Pass        G G A A C T G G T C T T A G T A T A C T T A T T C G A A T T G A A T T A G G T C A A C C T G G A G G T T T A A T T T G T
Grootvadersbos      G G A A C T G G T C T T A G T A T A C T T A T T C G A A T T G A A T T A G G T C A A C C T G G T G G T T T A A T T T G T
Riversdale          G G A A C T G G T C T T A G T A T A C T T A T T C G A A T T G A A T T A G G T C A A C C T G G T G G T T T A A T T T G T
Tsitsikamma         G G T A C T G G A T T A A G T A T A C T T A T T C G A A T T G A A T T A G G T C A G C C A G G C T C A T T T A T T G G C
M. penicillatus     G G T A C T G G T T T A A G A A T A A T T A T T C G T A C T G A G T T A G G T C A G C C T G G T A A G T T T A T T G G T
Mesamphisopus n. sp. G G T A C T G G G T T A A G A A T A A T T A T T C G T A C C G A G T T A G G T C A G C C T G G G A A G T T T A T T G G A

                                                                                                                1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
                    6 6 6 6 6 6 6 6 7 7 7 7 7 7 7 7 7 8 8 8 8 8 8 8 8 8 9 9 9 9 9 9 9 9 9 0 0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1 1 2
                    1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0

Betty's Bay A       G A T G A T C A A A T T T A T A A T G T T G T A G T A A C T G C T C A C G C T T T G T T A T A A T T T T T T T T A T A
Betty's Bay B       G A T G A T C A A A T T T A T A A T G T T G T A G T A A C T G C T C A C G C T T T G T T A T A A T T T T T T T T A T A
Wemmershoek         G A T G A T C A A A T T T A T A A T G T T G T A G T A A C T G C T C A T G C T T T G T T A T A A T T T T C T T T A T A
Steenbras A         G A T G A T C A A A T T T A T A A T G T T G T A G T A A C T G C T C A T G C T T T G T A A T A A T T T T T T T T A T A
Steenbras B         G A T G A T C A A A T T T A T A A T G T T G T A G T A A C T G C T C A T G C T T T G T T A T A A T T T T T T T T A T A
Steenbras C         G A T G A T C A A A T T T A T A A T G T T G T A G T A A C T G C T C A T G C T T T G T T A T A A T T T T T T T T A T A
Kogelberg           G A T G A T C A A A T T T A T A A T G T T G T A G T A A C T G C T C A T G C T T T G T A A T A A T T T T T T T T A T A
Grabouw             G A T G A C C A A A T T T A T A A T G T T G T A G T A A C T G C T C A T G C T T T G T T A T A A T T T T T T T T A T A
Greyton             G A T G A T C A A A T T T A T A A T G T T G T A G T A A C T G C T C A T G C T T T G T A A T A A T T T T T T T T A T A
Protea Valley 1     G A T G A T C A A A T T T A T A A T G T T G T A G T A A C T G C T C A T G C T T T G T A A T A A T T T T T T T T A T A
Protea Valley 2     G A T G A T C A A A T T T A T A A T G T T G T A G T A A C T G C T C A T G C T T T G T A A T A A T T T T T T T T A T A
Barrydale           G A T G A T C A A A T T T A T A A T G T T G T A G T A A C T G C T C A T G C T T T G T A A T A A T T T T T T T T A T A
Tradouw Pass        G A T G A T C A A A T T T A T A A T G T T G T A G T A A C T G C T C A T G C T T T G T A A T A A T T T T T T T T A T A
Grootvadersbos      G A T G A T C A A A T T T A T A A T G T T G T A G T A A C T G C T C A T G C T T T G T A A T A A T T T T T T T T A T A
Riversdale          G A T G A T C A A A T T T A T A A T G T T G T A G T A A C T G C T C A T G C T T T G T A A T A A T T T T T T T T A T A
Tsitsikamma         G A T G G T C A G A T C T A T A A T G T T A T T G T T A C T G C T C A T G C T T T T A T T A T A A T T T T T T T T A T A
M. penicillatus     G A T G A T C A A A T T T A T A A T G T T A T T G T A A C T G C T C A T G C T T T G T T A T A A T T T T T T T T A T A
Mesamphisopus n. sp. G A T G A C C A A A T T T A T A A T G T T A T T G T A A C T G C T C A T G C T T T G T T A T A A T T T T T T T T A T A
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Betty's Bay A	GTTATACCAATTATAATTGGTGGTTTTGGTAACTGGTTAATGCCTTTAATACTTGGTGCT
Betty's Bay B	GTTATACCAATTATAATTGGTGGTTTTGGTAACTGGTTAATGCCTTTAATACTTGGTGCT
Wemmershoek	GTTATACCAATTATAATTGGAGGGTTTGGTAATTGGTTGATACCTTTAATACTTGGTGCT
Steenbras A	GTTATACCAATTATGATTGGGGGTTTTGGTAATTGGTTAATGCCTTTAATACTTGGTGCC
Steenbras B	GTTATACCTATTATAATTGGGGGTTTTGGTAATTGGTTAATGCCTTTAATACTTGGTGCC
Steenbras C	GTTATACCTATTATAATTGGAGGTTTTGGTAATTGGTTAATGCCTTTAATACTTGGTGCT
Kogelberg	GTTATACCAATTATGATTGGGGGTTTTGGTAATTGGTTAATACCATTAATACTTGGTGCT
Grabouw	GTTATACCAATTATGATTGGGGGTTTTGGTAATTGATTAATGCCTTTAATACTTGGCGCT
Greyton	GTTATACCAATTATAATTGGTGGTTTTGGAAATTGGTTAATACCTTTAATACTTGGTGCT
Protea Valley 1	GTTATACCAATTATGATTGGAGGTTTTGGTAATTGGTTAATGCCTTTAATACTTGGTGCT
Protea Valley 2	GTTATACCAATTATGATTGGAGGTTTTGGTAATTGGTTAATGCCTTTAATACTTGGTGCT
Barrydale	GTTATGCCAATTATGATTGGGGGTTTTGGTAATTGGTTAATGCCTTTAATACTTGGTGCT
Tradouw Pass	GTTATACCAATTATGATTGGTGGTTTTGGTAATTGGTTAATGCCTTTAATACTTGGTGCT
Grootvadersbos	GTTATACCAATTATAATTGGGGGTTTTGGTAATTGATTAATGCCTTTAATACTTGGTGCT
Riversdale	GTTATACCAATTATGATTGGGGGTTTTGGTAATTGATTAATGCCTTTAATACTTGGTGCT
Tsitsikamma	GTAATACCTATTATAATTGGTGGATTTGGAAATTGATTAATACCTTTAATACTTGGAGCT
M. penicillatus	GTTATACCTATTATGATTGGTGGTTTTGGTAATTGGTTAATGCCTTTAATACTTGGTGCT
Mesamphisopus n. sp.	GTTATACCTATCATGATTGGTGGGTTTGGTAATTGGTTGATACCTTTAATGCTTGGTGCT

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Betty's Bay A	CCTGATATAGCTTTTCCTCGAATAAATAATATAAGGTTTTGGTTACTTGTTCCTTCTTTA
Betty's Bay B	CCTGATATAGCTTTTCCTCGAATAAATAATATAAGGTTTTGGTTACTTGTTCCTTCTTTA
Wemmershoek	CCGGATATAGCTTTTCCTCGAATAAACAATATAAGATTTTGATTACTTGTTCCTTCTTTA
Steenbras A	CCTGATATAGCTTTTCCTCGAATAAACAATATGAGATTTTGGTTACTTGTACCTTCTTTG
Steenbras B	CCTGATATAGCATTTCCTCGTATAAATAATATAAGTTTTTGGTTACTTGTTCCTTCTTTG
Steenbras C	CCTGATATAGCATTTCCTCGTATAAATAATATAAGTTTTTGGTTACTTGTTCCTTCTTTG
Kogelberg	CCTGATATAGCTTTTCCTCGAATAAACAATATGAGGTTTTGGTTACTTGTACCTTCTTTG
Grabouw	CCAGATATAGCTTTTCCTCGAATAAACAATATAAGATTTTGGTTACTTGTTCCATCTTTA
Greyton	CCTGATATAGCTTTTCCTCGTATAAATAATATGAGGTTTTGGTTACTTGTGCCTTCTTTG
Protea Valley 1	CCTGATATAGCTTTTCCTCGAATAAATAATATAAGGTTTTGGTTACTTGTGCCTTCTTTG
Protea Valley 2	CCTGATATAGCTTTTCCTCGAATAAATAATATGAGGTTTTGGTTACTTGTGCCTTCTTTG
Barrydale	CCTGATATAGCTTTTCCTCGCATAAATAATATAAGGTTTTGGTTACTTGTACCTTCTTTG
Tradouw Pass	CCTGATATAGCTTTTCCTCGAATAAATAATATGAGATTTTGGTTACTTGTACCTTCTTTA
Grootvadersbos	CCTGATATAGCTTTTCCTCGAATAAATAATATGAGGTTTTGGTTACTTGTACCTTCTTTG
Riversdale	CCTGATATAGCTTTTCCTCGAATAAACAATATAAGATTTTGGTTACTTGTACCTTCTTTA
Tsitsikamma	CCTGATATAGCTTTTCCTCGTATAAATAATTTGAGATATTTATTACTTATTCCTTCTTTA
M. penicillatus	CCTGATATAGCGTTTCCTCGAATAAATAATATAAGATTTTGATTGCTTGTTCCTTCTTTA
Mesamphisopus n. sp.	CCTGATATAGCATTTCCTCGGATGAATAATATAAGATTTTGATTGTTAGTTCCTTCTTTA

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Betty's Bay A	C T A T T A T T A C T T G G T A G T G G T T T G G T T G A A A G T G G A A T T G G T A C A G G T T G A A C T G T T T A T
Betty's Bay B	C T A T T A T T A C T T G G T A G T G G T T T G G T T G A A A G T G G A A T T G G T A C A G G T T G A A C T G T T T A T
Wemmershoek	T T A T T A T T A C T T G G T A G A G G T T T A G T T G A A A G T G G T A T T G G T A C A G G T T G A A C T G T T T A T
Steenbras A	T T A T T G T T A C T T G G T A G T G G T T T A G T T G A A A G T G G G A T T G G T A C A G G T T G A A C T G T T T A T
Steenbras B	T T A T T A T T A C T T G G T A G T G G T T T A G T T G A A A G T G G A A T T G G T A C A G G T T G A A C T G T T T A T
Steenbras C	T T A T T A T T A C T T G G T A G T G G T T T A G T T G A A A G T G G A A T T G G A A C A G G T T G A A C T G T T T A T
Kogelberg	T T A T T G T T A C T T G G T A G T G G T T T A G T T G A A A G T G G G A T T G G T A C A G G T T G A A C T G T T T A T
Grabouw	T T A T T A T T A C T T G G A A G T G G T T T A G T T G A A A G T G G T A T T G G T A C A G G T T G A A C T G T T T A T
Greyton	T T A T T G T T G T T A G G T A G T G G T T T A G T T G A A A G T G G A A T T G G T A C A G G T T G A A C T G T T T A T
Protea Valley 1	T T A T T A T T A C T T G G T A G A G G A T T A G T T G A A A G T G G A A T T G G T A C A G G T T G A A C T G T T T A T
Protea Valley 2	T T A T T A T T A C T T G G T A G A G G A T T A G T T G A A A G T G G A A T T G G T A C A G G T T G A A C T G T T T A T
Barrydale	T T A T T G T T A C T T G G T A G T G G T C T A G T T G A A A G T G G A A T T G G T A C A G G T T G A A C T G T T T A T
Tradouw Pass	T T G T T A T T A C T T G G T A G T G G A T T A G T T G A A A G T G G A A T T G G T A C A G G T T G A A C T G T T T A T
Grootvadersbos	T T A T T G T T A C T T G G T A G T G G T T T A G T T G A A A G T G G A A T T G G T A C A G G T T G A A C T G T T T A T
Riversdale	T T A T T G T T A C T T G G T A G T G G T T T A G T T G A A A G T G G A A T T G G T A C A G G T T G A A C T G T T T A T
Tsitsikamma	G T A T T G T T A C T T T G A A G T G G A A T A G T T G A G G G G G G G A T T G G T A C T G G T T G A A C T G T T T A T
M. penicillatus	G G T T T G T T A C T T G G T A G A G G T T T A G T T G A A G G A G G T G T A G G T A C T G G T T G G A C T G T T T A T
Mesamphisopus n. sp.	G G T T T A T T A C T T G G T A G A G G T T T A G T T G A A G G A G G T G T A G G T A C T G G T T G G A C T G T T T A T

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Betty's Bay A	C C T C C T T T A G C T T C T G G A G T G T T T C A T A G T G G G T C T T C A G T T G A T T T G G G A A T T T T T T C T
Betty's Bay B	C C T C C T T T A G C T T C T G G A G T G T T T C A T A G T G G G T C T T C A G T T G A T T T G G G A A T T T T T T C T
Wemmershoek	C C T C C T T T G G C T T C T G G A A G T T T T C A T A G T G G G T C T T C A G T T G A C T T A G G G A T T T T T T C T
Steenbras A	C C T C C T T T A G C T T C T G G G G T A T T T C A T A G T G G A T C T T C A G T A G A T T T A G G T A T T T T T T C T
Steenbras B	C C T C C T T T A G C T T C T G G A G T A T T T C A T A G T G G T T C T T C G G T T G A T T T A G G A A T T T T T T C T
Steenbras C	C C T C C T T T A G C T T C T G G A G T A T T T C A T A G T G G T T C T T C G G T T G A T T T A G G A A T T T T T T C T
Kogelberg	C C T C C T T T A G C T T C T G G G G T A T T T C A T A G T G G A T C T T C A G T A G A T T T A G G G A T T T T T T C T
Grabouw	C C T C C T T T G G C C T C T G G T G T T T T T C A T A G T G G T T C T T C G G T T G A T T T A G G A A T T T T T T C T
Greyton	C C T C C T T T A G C T T C T G G G G T A T T T C A T A G T G G A T C T T C G G T A G A T T T A G G G A T T T T T T C T
Protea Valley 1	C C T C C T T T A G C T T C T G G G G T G T T T C A T A G T G G A T C T T C A G T A G A T T T A G G G A T T T T T T C T
Protea Valley 2	C C T C C T T T A G C T T C T G G G G T G T T T C A T A G T G G A T C T T C A G T A G A T T T A G G G A T T T T T T C T
Barrydale	C C T C C T T T A G C T T C T G G A G T G T T T C A T A G T G G A T C T T C A G T A G A C T T A G G A A T T T T T T C T
Tradouw Pass	C C T C C T T T A G C T T C T G G G G T G T T T C A T A G T G G A T C T T C A G T A G A C T T A G G G A T T T T T T C T
Grootvadersbos	C C T C C T T T A G C T T C T G G G G T G T T T C A T A G T G G A T C T T C A G T A G A T T T A G G A A T T T T T T C T
Riversdale	C C T C C T T T A G C T T C T G G T A T G T T T C A T A G T G G A T C T T C A G T A G A T T T A G G G A T T T T T T C T
Tsitsikamma	C C T C C G T T A T C T T C T G G T A T T G C T C A T A G T G G T T C T T C A G T T G A T T T A G G T A T T T T T T C A
M. penicillatus	C C T C C T T T A G C T T C T G T A A T T G C T C A T A G T G G A T C T T C T G T A G A T T G G G G T A T T T T T T C T
Mesamphisopus n. sp.	C C T C C T T T A G C T T C T G T G A T T G C T C A T A G T G G A T C T T C T G T G G A T T G A G G G A T T T T T T C T

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Betty's Bay A	C T T C A T T T A G C T G G T G C T T C T T C T A T T C T T G G T G C A G T A A A T T T T A T G T C T A C T G T T T G T
Betty's Bay B	C T T C A T T T A G C T G G T G C T T C T T C T A T T C T T G G T G C A G T A A A T T T T A T G T C T A C T G T T T G T
Wemmershoek	C T T C A T T T A G C T G G T G C C T C T T C T A T T C T C G G T G C A G T A A A T T T T A T A T C T A C T G T A T G T
Steenbras A	C T T C A T T T A G C T G G T G C T T C T T C T A T T C T T G G T G C A G T A A A T T T T A T G T C T A C T G T T T G T
Steenbras B	C T T C A T T T G G C T G G T G C T T C T T C T A T T C T T G G T G C G G T A A A T T T T A T G T C T A C T G T A T G T
Steenbras C	C T T C A T T T G G C T G G T G C T T C T T C T A T T C T T G G T G C A G T A A A T T T T A T G T C T A C T G T A T G T
Kogelberg	C T T C A T T T A G C T G G T G C T T C T T C T A T T C T T G G T G C A G T A A A T T T T A T G T C T A C T G T T T G T
Grabouw	C T T C A T T T A G C T G G T G C T T C T T C T A T T C T T G G C G C A G T A A A T T T T A T A T C T A C T G T A T G T
Greyton	C T T C A T T T A G C T G G T G C T T C T T C T A T T C T T G G T G C A G T A A A T T T T A T G T C T A C T G T T T G T
Protea Valley 1	C T T C A T T T A G C T G G T G C T T C T T C T A T T C T T G G T G C A G T A A A T T T T A T G T C T A C T G T T T G T
Protea Valley 2	C T T C A T T T A G C T G G T G C T T C T T C T A T T C T T G G T G C A G T A A A T T T T A T G T C T A C T G T T T G T
Barrydale	C T T C A T T T A G C T G G T G C T T C T T C T A T T C T T G G T G C A G T A A A T T T T A T G T C T A C T G T T T G T
Tradouw Pass	C T T C A T T T A G C T G G T G C T T C T T C T A T T C T T G G C G C A G T A A A T T T T A T G T C T A C T G T T T G T
Grootvadersbos	C T T C A T T T A G C T G G T G C T T C T T C T A T T C T T G G T G C A G T A A A T T T T A T G T C T A C T G T T T G T
Riversdale	C T T C A T T T A G C T G G T G C T T C T T C T A T T C T T G G T G C A G T A A A T T T T A T G T C T A C T G T T T G T
Tsitsikamma	C T T C A T T T G G C T G G G G C T T C T T C T A T T T T A G G T G C T G C A A A T T T T A T G T C A A C T T T T T T G
M. penicillatus	C T T C A T T T A G C T G G T G C T T C T T C T A T T C T T G G T G C T G T T A A C T T T A T G T C G A C T G T T T T T
Mesamphisopus n. sp.	C T T C A T T T A G C T G G T G C T T C T T C T A T T C T T G G T G C T G T A A A T T T T A T G T C A A C T G T T T T T

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Betty's Bay A	A A T G T T C G T T T A A A G T G T A T G A A T T T T G A T T C T A T T T C T T T A T T T T C A T G G T C T G T T T T T
Betty's Bay B	A A T G T T C G T T T A A A G T G T A T G A A T T T T G A T T C T A T T T C T T T A T T T T C A T G G T C T G T T T T T
Wemmershoek	A A T G T T C G T T T A A A G T G T A T A A A T T T T G A T T G T A T T T C T T T A T T T T C T T G A T C T G T A T T T
Steenbras A	A A T G T T C G T T T A A A A T G T A T A A A T T T T G A T T C T A T T T C T T T A T T T T C A T G A T C T G T T T T T
Steenbras B	A A T G T T C G T T T G A A A T G T A T A A A T T T T G A T T G T A T C T C T T T A T T T T C A T G G T C T G T T T T T
Steenbras C	A A T G T T C G T T T A A A A T G T A T A A A T T T T G A T T G T A T T T C T T T A T T T T C A T G G T C T G T T T T T
Kogelberg	A A T G T T C G T T T A A A A T G T A T A A A T T T T G A T T C T A T T T C T T T A T T T T C A T G A T C T G T T T T T
Grabouw	A A T G T T C G T T T A A A A T G T A T G A A T T T T G A T T G T A T T T C T T T A T T T T C T T G A T C T G T A T T T
Greyton	A A T G T T C G T T T A A A A T G T A T A A A T T T T G A T T C T A T T T C T T T A T T T T C A T G A T C T G T T T T T
Protea Valley 1	A A T G T T C G T T T A A A A T G T A T A A A T T T T G A T T C T A T T T C T T T A T T T T C A T G A T C T G T T T T T
Protea Valley 2	A A T G T T C G T T T A A A A T G T A T A A A T T T T G A T T C T A T T T C T T T A T T T T C A T G A T C T G T T T T T
Barrydale	A A T G T T C G T T T A A A A T G T A T G A A T T T T G A T T C T A T T T C T T T A T T T T C A T G A T C T G T T T T T
Tradouw Pass	A A T G T T C G T T T A A A A T G T A T A A A T T T T G A T T C T A T T T C T T T A T T T T C A T G A T C T G T T T T T
Grootvadersbos	A A T G T T C G T T T A A A A T G T A T A A A T T T T G A T T C T A T T T C T T T A T T T T C A T G A T C T G T T T T T
Riversdale	A A T G T T C G T T T A A A A T G T A T G A A T T T T G A T T C T A T T T C T T T A T T T T C A T G A T C T G T T T T T
Tsitsikamma	A A C G T T C G T T T A A A G T C T A T A G A A T T A A G A C A T A T T T C T T T A T T T T C T T G A T C T G T A T T T
M. penicillatus	A A T G T T C G T T T G A A A A G T A T A A A A T T T G A T C A A A T T T C T T T G T T T T C T T G A T C T G T T T T T
Mesamphisopus n. sp.	A A T G T T C G T T T G A A A A G T A T A A A A T T T G A T C A A A T T T C T T T G T T T T C T T G A T C T G T T T T T

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Betty's Bay A	A T T A C T G T A A T T C T T T T A T T A T T A T C T C T T C C A G T T T T A G C T G G T G C T A T T A C T A T A T T A
Betty's Bay B	A T T A C T G T A A T T C T T T T A T T A T T A T C T C T T C C A G T T T T A G C T G G T G C T A T T A C T A T A T T A
Wemmershoek	A T T A C A G T A A T T C T T T T A T T A T T A T C T C T T C C G G T T T T A G C T G G T G C T A T T A C T A T A T T A
Steenbras A	A T T A C T G T A A T T C T T T T A T T A T T A T C T C T T C C A G T T T T A G C T G G T G C T A T T A C T A T A T T A
Steenbras B	A T T A C T G T T A T T C T T T T A T T A T T A T C T C T T C C A G T T T T A G C T G G T G C T A T C A C T A T G T T A
Steenbras C	A T T A C T G T T A T T C T T T T G T T G C T A T C T C T T C C A G T T T T A G C T G G T G C T A T T A C T A T G T T A
Kogelberg	A T T A C T G T A A T T C T T T T A T T A T T A T C T C T T C C A G T T T T A G C T G G T G C T A T T A C T A T A T T A
Grabouw	A T T A C T G T A A T T C T T T T A T T A T T A T C T C T T C C G G T T T T A G C C G G T G C T A T C A C T A T A T T A
Greyton	A T T A C T G T A A T T C T T T T A T T A T T A T C T C T T C C A G T T T T A G C T G G T G C T A T T A C T A T A T T A
Protea Valley 1	A T T A C T G T A A T T C T T T T A T T A T T A T C T C T T C C A G T T T T A G C T G G T G C T A T T A C T A T A T T A
Protea Valley 2	A T T A C T G T A A T T C T T T T A T T A T T A T C T C T T C C A G T T T T A G C T G G T G C T A T T A C T A T A T T A
Barrydale	A T T A C T G T A A T T C T T T T A T T A T T A T C T C T T C C A G T T T T A G C T G G T G C T A T T A C T A T G T T A
Tradouw Pass	A T C A C T G T A A T T C T T T T A T T A T T A T C T C T T C C A G T T T T A G C T G G T G C T A T T A C T A T G T T A
Grootvadersbos	A T T A C T G T A A T T C T T T T A T T A T T A T C T C T T C C A G T T T T A G C T G G T G C T A T T A C T A T A T T A
Riversdale	A T T A C T G T A A T T C T T T T A T T A T T A T C T C T T C C A G T T T T A G C T G G T G C T A T T A C T A T A C T A
Tsitsikamma	A T T A C T G T A G T T C T T T T G T T A T T A T C T C T T C C A G T T T T A G C T G G T G C A A T T A C T A T G T T A
M. penicillatus	A T T A C T G T T A T T C T T T T A T T A C T A T C T C T T C C G G T T T T A G C C G G T G C T A T T A C T A T G T T G
Mesamphisopus n. sp.	A T T A C T G T T A T T C T T T T A T T A T T A T C T C T T C C A G T T T T A G C T G G T G C T A T T A C T A T A T T G

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	4 4 4 4 4 4 4 4 4 5 5 5 5 5 5 5 5 5 5 6 6 6 6 6 6 6 6 6 6 7 7 7 7 7 7 7 7 7 7 8 8 8 8 8 8 8 8 8 8 9 9 9 9 9 9 9 9 9 0
	1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0
Betty's Bay A	T T A A C T G A T C G A A A C T T T A A T A C T T C T T T T T T T T G A T C C A A G A G G A G G T G G T G A T C C T G T T
Betty's Bay B	T T A A C T G A T C G A A A C T T T A A T A C T T C T T T T T T T T G A T C C A A G A G G A G G T G G T G A T C C T G T T
Wemmershoek	T T A A C T G A T C G A A A T T T T A A T A C T T C T T T T T T T T G A T C C T A G T G G T G G A G G T G A C C C T G T T
Steenbras A	T T A A C T G A T C G A A A T T T T A A T A C T T C T T T T T T T T G A C C C A A G A G G T G G G G G T G A T C C T G T T
Steenbras B	T T G A C T G A T C G A A A T T T T A A T A C T T C T T T T T T T T G A T C C T A G T G G T G G G G G T G A T C C T G T T
Steenbras C	T T A A C T G A T C G A A A T T T T A A C A C T T C T T T T T T T T G A T C C T A G T G G T G G G G G T G A T C C T G T T
Kogelberg	T T A A C T G A T C G A A A T T T T A A T A C T T C T T T T T T T T G A C C C G A G A G G T G G G G G T G A T C C T G T T
Grabouw	T T A A C T G A T C G A A A T T T T A A T A C T T C T T T T T T T T G A T C C T A G T G G T G G T G G T G A T C C T G T T
Greyton	T T A A C T G A T C G A A A T T T T A A T A C T T C T T T T T T T T G A C C C A A G A G G T G G G G G T G A T C C T G T T
Protea Valley 1	C T G A C T G A T C G A A A T T T T A A T A C T T C T T T T T T T T G A T C C A A G A G G T G G G G G T G A T C C T G T T
Protea Valley 2	C T G A C T G A T C G A A A T T T T A A T A C T T C T T T T T T T T G A T C C A A G A G G T G G G G G T G A T C C T G T T
Barrydale	T T A A C T G A T C G A A A T T T T A A T A C T T C T T T T T T T T G A C C C A A G A G G T G G G G G T G A T C C T G T T
Tradouw Pass	T T G A C T G A T C G A A A T T T T A A T A C T T C T T T T T T T T G A C C C T A G G G G T G G G G G T G A T C C T G T T
Grootvadersbos	C T G A C T G A T C G A A A T T T T A A T A C T T C T T T T T T T T G A C C C A A G A G G T G G G G G T G A T C C T G T T
Riversdale	T T A A C T G A T C G A A A T T T T A A T A C T T C T T T T T T T T G A T C C G A G A G G T G G T G G T G A T C C T G T T
Tsitsikamma	C T T A C C G A T C G A A A T T T T A A T A C T T C T T T T T T T T G A T C C T A G T G G T G G T G G A G A T C C T G T A
M. penicillatus	T T A A C T G A T C G A A A T T T A A A T A C T T C T T T T T T T T G A A C C T A G A G G G G G A G G A G A T C C T G T T
Mesamphisopus n. sp.	T T A A C T G A T C G A A A C T T A A A T A C T T C T T T T T T T T G A A C C T A G A G G G G G A G G A G A T C C T G T T

Appendix 8: Summary of the characters (mostly external) used, in combination, to distinguish the four known *Mesamphisopus* species and the six species described in Chapter 4. Character states of individuals from the additional populations examined in Chapters 2, 3 and 4 are also tabulated. NA = not examined/unknown.

Species/population	Characters			
	Coloration	Setation[4]		
		Antennal peduncles	Head	Pereon
M. abbreviatus[1]	dull, pale grey	sparse	sparse to common, short	sparse to common; laterally sparse, short
M. albidus	lacking pigmentation	common	sparse, short	sparse, short; common laterally
M. baccatus	dark brown-grey to slate-grey	sparse to common	sparse, short	common, dorsally and laterally
M. capensis[1,2]	pale grey to dark slate-grey	sparse to common	sparse, short	sparse, short
M. depressus[1]	pale grey to dark slate-grey	sparse	abundant laterally, elongate	common to abundant dorsally; abundant laterally, elongate
M. kensleyi	dark brown-grey to slate-grey	common	common, short	common, dorsally and laterally
M. paludosus	dark brown to brownish black	very dense	sparse, short	sparse, dorsally and laterally
M. penicillatus[1,3]	light brown-grey to slate-grey	very dense	sparse, more common laterally	sparse, more common laterally; short to elongate
M. setosus	lightly pigmented, orange-brown	common, elongate	absent	sparse dorsally and laterally
M. tsitsikamma	dark brown to dark slate-grey	abundant to dense	sparse	sparse dorsally, abundant laterally
Red Gods Valley	dark brown-grey	sparse	sparse	sparse dorsally and laterally
Kasteelspoort	grey to brown-grey	very sparse	sparse	sparse dorsally and laterally
Nursery Ravine	grey to brown-grey	very sparse	absent	absent or very sparse
Smitswinkelbaai	dark brown to red-grey or silver-grey	absent or very sparse	sparse, short	sparse
Krom River	dark brown-grey	sparse to common	very sparse	sparse
Schusters River	dark brown-grey to slate-grey	sparse to common	very sparse	sparse
Betty's Bay A	dark brown-grey to brownish black	common	absent	absent or very sparse
Betty's Bay B	dark brown to brownish black	common	sparse	sparse
Wemmershoek	lightly pigmented, yellowish brown	sparse to common	common	common, short to elongate
Steenbras A	dark brown	sparse	sparse	common dorsally and laterally
Steenbras B	light brown-grey to dark brown	common	sparse to common	common dorsally, short; elongate laterally
Steenbras C	gold-brown to dark brown	sparse to common	sparse	absent dorsally; abundant, elongate laterally
Kogelberg	light brown to dark brown-grey	very sparse	absent	absent or very sparse dorsally and laterally
Grabouw	yellow-brown to darker brown	sparse to common	absent	sparse dorsally and laterally
Greyton	brown-grey to slate-grey	sparse	sparse	sparse dorsally and laterally
Protea Valley	light slate-grey-brown	sparse	very sparse	sparse dorsally; sparse, elongate laterally
Barrydale	light grey-brown	abundant	common	common, elongate dorsally; laterally abundant, elongate
Tradouw Pass	dark red-brown to chocolate-brown	sparse to common	sparse to common	common, short dorsally; elongate laterally
Grootvadersbos	light grey-brown to bronze-brown	sparse	sparse to common	abundant dorsally; laterally common; short to elongate
Riversdale	yellow-brown, orange-brown to greyish	very sparse	absent or very sparse	sparse dorsally; sparse to common laterally; short to elongate

Species/population	Setation[4]	Characters				
		Antennule		Antenna		Eyes
	Pleon and pleotelson	Number of articles	Length/body length	Length/body length	Articles shape	Diameter/head depth
M. abbreviatus[1]	common to abundant	5 – 6	NA	NA	short, wide	~ 0.12
M. albidus	sparse	7 – 8	0.13 – 0.14	0.49 – 0.55	short, wide	0.08
M. baccatus	abundant, elongate	6 – 9	0.17	0.54	short, bulbous	0.14 – 0.16
M. capensis[1,2]	sparse, short	7 – 9	0.15	0.57	long, slender	~ 0.18
M. depressus[1]	abundant, elongate	7 – 8	NA	NA	short, wide	~ 0.11
M. kensleyi	abundant, elongate	7 – 8	0.15	0.51	short, wide	0.11
M. paludosus	sparse, short	9 – 10	0.23	0.78	short, wide	0.15 – 0.18
M. penicillatus[1,3]	common to abundant; laterally more elongate	8	0.17 – 0.21	0.54 – 0.76	short, wide	0.17
M. setosus	sparse, short to elongate	7	0.13	0.65	short, wide	0.10 – 0.12
M. tsitsikamma	common	7 – 8	0.16 – 0.18	0.62	short, inflated	0.17 – 0.22
Red Gods Valley	sparse, short to elongate	7	0.12 – 0.13	0.61	long, slender	0.16
Kasteelspoort	sparse, short to elongate	6 – 8	0.13 – 0.14	0.55 – 0.69	long, slender	0.15
Nursery Ravine	very sparse	6 – 7	0.13	0.51 – 0.57	long, slender	0.17
Smitswinkelbaai	common	6 – 7	0.14 – 0.15	0.46	short, inflated	0.11
Krom River	common	7 – 9	0.13 – 0.15	0.50 – 0.63	short, inflated	0.17
Schusters River	sparse to common	7 – 8	0.11 – 0.13	0.32 – 0.43	short, inflated	0.15
Betty's Bay A	sparse; dense, elongate postero-laterally	8 – 11	0.17 – 0.18	0.56 – 0.66	long, slender	0.15
Betty's Bay B	common; dense, elongate postero-laterally	8	0.16	0.69	long, slender	0.15
Wemmershoek	abundant, short to elongate	7 – 8	0.12 – 0.13	0.44 – 0.49	short, inflated	0.12
Steenbras A	sparse to common, more elongate	9	0.18 – 0.19	0.71 – 0.85	long, slender	0.14
Steenbras B	common to abundant, short; elongate ventrally	8	0.15	0.53 – 0.65	long, slender	0.17
Steenbras C	absent or sparse, more elongate	8	0.15 – 0.17	0.71 – 0.77	long, slender	0.17
Kogelberg	sparse, short	8 – 9	0.14 – 0.15	0.64 – 0.67	long, slender	0.14
Grabouw	absent or very sparse; longer ventrally	7	0.15 – 0.16	0.74 – 0.85	short, slender	0.17
Greyton	sparse to common, more elongate	7 – 8	0.14 – 0.17	0.53 – 0.63	short, slender	0.15
Protea Valley	sparse to common, short	7	0.14 – 0.15	0.59	short, inflated	0.14
Barrydale	common to abundant, elongate	7	0.16	0.51 – 0.53	short, inflated	0.17
Tradouw Pass	common, more elongate	7 – 9	0.14 – 0.16	0.67 – 0.72	short, inflated	0.10
Grootvadersbos	abundant, more elongate	7	0.13 – 0.16	0.51 – 0.53	short, inflated	0.12
Riversdale	common, more elongate	7	0.12 – 0.15	0.39 – 0.45	short, wide	0.10

Species/population	Characters				
	Maxillula medial lobe	Maxilla medial lobes		Pereopod I dactylus	Pereopod I propodus
	Accessory setae	Ventral basal setae	Proximal and distal setal rows	Distoventral row of scale-like spines	Distoventral cuticular process
M. abbreviatus[1]	2	single row	separated by gap	well developed	low, absent
M. albidus	2	single row	separated by gap	well developed	well developed
M. baccatus	2	single row	separated by gap	weakly developed	absent
M. capensis[1,2]	2 – 3	single row	separated by gap	well developed	absent
M. depressus[1]	2	two rows	separated by gap	well developed	absent
M. kensleyi	2	single row	separated by gap	absent	absent
M. paludosus	2	single row	separated by gap	weakly developed	absent
M. penicillatus[1,3]	NA	NA	NA	weakly developed	low, small
M. setosus	4	two rows	continuous	well developed	well developed
M. tsitsikamma	2	single row	separated by gap	well developed	well developed
Red Gods Valley	NA	NA	NA	well developed	absent
Kasteelspoort	NA	NA	NA	well developed	absent
Nursery Ravine	NA	NA	NA	well developed	absent
Smitswinkelbaai	NA	NA	NA	absent	present
Krom River	NA	NA	NA	well developed	small
Schusters River	NA	NA	NA	absent	small
Betty's Bay A	NA	NA	NA	weakly developed	well developed
Betty's Bay B	NA	NA	NA	weakly developed	present
Wemmershoek	NA	NA	NA	absent	low
Steenbras A	NA	NA	NA	well developed	absent
Steenbras B	NA	NA	NA	well developed	absent
Steenbras C	NA	NA	NA	well developed	absent
Kogelberg	NA	NA	NA	well developed	absent
Grabouw	NA	NA	NA	absent	short, low
Greyton	NA	NA	NA	well developed	long, low
Protea Valley	NA	NA	NA	well developed	long, low
Barrydale	NA	NA	NA	well developed	long, low
Tradouw Pass	NA	NA	NA	absent	long, low
Grootvadersbos	NA	NA	NA	well developed	well developed
Riversdale	NA	NA	NA	well developed	low

Species/population	Characters		
	Pereopod I – VII	Pleotelson	
	Setation	Dorsal margin and apex	Subapical dorsal robust setae
M. abbreviatus[1]	moderately setose, fine to fairly robust	steep, but shallow ventral inflection; apex indefinite, stubby, hardly upturned	present
M. albidus	moderately robust, fine to heavily robust	abrupt, sharp, deep ventral inflection; short apex upturned	absent
M. baccatus	moderately setose, fine to fairly robust	gradually curving, shallow ventral inflection; apex upturned	absent
M. capensis[1,2]	moderately setose, mostly fine to fairly robust	ventral inflection abrupt, convex, deep; apex slender, long, upturned	absent
M. depressus[1]	moderately setose, mostly fine to fairly robust	ventral inflection gradual, deep; apex broad, small upturned	present
M. kensleyi	heavily setose, fine to fairly robust	abrupt, straight, deep ventral inflection; stubby apex upturned	absent
M. paludosus	abundant, mostly fine to fairly robust	margin straight, ventral inflection absent/very shallow; apex not upturned	present or absent
M. penicillatus[1,3]	abundant, mostly fine	gentle, straight, deep ventral inflection; long apex upturned	present
M. setosus	abundant, mostly strongly robust	abrupt, sharp, deep ventral inflection; slight apex upturned	absent
M. tsitsikamma	abundant, mostly fairly robust to robust	gently curving, shallow ventral inflection; small apex upturned	present or absent
Red Gods Valley	setose, fine	abrupt, sharp, deep ventral inflection; apex upturned	absent
Kasteelspoort	setose, fine to fairly robust	abrupt, sharp, deep ventral inflection; apex upturned	absent
Nursery Ravine	moderately setose, fine to fairly robust	abrupt, sharp, deep ventral inflection; apex upturned	absent
Smitswinkelbaai	moderately setose, fine to fairly robust	abrupt, sharp, deep ventral inflection; apex upturned	absent
Krom River	common to abundant, fine to fairly robust	sharp ventral inflection, not deep; apex upturned	absent
Schusters River	moderately setose, fine to robust	abrupt, sharp, deep ventral inflection; apex upturned	absent
Betty's Bay A	common to abundant, fine to robust	margin straight, not ventrally inflected; apex not upturned	present
Betty's Bay B	common to abundant, fine to robust	very slight ventral inflection before upturned apex	present
Wemmershoek	common to abundant, fine to robust	abrupt, sharp, deep ventral inflection; apex broad, upturned	present
Steenbras A	common to abundant, fine to robust	abrupt, sharp ventral inflection, not deep; apex upturned	present
Steenbras B	common, fine to fairly robust	sharp, shallow ventral inflection; apex upturned	present
Steenbras C	common, fine to fairly robust	abrupt, sharp, deep ventral inflection; apex short, upturned	present
Kogelberg	sparse to common, mostly fine	gentle, slight ventral inflection; apex stubby, slight upturn	present
Grabouw	sparse to common, mostly fine	margin horizontal, sudden, very deep ventral inflection; apex upturned	present or absent
Greyton	common, most fairly robust	ventral inflection not deep; apex upturned	present or absent
Protea Valley	sparse to common, most fairly robust	sharp, steep ventral inflection, not too deep; short apex upturned	present or absent
Barrydale	common to abundant, most fine	abrupt, sharp, deep ventral inflection; short apex upturned	present or absent
Tradouw Pass	common to abundant, fine to fairly robust	gradually curving, shallow ventral inflection; broad apex upturned	present or absent
Grootvadersbos	common, mostly fine to fairly robust	gradually curving, shallow ventral inflection; broad apex upturned	present or absent
Riversdale	common, mostly strongly robust	abrupt, sharp, deep ventral inflection; broad apex upturned	present or absent

Species/population	Characters					
	Pleotelson	Pleopod I – V endopods			Pleopod II	Uropod
	Lateral uropodal ridge	With setae on	Plumose setae on	Distomedial margins	Extension of appendix masculina[5]	Peduncle dorsomedial ridge
M. abbreviatus[1]	well developed	I – V?	I – IV?	entire	to margin	produced, plate like
M. albidus	weak/absent	I – V	I – IV	entire	beyond margin	produced, plate-like
M. baccatus	well developed	I – V	I – V	entire	to margin	produced, plate-like
M. capensis[1,2]	well developed	I – V	I – IV	entire	beyond margin	excessively produced, plate-like
M. depressus[1]	well developed	I – V	I – IV	entire	to margin	weakly produced, plate-like
M. kensleyi	weak/absent	I – V	I – IV	III – V shallowly cleft	beyond margin	produced, plate-like
M. paludosus	well developed	I – II	I – II	entire	not to margin	produced, plate-like
M. penicillatus[1,3]	well developed	I – III	I – III	entire	to margin	produced, plate-like
M. setosus	weak/absent	I – V	I – IV	V shallowly cleft	beyond margin	weakly produced, plate-like
M. tsitsikamma	weak/absent	I – V	I – V	entire	to margin	not produced, linear
Red Gods Valley	well developed	I – V	I – IV	entire	to margin	excessively produced, plate-like
Kasteelspoort	well developed	I – V	I – IV	entire	to margin	excessively produced, plate-like
Nursery Ravine	well developed	I – V	I – IV	entire	to margin	excessively produced, plate-like
Smitswinkelbaai	weak	I – V	I – IV	entire	beyond margin	produced, plate-like
Krom River	weak	I – V	I – V	entire	to margin	produced, plate-like
Schusters River	weak	I – V	I – V	entire	to margin	produced, plate-like
Betty's Bay A	weak	I – V	I – V	entire	not to margin	produced, plate-like
Betty's Bay B	weak	I – V	I – V	entire	to margin	produced, plate-like
Wemmershoek	well developed	I – V	I – IV	III shallowly cleft	beyond margin	produced, plate-like
Steenbras A	well developed	I – V	I – IV	III – V shallowly cleft	NA	produced, plate-like
Steenbras B	weak	I – V	I – IV	III – V shallowly cleft	beyond margin	weakly produced, plate-like
Steenbras C	weak absent	I – V	I – IV	III – V shallowly cleft	beyond margin	weakly produced, plate like
Kogelberg	well developed	I – V	I – IV	entire	NA	produced, plate-like
Grabouw	weak	I – V	I – IV	entire	beyond margin	produced, plate-like
Greyton	weak	I – V	I – V	entire	to margin	produced, plate-like
Protea Valley	weak to absent	I – V	I – V	entire	beyond margin	slightly produced, plate-like
Barrydale	well developed	I – V	I – IV	entire	beyond margin	strongly produced, lobe-like
Tradouw Pass	well developed	I – V	I – IV	III – V shallowly cleft	to margin	produced, plate-like
Grootvadersbos	well developed	I – V	I – IV	III – V shallowly cleft	to margin	produced, plate-like
Riversdale	well developed	I – V	I – IV	III – V shallowly cleft	beyond margin	produced, plate-like

Species/population	Characters		
	Uropod		
	Endopod robust setae	Exopod robust setae	Elongate fine setae
M. abbreviatus[1]	variable	variable	moderately abundant
M. albidus	9 – 10	7 – 8	moderately abundant
M. baccatus	6	4	common
M. capensis[1,2]	4 – 6	3 – 5	sparse to common
M. depressus[1]	6 – 9	~ 5	absent to sparse
M. kensleyi	6	4	abundant
M. paludosus	11	5	moderately abundant
M. penicillatus[1,3]	3 – 11	3 – 7	very dense
M. setosus	10	11	common
M. tsitsikamma	6	6	sparse
Red Gods Valley	9	5	sparse
Kasteelspoort	7	5	sparse
Nursery Ravine	8	5	sparse
Smitswinkelbaai	5	4	sparse
Krom River	6 – 9	4	sparse
Schusters River	6 – 7	4	sparse
Betty's Bay A	7 – 8	4 – 6	dense
Betty's Bay B	10	6 – 7	dense
Wemmershoek	7	4	common
Steenbras A	10 – 12	6 – 7	sparse
Steenbras B	8 – 9	5 – 7	sparse
Steenbras C	8 – 11	6 – 7	sparse
Kogelberg	11	6	absent to sparse
Grabouw	10	5 – 6	sparse
Greyton	9 – 11	6	common
Protea Valley	7 – 9	4 – 6	absent to sparse
Barrydale	8 – 9	4 – 6	common
Tradouw Pass	8 – 10	5 – 8	sparse to common
Grootvadersbos	10	7	common
Riversdale	7	4	sparse

[1]Character summary compiled from the descriptions and diagnoses provided by Barnard (1914, 1927, 1940), Nicholls (1943) and Kensley (2001).
[2]Summary supplemented by examination of individuals collected from the type locality (Echo Valley, Table Mountain) of *Mesamphisopus capensis*.
[3]Summary supplemented by examination of individuals identified as *M. penicillatus*, collected from Stanford, near Barnard's (1940) type locality for the species (Hermanus).
[4]Setation refers to the abundance and length of the fine setae. Robust setae of the pleotelson are ignored.
[5]Relative to the distal margin of the pleopodal endopod.

Appendix 9: Clustal X sequence alignment (328bp) of the fragment of the 12S rRNA mtDNA gene used to determine phylogenetic relationships within *Mesamphisopus* (Chapter 5). This alignment was also used in the combined analysis of the mtDNA data and the total analysis (including recoded allozyme data). Gaps (indels) are represented by hyphens. The ambiguous alignment region, omitted in preliminary analyses, is indicated by square parentheses.

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	1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0
Colubotelson	A T T T T C T T T A A A C C C A A A T A A T T T G G C G G T G T T T A - C A A G A A T C A G A G G A A C C T G T C T A T
Amphisopus	A T A A T T T T C A A A C T T A A A G A A T T T G G C G G T G T T T T - T T C T A A T C A G A G G A A C C T G T C T A T
Paramphisopus	A T G A T C T T T C A A A C T T C A A A G A A T T T G G C G G T A T T T T - A T C T A A T C A G A G G A A C C T G T C T A G
Barrydale	A T G T T C T T T C A A - C C T A A A G A A T A T G G C G G T G T T T A A T T A T A T A A T T A G A G G A A C C T G T T T A T
Betty's Bay	A T G G T C T T T C A A - C C T A A A G A A T A T G G C G G T G T T T A A T T A T A T A A T T A G A G G A A C C T G T T T A T
Grabouw	A T G T T C T T T C A A A C C T A A A G A A T A T G G C G G T G T T T A A T T A T A T A A T T A G A G G A A C C T G T T T A T
Greyton	A T G T T C T T T C A A A C C T A A A G A A T A T G G C G G T G T T T A A T T A T A T A A T T A G A G G A A C C T G T T T A T
Grootvadersbos	A T G T T C T T T C A A - C C T A A A G A A T A T G G C G G T G T T T A A T T A T A T A A T T A G A G G A A C C T G T T T A T
Kogelberg	A T G T T C T T T C A A A C C T A A A G A A T A T G G C G G T G T T T A A T T A T A T A A C T A G A G G A A C C T G T T T A T
Protea Valley	A T G T T C T T T T A A A C C T A A A G A A T A T G G C G G T G T T T A A T T A T A T A A T T A G A G G A A C C T G T T T A T
Riversdale	A T G T T C T T T C A A - C C T A A A G A A T A T G G C G G T G T T T A A T T A T A T A A T T A G A G G A A C C T G T T T A T
Steenbras 1	A T G T T C T T T C A A A C C T A A A G A A T A T G G C G G T G T T T A A T T A T A T A A C T A G A G G A A C C T G T T T A T
Steenbras 2	A T G T C C T T T C A A A C C T A A A G A A T A T G G C G G T G T T T A A T T A T A T A A T T A G A G G A A C C T G T T T A T
Steenbras 3	A T G G C C T T T C A A - C C T A A A G A A T A T G G C G G T G T T T A A T T A T A T A A T T A G A G G A A C C T G T T T A T
Tradouw Pass	A T G T T C T T T C A A A C C T A A A G A A T A T G G C G G T G T T T A A T T A T A T A A T T A G A G G A A C C T G T T T A T
Wemmershoek	A T G T T C T T T C A A A C C T A A A G A A T A T G G C G G T G T T T A A T T A T A T A A T T A G A G G A A C C T G T T T A T
M. albidus	A T G T T C T T T C A A A C C T A A A G A A T A T G G C G G T G T T T A A T T A T A T A A T T A G A G G A A C C T G T T T A T
M. baccatus	A T G A T T T T C A A A C C T A A A G A A T A T G G C G G T G T T T A A C T A T A A T T A G A G G A A C C T G T T T A T
M. capensis 1	A T G A T T T T C A A A C C T A A A G A A T A T G G C G G T G T T T T A T T A T A A T T A G A G G A A C C T G T T T A T
M. capensis 2	A T G A T T T T C A A A C C T A A A G A A T A T G G C G G T G T T T T A T T A T A A T T A G A G G A A C C T G T T T A T
M. kensleyi	A T G T T C T T T C A A A C C T A A A G A A T A T G G C G G T G T T T A A T T A T A A T T A G A G G A A C C T G T T T A C
M. paludosus 1	A C G T T C T T T C A A A C C C A A A G A A T A T G G C G G T G T T T T T T C A T A A T T A G A G G A A C C T G T C T A T
M. paludosus 2	A C G T T C T T T C A A - C C C A A A G A A T A T G G C G G T G T T T T T T C A T A A T T A G A G G A A C C T G T C T A T
M. penicillatus	A T A T T C T T T C A A A C C C A A A G A A T A T G G C G G T G T T T T T T C A T A A T T A G A G G A A C C T G T C T A T
M. setosus	A T G T T C T T T C A A A C C T A A A G A A T A T G G C G G T G T T T A A T T A T A T A A T T A G A G G A A C C T G T T T A T
M. tsitsikamma	A A A T T C T T T C A A A C C C A A A G A A T T T G G C G G T G T T T T - T T A T A A T T A G A G G A A C C T G T T T A T

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Colubotelson	T A A T - C G A T G A T C C A C G A A T A T C T T T C T T G C A T T - - - - - - - - - - T A T A G T T T G T A T A C C A
Amphisopus	T A A A - C G A T A A T C C A C G A A T A T C T T A C T T A A T T A - - - - - - - - - - A G A A G T T T G T A T A C C G
Paramphisopus	T A A A - C G A T G A T C C A C G A A T A T C T T A C T T A G T T T - - - - - - - - - - A A A A G T T T G T A T A C C G
Barrydale	T A A T - C G A T A A T C C A C G A A A A T C T T A C T T A A A T T - - - - - - - - - - A A A A G T T T G T A T A C C A
Betty's Bay	T A A T - C G A T A A T C C A C G A A A A T C T T A C T T A A A T T - - - - - - - - - - A A A A G T T T G T A T A C C A
Grabouw	T A A T - C G A T A A T C C A C G A A A A T C T T A C T T A A A T T - - - - - - - - - - A A A A G T T T G T A T A C C A
Greyton	T A A T - C G A T A A T C C A C G A A A A T C T T A C T T A A A T T - - - - - - - - - - A A A A G T T T G T A T A C C A
Grootvadersbos	T A A T - C G A T A A T C C A C G A A A A T C T T A C T T A A A T T - - - - - - - - - - C A A A G T T T G T A T A C C A
Kogelberg	T A A T - C G A T A A T C C A C G A A A A T C T T A C T T A A A T T - - - - - - - - - - A A A A G T T T G T A T A C C A
Protea Valley	T A A T - C G A T A A T C C A C G A A A A T C T T A C T T A A A T T - - - - - - - - - - A A A A G T T T G T A T A C C A
Riversdale	T A A T - C G A T A A T C C A C G A A A A T C T T A C T T A A A T T - - - - - - - - - - A A A A G T T T G T A T A C C A
Steenbras 1	T A A T - C G A T A A T C C A C G A A A A T C T T A C T T A A A T T - - - - - - - - - - A A A A G T T T G T A T A C C A
Steenbras 2	T A A T - C G A T A A T C C A C G A A A A T C T T A C T T A A A T T - - - - - - - - - - A A A A G T T T G T A T A C C A
Steenbras 3	T A A T - C G A T A A T C C A C G A A A A T C T T A C T T A A A T T - - - - - - - - - - A A A A G T T T G T A T A C C A
Tradouw Pass	T A A T - C G A T A A T C C A C G A A A A T C T T A C T T A A A T T - - - - - - - - - - A A A A G T T T G T A T A C C A
Wemmershoek	T A A T - C G A T A A T C C A C G A A A A T C T T A C T T A A A T T - - - - - - - - - - A A A A G T T T G T A T A C C A
M. albidus	T A A T - C G A T A A T C C A C G A A A A T C T T A C T T A A A T T - - - - - - - - - - A A A A G T T T G T A T A C C A
M. baccatus	T A A T - C G A T A A T C C A C G A A A A T C T C A C T T A A A T T T - - - - - - - - - A A A A G T T T G T A T A C C G
M. capensis 1	T A A T - C G A T A A T C C A C G A A A A T C T C A C T T A A A T T T - - - - - - - - - C A A A G T T T G T A T A C C G
M. capensis 2	T A A T - C G A T A A T C C A C G A A A A T C T C A C T T A A A T T T - - - - - - - - - A A A A G T T T G T A T A C C A
M. kensleyi	T A A T - C G A T A A T C C A C G A A A A T C T T A C T T A A A T T - - - - - - - - - - A A A A G T T T G T A T A C C A
M. paludosus 1	T A A T T C G A T A A T C C A C G A A A A T C T T A C T T A A A T T - A A A A G A A T T A G A A G C T T G T A T A C C G
M. paludosus 2	T A A T T C G A T A A T C C A C G A A A A T C T T A C T T A A A T T - A A A A G A A T T A G A A G C T T G T A T A C C G
M. penicillatus	T A A T T C G A T A A T C C A C G A A A A T C T T A C T T A A A T T T A A A A A A A T T A A A A G C T T G C A T A C C G
M. setosus	T A A T - C G A T A A T C C A C G A A A A T C T T A C T T A A A T T - - - - - - - - - - A A A A G T T T G T A T A C C A
M. tsitsikamma	T A A T - C G A T G A T C C A C G A A A A T C T T A C T T A A A T T - - - - - - - - - - A A A A G C T T G T A T A C C G

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Colubotelson	T C G T T T A T A G T A T C A T T T T A A A G T A T A T A C T G [- A A A T T A T T T - - A A G - - - T T A A - - A A] A T
Amphisopus	T C G T T T G T A A T T G T G T T A G T A G A T A A A T G T T G [- A A A T G G A A T A A A A T - - - T C A T - - A A] A T
Paramphisopus	T C G T T T G T A A T T T T G C T T G T A G G T A T T T G T T A [- T A A T G A A A - - - G A T - - - T T A A - - T G] A T
Barrydale	T C G T T T G A A A T A A T A T T T A A A A A T - T C T A T T T [- T T A T A T A T A T A C A - - - - A A T A T - - A] A T
Betty's Bay	T C G T T T G A A A T A A T A T T A A A A A A T - T C T A T T T [- C T A T A T A T - - A C A - - - - T A A A T A A G] A T
Grabouw	T C G T T T G A A A T A A T A T T A A A A A A T - T C T A T T T [- T T A C A T A T - - A C A - - - - T A A A T A A A] A T
Greyton	T C G T T T G A A A T A A T A T T A A A A A A T - T C T A T T T [- A C A C A T A T - - A T A - - - - A A A A T A A A] A T
Grootvadersbos	T C G T T T G A A A T A A T A T T A A A A A A T - T C T A T T T [- C C A C A T A T - - A C A - - - - A A A A T A A A] A T
Kogelberg	T C G T T T G A A A T A A T A T T A A A A A A T - T C T A T T T [- C C A T A T A T - - A C A - - - - A A A A T A A A] A T
Protea Valley	T C G T T T A A A A T A A T A T T A A A A A A T - T C T A T T T [- C C A C A T A T G T A T A - - - - A A A A T A A A] A T
Riversdale	T C G T T T G A A A T A A T A T T A A A A A A T - T C T A T T T [- C C A T A T A T G T G T A - - - - T A A A T A A A] A T
Steenbras 1	T C G T T T G A A A T A A T A T T A A A A A A T - T C T A T T T [- C C A T A T A T - - A T A - - - - A A A A T A A A] A T
Steenbras 2	T C G T T T G A A A T A A T A T T A A A A G A T - T C T A T T T [- C T A T A T A T - - A T A - - - - T A A A T A A A] A T
Steenbras 3	T C G T T T G A A A T A A T A T T A A A A A A T - T C T A T T T [- C T A T A T A T - - A C A - - - - T A A A T A A A] A T
Tradouw Pass	T C G T T T G A A A T A A T A T T A A A A A A T - T C T A T T T [- C T A T A T A T A T A C A T A C A A A A A T T A A] A T
Wemmershoek	T C G T T T G A A A T A A T A T T T A A A A A T - C T T A T T T [- A T A T A T A T T C A C A - - - - - - A A T A A A] A T
M. albidus	T C G T T T G A A A T A A T A T T T A A A A A T - T C T A T T T [- G T A C A T A T - - A C A - - - - T A A A T A A A] A T
M. baccatus	T C G T C T A A A A T A A T A T C T A A A A A T - T T T A T T G [- C C A A A T A C - - A C A - - - - - A A A T A A A] A T
M. capensis 1	T C G T C T A A A A T A A T A T C T A A A A A T - T T T A T T A [- C C A A A T A T - - A C A - - - - - A A A T A A A] A T
M. capensis 2	T C G T C T A A A A T A A T A T C T A A A A A T - T T T A T T A [- C C A A A T A T - - A C A - - - - - A A A T A A A] A T
M. kensleyi	T C G T T T G A A A T A A T A T T A A A A G A T - T C T A T T T [- T C A C A T A T - - A C A - - - - T A A A T A A A] A T
M. paludosus 1	T C G T T T G A A A T A A T A T T T A A A A A A T - C T T A T T A [- C C A T A T A T - T A T A A A - - T A A A T T A A] A G
M. paludosus 2	T C G T T T G A A A T A A T A T T T A A A A A A T - C T T A T T A [- C C A T A T A T - T A T A A A - - T A A A T T A A] A G
M. penicillatus	T C G T T T G A A A T A A T A T T C G A A A A T - C T T A T T A [- T C A C A T A C - C A - A T A - - T A A A T T T A] A G
M. setosus	T C G T T T G A A A T A A T A T T T A A A A A A T - T C T A T T T [- C T A C A T A T - - A C A - - - - T A A A T A A A] A T
M. tsitsikamma	T C G T T T G A A G T G A C A T T T T T A A A T - A T C A T T T [G A C A T A T A T - T T T A T T A A T T T A T A A A] A T

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	1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0
Colubotelson	A T C A G A T C A A G G T G C A G C T T A T A T G T A A G G T T A G A T G G G T T A C A T T T T T T A G T - A A T - - A
Amphisopus	G T C A G A T C A A G G T G C A G C T A A A A A T T A A G T T A A G A T G G G T T A C A T T G A G C T A T - T G T - - G
Paramphisopus	G A C A G A T C A A G G T G C A G C A A A T A G C T A T G A T T G G A T G G G T T A C A T T G T A A T A T - A G T - - G
Barrydale	G T C A G A T C A T G G T G C A G C - A A T A T T T A A G A T T A A A T T G G T T A C A T T C T A A A A T C T A T T G A
Betty's Bay	G T C A G A T C A T G G T G C A G C - A A T A T T T A A G A T T A A A T T G G T T A C A T T C T A A A A T C T A T T G A
Grabouw	G T C A G A T C A T G G T G C A G C - A A T A T T T A A G A T T A A A T T G G T T A C A T T C T A T A A T C T A T T G A
Greyton	G T C A G A T C A T G G T G C A G C - A A T A T T T A A G A T T A A A T T G G T T A C A T T C T A A A A T C T A T T G A
Grootvadersbos	G T C A G A T C A T G G T G C A G C - A A T A T T T A A G A T T A A A T T G G T T A C A T T C T A A A A T C T A T T G A
Kogelberg	G T C A G A T C A T G G T G C A G C - A A T A T T T A A G A T T A A A T T G G T T A C A T T C T A A A A T C T A T T G A
Protea Valley	G T C A G A T C A T G G T G C A G C - A A T A T T T A A G A T T A A A T T G G T T A C A T T C T A A A A T C T A T T G A
Riversdale	G T C A G A T C A T G G T G C A G C - A A T A T T T A A G A T T A A A T T G G T T A C A T T C T A A A A T C T A T T G A
Steenbras 1	G T C A G A T C A T G G T G C A G C - A A T A T T T A A G A T T A A A T T G G T T A C A T T C T A A A A T C T A T T G A
Steenbras 2	G T C A G A T C A T G G T G C A G C - A A T A T T T A A G A T T A A A T T G G T T A C A T T C T A C A A T C T A T T G A
Steenbras 3	G T C A G A T C A T G G T G C A G C - A A T A T T T A A G A T T A A A T T G G T T A C A T T C T A T A A T C T A T T G A
Tradouw Pass	G T C A G A T C A T G G T G C A G C - A A T A T T T A A G A T T A A A T T G G T T A C A T T C T A A A A T C T A T T G A
Wemmershoek	G T C A G A T C A T G G T G C A G C - A A T A T T T A A G A T T A A A T T G G T T A C A T T C T A C A A T C T A T T G A
M. albidus	G T C A G A T C A T G G T G C A G C - A A T A T T T A A G A T T A A A T T G G T T A C A T T C T G T A A T C T A T C G A
M. baccatus	G T C A G A T C A T G G T G C A G C - A A T A T T T A A G A T T A A A T T G G T T A C A T T C T A C A A T C T A T T G A
M. capensis 1	G T C A G A T C A T G G T G C A G C - A A T A T T T A A G A T T A A A T T G G T T A C A T T C T A T A A T C T A T C G A
M. capensis 2	G T C A G A T C A T G G T G C A G C - A A T A T T T A A G A T T A A A T T G G T T A C A T T C T A T A A T C T A T C G A
M. kensleyi	G T C A G A T C A T G G T G C A G C - A A T A T T T A A G A T T A A A T T G G T T A C A T T C T A C A A T C T A T T G A
M. paludosus 1	G T C A G A T C A T G G C A C A G C - T A T A T T T A A G G T C A A A T T G G T T A C A T T C T A T A A T C T A T C G A
M. paludosus 2	G T C A G A T C A T G G C A C A G C - T A T A T T T A A G G T C A A A T T G G T T A C A T T C T A T A A T C T A T C G A
M. penicillatus	G T C A G A T C A T G G C G C A G C - T A T A T T T A A G G T T A A A T T G G T T A C A T T C T A A A A T C T A T T G A
M. setosus	G T C A G A T C A T G G T G C A G C - A A T A T T T A A G A T T A A A T T G G T T A C A T T C T A T A A T C T A T C G A
M. tsitsikamma	G T C A G A T C A T G G T G C A G T - A A T A T T T A A G A A T A A A T T G G T T A C A T T C T A T A A A C T T T A G A

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	1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0
Colubotelson	C G T T A A A T A T T A - G G A A G - T T A A A T T A A A G G A G G A T T T G A A A G T A A T T - - T A G A A T T A A A
Amphisopus	G T T A A A A T T T T A - T G C A A - A T T T A T T A T T A T T G G A T T T G A A A T T A A T T - - T A A A A T T A T A
Paramphisopus	G T T T A A T T A A A A - T G A A A T A T T T A T T A A G G A T G G A T T T G A A A G T A A T T - - T C A A A - - - - -
Barrydale	C G T T A T C T G A A T - T T A A A A A T C A A T A - A A G T A G A A T T T A A A T G T A A T T - - A C A A A A T A T A
Betty's Bay	C G T T A T C T G A A T - T T A A A A A T C A C T A - A A G C A G A A T T T A A A T G T A A T T - - A T A A A A T A T A
Grabouw	C G T T A T A T G A A T - T T A A A A A T C A T T A - A A G C A G A A T T T A A A T G T A A T T - - A T A A A C T A T A
Greyton	C G T T A T C T G A A T - T T A A A A T G C A G T A - A A G C A G A A T T T A A A T G T A A T T - - A T A A A C T A T A
Grootvadersbos	C G T T A T C T G A A T - T T A A A A A T C A A T A - A A G C A G A A T T T A A A T G T A A T T - - A C A A A C T A T A
Kogelberg	C G T T A T C T G A A T - T T A A A A A T C A G T A - A A G C A G A A T T T A A A T G T A A T T - - A T A A A C T A T A
Protea Valley	C G T T A T C T G A A T - T T A A A A A T C A A C A - A A G C A G A A T T T A A A T G T A A T T - - A C A A A C T A T A
Riversdale	C G T T A T C T G A A T - T T A A A A A T C A A T A - A A G C A G A A T T T A A A T G T A A T T - - A T A A A C T A T A
Steenbras 1	C G T T A T C T G A A T - T T A A A A A T C A G T A - A A G C A G A A T T T A A A T G T A A T T - - A T A A A C T A T A
Steenbras 2	C G T T A C C T G A A T - T T A A A A A T C A C A A - A A G C A G A A T T T A A A T G T A A T T - - A T A A A C T A T A
Steenbras 3	C G T T A C C T G A A T - T T A A A A A T C A C A A - A A G C A G A A T T T A A A T G T A A T T - - A T A A A C T A T A
Tradouw Pass	C G T T A T T T G A A T - T T A A A A A T C A A T A - A A G A A G A A T T T A A A T G T A A T T - - A T A A A T T A T A
Wemmershoek	C G T T A T C T G A A T - T T A A A A A T T A C T A - A A G C A G A A T T T A A A T G T A A T T - - A T A A A T T A T A
M. albidus	C G T T A T C T G A A T - T T A A A A A T C A C A A - A A G C A G A A T T T A A A T G T A A T A - - A T A A A C T A T A
M. baccatus	C A T T A T T T G A A T - T T A A A A T T C A T T A - A A G T A G G A T T T A A A T G T A A T T - - A A A A A C T A T A
M. capensis 1	C A T T A T T T G A A T - T T T A A A C T C A T T A - A A G T A G G A T T T A A A T G T A A T T - - A A A A A C T A T A
M. capensis 2	C A T T A T T T G A A T - T T A A A A C T C A T T A - A A G T A G G A T T T A A A T G T A A T T - - A A A A A C T A T A
M. kensleyi	C G T T A C A T G A A T - T T A A A A A T C A A T A - A A G C A G A A T T T A A A T G T A A T T - - A C A A A C T A T A
M. paludosus 1	C A G A A A A T A A A A - T G A A A A A T T A T T T C A A G C C G A A T C T A A A C G T A A T T A A A T A A G T T A T A
M. paludosus 2	C A G A A A A T A A A A - T G A A A A A T T A T T T C A A G C C G A A T C T A A A C G T A A T T A A A T A A G T T A T A
M. penicillatus	C A G A A A A T A A A A - T G A A A A A T T A T T T T A A G C C G A A T C T A A A C G T A A T T T A A C A A G T T A T A
M. setosus	C G T T A T C T G A A T - T T A A A A A T C A C A A - A A G C A G A A T T T A A A T G T A A T A - - A T A A A C T A T A
M. tsitsikamma	C G T T A C A T T T G T A T G A A A A A A T A A T T A - A A G C T G A A T C T A A A T G T A A T T - - A T A A A C T A T A

	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3
	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8
Colubotelson	C	A	A	T	T	A	A	G	A	T	G	A	T	T	A	A	A	T	T	T	G	T	T	A	A	C	A	T	G	C	A	C	A	T	A	T	C	G
Amphisopus	T	T	T	T	T	A	T	G	A	-	-	A	T	T	T	G	A	A	T	T	-	T	A	A	A	C	A	T	G	T	A	C	A	T	A	T	C	G
Paramphisopus	C	T	T	T	T	A	T	G	A	-	-	A	T	T	T	G	G	T	T	T	-	A	A	A	A	T	A	T	G	T	A	C	A	T	A	T	C	G
Barrydale	A	T	T	T	T	A	T	A	A	T	G	A	A	T	-	A	T	T	T	A	C	A	A	A	A	C	A	T	G	C	A	C	A	T	A	T	C	G
Betty's Bay	A	T	T	T	T	A	T	A	A	T	G	A	A	T	-	A	C	T	T	C	C	A	A	A	A	C	A	T	G	C	A	C	A	T	A	T	C	G
Grabouw	A	T	T	T	T	T	T	A	A	T	G	A	A	T	-	A	T	T	T	A	C	A	A	A	A	C	A	T	G	C	A	C	A	T	A	T	C	G
Greyton	A	T	T	T	T	A	T	A	A	T	G	A	A	T	-	A	T	T	T	A	C	A	A	A	A	C	A	T	G	C	A	C	A	T	A	T	C	G
Grootvadersbos	A	T	T	T	T	A	T	A	A	T	G	A	A	T	-	A	T	T	C	A	C	A	A	A	A	C	A	T	G	C	A	C	A	T	A	T	C	G
Kogelberg	A	T	T	T	C	A	T	A	A	T	G	A	A	T	-	A	T	T	T	A	C	A	A	A	A	C	A	T	G	C	A	C	A	T	A	T	C	G
Protea Valley	A	T	T	T	T	G	T	A	A	T	G	A	A	T	-	A	T	T	T	A	C	A	A	A	A	C	A	T	G	C	A	C	A	T	A	T	C	G
Riversdale	A	T	T	T	T	A	T	A	A	T	G	A	A	T	-	A	T	T	T	A	C	A	A	A	A	C	A	T	G	C	A	C	A	T	A	T	C	G
Steenbras 1	A	T	T	T	C	A	T	A	A	T	G	A	A	T	-	A	T	T	T	A	C	A	A	A	A	C	A	T	G	C	A	C	A	T	A	T	C	G
Steenbras 2	A	C	T	T	T	A	T	A	A	T	G	A	A	T	-	A	T	T	T	A	C	A	A	A	A	C	A	T	G	C	A	C	A	T	A	T	C	G
Steenbras 3	A	C	T	T	T	A	T	A	A	T	G	A	A	T	-	A	T	T	T	A	C	A	A	A	A	C	A	T	G	C	A	C	A	T	A	T	C	G
Tradouw Pass	A	T	T	T	T	A	T	A	A	T	G	A	A	T	-	A	T	T	T	A	C	A	A	A	A	C	A	T	G	C	A	C	A	T	A	T	C	G
Wemmershoek	A	T	T	T	T	T	T	A	A	T	G	A	A	T	-	A	T	T	T	T	C	A	A	A	-	C	A	T	G	C	A	C	A	T	A	T	C	G
M. albidus	A	T	T	T	T	A	T	A	A	T	G	A	A	T	-	A	T	T	T	A	C	A	A	A	A	C	A	T	G	C	A	C	A	C	A	T	C	G
M. baccatus	A	A	T	T	T	A	T	A	A	T	G	A	A	T	-	A	T	T	T	T	C	A	A	A	A	C	A	T	G	T	A	C	A	T	A	T	C	G
M. capensis 1	A	A	T	T	T	A	T	A	A	T	G	A	A	T	-	A	T	T	T	C	C	A	A	A	-	C	A	T	G	T	A	C	A	C	A	T	C	G
M. capensis 2	A	A	T	T	T	A	T	A	A	T	G	A	A	T	-	A	T	T	T	C	C	A	A	A	-	C	A	T	G	T	A	C	A	C	A	T	C	G
M. kensleyi	A	T	T	T	T	T	T	A	A	T	G	A	A	T	-	A	T	T	T	A	C	A	A	A	A	C	A	T	G	C	A	C	A	T	A	T	C	G
M. paludosus 1	A	A	C	T	T	T	T	A	A	T	G	A	A	T	-	A	T	T	-	A	C	A	A	A	A	C	A	T	G	C	A	C	A	T	A	T	C	G
M. paludosus 2	A	A	C	T	T	T	T	A	A	T	G	A	A	T	-	A	T	T	-	A	C	A	A	A	A	C	A	T	G	C	A	C	A	T	A	T	C	G
M. penicillatus	A	A	C	T	T	T	T	A	A	T	G	A	A	T	-	A	C	T	-	A	C	A	A	A	A	C	A	T	G	C	A	C	A	T	A	T	C	G
M. setosus	A	T	T	T	T	A	T	A	A	T	G	A	A	T	-	A	T	T	T	A	C	A	A	A	A	C	A	T	G	C	A	C	A	T	A	T	C	G
M. tsitsikamma	A	C	T	T	T	A	T	A	A	T	G	A	A	T	-	A	T	T	T	C	C	A	A	A	A	C	A	T	G	C	A	C	A	T	A	T	C	G

Appendix 10: Sequence alignment (585 bp) of the COI mtDNA fragment used to examine phylogenetic relationships within *Mesamphisopus* (Chapter 5). Missing data are represented by N. This alignment was used in combination with the 12S rRNA sequence data alignment (Appendix 6) in the combined analyses of mtDNA data, and in the total analysis, where it was combined with the 12S rRNA partition and the recoded allozyme data set (Appendix 8).

	1 1 1 1 1 1 1 1 1 1 2 2 2 2 2 2 2 2 2 3 3 3 3 3 3 3 3 3 4 4 4 4 4 4 4 4 4 5 5 5 5 5 5 5 5 5 6
	1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0
Colubotelson	G G T A T G G G T C T T A G C A T A A T T A T T C G T G T T G A G T T A G G T C A A C C T G G A A G A T T T A T T G G T
Amphisopus	G G T A T A G G C T T A A G T A T A C T A A T T C G A A C A G A A T T A G G A C A A C C C A G G A A G A T T T A T T G G A
Paramphisopus	N N N N N N N G G A T A A G T A T A C T A A T T C G A A C T G A A C T A G G A C A A C C C A G G A A G A T T T A T T G G C
Barrydale	G G A A C T G G T C T T A G T A T A C T T A T T C G A A T T G A A T T A G G T C A A C C T G G T G G T T T A A T T T G T
Betty's Bay	G G A A C T G G T C T T A G T A T A C T T A T T C G A A T T G A A T T A G G T C A A C C T G G T G G T T T A A T T T G T
Grabouw	G G A A C T G G T C T C A G T A T A C T T A T C C G A A T T G A G T T A G G T C A A C C T G G T G G T T T A A T T T G T
Greyton	G G A A C T G G T C T T A G T A T A C T T A T T C G A A T T G A A T T A G G T C A A C C T G G T G G C T T A A T T T G T
Grootvadersbos	G G A A C T G G T C T T A G T A T A C T T A T T C G A A T T G A A T T A G G T C A A C C T G G T G G T T T A A T T T G T
Kogelberg	G G A A C T G G G C T T A G T A T G C T T A T T C G A A T T G A A T T A G G T C A A C C T G G T G G T T T A A T T T G T
Protea Valley	G G A A C T G G T C T T A G T A T A C T T A T T C G A A T T G A A T T A G G T C A A C C T G G T G G T T T A A T T T G T
Riversdale	G G A A C T G G T C T T A G T A T A C T T A T T C G A A T T G A A T T A G G T C A A C C T G G T G G T T T A A T T T G T
Steenbras 1	G G A A C T G G T C T T A G T A T G C T T A T T C G A A T T G A A T T A G G T C A A C C T G G T G G T T T A A T T T G T
Steenbras 2	G G A A C T G G T C T T A G T A T A C T T A T T C G A A T T G A A T T G G G T C A A C C T G G T G G C T T A A T T T G T
Steenbras 3	G G A A C T G G T C T T A G T A T G C T T A T T C G A A T T G A A T T G G G T C A A C C T G G C G G T T T A A T T T G T
Tradouw Pass	G G A A C T G G T C T T A G T A T A C T T A T T C G A A T T G A A T T A G G T C A A C C T G G A G G T T T A A T T T G T
Wemmershoek	G G A A C T G G T C T T A G T A T A C T T A T T C G A A T T G A G T T A G G T C A A C C T G G T G G T T T A A T T T G T
M. albidus	G G G A C T G G T C T T A G T A T A C T T A T T C G A A T T G A A T T A G G T C A A C C T G G G G G T T T G A T T T G T
M. baccatus	G G T A C T G G T C T T A G A A T G C T T A T T C G T A T T G A A T T A G G T C A G C C T G G T G G T T T A A T A T G T
M. capensis 1	G G C A C T G G T C T T A G A A T A C T T A T T C G T A T T G A A T T A G G T C A A C C T G G T G G T T T A A T T T G T
M. capensis 2	G G C A C T G G T C T T A G A A T G C T T A T T C G T A T T G A A T T A G G T C A A C C T G G T G G T T T A A T T T G T
M. kensleyi	G G A A C T G G T C T T A G T A T A C T T A T T C G A A T T G A A T T A G G T C A G C C T G G T G G T T T A A T T T G T
M. paludosus 1	G G T A C T G G G T T A A G A A T A A T T A T T C G T A C C G A G T T A G G T C A G C C T G G G A A G T T T A T T G G A
M. paludosus 2	G G T A C T G G G T T A A G A A T A A T T A T T C G T A C C G A G T T A G G T C A G C C T G G G A A G T T T A T T G G A
M. penicillatus	G G T A C T G G T T T A A G A A T A A T T A T T C G T A C T G A G T T A G G T C A G C C T G G T A A G T T T A T T G G T
M. setusos	G G A A C T G G T C T T A G T A T A C T T A T T C G A A T T G A A T T A G G T C A A C C T G G T G G T T T G A T T T G T
M. tsitsikamma	G G T A C T G G A T T A A G T A T A C T T A T T C G A A T T G A A T T A G G T C A G C C A G G C T C A T T T A T T G G C

	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
	6 6 6 6 6 6 6 6 6 7 7 7 7 7 7 7 7 7 7 8 8 8 8 8 8 8 8 8 8 9 9 9 9 9 9 9 9 9 9 0 0 0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1 1 1 2
	1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0
Colubotelson	G A T G A C C A A A T T T A T A A C G T T A T T G T T A C G G C T C A T G C T T T T G T A A T A A T T T T T T T T A T A
Amphisopus	A A T G A T C A A A T T T A T A A T G T A A T T G T T A C A G C A C A T G C T T T T G T T A T A A T T T T T T T T A T A
Paramphisopus	A A C G A C C A G A T T T A T A A C G T A A T T G T G A C A G C A C A T G C A T T T G T A A T A A T T T T C T T T A T A
Barrydale	G A T G A T C A A A T T T A T A A T G T T G T A G T A A C T G C T C A T G C T T T T G T A A T A A T T T T T T T T A T A
Betty's Bay	G A T G A T C A A A T T T A T A A T G T T G T A G T A A C T G C T C A C G C T T T T G T T A T A A T T T T T T T T A T A
Grabouw	G A T G A C C A A A T T T A T A A T G T T G T A G T A A C T G C T C A T G C T T T T G T T A T A A T T T T T T T T A T A
Greyton	G A T G A T C A A A T T T A T A A T G T T G T A G T A A C T G C T C A T G C T T T T G T A A T A A T T T T T T T T A T A
Grootvadersbos	G A T G A T C A A A T T T A T A A T G T T G T A G T A A C T G C T C A T G C T T T T G T A A T A A T T T T T T T T A T A
Kogelberg	G A T G A T C A A A T T T A T A A T G T T G T A G T A A C T G C T C A T G C T T T T G T A A T A A T T T T T T T T A T A
Protea Valley	G A T G A T C A A A T T T A T A A T G T T G T A G T A A C T G C T C A T G C T T T T G T A A T A A T T T T T T T T A T A
Riversdale	G A T G A T C A A A T T T A T A A T G T T G T A G T A A C T G C T C A T G C T T T T G T A A T A A T T T T T T T T A T A
Steenbras 1	G A T G A T C A A A T T T A T A A T G T T G T A G T A A C T G C T C A T G C T T T T G T A A T A A T T T T T T T T A T A
Steenbras 2	G A T G A T C A A A T T T A T A A T G T T G T A G T A A C T G C T C A T G C T T T T G T T A T A A T T T T T T T T A T A
Steenbras 3	G A T G A T C A A A T T T A T A A T G T T G T A G T A A C T G C T C A T G C T T T T G T T A T A A T T T T T T T T A T A
Tradouw Pass	G A T G A T C A A A T T T A T A A T G T T G T A G T A A C T G C T C A T G C T T T T G T A A T A A T T T T T T T T A T A
Wemmershoek	G A T G A T C A A A T T T A T A A T G T T G T A G T A A C T G C T C A T G C T T T T G T T A T A A T T T T C T T T A T A
M. albidus	G A T G A T C A G A T T T A T A A T G T T G T A G T A A C T G C T C A T G C T T T T G T T A T A A T T T T C T T T A T A
M. baccatus	G A T A G T C C G A T T T A T A A T G T T G T A G T A A C T G C T C A T G C C T T T G T T A T G A T T T T T T T T A T A
M. capensis 1	G A T A G T C A A A T T T A T A A T G T T A T T G T A A C T G C T C A T G C T T T T G T T A T A A T T T T C T T T A T A
M. capensis 2	G A C A G T C A A A T T T A T A A T G T T A T T G T A A C T G C T C A T G C T T T T G T T A T A A T T T T C T T T A T A
M. kensleyi	G A T G A C C A A A T T T A T A A T G T T G T A G T A A C T G C T C A T G C T T T T G T T A T A A T T T T T T T C A T A
M. paludosus 1	G A T G A C C A A A T T T A T A A T G T T A T T G T A A C T G C T C A T G C T T T T G T T A T A A T T T T T T T T A T A
M. paludosus 2	G A T G A C C A A A T T T A T A A T G T T A T T G T A A C T G C T C A T G C T T T T G T T A T A A T T T T T T T T A T A
M. penicillatus	G A T G A T C A A A T T T A T A A T G T T A T T G T A A C T G C T C A T G C T T T T G T T A T A A T T T T T T T T A T A
M. setusos	G A T G A T C A G A T T T A T A A T G T T G T A G T A A C T G C T C A T G C T T T T G T A A T A A T T T T C T T T A T A
M. tsitsikamma	G A T G G T C A G A T C T A T A A T G T T A T T G T T A C T G C T C A T G C T T T T A T T A T A A T T T T T T T T A T A

	Position
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Colubotelson	G T T A T A C C T G T A A T A A T T G G T G G A T T T G G A A A T T G G T T G G T T C C T C T A A T A C T T G G G G C A
Amphisopus	G T T A T A C C T A T T T T A A T T G G A G G A T T T G G A A A C T G A T T A G T A C C A T T A A T A T T A G G A G C C
Paramphisopus	G T T A T A C C T A T A C T A A T T G G G G G G T T C G G N A A C T G A C T A G T C C C A C T G A T A T T A G G A G C T
Barrydale	G T T A T G C C A A T T A T G A T T G G G G G G T T T T G G T A A T T G G T T A A T G C C T T T A A T A C T T G G T G C T
Betty's Bay	G T T A T A C C A A T T A T A A T T G G T G G T T T T G G T A A C T G G T T A A T G C C T T T A A T A C T T G G T G C T
Grabouw	G T T A T A C C A A T T A T G A T T G G G G G T T T T G G T A A T T G A T T A A T G C C T T T A A T A C T T G G C G C T
Greyton	G T T A T A C C A A T T A T A A T T G G T G G T T T T G G A A A T T G G T T A A T A C C T T T A A T A C T T G G T G C T
Grootvadersbos	G T T A T A C C A A T T A T A A T T G G G G G T T T T G G T A A T T G A T T A A T G C C T T T A A T A C T T G G T G C T
Kogelberg	G T T A T A C C A A T T A T G A T T G G G G G T T T T G G T A A T T G G T T A A T A C C A T T A A T A C T T G G T G C T
Protea Valley	G T T A T A C C A A T T A T G A T T G G A G G T T T T G G T A A T T G G T T A A T G C C T T T A A T A C T T G G T G C T
Riversdale	G T T A T A C C A A T T A T G A T T G G G G G T T T T G G T A A T T G A T T A A T G C C T T T A A T A C T T G G T G C T
Steenbras 1	G T T A T A C C A A T T A T G A T T G G G G G T T T T G G T A A T T G G T T A A T G C C T T T A A T A C T T G G T G C C
Steenbras 2	G T T A T A C C T A T T A T A A T T G G G G G T T T T G G T A A T T G G T T A A T G C C T T T A A T A C T T G G T G C C
Steenbras 3	G T T A T A C C T A T T A T A A T T G G A G G T T T T G G T A A T T G G T T A A T G C C T T T A A T A C T T G G T G C T
Tradouw Pass	G T T A T A C C A A T T A T G A T T G G T G G T T T T G G T A A T T G G T T A A T G C C T T T A A T A C T T G G T G C T
Wemmershoek	G T T A T A C C A A T T A T A A T T G G A G G G T T T G G T A A T T G G T T G A T A C C T T T A A T A C T T G G T G C T
M. albidus	G T T A T A C C T A T T A T A A T T G G G G G T T T T G G T A A T T G A C T A A T A C C T T T A A T A C T T G G T G C C
M. baccatus	G T T A T A C C T A T C A T G A T T G G T G G G T T T G G A A T T T G G G T A A T G C C T T T A A T A C T T G G G G C T
M. capensis 1	G T C A T G C C T A T T A T G A T T G G T G G A T T T G G A A A T T G G T T A A T G C C T T T A A T A C T T G G G G C G
M. capensis 2	G T T A T G C C T A T T A T G A T T G G T G G A T T T G G G A A T T G G T T A A T G C C T T T A A T A C T T G G G G C G
M. kensleyi	G T T A T A C C A A T T A T A A T T G G G G G A T T T G G T A A T T G G T T A A T G C C T T T A A T A C T T G G T G C T
M. paludosus 1	G T T A T A C C T A T C A T G A T T G G T G G G T T T G G T A A T T G G T T G A T A C C T T T A A T G C T T G G T G C T
M. paludosus 2	G T T A T A C C T A T C A T G A T T G G T G G G T T T G G T A A T T G G T T G A T A C C T T T A A T G C T T G G T G C T
M. penicillatus	G T T A T A C C T A T T A T G A T T G G T G G T T T T G G T A A T T G G T T A A T G C C T T T A A T A C T T G G T G C T
M. setusos	G T T A T A C C T A T T A T A A T T G G A G G C T T T G G T A A T T G G T T G A T A C C T T T A A T A C T T G G T G C T
M. tsitsikamma	G T A A T A C C T A T T A T A A T T G G T G G A T T T G G A A A T T G A T T A A T A C C T T T A A T A C T T G G A G C T

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Colubotelson	C C T G A T A T A G C A T T T C C T C G A A T A A A T A A T A T G A G A T T T T G A C T T T T A C C A C C T T C T T T A
Amphisopus	C C A G A T A T A G C A T T C C C A C G T A T A A A T A A T A T A A G A T T T T G A C T T C T A C C A C C A T C T C T T
Paramphisopus	C C A G A T A T A G C T T T C C C A C G C A T A A A T A A T A T A A G A T T T T G A C T A C T A C C T C C T T C A T T A
Barrydale	C C T G A T A T A G C T T T T C C T C G C A T A A A T A A T A T A A G G T T T T G G T T A C T T G T A C C T T C T T T G
Betty's Bay	C C T G A T A T A G C T T T T C C T C G A A T A A A T A A T A T A A G G T T T T G G T T A C T T G T T C C T T C T T T A
Grabouw	C C A G A T A T A G C T T T T C C T C G A A T A A A C A A T A T A A G A T T T T G G T T A C T T G T T C C A T C T T T A
Greyton	C C T G A T A T A G C T T T T C C T C G T A T A A A T A A T A T G A G G T T T T G G T T A C T T G T G C C T T C T T T G
Grootvadersbos	C C T G A T A T A G C T T T T C C T C G A A T A A A T A A T A T G A G G T T T T G G T T A C T T G T A C C T T C T T T G
Kogelberg	C C T G A T A T A G C T T T T C C T C G A A T A A A C A A T A T G A G G T T T T G G T T A C T T G T A C C T T C T T T G
Protea Valley	C C T G A T A T A G C T T T T C C T C G A A T A A A T A A T A T G A G G T T T T G G T T A C T T G T G C C T T C T T T G
Riversdale	C C T G A T A T A G C T T T T C C T C G A A T A A A C A A T A T A A G A T T T T G G T T A C T T G T A C C T T C T T T A
Steenbras 1	C C T G A T A T A G C T T T T C C T C G A A T A A A C A A T A T G A G A T T T T G G T T A C T T G T A C C T T C T T T G
Steenbras 2	C C T G A T A T A G C A T T T C C T C G T A T A A A T A A T A T A A G T T T T T G G T T A C T T G T T C C T T C T T T G
Steenbras 3	C C T G A T A T A G C A T T T C C T C G T A T A A A T A A T A T A A G T T T T T G G T T A C T T G T T C C T T C T T T G
Tradouw Pass	C C T G A T A T A G C T T T T C C T C G A A T A A A T A A T A T G A G A T T T T G G T T A C T T G T A C C T T C T T T A
Wemmershoek	C C G G A T A T A G C T T T T C C T C G A A T A A A C A A T A T A A G A T T T T G A T T A C T T G T T C C T T C T T T A
M. albidus	C C T G A T A T A G C A T T T C C T C G T A T A A A C A A T A T A A G G T T T T G A T T A C T T G T C C C T T C T T T A
M. baccatus	C C A G A T A T G G C T T T T C C T C G T A T A A A C A A T A T A A G A T T T T G G T T A C T T G T T C C T T C T T T G
M. capensis 1	C C G G A T A T G G C T T T T C C A C G A A T A A A T A A T A T G A G A T T T T G G T T A C T T G T T C C T T C T T T A
M. capensis 2	C C G G A T A T G G C T T T T C C A C G A A T A A A T A A T A T G A G A T T T T G G T T G C T T G T T C C T T C T T T A
M. kensleyi	C C G G A T A T A G C T T T T C C T C G A A T A A A T A A T A T A A G A T T T T G A T T A C T T G T T C C T T C T T T A
M. paludosus 1	C C T G A T A T A G C A T T T C C T C G G A T G A A T A A T A T A A G A T T T T G A T T G T T A G T T C C T T C T T T A
M. paludosus 2	C C T G A T A T A G C A T T T C C T C G G A T G A A T A A T A T A A G A T T T T G A T T G T T A G T T C C T T C T T T A
M. penicillatus	C C T G A T A T A G C G T T T C C T C G A A T A A A T A A T A T A A G A T T T T G A T T G C T T G T T C C T T C T T T A
M. setusos	C C T G A T A T A G C A T T T C C T C G T A T G A A C A A T A T A A G G T T T T G A T T A C T T G T C C C T T C T C T A
M. tsitsikamma	C C T G A T A T A G C T T T T C C T C G T A T A A A T A A T T T G A G A T A T T T A T T A C T T A T T C C T T C T T T A

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Colubotelson	A C T T T A T T G T T A G G T A G A G G T C T G G T T G A A A G T G G G G T T G G T A C A G G T T G G A C A G T A T A T
Amphisopus	A C A T T A T T A T T A A G C A G A G G A C T A G T T G A A A G A G G A G C A G G A A C A G G A T G A A C A G T C T A C
Paramphisopus	A C C C T A T T A T T A A G A A G A G G C T T A G T A G A A A G T G G T G C A G G A A C A G G A T G A A C A G T G T A T
Barrydale	T T A T T G T T A C T T G G T A G T G G T C T A G T T G A A A G T G G A A T T G G T A C A G G T T G A A C T G T T T A T
Betty's Bay	C T A T T A T T A C T T G G T A G T G G T T T G G T T G A A A G T G G A A T T G G T A C A G G T T G A A C T G T T T A T
Grabouw	T T A T T A T T A C T T G G A A G T G G T T T A G T T G A A A G T G G T A T T G G T A C A G G T T G A A C T G T T T A T
Greyton	T T A T T G T T G T T A G G T A G T G G T T T A G T T G A A A G T G G A A T T G G T A C A G G T T G A A C T G T T T A T
Grootvadersbos	T T A T T G T T A C T T G G T A G T G G T T T A G T T G A A A G T G G A A T T G G T A C A G G T T G A A C T G T T T A T
Kogelberg	T T A T T G T T A C T T G G T A G T G G T T T A G T T G A A A G T G G G A T T G G T A C A G G T T G A A C T G T T T A T
Protea Valley	T T A T T A T T A C T T G G T A G A G G A T T A G T T G A A A G T G G A A T T G G T A C A G G T T G A A C T G T T T A T
Riversdale	T T A T T G T T A C T T G G T A G T G G T T T A G T T G A A A G T G G A A T T G G T A C A G G T T G A A C T G T T T A T
Steenbras 1	T T A T T G T T A C T T G G T A G T G G T T T A G T T G A A A G T G G G A T T G G T A C A G G T T G A A C T G T T T A T
Steenbras 2	T T A T T A T T A C T T G G T A G T G G T T T A G T T G A A A G T G G A A T T G G T A C A G G T T G A A C T G T T T A T
Steenbras 3	T T A T T A T T A C T T G G T A G T G G T T T A G T T G A A A G T G G A A T T G G A A C A G G T T G A A C T G T T T A T
Tradouw Pass	T T G T T A T T A C T T G G T A G T G G A T T A G T T G A A A G T G G A A T T G G T A C A G G T T G A A C T G T T T A T
Wemmershoek	T T A T T A T T A C T T G G T A G A G G T T T A G T T G A A A G T G G T A T T G G T A C A G G T T G A A C T G T T T A T
M. albidus	C T G T T A T T A C T T G G C A G T G G C T T A G T T G A A A G T G G A A T T G G T A C A G G T T G G A C T G T T T A T
M. baccatus	A T A T T G T T G C T T G G T A G A G G A T T A G T T G A A A G A G G G A T T G G T A C A G G T T G A A C T G T T T A T
M. capensis 1	A T G T T A T T G C T T G G A A G A G G T T T A G T A G A A A G A G G G A T T G G C A C A G G T T G A A C T G T T T A T
M. capensis 2	A T A T T A C T G C T C G G A A G A G G T T T A G T A G A A A G A G G G A T T G G T A C G G G T T G A A C T G T T T A T
M. kensleyi	T T A T T A T T A C T T G G T A G A G G T T T A G T T G A G A G T G G A A T T G G T A C A G G T T G A A C A G T T T A T
M. paludosus 1	G G T T T A T T A C T T G G T A G A G G T T T A G T T G A A G G A G G T G T A G G T A C T G G T T G G A C T G T T T A T
M. paludosus 2	G G T T T A T T A C T T G G T A G A G G T T T A G T T G A A G G A G G T G T A G G T A C T G G T T G G A C T G T T T A T
M. penicillatus	G G T T T G T T A C T T G G T A G A G G T T T A G T T G A A G G A G G T G T A G G T A C T G G T T G G A C T G T T T A T
M. setusos	T T A T T A T T A C T T G G T A G T G G T T T A G T T G A A A G T G G A A T T G G C A C A G G T T G G A C T G T C T A T
M. tsitsikamma	G T A T T G T T A C T T T G A A G T G G A A T A G T T G A G G G G G G G G A T T G G T A C T G G T T G A A C T G T T T A T

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Colubotelson	C C A C C T T T A G C G G C A A G A A T T G C A C A T A G G G G T G G A T C A G T A G A T A T A G G T A T T T T T T C T
Amphisopus	C C A C C A T T A G C A G C A A A T A C A G C C C A T A G A G G A G C A T C A G T T G A T C T T G C A A T C T T T T C A
Paramphisopus	C C T C C C C T T G C T G C T A G T A C A G C A C A T A G A G G A G C A T C A G T A G A T T T A G C A A T T T T T T C A
Barrydale	C C T C C T T T A G C T T C T G G A G T G T T T C A T A G T G G A T C T T C A G T A G A C T T A G G A A T T T T T T C T
Betty's Bay	C C T C C T T T A G C T T C T G G A G T G T T T C A T A G T G G G T C T T C A G T T G A T T T G G G A A T T T T T T C T
Grabouw	C C T C C T T T G G C C T C T G G T G T T T T T C A T A G T G G T T C T T C G G T T G A T T T A G G A A T T T T T T C T
Greyton	C C T C C T T T A G C T T C T G G G G T A T T T C A T A G T G G A T C T T C G G T A G A T T T A G G G A T T T T T T C T
Grootvadersbos	C C T C C T T T A G C T T C T G G G G T G T T T C A T A G T G G A T C T T C A G T A G A T T T A G G A A T T T T T T C T
Kogelberg	C C T C C T T T A G C T T C T G G G G T A T T T C A T A G T G G A T C T T C A G T A G A T T T A G G G A T T T T T T C T
Protea Valley	C C T C C T T T A G C T T C T G G G G T G T T T C A T A G T G G A T C T T C A G T A G A T T T A G G G A T T T T T T C T
Riversdale	C C T C C T T T A G C T T C T G G T A T G T T T C A T A G T G G A T C T T C A G T A G A T T T A G G G A T T T T T T C T
Steenbras 1	C C T C C T T T A G C T T C T G G G G T A T T T C A T A G T G G A T C T T C A G T A G A T T T A G G T A T T T T T T C T
Steenbras 2	C C T C C T T T A G C T T C T G G A G T A T T T C A T A G T G G T T C T T C G G T T G A T T T A G G A A T T T T T T C T
Steenbras 3	C C T C C T T T A G C T T C T G G A G T A T T T C A T A G T G G T T C T T C G G T T G A T T T A G G A A T T T T T T C T
Tradouw Pass	C C T C C T T T A G C T T C T G G G G T G T T T C A T A G T G G A T C T T C A G T A G A C T T A G G G A T T T T T T C T
Wemmershoek	C C T C C T T T G G C T T C T G G A A G T T T T C A T A G T G G G T C T T C A G T T G A C T T A G G G A T T T T T T C T
M. albidus	C C A C C T T T A G C T T C T G G G A T A T T T C A T A G T G G A T C T T C A G T T G A T T T A G G T A T T T T T T C T
M. baccatus	C C T C C T T T G T C T T C T G G G G T G T A T C A T A G T G G A T C C T C T G T T G A T T T A G G T A T T T T T T C T
M. capensis 1	C C T C C T T T A G C T T C T G G T G T G T A T C A T A G T G G A T C T T C T G T T G A T C T A G G T A T T T T T T C T
M. capensis 2	C C T C C T T T A G C T T C T G G G G T G T A T C A T A G T G G A T C T T C T G T T G A T T T A G G T A T T T T T T C T
M. kensleyi	C C T C C T T T A G C T T C T G G A G T T T T T C A T A G G A G G T C T T C A G T T G A T T T A G G A A T T T T T T C T
M. paludosus 1	C C T C C T T T A G C T T C T G T G A T T G C T C A T A G T G G A T C T T C T G T G G A T T G A G G G A T T T T T T C T
M. paludosus 2	C C T C C T T T A G C T T C T G T G A T T G C T C A T A G T G G A T C T T C T G T G G A T T G A G G G A T T T T T T C T
M. penicillatus	C C T C C T T T A G C T T C T G T A A T T G C T C A T A G T G G A T C T T C T G T A G A T T G G G G T A T T T T T T C T
M. setusos	C C T C C T T T A G C T T C T G G A A T A T T T C A T A G T G G A T C T T C A G T T G A T T T A G G A A T T T T T T C T
M. tsitsikamma	C C T C C G T T A T C T T C T G G T A T T G C T C A T A G T G G T T C T T C A G T T G A T T T A G G T A T T T T T T C A

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	1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0
Colubotelson	T T A C A T C T T G C G G G A G C T T C A T C T A T T T T A G G G G C T G T T A A C T T T A T T A C T A C T G T A A T T
Amphisopus	T T A C A T T T A G C T G G T G T A T C T T C T A T T T T A G G A G C A G T A A A C T T T A T T A C A A C A G T A A T T
Paramphisopus	C T T C A T C T A G C T G G A G T C T C C T C T A T T T T A G G A G C A G T A A A T T T T A T C A C T A C A G T A A T C
Barrydale	C T T C A T T T A G C T G G T G C T T C T T C T A T T C T T G G T G C A G T A A A T T T T A T G T C T A C T G T T T G T
Betty's Bay	C T T C A T T T A G C T G G T G C T T C T T C T A T T C T T G G T G C A G T A A A T T T T A T G T C T A C T G T T T G T
Grabouw	C T T C A T T T A G C T G G T G C T T C T T C T A T T C T T G G C G C A G T A A A T T T T A T A T C T A C T G T A T G T
Greyton	C T T C A T T T A G C T G G T G C T T C T T C T A T T C T T G G T G C A G T A A A T T T T A T G T C T A C T G T T T G T
Grootvadersbos	C T T C A T T T A G C T G G T G C T T C T T C T A T T C T T G G T G C A G T A A A T T T T A T G T C T A C T G T T T G T
Kogelberg	C T T C A T T T A G C T G G T G C T T C T T C T A T T C T T G G T G C A G T A A A T T T T A T G T C T A C T G T T T G T
Protea Valley	C T T C A T T T A G C T G G T G C T T C T T C T A T T C T T G G T G C A G T A A A T T T T A T G T C T A C T G T T T G T
Riversdale	C T T C A T T T A G C T G G T G C T T C T T C T A T T C T T G G T G C A G T A A A T T T T A T G T C T A C T G T T T G T
Steenbras 1	C T T C A T T T A G C T G G T G C T T C T T C T A T T C T T G G T G C A G T A A A T T T T A T G T C T A C T G T T T G T
Steenbras 2	C T T C A T T T G G C T G G T G C T T C T T C T A T T C T T G G T G C G G T A A A T T T T A T G T C T A C T G T A T G T
Steenbras 3	C T T C A T T T G G C T G G T G C T T C T T C T A T T C T T G G T G C A G T A A A T T T T A T G T C T A C T G T A T G T
Tradouw Pass	C T T C A T T T A G C T G G T G C T T C T T C T A T T C T T G G C G C A G T A A A T T T T A T G T C T A C T G T T T G T
Wemmershoek	C T T C A T T T A G C T G G T G C C T C T T C T A T T C T C G G T G C A G T A A A T T T T A T A T C T A C T G T A T G T
M. albidus	C T T C A T T T A G C C G G T G C T T C T T C T A T T C T T G G T G C G G T A A A T T T T A T A T C T A C T G T C T G T
M. baccatus	C T T C A T T T G G C T G G T G C T T C T T C T A T T C T A G G G G C A G T T A A T T T T A T A T C T A C T G T T T G G
M. capensis 1	C T T C A T T T G G C T G G T G C T T C T T C T A T T C T T G G A G C A G T T A A T T T T A T A T C T A C T G T T T G A
M. capensis 2	C T T C A T T T G G C T G G T G C T T C T T C T A T T C T T G G A G C A G T T A A T T T T A T A T C T A C T G T T T G A
M. kensleyi	C T T C A T T T A G C T G G T G C T T C T T C T A T T C T T G G T G C A G T A A A T T T T A T A T C T A C T G T A T G T
M. paludosus 1	C T T C A T T T A G C T G G T G C T T C T T C T A T T C T T G G T G C T G T A A A T T T T A T G T C A A C T G T T T T T
M. paludosus 2	C T T C A T T T A G C T G G T G C T T C T T C T A T T C T T G G T G C T G T A A A T T T T A T G T C A A C T G T T T T T
M. penicillatus	C T T C A T T T A G C T G G T G C T T C T T C T A T T C T T G G T G C T G T T A A C T T T A T G T C G A C T G T T T T T
M. setusos	C T T C A T T T A G C T G G T G C T T C T T C T A T T C T T G G T G C A G T A A A T T T T A T A T C T A C T G T C T G T
M. tsitsikamma	C T T C A T T T G G C T G G G G C T T C T T C T A T T T T A G G T G C T G C A A A T T T T A T G T C A A C T T T T T T G

	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480
Colubotelson	A	A	T	A	T	A	C	G	T	A	T	G	G	T	T	G	G	T	A	T	A	A	G	G	T	T	T	G	A	T	C	G	C	G	T	C	C	C	T	T	T	G	T	T	T	G	T	T	T	G	A	T	C	T	G	T	C	T	T	T
Amphisopus	A	A	T	A	T	A	C	G	A	A	C	T	T	A	C	A	A	T	A	T	A	A	G	A	T	T	T	G	A	T	C	G	G	G	T	A	C	C	T	T	T	A	T	T	T	G	T	A	T	G	A	T	C	A	G	T	A	T	T	A
Paramphisopus	A	A	T	A	T	A	C	G	A	A	C	A	T	A	C	A	A	T	A	T	A	T	C	A	T	T	T	G	A	C	C	G	A	G	T	T	C	C	C	T	T	A	T	T	T	G	T	A	T	G	A	T	C	A	G	T	A	C	T	A
Barrydale	A	A	T	G	T	T	C	G	T	T	T	A	A	A	A	T	G	T	A	T	G	A	A	T	T	T	T	G	A	T	T	C	T	A	T	T	T	C	T	T	T	A	T	T	T	T	C	A	T	G	A	T	C	T	G	T	T	T	T	T
Betty's Bay	A	A	T	G	T	T	C	G	T	T	T	A	A	A	G	T	G	T	A	T	G	A	A	T	T	T	T	G	A	T	T	C	T	A	T	T	T	C	T	T	T	A	T	T	T	T	C	A	T	G	G	T	C	T	G	T	T	T	T	T
Grabouw	A	A	T	G	T	T	C	G	T	T	T	A	A	A	A	T	G	T	A	T	G	A	A	T	T	T	T	G	A	T	T	G	T	A	T	T	T	C	T	T	T	A	T	T	T	T	C	T	T	G	A	T	C	T	G	T	A	T	T	T
Greyton	A	A	T	G	T	T	C	G	T	T	T	A	A	A	A	T	G	T	A	T	A	A	A	T	T	T	T	G	A	T	T	C	T	A	T	T	T	C	T	T	T	A	T	T	T	T	C	A	T	G	A	T	C	T	G	T	T	T	T	T
Grootvadersbos	A	A	T	G	T	T	C	G	T	T	T	A	A	A	A	T	G	T	A	T	A	A	A	T	T	T	T	G	A	T	T	C	T	A	T	T	T	C	T	T	T	A	T	T	T	T	C	A	T	G	A	T	C	T	G	T	T	T	T	T
Kogelberg	A	A	T	G	T	T	C	G	T	T	T	A	A	A	A	T	G	T	A	T	A	A	A	T	T	T	T	G	A	T	T	C	T	A	T	T	T	C	T	T	T	A	T	T	T	T	C	A	T	G	A	T	C	T	G	T	T	T	T	T
Protea Valley	A	A	T	G	T	T	C	G	T	T	T	A	A	A	A	T	G	T	A	T	A	A	A	T	T	T	T	G	A	T	T	C	T	A	T	T	T	C	T	T	T	A	T	T	T	T	C	A	T	G	A	T	C	T	G	T	T	T	T	T
Riversdale	A	A	T	G	T	T	C	G	T	T	T	A	A	A	A	T	G	T	A	T	G	A	A	T	T	T	T	G	A	T	T	C	T	A	T	T	T	C	T	T	T	A	T	T	T	T	C	A	T	G	A	T	C	T	G	T	T	T	T	T
Steenbras 1	A	A	T	G	T	T	C	G	T	T	T	A	A	A	A	T	G	T	A	T	A	A	A	T	T	T	T	G	A	T	T	C	T	A	T	T	T	C	T	T	T	A	T	T	T	T	C	A	T	G	A	T	C	T	G	T	T	T	T	T
Steenbras 2	A	A	T	G	T	T	C	G	T	T	T	G	A	A	A	T	G	T	A	T	A	A	A	T	T	T	T	G	A	T	T	G	T	A	T	C	T	C	T	T	T	A	T	T	T	T	C	A	T	G	G	T	C	T	G	T	T	T	T	T
Steenbras 3	A	A	T	G	T	T	C	G	T	T	T	A	A	A	A	T	G	T	A	T	A	A	A	T	T	T	T	G	A	T	T	G	T	A	T	T	T	C	T	T	T	A	T	T	T	T	C	A	T	G	G	T	C	T	G	T	T	T	T	T
Tradouw Pass	A	A	T	G	T	T	C	G	T	T	T	A	A	A	A	T	G	T	A	T	A	A	A	T	T	T	T	G	A	T	T	C	T	A	T	T	T	C	T	T	T	A	T	T	T	T	C	A	T	G	A	T	C	T	G	T	T	T	T	T
Wemmershoek	A	A	T	G	T	T	C	G	T	T	T	A	A	A	G	T	G	T	A	T	A	A	A	T	T	T	T	G	A	T	T	G	T	A	T	T	T	C	T	T	T	A	T	T	T	T	C	T	T	G	A	T	C	T	G	T	A	T	T	T
M. albidus	A	A	T	G	T	T	C	G	T	T	T	A	A	A	A	T	G	T	A	T	G	A	G	T	T	T	T	G	A	T	T	G	T	A	T	T	T	C	T	T	T	A	T	T	T	T	C	A	T	G	G	T	C	T	G	T	T	T	T	T
M. baccatus	A	A	T	G	T	T	C	G	G	T	T	A	A	A	A	A	T	A	A	T	A	A	A	T	T	T	T	G	A	T	T	G	T	A	T	T	T	C	T	T	T	A	T	T	T	T	C	G	T	G	A	T	C	C	G	T	A	T	T	T
M. capensis 1	A	A	T	G	T	T	C	G	T	C	T	T	A	A	A	A	T	A	A	T	G	A	A	T	T	T	T	G	A	T	T	G	T	A	T	T	T	C	T	T	T	A	T	T	T	T	C	A	T	G	A	T	C	T	G	T	A	T	T	T
M. capensis 2	A	A	T	G	T	A	C	G	G	C	T	T	A	A	A	A	T	A	A	T	G	A	A	T	T	T	T	G	A	T	T	G	T	A	T	T	T	C	T	T	T	A	T	T	T	T	C	A	T	G	A	T	C	T	G	T	A	T	T	T
M. kensleyi	A	A	C	G	T	T	C	G	T	T	T	G	A	A	G	T	G	T	A	T	G	A	A	T	T	T	T	G	A	T	T	G	T	A	T	T	T	C	T	T	T	A	T	T	T	T	C	T	T	G	A	T	C	T	G	T	A	T	T	T
M. paludosus 1	A	A	T	G	T	T	C	G	T	T	T	G	A	A	A	A	G	T	A	T	A	A	A	A	T	T	T	G	A	T	C	A	A	A	T	T	T	C	T	T	T	G	T	T	T	T	C	T	T	G	A	T	C	T	G	T	T	T	T	T
M. paludosus 2	A	A	T	G	T	T	C	G	T	T	T	G	A	A	A	A	G	T	A	T	A	A	A	A	T	T	T	G	A	T	C	A	A	A	T	T	T	C	T	T	T	G	T	T	T	T	C	T	T	G	A	T	C	T	G	T	T	T	T	T
M. penicillatus	A	A	T	G	T	T	C	G	T	T	T	G	A	A	A	A	G	T	A	T	A	A	A	A	T	T	T	G	A	T	C	A	A	A	T	T	T	C	T	T	T	G	T	T	T	T	C	T	T	G	A	T	C	T	G	T	T	T	T	T
M. setusos	A	A	T	G	T	T	C	G	T	T	T	A	A	A	A	T	G	T	A	T	G	A	A	T	T	T	T	G	A	T	T	G	T	A	T	T	T	C	T	T	T	A	T	T	T	T	C	A	T	G	A	T	C	T	G	T	T	T	T	T
M. tsitsikamma	A	A	C	G	T	T	C	G	T	T	T	A	A	A	G	T	C	T	A	T	A	G	A	A	T	T	A	A	G	A	C	A	T	A	T	T	T	C	T	T	T	A	T	T	T	T	C	T	T	G	A	T	C	T	G	T	A	T	T	T

	4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5
	8 8 8 8 8 8 8 8 8 9 9 9 9 9 9 9 9 9 9 0 0 0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1 1 1 2 2 2 2 2 2 2 2 2 2 3 3 3 3 3 3 3 3 3 3 4
	1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0
Colubotelson	A T T A C T G C T A T T T T G T T A C T T C T A T C T C T T C C T G T T C T T G C T G G A G C A A T T A C T A T A T T T
Amphisopus	A T T A C A G C A A T C T T A T T A T T A T T A T C A C T A C C A G T A C T A G C A G G A G C A A T T A C C A T G C T A
Paramphisopus	A T T A C A G C A G T T C T A T T T T T A N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N N
Barrydale	A T T A C T G T A A T T C T T T T A T T A T T A T C T C T T C C A G T T T T A G C T G G T G C T A T T A C T A T G T T A
Betty's Bay	A T T A C T G T A A T T C T T T T A T T A T T A T C T C T T C C A G T T T T A G C T G G T G C T A T T A C T A T A T T A
Grabouw	A T T A C T G T A A T T C T T T T A T T A T T A T C T C T T C C G G T T T T A G C C G G T G C T A T C A C T A T A T T A
Greyton	A T T A C T G T A A T T C T T T T A T T A T T A T C T C T T C C A G T T T T A G C T G G T G C T A T T A C T A T A T T A
Grootvadersbos	A T T A C T G T A A T T C T T T T A T T A T T A T C T C T T C C A G T T T T A G C T G G T G C T A T T A C T A T A T T A
Kogelberg	A T T A C T G T A A T T C T T T T A T T A T T A T C T C T T C C A G T T T T A G C T G G T G C T A T T A C T A T A T T A
Protea Valley	A T T A C T G T A A T T C T T T T A T T A T T A T C T C T T C C A G T T T T A G C T G G T G C T A T T A C T A T A T T A
Riversdale	A T T A C T G T A A T T C T T T T A T T A T T A T C T C T T C C A G T T T T A G C T G G T G C T A T T A C T A T A C T A
Steenbras 1	A T T A C T G T A A T T C T T T T A T T A T T A T C T C T T C C A G T T T T A G C T G G T G C T A T T A C T A T A T T A
Steenbras 2	A T T A C T G T T A T T C T T T T A T T A T T A T C T C T T C C A G T T T T A G C T G G T G C T A T C A C T A T G T T A
Steenbras 3	A T T A C T G T T A T T C T T T T G T T G C T A T C T C T T C C A G T T T T A G C T G G T G C T A T T A C T A T G T T A
Tradouw Pass	A T C A C T G T A A T T C T T T T A T T A T T A T C T C T T C C A G T T T T A G C T G G T G C T A T T A C T A T G T T A
Wemmershoek	A T T A C A G T A A T T C T T T T A T T A T T A T C T C T T C C G G T T T T A G C T G G T G C T A T T A C T A T A T T A
M. albidus	A T T A C T G T T A T T C T C T T A T T A T T A T C T C T T C C G G T T T T A G C T G G T G C T A T T A C T A T A C T A
M. baccatus	A T T A C T G T A A T T T T A T T A T T G T T A T C T T T A C C T G T T T T G G C A G G T G C T A T T A C T A T A T T A
M. capensis 1	A T T A C T G T A A T T T T A T T A T T G T T A T C T T T G C C T G T T T T G G C A G G T G C T A T T A C T A T A T T A
M. capensis 2	A T T A C T G T A A T T T T A T T G T T G T T A T C T T T A C C T G T T T T G G C T G G T G C C A T C A C T A T A C T A
M. kensleyi	A T T A C T G T A A T T C T T T T A T T A T T A T C T C T T C C A G T T T T A G C T G G C G C T A T C A C C A T A T T A
M. paludosus 1	A T T A C T G T T A T T C T T T T A T T A T T A T C T C T T C C A G T T T T A G C T G G T G C T A T T A C T A T A T T G
M. paludosus 2	A T T A C T G T T A T T C T T T T A T T A T T A T C T C T T C C A G T T T T A G C T G G T G C T A T T A C T A T A T T G
M. penicillatus	A T T A C T G T T A T T C T T T T A T T A C T A T C T C T T C C G G T T T T A G C C G G T G C T A T T A C T A T G T T G
M. setusos	A T T A C T G T T A T T C T T T T G T T A T T A T C T C T T C C G G T T T T A G C C G G T G C T A T T A C T A T A T T A
M. tsitsikamma	A T T A C T G T A G T T C T T T T G T T A T T A T C T C T T C C A G T T T T A G C T G G T G C A A T T A C T A T G T T A

	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	4	4	4	4	4	4	4	4	4	5	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	8	8	8	8	8	8
	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5
Colubotelson	T	T	A	A	C	A	A	A	T	C	G	T	A	A	T	T	T	A	A	A	T	A	C	T	T	C	T	T	T	T	T	T	T	G	A	T	C	C	T	A	N	N	N	N	N
Amphisopus	T	T	A	A	C	T	G	A	T	C	G	T	A	A	T	T	T	A	A	A	T	A	C	A	T	C	A	T	T	T	T	T	T	G	A	T	C	C	T	A	G	A	C	C	C
Paramphisopus	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Barrydale	T	T	A	A	C	T	G	A	T	C	G	A	A	A	T	T	T	T	A	A	T	A	C	T	T	C	T	T	T	T	T	T	T	G	A	C	C	C	A	A	G	A	G	G	T
Betty's Bay	T	T	A	A	C	T	G	A	T	C	G	A	A	A	C	T	T	T	A	A	T	A	C	T	T	C	T	T	T	T	T	T	T	G	A	T	C	C	A	A	G	A	G	G	A
Grabouw	T	T	A	A	C	T	G	A	T	C	G	A	A	A	T	T	T	T	A	A	T	A	C	T	T	C	T	T	T	T	T	T	T	G	A	T	C	C	T	A	G	T	G	G	T
Greyton	T	T	A	A	C	T	G	A	T	C	G	A	A	A	T	T	T	T	A	A	T	A	C	T	T	C	T	T	T	T	T	T	T	G	A	C	C	C	A	A	G	A	G	G	T
Grootvadersbos	C	T	G	A	C	T	G	A	T	C	G	A	A	A	T	T	T	T	A	A	T	A	C	T	T	C	T	T	T	T	T	T	T	G	A	C	C	C	A	A	G	A	G	G	T
Kogelberg	T	T	A	A	C	T	G	A	T	C	G	A	A	A	T	T	T	T	A	A	T	A	C	T	T	C	T	T	T	T	T	T	T	G	A	C	C	C	G	A	G	A	G	G	T
Protea Valley	C	T	G	A	C	T	G	A	T	C	G	A	A	A	T	T	T	T	A	A	T	A	C	T	T	C	T	T	T	T	T	T	T	G	A	T	C	C	A	A	G	A	G	G	T
Riversdale	T	T	A	A	C	T	G	A	T	C	G	A	A	A	T	T	T	T	A	A	T	A	C	T	T	C	T	T	T	T	T	T	T	G	A	T	C	C	G	A	G	A	G	G	T
Steenbras 1	T	T	A	A	C	T	G	A	T	C	G	A	A	A	T	T	T	T	A	A	T	A	C	T	T	C	T	T	T	T	T	T	T	G	A	C	C	C	A	A	G	A	G	G	T
Steenbras 2	T	T	G	A	C	T	G	A	T	C	G	A	A	A	T	T	T	T	A	A	T	A	C	T	T	C	T	T	T	T	T	T	T	G	A	T	C	C	T	A	G	T	G	G	T
Steenbras 3	T	T	A	A	C	T	G	A	T	C	G	A	A	A	T	T	T	T	A	A	C	A	C	T	T	C	T	T	T	T	T	T	T	G	A	T	C	C	T	A	G	T	G	G	T
Tradouw Pass	T	T	G	A	C	T	G	A	T	C	G	A	A	A	T	T	T	T	A	A	T	A	C	T	T	C	T	T	T	T	T	T	T	G	A	C	C	C	T	A	G	G	G	G	T
Wemmershoek	T	T	A	A	C	T	G	A	T	C	G	A	A	A	T	T	T	T	A	A	T	A	C	T	T	C	T	T	T	T	T	T	T	G	A	T	C	C	T	A	G	T	G	G	T
M. albidus	T	T	A	A	C	T	G	A	T	C	G	A	A	A	T	T	T	T	A	A	T	A	C	T	A	G	T	T	T	T	T	T	T	G	A	T	C	C	T	A	G	T	G	G	G
M. baccatus	T	T	A	A	C	T	G	A	T	C	G	A	A	A	T	T	T	T	A	A	T	A	C	T	T	C	T	T	T	T	T	T	T	G	A	T	C	C	T	A	G	T	G	G	G
M. capensis 1	T	T	A	A	C	T	G	A	T	C	G	A	A	A	T	T	T	T	A	A	T	A	C	T	T	C	T	T	T	T	T	T	T	G	A	C	C	C	T	A	G	T	G	G	T
M. capensis 2	T	T	A	A	C	T	G	A	T	C	G	A	A	A	T	T	T	T	A	A	T	A	C	T	T	C	T	T	T	T	T	T	T	G	A	T	C	C	T	A	G	T	G	G	T
M. kensleyi	T	T	A	A	C	T	G	A	T	C	G	A	A	A	T	T	T	T	A	A	C	A	C	T	T	C	T	T	T	T	T	T	T	G	A	T	C	C	T	A	G	T	G	G	T
M. paludosus 1	T	T	A	A	C	T	G	A	T	C	G	A	A	A	C	T	T	A	A	A	T	A	C	T	T	C	T	T	T	T	T	T	T	G	A	A	C	C	T	A	G	A	G	G	G
M. paludosus 2	T	T	A	A	C	T	G	A	T	C	G	A	A	A	C	T	T	A	A	A	T	A	C	T	T	C	T	T	T	T	T	T	T	G	A	A	C	C	T	A	G	A	G	G	G
M. penicillatus	T	T	A	A	C	T	G	A	T	C	G	A	A	A	T	T	T	A	A	A	T	A	C	T	T	C	T	T	T	T	T	T	T	G	A	A	C	C	T	A	G	A	G	G	G
M. setusos	T	T	A	A	C	T	G	A	T	C	G	A	A	A	T	T	T	T	A	A	T	A	C	T	A	G	T	T	T	T	T	T	T	G	A	T	C	C	T	A	G	T	G	G	G
M. tsitsikamma	C	T	T	A	C	C	G	A	T	C	G	A	A	A	T	T	T	T	A	A	T	A	C	T	T	C	T	T	T	T	T	T	T	G	A	T	C	C	T	A	G	T	G	G	T

Appendix 11: Matrix of the presence (1) and absence (0) of alleles used in the cladistic analysis of allozyme data from 23 *Mesamphisopus* taxa. Alleles were regarded as present if they occurred at a frequency greater than 0.05 in any taxon/population. Two null alleles (characters 55 and 56) were each regarded as being identical in the populations in which they were fixed, following the "minimizing" approach of Berrebi *et al.* (1990).

Taxon/Population	Characters																												
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Barrydale	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1
Betty's Bay	1	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
Grabouw	0	0	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0	1
Greyton	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1
Grootvadersbos	1	0	0	0	1	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1
Kogelberg	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
Protea Valley	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
Riversdale	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
Steenbras 1	1	0	1	0	0	1	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	0	0	0	1
Steenbras 2	1	0	0	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	1
Steenbras 3	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1
Tradouw Pass	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	1	0	0	0	0	1
Wemmershoek	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	1
Mesamphisopus albidus	1	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0
Mesamphisopus baccatus	1	0	0	1	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	1	0
Mesamphisopus capensis 1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
Mesamphisopus capensis 2	1	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	1	1	0	0	0
Mesamphisopus kensleyi	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0
Mesamphisopus paludosus 1	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	0
Mesamphisopus paludosus 2	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	0
Mesamphisopus penicillatus	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	1	0	1	0	0
Mesamphisopus setosus	1	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	1
Mesamphisopus tsitisikamma	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0

	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56
Barrydale	1	0	0	0	0	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0
Betty's Bay	0	0	0	1	1	1	0	0	1	0	0	1	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
Grabouw	0	0	0	0	1	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0
Greyton	0	0	0	0	0	1	0	0	1	0	1	1	1	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0
Grootvadersbos	1	0	0	0	1	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0
Kogelberg	0	0	0	0	0	1	0	0	1	0	0	1	1	0	1	0	0	0	1	0	0	0	0	0	1	0	1	0
Protea Valley	1	0	0	0	0	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	1	1	1	0	1	0
Riversdale	0	0	0	0	0	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	1	0	1	1	0
Steenbras 1	0	0	0	0	1	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	1	1	1	0	0	0
Steenbras 2	0	0	0	1	0	1	0	0	1	0	1	0	1	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0
Steenbras 3	0	0	0	1	0	1	0	0	1	0	1	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0
Tradouw Pass	0	0	0	0	1	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0
Wemmershoek	0	0	0	0	1	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0
Mesamphisopus albidus	1	0	1	0	0	1	0	0	1	0	1	0	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
Mesamphisopus baccatus	0	0	0	1	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0
Mesamphisopus capensis 1	0	0	0	1	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
Mesamphisopus capensis 2	0	0	1	0	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
Mesamphisopus kensleyi	0	1	0	0	1	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0
Mesamphisopus paludosus 1	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	1	1	0	0	0	1	0	1	0	0	0	0	1
Mesamphisopus paludosus 2	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	1	0	0	0	1	1	1	0	0	0	0	1
Mesamphisopus penicillatus	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	1	0	0	1	1	0	1	0	0	0	0	1
Mesamphisopus setosus	0	0	1	0	0	0	1	0	1	0	1	0	1	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0
Mesamphisopus tsitisikamma	1	0	1	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	1

Characters: (1) *Ao*^{100}; (2) *Ao*^{95}; (3) *Ao*^{90}; (4) *Ark*^{115}; (5) *Ark*^{105}; (6) *Ark*^{100}; (7) *Gpi*^{160}; (8) *Gpi*^{145}; (9) *Gpi*^{140}; (10) *Gpi*^{125}; (11) *Gpi*^{120}; (12) *Gpi*^{115}; (13) *Gpi*^{110}; (14) *Gpi*^{105}; (15) *Gpi*^{100}; (16) *Gpi*^{90}; (17) *Gpi*^{80}; (18) *Gpi*^{70}; (19) *Gpi*^{60}; (20) *Gpi*^{40}; (21) *Gpi*^{5}; (22) *Hk*^{100};(23) *Hk*^{95}; (24) *Hk*^{85}; (25) *Idh*^{170}; (26) *Idh*^{135}; (27) *Idh*^{125}; (28) *Idh*^{120}; (29) *Idh*^{100}; (30) *Idh*^{90}; (31) *Ldh*^{100}; (32) *Ldh*^{80}; (33) *Ldh*^{70}; (34) *Lt-1*^{100}; (35) *Lt-1*^{95}; (36) *Lt-1*^{90}; (37) *Lt-2*^{100}; (38) *Mdh-1*^{130}; (39) *Mdh-1*^{100}; (40) *Mdh-1*^{80}; (41) *Mdh-2*^{100}; (42) *Mdh-2*^{40}; (43) *Mdh-2*^{15}; (44) *Mdh-2*$^{-50}$; (45) *Mdh-2*$^{-110}$; (46) *Me*^{100}; (47) *Me*^{90}; (48) *Me*^{75}; (49) *Me*^{65}; (50) *Pgm*^{110}; (51) *Pgm*^{100}; (52) *Pgm*^{90}; (53) *Pgm*^{80}; (54) *Pgm*^{70}; (55) *Ldh*^{null}; (56) *Lt-2*^{null}

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Cryptic species within the freshwater isopod *Mesamphisopus capensis* (Phreatoicidea: Amphisopodidae) in the Western Cape, South Africa: allozyme and 12S rRNA sequence data and morphometric evidence

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The freshwater phreatoicidean isopod *Mesamphisopus capensis* has been regarded as the most widespread of the four *Mesamphisopus* species occurring in the Western Cape, South Africa. To determine whether this species was monotypic across its distribution over two mountainous regions, separated by a low-lying coastal plain remnant, genetic differentiation among populations from 11 localities was studied through allozyme electrophoresis of 12 loci and sequencing of a 338-bp 12S rRNA mtDNA fragment from representative individuals. Populations of the two regions were separated by a mean identity value of 0.477. Fixed allele differences at two loci distinguished these regions. Estimates of θ indicated substantial differentiation among populations across the entire sample, as well as within each of the regions. Topologies derived through parsimony and neighbour joining supported the monophyly of the two regions. On the basis of these topologies, allele frequencies and an allozyme dendrogram, five groups were identified. Discriminant function analyses, performed on body and pereopod variables independently, revealed these groups to be well differentiated with a high rate of correct a posteriori reclassification. Using genetic distance criteria these five distinct forms may be considered to be putative species. From a conservation perspective, the two regions can be seen to represent two evolutionarily significant units, while the five groups should be regarded as management units.

ADDITIONAL KEYWORDS: Cape Peninsula – conservation – evolutionarily significant units – management units.

INTRODUCTION

The freshwater isopod *Mesamphisopus capensis* was initially described from Table Mountain (Cape Town, South Africa) by Barnard (1913, 1914) and placed in the genus *Phreatoicus*, which then included species described from Australia and New Zealand. *Phreatoicus capensis* was regarded to be widespread and morphological variation among populations from only three localities warranted the later description of varieties (Barnard, 1927, 1940). These varieties were subsequently afforded specific status and included, together with *P. capensis*, in the endemic South African genus *Mesamphisopus* (Nicholls, 1943; Kensley, 2001). Limited collection records (South African Museum, Cape Town) and sparse literature (Barnard, 1927, 1940) suggest that *M. capensis* is distributed across the south-western portion of the Western Cape province and extends eastwards towards the temperate forests, some 500 km east of Cape Town, along the south coast of South Africa. The identification of specimens from many of the more eastern localities predates, and is questionable in light of, the most recently compiled key (Kensley, 2001). Harrison and Barnard (1972) had regarded populations of *M. capensis* from the mountains of the Cape Peninsula and the Hotten-

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tots Holland Mountains, separated by the low-lying Cape Flats, to be conspecific, although these mountains have been separated since the late Tertiary. These authors stated that slight, consistent, morphological differences were observed, but provided no further information. Harrison, working from the late Keppel Barnard's notes, could possibly have been referring to Barnard's (1927, 1940) varieties, and eventual species (Nicholls, 1943; Kensley, 2001).

M. capensis is defined in Kensley's (2001) key by the absence of a pair of dorsal subapical robust setae, Kensley's (2001: 70) 'spines', on the pleotelson, typical of other species within *Mesamphisopus*. The typical morphological conservatism of the Phreatoicidea, coupled with intraspecific variation (Wilson & Ho, 1996), makes cursory identification of specimens problematic, however. For example, Barnard (1927) highlighted considerable variation with regard to pleotelson and gnathopod shape within individual *M. capensis* populations (e.g. Barnard, 1927: fig. 5). This within-population variation may have been underlying Nicholl's (1944: 154) hesitancy to discuss or identify a single specimen collected from Table Mountain. While completing the last revision of the Phreatoicidea, Nicholls (1943, 1944) had examined numerous, presumably mature, individuals received from Barnard (see Nicholls, 1943: 31). His hesitancy to comment on this specimen indicates that the specimen was immature, damaged, or represented an unknown morphotype for which he had no further access to material. Unrecognized diagnostic characters may thus possibly be obscured by this variation, with geographically disjunct populations initially identified as *M. capensis* representing a complex of cryptic species.

Against a backdrop of increasing anthropogenic threat to both fauna and habitat (see Barnard, 1927; Rebelo, 1992; Cowling, MacDonald & Simmons, 1996; Picker & Samways, 1996), it becomes imperative that the diversity within *M. capensis* (as well as other similarly unique, narrowly endemic, or poorly dispersing invertebrate species) be documented and conservation units identified. Accurate identification of biological diversity is paramount to its conservation (Roe & Lydeard, 1998). Genetic diversity is also increasingly being emphasized as a prerequisite for adaptation, evolutionary success and survival (Mulvey, Liu & Kandl, 1998), a fact recognized in South African conservation policy (DEAT, 1997). Thus, the description of population differentiation serves to identify more populations to be conserved for the maintenance of sufficient variation for species survival (Newton *et al*., 1999). Furthermore, the geographical distributions and demographic and ecological characteristics and requirements of widespread species are very different from those of the independent, constituent species of a species complex. The latter are more likely to be negatively affected by environmental perturbations and habitat destruction (Duffy, 1996). This would have significant conservation and management implications.

In this study, genetic differentiation, using both allozyme and mtDNA 12S rRNA sequence data as well as morphometric variation were examined within *M. capensis* across two mountain ranges, to determine whether disjunct populations were indeed conspecific. A further aim was to discern distinct lineages or identify units for conservation, in light of widely applied evolutionarily significant unit (ESU) and management unit (MU) criteria (e.g. Ryder, 1986; Waples, 1991; Moritz, 1994). Lastly, collections made from Table Mountain were considered further to determine whether more than one species was present.

MATERIAL AND METHODS

Collections

Isopods were collected from the shallow pools and slow-flowing seepages of upper catchments, by sifting through the sand and mud sediment using hand-nets, or by picking individuals from matted plant material. Eleven localities were sampled (Fig. 1), eight from the Cape Peninsula (including four from Table Mountain) and three from the Hottentots Holland Mountains, all within conservation areas. Using the key compiled by Kensley (2001), individuals were regarded as *M. capensis* if the pair of subapical dorsal robust setae was lacking. Individuals to be used in genetic analyses were snap frozen, while remaining individuals (voucher specimens and specimens for morphometric examination) were placed in absolute ethanol.

Allozyme electrophoresis

Between 19 and 70 individuals from each sampling locality were individually homogenized using a glass rod attached to a variable-speed, electric motor in 20–50 μL 0.01 M Tris pH 8.0 extraction buffer. Prior to electrophoresis water soluble proteins were separated from the homogenate by centrifugation at 10 000 *g* for 3 min. Filter paper wicks (Whatman #3) were dipped in the supernatant and inserted into the origin cut in the 13% hydrolysed starch gel (Aldrich Chemical Co.).

Gels were run (at 2–4°C) at 40 mA for 5 h, using two standard electrophoretic buffer systems (Markert & Faulhaber, 1965; Ridgeway, Sherburne & Lewis, 1970). A third buffer system, with a gel pH of 6.5 and an electrode pH of 6.3, modified from Clayton & Tretiak (1972), was also used. Staining for enzymatic activity followed standard protocols (Shaw & Prasad, 1970) with histochemical reagents being applied in a 2% agar overlay. Enzymatic activity was examined in

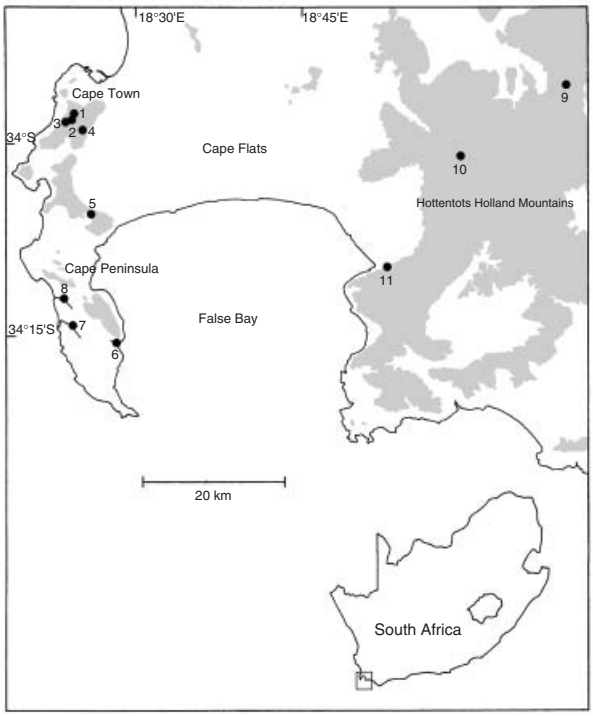

Figure 1. Collection localities of *Mesamphisopus capensis* from the Cape Peninsula and Hottentots Holland Mountains in the Western Cape, South Africa: (1) Echo Valley, (2) Valley of the Red Gods, (3) Kasteelspoort, (4) Nursery Ravine, (5) Silvermine, (6) Smitswinkelbaai, (7) Krom River, (8) Schusters River, (9) Franschhoek, (10) Jonkershoek and (11) Gordon's Bay. Shaded areas represent areas of >300 m elevation.

10 enzyme systems. These included: aldehyde oxidase (*Ao*, EC 1.2.3.1), arginine kinase (*Ark*, EC 2.7.3.3), glucose-6-phosphate isomerase (*Gpi*, EC 5.3.1.9), hexokinase (*Hk*, EC 2.7.1.1), isocitrate dehydrogenase (*Idh*, EC 1.1.1.42), lactate dehydrogenase (*Ldh*, EC 1.1.1.27), malate dehydrogenase (*Mdh*, EC 1.1.1.37), malic enzyme (*Me*, EC 1.1.1.40), peptidase with leucine-tyrosine as substrate (*Lt*, EC 3.4.11.–), and phosphoglucomutase (*Pgm*, EC 2.7.5.1). At each locus, the mobility of each electromorph was expressed relative to the mobility of the most common allele, designated a value of 100, in the Franschhoek (Hottentots Holland Mountains) population, arbitrarily chosen as the reference population. When more than one locus was expressed for a specific enzyme, the most anodally migrating locus was numbered one, with the remaining loci being labelled sequentially.

Allozyme data were analysed numerically using the BIOSYS-1 package (Swofford & Selander, 1981). Allele and genotype frequencies were calculated for the 11 populations. A χ^2 goodness-of-fit test was used to test for significant deviation of observed genotype frequencies from those expected under Hardy–Weinberg equilibrium in each population for each case of polymorphism. Observed (H_O) and expected (H_E) heterozygosities were calculated using Nei's (1978) unbiased estimates. The percentage of polymorphic loci was determined using a 95% criterion (loci were regarded as polymorphic if the frequency of the most common allele was <0.95). Nei's (1978) mean unbiased genetic identity (*I*) and genetic distance (*D*) were calculated among populations from the allele frequencies. The genetic identity values were used to construct a dendrogram of genetic similarity among populations using the UPGMA algorithm (Sneath & Sokal, 1973). In the majority of cases, the combination of Nei's (1978) distance measure (and, hence, identity measure) and the UPGMA algorithm retrieves dendrogram topologies that are congruent to topologies derived by cladistic analyses of other datasets, for example morphological or sequence data (Wiens, 1999). In addition, a principal component analysis was performed, with sampling localities as cases and the frequencies of alleles occurring at the polymorphic loci as variables. All principal components (factors) with eigenvalues >1 were extracted, and preliminary ordination of populations visualized by plotting cases according to their respective scores along the first three principal components extracted.

Partitioning of genetic variation was examined both across the entire sample and within regions (Cape Peninsula and Hottentots Holland Mountains), using the θ-estimates of Weir & Cockerham (1984). These were calculated for individual loci and across all loci, using FSTAT 2.9.3 (Goudet, 2001). Sampling localities were also pooled within regions, enabling a direct comparison between the Cape Peninsula and Hottentots Holland Mountains.

DNA sequencing and sequence analysis

Preliminary sequencing of the 12S rRNA gene region of five individuals from each of the Echo Valley and Franschhoek populations revealed a single haplotype to be present within each of these sampling localities, while the near fixation of cytochrome oxidase subunit I (COI) haplotypes has been observed in several examined populations (G. Gouws, unpubl. data). Similarly, Wetzer (2001) found, albeit with very limited sampling, single 12S rRNA and COI haplotypes to be present in individual phreatoicidean populations. Consequently, total genomic DNA was extracted from one individual per locality, as well as from one specimen of *M. penicillatus*, which was used as an outgroup, using a Qiagen DNEasy tissue extraction kit, following the manufacturer's instructions. The choice of outgroup was determined by the species's basal

position within a molecular phylogeny for *Mesamphisopus* (G. Gouws, unpubl. data).

Polymerase chain reactions (PCRs) were set up in 25 μL volumes, including millipore water, ~5 ng·μL^{-1} template DNA, 10× magnesium- (Mg^{2+})-free buffer, 3 mM·μL^{-1} magnesium choloride ($MgCl_2$), 0.2 mM·μL^{-1} each dNTP, 0.2 μM·μL^{-1} each of the peracarid-specific 12S primer pair (12SCRF and 12SCRR; Wetzer, 2001), and 0.5 units super-thermal DNA polymerase (Southern Cross Biotechnologies). The PCR-regime included an initial denaturing step at 94°C for 5 min, followed by 35 cycles of denaturing (94°C) for 15 s, annealing (52°C) for 1 min, and extension (72°C) for 1.5 min. This was followed by a final cycle of annealing (52°C) for 5 min and extension (72°C) for 15 min. Each series of PCR reactions included a template-free negative control to test for contamination. PCR products were visualized under UV light after electrophoresis in a 1% agarose gel containing ethidium bromide. Products were purified using a Qiagen QiaQuick purification kit, following the manufacturer's directions. Purified products were cycle-sequenced (both forward and reverse strands) following standard protocols, using 3 μL purified PCR product, 3 μL of a 1 μM solution of the appropriate primer, and 4 μL fluorescent-dye terminators (ABI PRISM Dye Terminator Cycle Sequencing Reaction Kit, Perkin Elmer). Samples were analysed using an ABI 3100 automated sequencer.

Each sequence was visually inspected and checked for base ambiguity against its respective electropherogram using Sequence Navigator (Applied Biosystems) and a consensus sequence was created for each sample. Sequences were aligned using ClustalX 1.81 (Thompson *et al*., 1997) with the default parameters applied. Alignments were subsequently inspected manually.

Phylogenetic analyses were performed using PAUP*4b10 (Swofford, 2001). Maximum parsimony (MP) analysis was performed regarding gaps (indels) as missing data, with the heuristic search option and the tree-bisection-reconnection (TBR) branch-swapping algorithm employed to find the most parsimonious trees. Characters were unweighted in all analyses. Phylogenetic support for nodes was determined by performing 1000 bootstrap replicates (Felsenstein, 1985) on the dataset, using a random addition of sequences (1000 iterations).

To determine the appropriate model of nucleotide substitution within the dataset for the maximum likelihood (ML) analysis, Modeltest 3.06 (Posada & Crandall, 1998) was used. A neighbour-joining (NJ) tree was also constructed using the 'uncorrected p' sequence divergence obtained from pair-wise comparisons of haplotypes. In the ML and NJ analyses, bootstrap support was calculated using 100 and 10 000 resampling replicates, respectively, together with a random addition of sequences (100 replicates) in the case of ML analysis.

Additionally, the log-likelihood scores of the unconstrained ML tree and an ML tree with a molecular clock enforced (under the determined model) were compared, using a likelihood ratio test (Felsenstein, 1981). This tests for rate constancy among lineages to determine whether a molecular clock can be applied to the dataset.

Morphometric analysis

To determine the extent to which operational taxonomic units (OTUs) identified by genetic analysis could be ordinated or discriminated, five of the largest ethanol-preserved males from each locality were dissected and digitally photographed using a Leitz stereoscopic dissection microscope and a JVC TK-C1381 digital camera. The largest individuals in each population were taken in order to minimize within-group variation attributable to immature individuals and possible patterns of allometric growth. In the case of the sample from the Valley of the Red Gods, two individuals were examined as only these were appreciably larger than the remaining males and they were thought to belong to the largest size class. Following calibration under different magnifications, measurements were taken from the captured images using Leica QWin and Leica Lida software (Leica Imaging Systems, 1996). Forty-seven variables were measured to incorporate possible variation in overall body (cephalon, pereon, pleon and pleotelson) shape and pereopod dimensions.

To eliminate possible confounding effects of asymmetry, insofar as was possible, right limbs and uropods were measured. If these were missing, damaged or incomplete, they were substituted with the corresponding left limb. Although as yet no evidence has suggested the presence of heterochely, and substantial differences between right and left gnathopods were only observed when these limbs were damaged and regenerated, only the right pereopod 1 (gnathopod) was included in the analysis. Further missing data were substituted with the mean for the respective group, in order to maximize the number of cases.

Morphometric discrimination among the identified groups was investigated by means of standard discriminant function analyses, performed using the body and pereopod variables independently. All data were log-transformed (common logarithms) prior to analysis and all analyses were performed using STATISTICA 6.0 (Statsoft Inc, 2001).

For each analysis, classification functions (linear combinations of variables that optimally differentiate a priori determined groups) were calculated, using a jack-knife procedure. Classification functions were

then used to reassign individuals to groups, based on a posteriori probabilities. Prior classification probabilities were kept equal for all groups. Scatterplots of scores for all individuals for the first two canonical (discriminant) functions were produced to visualize the extent of differentiation between groups.

RESULTS

Allozyme electrophoresis

Of an initial array of 29 enzyme systems screened, only 12 loci provided reliably interpretable zymograms and were included in the study. Eleven of the 12 loci were polymorphic, with *Lt-2* being monomorphic within and across all populations. Allele frequencies at the polymorphic loci and genetic variability measures are presented in Table 1. The number of alleles per polymorphic locus varied between two (*Ao*, *Lt-1*, *Mdh-1* and *Mdh-2*) and ten (*Gpi*). While the mean (± SD) number of alleles per locus varied between 1.083 ± 0.289 (Nursery Ravine) and 1.667 ± 1.155 (Silvermine), the largest number of alleles found at a locus in a single population was five, at the *Gpi* locus in the Silvermine population. Both observed (direct-count) heterozygosity (H_O) and expected heterozygosity (H_E) varied greatly among populations, ranging from 0.003 ± 0.010 to 0.088 ± 0.197, and from 0.003 ± 0.010 to 0.133 ± 0.218, respectively. The percentage of polymorphic loci (95% criterion) varied between 0% (Echo Valley and Nursery Ravine populations) and 25.00% (Silvermine and Jonkershoek populations). No loci were found to be polymorphic across all sampling localities, while the *Lt-1* and *Mdh-1* loci, although polymorphic within the entire dataset, were monomorphic within individual populations.

Of 34 cases of polymorphism involving all populations and loci, six (17.65%) were found not to conform to Hardy–Weinberg expected frequencies, due to a deficit of heterozygous individuals (Table 1). When more than two alleles were present at a particular locus within a population, the pooling of common/rare-allele heterozygotes, and rare-allele homozygotes with rare-allele heterozygotes brought about conformance to Hardy–Weinberg expectations at the *Hk* locus in the Schusters River population ($\chi^2 = 0.065$, $P = 0.799$), but failed to do so at the *Pgm* locus in the Franschhoek population.

The dendrogram (Fig. 2) constructed from the matrix of genetic identities (*I*) for among-population comparisons (Table 2) revealed a marked divergence between the Gordon's Bay population and the remain-

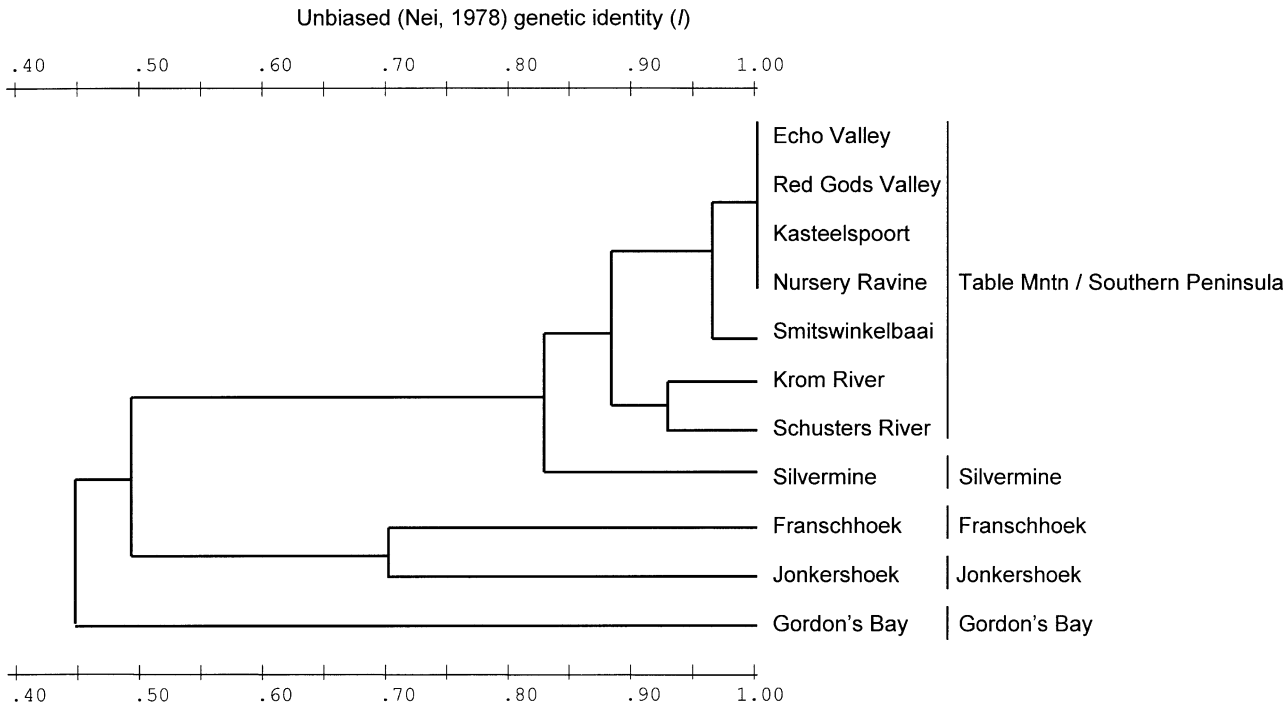

Figure 2. UPGMA dendrogram of genetic similarity between the 11 *Mesamphisopus capensis* populations studied, constructed from the matrix of Nei's (1978) unbiased genetic identities obtained in pair-wise comparison among populations. Text labels to the right of the dendrogram indicate the five groups identified on the basis of allele frequency and sequence data.

Table 1. Allele frequencies at the 11 polymorphic loci and genetic variability measures for the 11 populations of *Mesamphisopus capensis*

	Population										
Locus	1	2	3	4	5	6	7	8	9	10	11
Ao (*N*)	62	45	44	30	38	19	35	26	70	19	25
100	0.992	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.764*	0.605	1.000
95	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.236*	0.395	0.000
Ark (*N*)	57	39	40	30	30	19	35	29	58	20	30
130	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
115	0.991	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000
Gpi (*N*)	60	42	43	30	33	19	35	29	64	20	30
170	0.017	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000
145	0.000	0.000	0.000	0.000	0.682	0.000	0.000	0.000	0.000	0.000	0.000
140	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.033
125	0.983	1.000	1.000	1.000	0.212	0.395	0.043	0.707	0.000	0.000	0.000
115	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000
105	0.000	0.000	0.000	0.000	0.061	0.000	0.000	0.000	0.000	0.000	0.000
100	0.000	0.000	0.000	0.000	0.000	0.447	0.957	0.293	1.000	0.000	0.967
95	0.000	0.000	0.000	0.000	0.030	0.000	0.000	0.000	0.000	0.000	0.000
70	0.000	0.000	0.000	0.000	0.000	0.132	0.000	0.000	0.000	0.000	0.000
40	0.000	0.000	0.000	0.000	0.000	0.026	0.000	0.000	0.000	0.000	0.000
Hk (*N*)	61	45	49	30	36	17	35	28	63	20	29
125	0.000	0.000	0.000	0.000	0.000	0.000*	0.000	0.000*	0.008	0.000	0.000
100	0.000	0.000	0.000	0.000	0.000	0.000*	0.000	0.036*	0.992	0.400	0.931
95	0.000	0.011	0.010	0.000	0.708	0.412*	0.800	0.607*	0.000	0.450	0.069
85	0.992	0.989	0.990	1.000	0.292	0.588*	0.200	0.357*	0.000	0.150	0.000
75	0.008	0.000	0.000	0.000	0.000	0.000*	0.000	0.000*	0.000	0.000	0.000
Idh (*N*)	59	48	40	30	39	19	32	29	41	20	30
170	1.000	1.000	1.000	1.000	0.000	1.000	0.984	1.000	0.000	0.000	0.000
125	0.000	0.000	0.000	0.000	0.987	0.000	0.000	0.000	0.000	0.000	0.000
120	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	1.000	0.000
100	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	1.000	0.000	0.000
90	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
Ldh (*N*)	62	43	49	30	39	15	32	28	67	20	30
100	0.024*	0.000	0.000	0.017	0.000	0.000	0.422	0.964*	1.000	0.975	0.000
80	0.976*	1.000	1.000	0.983	1.000	1.000	0.578	0.036*	0.000	0.025	0.000
70	0.000*	0.000	0.000	0.000	0.000	0.000	0.000	0.000*	0.000	0.000	1.000
Lt-1 (*N*)	56	34	41	10	35	19	15	29	65	20	30
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	1.000
95	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000
Mdh-1 (*N*)	64	48	48	30	39	19	35	29	69	20	30
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000
80	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
Mdh-2 (*N*)	64	48	49	30	39	19	35	29	69	20	30
190	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000	0.000
100	1.000	1.000	1.000	1.000	0.987	1.000	1.000	1.000	1.000	1.000	1.000
Me (*N*)	60	45	46	30	36	19	35	29	67	20	30
115	0.000	0.056	0.054	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000
75	1.000	0.944	0.946	1.000	1.000	1.000	1.000	1.000	0.000	0.000	1.000

Table 1. *Continued*

	Population										
Locus	1	2	3	4	5	6	7	8	9	10	11
Pgm (*N*)	58	42	43	30	33	19	35	29	67	20	30
120	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000*	0.000	0.000
105	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.015*	0.000	0.000
100	1.000	1.000	1.000	1.000	0.894	1.000	1.000	0.983	0.963*	0.900	0.000
90	0.000	0.000	0.000	0.000	0.106	0.000	0.000	0.000	0.022*	0.000	0.000
80	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000*	0.100	1.000
A	1.417	1.167	1.167	1.083	1.667	1.333	1.333	1.417	1.333	1.417	1.167
	(0.515)	(0.389)	(0.389)	(0.289)	(1.155)	(0.888)	(0.492)	(0.669)	(0.651)	(0.669)	(0.389)
H_O	0.008	0.011	0.011	0.003	0.088	0.063	0.058	0.088	0.013	0.085	0.017
	(0.011)	(0.032)	(0.031)	(0.010)	(0.171)	(0.161)	(0.113)	(0.197)	(0.030)	(0.151)	(0.043)
H_E	0.011	0.011	0.010	0.003	0.096	0.095	0.078	0.087	0.038	0.133	0.016
	(0.016)	(0.031)	(0.030)	(0.010)	(0.177)	(0.224)	(0.161)	(0.180)	(0.105)	(0.218)	(0.041)
$P_{95\%}$	0.00	8.33	8.33	0.00	25.00	16.67	16.67	16.67	8.33	25.00	8.33

Genetic variability measures include the mean number of alleles per locus (A), mean observed (H_O) and expected (H_E) heterozygosities, and the percentage of polymorphic loci ($P_{95\%}$) using a 95% criterion. Standard deviations are presented in parentheses under the respective variability estimates. *N* = sample size. *Cases where genotype frequencies were found not to conform to Hardy–Weinberg expectations (all at $P < 0.05$). Refer to Fig. 1 for population names.

Table 2. Nei's (1978) unbiased genetic identity (above diagonal) and unbiased genetic distance (below diagonal) obtained from pair-wise comparison among the 11 *Mesamphisopus capensis* populations studied

	Population										
	1	2	3	4	5	6	7	8	9	10	11
Echo Valley (1)	–	1.000	1.000	1.000	0.823	0.963	0.854	0.883	0.491	0.418	0.422
Valley of the Red Gods (2)	0.000	–	1.000	1.000	0.823	0.962	0.851	0.879	0.489	0.417	0.418
Kasteelspoort (3)	0.000	0.000	–	1.000	0.823	0.962	0.851	0.879	0.489	0.417	0.418
Nursery Ravine (4)	0.000	0.000	0.000	–	0.822	0.962	0.852	0.882	0.489	0.416	0.421
Silvermine (5)	0.195	0.195	0.195	0.196	–	0.869	0.830	0.787	0.503	0.445	0.445
Smitswinkelbaai (6)	0.038	0.038	0.038	0.039	0.141	–	0.954	0.906	0.551	0.447	0.482
Krom River (7)	0.158	0.161	0.161	0.160	0.186	0.047	–	0.932	0.629	0.492	0.523
Schusters River (8)	0.124	0.129	0.129	0.126	0.239	0.098	0.071	–	0.622	0.534	0.471
Franschhoek (9)	0.712	0.715	0.715	0.716	0.687	0.595	0.464	0.474	–	0.703	0.570
Jonkershoek (10)	0.872	0.876	0.876	0.877	0.809	0.806	0.710	0.627	0.352	–	0.367
Gordons Bay (11)	0.863	0.873	0.873	0.866	0.809	0.729	0.648	0.753	0.562	1.004	–

ing populations. The Gordon's Bay population was separated from the others by a mean *I* of 0.454 ± 0.059, with fixed allelic differences observed at the *Idh* and *Mdh-1* loci.

The remaining Hottentots Holland Mountain populations (Franschhoek and Jonkershoek) were next separated from the Peninsula populations at a mean *I*-value of 0.491 ± 0.067. These three populations from the Hottentots Holland Mountains were separated by identity values of between 0.367 and 0.703, while fixed allelic differences at the *Gpi*, *Idh*, *Ldh*, *Lt-1* and *Me* loci identified individual populations or distinguished a pair of populations from the third.

Among the populations collected from the Cape Peninsula, the Silvermine population was shown to be genetically distinct, separated ($I = 0.825 \pm 0.024$) from the remaining Peninsula populations by a fixed allelic difference at the *Idh* locus, and by significant heterogeneity at the *Gpi*, *Hk*, *Ldh*, *Mdh-2* and *Pgm* loci (all $P < 0.01$). Allele frequency differences rather than qualitatively different sets of alleles and the presence of unique rare alleles led to the distinction of the

Smitswinkelbaai, Krom River, Schusters River and Table Mountain (Echo Valley, Valley of the Red Gods, Kasteelspoort and Nursery Ravine) populations. The Krom River and Schusters River populations, clustering together ($I = 0.932$), were separated from the remaining populations ($I = 0.879 \pm 0.032$) due to the high frequencies of the Hk^{95} and Ldh^{100} alleles in these two populations. The Hk^{85} and Ldh^{80} alleles were more abundant in the remaining populations. While the Smitswinkelbaai population clustered with the Table Mountain populations at an identity-value of 0.962 ± 0.001, the populations collected from Table Mountain itself were genetically homogenous, with *I*-values of 1.000 obtained in all among-population comparisons.

Comparison between the two regions (Cape Peninsula and Hottentots Holland Mountains) resulted in a mean identity value 0.477 ± 0.062. The two regions could be distinguished, primarily by the *Ark* locus. Populations of the Hottentots Holland Mountains were fixed for the allele Ark^{100}, with Ark^{115} and the rare allele Ark^{130}, unique to the Echo Valley population, occurring in the Peninsula populations. Contingency χ^2-analyses revealed highly significant ($P < 0.001$) heterogeneity between the two regions at all polymorphic loci with the exception of *Mdh-2*.

In the principal component analysis of allele frequencies, seven factors were extracted from the 42 variables (alleles occurring at polymorphic loci). The first three factors, along which the populations were plotted, had eigenvalues of 12.732, 8.459 and 8.019, respectively, and accounted for 69.55% of the variation observed (30.32%, 20.14% and 19.09%, respectively). The scatterplot (Fig. 3) firstly revealed the similarity of populations from Table Mountain (localities 1–4), Smitswinkelbaai (6), Krom River (7) and Schusters River (8), along these three principal components. Secondly, the distinction between the Silvermine (5) population and the remaining Peninsula populations was substantiated. Thirdly, the three Hottentots Holland Mountain populations were distinguished from the Peninsula populations by higher scores along the first principal component, while they were individually distinct.

The θ-estimates of Weir & Cockerham (1984) (Table 3) indicated substantial structuring among individual populations across the entire sample. This was evident considering all loci ($\theta = 0.871$), as well as all individual polymorphic loci, with the exception of *Mdh-2* ($\theta = 0.000$). While the overall estimate ($\theta = 0.688$) and individual estimates at certain loci (e.g. *Gpi, Hk*, *Idh* and *Ldh*) indicated substantial differentiation among populations sampled from the Cape Peninsula (Table 3), estimates from other loci indicated only slight to moderate differentiation. Populations of the Hottentots Holland Mountain region

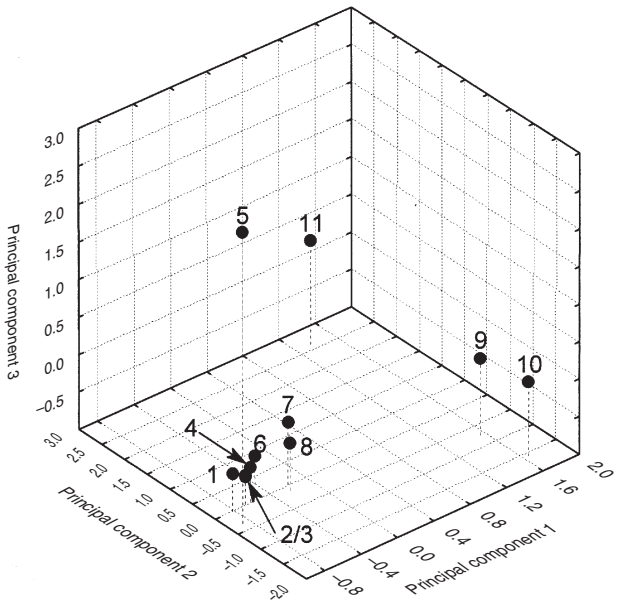

Figure 3. Populations of *Mesamphisopus capensis* plotted according to scores along the first three principal components extracted in the principal component analysis from the frequencies of 42 alleles occurring at the 11 polymorphic loci. Numbering of populations follows the numbers allocated in Fig. 1.

showed very great population differentiation overall ($\theta = 0.895$) and at all individual polymorphic loci (Table 3), with the exception of the *Ao* locus, where differentiation was moderate. Direct comparison of the two regions, by pooling sampling localities within each, yielded an overall θ of 0.673 (Table 3). Individual loci showed θ-estimates typical of greatly differentiated populations, with the exception of the *Mdh-2* locus ($\theta = -0.002$).

In combination, these data supported the recognition of five OTUs (Fig. 2) for further examination. These included the individual Silvermine, Franschhoek, Jonkershoek and Gordon's Bay populations, and a large group formed by the Table Mountain (Echo Valley, Valley of the Red Gods, Kasteelspoort and Nursery Ravine) and southern Peninsula (Smitswinkelbaai, Krom River and Schusters River) populations.

Sequence data analysis

The 328-bp region of the 12S rRNA gene could be unambiguously aligned for the ingroup and outgroup (*M. penicillatus*) specimens. Sequences, with individual lengths of 319–337 bp, have been deposited in

Table 3. Weir & Cockerham's (1984) θ estimates for comparisons among the 11 *Mesamphisopus capensis* populations studied, populations of the Cape Peninsula, populations of the Hottentots Holland Mountains and the two regions with populations pooled within each

Hierarchical level	Weir & Cockerham's (1984) θ										
	Overall	*Ao*	*Ark*	*Gpi*	*Hk*	*Idh*	*Ldh*	*Lt-1*	*Mdh-1*	*Mdh-2*	*Me*	*Pgm*
All populations	0.871 (0.786–0.947)	0.239	0.994	0.822	0.742	0.991	0.904	1.000	1.000	0.000	0.941	0.793
Cape Peninsula	0.688 (0.532–0.833)	–0.006	–0.007	0.679	0.596	0.975	0.731	–	–	0.000	0.032	0.080
Hottentots Holland	0.895 (0.724–0.991)	0.139	–	0.966	0.464	1.000	0.987	1.000	1.000	–	1.000	0.893
Two regions (pooled)	0.673 (0.544–0.798)	0.313	0.997	0.545	0.645	0.630	0.667	0.240	0.376	–0.002	0.805	0.347

Estimates are given over all loci and at individual polymorphic loci. 95% CIs (determined by 1000 bootstrap replicates) are presented in parentheses for θ estimates calculated over all loci.

Table 4. Sequence divergence (uncorrected p) among representative individuals of 11 putative *Mesamphisopus capensis* populations and one outgroup (*M. penicillatus*) individual

	Representative haplotype										
	Outgroup	1	2	3	4	5	6	7	8	9	10	11
M. penicillatus (outgroup)	–											
Echo Valley (1)	0.177	–										
Valley of the Red Gods (2)	0.177	0.006	–									
Kasteelspoort (3)	0.177	0.006	0.000	–								
Nursery Ravine (4)	0.177	0.006	0.006	0.006	–							
Silvermine (5)	0.170	0.034	0.034	0.034	0.034	–						
Smitswinkelbaai (6)	0.171	0.006	0.006	0.006	0.006	0.028	–					
Krom River (7)	0.180	0.016	0.016	0.016	0.016	0.038	0.009	–				
Schuster River (8)	0.173	0.009	0.016	0.016	0.016	0.031	0.009	0.019	–			
Franschhoek (9)	0.161	0.097	0.097	0.097	0.104	0.103	0.097	0.107	0.088	–		
Jonkershoek (10)	0.155	0.094	0.088	0.088	0.094	0.094	0.088	0.097	0.085	0.009	–	
Gordon's Bay (11)	0.136	0.107	0.107	0.107	0.107	0.094	0.101	0.110	0.098	0.050	0.043	–

GenBank (accession numbers AY322172–AY322183 inclusive). The base frequencies (A = 0.406, C = 0.129, G = 0.112, T = 0.353) were characteristic of the 12S gene region in other isopods, and typically adenine- and thymine-rich (Wetzer, 2001).

The mean sequence divergence (uncorrected p distances; Table 4) between the outgroup and ingroup sequences was 16.85 ± 1.31%. Sequence divergence among the ingroup haplotypes ranged from 0.0% to 11.01%, with a mean sequence divergence of 9.79 ± 0.74% separating representative individuals from the Cape Peninsula and Hottentots Holland Mountains. Grouped according to the units identified by the allozyme analyses, a mean sequence divergence of 3.36 ± 0.30% distinguished the Silvermine individual from the remaining Cape Peninsula individuals, while sequence divergences of 0.93–4.99% were found among the Hottentots Holland Mountain individuals.

Thirty-one of 44 variable characters were parsimony-informative within the ingroup and yielded a single tree of 52 steps (CI = 0.808, RI = 0.878, rescaled CI = 0.709) in the MP analysis. Modeltest revealed that the use of the Tamura & Nei (1993) model of nucleotide substitution together with a gamma-distribution, among-site rate variation model (TrN + Γ) resulted in a significantly improved likelihood score for ML analyses compared with other less parameter-rich models. Estimated base frequencies (A = 0.417, C = 0.127, G = 0.108, T = 0.348) were inputted, together with the following rate matrix: $R_1 = R_3 = R_4 = R_6 = 1.000$,

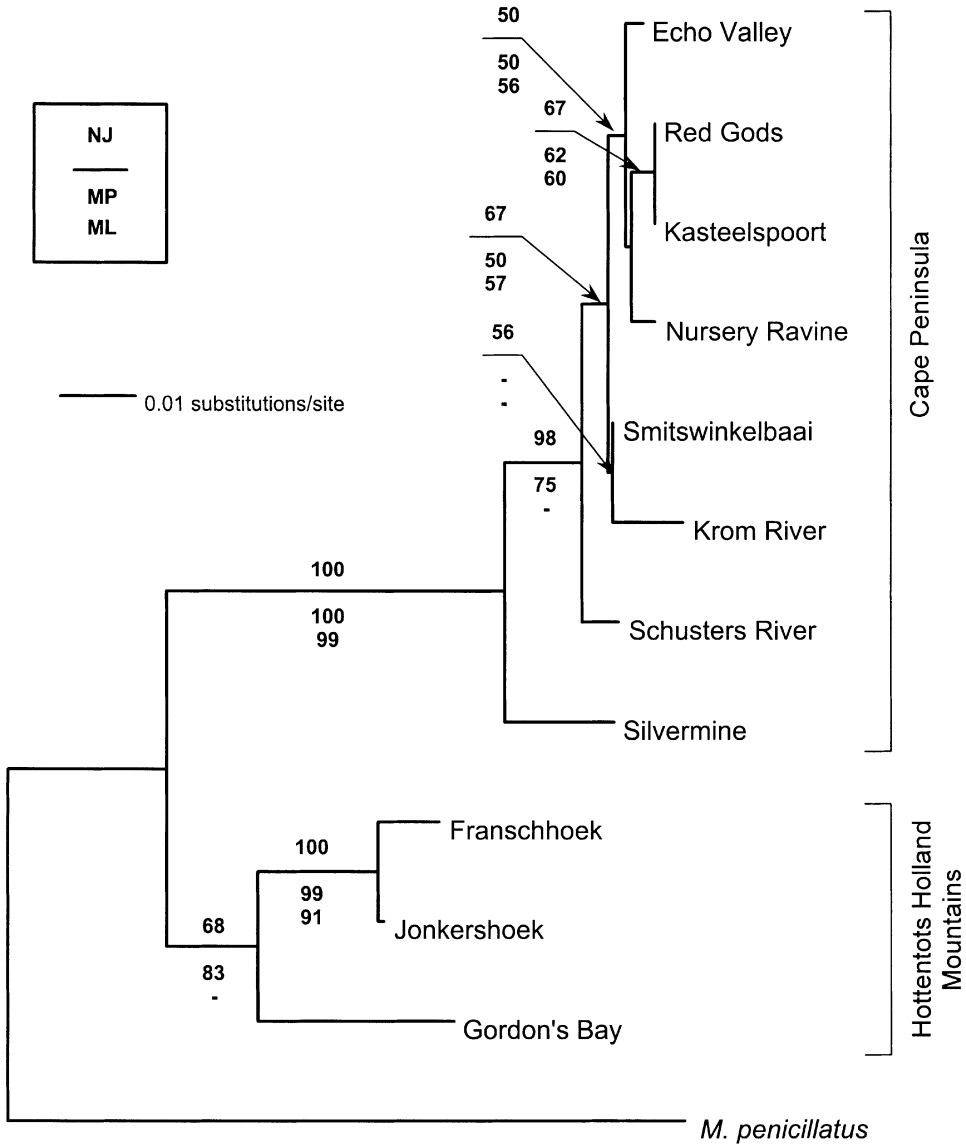

Figure 4. Neighbour-joining (NJ) phylogram, based on uncorrected p sequence divergence, from an analysis of 328 bp of the 12S rRNA gene region in individuals from 11 putative *Mesamphisopus capensis* populations and one outgroup (*M. penicillatus*) individual. Numbers above the branches indicate bootstrap support (10 000 replicates). Numbers below the branches represent bootstrap support from the maximum parsimony (MP, 1000 replicates) and maximum likelihood (ML, 100 replicates) analyses. Bootstraps <50% are not indicated, or are indicated by hyphens if nodes were supported in other analyses.

$R_2 = 3.586$, and $R_5 = 12.600$. The proportion of invariant sites was set to zero and the α-shape parameter was estimated at 0.271.

Identical tree topologies were obtained in the MP analysis and by NJ using uncorrected p sequence divergences. These retrieved two monophyletic clades (Fig. 4), comprising individuals sampled from the Cape Peninsula and Hottentots Holland Mountains, respectively. While the Hottentots Holland clade received fair bootstrap support (>68%), the clade comprising the Cape Peninsula haplotypes was supported by 100% bootstrap in both analyses. Within the Cape Peninsula clade, the Silvermine representative was placed as a sister taxon to the well-supported (>75%) clade formed by the Table Mountain and remaining Peninsula representatives. Further relationships within the Cape Peninsula clade reflected those obtained in the allozyme analysis. The ML and NJ analyses using the distance parameters estimated from the ML analysis retrieved topologies (trees not

shown) identical to the allozyme dendrogram, with the Gordon's Bay population occurring basally as a sister taxon to the clade (bootstrap support of >69%) of remaining representatives. Within this clade, the relationship of the remaining two Hottentots Holland Mountain representatives (Franschhoek and Jonkershoek) was well supported (>87%). Again, the Peninsula representatives formed a well-supported (>99%) monophyletic clade, with the individual relationships reflecting those revealed by the MP and allozyme analyses. A topology constrained to reflect the monophyly of representatives from the Hottentots Holland Mountains had a higher log-likelihood score (–lnL = 872.325) than did the unconstrained tree (–lnL = 871.429), but it was not significantly less likely (Shimodaira & Hasegawa (1999) test: $\ln L_1 - \ln L_0 = 0.896$, $P = 0.257$). The monophyly of the Hottentots Holland Mountain individuals, supported in the MP analysis, could not be rejected.

No significant difference was observed between the log-likelihood scores of the unconstrained ML tree and those obtained with a molecular clock enforced (likelihood ratio test: $2(\ln L_1 - \ln L_0) = 1.791$; d.f. = 10; $P > 0.995$). A molecular clock could thus be tentatively applied.

Morphometric analysis

The 47 variables included in the morphometric analysis are indicated in Table 5. In the discriminant function analysis involving the body variables only (Table 5, variables 1–22), significant discrimination was obtained among the five defined groups (Wilks' lambda = 0.012, $F_{(88, 105)} = 2.431$, $P < 0.001$). Similarly, groups were significantly discriminated (Wilks' lambda = 0.004, $F_{(100, 93)} = 2.913$, $P < 0.001$) using the 25 pereopod variables (Table 5, variables 23–47).

Groups appeared to be well differentiated in both analyses, as evident from the reclassification matrices (Table 6). In the analysis based on body variables, 96.88% correct reclassification was obtained for the Table Mountain–Southern Peninsula group, with one of the 32 individuals being incorrectly reassigned to the Silvermine group. The Silvermine, Franschhoek, Jonkershoek and Gordon's Bay groups all had 100% correct reassignment. In the analysis based on pereopod variables, all individuals were correctly reassigned to their respective groups.

Plots of individuals along the first two canonical variables in both analyses (Fig. 5) revealed the Gordon's Bay group to be markedly distinct from the remaining groups. In the analysis of body variables, the Silvermine group overlapped the Table Mountain–Southern Peninsula, Franschhoek and Jonkershoek groups slightly. The first two canonical variables accounted for 85.18% of the variation among groups and had eigenvalues of 6.542 and 2.400, respectively. In the analysis of pereopod variables, the two canonical variables, with eigenvalues of 10.737 and 4.572, accounted for 87.28% of the between-group variation. Here, the Jonkershoek group overlapped the Table Mountain–Southern Peninsula and Franschhoek groups slightly, while the Silvermine and Table Mountain–Southern Peninsula groups also showed limited overlap.

The factor structure (loading) matrices, representing the correlations between the variables and the functions, are summarized for the first two discriminant functions (canonical variables) in both analyses in Table 5. In the analysis of body variables, the first canonical variable had highest correlation with HD (4), P5L (12), PL4D (19) and P3L (9) (for definitions see Table 5). For the second canonical variable HD, P5W (11), PL4D and PL4W (17) had the highest loadings. While it appeared as though dimensions of the fifth pereonite and fourth pleonite specifically contributed to the discrimination of the groups, the width and depth variables were generally less important in discriminating groups along the first and second canonical variables, respectively. The first canonical variable in the analysis of pereopod variables was correlated most highly (albeit negatively) with Pe1L (23), Pe1PL (26), Pe3L (28) and Pe3PL (31). The width of individual pereopod articles was less important in distinguishing groups than were limb and article length, and thus generally carried the lowest loadings along this function. Along the second discriminant function the opposite was apparent, with width variables carrying the highest loadings. The highest correlations were observed with Pe1PW (27), Pe7BW (45) and Pe7PW (47), while Pe5PL (41) showed a high negative correlation.

DISCUSSION

Generally congruent patterns of population differentiation were observed in the two independent molecular markers examined. Additionally, five distinct groups (Table Mountain–Southern Peninsula, Silvermine, Franschhoek, Jonkershoek and Gordon's Bay), distinguished on the basis of fixed allele differences or significant allele frequency heterogeneity, were morphometrically distinct. Importantly, a large genetic divergence was seen between the Cape Peninsula and Hottentots Holland populations in the allozyme data, while the 12S sequence data supported the monophyly of each of the two regions.

Genetic evidence of specific status

Genetically divergent populations occurring allopatrically are problematic when morphological or other cri-

Table 5. The 47 body and pereopod variables used to examine morphometric differentiation among 11 putative populations of *Mesamphisopus capensis* and summary of the factor structure (loading) matrices

		Structure matrix	
Abbreviation	Measurement	CV1	CV2
(1) BL	Body length	0.224	0.202
(2) HW	Head (cephalon) width	0.184	0.092
(3) HL	Head (cephalon) length	0.289	0.209
(4) HD	Head (cephalon) depth	0.322	0.256
(5) P1W	Pereonite 1 width	0.187	0.106
(6) P1L	Pereonite 1 length	0.243	0.156
(7) P1D	Pereonite 1 depth	0.225	–0.014
(8) P3W	Pereonite 3 width	0.180	0.221
(9) P3L	Pereonite 3 length	0.299	0.216
(10) P3D	Pereonite 3 depth	0.261	0.009
(11) P5W	Pereonite 5 width	0.190	0.249
(12) P5L	Pereonite 5 length	0.310	0.142
(13) P5D	Pereonite 5 depth	0.222	–0.029
(14) P7W	Pereonite 7 width	0.197	0.233
(15) P7L	Pereonite 7 length	0.205	0.075
(16) P7D	Pereonite 7 depth	0.223	0.073
(17) PL4W	Pleonite 4 width	0.195	0.243
(18) PL4L	Pleonite 4 length	0.262	0.066
(19) PL4D	Pleonite 4 depth	0.301	0.246
(20) TW	Pleotelson width	0.263	0.188
(21) TL	Pleotelson length	0.016	0.226
(22) TD	Pleotelson depth	0.207	0.027
(23) Pe1L	Pereopod 1 (gnathopod) length	–0.314	0.136
(24) Pe1BL	Pereopod 1 (gnathopod) basis length	–0.267	0.014
(25) Pe1BW	Pereopod 1 (gnathopod) basis width	–0.165	0.050
(26) Pe1PL	Pereopod 1 (gnathopod) propodus length	–0.327	0.148
(27) Pe1PW	Pereopod 1 (gnathopod) propodus width	–0.291	0.252
(28) Pe3L	Pereopod 3 length	–0.317	0.003
(29) Pe3BL	Pereopod 3 basis length	–0.307	0.020
(30) Pe3BW	Pereopod 3 basis width	–0.175	0.138
(31) Pe3PL	Pereopod 3 propodus length	–0.312	–0.037
(32) Pe3PW	Pereopod 3 propodus width	–0.160	0.135
(33) Pe4L	Pereopod 4 length	–0.184	–0.032
(34) Pe4BL	Pereopod 4 basis length	–0.203	–0.079
(35) Pe4BW	Pereopod 4 basis width	–0.140	0.051
(36) Pe4PL	Pereopod 4 propodus length	–0.247	0.036
(37) Pe4PW	Pereopod 4 propodus width	–0.213	0.064
(38) Pe5L	Pereopod 5 length	–0.232	–0.094
(39) Pe5BL	Pereopod 5 basis length	–0.273	0.040
(40) Pe5BW	Pereopod 5 basis width	–0.132	0.150
(41) Pe5PL	Pereopod 5 propodus length	–0.164	–0.232
(42) Pe5PW	Pereopod 5 propodus width	–0.061	–0.005
(43) Pe7L	Pereopod 7 length	–0.296	–0.033
(44) Pe7BL	Pereopod 7 basis length	–0.268	0.030
(45) Pe7BW	Pereopod 7 basis width	–0.138	0.216
(46) Pe7PL	Pereopod 7 propodus length	–0.237	–0.124
(47) Pe7PW	Pereopod 7 propodus width	–0.089	0.207

Correlations for the first two canonical variables, CV1 and CV2, from two independent discriminant function analyses are given, i.e. using body variables (variables 1–22), and pereopod variables (23–47), respectively.

Table 6. A posteriori reclassification of individuals to groups, based on classification functions determined in the discriminant function analyses of body variables and pereopod variables

	A posteriori reclassification					
	Correctly reclassified (%)	Table Mntn–Southern Peninsula	Silvermine	Franschhoek	Jonkershoek	Gordon's Bay
Body variables						
Table Mntn–Southern Peninsula	96.88	31	1	–	–	–
Silvermine	100.0	–	5	–	–	–
Franschhoek	100.0	–	–	5	–	–
Jonkershoek	100.0	–	–	–	5	–
Gordon's Bay	100.0	–	–	–	–	5
Pereopod variables						
Table Mntn–Southern Peninsula	100.0	32	–	–	–	–
Silvermine	100.0	–	5	–	–	–
Franschhoek	100.0	–	–	5	–	–
Jonkershoek	100.0	–	–	–	5	–
Gordon's Bay	100.0	–	–	–	–	5

teria, which may be instructive of the taxonomic status of the populations, are absent (Thorpe, 1983) and species concepts based on reproductive compatibility cannot be tested (Butlin & Tregenza, 1998). Several authors have cautioned against the use of genetic distance measures in making taxonomic inferences, principally because such estimates are not equivalent at equivalent taxonomic hierarchies within different classes (Avise & Aquadro, 1982; Sites & Crandall, 1997; Butlin & Tregenza, 1998; Johns & Avise, 1998; Avise & Johns, 1999). These estimates do, however, provide a guideline, but corroborative evidence of taxonomic status should be sought in other datasets (Bradley & Baker, 2001).

While no allozyme studies on phreatoicidean isopods have yet been published, identity values obtained in comparisons of valid congenerics or putatively new species of other freshwater, terrestrial, marine and troglobitic isopods range from 0.159 to 0.816 (Garthwaite, Lawson & Taiti, 1992; Lessios & Weinberg, 1994; Cobolli Sbordoni *et al*., 1997; Ketmaier *et al*., 1998, 2000). Intraspecific identity values obtained in these studies varied between 0.656 and 1.000. Similarly, surveys of electrophoretic studies involving a range of invertebrate taxa led Thorpe (1982, 1983), Skibinski, Woodwark & Ward (1993) and Thorpe & Solé-Cava (1994) to conclude that identity values for comparisons among congeneric species typically fall between 0.25 and 0.85, while intraspecific values are generally greater than 0.91. Furthermore, they considered it unlikely for allopatric populations with identity values less than 0.85 to be conspecific.

Using these genetic distances as broad criteria, five putative species may be recognized from the allozyme data presented here: the Franschhoek, Jonkershoek, Gordon's Bay and Silvermine populations may be recognized as separate species, while the Table Mountain and southern Peninsula populations may be considered conspecific to each other. Mean identity values obtained in comparisons among these putative species ranged from 0.367 to 0.825, while (intraspecific) comparisons of the Table Mountain–Southern Peninsula populations resulted in *I*-values between 0.851 and 1.000.

From the sequence data, a mean sequence divergence of 7.90% was observed among these putative species. Individual comparisons among these different species ranged from 0.93% to 11.01%, while intraspecific sequence divergence (among Table Mountain and Southern Peninsula representatives) ranged between 0.0% and 1.88%. With the exception of the comparison between the Franschhoek and Jonkershoek sequences (0.93%), mean interspecific sequence divergence estimates among any two groups (between 3.36% and 10.52%) were greater than those reported for the 12S region in phreatoicidean isopods (Wetzer, 2001), where congeneric phreatoicidean species showed approximately 2% sequence divergence. These values are, however, lower than those reported for interspecific comparisons within other isopod suborders, for example the Valvifera and Flabellifera (Wetzer, 2001).

Based on this data, only two putative *Mesamphisopus* species may be recognized from the Cape Peninsula. The diversity of the phreatoicideans on the Cape Peninsula appears to be considerably less than the region's 11 paramelitid amphipod species (Stewart &

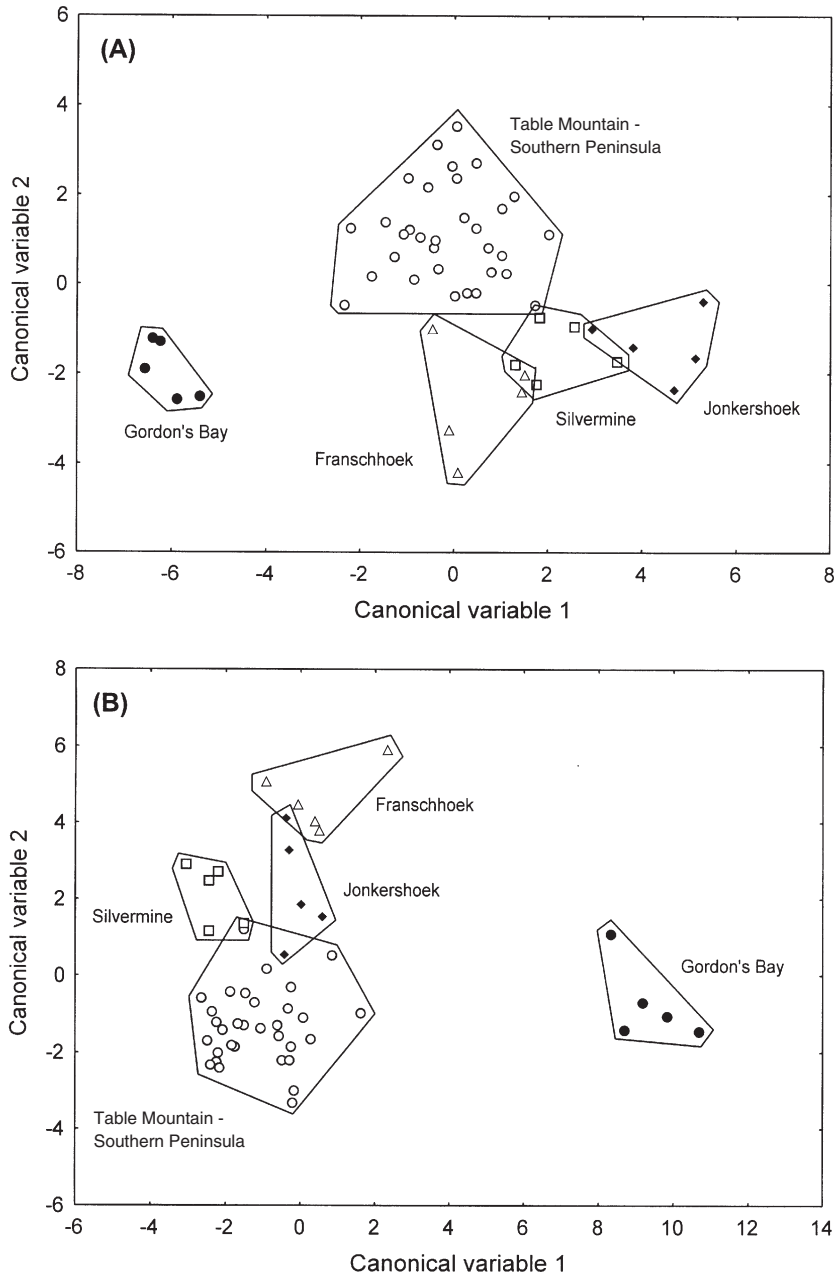

Figure 5. Individuals belonging to the five identified groups of *Mesamphisopus capensis* plotted along scores for the first two canonical variables derived from the discriminant function analyses of 22 body variables (A) and 25 pereopod variables (B).

Griffiths, 1995), some of which were brought to light using a similar combination of techniques (e.g. Stewart, 1992). The presence of another species on Table Mountain is also not supported. Indeed, populations collected from Table Mountain were genetically identical in terms of allozyme data, with no evidence (significant deviations from Hardy–Weinberg expectations at polymorphic loci) suggesting separate but sympatric gene-pools at any locality. The three 12S rRNA haplotypes from Table Mountain were also similar and could be considered to be from conspecific individuals.

EVOLUTIONARILY SIGNIFICANT UNITS OR SPECIES?

The formulation of the ESU and MU concepts and their application aim to identify populations (or population groups) with independent and unique evolu-

tionary trajectories for conservation purposes (Moritz, 1994). Although these concepts aimed to negate the reliance on formal taxonomic designations, great conceptual overlap exists between various species concepts and ESU definitions and these may represent equivalent entities as far as the criteria used to identify each is concerned (Roe & Lydeard, 1998).

The five groups initially identified above may qualify as ESUs under Ryder's (1986) initial broad definition. Under that definition, populations (subspecies) that showed significant adaptive variation, based on concordant datasets, would be recognized as discrete units. While reproductive isolation, a criterion under Waples's (1991) expanded ESU definition, cannot be demonstrated empirically among allopatric populations, a lack of gene flow is apparent and reproductive isolation between groups may be inferred on the basis of fixed allele differences revealed by the allozyme data. However, as highlighted by Roe & Lydeard (1998), reproductive isolation may also be invoked to argue for specific status under the biological species concept (Mayr, 1963).

Moritz (1994) defined ESUs as being reciprocally monophyletic for mtDNA alleles and showing significant divergence in allele frequency at nuclear loci. Significant differences in allele frequency have been identified at numerous loci between the five groups identified as putative species. However, the inclusion of only one individual per population in the DNA sequence analyses precludes the identification of ESUs at the population (locality) level. Thus, only the two regions could be regarded as ESUs under Moritz's (1994) strictest definition, with the monophyly of each demonstrated by parsimony analysis, and not rejected with ML. Again, as highlighted by Roe & Lydeard (1998), diagnostic (nucleotide) characters bringing about monophyly of the two groups may be used to diagnose two species under a phylogenetic species concept. Significant differences in allele frequency at allozyme loci between the five identified groups do, however, satisfy Moritz's (1994) criteria for each to be recognized as an MU, these being functionally independent populations with significantly different allele frequencies at nuclear or mitochondrial loci.

Despite the identification of ESUs and MUs in a number of South African taxa (e.g. Matthee & Robinson, 1999; Bloomer & Impson, 2000; Daniels *et al*., 2003; Stewart *et al*., 2004), these concepts have so far found only limited application in South African conservation. These cases have typically involved only enigmatic taxa of economic importance (e.g. Matthee & Robinson, 1999). This is of concern, as the best biological information is of little consequence if the legal framework does not exist to use this information in the implementation of sound conservation policy (Rohlf, 1991). Of greater concern is that only two of the presently used provincial ordinances within South African conservation include schedule provisions for invertebrate species (Bürgener, Snyman & Hauck, 2001).

Concordant patterns and historical narrative

Moritz (1994) alluded to a possible extension of the ESU concept whereby whole communities are examined and a comparative phylogeographical approach taken to define ESUs in terms of geographical areas, in which allopatric populations of different taxa are distinct. In this regard, two genetic studies on freshwater invertebrates of the Western Cape provide useful comparison with the data presented above. Daniels, Stewart & Burmeister (2001) found marked divergence between freshwater crab populations initially regarded as *Potamonautes brincki* (Bott 1960) collected from the Cape Peninsula and the Hottentots Holland Mountains. Wishart & Hughes (2001) found an identical pattern of divergence between populations of the lotic, net-winged midge, *Elporia barnardi* (Edwards). This divergence was, to a large extent, also seen among populations of freshwater amphipods formerly believed to be *Paramelita capensis* (Barnard 1916) conspecifics (Stewart, 1992).

The marked divergence among the freshwater fauna of the two regions can be attributed to the Cape Flats. This coastal plain remnant stretches from False Bay to the west coast with elevations of less than 50 m, separating the Hottentots Holland Mountains of the Cape Fold Belt from their outliers on the Cape Peninsula (Harrison & Barnard, 1972; Lambrechts, 1979; Cowling *et al*., 1996). Although the Cape Flats are exposed, gene flow between *Mesamphisopus* populations across them is unlikely, as present conditions have prohibited the establishment of viable populations (Harrison & Barnard, 1972). Indeed, Harrison & Barnard (1972) believed this current 'land bridge' to be as insurmountable as are the marine transgressions. Although the sandy Cape Flats were periodically covered by forest during mesic periods in the late Pleistocene (Hendey, 1983a), they are presently dry, receiving less precipitation annually than do the surrounding mountainous areas from the mist belt alone (Fuggle & Ashton, 1979). Flowing water on the Cape Flats is also strongly alkaline or brackish, while the water of the mountain streams, in which the phreatoicideans are abundant, is highly acidic (Harrison & Barnard, 1972).

Although geologically stable throughout the Cenozoic (the last 65 Myr), the Western Cape has experienced substantial and rapid climatic change (Hendey, 1983a,b; Cowling *et al*., 1996). While tectonically induced sea-level changes occurred throughout the Cenozoic to the middle Miocene, glacial and intergla-

cial cycles became established during the Pliocene, during which time marine transgressions and regressions exposed and inundated the coastal platform and low-lying areas (Deacon, 1983; Hendey, 1983b) including the Cape Flats and 'gaps' interrupting the mountain range of the Peninsula (Cowling *et al*., 1996). Repeated marine transgressions have also been invoked to account for the general lack of invertebrates endemic to the southern Peninsula (Picker & Samways, 1996). While the magnitude of these transgressions and regressions is unknown, sea levels are thought to have dropped (through glacioeustatic change) by 200 m towards the end of the Miocene, and may have risen substantially in the Tertiary (200 m), middle Miocene (150 m) and early Pliocene (100 m) (Hendey, 1983b; Linder, Meadows & Cowling, 1992). Sea levels have not risen more than 6 m during the more recent Pleistocene and Quarternary interglacials (Hendey, 1983b).

While the most important impact of these cycles is the inundation or exposure of coastal platforms, the changes between warm, mesic, interglacial conditions and cold, xeric, glacial conditions bring about concomitant changes in weathering, erosion and deposition regimes and can significantly alter river courseways, flow regimes and drainage patterns (Hendey, 1983a,b). These Pleistocene climatic oscillations (and induced environmental changes) have been cited as a major driving force in the speciation and differentiation of the flora of the region (Richardson *et al*., 2001).

Applying a protein clock calibrated for isopods (Ketmaier *et al*., 1999) to the mean allozyme divergence between populations of the two regions ($D = 0.748 \pm 0.123$) indicates a divergence time of approximately 14 Myr. This estimate would attribute the separation to a significant sea-level rise occurring in the middle Miocene (see Hendey, 1983b: fig. 2). Although no molecular clocks have been specifically calibrated for the 12S gene region in isopods, several mtDNA clocks calibrated for Crustacea (Cunningham, Blackstone & Buss, 1992; Knowlton *et al*., 1993), including isopods (Ketmaier, Argano & Caccone, 2003), and other arthropods (Brower, 1994) have suggested a rate of sequence divergence of between 2.2 and 2.6% per Myr. Applying this to the mean maximum-likelihood corrected sequence divergence ($17.67 \pm 2.03\%$) obtained from comparison among individuals of the two regions suggests that the lineages of the two regions diverged between approximately 6.8 and 8 Mya. This lends credence to the faunistic separation of the regions through marine transgressions and regressions, discussed above, and is entirely consistent with the view of Harrison & Barnard (1972), who believed that *M. capensis* has existed as separate gene-pools in each of the regions since the late Tertiary. These differences in estimates of divergence times may well be due to differing evolutionary rates of the markers examined, specifically the allozyme loci included. The later divergence times estimated for other taxa (e.g. Daniels *et al*., 2001) could also reflect differences in dispersal capacity.

While the origin and nature of the Cape Flats may explain the differentiation between populations between the two regions, patterns of differentiation within each region may well be attributed to drainage evolution and local extinctions and recolonization. This possibility, however, remains to be tested with data from a wide variety of aquatic invertebrates from both regions.

CONCLUSIONS

While fixed allele differences and large sequence divergence values can be considered character differences, an essentially tree-based approach to species delimitation (see Wiens, 1999) has led to the identification of five groups within *M. capensis*, with four of these possibly representing undescribed species. Genetic distance and similarity data formed the basis of this delimitation, although morphometric analyses had also shown these putative taxa to be distinguishable. Wiens (1999) stated that the congruence (or incongruence) of multiple datasets is instructive of the extent of species boundaries. Thus, further work should focus on intensive morphological examination of individuals of the putative species identified above, as cryptic species are often revealed to be diagnosable by consistent differences in morphology, once initially identified using genetic or morphometric data (Duffy, 1996).

From a conservation point of view, prudence dictates the consideration of the five identified population groups as MUs. Due to the limitations of the mtDNA study, only two ESUs (the Cape Peninsula and Hottentots Holland Mountain groups) could be defined using Moritz's (1994) criteria. As all populations sampled fall within existing conservation areas, it is hoped that this study, in conjunction with further studies on endemic freshwater fauna, may contribute towards a management strategy for the conservation of aquatic invertebrates within the Western Cape.

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