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Systematics of the Australasian Lymnaeidae

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CERTIFICATION

I, Louise Puslednik, declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Biological Sciences, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution.

Abstract

The Lymnaeidae Rafinesque, 1815 are one of the most widespread groups of freshwater snails, however, they are characterised by a long and confused systematic history largely due to problems associated with shell plasticity. Recent molecular studies that have utilised DNA sequences have failed to adequately represent the Australasian lymnaeids. The aim of this study was to understand the systematics of the Australasian Lymnaeidae, using 16S and ITS-2 sequences in tandem with a anatomical and shell studies.

The native Australian and New Zealand lymnaeids are currently attributed to *Austropeplea* Cotton, 1942 and *Kutikina* Ponder and Waterhouse 1997, which are thought to be represented by three and one species, respectively. Results of this study indicate there are 5 distinct species across three genera. Phylogenetic analyses of the *A. tomentosa* (Pfeiffer, 1855) complex recovered two distinct species, *A. tomentosa* in New Zealand and *A. huonensis* (Tenison-Woods, 1876) in southern Australia. There was however incongruence between the anatomical and molecular phylogenies. *Kutikina hispida* was suggested to be closely related to the *A. tomentosa complex*, however, molecular phylogenies genes resolved *K. hispida* as sister to *A. huonensis*, with *A. tomentosa* being resolved as sister to the *A. huonensis* + *Kutikina* clade. *Kutikina* was therefore synonymised into *Austropeplea* based on the molecular phylogenies. Based on molecular and anatomical phylogenies, the more northern complex, *A. lessoni* (Deshayes, 1830) was more appropriately placed in the *Peplimnea* (Iredale, 1943), and was found to be represented by two distinct taxa, *P. lessoni* and *P. affinis* (Küster, 1862). Phylogenetic analysis of 16S, ITS-2 and anatomical characters recovered *A. viridis* (Quoy and Gaimard, 1832) as relatively divergent from other members of *Austropeplea*. Therefore, *A. viridis* was placed into *Viridigalba* Kruglov and Starobogatov, 1985.

Using 16S sequences and anatomical characters, a phylogeny of the Lymnaeidae was produced. The Australasian lymnaeids represented one of the most derived groups within the family in both the 16S and anatomical phylogenies. The North American and European lymnaeids were resolved at the base of the lymnaeid phylogeny, suggesting that these taxa represent the older groups within the family.

Phylogenies based on molecular sequences suggest that the *Austropeplea lessoni* complex is more closely related to lymnaeids from South East Asia than to other Australian lymnaeids. Furthermore, based on molecular and anatomical phylogenies, *A. viridis* is suggested as sister to the *A. tomentosa* complex. Therefore it is highly likely the *A. lessoni* complex and *A. tomentosa* complex have separate derivations. The monophyly of *Radix* Montfort, 1810 remains however unresolved.

Two theories of biogeography of the Australasian Lymnaeidae have been recently proposed and were examined in light of the new phylogeny. While it seems certain that the *Austropeplea lessoni* complex had a South East Asian origin, the origin of the *A. tomentosa* complex is still unclear. The close relationship of the *A. tomentosa* complex with Asian *A. viridis* plus the derived position of the group in the family, suggest a second invasion of Australia by lymnaeids from South East Asia. However, the basal position of the New Zealand *A. tomentosa* would suggest the group occurred here first and moved into Australia, thus suggesting a Gondwanan radiation of the *A. tomentosa* complex. The discovery of a lymnaeid fossil in Antarctica lends further weight to this theory.

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Chapter 1   Introduction

The Lymnaeidae Rafinesque, 1815 classically belong to the Hygrophila in the pulmonate order Basommatophora . Recent molecular evidence (Dayrat *et al.* 2001) supports the monophyly of the Hygrophila, which is divided into seven families; the Chilinidae, Latiidae, Acroloxidae, the Lymnaeidae, Planorbidae, Ancylidae and Physidae (Jørgensen *et al.* 2004). The Lymnaeidae, Planorbidae, Physidae and Ancylidae are thought to have a common ancestor and are considered the most advanced of the Hygrophila (Hubendick, 1978), with recent molecular studies suggesting these four families are monophyletic (Remigio and Hebert 2003). The relationship between the Lymnaeidae, Planorbidae, Physidae and Ancylidae is currently unclear. The Lymnaeidae were previously thought to be the most primitive of the four families, with the Ancyloplanorbidae (Planorbidae + Ancylidae) representing the most advanced (Hubendick, 1978). Additional phylogenetic analyses resolved the Ancyloplanorbidae and Lymnaeidae as the most basal groups, and the Physidae as the most derived group (Swiderski 1990), while recent molecular data suggests that the Planorbidae may represent the oldest lineage in the group, with Physidae being sister to Lymnaeidae (Remigio and Hebert 2003).

The Lymnaeidae are characterised by flat triangular tentacles. The shell is generally dextral and rounded, with a wide last whorl and a large aperture. Shell sculpture or colour is usually lacking, and the shell is thin and fragile. The Lymnaeidae, like other basommatophorans, are simultaneous hermaphrodites with internal fertilisation and separate gonophores. Lymnaeids are capable of sperm storage, both of their own and that received in copulation. Individual eggs are formed in complex egg masses (Geraerts and Joosse 1984).

The Lymnaeidae inhabit a wide variety of freshwater habitats, and as such display a tremendous morphological diversity. This high level of diversity makes phylogenetic studies of the Lymnaeidae difficult. Despite this, interest in the phylogenetic problem of the lymnaeids is important for a number of reasons. Firstly, many lymnaeid species are intermediate hosts for trematode parasites and secondly,

lymnaeids are part of a growing number of freshwater taxa that are facing the threat of extinction.

1.1 Importance of the Lymnaeidae

1.1.1 Parasite host interactions

The Lymnaeidae are an important group of freshwater snails due to their crucial role in the life cycle of digenean trematodes. Lymnaeids act as intermediate hosts to 13 families of digenean trematodes, with some lymnaeid species acting as hosts for multiple digenean trematode species (Table 1.1). Research has largely been focused on economically important trematodes, including the liver flukes (*Fasciola*), the avian blood flukes (*Schistosoma*) and the intestinal flukes (Echinostamatidea). Of these three, *Fasciola* has the greatest impact, medically and economically in Australia (Ponder *et al.* 2006)

Fasciola is specific to lymnaeids, with at least 12 named species acting as natural intermediate hosts for one or both of the two species of *Fasciola* (Table 1.1). Both *Fasciola* species have large distributions; *F. hepatica* has a cosmopolitan distribution, while *F. gigantica* is limited to more tropical regions (Brown 1978; Torgerson and Claxton 1999). World-wide, fascioliasis is one of the most economically important diseases within the agricultural sector, with over 600 million animals infected world-wide and a significant economic loss of over US$200 million per annum (Spithill *et al.* 1999b; Torgerson and Claxton 1999). In some countries, up to 80-100% of ruminants are estimated to be infected with *F. gigantica*, a significant constraint on productivity (Spithill *et al.* 1999a). While fascioliasis has traditionally been a vetinary problem, there have been increasing numbers of outbreaks in humans. This has lead to fascioliasis being listed as a significant medical disease. It has been estimated that between 2.4 and 17 million people are infected throughout the word (Hopkins 1992; Rim *et al.* 1994). The largest problem areas are the Caribbean Islands and South America, with the highest levels of infection rates being reported from the Bolivian Antiplano region, with up to 66.7% of individuals from the region being infected (Mas-Coma *et al.* 1995; Esteban *et al.* 1997; Mas-Coma *et al.* 1999).

Fascioliasis in humans is largely due to *F. hepatica*, although some areas in Africa and Asia have reported cases of fasciolosis resulting from *F. gigantica* (Hafeez 2003).

The other two groups of trematodes important in relation to the Lymnaeidae are the schistosomes and Echinostomata. Schistosomatids parasitise the blood vessels of mammals (other than humans), birds and reptiles. Lymnaeids throughout the world act as intermediate hosts to schistosomes (Table 1.1). However, sometimes a non-specific host is targeted, such as a human, resulting in cercarial dermatitis, an inflammatory skin disease (Horak and Kolarova 2001; Hafeez 2003). Cercarial dermatitis, or 'swimmers itch', occurs in freshwater areas throughout the world (Hunter 1998). Cercarial dermatitis does not cause a transmissible infection, although there is some concern that these parasites may be able to adapt to new vertebrate hosts (Horak and Kolarova 2001).

The Echinostomatidae are a group of intestinal flukes that infect birds and mammals, with up to 11 species reported to infect humans (Neva and Brown 1994). Several lymnaeid species are known as intermediate hosts for this group of intestinal flukes (Table 1.1). Infections generally occur throughout Asia, and directly result from eating raw snails, fish and amphibians (Monzon and Kitikoon 1989; Hafeez 2003). Little research has been undertaken in this area due to the patchy distribution of the disease throughout Asia.

Snails and digenean trematodes have a long evolutionary association, a relationship that is estimated to be at least 200 million years old (Blair *et al.* 2001). It has been suggested that such a long and intimate association has lead to an evolutionary 'arms race' between snail host and trematode, resulting in complex developments in both parasite and snail host (Lockyer *et al.* 2004). Understanding these complex host-parasite interactions is essential in the control of the above discussed diseases (Lockyer *et al.* 2004). Central to this understanding is a sound knowledge of snail host phylogenies.

Comparative examination of phylogenies for both snail host and parasites can provide insights into parasite origin and colonisation episodes, thus explaining their extant distributions (Blair *et al.* 2001; Morgan *et al.* 2001; Morgan *et al.* 2002)**.** Host extensions are more likely to occur in closely related taxa (Blair *et al.* 2001), such that snail phylogenies could be used as a predictive tool to identify future interchange between possible snail hosts and parasites. A thorough phylogenetic investigation of the planorbid snail host, *Biomphalaria*, was instrumental in understanding why the blood fluke, *Schistosoma mansoni*, easily switched hosts in only a few hundred years (Lockyer *et al.* 2004 and ref's therein). To date no such detailed study has been undertaken for any lymnaeids. Therefore a solid systematic study of the Lymnaeidae could be critical in relieving both humans and domesticated animals from the medical and economic burden of trematode worm infections.

Table 1.1 Lymnaeid species as hosts to digenean trematode parasites, showing parasite species, definitive host and group of digenean trematode

Lymnaeid species	Parasite	Definitive host	Reference	Digenean Family
Austropeplea lessoni	*Trichobilharzia* spp.	Birds	Hurley *et al.* (1994)	Schistosomatidae
Austropeplea ollula	*Echinostoma cinetorchis*	Mammals	Chung *et al.* (1998)	Echinostomatidae
	Fasciola gigantica	Mammals	Alicata (1938)	Fasciolidae
	Fasciola hepatica	Mammals	Boray (1966)	Fasciolidae
Austropeplea tomentosa	*Fasciola gigantica**	Mammals	Boray (1966)	Fasciolidae
	Fasciola hepatica	Mammals	Boray (1966)	Fasciolidae
	Trichobilharzia spp.	Birds	Boray (1998)	Schistosomatidae
Fossaria cubensis	*Fasciola hepatica*	Mammals	Gutierrez *et al.*(2000)	Fasciolidae
Galba truncatula	*Echinostoma echinatum*	Birds	Toledo *et al* (2000)	Echinostomatidae
	Fasciola gigantica	Mammals	Brown *et al.* (1957)	Fasciolidae
	Fasciola hepatica	Mammals	Brown *et al.* (1957)	Fasciolidae
Lymnaea stagnalis	*Echinostoma audyi*	Birds	Toledo *et al.* (2000)	Echinostomatidae
	Echinostoma revolutum	Birds & mammals	Faltýnková (2005)	Echinostomatidae
	Echinostoma paraulum	Birds	Toledo *et al.* 2000	Echinostomatidae
	Fasciola gigantica*	Mammals	Boray (1966)	Fasciolidae
	Fasciola hepatica*	Mammals	Boray (1966)	Fasciolidae
	Trichobilharzia szidati	Birds	Faltýnková (2005)	Schistosomatidae
Pseudosuccinea columella	*Fasciola hepatica*	Mammals	Boray (1985)	Fasciolidae

Radix auricularia	*Echinostoma revolutum*	Birds	Toledo *et al.* (2000)	Echinostomatidae
	Fasciola gigantica	Mammals	Brown (1978)	Fasciolidae
	Trichobilharzia szidatti	Birds	Faltýnková (2005)	Schistosomatidae
Radix luteola	*Fasciola gigantica*	Mammals	Brown (1978)	Fasciolidae
Radix natalensis	*Fasciola gigantica*	Mammals	Brown (1978)	Fasciolidae
Radix peregra	*Echinostoma revolutum*	Birds & mammals	Faltýnková (2005)	Echinostomatidae
	Echinostoma friedi	Birds & mammals	Toledo *et al.* (2000)	Fasciolidae
	Fasciola gigantica*	Mammals	Boray (1966)	Fasciolidae
	Fasciola hepatica*	Mammals	Boray (1966)	Fasciolidae
	Hypoderaeum conoideum	Birds & mammals	Toledo *et al.* (1999)	Echinostomatidae
Radix rubiginosa	*Echinostoma audyi*	Birds	Toledo *et al.* (2000)	Echinostomatidae
	Echinostoma malayanum	Mammals	Lie (1963)	Echinostomatidae
	Fasciola gigantica	Mammals	Brown (1978)	Fasciolidae
Stagnicola corvus	*Echinostoma revolutum*	Birds & mammals	Faltýnková (2005)	Echinostomatidae
	Echinostoma friedi*	Birds & mammals	Toledo *et al.* (2000)	Echinostomatidae
Stagnicola palustris	*Fasciola hepatica**	Mammals	Boray (1966)	Fasciolidae
	Hypoderaeum conoideum*	Birds & mammals	Toledo *et al.* (1999)	Echinostomatidae
Bullastra cumingiana	*Echinistoma malayanum*	Mammals	Monzon and Kitikoon (1989)	Echinostomatidae
Radix cubensis	*Fasciola hepatica*	Mammals	Boray *et al.* (1985)	Fasciolidae
Radix viatrix	*Fasciola hepatica*	Mammals	Boray *et al.* (1985)	Fasciolidae
Radix quadrasi	*Fasciola hepatica*	Mammals	Monzon and Kitikoon (1989)	Fasciolidae
Lymnaea cousini	*Fasciola hepatica*	Mammals	Graczyk and Fried (1999)	Fasciolidae
Fossaria bulimoides	*Fasciola hepatica*	Mammals	Torgerson and Claxton (1999)	Fasciolidae

* Experimentally infected with trematode

1.1.2 Conservation status

Freshwater molluscs represent one of the most threatened groups of animals in the world, due to the increasing destruction to freshwater ecosystems through diversion and damming (Saunders *et al.* 1999). In North America, the growing number of species being listed as endangered or threatened reflects this growing crisis. For example, 72% of North America's recognised mussel species are listed as endangered, threatened or of special concern (Riccardi *et al.* 1998). Members of the

Lymnaeidae also face the possibility of extinction due to the major changes taking place in freshwater habitats. In North America, 23% of lymnaeids are considered to be imperilled, with six species federally listed as endangered or threatened from Idaho alone (Brown and Johnson 2004). In Europe, *Myxas glutinosa* is considered to be one of the rarest freshwater molluscs. The decline of this once widespread species has been attributed to eutrophication, increased turbidity and the regulation of water flow in lakes (Whitfield *et al.* 1998). Despite the above, only four lymnaeid species have been listed as threatened on the IUCN Red List (IUCN 2004). Two of these (*Stagnicola bonnevillensis* and *S. utahensis*) are North American taxa, one is European (*Myxas glutinosa*), and the fourth (*Erinna newcombi*) is restricted to one island of Hawaii (IUCN 2004).

This indicates that the threat to lymnaeids is not a localised problem, but a phenomenon that is occurring across the world. As discussed previously, many lymnaeids are hosts for *Fasciola*, which has a large economic impact on agriculture. Control measures of *Fasciola* include targeting the intermediate host, by draining wet areas or using broadcast molluscicides. These methods of eradication kill over 90% of snail populations in the targeted area (Graczyk and Fried 1999). Such wide scale destruction of temporary ponds and the snails within them poses a potential threat to native species.

Within Australia, there are 44 freshwater molluscs listed as threatened under state legislation. One well documented case is that of the Murray River in South Australia, where nearly all the natural populations of 18 gastropod species inhabiting the river have declined in the last 50 years. This decline corresponds with the increasing flow regulation of the river. Moreover, *Notopala sublineata* and *N. hanleyi* are extinct from the Murray Darling Basin, other than a few surviving populations in irrigation pipelines in the South Australian Riverland (Sheldon and Walker 1993; Ponder and Walker 2004). The decline of these freshwater species has been attributed to a shift in diet from detritus to algae in water flow regulated areas. Algae however has low levels of nitrogen, which are essential for egg production in freshwater snails (Sheldon and Walker 1997). These findings could have important implications for Australian lymnaeids, as lymnaeids require high levels of nitrogen for growth and reproduction (McMahon *et al.* 1974).

It has been previously thought that the Australian freshwater molluscan fauna was composed of a small number of widely distributed species. However, a recent review described a significant freshwater molluscan fauna composed of over 430 species, of which 99% are endemic and 42% undescribed (Ponder and Walker 2004). Indeed, there are a large number of small range endemics associated with springs in arid Australia that have only recently been discovered (Ponder and Clark 1990; Ponder 1995; Ponder *et al.* 1996). These recent discoveries suggest that further taxonomic investigation of the Australian freshwater fauna is needed. Systematic studies underpin biodiversity in two key areas. Firstly, by providing formal names for species, which is imperative as most protective legislation is species-based and species-focussed. Secondly by producing phylogenies that can be used as predictive tools by biodiversity managers (Ponder 2004). Therefore, in order to manage biodiversity properly, it is essential that there is a good understanding of Lymnaeidae systematics.

1.2 *Lymnaeidae biogeography and classification*

1.2.1 Classification of Lymnaeidae

There has been disagreement among workers as to the level of generic differentiation within the Lymnaeidae. Due to the morphological and anatomical uniformity throughout the family, Hubendick (1951) synonymised all the available generic names into just two; *Lymnaea* Lamark, 1799 and *Lanx* Clessin, 1882. *Lymnaea* applied to the 40 coiled species while *Lanx* represented limpet shaped species. This classification of the Lymnaeidae was challenged by subsequent workers who favoured more restricted genera. Based on chromosome number, shell characters, reproductive anatomy and immunology studies, several genera were recognised; *Lymnaea, Stagnicola* Leach, 1830*, Fossaria* (=*Galba*; Westerlund 1855)*, Radix* Montfort, 1810*, Austropeplea* Cotton, 1942*, Pseudosuccinea* Baker, 1908*,* and *Bulimnea* Halderman, 1841(Burch and Lindsay 1968; Inaba 1969; Patterson 1969; Burch and Lindsay 1972; Burch and Lindsay 1973; Patterson and Burch 1978). However, some workers were reticent to accept this multigeneric scheme (Walter 1968b), while other workers recognised up to 26 different genera within the family

(Kruglov and Starboratov 1993b, a). Recent molecular studies have demonstrated that the multigeneric scheme should be used (Remigio and Blair 1997a; Bargues *et al.* 2001; Remigio 2002; Bargues *et al.* 2003). Phylogenetic analyses using 16S and ITS-2 sequences have showed that distinct clades can be recognised within the Lymnaeidae that largely correspond to certain previously described. Therefore the multigeneric classification system endorsed by Burch and co-workers (Burch and Lindsay 1968; Inaba 1969; Patterson 1969; Burch and Lindsay 1972; Burch and Lindsay 1973; Patterson and Burch 1978) should remain in use and will be followed in this study.

1.2.2 Lymnaeidae biogeography and evolution

Studies aimed at understanding the evolutionary history of the Lymnaeidae during the past 50 years have lead to a number of theories relating to their biogeography (Walter 1968; Inaba 1969; Ponder and Waterhouse 1997; Remigio and Blair 1997a; Bargues *et al.* 2001; Remigio 2002; Bargues *et al.* 2003). These five theories can be divided into two groups based on whether northern hemisphere taxa are considered to be the most recently diverged lymnaeids (Walter 1968; Inaba 1969; Bargues *et al.* 2001; Bargues *et al.* 2003), or the oldest lineages within the Lymnaeidae (Ponder and Waterhouse 1997; Remigio and Blair 1997a; Remigio 2002). These theories of lymnaeid evolution are based on a range of characters, including anatomical characters, radula dentition, chromosome number, and gene sequences.

One of the theories of lymnaeid evolution and biogeography based on anatomical characters considered the prostate, distal genitalia, the uterine caecum and the lateral teeth of the radula to be the most important in lymnaeid evolution (Walter 1968, 1969). Based on these characters three main lymnaeid groups were recognised; the radicine, the prostagnicoline and the stagnicoline (Table 1.2). The radicine lymnaeids were considered the most primitive, originating in the southern hemisphere. The radicine group then gave rise to the prostagnicoline group, which had an Asian distribution. The stagnicoline were thought to be the most advanced of all lymnaeids and to have evolved in North America in the mid-Pliocene (Walter 1968; Figure 1.1). The stagnicoline group was further divided into three subgroups, the primitive

stagnicoline, the intermediate stagnicoline and the advanced stagnicoline (Walter 1968; Table 1.2).

Anatomically, the radicine lymnaeids were thought to be characterised by a unifolded prostate, a long penis, a uterine caecum and tricuspid lateral teeth (Table 1.2). In the prostagnicoline group, the simple prostate had developed into a multifolded prostate, and there was a decrease in penis size and a loss of the uterine caecum (Table 1.2). The stagnicoline group, were considered the most advanced lymnaeids, due to the development of the prostate pouch, penal knot, vaginal bulb, and bicuspid lateral teeth. However there was also a reversal to the simple prostate, a long penis and a uterine caecum (Table 1.2)

Table 1.2 Classification of the Lymnaeidae based on anatomical characters (Walter 1968).

Morphological types	Species typified	Distal genitalia	Prostate	Uterine caecum	Lateral teeth
Radicine	*Austropeplea ollula*	long and simple	unifolded	present	tricuspid
Prostagnicoline	*Radix luteola*	vestigal state	multifolded	absent	tricuspid
Primitive stagnicoline	*Stagnicola corvus*	long and simple with penal knot	multifolded	present	bicuspid
Intermediate stagnicoline	*Lymnaea stagnalis* *Stagnicola* cf. *palustris*	long and simple with penal knot	unifolded	present	bicuspid
Advance stagnicolicine	*Lymnaea catascopium*	penal knot, narrow vagina and vaginal bulb	unifolded prostate with prostate pouch	present	bicuspid

Not all lymnaeid species can be easily classified into this system. For example, *Pseudosuccinea columella* (Say, 1817), an endemic North American lymnaeid, possesses a prostate that has no fold, a prostate pouch, a weakly developed uterine caecum and tricuspid lateral teeth. Therefore, it is possible to align this species with the advanced stagnicolines on the basis of possessing a prostate pouch and a uterine caecum (Rudolph 1983). Walter (1968) states that *P. columella* has affinities with the prostagnicoline group, probably due to the tricuspid lateral teeth and lack of a penal

knot and vagina sphincter. Therefore the position of *P. columella* is unclear according to Walter's (1968) classification system.
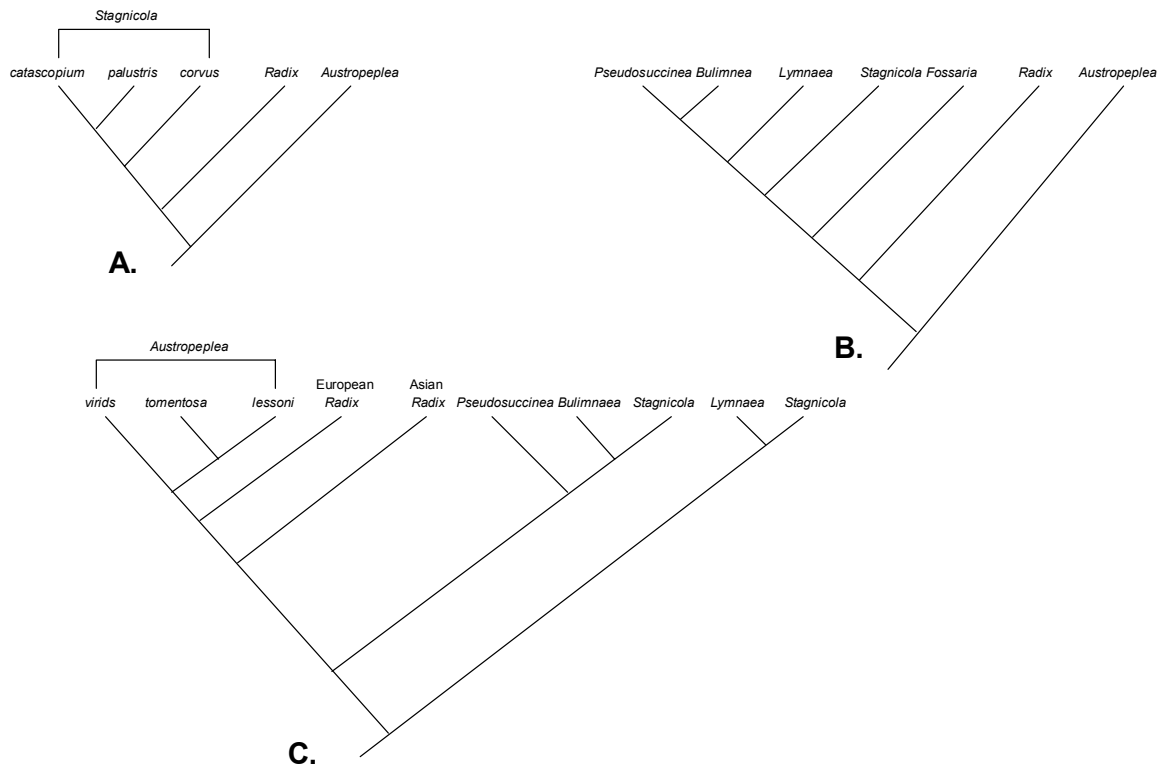
Figure 1.1 Evolutionary theories of the Lymnaeidae. A. Based on anatomical characters of Walter (1968). B. Based on chromosome and anatomical characters of Inaba (1969). C. Based on molecular phylogenies of Remigio and Blair 1997a, Remigio 2002.

Based on Walter's (1968) anatomical theory of lymnaeid evolution, *Stagnicola corvus* and *Lymnaea stagnalis* should be closely related, with *R. luteola* (Lamark 1822) possibly representing a basal sister group to these two species. While studies of DNA sequences show a reasonably close relationship between *L. stagnalis* and *S. corvus*, *Radix luteola* is not resolved as sister to this group (Remigio 2002). Moreover, the theory of lymnaeid evolution according to Walter (1968) relies on only seven characters, three of which undergo reversals.

Theories of lymnaeid evolution based on chromosome number supported the theory of lymnaeid evolution based on anatomical characters (Burch 1965, 1967; Inaba 1969; Patterson and Burch 1978). This theory was based on the assumption that variation in chromosome numbers usually reflects addition rather than loss of

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chromosomes. Therefore it was expected that the more primitive molluscs have lower chromosome numbers (Burch 1965). Thus, *Austropeplea* with 16 pairs of chromosomes was thought to be the most primitive, followed by *Radix* with 17 pairs and *Stagnicola* with 18 pairs, the most recently derived group (Burch 1965, 1967; Inaba 1969). The hypothetical pro-lymnaeid stock is thought to have appeared in the Palaeozoic, with *Austropeplea* appearing in the late Palaeozoic to the early Mesozoic (Fig 1.1). *Radix* diverged from this stock. *Fossaria* diverged from *Radix* in the Jurassic and *Stagnicola* later in the Cretaceous era (Fig 1.1). *Acella* (Halderman, 1841) then diverged from *Stagnicola* in the Cretaceous period. In the Paleocene *Lymnaea* branched from *Radix* (Fig 1.1), while *Polyrythis* stemmed from *Stagnicola* in the Pliocene. *Hinkleyia* and *Bakerilymnaea* evolved from the *Stagnicola* stem in the later Pliocene, as did *Bulimnea* and *Pseudosuccinea* from the *Radix* stem (Fig 1.1; Inaba 1969). Some species of *Fossaria* have been identified as having 19 pairs of chromosomes, and it is thought they diverged within the Quaternary from other members of *Fossaria* with 18 pairs of chromosomes (Inaba 1969).

A molecular study using DNA sequences of the ITS-2 region (Bargues *et al.* 2001; 2003) supported the anatomical and chromosomal theories of lymnaeid evolution to some degree. European *Radix* was the most basal group of the molecular phylogeny, followed by the North American *Stagnicola*. *Lymnaea*, the European *Stagnicola* and *Omphiscola* were the most recently diverged lineages. The basal position of *Radix* agrees with the previous two theories, as does the position of *Stagnicola* as the most recent group. Based on the assumption that the oldest taxa have the shortest gene length, the authors propose *Fossaria* (=*Galba*) *truncatula* along with *Radix* as the oldest lymnaeid group (Bargues *et al.* 2001). However, *F. truncatula* has 18 pairs of chromosomes, and Inaba (1969) states that the oldest lymnaeid taxa had n=16 or n=17. In addition, the phylogenetic tree shows *Radix* to be the most basal group, with *F. truncatula* more recently derived than *Radix*.

A third theory of lymnaeid evolution and biogeography, based on the 16S gene contradicts all of the previous discussed theories. *Austropeplea* was the most recently diverged group followed by *Radix* (Fig1.1). *Lymnaea* and *Stagnicola*, from Europe and North America, are recovered as the oldest taxa within the family (Fig 1.1; Remigio and Blair 1997a; Remigio 2002). This theory suggested the lymnaeids had a

Laurasian origin in the late Jurassic. The subsequent split of Laurasia into the Paleartic and Neartic region resulted in a split of the ancestral taxa between the landmasses. Contact between North America and Northeast Asia in the late Cretaceous facilitated dispersal of the stock that gave rise to *Radix* and *Austropeplea*. Rapid radiation resulted in the dispersal of lymnaeids into parts of Asia, Europe, Africa and the west Pacific, resulting in overlap with *Stagnicola* in Europe. South American lymnaeids are thought to have been derived from North America, via the isthmus of Panama in the late Pliocene (Remigio and Blair 1997a; Remigio 2002). Support of this theory comes from outgroup comparison with Lancinae, Chilinidae and Latiidae, which suggest that 18 pairs of chromosomes is likely to be pleisomorphic (Ponder and Waterhouse 1997; Remigio 2002).

Differences between the two molecular phylogenies could be attributed to the European sampling bias in the ITS-2 molecular study. Of the 37 samples used in the study, only 8 are from North America, the rest representing Europe. *Radix* has a distribution throughout Europe and Asia, although only members of *Radix* from Europe are represented in the ITS-2 study (Bargues *et al.* 2001; Bargues *et al.* 2003). The 16S molecular study, while still largely focused on northern hemisphere lymnaeids, sampled more lymnaeids from across their distribution than the ITS-2 study. Inadequate sampling could be producing misleading results in the ITS-2 study. The 16S gene also has a more broad phylogenetic utility than the ITS-2 region, resolving lineages at both recent and deeper levels of divergence (Hillis and Dixon 1991; Simon 1991). The ITS-2 region is generally much more variable than 16S (Coleman 2003), and may be less reliable for resolving phylogenetic relationships.

An issue raised by the molecular phylogenies is whether *Fossaria* really is the oldest member of the Lymnaeidae. The oldest fossil of the family has been identified as *Fossaria* from Jurassic deposits (Benton 1993). However, both the 16S and ITS-2 sequence analyses place *Fossaria* (=*Galba*) at an intermediate level of the tree, not basally. This raises the possibility that the Lymnaeidae arose earlier than the Jurassic. Alternatively, the oldest fossil has simply been misidentified (Remigio 2002).

These previous theories of biogeography are currently limited due to their northern hemisphere bias. In order to understand the biogeography of the

Lymnaeidae, a world wide approach must be taken. Taxa from Australia, South American, African and South East Asian need to be included to obtain a better understanding of the biogeography of the Lymnaeidae.

The biogeography of the Australasian lymnaeids was recently revised (Ponder and Waterhouse 1997). This revision supported the biogeographic theory based on the 16S gene sequences, with *Stagnicola* as a basal lymnaeid group and 18 pairs of chromosomes being the pleisomorphic state. *Austropeplea* emerged in the late Cretaceous from a *Radix* ancestor by undergoing chromosome reduction from 17 pairs to 16 pairs. Based on anatomical characters and the current distribution of the Australian lymnaeids three separate theories of Australian lymnaeid evolution were suggested. The first suggested the *Austropeplea* derived from South East Asia as previously suggested by Remigio and Blair (1997a). The second theory suggested that *Austropeplea* had a common Gondwanan ancestry, and dispersed into South East Asia. The third, and most favoured theory by the authors, suggested that the southern Australian groups, *A. tomentosa* (Pfeiffer, 1855) and *K. hispida* Ponder and Waterhouse 1997, derived from a Gondwanan *Radix* ancestor before the break-up of Gondwanaland. The northern species, *A. lessoni* (Deshayes, 1830) is thought to be derived from South East Asia. The other member of *Austropeplea*, *A. viridis* (Quoy and Gaimard, 1833) is thought to be derived from European lymnaeids. Thus the third theory suggests that *Austropeplea* represents a polyphyletic group. These theories however, have yet to be formally tested.

1.3 Previous systematic studies of the Lymnaeidae

The phylogeny and classification of Lymnaeidae has traditionally been based on shell characters. Subsequently, the variable nature of the shell was demonstrated (e.g. Hubendick 1951) and lead workers to take a more anatomical focus (e.g. Jackiewicz 1993a). DNA gene sequencing has also proven to be a very useful tool in lymnaeid phylogenetics.

1.3.1 Shell plasticity

Shell characters and shape were the traditional characters used to distinguish species and define genera. Shell shape, sculpture and colour pattern were considered

significant in the identification and classification. The shells of the Lymnaeidae are rather uniform throughout the family, as most shells are high spired, dextral with a closed umbilicus and uniformally coloured. The shell is also usually thin with no shell sculpture. Historically, the lymnaeid shell characters have been considered consistent characters and were generally the primary characteristic considered in species designations. However, the fact that shell shape within the group is ecophenotypically plastic has lead to the description of more species of lymnaeids (around 1800) than actually exist (Hubendick, 1951).

The use of shell shape as the major characteristic in determining the taxonomy of the group has proved problematic (Evans 1989). Intraspecific variation of shell shape is common throughout the Lymnaeidae and is thought to be a response to the relative transience of most freshwater habitats (Russell-Hunter 1978). Freshwater environments are dominated by often short-term, small scale isolation. In such unpredictable and heterogeneous environments, different populations of a given species may be subject to different environmental conditions and/ or selection pressures. This results in much inter-population diversity, although very little of this diversity becomes fixed and results in speciation (Russell-Hunter 1978; Britton and McMahon 2004). Phenotypic plasticity is therefore an important adaptive trait in heterogeneous environments (Via *et al.* 1995). This theory is supported by several studies within the lymnaeid family, whereby observed shell variation within a species is due to direct environmental effects on the phenotype (Hubendick 1951; Arthur 1982; Lam and Calow 1988; Evans 1989; Oviedo *et al.* 1995; Ward *et al.* 1997; Wullschleger and Jokela 2002). Shell morphology can depend on several environmental factors, such as habitat type, water movement, and predation (Arthur 1982; Lam and Calow 1988; Crowl 1990; De Witt 1998)

The exclusive use of shell characters to understand evolutionary relationships can also be problematic when differentiation is limited. An absence of obvious shell diversification can result in the incorrect assumption of a single evolutionary lineage. Cryptic speciation has been demonstrated in a number of freshwater molluscs (Baker *et al.* 2003; Liu *et al.* 2003; Pfenninger *et al.* 2003), although less frequently than phenotypic plasticity. An example from within the Lymnaeidae concerns two South American taxa, '*Lymnaea' viatrix* Orbigny, 1835and '*Lymnaea' cubensis* . These

species are genetically and morphologically distinguishable, but have identical shells (Jabbour-Zahab *et al.* 1997; Samadi *et al.* 2000; Durand *et al.* 2002).

1.3.2 Anatomical variation

Anatomical studies of the soft bodied parts of snails have proved useful in the past for identifying and separating species. However, internal anatomical characteristics are also thought to be problematic in their application to lymnaeid systematics. Some workers advocate that only a small, limited set of anatomical characters are useful in determining species, such as the distal genitalia, prostate and radula teeth (Hubendick 1951; Walter 1968, 1969). Other workers have shown that characteristics of the outer body, kidney, nervous system and digestive system are useful in distinguishing lymnaeid species (Paraense 1976, 1982, 1984, 1994a, 1995; Ponder and Waterhouse 1997).

Very few lymnaeid taxa are well described anatomically, with the exception of the South American taxa (Paraense 1976, 1982, 1983, 1984, 1994a, 1995), some native and introduced Japanese taxa (Itagaki and Itagaki 1955; Itagaki 1956, 1959) and two North American taxa (Baker 1900; Walter 1969). Most species were superficially described in a review of the family, although the focus was largely on the distal end of the reproductive system and the radula (Hubendick 1951). Other anatomical characters, such as the shape and form of the kidney were considered either too variable, or not variable at all (e.g. nervous system) by Hubendick (1951). However, very little data was presented to support these statements.

The anatomy of the European and Asian lymnaeids has been studied to some degree. A number of anatomical studies have been undertaken by Russian workers (Kruglov and Starboratov 1993b; a for summary). Their designations are, however, based on very minor differences in the reproductive system, such as small changes in the shape of the oothecal gland (Kruglov and Starboratov 1981, 1989) and the taxonomic utility of these characters is questionable. In addition, the species *Polyrhytis kukenkovi* has a similar shell to *Pseudosuccinea columella* and may have been misdiagnosed. Anatomical descriptions of European lymnaeids are available (Jackiewicz 1959, 1984, 1986, 1988a, b, 1989, 1990a), although there is a strong

focus on only the male and female reproductive systems. Some more recent studies have also examined the utility of other characters situated on the Head-foot region of the animals (Jackiewicz 1993b; Jackiewicz and Buksalewicz 1998; Jackiewicz and Dudzien 1998).

The purpose of the above mentioned studies has generally been to separate species, rather than to understand their evolutionary relationships. Just one cladistic analysis of anatomical characters has been performed within the Lymnaeidae (Jackiewicz 1993a). This included only European lymnaeids and based on 11 reproductive characters. While there is variation of anatomical characters within populations, some characters have proven to be more useful in discriminating taxa than shell morphology (Samadi *et al.* 2000). Thus, the value of anatomical characters in understanding the systematics of the Lymnaeidae should not be underestimated. The lack of critical comparative anatomical studies across the family is limiting our understanding of differences between groups, as well as the role certain anatomical traits have played in lymnaeid evolution.

1.3.3 Genetic studies

In order to overcome problems associated with phenotypic plasticity of the shell and anatomical variation within the lymnaeids, various genetic studies have been carried out within the Lymnaeidae. Numerous techniques have been used in an attempt to understand lymnaeid taxonomy and phylogenetics, including cross-breeding experiments (Pagulayan and Enriquez 1983; Kruglov and Starbogatov 1985), enzyme electrophoresis (Evans 1989; Monzon *et al.* 1994; Jabbour-Zahab *et al.* 1997; Durand *et al.* 2002), body surface chromatography (Wright 1964), cytology (Burch and Lindsay 1969; Garbar and Korniushin 2002; 2003), immunological studies (Burch 1973; Burch and Lindsay 1973; Burch and LoVerde 1973; Burch and Hadzisce 1974), allozymes (Coutellec- Vreto *et al.* 1994), PCR-RFLP's (Carvalho *et al.* 2004), RAPD analysis (Rybska *et al.* 2000) and DNA sequencing (Marquez *et al.* 1995; Bargues *et al.* 1997; Bargues and Mas-Coma 1997; Remigio and Blair 1997a, b; Stothard *et al.* 2000; Bargues *et al.* 2001; Remigio 2002; Bargues *et al.* 2003). The majority of studies listed above have generally been focussed on defining species

limits, and understanding the taxonomy and distribution of species. Of the above techniques, DNA sequencing has been the most successful tool in determining species boundaries and taxonomy. Whilst the main focus has been on species distinctions within the Lymnaeidae, there has only been a small number of studies (Remigio and Blair 1997a; Bargues *et al.* 2001; Remigio 2002; Bargues *et al.* 2003) focussing on phylogenetic relationships within the family.

DNA gene sequencing has also been an important tool for providing insights into traditional lymnaeid taxonomy based on shell and anatomical characters. There are major inconsistencies between relationships predicted from DNA gene sequencing compared to those predicted from shell and anatomical characters. Designations of groups of lymnaeids (particularly genera) have been based largely on shell morphology, radula dentition, male reproductive systems, haploid chromosome number and immunological data (Hubendick 1951; Burch and Lindsay 1968; Burch and Lindsay 1969; Inaba 1969; Patterson 1969; Burch and Lindsay 1972; Patterson and Burch 1978). However, recent molecular phylogenies suggest that these characters are the result of convergent evolution, as demonstrated by the paraphyletic clades of both *Stagnicola* and *Radix* (Remigio and Blair 1997a; Remigio 2002). Molecular phylogenies also conflict with the morphology based phylogeny of European lymnaeids (Jackiewicz 1993a). Morphological phylogenies indicate that *S. palustris* (Müller, 1774) is distantly related to *S. corvus* and *L. stagnalis* , whereas molecular relationships show *S. palustris* and *S. corvus* are sister taxa, with *L. stagnalis* sister to the *S. corvus* and *S. palustris* clade (Remigio 2002). Molecular phylogenies also show that taxa with the same number of prostate folds or identical radula dentition (previously considered to be two phylogenetically important characters within the family), are not necessarily closely related (Remigio and Blair 1997a; Remigio 2002). However, chromosome numbers, which have been previously used to determine generic classifications, are generally congruent with the molecular phylogenies (Remigio 2002).

1.4 Australian lymnaeids

1.4.1 Key gap in biogeography

The southern hemisphere taxa have been poorly represented, in previous studies of lymnaeid systematics. To gain a world-wide view of Lymnaeidae biogeography we need to understand the phylogenetic relationships of the Australian, South American, African, and Asian taxa. Previous theories of lymnaeid evolution have either placed the Australian lymnaeids as the oldest lineage (Inaba 1969) or as the most recently diverged lineage (Remigio and Blair 1997a; Remigio 2002). Clearly the position of the Australian lymnaeids within the family remains unresolved.

1.4.2 History of *Austropeplea*

The genus *Austropeplea* was introduced for Australian lymnaeids with distinctive shell characters and a wide mantle border (Cotton 1942). Based on shell characters alone, four lymnaeid genera were later recognised within Australia; *Peplimnea, Austropeplea, Simlimnea* and *Glacilimnea* (Iredale 1943, 1944). *Peplimnea* represented what is now known as *A. lessoni*, while the other three genera represented what is currently called *A. tomentosa*. The three species representing *Austropeplea* were restricted to South Australia and Tasmania. *Simlimnea* had a broader distribution (recorded from New South Wales and Victoria as well as South Australia and Tasmania), with some species having sympatric distributions (Iredale 1943, 1944). The third genus (*Glacilimnea*) was monotypic, being represented by a species only known to occur in Blue Lake on the Kosciuszko Plateau and was quite divergent in shell shape (Iredale 1944). All four genera were later synonymised with *Lymnaea* due to the recognition of only two genera within the Lymnaeidae, *Lanx* and *Lymnaea*. Hubendick (1951) described the diagnosis of the *Peplimnea, Simlimnea* and *Glacilimnea* as either absent or totally inadequate to distinguish the groups as separate from other lymnaeids. *Austropeplea* was synonymised due to the independent evolution of the expanded mantle border in a number of taxa that were considered not closely related. In addition the shell characters that the genus was based upon were determined to be insufficient to distinguish a separate group (Hubendick 1951).

Austropeplea was later resurrected, based on chromosome numbers, with all members having 16 pairs of chromosomes. Members of the genus include three species, *A. lessoni*, *A. tomentosa* and *A. viridis* (Inaba 1969). However, recent molecular studies suggest that *Austropeplea* is not a monophyletic group, as *A. lessoni* was recovered as sister to *Bullastra cumingiana* (Remigio and Blair 1997a; Remigio 2002). The inclusion of *A. viridis* in the genus is also questionable, given the large amount of sequence, shell and anatomical divergence exhibited between this specie and other members of *Austropeplea* (Ponder and Waterhouse 1997; Remigio 2002).

1.4.3 Testing current taxonomy

Under the current classification, there are three Australian lymnaeids recognised, *Austropeplea lessoni* (Deshayes 1830), *A. tomentosa* (Pfeiffer 1855) and *Kutikina hispida* Ponder and Waterhouse, 1997 (Smith 1992; Ponder and Waterhouse 1997). As currently recognised *Austropeplea lessoni* and *A. tomentosa* both have wide distributions across the Australian continent. *Austropeplea lessoni*, the larger of the two species, has a northern Australian distribution and has also been recorded from New Guinea (Benthem-Jutting 1963; Smith 1992). *Austropeplea tomentosa* is found in south eastern areas of Australia, including Tasmania (Smith 1992) and on both the North and South Islands of New Zealand (Pullan *et al.* 1972).

Austropeplea lessoni, as it is currently, has 19 available names as synonyms (Boray and McMichael 1961). Numerous localities across the Australian continent have been identified as type localities for the various forms of previously recognised *A. lessoni* (See Smith, 1992 for review). Indeed, 13 species were considered valid under what is now known as *A. lessoni* even up until the 1950's (Iredale 1943, 1944). A review of the lymnaeid family, however, reduced these 13 taxa to just one widely distributed species (Hubendick 1951).

A similar pattern is observed with *A. tomentosa*, in which numerous Australian and New Zealand forms (Boray and McMichael 1961), which were thought to be distinct species based on divergent shell morphologies. Within Australia, *A. tomentosa* was thought to comprise nine separate species divided into three distinct genera (Iredale 1943, 1944), prior to a review of the lymnaeid family (Hubendick

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1951). These nine species were condensed into just two species (Hubendick 1951), and then later reduced to one species (Boray and McMichael 1961).

The review of the Australian lymnaeids was based on a small amount of material, with only three samples examined for *A. lessoni* and four for *A. tomentosa* (Hubendick, 1951). Considering the expansive area from which these species are recorded, the validity of conclusions drawn from such limited sampling is questionable. Indeed, the author concedes that insufficient material was examined to provide a thorough understanding of the Australian group. A review of the taxonomic status of these two groups is warranted.

Kutikina hispida, the third native Australian lymnaeid, was only recently discovered, and differs from the other two Australian lymnaeids in distribution, habitat and anatomy. *Kutikina hispida* has a very limited distribution; it has been recorded from only the lower section of the Franklin River in the south west of Tasmania, in marked contrast to the broad distributions of *A. lessoni* and *A. tomentosa* (Ponder and Waterhouse 1997). *Kutikina hispida* inhabits fast-flowing regions of the river, being found attached to rocks, boulders or rock faces on the edge of the river. This contrasts with *Austropeplea lessoni* and *A. tomentosa* that inhabit the slow-flowing parts of rivers and streams as well as standing water. *Austropeplea tomentosa* is generally confined to the edges of smaller creeks and swamps and found sitting in algae or on mud. *Austropeplea lessoni* inhabits temporary billabongs and creeks, floating on top of the water or attached to macrophytes.

Anatomically, *K. hispida* is divergent from the other two Australian lymnaeid species, with several features not seen in any other lymnaeids. Some of these morphological differences may be adaptations for life in turbulent waters. Anatomical comparisons with other lymnaeids suggested that *K. hispida* is most closely related to *A. tomentosa* and may be a Gondwanan relict derived from a *Radix* ancestor (Ponder and Waterhouse 1997). The phylogenetic relationships of *K. hispida* to the Australian lymnaeids or world lymnaeids have not been formally tested.

1.5 Methodology and Aims

1.5.1 Methodological approach

Some authors have proposed that both shell and anatomical characters are too variable and should be avoided in phylogenetic studies, being more prone to selective processes and hence more homoplastic than other characters (Hubendick 1951; Bargues *et al.* 2001; Remigio 2002). Homologous and nonhomologous similarity cannot be distinguished from observations alone; instead they have to be recognised in light of an inferred phylogeny. However, other authors position is that all available information may be useful in the reconstruction of phylogenies and characters should not be dismissed before hand as useless or bad characters (Schander and Sunberg 2001).

Therefore, the approach of this study was to use shell morphology, anatomical characters and DNA sequencing to elucidate the taxonomy and phylogenetic relationships within the Australasian Lymnaeidae. Separate phylogenetic analyses of DNA sequences and anatomical characters will be undertaken so there can be a direct comparison between the results of the separate the datasets. Utilising DNA phylogenies to comprehend shell and anatomical variation within the Lymnaeidae is an area of research that has largely been unexploited. In undertaking such an approach, an assessment can be made of the usefulness of shell and anatomical characters in phylogenetic studies of lymnaeids.

1.5.2 Aims and objectives

The major aims of this study are;
1. to delimit species within the Australian *Austropeplea*,
2. to reconstruct their phylogenetic relationships,
3. to propose a theory for the biogeography of the Australasian Lymnaeidae, and
4. to assess the usefulness of shell and anatomical characters in phylogenetic studies of the Lymnaeidae.

The results of these four aims will be achieved by using a number of separate approaches. The first approach will be to use DNA and anatomical-based phylogenies

to delimit species (Aim 1), and to reconstruct the phylogeny and biogeography of Austropeplea (Aims 2 and 3). A direct comparison between the DNA and anatomical based phylogenies will then be used to assess the value of anatomical characters in future systematic studies of the Lymnaeidae (Aim 4). Shell morphology is a character that is broadly used to identify and distinguish molluscan species. Therefore measurements of shell morphology will also be used to assess whether this technique can reliably be used to distinguish phylogenetic groups of closely related lymnaeids (Aims 1 and 4).

1.5.3 Structure of Thesis

The systematic study of *Austropeplea tomentosa* and *A. lessoni* is investigated in Chapter 2 and 3, respectively. Chapter 4 will investigate the phylogenetic relationships of *Austropeplea* and the Australian lymnaeids to other members of the family. Chapter 5 is a taxonomic description of the Australian and New Zealand lymnaeids. Chapter 6 is a general discussion and overview of the entire study. Chapters 2, 3 and 4 have been designed for submission as papers to scientific journals; therefore, the results of these sections will be discussed at length at the end of each chapter. Chapter 6 will therefore only be a short overview of the results obtained.

Chapter 2 Systematics of the *Austropeplea tomentosa* complex

2.1 Introduction

2.1.1 Geographic isolation in freshwater gastropods

The geographic isolation of populations has long been recognized as one of the major mechanisms driving the process of speciation (Mayr 1942). The erection of geographical barriers not only prevents gene exchange between populations, but also results in each population being subject to different selection pressures, genetic drift and mutation (Templeton 1989). Freshwater gastropods that exhibit a wide geographic distribution are subject to such population isolation, as freshwater environments. Hence, gene flow between freshwater gastropod populations depends on the degree of dispersal of individuals between freshwater 'islands'. Many freshwater gastropods display passive dispersal life histories, relying on agents such as animal vectors, wind or water flow, to be transported from one freshwater biota to another (Russell-Hunter 1978; Bilton *et al.* 2001). Limited dispersal ability coupled with the hydrographic isolation of many freshwater habitats, can lead to subdivision and isolation of gastropod populations, the prerequisites for allopatric speciation. Indeed, there is evidence to suggest that gene flow between isolated populations of freshwater gastropods is low (Gow *et al.* 2001; Charbonnel *et al.* 2002; Emery *et al.* 2003).

2.1.2 Distribution of *Austropeplea tomentosa*

Austropeplea tomentosa (Pfeiffer 1855) is a member of the cosmopolitan freshwater pulmonate family Lymnaeidae, and has an extensive distributional range throughout southeastern Australia, Tasmania and New Zealand (Figure 2.1; Boray 1964; Smith 1992). This small gastropod reaches a maximum shell height of 18 mm and inhabits slow moving waters, pools and ponds, thriving in eutrophic conditions (Boray 1964; Smith and Kershaw 1979). Like other members of the Lymnaeidae, this species has direct development (no larval swimming stage) and is thought to be

passively dispersed between isolated habitats, for example by water current and water fowl (Boray 1969).
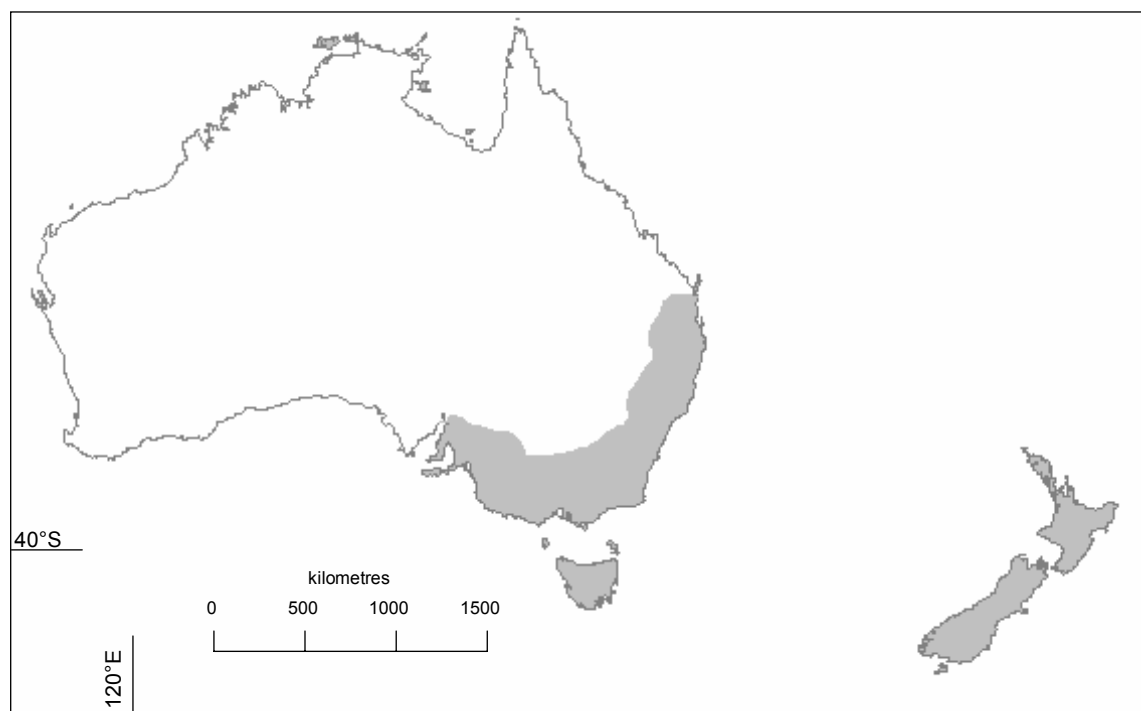
Figure 2.1 Distribution of the *Austropeplea tomentosa* complex within Australia and New Zealand, represented by grey shading.

Australian and New Zealand populations of *Austropeplea tomentosa* may well have been reproductively isolated for millions of years by the Tasman Sea. Sea floor spreading between Australia and New Zealand is estimated to have initiated 83 − 79 Mya, with the spreading of the Tasman Sea persisting until the early Eocene (55.5 MyaVeevers *et al.* 1991; Sutherland 1999) It seems highly unlikely, that over such a distance and time, this freshwater gastropod would have been able to maintain sufficient gene flow to consider the two populations synonymous. Fossil evidence of *A. tomentosa*, in both Australia and New Zealand, dates back to the Pleistocene, thus effectively ruling out the possibility of European introductions (Gill and Banks 1956; Climo and Pullan 1972; Climo 1984). However, the possibility of a recent dispersal event, possibly by birds, could also account for the current distribution of *A. tomentosa*, considering the recent age of fossils.

Within Australia, *A. tomentosa* has a wide distribution both on the mainland and throughout Tasmania, however within this distribution there are a number of geographical barriers that could potentially limit gene exchange between populations.

For example, eastern mainland Australia is divided by a mountain range, which forms a watershed running largely the length of the coastline. This Great Divide, which typically lies 150km inland, separates eastern and western populations of *A. tomentosa*. Tasmanian and mainland Australian populations of *A. tomentosa* are separated by Bass Strait, a stretch of sea 250 kilometres wide. The passive dispersal life history of *A. tomentosa* coupled with these natural barriers to gene flow and the large areas of uninhabitable environments separating isolated populations it is plausible that this complex represents more than one genetically distinct species.

2.1.3 Previous studies

Austropeplea tomentosa has been identified as Australia's most important intermediate host of *Fasciola hepatica*. However, the taxonomy of *A. tomentosa* has never been studied comprehensively. Under the current classification, *A. tomentosa* has 16 synonyms, although these synonymies have never been tested using molecular or even anatomical methods. *Austropeplea tomentosa* was originally described from Auckland, New Zealand (Pfeiffer 1855). Subsequently seven new lymnaeid species from New Zealand were described, under what is now known as *A. tomentosa* (Hutton 1885; Suter 1913). These eight species was later reduced to just two species (give he names here) by Dell (1956), who recognised some similarities between the New Zealand and Australian lymnaeids.

Within Australia, the first recorded specimens of *Austropeplea tomentosa* were from Sydney in 1864 (AM C.42272), and the earliest described taxa was *Limnaea huonensis* in 1876 from the Huon River, Tasmania (Tenison Woods 1876). Numerous other taxa were subsequently described (Tenison Woods 1876; Tate 1880; Smith 1882; Petterd 1889). Iredale (1943, 1944) recognised 11 lymnaeid species under what is now currently known as *A. tomentosa*. Virtually all of these early descriptions are loosely based on shell shape, and are poorly justified. Some descriptions did not even illustrate shells (Pfeiffer 1855; Tenison Woods 1876; Cherry 1896), whilst other descriptions are based on only a few shells alone (Tenison Woods 1876; Smith 1882; Hutton 1885; Petterd 1889). Furthermore, consistency between species descriptions was lacking, making comparisons between the nominal species

difficult (e.g. Petterd 1888; Iredale 1943, 1944). Early studies of *A. tomentosa* are therefore of little help in trying to solve this taxonomic problem.

Hubendick (1951) undertook a taxonomic review of the entire Lymnaeidae, although the review focused largely on northern hemisphere taxa and the Australian lymnaeids were only dealt with briefly. Three samples of *A. tomentosa* were studied from within its large distribution, (one from New Zealand and two from Australia) resulting in the recognition of two species *Lymnaea tomentosa* and *L. tasmanica* (Tenison-Woods, 1876). *Lymnaea tomentosa* was distributed across Australia and New Zealand, while *L. tasmanica* was confined to Tasmania and South Australia (Hubendick 1951).These findings were largely based upon differences in the distal parts of the genitalia, radula and shell shape. The author realised his inadequacies in trying to solve the taxonomic problem of this species, as he notes that his designations are not definitive conclusions but simply theories based on limited material. Hubendick (1951) also alluded to the fact that the Australian and New Zealand taxa may, with further study, prove to be separate species.

Boray and McMichael (1961) later reduced Hubendick's (1951) two species to just one, largely from a parasitological perspective. Whilst the study sampled numerous populations within the distribution of *A. tomentosa,* the status of *A. tomentosa* was largely based upon the susceptibility of the populations to the digenean trematode, *Fasciola hepatica*. However, *F. hepatica* was introduced to Australia with domestic livestock from Europe and is compatible with a wide variety of lymnaeids. Host expansion has occurred in New Zealand, North and South America and some Pacific Islands, whereby *F. hepatica* has used endemic lymnaeids as hosts in its life cycle (Boray 1966, 1969). While Boray and McMichael (1961) also used some morphological measures in their study, the lack of quantifiable evidence presented in the paper does not strongly support their conclusions that all populations are variants of the one species. Therefore, the status of the Australian and New Zealand taxa attributed to *A. tomentosa* requires re-examination.

Finally, a full description of the anatomy of *A. tomentosa* has not been published. Early papers of original descriptions are largely of the shell and outer body of the animal. Hubendick (1951) published some drawing of the male reproductive

system and radula, as did Climo and Pullan (1972). Ponder and Waterhouse (1997) expanded on this by describing some anatomical features of *A. tomentosa* including the pallial cavity, female reproductive system and nervous system.

2.1.4 Fascioliasis

Understanding the taxonomy of *Austropeplea tomentosa* could have important implications for the Australian and New Zealand agricultural industries. Within Australia, and possibly New Zealand, *A. tomentosa* is the primary intermediate host for *Fasciola hepatica*. It is estimated that, within Australia alone, fascioliasis costs the agricultural industry $A100 million annually in wool, meat and milk production (Boray 1998). An important component of understanding the evolution and biology of this parasite lies in understanding the taxonomy of their snail intermediate hosts. Therefore, the taxonomy of *A. tomentosa* will aid future studies of fascioliasis.

2.1.5 Phylogenetic relationship of *Kutikina hispida*

The recent discovery of another indigenous lymnaeid within Australia resulted in a reconsideration of the relationships of the Australian lymnaeids (Ponder and Waterhouse 1997). *Kutikina hispida* Ponder and Waterhouse, 1997, a morphologically distinct species, is confined to the lower regions of the Franklin River in south western Tasmania. Morphologically, *K. hispida* is thought to be more closely related to *A. tomentosa* than to the other Australian lymnaeid *A. lessoni* (Deshayes, 1830). Moreover, it has been suggested that *K. hispida* could be a Gondwanan relict derived from a *Radix* Montfort, 1810 ancestor (Ponder and Waterhouse 1997). However, this hypothesis has not been tested.

2.1.6 Methodological Approach

An understanding of the speciation and taxonomy of freshwater gastropods is often hampered by the morphological problems associated with shell shape variation. High levels of phenotypic plasticity, which have been demonstrated within the lymnaeids (Hubendick 1951; Arthur 1982; Evans 1989; Ward *et al.* 1997; Lam and Calow 1998; Wullschleger and Jokela 2002), can have confounding effects on

speciation studies when the traditional shell shape approach is used as the sole indicator of taxonomic status. However, understanding the differences in shell morphologies in relation to other anatomical characters and genetic differences can be a useful tool. Therefore, this study explores differences in the shell morphology of *Austropeplea tomentosa*.

While anatomical studies of the soft-bodied parts of snails have proved useful for identifying and separating species, the utility of such anatomical characters is disputed within the Lymnaeidae (Hubendick 1951; Ponder and Waterhouse 1997; Remigio and Blair 1997a; Remigio 2002). However, this utility has never been tested with reference to an inferred phylogeny. Therefore, this study includes anatomical examination of specimens for any distinguishing characters that can be useful in understanding the taxonomy and evolutionary history of the group.

DNA sequencing has proven to be a useful tool in understanding speciation within freshwater molluscs. Sequence analysis of the large subunit (16S) mitochondrial ribosomal DNA successfully distinguished several species in previous lymnaeid studies (Remigio and Blair 1997a; Remigio 2002). This gene region has both rapidly and slowly evolving regions, making it suitable for examining both ancient and recent divergences (Hillis and Dixon 1991; Simon 1991). In addition, the second nuclear internal transcribed spacer (ITS-2) region has been utilised in the Mollusca to understand the relationships of recently diverged organisms, < 50 million years (Coleman and Vacquier; Oliverio *et al.* 2002; Insua *et al.* 2003). Studies within the Lymnaeidae and the closely related Planorbidae show that ITS-2 is a reliable indicator of closely related snails at the species and genus level (Vidigal *et al.* 2000; Bargues *et al.* 2001; Mavarez *et al.* 2002; Bargues *et al.* 2003). Therefore, molecular studies performed in tandem with both shell and anatomical studies, are a powerful approach to understanding speciation in *Austropeplea tomentosa*.

2.1.7 Aims

The primary objectives of this study were to determine,
1.   the species status of the Australian and New Zealand populations referred to *Austropeplea tomentosa*,

2.	whether Australian populations of *A. tomentosa* are represented by more than one species,

3.	the phylogenetic relationship of *Kutikina hispida* to *Austropeplea tomentosa*.

These objectives were met by using the sequences of the partial mitochondrial 16S gene and ITS-2 region in conjunction with anatomical studies and measurements of shell morphology.

2.2 Materials and Methods

2.2.1 Material examined

Twenty eight populations of *Austropeplea tomentosa* were sampled for this study, representing 23 distinct geographic areas and encompassing the entire range of this species. Under the current classification *A. tomentosa* is represented by 24 synonymies. Populations were sampled from these type localities where possible (Table 2.1, 2.2) in addition to other areas within the range of the complex. However *A. tomentosa* was not found at a number of type localities. This is probably due to extensive habitat change in the areas from which they were described. Several other taxa were included in this study; four populations of *A. lessoni* and one population each of *A. viridis* (Quoy and Gaimard 1832), *Bullastra cumingiana* (Pfeiffer 1839), *Kutikina hispida*, *Radix auricularia* (Linnaeus 1758) and *R. peregra* (Müller 1774; Table 2.2).

All specimens were collected live in the field between 2002 and 2004, with the exception of two populations, NSW 3 (2) and NSW 4 (2), that were from the Australian Museum collection. After collection, if there were a sufficient number of specimens, samples were split into two portions, one portion was used shell and anatomical examination and the other for DNA sequencing. Specimens used for morphological examinations were relaxed overnight in menthol, and fixed in 10% saltwater formalin. This was subsequently changed to 5% saltwater formalin a few days later. Specimens to be used for DNA sequencing were fixed in either absolute ethanol or 95% ethanol, with the ethanol changed 12 hours later. If there were insufficient specimens to permit splitting, all specimens were preserved for DNA

analysis. All specimens have subsequently been lodged with the Australian Museum, Sydney (See Table 2.2).

For the samples where only DNA material was able to be collected or where only a small number of individuals were available for shell and anatomical studies, a second population that was within a 30 km radius of the first population was also included in the study. These populations have the same code as the original population, but have (2) following the code (Table 2.2).

2.2.2 DNA sequencing

2.2.2.1 Material examined

A total of 25 specimens representing 23 samples of *Austropeplea tomentosa* were sequenced for the 16S gene and ITS-2 region (Table 2.1). One individual from each sample was sequenced, except for two populations (NSW 6 and VIC 1) where two individuals were sequenced. In addition, two specimens of *Kutikina hispida* were genotyped as well as four specimens of *A. lessoni*, and one individual of *A. viridis* and *Bullastra cumingiana* (Table 2.1). Sequences for other outgroup taxa were obtained from Genbank, *Radix auricularia* 16S: AF485646, ITS-2: AJ319628, *R. peregra* 16S: U82074, ITS-2: AJ319633 and also 16S for *B. cumingiana* U82068.

Table 2.1 Synonymies of *Austropeplea tomentos*a, the type locality and the sample number from this study that equates to the type locality.

Name	Author and Date	Type Locality	Sample Numbers
Succinea tomentosa	Pfeiffer 1855	NZ	C.422731
Lymnaea huonensis	Tenison-Woods 1876	Upper Huon River, TAS, AUS	Not found
Lymnaea hobartonensis	Tenison-Woods 1876	Hobart, TAS, AUS	Not found
Lymnaea launcestonensis	Tenison-Woods 1876	Launceston, TAS, AUS	C.422098
Lymnaea subaquatilis	Tate 1880	Torrens River, Adelaide, SA, AUS	Not found
Lymnaea papyeracea	Tate 1880	Penola, SA, AUS	C.427947
Succinea johnstoni	Tate 1880	Tasmania	No type locality given
Lymnaea brazieri	Smith 1882	Sydney, NSW, AUS	C.431874, C.407263, C.431876
Lymnaea victoriae	Smith 1882	Bairnsdale, VIC, AUS	Not found
Lymnaea viridula	Smith 1882	Hamilton, VIC, AUS	Not found
Lymnaea ampulla	Hutton 1885	Arthurs Pass, NZ	C.433250
Lymnaea arguta	Hutton 1885	Avon River, Christchurch, NZ	C.433525
Lymnaea leptosoma	Hutton 1885	Wellington, NZ	Not found
Lymnaea pucilla	Hutton 1885	Auckland, NZ	Not found
Lymnaea tenella	Hutton 1885	Heathcote River, Christchurch, NZ	Not found
Lymnaea subaquatilis neglecta	Petterd 1889	Swamp near Launcetson, TAS, VIC	Not found
Lymnaea gunni	Petterd 1889	South Esk River, TAS, AUS	C.422104
Lymnaea lutosa	Petterd 1889	Jordan River, Brighton, TAS, AUS	Not found
Lymnaea alfredi	Suter 1890	Hooker Valley, NZ	Not found
Lymnaea ampulla globosa	Suter 1891	Tasman Valley, NZ	Not found
Lymnaea venustula	Cherry 1896	Wimmera River, VIC	Not found
Glacilimnea gelida	Iredale 1943	Blue Lake, Mt. Kosciuszko, NSW, AUS	C.436026
Simlimnea aegrifer	Iredale 1944	Bombala, NSW, AUS	EBU.35591
Simlimnea morbida	Iredale 1944	Walcha, NSW, AUS	C.431248, C.442100, C.431236

AUS= Australia, NSW= New South Wales, Australia, NZ= North Island, TAS= Tasmania, Australia, SA= South Australia, Australia, VIC= Victoria, Australia

Table 2.2Summary of taxa and voucher numbers for material used in the systematic study of the *Austropeplea tomentosa* complex.

Code	Australian Museum Accession No.	Latitude	Longitude	Locality	Shells measured	Anatomical examination	16S sequenced	ITS-2 sequenced
Austropeplea tomentosa								
NSW-1	C.442100	30º 13.330' S	151º 40.170' E	Guyra, NSW, AUS	Yes	Yes	Yes	Yes
NSW-1 (2)	C.431236	30º 27.649' S	151º 21.392' E	Guyra, NSW, AUS	Yes	Yes	No	No
NSW-2	C.431248	30º 54.586' S	151º 17.306' E	Walcha, NSW, AUS	Yes	Yes	Yes	Yes
NSW-3	C.431874	33º 38.500' S	150º 41.500' E	Penrith, NSW, AUS	No	No	Yes	Yes
NSW- 3(2)	C.407263	33º 46.230' S	150º 45.660' E	Penrith, NSW, AUS	Yes	Yes	No	No
NSW-4	C.431876	33º 37.000' S	150º 49.000' E	Windsor, NSW, AUS	No	No	Yes	Yes
NSW-4 (2)	C.309424	33º 38.500' S	150º 45.660' E	Windsor, NSW, AUS	Yes	Yes	No	No
NSW-5	C.442102	35º 31.483' S	149º 31.700' E	Braidwood, NSW, AUS	Yes	Yes	Yes	Yes
NSW-6	C.436026	36° 24.368' S	148° 19.064' E	Kosciuszko Plateau, NSW, AUS	Yes	Yes	Yes	Yes
NSW-7	EBU.35591	37º 08.783' S	149º 28.087' E	Bombala, NSW, AUS	No	No	Yes	Yes
NSW-8	EBU.35582	36º 34.500' S	149º 41.467' E	Bemboka, NSW, AUS	Yes	Yes	Yes	Yes
NZn-1	C.422732	37º 39.400' S	178º 29.610' E	East Cape, North Island, NZ	Yes	Yes	Yes	Yes
NZn-2	C.422731	39º 13.040' S	176º 53.380' E	North of Napier, North Island, NZ	Yes	Yes	Yes	Yes
NZs-1	C.433250	42º 54.340' S	171º 33.618' E	Arthur's Pass, South Island NZ	No	No	Yes	Yes
NSs-2	C.433525	43º 32.000' S	172º 38.000' E	Avon River, Christchurch, NZ	No	No	Yes	Yes
NZs-3	C.433513	43º 44.929' S	172º 49.450' E	Little River, South Island, NZ	Yes	Yes	Yes	Yes
NZs-4	C.433524	45º 00.437' S	168º 34.384' E	Mole Lake, South Island NZ	Yes	Yes	Yes	Yes

SA-1	C.427947	37º 15.299' S	140º 26.114' E	Penola, SA, AUS	Yes	Yes	Yes	Yes
SA-2	C.428299	37º 22.301' S	140º 12.624' E	Mt Gambier, SA, AUS	No	Yes	Yes	Yes
SA-2 (2)	C.427946	37º 27.051' S	140º 14.651' E	Mt Gambier, SA, AUS	Yes	Yes	No	No
SA-3	C.427948	37º 09.688' S	140º 06.075' E	Millicent, SA, AUS	Yes	Yes	Yes	Yes
SA-3 (2)	C.427949	37º 04.927' S	140º 04.927' E	Millicent, SA, AUS	Yes	Yes	No	No
TAS-1	C.422098	41º 26.873' S	147º 07.286' E	Launceston, TAS, AUS	No	Yes	Yes	Yes
TAS-2	C.422104	42º 16.800' S	147º 35.400' E	South Esk River, TAS, AUS	Yes	Yes	Yes	Yes
TAS-3	C.422096	41º 52.340' S	146º 30.779' E	Lake Augusta, TAS, AUS	Yes	Yes	Yes	Yes
TAS-4	C.422102	42º 21.385' S	147º 01.395' E	Clyde River, TAS, AUS	No	No	Yes	Yes
TAS-5	C.422101	42º 16.807' S	147º 35.411' E	Lemont, TAS, AUS	Yes	Yes	Yes	Yes
VIC-1	C.422092	37º 19.277' S	144º 21.777' E	Castlemaine, VIC, AUS	Yes	Yes	Yes	Yes
Kutikina hispida	C.422107	42º 32.450' S	145º 45.202' E	Franklin River, TAS, AUS	n/a	Yes	Yes	Yes
Austropeplea viridis	C. 449003	31º 56.000' S	115º 50.000' E	Perth, WA, AUS	n/a	Yes	Yes	Yes
Austropeplea lessoni WA	C.439182	16° 58.460' S	122° 40.070' E	Beagle Bay, Broome, WA, AUS	n/a	Yes	Yes	Yes
Austropeplea lessoni NT	C.436053	12° 33.940' S	131° 18.380' E	Humpty Doo, NT, AUS	n/a	Yes	Yes	Yes
Austropeplea lessoni QLD	C.451980	19° 24.000' S	146° 44.000' E	Ross River, QLD, AUS	n/a	Yes	Yes	Yes
Austropeplea lessoni NSW	C.431243	29º 27.873' S	151º 37.180' E	Glenn Innes, NSW, AUS	n/a	Yes	Yes	Yes
Bullastra cumingiana	C.416760	14º 05.000' S	121º 11.000' E	Luzon, Philippines	n/a	Yes	Yes	Yes
Radix auricularia	C.449004	50º 0 8.000' S	167º 44.000' E	North Island, NZ	n/a	Yes	Yes	Yes
Radix peregra	C.428190	60º 10.000' N	24º 27.000' E	Finland	n/a	Yes	Yes	Yes

AUS= Australia, NSW= New South Wales, Australia, NT= Northern Territory, NZn= North Island, New Zealand, NZs= South Island, New Zealand QLD= Queensland, Australia, TAS= Tasmania, Australia, SA= South Australia, Australia, WA= Western Australia, Australia, VIC= Victoria, Australia

2.2.2.2 DNA extraction, PCR amplification, and sequencing

Lymnaeids are intermediate hosts to a number of parasitic trematodes (Brown 1978). Development and multiplication of the parasites take place inside the body cavity of the snail, and usually within the digestive gland (which is situated in the upper spirals of the shell). In order to ensure that no parasite DNA was extracted from the snails, only a small piece of foot tissue was used for the extraction of DNA. A CTAB method was employed for the extraction of the DNA. A small piece of foot tissue was sliced from the animal and this tissue placed in a solution of 200 μl of 2% CTAB and 100 μg proteinase K. The tissue was then broken up by grinding with a plastic pestle and digested for two hours at 55°C, with inversion every 30 minutes. Polymuccosaccarides were extracted from the sample in four steps; (1) 200 μl of chloroform/ isoamylalcohol (24:1) was added to the solution; (2) mixing was carried out by repeated inversion for two minutes; (3) centrifuge for four minutes at 13 200 rpm to separate the two phases (an upper and lower phase); and (4) the upper phase (containing the DNA) was carefully removed. The lower phase, resulting from the centrifuging, is distinguished by the white polymucosacharide layer that forms above the polymucosacharide-containing supernatant. These extraction steps were repeated three times to ensure all polymuccosaccarides had been removed from the sample. Genomic DNA was precipitated by the addition of two volumes of absolute ethanol and incubated at -20ºC for 20 minutes. The DNA pellet was then centrifuged for 15 minutes at 13 200 rpm, the supernatant removed and the pellet washed with 70% ethanol at -20ºC. The genomic DNA was dissolved in 50 ul of 1 mM Tris-HCl (pH 8) and stored at 4°C. This genomic DNA solution was used directly in the PCR reaction.

The primers used to amplify 16S were 5'-CCG GTC TGA ACT CAG ATC ACG T-3' and 5'-CGC CTG TTT AAC AAA AAC AT-3' (Simon *et al.*, 1994). Reactions were performed in a total volume of 20 μl. Reactions contained 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 1.25 mM $MgCl_2$, 25 μM of each dNTP, 60 nM of each primer, 0.75 U of *Taq* DNA polymerase (Promega) and 0.5 μl of DNA template. A negative control was also prepared with the above constituents but without DNA, so that if contamination was present, it could be easily detected. Amplifications were performed in a gradient thermocycler with an initial denaturation

step of 94°C for 2 min; then 35 cycles of 94°C for 30 sec, 45-65°C for 30 sec,72°C for 1 min; one cycle at 72°C for 5 min, and one cycle at 30°C for1 min. Reaction conditions for each taxon were optimised with respect to $MgCl_2$ concentration and annealing temperature. The same procedure was followed for the amplification of the ITS-2 rDNA region using primers LT1 5'-TCG TCT GTG TGA GGG TCG-3' (Bargues *et al.* 2001) and BD2 5'-TAT GCT TAA ATT CAG CGG GT-3' (Remigio and Blair 1997b).

Amplification products were purified using a polylene glycol precipitation method, whereby 66 μl of PEG (30% w/v in 1.5 M NaCl) was added to 110 μl of PCR product. This mixture was incubated at room temperature for one hour, centrifuged for 15 minutes at 13 200 rpm and the supernatant removed. The pellet was washed with 70% ethanol at -20ºC and centrifuged for 10 minutes at 13 200 rpm. The supernatant was removed and the pellet again washed and recentrifuged for 5 minutes at 13 200 rpm. The supernatant was then removed and the pellet resuspended in 15 μl TE buffer (1 mM Tris-HCl pH 8, 0.1mM EDTA). The final concentration required for subsequent sequencing reactions was determined by visualizing 1 μl of the purified product on a 1% agarose gel and comparison with known amount of DNA from a molecular weight marker preparation.

Direct sequencing of PCR products from a portion of the 16S rDNA and ITS-2 rDNA was performed using the Big Dye® Terminator v.3.1 cycle sequencing kit as described by the manufacturer. Sequencing reactions were made up to 12μL with 40nM of primer, 4 μL of a Dye Terminator and 1 μL of template DNA. Conditions for cycling were 30 cycles of: 96°C for 30 sec; 50°C for 15 sec; 60°C for 4 min. Sequencing products were purified and precipitated by adding 8 μL of nuclease free water, 2 μL of 125 mM EDTA (pH=8), 2 μL of 3M sodium acetate (pH 4.5), 50 μL of absolute ethanol and leaving for 15 minutes at room temperature. The products were centrifuged for 15 minutes at 13 200 rpm and the supernatant removed. The pellet was washed with 70% ethanol at -20ºC, allowed to air dry, and stored at -20°C until analysis. Both DNA strands were sequenced.

Sequence electropherograms were edited manually by comparing both strands for all taxa using Bioedit v.3.0.9 (Hall, 1999). Prior to alignment, a blast search was carried out on Genbank to ensure that the all sequences were free of parasite contamination. Sequences were aligned using CLUSTAL W (Thompson *et al.* 1994), as distributed with the Bioedit program (Hall 1999). Multiple alignments were further improved by manual adjustment. Gene sequence length, base frequencies, and genetic distances were calculated in PAUP* 4.08b (Swofford 1998).

2.2.2.3 Phylogenetic analysis

Phylogenetic analyses were performed on the individual datasets, 16S and ITS-2, to assess congruence of the phylogenetic trees produced. The data were then combined for the final analysis. To determine whether significant incongruence existed between the 16S and ITS-2 datasets, incongruence length difference (ILD) tests (Farris *et al.* 1995) were conducted by excluding all invariant sites and using the partition homogeneity test in PAUP*4.08b (Swofford 2002) with 100 iterations.

To reconstruct phylogenies, I used both maximum parsimony (MP) as implemented in PAUP* 4.08b (Swofford 2002) and Bayesian inference using Markov chain Monte Carlo in the program MrBayes 3.1 (Huelsenbeck *et al.* 2001; Ronquist and Huelsenbeck 2003). MP searches were heuristic with 100 random-taxon-addition replicates, TBR branch swapping, and no maxtrees restrictions. All characters were treated as equal and unordered, with gaps treated as missing data. Clade support was assessed with 1000 bootstrap replicates, each with 100 random-addition heuristic searches (Felsenstein 1985). *Radix peregra* and *R. auricularia* were selected as the outgroup taxa in the MP analyses based on Remigio (2002).

For Bayesian inference, hierarchical likelihood ratio tests were performed as implemented in the program MrModelTest, in order to determine which nucleotide substitution model best fit each data set (Nylander *et al.* 2004). A general time reversal model of molecular evolution (nst=6), with the rates across sites being subject to a gamma distribution (rates=gamma), was selected for the 16S dataset. The alignment of the ITS-2 region resulted in large indel regions. Due to the variable nature of these regions, they were excluded from the phylogenetic analysis (see

Appendix 2.4). For this dataset, the best fit model was a HKY model (nst=2) with the rates across sites being subject to a gamma distribution (rates=gamma). I ran three independent simulations of four simultaneous chains of Markov Chain Monte Carlo for 3 million generations for the single datasets (16S, ITS-2) sampling every 100 generations. Each independent simulation was started from different randomly chosen trees. Stationarity was defined as when the standard deviation reached 0.01, which was reached at 500 000 generations in the 16S dataset and 250 000 generations in the ITS-2 dataset. Therefore, burnin discarded the first 5000 and 2500 trees for the 16S and ITS-2 datasets, respectively. *Radix peregra* was selected as the outgroup taxa in the Bayesian analyses based on Remigio (2002).

The combined dataset (16S + ITS-2) was partitioned for the Bayesian analysis so that the best fit models were applied separately to the 16S and ITS-2 data (unlink command). The best fit models for the 16S and ITS-2 data were the same as those used in the single dataset analyses. For the combined dataset, I ran three independent simulations of four simultaneous chains of Markov Chain Monte Carlo for 5 million generations. Sampling occurred every 100 generations and each independent simulation was started from different randomly chosen trees. Stationarity was defined as when the standard deviation reached 0.01, which was reached at 400000 generations. Therefore the first 4000 trees were discarded as the burn-in for the combined molecular dataset.

2.2.3 Anatomical morphology

I examined formalin and/or ethanol preserved material of 19 samples of *Austropeplea tomentosa,* four samples of *A. lessoni*, and one sample each of *A. viridis*, *Bullastra cumingiana*, *Radix auricularia*, and *R. peregra* (Table 2.1). *Kutikina hispida* was coded using images and descriptions from Ponder and Waterhouse (1997). For each population at least three adult and parasite free specimens were examined. I examined the internal body and dissected all animals under a Wild M3C Leica dissecting microscope. All morphological features that were identified as differing between samples were coded.

A total of three radulae from each sample were examined using a scanning electron microscope. The extracted radulae were cleaned by heating to 60-80ºC in 5% NaOH solution overnight. Each radula was then rinsed in distilled water, and subject to ultrasound to remove any debris. Radulae were mounted on specimen stubs using a dry method, whereby radulae were allowed to dry at room temperature on a glass cover slip that was attached to the stub with double sided tape. The radulae were then coated with gold for examination with the scanning electron microscope.

A total of 57 characters from the shell, outer body, pallial cavity, nervous system, reproductive systems and the radula were identified as variable between samples and were employed in the phylogenetic analysis, and are summarised in Table 2.3. A full description of these characters and their respective states are in Appendix 2.1. The full dataset for the 27 taxa is presented in Appendix 2.2. Maximum parsimony analyses of the data were performed using PAUP* 4.0b8 (Swofford 2002). A heuristic search was performed with 100 random addition sequence replicates, whereby all characters were treated as equal and unordered. To estimate tree support, bootstrap analysis was performed, with 1000 replicates. The distribution of character states on the trees was examined using McClade 4.0 (Maddison and Maddison 2000).

Table 2.3 Characters and character states used in anatomical analysis of the *Austropeplea tomentosa* complex. See Appendix 2.1 for a full description of these characters

Character number	Character	Character state and codification
1	Shell umbilicus	Closed (1); half open (2); open (3)
2	Shell thickness	Thin (1); thick (2)
3	Number of whorls	2.5 (1); three (2); 3.5 (3); four (4); 4.5 (5);five (6)
4	Columella fold	Absent (1); slight (2); distinct (3)
5	Shell sculpture	Absent (1); present (2)
6	Periostracum ornamentation	Absent (1); hairy (2)
7	Broadest area of foot	Anterior end of foot (1); same width along length (2)
8	Foot shape at posterior end	Tapering to a point (1); rounded (2)
9	Foot width to length ratio	2:1 (1); less than 2:1 (2); greater than 2:1 (3)
11	Eye lobe	Absent (1); well developed (2); undeveloped (3)
11	Tentacle shape	Wider than long (1); width equal to length (2); longer than wide (3); twice as long as wide (4)
12	Lateral sides of snout	Developed (1); undeveloped (2)

13	Pallial roof pigmentation	Mottled black and white (1); black (2)
14	Visceral coil pigmentation	Absent (1); present (2)
15	Mantle expansion	Absent (1); just outside of shell (2); covering some parts of the shell (3); covering large parts of the shell (4)
16	Expanded mantle pigmentation	Absent (1); present (2)
17	Number of pneumostomal ridges	One (1); two (2)
18	Outer lobe	Absent (1); present (2)
19	Upper plate of pneumostome	Thin (1); thick (2)
20	Broadest area of kidney	Anterior end (1); same width along length (2); posterior end (3); middle (4)
21	Kidney width to length ratio	3:1 (1); 2:1 (2); greater than 3:1 (3)
22	Right lobe of kidney	Absent (1); present (2)
23	Position of pulmonary vein	To the right of kidney (1); inside right lobe (2)
24	Pulmonary vein length	One third the length of the kidney (1); less than one third the length of the kidney (2); greater than one third the length of the kidney (3)
25	Ureter	Absent (1); present (2)
26	Opening of kidney	Inside pnuemostome (1); anterior to the pnuemostome (2)
27	Buccal mass shape	Longer than wide (1); width equal to length (2)
28	Cerebral commissure length	Half as long as distance between cerebral ganglion (1); one third the distance between cerebral ganglion (2); less than a third the distance between cerebral ganglion (3)
29	Pedal commissure	Absent (1); short (2)
31	Pedal commissure extra lobe	Normal (1); enlarged(2)
30	Statocysts	Absent (1); present (2)
32	Radula sac	Equal in length to buccal mass (1); longer than buccal mass (2); shorter than buccal mass (3)
33	Salivary glands relative size	Equal size (1); right longest(2); left longest (3)
34	Uterus/ vagina length relative to oothecal gland length	greater than half the length (1); less than half the length (2); equal or longer (3)
35	Spermathecal duct length	Shorter than uterus/ vagina (1); equal to uterus/ vagina (2); longer than uterus/ vagina (3)
36	Spermathecal duct width	Equal to uterus/ vagina (1); thinner than uterus/ vagina (2)
37	Uterus shape	Parallel (1); tapering distally (2)
38	Oviducal caecum size relative to oothecal gland	¼ width (1); ½ width (2); between ½ and one width (3); wider (4); absent (5)
39	Oothecal gland shape	Globular (1); pyriform (2); rectangular (3); square (4)
40	Oviduct 1	With brain like convolutions (1); with radial ridges (2); bosselated wall (3)
41	Position of uterus relative to oothecal	At right angles (1); greater than right angles (2); less than right

	gland	angles (3)
42	Velum shape	Horse-shoe shaped (1); circular (2); absent (3)
43	Penis sheath length relative to praeputium	Less than half the length (1); Greater than half the length (2); Equal in length (3); Half the length (4); longer than praeputium (5)
44	Penis in penis sheath head	Looped (1); straight (2)
45	Seminal vesicle	Pockets present (1); low blisters (2)
46	Seminal vesicle shape	Short and wide (1); long and narrow (2)
47	Seminal vesicle form	U shaped (1); convoluted (2); straight (3); looped (4)
48	Junction of vas deferens and prostate	Simple (1); small sac (2)
49	Prostate ventral wall	Large fold present (1); slightly concave (2)
50	Upper prostate	Thin (1); wide (2)
51	Length of prostate relative to female reproductive system	Equal in length (1) longer (2); much longer (3); shorter (4)
52	Shape of lower prostate	Straight (1); bent to left (2)
53	Central tooth	Bicuspid (1); unicuspid (2); tricuspid (3); multicuspid (4)
54	Position of small cusp on central tooth	Left (1); right (2)
55	Radula teeth shape	Blunt (1); sharp (2)
56	Lateral teeth	Bicuspid (1); tricuspsid (2); unicuspid (3); multicuspid (4)
57	Marginal teeth	Bicuspid (1); tricuspid (2); tetracuspid (3) , 5 cups (4), greater than 5 cusps (5)

2.2.4 Combined anatomical and molecular analyses

Phylogenetic analyses were performed on a combined dataset consisting of the molecular and anatomical data. Taxa for which all datasets were not complete were deleted, creating a dataset of 30 samples (twenty *Austropeplea tomentosa*, two *Kutikina hispida*, four *A. lessoni*, one each of *A. viridis*, *Bullastra cumingiana*, *Radix auricularia* and *R. peregra*). To determine whether significant incongruence existed between the molecular and anatomical datasets, incongruence length difference (ILD) tests (Farris *et al.* 1995) were conducted by excluding all invariant sites and using the partition homogeneity test in PAUP*4.08b (Swofford 2002) with 100 iterations. A maximum parsimony analysis was performed as described in Section 2.2.4.3. A partitioned Bayesian analysis was performed with the 16S, ITS-2 and anatomical data.

The best fit models for the 16S and ITS-2 data were the same as used in previous analyses. The combined dataset (16S + ITS-2 + anatomical) was partitioned

for the Bayesian analysis so that the best fit models were applied separately to the 16S and ITS-2 data (unlink command) and the anatomical data were subject to a gamma distribution rate model. For the combined molecular and anatomical dataset, I ran three independent simulations of four simultaneous chains of Markov Chain Monte Carlo for 5 million generations. Sampling occurred every 100 generations and each independent simulation was started from different randomly chosen trees. Stationarity was defined as when the standard deviation reached 0.01, which was reached at 1 000 000 generations. Therefore the first 1000 trees were discarded as the burn-in for the combined molecular and anatomical dataset.

2.2.5 Shell morphometrics

Nineteen samples (representing 134 individuals) of the *Austropeplea tomentosa* complex were measured in the shell morphometrics analysis (Table 2.4). Up to ten adult shells of each sample were measured. Prior to measurement, each specimen was checked to ensure that it was parasite-free and was a mature reproductive adult. Each specimen was drawn with the aid of a camera lucida, with shell measurements taken from these drawings. Shell measurements included shell length, shell width, last whorl length, aperture length, aperture width and spire height, as shown in Figure 2.2. Each population was assigned to one of the geographic regions, as shown in Table 2.4. The sample of *A. tomentosa* from Blue Lake in the Kosciuszko Plateau region of Australia was treated as a separate geographic group in the shell morphometrics study. Individuals of *Austropeplea tomentosa* from Blue Lake have a very distinct shell shape, and this group have previously been described as a monotypic genus (Iredale 1943, 1944). Moreover, it is isolated from other populations of *A. tomentosa*, and Blue Lake has a high level of invertebrate endemicity (Hancock *et al.* 2000).
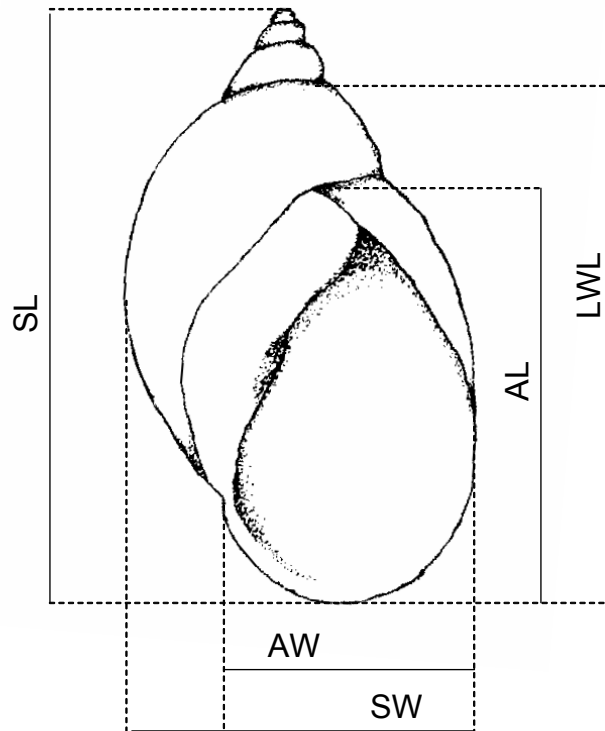
Figure 2.2 Five shell measurements taken in the shell morphometrics analysis of the *Austropeplea tomentosa* complex. AL= aperture length, AW= aperture width, LWL= last whorl length, SL= shell length, SW= shell width.

A discriminant function analysis (DFA) was used to assess whether populations of *Austropeplea tomentosa* from different geographic regions had significantly different shell morphologies, based on the five variables measured. This was performed using the discriminant function platform in SPSS 11.5. All assumptions required for the DFA to be performed were tested. These assumptions are that no two morphometric variables were highly correlated, and therefore measured essentially the same trait; that there was no significant deviations from multivariate normality; and that there was equality in group covariance matrices (Klecka 1980; Hair *et al.* 1998). An initial DFA analysis showed that the New South Wales and Victorian populations were not significantly different, so they have been pooled together to form the East Australian group (EAUST). *A priori* groups for the DFA were the geographic regions as shown in Table 2.2.

Table 2.4 Populations of the *Austropeplea tomentosa* complex sampled for shell morphometrics study, showing number of shells measured for each population and the *a priori* geographic region assigned to each population.

Population code	Australian Museum Accession No.	Latitude	Longitude	No. of shells measured	Geographic Region
Austropeplea tomentosa					
NSW-1	C.442100	30° 13.330'	151° 40.170'	3	EAUST
NSW-1 (2)	C.431236	30° 13.330'	151° 40.170'	10	EAUST
NSW-2	C.431248	43° 02.000'	146° 17.800'	1	EAUST
NSW-3 (2)	C.407263	33° 38.500'	150° 41.500'	8	EAUST
NSW-4 (2)	C.309424	33° 37.000'	150° 49.000'	10	EAUST
NSW-5	C.442102	35° 31.483'	149° 31.700'	10	EAUST
KOS-1	C.436026	36° 24.368'	148° 19.064'	7	KOS
NSW-8	EBU.35582	36° 34.500'	149° 41.467'	10	EAUST
NZn-1	C.422732	37° 39.400'	178° 29.610'	3	NZ
NZs-3	C.433513	43° 44.929	172° 49.450'	10	NZ
NZs-4	C.433524	45° 00.437'	168° 34.384'	5	NZ
SA-1	C.427947	37° 15.299'	140° 26.114'	7	SA
SA-2 (2)	C.427946	37° 27.051'	140° 14.651	2	SA
SA-3	C.427949	37° 22.301	140° 12.624'	2	SA
SA-3 (2)	C.427948	37° 09.688'	140° 06.075'	6	SA
TAS-2	C.422104	42° 16.800'	147° 35.400'	10	TAS
TAS-3	C.422096	41° 52.340'	146° 30.779'	10	TAS
TAS-5	C.422101	42° 16.807'	147° 35.411'	10	TAS
VIC-1	C.422092	37° 19.277'	144° 21.777'	10	EAUST

EAUST= eastern Australia, KOS= Kosciuszko Plateau, NZ= New Zealand, SA= South Australia, TAS= Tasmania, Australia.

2.3 Results

In this section I will first present the molecular analyses including sequence variation and phylogenies. This will be followed by the anatomical analyses and lastly the shell moprhometrics study will be presented.

2.3.1 Sequence variation

The alignment of the16S and ITS-2 regions resulted in aligned data matrices of 446 bp and 559 bp including indels, respectively (Table 2.5; Appendix 2.3, 2.4).

The combined 16S and ITS-2 data matrix was 1005 bp long. The alignment of the ITS-2 sequences resulted in large insert regions, and were largely due to outgroup members having divergent sequences. These regions were excluded from all phylogenetic analyses, although pilot analyse including these produced trees largely congruent with those excluding such regions. Characteristics of the three molecular datasets are shown in Table 2.5. The ITS-2 alignment (excluding variable regions) had the least number of parsimony informative characters and the largest number of equally parsimonious trees (Table 2.5).

Table 2.5 Descriptive statistics for molecular data sets and indices for the trees analysed

Data set	16S	ITS-2	16S + ITS-2
Number of characters	446 bp	559 bp	1005 bp
Number of variable sites (% of data partition)	132 (30)	171 (48)	303 (38)
Number parsimony informative sites (% of data partition)	90 (20)	82 (15)	172 (17)
% A	35	17	26
% C	16	32	24
% G	13	28	20
% T	36	23	30
Test of homogeneity	n.s.	n.s.	n.s.
Sequence divergence (%)	0-15	0-35	0-35
Tree L	234	249	488
CI*	0.65	0.77	0.70
RI*	0.63	0.88	0.86
RC*	0.86	0.79	0.69
Number of MP trees	22	72	36

*excluding uninformative characters. n.s. = not significant

There was little variation in the length of the 16S gene between samples with sequences ranging between 427 and 438 base pairs (Table 2.6). Sequence lengths for ITS-2 showed a much greater level of variation than the 16S gene (Table 2.6). The New Zealand samples of *A. tomentosa* had the shortest ITS-2 sequence lengths (382-393bp) and were quite different from the Australian samples of *A. tomentosa* (404-414bp). *Kutikina hispida* was much longer than either the Australian or New Zealand samples of *A. tomentosa* (430bp).

Table 2.6 16S and ITS-2 sequence length measured in number of base pairs.

Taxa	16S gene length (base pairs)	ITS-2 region length (base pairs)
Austropeplea tomentosa		
New South Wales and Victoria	427-429	411-414
South Australia	428	404
Tasmania	427-428	407-414
New Zealand	428-429	382-393
Kutikina hispida	427	430
Austropeplea viridis	431	397
Austropeplea lessoni	429-430	472-495
Bullastra cumingiana	431	454
Radix auricularia	438	401
Radix peregra	422	395

Sequence divergence between the 35 samples varied from zero to 15% difference in the 16S dataset (Appendix 2.5). The Australian samples of *A. tomentosa* compared to the New Zealand samples had a 4.9 to 7.0% difference. Within the New Zealand, samples of *A. tomentosa* sequence divergence ranged from 0.7 to 2.3%. *Kutikina hispida* had a sequence difference of between 5.2 and 6.8% with the New Zealand samples of *A. tomentosa*, and between 1.9 and 3.3% with the Australian samples of *A. tomentosa*. Between the Australian populations of *A. tomentosa*, sequence divergence ranged from 0 to 2.3%.

Sequence divergence within the ITS-2 dataset showed an overall similar pattern of genetic divergence as the 16S gene (Appendix 2.6). The New Zealand and Australian samples of *A. tomentosa* had sequence divergences ranging from 2.3 to 5.1% and within the New Zealand samples divergence was between 0.3 to 1.3%. *Kutikina hispida* was 3 to 4% divergent from the New Zealand samples of *A. tomentosa* and 1.1 to 4.2% divergent with the Australian samples of *A. tomentosa*. Within the Australian samples of *A. tomentosa* sequence divergence was between 0 and 4.0%.

2.3.2 Molecular Phylogenies

2.3.2.1 16S

Bayesian and MP analyses of the 16S dataset resulted in similar tree topologies as shown in Figures 2.3, 2.4. The 16S phylogenies divide the samples of *Austropeplea tomentosa* into two well supported distinct lineages (Figs 2.3, 2.4). The New Zealand samples of *A. tomentosa* are shown as sister to the Australian samples. Furthermore, *A. tomentosa* is not monophyletic due to the inclusion of *Kutikina hispida* within the clade of the Australian samples of *A. tomentosa*. The branch length of *K. hispida* is longer than any of the other Australian samples of *A. tomentosa*, indicating a larger level of divergence (Fig 2.4). The New Zealand populations of *A. tomentosa* are further divided into clades that represent the geographic separation of the North and South Islands, with reasonable levels of support (Figs 2.3, 2.4). The Australian samples of *A. tomentosa* show some geographic separation. In the MP analysis, the Victorian sample forms a basal clade to the other Australian populations of *A. tomentosa* and *K. hispida* (Fig 2.3). However this relationship does not have strong bootstrap support and is not supported by the Bayesian analysis (Fig 2.4). Other small clades of the New South Wales samples form within the polytomy, although the branch lengths are short (Fig 2.4).

The MP analysis shows *Austropeplea lessoni* plus *Bullastra cumingiana* as sister to the *A. tomentosa* and *Kutikina hispida* clade (Fig 2.3). However, this relationship has less than 50% bootstrap support (Fig 2.3). The sister taxon to the *A. tomentosa* and *Kutikina hispida* clade remains unresolved in the Bayesian analysis, as both *A. viridis* and the *A. lessoni* and *B. cumingiana* are shown to be equally likely as sister taxa (Fig 2.4).
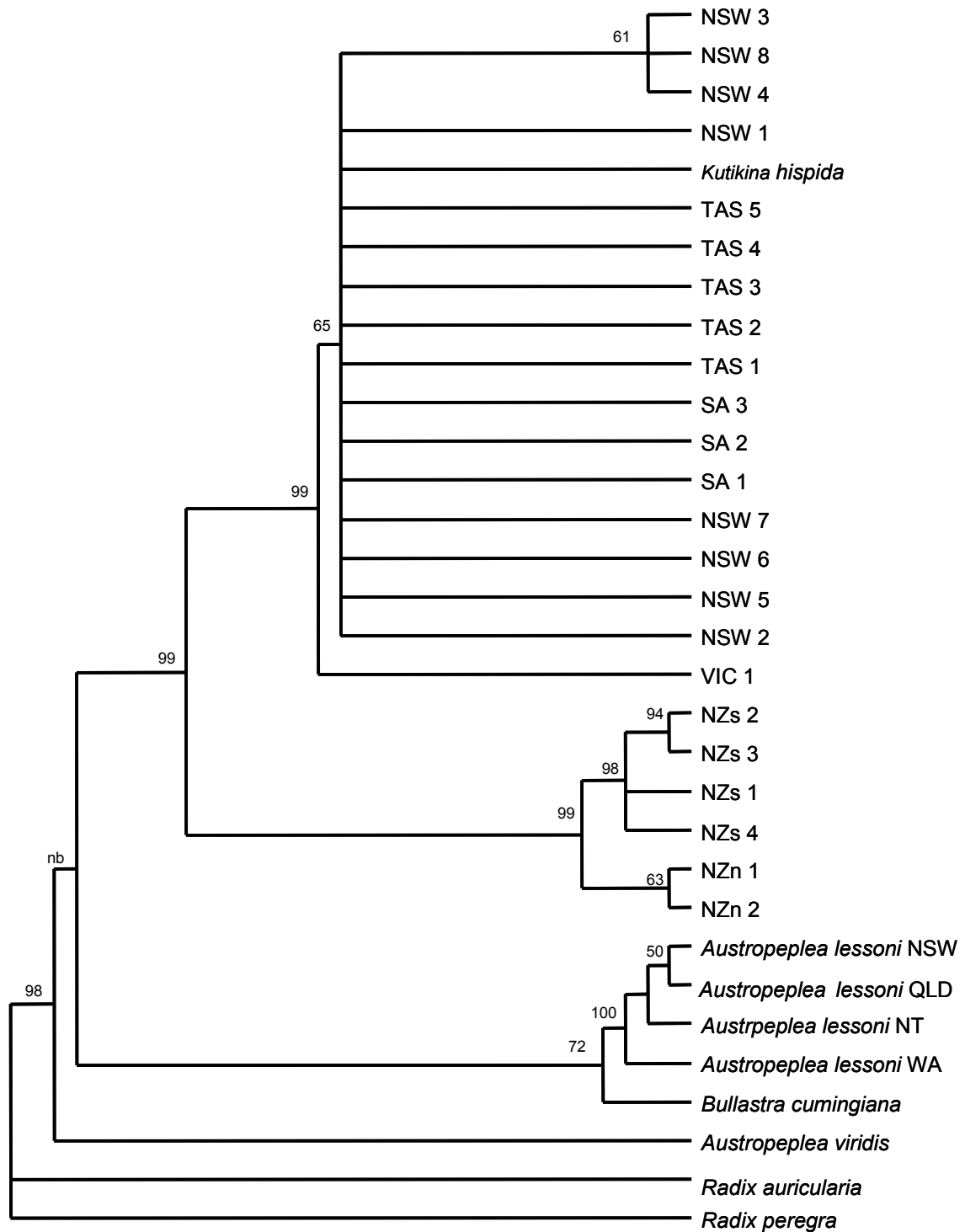
Figure 2.3 Phylogeny of the *Austropeplea tomentosa* complex based on 16S rRNA sequences. Strict consensus tree of 22 maximum parsimony trees with tree length 234. Numbers above the branches are bootstrap scores, with only bootstrap >50% given. nb= no bootstrap support, NSW= New South Wales, Australia, NZn= New Zealand, North Island, NZs= New Zealand, South Island, SA= South Australia, TAS= Tasmania, Australia, VIC= Victoria, Australia. Taxa without names are currently recognised as *A. tomentosa*.
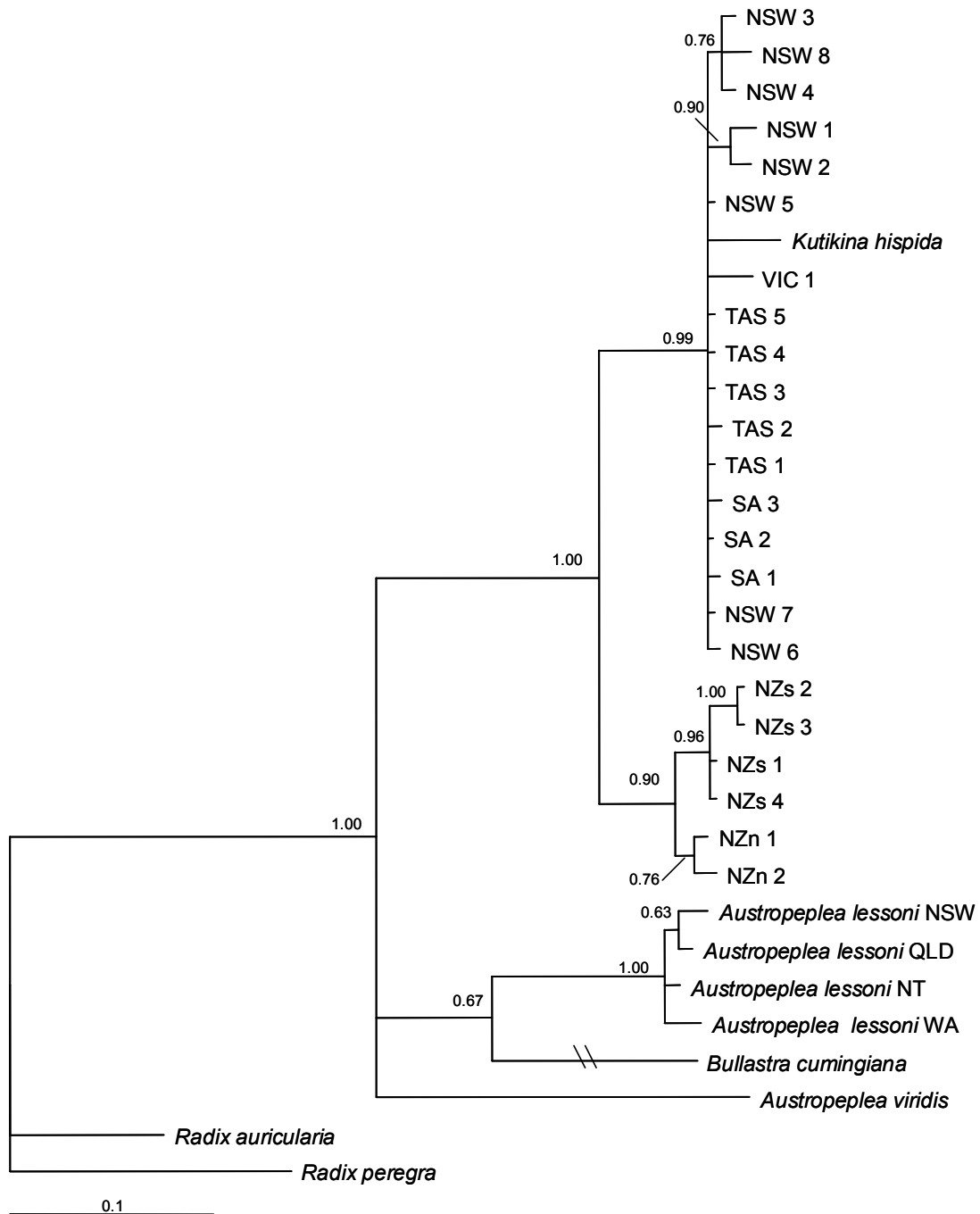
Figure 2.4 Phylogeny of the *Austropeplea tomentosa* complex based on 16S rRNA sequences. Majority rule tree based on Bayesian inference, with maximum likelihood setting under GTR for DNA substitution. Bayesian analysis based on 3 000 000 generation replicates, with posterior probabilities indicated above the branch, only >50% posterior probability given. NSW= New South Wales, Australia, NZn= New Zealand, North Island, NZs= New Zealand, South Island, Australia, SA= South Australia, TAS= Tasmania, VIC= Victoria, Australia, // = branch length shortened. Taxa without names are currently recognised as *A. tomentosa*.

2.3.2.2 ITS-2

Separate analysis of the ITS-2 sequences resulted in trees of slightly different topologies (Figs 2.5, 2.6). Moreover, the relationships shown in the ITS-2 phylogenies are different to the 16S phylogenies. The MP analysis divides the samples of *Austropeplea tomentosa* into two polytomous clades. The basal polytomy is composed of the Tasmanian and northern New South Wales samples. The more derived group is a polytomy of all the other samples of *A. tomentosa* and *K. hispida*. This relationship, however, has less than 50% bootstrap support (Fig 2.5). The Bayesian analysis showed a similar result to the MP analysis, although the polytomy of *A. tomentosa* and *K. hispida* is further divided into a clade of the New Zealand and South Australian samples, with intermediate support (Fig 2.6).

Unlike the 16S phylogeny, the ITS-2 phylogeny did not distinguish between the Australian and New Zealand samples of *A. tomentosa*. The New Zealand samples do however form a well-supported clade within the polytomy. Furthermore, the New Zealand clade has a longer branch length than any of the smaller Australian *A. tomentosa* clades within the polytomy (Fig 2.6). In the MP and Bayesian analysis, small geographical clades form within the polytomies, including a southern New South Wales (NSW 5, NSW 6, NSW 7) and Victorian clade, and a Tasmanian clade. Support for these small clades varies (Figs 2.5, 2.6).

The basal clades of the ITS-2 phylogenies from the MP analysis are largely unresolved (Fig 2.5). *Austropeplea viridis* is shown as sister to the *A. tomentosa* and *Kutikina hispida* clade, although both bootstrap and posterior probability support is low (Figs 2.5, 2.6).
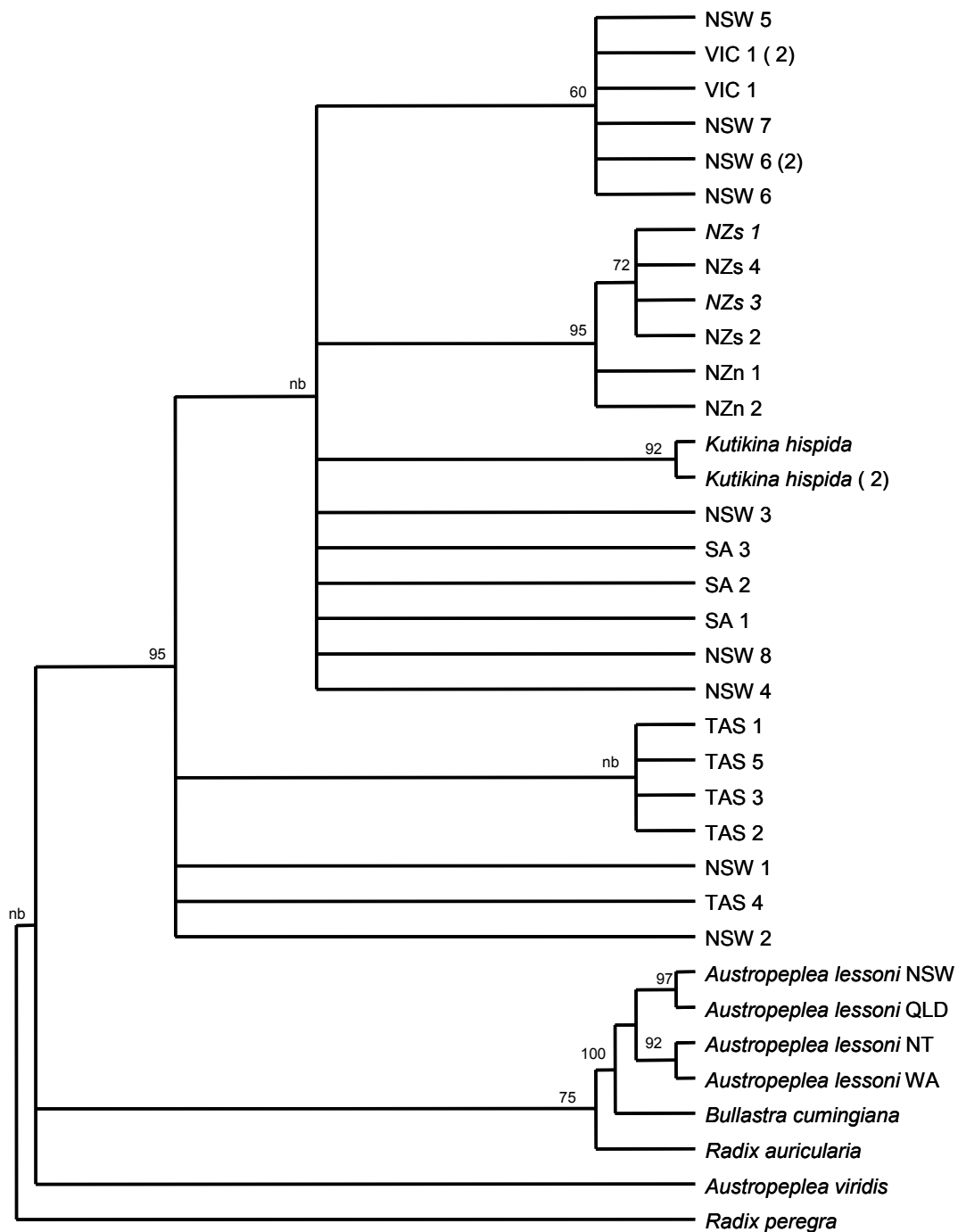
Figure 2.5 Phylogeny of the *Austropeplea tomentosa* complex based on ITS-2 rRNA sequences. Strict consensus tree of 72 maximum parsimony trees with tree length 249. Numbers above the branches are bootstrap scores, with only bootstrap >50% given. nb= no bootstrap support, NSW= New South Wales, Australia, NZn= New Zealand, North Island, NZs= New Zealand, South Island, SA= South Australia, TAS= Tasmania, Australia, VIC= Victoria, Australia. Taxa without names are currently recognised as *A. tomentosa*.
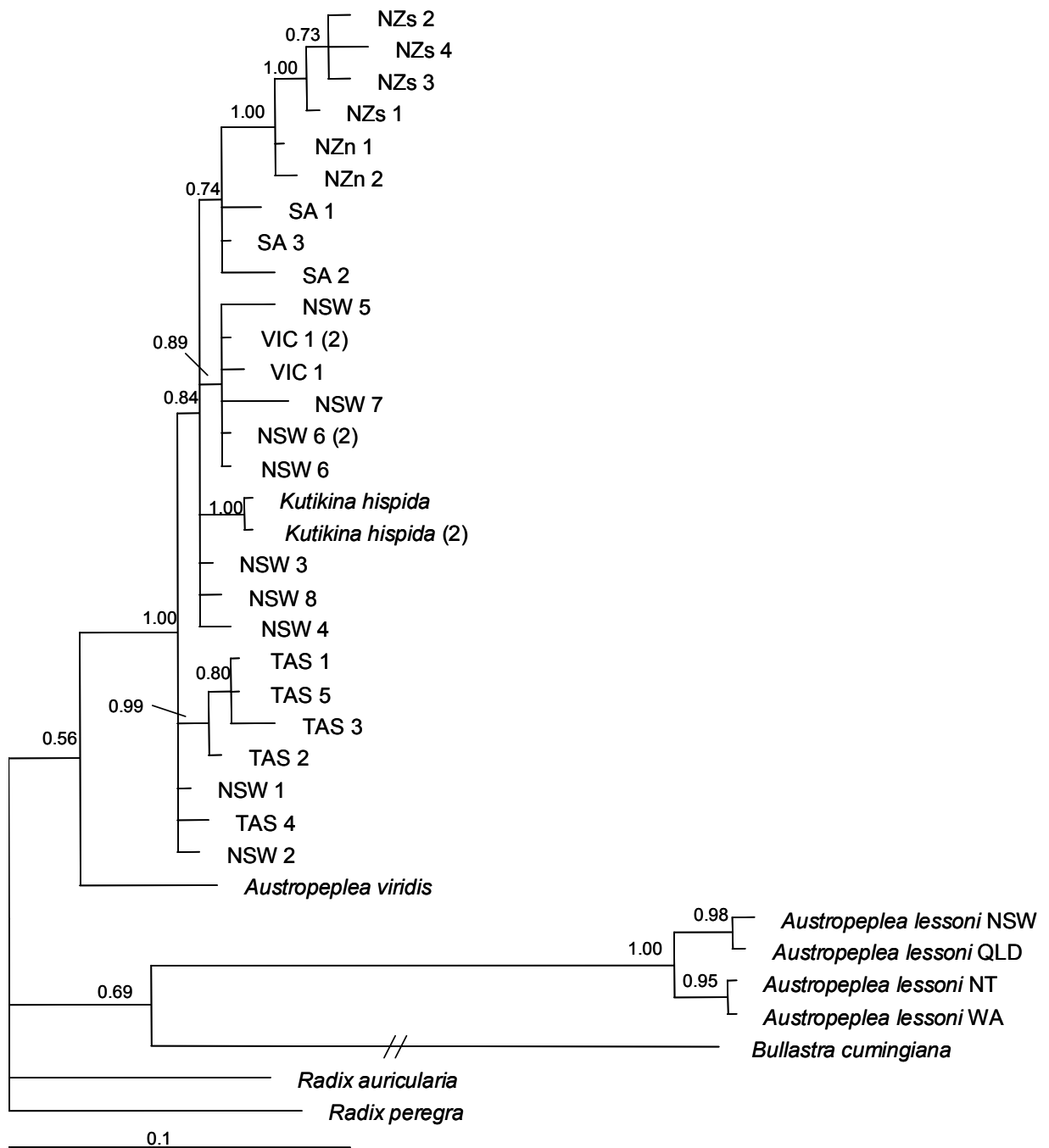
Figure 2.6 Phylogeny of the *Austropeplea tomentosa* complex based on ITS-2 rRNA sequences.
Majority rule tree based on Bayesian inference with maximum likelihood setting under HKY
model for DNA substitution. Bayesian analysis is based on the 5 000 000 generations replicates,
with the posterior possibilities indicated above the branch, only posterior probability >50%
given. NSW= New South Wales, Australia, NZn= New Zealand, North Island, NZs= New
Zealand, South Island, SA= South Australia, TAS= Tasmania, Australia, VIC= Victoria,
Australia, // = branch length shortened. Taxa without names are currently recognised as *A.*
tomentosa.

2.3.2.3 Combined 16S and ITS-2 Phylogenies

The ILD test demonstrated no conflict between the 16S and ITS-2 datasets (p= 0.20). Bayesian and MP analyses of the combined molecular dataset resulted in very similar tree topologies (Figs 2.7, 2.8). *Austropeplea tomentosa* diverges into two separate lineages, New Zealand samples are sister to the Australian samples. This relationship has strong support (Figs 2.7, 2.8) and was recovered in the 16S phylogenies. The New Zealand lineage is further divided into North and South Island samples, with reasonable levels of support (Figs 2.7, 2.8). *Austropeplea tomentosa* is not monophyletic, due to *Kutikina hispida* being placed as sister to the Australian samples of *A. tomentosa*. This relationship, while not shown in previous single gene analyses, has low bootstrap and posterior probability support (Figs 2.7, 2.8). All analyses show the Australian samples of *A. tomentosa* as a large polytomy, thereby providing no resolution for this group. Within the polytomy there is a small amount of geographical separation amongst the Australian samples of *A. tomentosa*, although the branch lengths are only short (Fig 2.8). The Tasmanian populations form a clade, with reasonable posterior probability support but low bootstrap support (Figs 2.7, 2.8). A similar pattern is seen with the northern New South Wales populations (NSW 1, NSW 2) and the more southern New South Wales populations (NSW 3, NSW 4, NSW 8).

The basal relationships shown from the combined molecular analyses are clearer than in the single gene phylogenies. *Austropeplea lessoni* and *Bullastra cumingiana* form a well supported basal clade, with *A. viridis* placed as sister to the *A. tomentosa* complex (Figs 2.7, 2.8). *Austropeplea lessoni* forms a well supported monophyletic group, that is further divided into well supported geographic clades of eastern (NSW and QLD) and western (NT and WA) Australia (Figs 2.7, 2.8). These relationships will be dealt with further in Chapter Three.
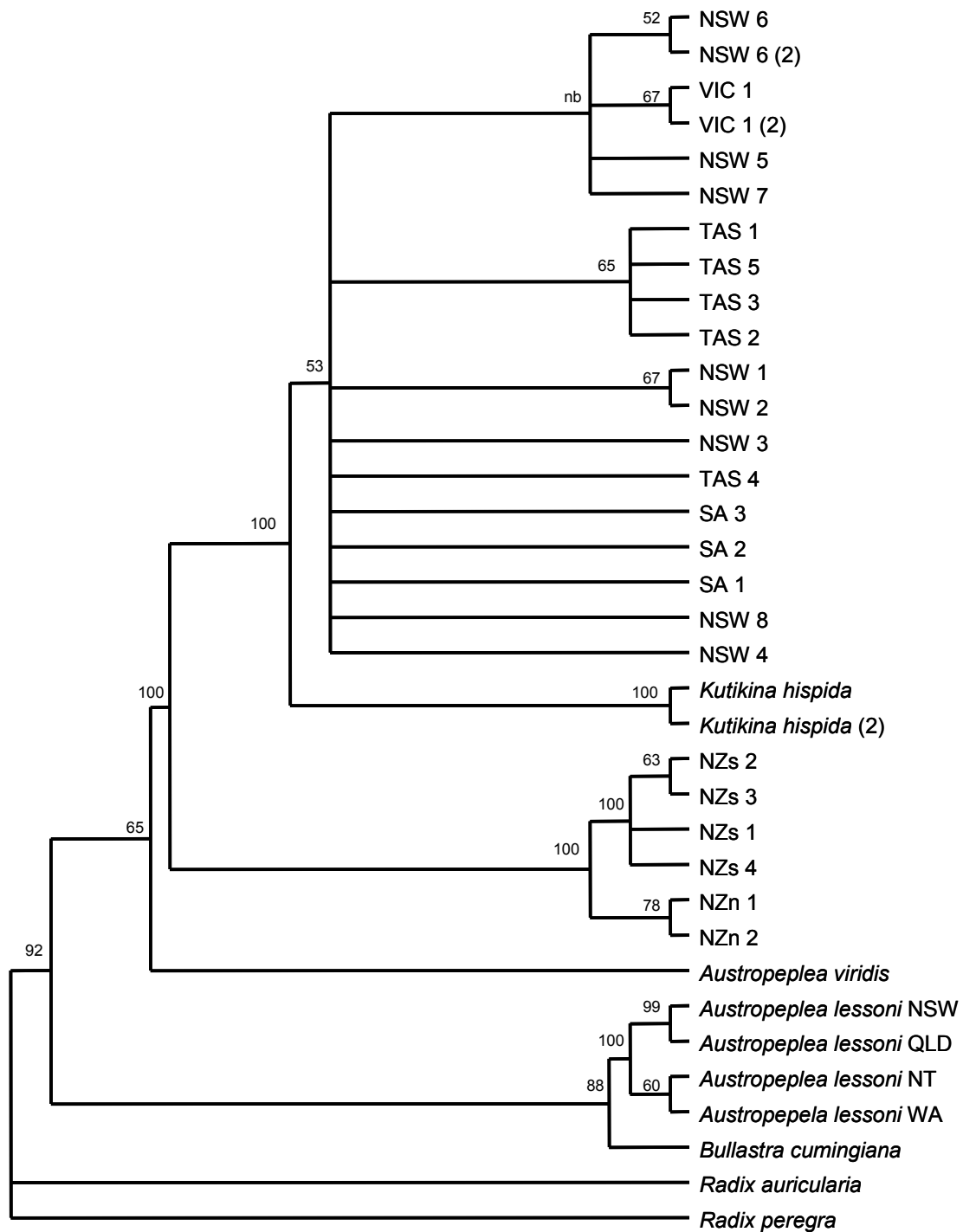
Figure 2.7 Phylogeny of the *Austropeplea tomentosa* complex based on combined 16S and ITS-2 sequences. Strict consensus tree of 36 maximum parsimony trees, with tree length 488. Numbers above the branches are bootstrap scores, with only bootstrap >50% given. nb= no bootstrap support, NSW= New South Wales, Australia, NZn= New Zealand, North Island, NZs= New Zealand, South Island, SA= South Australia, TAS= Tasmania, Australia, VIC= Victoria, Australia. Taxa without names are currently recognised as *A. tomentosa*.
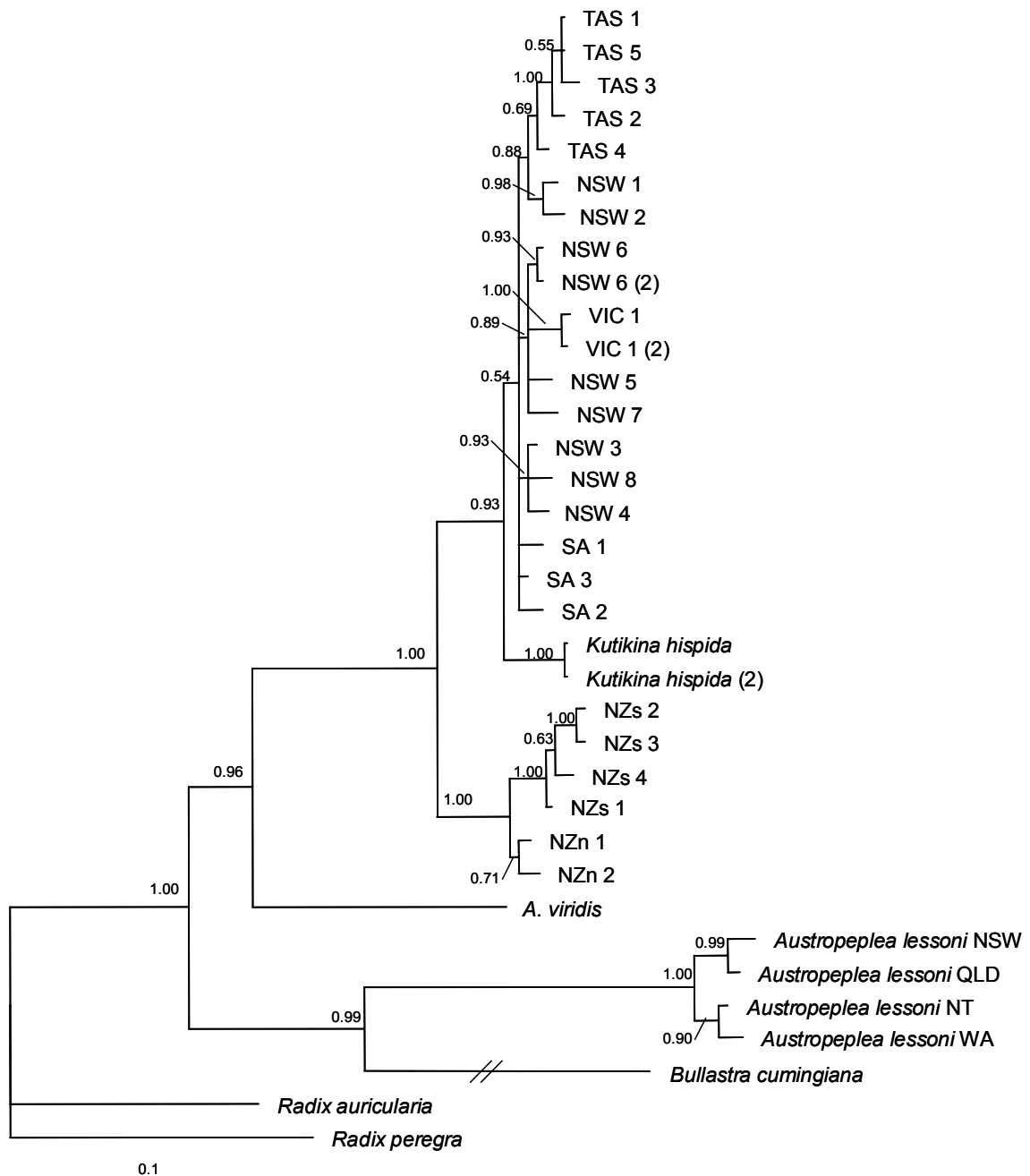
Figure 2.8 Phylogeny of the *Austropeplea tomentosa* complex based on combined 16S and ITS-2 sequences. Majority rule consensus tree based on Bayesian inference with maximum likelihood setting under a partitioned model for DNA substitution. Bayesian analysis is based on the 5 000 000 generations replicates, with the posterior possibilities indicated above the branch, only posterior probabilities >50% are given. NSW= New South Wales, Australia, NZs= New Zealand, South Island, NZn= New Zealand, North Island Australia, SA= South Australia, TAS= Tasmania, VIC= Victoria, Australia, // = branch length shortened. Taxa without names are currently recognised as *A. tomentosa*.

2.3.3 Anatomical phylogeny and variation

Of the 57 characters used in the MP analyses, 45 were parsimony informative, resulting in 54 equally parsimonious trees with a tree length of 162 (CI=0.49, RI=0.78, RC=0.41). A strict consensus of these 54 trees is shown in Figure 2.9. A list of the character statistics for the anatomical analysis can be found in Appendix 2.7.

There are striking differences of the topology of the anatomical phylogeny compared to the molecular phylogenies. *Austropeplea tomentosa* forms a monophyletic group that is characterised by two large polytomies (Fig 2.9). The basal polytomy includes the New South Wales samples of *A. tomentosa*, and the second polytomy contains the Tasmanian, South Australian and New Zealand samples. Within *A. tomentosa*, the most northern New South Wales samples form a well supported clade (bp=98) as do the South Australian (bp=100) and New Zealand (bp=93) samples (Figure 2.9). Very few of the higher nodes are well supported in the anatomical phylogeny (Figure 2.9). The node supporting the monophyly of *A. tomentosa* has very low bootstrap support (bp=56), as does the polytomy supporting the Tasmanian, South Australian and New Zealand samples (bp=58).

Kutikina hispida and *A. viridis* were recovered as sister taxa to the *A. tomentosa* complex, although support for this relationship is low. *Bullastra cumingiana* forms the most basal clade in the anatomical phylogeny. This relationship has high bootstrap support and is further supported by four synapmorphies. *Bullastra cumingiana* is sister to the reasonably supported and monophyletic *Austropeplea lessoni*. Moreover, *A. lessoni* is supported by two synapomorphies.

Examination of the parsimony informative anatomical characters revealed all of the shell and female reproductive characters to be homoplastic when traced on the tree topology. Moreover, several characters were polymorphic within samples from the same geographic area, indicating they are not very useful for distinguishing (character numbers 9, 11, 14, 27, 29, 33, 38, 44, and 47). Characters from the kidney, nervous system, male and reproductive system were useful in defining phylogenetic relationships. Of the 45 parsimony informative characters used in the anatomical analysis, 13 formed synapomorphies on the strict consensus tree (Fig 2.9). Two synapomorphies support the monophyly of *A. tomentosa*, a short cerebral commissure

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and a broad anterior end of the kidney (Fig 2.9). A wide upper prostate and short pulmonary vein supports the Tasmanian, South Australian and New Zealand clade (Fig 2.9). *Kutikina hispida* and the South Australian samples displayed a number of autapomorphies (Fig 2.9.).

The *Bullastra*, *Austropeplea*, and *Kutikina* clade is supported by four synapmorphies; the absence of an outer lobe and thin upper plate of pneumostome, a long cerebral commissure and a straight lower prostate (Fig 2.9). The *A. lessoni* group are characterised by an enlarged pedal commissure and a looped seminal vesicle. The clade comprising *Austropeplea tomentosa*, *A. viridis* and *Kutikina* is characterised by the absence of a right lobe in the kidney and a pulmonary vein that runs along the right hand side of the kidney.

2.3.4 Combined molecular and anatomical phylogeny

The ILD test indicated significant incongruence between the molecular (16S + ITS-2) and anatomical datasets (p= 0.01). The combined molecular and anatomical dataset contained 217 parsimony informative characters and produced 4 equally parsimonious trees with tree length 673 (CI=0.63, RI=0.80, RC=0.57). A strict consensus of these 4 trees is shown in Figure 2.10. Bootstrap analysis and Bayesian inference produced trees with differing topologies to the strict consensus tree (Figs 2.10, 2.11).

The MP analysis divides the *Austropeplea tomentosa* complex into a number of separate lineages (Fig 2.10). The New Zealand samples are shown as a well supported basal lineage to the other samples of *A. tomentosa* (Fig 2.10). *Austropeplea tomentosa* is polyphyletic, as *Kutikina hispida* was shown as sister to the Australian samples of *A. tomentosa*. This relationship has minimal bootstrap support (bp=52).

Figure 2.9 Phylogeny of the *Austropeplea tomentosa* complex based on 45 anatomical characters. Strict consensus tree of 54 maximum parsimony trees with branch length 162. Below branches are autapomorphies and synapmorphies (in bold), numbers corresponding with characters and character states as listed in Table 2.3. Numbers above the branches are bootstrap scores, with only bootstrap >50% given. nb= no bootstrap support, NSW= New South Wales, Australia, NZn= New Zealand, North Island, NZs= New Zealand, South Island, SA= South Australia, TAS= Tasmania, Australia, VIC= Victoria, Australia. Taxa without names are currently recognised as *A. tomentosa*.

The Australian samples are further divided into distinct geographic regions, with the Victorian and southern New South Wales (NSW 6) samples at the base of the Australian *A. tomentosa* clade. The next clade is represented by northern New South Wales samples (NSW 1 and NSW 2). A polytomy of the other New South Wales samples then forms as sister to the most diverged group, the Tasmanian and South Australian samples of *A. tomentosa* (Fig 2.10).

Much of this geographical separation of *A. tomentosa* observed in the MP analysis has poor bootstrap support or less than 50% bootstrap support (Fig 2.10). Moreover, the Bayesian analysis recovers the Australian samples of *A. tomentosa* as a large polytomy. Included in the polytomy is *Kutikina hispida*. The branch length of *K. hispida* is longer than any other samples of the Australian *A. tomentosa*, indicating a greater level of divergence. The position of the New Zealand samples in the bootstrap and Bayesian inference trees is the same as that shown on the strict consensus. Support for the separation of the Australian and New Zealand samples of *A. tomentosa* is much weaker than in previous molecular analyses (Fig 2.10, 2.11).

The relationships of the basal clades in the combined molecular and anatomical phylogenies are the same as those shown in the combined molecular phylogenies. Support for these relationships is strong both in terms of bootstrap and posterior probability values (Fig 2.10, 2.11).

The addition of the anatomical data to the combined molecular analyses resulted in decreasing posterior probability support and changes to tree topology. Support for the Australian clade of *A. tomentosa* decreased and the branch supporting the position of *Kutikina hispida* as sister taxa to the Australian populations of *A. tomentosa* collapsed into the Australian group. Moreover, the CI and RI indices of the combined anatomical and molecular dataset are lower than those of the combined molecular dataset.
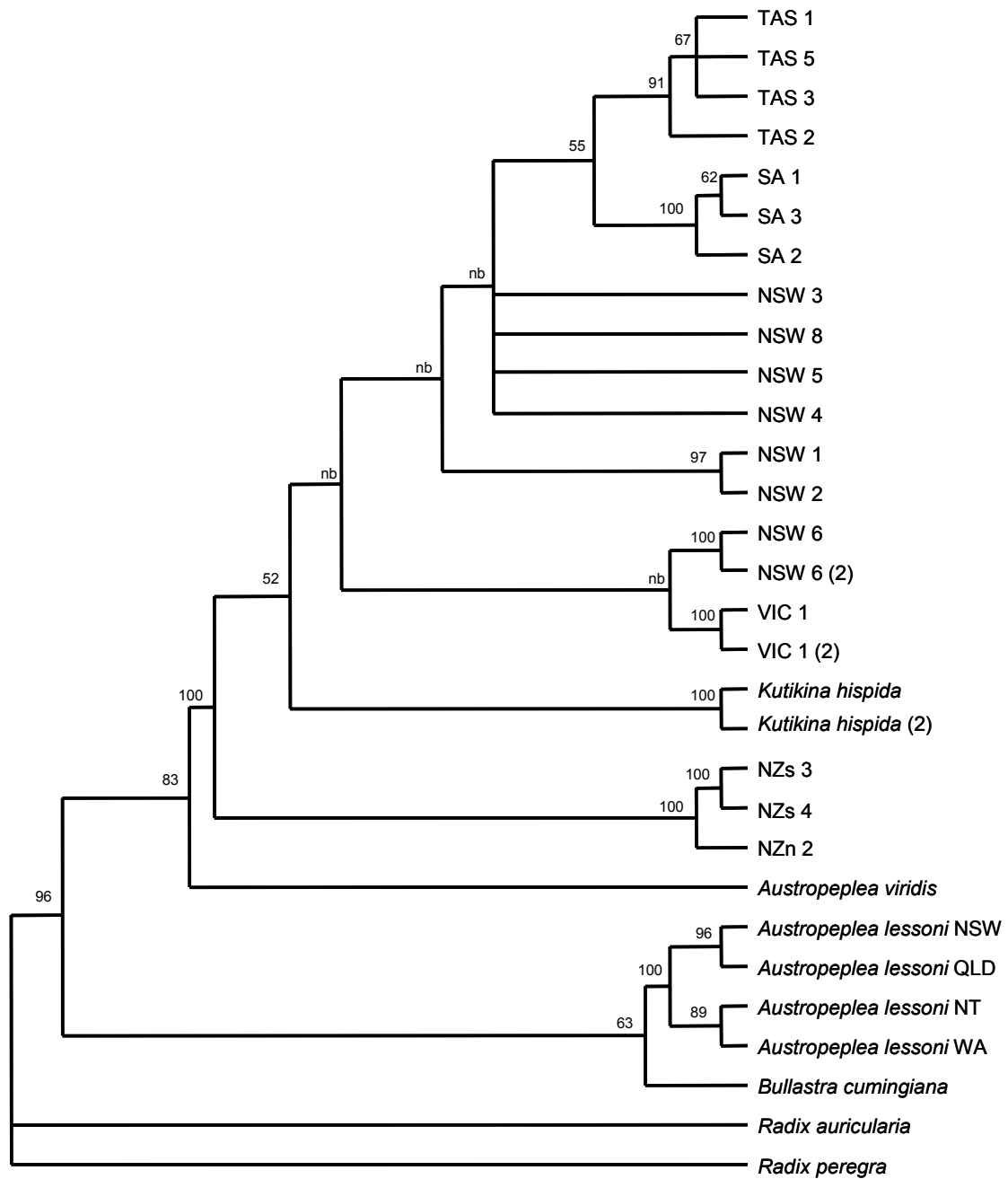
Figure 2.10 Phylogeny of the *Austropeplea tomentosa* complex, based on anatomical and molecular characters. Strict consensus tree of 4 maximum parsimony trees with branch length 673. Numbers above the branches are bootstrap scores, with only bootstrap >50% given. nb= no bootstrap support, NSW= New South Wales, Australia, NZn= New Zealand, North Island, NZs= New Zealand, South Island, SA= South Australia, TAS= Tasmania, Australia, VIC= Victoria, Australia. Taxa without names are currently recognised as *A. tomentosa*.
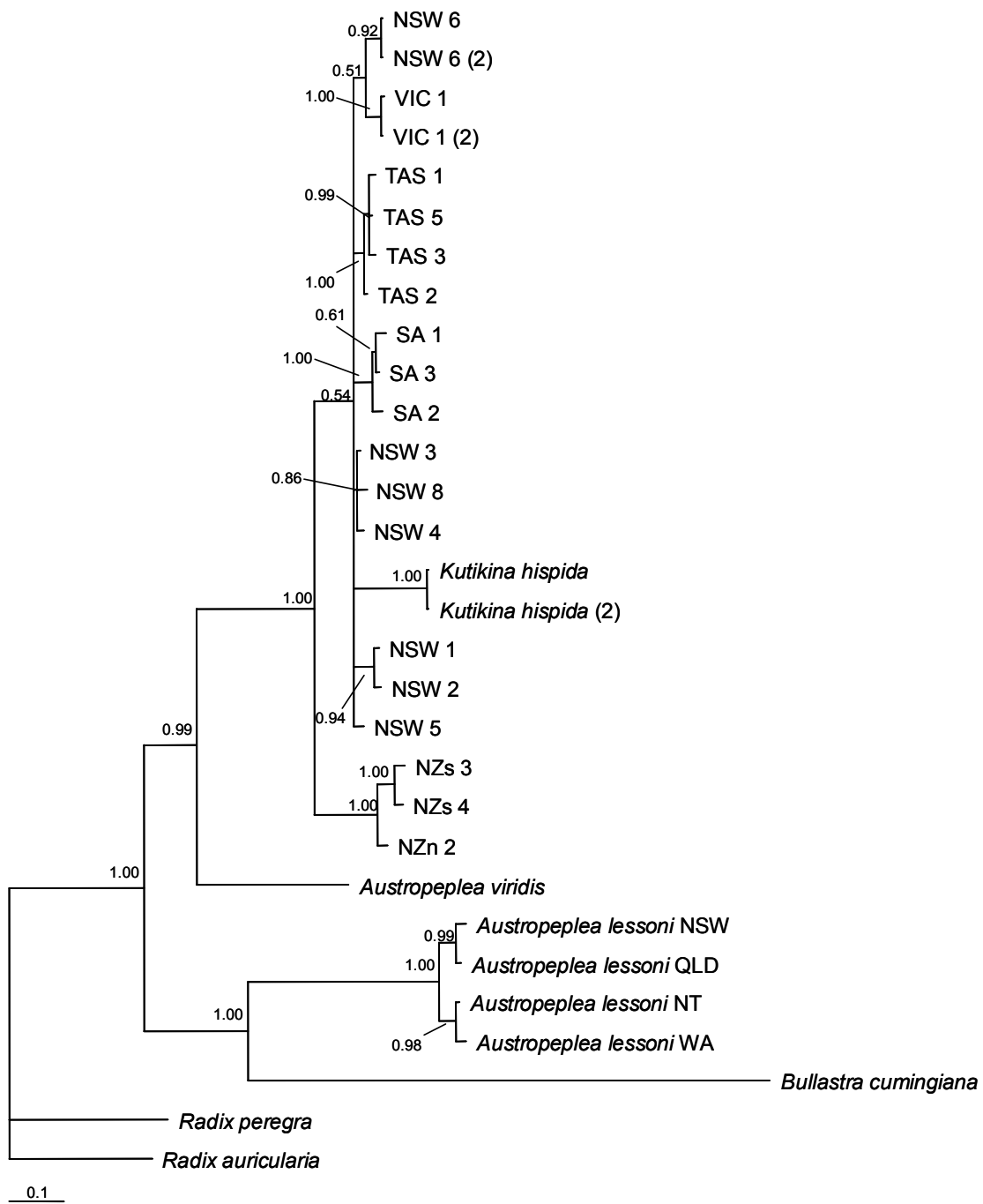
Figure 2.11 Phylogeny of the *Austropeplea tomentosa* **complex, based on anatomical and molecular characters. Majority rules consensus tree based on Bayesian inference, maximum likelihood setting under a partitioned model for DNA substitution, 5 000 000 generations. Posterior probabilities indicated above the branch, only posterior probabilities >50% given. NSW= New South Wales, Australia, NZs= New Zealand, South Island, NZn= New Zealand, North Island, SA= South Australia, TAS= Tasmania, Australia, VIC= Victoria, Australia. Taxa without names are currently recognised as** *A. tomentosa***.**

2.3.5 Shell morphometrics

Shell morphology is a character that is broadly used to identify and distinguish molluscan species. Therefore I examined whether this technique could reliably be used to distinguish the phylogenetic groups identified above.

The DFA showed that there was a significant difference in the shell morphology between the five *a priori* geographic regions of the *Austropeplea tomentosa* complex. (Wilk's Lambda= 0.084, χ^2=30.13, df = 16, p<0.0001). The South Australian, Kosciuszko and Tasmanian samples of *A. tomentosa* were significantly different from all other samples of *A. tomentosa*. Surprisingly, the New Zealand and East Australian samples of *A. tomentosa* were not significantly different to one another (Fig 2.12), given their distinct phylogenetic positions.
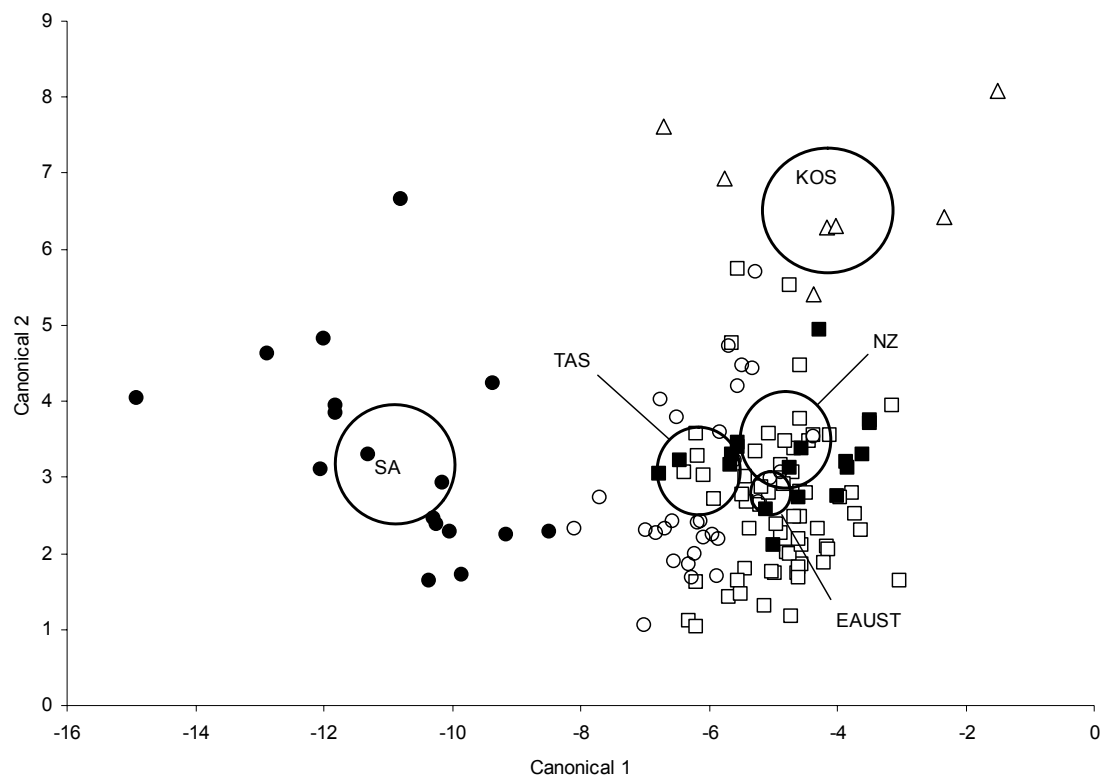
Figure 2.12 Canonical plot showing the point and multivariate means of DFA for shell measurements of populations of *Austropeplea tomentosa*. Circles correspond to multivariate mean of each group with a 95% confidence limit. EAUST= Eastern Australia (□), KOS= Kosciuszko Plateau (Δ), NZ= New Zealand (■), SA= South Australia (●),TAS= Tasmania (○).

The DFA based on shell morphology distinguished four groups of *Austropeplea tomentosa*, however, these groups do not represent the same phylogenetic groups

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identified in the molecular and anatomical analyses. Based on shell morphology the New Zealand samples of *A. tomentosa* are indistinguishable from New South Wales and Victorian populations of *A. tomentosa*, while in the 16S and combined molecular analyses the New Zealand samples of *A. tomentosa* form a distinct diverged lineage from the Australian samples of *A. tomentosa*. Moreover, in the anatomical phylogeny the New Zealand taxa are recovered as more closely related to the South Australian and Tasmanian samples of *A. tomentosa*. The Kosciuszko samples of *A. tomentosa*, while having distinct shell morphology, are not a distinct group based either molecular or anatomical phylogenies. The Tasmanian and South Australian samples of *A. tomentosa* form small clades within the molecular and anatomical analyses, however, there is not good support in either analyses to suggest that these are distinct groups as indicated by the shell morphology results.

All four of the shell variables contributed significantly to the analysis, with shell length and aperture length contributing the most to canonical function 1 and aperture width and shell width contributing the most to canonical function 2. The first and second canonical function account for 95.1% of the total variation, with respective eigen values of 4.17 and 0.820. The DFA classified 74.6% of the original grouped cases into their correct groups, and 73.1% of cross-validated grouped cases were correctly identified.

All five variables to be used in the discriminate function analysis (DFA) had a normal distribution. Last whorl length was very highly correlated with shell length and aperture length, with variance inflation factors of 50 and 25, respectively. Variance inflation factors greater than 10 are indicative of high correlation between variables (Hair et al. 1998). In order not to violate the assumption that two or more variables should not be highly correlated, last whorl length was excluded from the DFA. A Box's M test was used to test the assumption of homogeneity of covariance matrices. The significant result (Box's M= 269.308, approx. $F_{90, 5012}=2.469$, p=0.000), violates the assumption that covariance matrices of groups should not differ. However, DFA is robust even when the homogeneity of variances assumption is not met and the violation of this assumption is not likely to affect the conclusions of the DFA (Lachenbruch 1975; Klecka 1980).

2.4 Discussion

2.4.1 Species status of Australian and New Zealand samples of *Austropeplea tomentosa*

This study resolved the Australian and New Zealand samples of *Austropeplea tomentosa* as separate species, a finding that is in contrast to the currently accepted taxonomy (Boray and McMichael 1961; Climo and Pullan 1972), although suspected by other workers (Hubendick, 1951; Ponder and Waterhouse 1997). The two highly divergent clades in the 16S and combined molecular phylogenetic analysis indicate the presence of two independent evolving lineages. Sequence divergence between the Australian and New Zealand samples of *A. tomentosa* was as high as between other lymnaeid species (Remigio and Blair 1997a; Bargues *et al.* 2001; Remigio 2002; Bargues *et al.* 2003). Moreover, the length of the ITS-2 region of the New Zealand samples of *A. tomentosa* was up to 32 base pairs shorter than the Australian populations.

With the Tasman Sea acting as significant barrier to gene flow, Australian and New Zealand species of *Austropeplea tomentosa* may well have been reproductively isolated for millions of years. The Tasman Sea formed over 55 million years ago, as a result of sea floor spreading between Australia and New Zealand (Veevers *et al.* 1991; Sutherland 1999). Mitochondrial DNA divergence for freshwater molluscs is thought to range between 1-5% per million years (Pfenninger *et al.* 2003). Under a conservative assumption of 1%, the estimated age of separation between the Australian and New Zealand samples would be in the late Miocene (4.9 to 7 Mya; with a 4.9 to 7% sequence divergence). Therefore, divergence due to vicariance events seems unlikely. A more recent divergence of the Australian and New Zealand species of *A. tomentosa* is more probable, possibly due to dispersal by birds. Fossil evidence of *A. tomentosa* in both Australian and New Zealand only dates back 2 million years (Gill and Banks 1956; Climo and Pullan 1972; Climo 1984), thus is in accord with a dispersal event in the late Miocene. A more recent divergence of the Australian and New Zealand species of *A. tomentosa* also accords with the Australian

lymnaeids being one of the most recently derived lymnaeid groups (Remigio and Blair 1997a; Remigio 2002).

In addition, there is geographic structuring of the New Zealand species of *Austropeplea tomentosa* between the North and South Islands in all phylogenetic analyses. New Zealand has been subject to considerable geological change in the last 12 million years. Indeed, the New Zealand archipelago has been characterised by marine intrusions, uplift separating east and west coasts and glacial/ interglacial oscillations (Stevens *et al.* 1995). Such extensive geological changes, in addition to the Cook Strait acting as a barrier to gene flow, could result in the formation of distinct species on the two islands. Therefore, these taxa need more investigation. Examination of the anatomy and of the taxonomic status of the New Zealand species of *A. tomentosa* taxa is undertaken in Chapter 5.

The lack of ITS-2 divergences within the *Austropeplea tomentosa* complex contrasts strongly with the diversity evident from 16S. The inability of ITS-2 to resolve the New Zealand and Australian species of *A. tomentosa* into two divergent clades was surprising considering that ITS-2 has been successfully used in previous studies to identify groups that have diverged in the last 50 million years (Vidigal *et al.* 2000; Bargues *et al.* 2001; Coleman and Vacquier 2002; Mavarez *et al.* 2002; Oliverio *et al.* 2002; Bargues *et al.* 2003; Insua *et al.* 2003). The lack of ITS-2 diversity between the Australian and New Zealand clades may reflect transmission genetics, whereby coalescence theory predicts that mitochondrial organelles will evolve more quickly than nuclear loci (Simon 1991). A number of studies have shown that mitochondrial genes become monophyletic more quickly than nuclear loci, thus when mitochondrial loci reach monophyly, the average nuclear locus will still be either polyphyletic or paraphyletic (see Palumbi *et al.* 2001 for review). This theory accords with the smaller number of parsimony informative characters observed in the ITS-2 alignment as compared to 16S, and the previously suggested recent divergence time of the New Zealand and Australian samples of *A. tomentosa*.

Neither the anatomical nor shell morphological analyses reflected the separate species status of the Australian and New Zealand samples of *Austropeplea tomentosa,* as shown by 16S. Based on the anatomical phylogeny, the Australian and New

Zealand species of *A. tomentosa* were indistinguishable. Moreover, it was impossible to differentiate between the New Zealand and eastern Australian samples of *A. tomentosa* based on shell morphology. The lack of morphological differentiation among closely related species may suggest a recent divergence. Alternatively, morphological divergence may be slow due to habitat stability. Morphological differences are thought to reflect underlying genetic differences; however the incongruence between the molecular and morphological results for the *A. tomentosa* complex suggests that the New Zealand samples of *A. tomentosa* may represent a cryptic species. Cryptic speciation has been demonstrated in a number of freshwater molluscs (Jones *et al.* 2001; Baker *et al.* 2003; Liu *et al.* 2003; Pfenninger *et al.* 2003).

2.4.2 Species status of the Australian populations of *Austropeplea tomentosa*

The Australian samples of *Austropeplea tomentosa*, despite their large geographical separations, represent only one species. In all of the molecular analyses and the combined anatomical and molecular analysis, the Australian samples of *A. tomentosa* cannot be resolved, indicating that only one taxon is present. Moreover, genetic divergence of the 16S gene between the Australian samples of *A. tomentosa* is low compared to other species of Lymnaeidae (Remigio and Blair 1997a). The genetic divergence observed between the ITS-2 sequences of the Australian samples ranged from between zero and 4%. Studies of northern hemisphere lymnaeids have suggested that between proximal species, ITS-2 genetic divergence varies between 2.30 and 10.15% (Bargues *et al.* 2001). The Australian samples of *A. tomentosa* fall within the lower end of this range, however, there is a lack of consistent divergence levels between geographic areas.

However, both the anatomical data and shell morphologies showed distinct geographic groupings of the Australian species of *Austropeplea tomentosa*. The anatomical phylogeny indicates there are distinguishable groups of *A. tomentosa* within the Australian species. However, these groups have either weak bootstrap support or less than 50% bootstrap support at all, suggesting the anatomical phylogeny should be treated with some caution. Furthermore, the addition of the

anatomical dataset to the combined molecular dataset resulted in a destabilisation of relationships and support. Only a small number of anatomical characters were available relative to the molecular datasets, and the majority of these characters showed homoplasy in the tree topology. Moreover, only a small number of synapomorphies supported the geographical groups of *A. tomentosa*. This level of homoplasy is thought to be common in closely related species (Fukuda and Ponder 2005), indicating that anatomical characters should be used in conjunction with molecular methods when trying to understand the relationships of closely related species.

The shell morphologies of the Australian species of *Austropeplea tomentosa* indicate that the Tasmanian, South Australian, Eastern Australian and Kosciuszko samples are distinct. These distinctions lack support from both the molecular and anatomical data. The molecular data does not distinguish any of the Australian samples of *A. tomentosa*. The anatomical data does not distinguish the Kosciuszko samples as distinct from the other east Australian samples of *A. tomentosa*. The New Zealand species of *A. tomentosa* in the anatomical phylogeny are placed as more closely related to the South Australian and Tasmanian samples of *A. tomentosa*, but the shell morphology groups New Zealand with samples of *A. tomentosa* from New South Wales and Victoria. Moreover, only 73% of all the samples were correctly classified into their correct *a priori* geographical regions, indicating relatively high levels of shell shape variation within *A. tomentosa*. These findings are not in contrast with other members of the Lymnaeidae, who display large amounts of phenotypic variation (Hubendick 1951; Arthur 1982; Lam and Calow 1988; Evans 1989; Oviedo *et al.* 1995; Ward *et al.* 1997; Wullschleger and Jokela 2002). Moreover, it has been shown that shell morphology can depend on environmental factors such as habitat type and water movement (Arthur 1982; Lam and Calow 1988). Single species with polymorphic populations have been identified in other freshwater molluscs (Wilke and Falniowski 2001). The distinct morphologies of *A. tomentosa* that are observed in different geographic regions of Australia therefore may represent local adaptations in different habitats. Further examination of anatomy and the taxonomic status of the Australian *A. tomentosa* taxa will be carried out in Chapter 5.

The South Australian samples of *Austropeplea tomentosa* displayed a number of unique anatomical and molecular characteristics. Anatomical characters included longer shell, tentacles, praeputium and prostate, as compared to the other Australian samples of *A. tomentosa*. The penal differences observed in the South Australian samples have been used by other workers to distinguish species (Hubendick 1951; Jackiewicz 1993a). However, the autapomorphic nature of these characters means they are of little use in understanding phylogenetic relationships of Australian samples of *A. tomentosa*. The South Australian samples of *A. tomentosa* consistently had the shortest ITS-2 region of all the Australian samples. Moreover, the South Australian samples of *A. tomentosa* occur at the western margin of the range of *A. tomentosa* within Australia. Thus, it is possible that the South Australian samples of *A. tomentosa* may have recently diverged or are currently undergoing speciation. Future studies are needed to better define the relationship between the South Australian samples and other samples of the Australian *A. tomentosa*.

Surprisingly, geographical barriers, including the Great Dividing Range and the Tasman Sea do not seem to act as barriers to gene flow between samples of *Austropeplea tomentosa* within Australia. The Tasman Sea is a 200km wide stretch of sea separating mainland Australia and Tasmania. In the molecular phylogenetic analyses while Tasmania formed small clades, these clades were part of the larger polytomy formed by the Australian samples of *A. tomentosa*. Therefore, genetically, the Tasmanian and mainland Australian samples cannot be distinguished. Interestingly, the Cook Strait separating the North and South Islands of New Zealand is smaller than Bass strait, yet the North and South island samples of *A. tomentosa* form distinct clades in the molecular phylogenies. Due to dropping sea levels during the Pleistocne an intermittent land bridge between mainland Australia and Tasmania formed (Lamback and Chappell 2001). While it is though that the land bridge was never greater than 10 metres above sea level (Lamback and Chappell 2001), it is possible that birds dispersing between the mainland and Australia could have facilitated gene flow between mainland Australia and Tasmania.

2.4.3 Phylogenetic position of *Kutikina hispida*

Kutikina hispida was thought to have been a Gondwanan relic, derived from the *Austropeplea* group (Ponder and Waterhouse 1997). However, this study indicates that *K. hispida* is more closely related to the Australian species attributed to *A. tomentosa* than originally thought. *Kutikina hispida*, while morphologically distinct from the *A. tomentosa* complex, is more closely related to the Australian *A. tomentosa* species than to the New Zealand species. Both the 16S and ITS-2 phylogenetic analyses place *K. hispida* within the Australian *A. tomentosa* group, however the combined molecular analysis places *K. hispida* as sister to the Australian *A. tomentosa* species, albeit with weak support. These findings suggest that *K. hispida* and the Australian *A. tomentosa* species may be ecophenotypes, the distinctive shell and anatomical characters of *K. hispida* induced by the fast flowing habitat that it occurs in. Alternatively *K. hispida* and the Australian *A. tomentosa* could be distinct species that originated relatively recently, accounting for the small number of phylogenetically informative characters detected in the molecular datasets. The latter hypothesis appears to be more plausible, owing to other lines of evidence. During the Quaternary, south western regions of Tasmania were subject to a number of glacial periods (Kiernan 1983, 1989; Hannan *et al.* 1993), a process that could have facilitated the split between the *A. tomentosa* stock and *K. hispida*. The mitochondrial genetic divergence between *K. hispida* and the Australian *A. tomentosa* group (1.9 to 3.3%) is as high as between other lymnaeid species (Remigio and Blair 1997a). Moreover, mitochondrial DNA divergence for *Kutikina hispida* using a conservative estimate would place its divergence age within the late Pliocene (1.9 to 3 Mya).

Given the evidence of such a close phylogenetic relationship between the Australian samples of *Austropeplea tomentosa* and *Kutikina hispida*, their placement in the same genus is more suitable than the current taxonomy. Moreover, levels of 16S gene divergence greater than 10% are thought to represent separate genera (Remigio 2002); 16S gene divergence between the Australian samples of *A. tomentosa* and *K. hispida* were 8.2 to 9.4%, suggesting placement within the same genus. Designations will be discussed more in Chapter 5.

2.4.4 Previous studies of the taxonomy of *Austropeplea tomentosa*

The Australian samples of *Austropeplea tomentosa* display a large amount of shell and anatomical differences throughout their distribution. Conversely, the New Zealand species of *A. tomentosa* do not differ in any major anatomical details from the Australian species, but the molecular data shows that they represent a distinct evolutionary lineage. With anatomical differences between the Australian samples being greater than anatomical differences between Australian and New Zealand samples, and without information on DNA divergences, it is not surprising that previous workers (Hubendick 1951; Boray and McMichael 1961; Climo and Pullan 1972) have maintained that the New Zealand and Australian samples of *A. tomentosa* are variants of the one species.

The high susceptibility of both the Australian and New Zealand populations of *Austropeplea tomentosa* to *Fasciola hepatica* was used as evidence for the existence of a single species (Boray and McMichael 1961). However, infection from *Fasciola hepatica* has not been uniform. *Fossaria truncatula* (Müller, 1774) is the original host of *F. hepatica*. But as *F. hepatica* has been introduced throughout the world, it has used endemic lymnaeids as its host (Boray 1966, 1969). With host addition usually occurring in closely related groups (Blair *et al.*, 2001), it is not surprising that both the Australian and New Zealand species of *A. tomentos*a are susceptible to *F. hepatica*, considering their close phylogenetic relationship. Host parasite associations to appear unreliable for determining species boundaries, at least in the lymnaeids.

2.4.5 Phenotypic plasticity

The incongruence between shell morphology, anatomical variation and genetic divergence indicate that phenotypic plasticity is prevalent amongst the New Zealand and Australian species of *Austropeplea tomentosa*. Throughout its distribution, *A. tomentosa* inhabits slow flowing waters such as small creeks and streams, lagoons, dams, and swamps (Boray 1964; Pullan *et al.* 1972; Kershaw 1975; Smith and Kershaw 1979; Smith 1992). Such freshwater environments are heterogeneous, and the ability of one phenotype to display high fitness in all ranges of the environment is

unlikely. In such dynamic environments, phenotypic plasticity can be an advantageous trait, as it allows for modification of the phenotype to suit particular environments or habitats (Via *et al.* 1995; Yeap *et al.* 2001; Britton and McMahon 2004). The variation observed within the *A. tomentosa* complex may have evolved in response to variable local environmental conditions. Undertaking an analysis of the relationship between environmental conditions and morphological variation in *A. tomentosa* would be useful in understanding the role phenotypic plasticity plays in maintaining anatomical and shell variation.

2.4.6 Dispersal, isolation and speciation

Dispersal is thought to be associated with the long term persistence of freshwater taxa, due to range expansion and the transfer of genes from one population to another (Bilton *et al.* 2001). Assuming that the gene flow is greater than the differentiation arsing from contemporary evolutionary factors, gene flow increases the levels of genetic diversity within local populations and decreases levels of diversity between populations (Colgan and Ponder 1994; Bilton *et al.* 2001; Bohonak and Jenkins 2003). This study suggests that gene flow between the subdivided Australian samples of *Austropeplea tomentosa* may be large enough to overcome the evolutionary factors such as genetic drift, local selection pressures and mutation. Within Australia, gene flow between isolated populations of *A. tomentosa* may increase seasonally during periods of flood and high water flow and during seasonal migration of birds. Geographical barriers within Australia, such as the Great Dividing Range and Bass Strait, may not be large enough to stop passive dispersal by birds. The Tasman Sea, however, is 2000 km wide and represents a significant barrier to gene flow, as indicated by the diverged lineages of the Australian and New Zealand populations of *A. tomentosa*.

Chapter 3   Systematics of the *Austropeplea lessoni* complex

3.1 Introduction

There has been recent interest in the biogeography of widespread Australian freshwater species, largely due to the unique situation Australian freshwater systems present for such studies (Unmack 2001; Munasinghe *et al.* 2004; Nguyen *et al.* 2004). Australian freshwater systems are characterised by diverse organisms, one of which is the freshwater molluscs. While the Australian freshwater molluscan fauna is not as diverse as other regions of the world, a recent review demonstrated that it is composed of over 430 species, of which 99% are endemic and 42% undescribed (Ponder and Walker 2004). Indeed, there are a large number of small range endemics associated with arid zone springs (Ponder and Clark 1990; Ponder 1995; Ponder *et al.* 1996) or temperate- zone permanent streams (Ponder *et al.* 1993; Ponder and Waterhouse 1997). These recent discoveries indicate that further taxonomic investigation of the Australian freshwater molluscan fauna is required before it can be regarded as well documented.

Austropeplea lessoni (Deshayes, 1830) is the largest of the three lymnaeid species endemic to Australia (Boray and McMichael 1961; Smith 1992, Ponder and Waterhouse 1997). A certain degree of taxonomic uncertainty surrounds the group, due to three main factors. First its wide distribution throughout Australia, second the taxonomic status of the group has never been thoroughly investigated, and finally the group is characterised by numerous synonymies that have never been rigorously tested. Furthermore, some workers have recognised that *A. lessoni* may represent more than one species (Blair and Finlayson 1981).

3.1.1 Distribution of the taxon currently recognised as *Austropeplea lessoni*

As currently recognised, *Austropeplea lessoni* is one of Australia's most widespread freshwater molluscs, with a natural range of approximately two million

square kilometres. A significant portion of this distribution includes semi-arid and arid Australia (Figure 3.1). Moreover, its distribution encompasses a large number of major drainage basins and biogeographical regions identified for freshwater fish (Unmack 2001). The name *lessoni* has also been used for samples from parts of New Guinea (Benthem-Jutting 1963). It is thought to be an ecologically diverse species, as it occurs in ephemeral rivers and ponds of northern and central Australia, and the more permanent lakes and rivers of the much cooler southeast Australia (Boray and McMichael 1961; Smith and Kershaw 1979; Smith 1992).

Although a widespread and common freshwater mollusc, the dispersal mechanism of *Austropeplea lessoni* has not been investigated. Lymnaeids, like many other freshwater gastropods, are thought to be passively dispersed between isolated habitats via vectors, such as water flow and water birds (Boag 1986). However, reliance on passive dispersal may segregate isolate populations of this widespread species.

Because of the broad latitudinal and longitudinal area that *Austropeplea lessoni* encompasses, populations of *A. lessoni* experience considerable climatic differences between different regions. The most obvious climatic barrier to connectivity between populations is aridity, whereby populations between river basins and different regions have limited connectivity due to negligible surface runoff. While eastern Australia is typified by a temperate climate, with uniform rainfall throughout the year, central Australia (and some parts of Western Australia) are characterised by an arid climate, with ephemeral rivers connecting only during episodic flooding. In contrast, Northern and northwestern Australia is characterised by a monsoonal climate, with connectivity reaching a maximum in summer, when rivers flood. Winter is characterised by minimal rainfall and dry rivers.
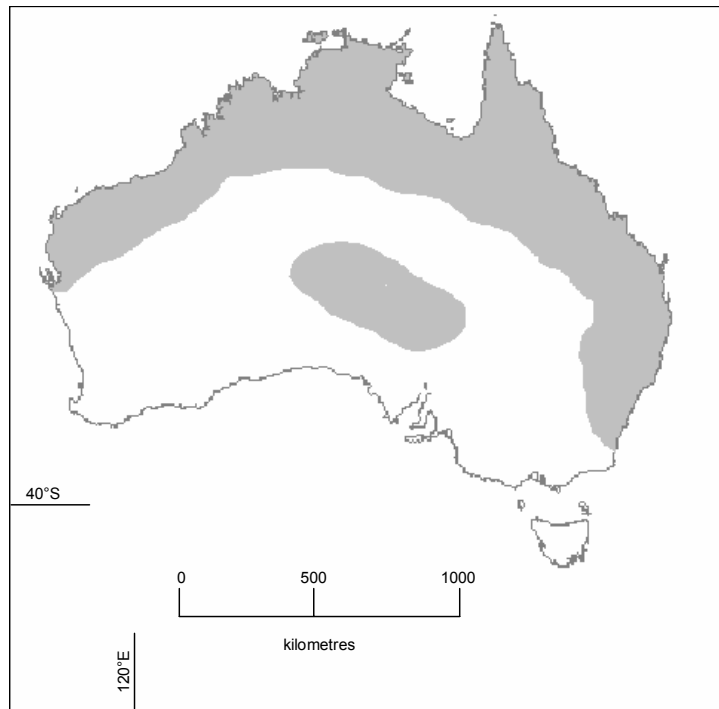
**Figure 3.1 Distribution of the *Austropeplea lessoni*
complex within Australia, represented by grey shading.**

Isolation due to erratic water flow patterns within and between major drainage systems and biogeographic regions could decrease gene flow between isolated populations. Indeed, recent studies of freshwater invertebrates within western Queensland have identified distinct lineages and population subdivisions between catchments. These studies were based on groups that actively disperse (Cook *et al.* 2002; Baker *et al.* 2003; Hughes and Hillyer 2003; Carini and Hughes 2004; Nguyen *et al.* 2004), and subdivision may be even more pronounced in passive dispersers, such as *A. lessoni*. It has been assumed that freshwater invertebrates disperse across catchment boundaries during flood events (Bilton *et al.* 2001). However, studies of even the highly mobile freshwater prawn, *Macrobrachum australiense*, suggest that almost no dispersal occurs during times of flood (Carini and Hughes 2004). Moreover, freshwater invertebrates have been shown to accumulate in areas of low hydraulic disturbance during floods, thereby avoiding dispersal (Winterbottom *et al.* 1997).

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The role of historical climatic patterns in shaping speciation patterns within the *Austropeplea lessoni* complex cannot be discounted. During the Quaternary, the Australian environment was dominated by glacial cycles. In the interglacial periods extensive freshwater lakes formed throughout Australia, whilst the environment was dominated by an arid climate during glacial maxima (Kershaw and Nanson 1993). These cycles could have had a significant effect upon the historical movement, distribution and isolation of populations of the *A. lessoni* complex throughout Australia. Historical climatic patterns are thought to account for speciation and population structure within other groups of Australian freshwater invertebrates (Cook *et al.* 2002; Hughes and Hillyer 2003; Carini and Hughes 2004; Nguyen *et al.* 2004).

Furthermore, selection pressures may differ between groups of the *Austropeplea lessoni* complex from different climatic regions, especially in relation to reproduction and growth. The northern and northwestern populations of the *A. lessoni* complex are dominated by monsoonal and arid climates, and therefore freshwater habitats are often seasonal or ephemeral. Selection in these populations may favour faster growing individuals that can reproduce before the end of the wet season. Different selection pressures between climatic regions, coupled with isolation, could result in speciation within the *A. lessoni* complex.

3.1.2 Previous taxonomic studies

The taxonomy of the *Austropeplea lessoni* complex is particularly difficult and uncertain owing to the nature of the group. Under the current classification, there are 19 synonyms for what is currently treated as a single species, *Austropeeplea lessoni* (Table 3.1). Virtually all of these names are based on shells alone and the type localities for a number of these synonyms are vague or unknown (Table 3.1). Lymnaeid shells have been shown to be highly plastic (Hubendick 1951; Arthur 1982; Lam and Calow 1988; Evans 1989; Oviedo *et al.* 1995; Ward *et al.* 1997; Wullschleger and Jokela 2002), with environmental factors have been identified as important factors in determining shell shape (Arthur 1982; Lam and Calow 1988).

In a review of the Lymnaeidae Rafinesque, 1815, Hubendick (1951) synonymised all 19 names into just one widely distributed species, *Austropeplea*

lessoni, which was thought to vary greatly under environmental stress. This conclusion was however, based on the examination of material from only two localities within Australia. Moreover, his conclusion was based largely on shell characteristics and on a limited number of distal reproductive characters. Little work with *A. lessoni* has since been carried out, although it has been suggested that the northern and southern distributed populations of *A. lessoni* may be distinct species based on electrophoretic evidence (Blair and Finlayson 1981), but supporting evidence for their suggestion has not been published. Furthermore, only small parts of the reproductive system, the radula and some other anatomical features of the *A. lessoni* complex have previously been described (Hubendick, 1951; Ponder and Waterhouse 1997).

3.1.3 Methodological Approach

An understanding of the speciation and taxonomy of freshwater pulmonates is often hampered by the phenotypic plasticity of their shells, this being particularly the case in lymnaeids (Hubendick 1951; Arthur 1982; Evans 1989; Ward *et al.* 1997; Lam and Calow 1998; Wullschleger and Jokela 2002). This can have confounding effects on speciation studies when the traditional shell shape approach is used as the primary indicator of taxonomic status. Thus, an understanding of the range of shell morphologies is needed in conjunction with anatomical characters and genetic differences to reliably discriminate species.

While anatomical studies of the soft bodied parts of snails have proved useful in the past for identifying and lymnaeid separating species, the utility of anatomical characters is disputed within the Lymnaeidae (Hubendick 1951; Ponder and Waterhouse 1997; Remigio and Blair 1997a; Remigio 2002). However, this utility has never been tested in light of an inferred phylogeny. This study will include an anatomical examination of specimens with the aim of identifying characters that can be useful in understanding the taxonomy of the group and their evolutionary history.

DNA sequencing has proven to be a useful tool in understanding speciation within freshwater molluscs. Sequence analysis of the large subunit (16S) mitochondrial ribosomal DNA successfully distinguished several species in previous

lymnaeid studies (Remigio and Blair 1997a; Remigio 2002). This gene region has both rapidly and slowly evolving regions, making it suitable for examining both ancient and recent divergences (Hillis and Dixon 1991; Simon 1991). In addition, the second nuclear internal transcribed spacer (ITS-2) region has been utilised in the Mollusca to understand the relationships of recently diverged (< 50 million years) organisms (Coleman and Vacquier 2002; Oliverio *et al.* 2002; Insua *et al.* 2003). Studies within the Lymnaeidae and the closely related Planorbidae show that ITS-2 is a reliable indicator of closely related snails at the level of species and genera (Vidigal *et al.* 2000; Bargues *et al.* 2001; Mavarez *et al.* 2002; Bargues *et al.* 2003). Therefore, molecular studies performed in tandem with both shell and anatomical studies, represents a powerful approach to understanding speciation in the *Austropeplea lessoni* complex.

3.1.4 Aims

The primary objective of this study was to determine whether Australian populations assigned to *Austropeplea lessoni* are represented by more than one species. This objective was met by using the partial mitochonrial gene sequences of 16S and the sequences of the ITS-2 region in conjunction with anatomical studies and measurements of shell morphometrics.

3.2 Methods

3.2.1 Material examined

Nineteen samples of the *Austropeplea lessoni* complex were used in this study, representing 18 distinct geographic areas. There are 19 names within the synonymy of *Austropeplea lessoni* as currently recognised (Table 3.1, 3.2), and, where possible, populations were sampled from their type localities, although in some cases attempts to collect material were unsuccessful (see Table 3.1). In a few cases type localities were not specified or not clearly defined. Several other taxa were included in this study, with one population of each species sampled; *A. viridis* (Quoy and Gaimard, 1832), *Bullastra cumingiana* (Pfeiffer, 1839), *'Lymnaea' brevispina* (Martin, 1897), *Radix auricularia* (Linnaeus, 1758), *R. peregra* (Müller, 1774), *R. quadrasi*

(Möllendorf, 1898), and *R. rubiginosa* (Michelin, 1831; Table 3.1). Samples from the *A. tomentosa* complex were not included in these analyses as previous results (see chapter 2) suggested that *A. lessoni* is ancestral to the *A. tomentosa* complex. Four species were used as outgroup taxa, *Stagnicola caperata* (Say, 1829), *S. catascopium* (Say, 1876), *S. elodes* (Say, 1821), and *S. emarginata* (Say, 1832). The outgroup taxa were however not directly sampled, with gene sequences were from Genbank and anatomical features coded from the literature.

All specimens were collected live in the field between 2002 and 2004, with the exception of five populations- NSW 4 (2), QLD 2 (2), QLD 4, WA 5 (2) and NT 5 (2)- which were from the Australian Museum collection. After collection, if there were a sufficient number of specimens, samples were split into two portions, one portion was used shell and anatomical examination and the other for DNA sequencing. Specimens used for morphological examination were relaxed overnight in menthol and fixed in 10% saltwater formalin. This was subsequently changed to 5% saltwater formalin a few days later. Specimens to be used for DNA sequencing were fixed in either absolute ethanol or 95% ethanol, with the ethanol changed 12 hours later. If there were insufficient specimens to permit splitting, all specimens were preserved for DNA analysis. All specimens have subsequently been lodged with the Australian Museum, Sydney (See Table 3.2).

For the samples where only DNA material was able to be collected or where only a small number of individuals were available for shell and anatomical studies, a second population that was geographically the closet to the first population was also included in the study. These populations have the same code as the original population, but have (2) following the code (Table 3.2).

Table 3.1 Synonymies of *Austropeplea lessoni*, the type locality and the sample number from this study that equates to the type locality.

Name	Author and Date	Type Locality	Sample Numbers
Limnaea lessoni	Deshayes (1830)	Australia	-
Limnaea perlevis	Conrad (1850)	Salamanca and Balonne Rivers, NSW	-
Amphipeplea strangei	Pfeiffer (1854)	Moreton Bay, QLD	C.431244
Amphipeplea melbournensis	Pfeiffer 1856	Near Melbourne, VIC	Not found
Limnaeus affinis	Küster (1862)	Australia	-
Amphipeplea vinosa	Adams and Angas (1864)	Tributary of Adelaide River, NT	C.436052
Amphipeplea phillipsi	Adams and Angas (1864)	Arnhem Land, NT	C.443999, C.439475
Amphipeplea iuvoluta	Schmeltz (1869)	Unknown	-
Limnaea angasi	Sowerby (1872)	Port Darwin, NT	C.436053
Limnaea cumingii	Sowerby (1872)	Australia	-
Limnaea globosa	Sowerby (1872)	Australia	-
Limnaea deshayesii	Sowerby (1872)	Corent Creek and Roper's Lake, NT	-
Limnaea spirulata	Sowerby (1872)	Australia	Not *A. lessoni*
Amphipeplea queenslandica	Clessin (1886)	Queensland	-
Peplimnea lilimera	Iredale (1943)	Burdekin River, QLD	C.451980
Peplimnea vinolenta	Iredale (1943)	Palm Creek, Darwent River, NT	-
Peplimnea caurina	Iredale (1943)	Lennard River, NT	C.436051
Peplimnea lessoni thelma	Iredale (1944)	Cobar, NSW	-
Peplimnea opima	Iredale (1944)	Hornsby, NSW	C.449005
Peplimnea spiriger	Iredale (1944)	Glenn Innes, NSW	C.431243

NSW= New South Wales, Australia, NT= Northern Territory, QLD= Queensland, Australia, WA= Western Australia, Australia

3.2.2 DNA sequencing

3.2.2.1 Material examined

A total of 18 specimens representing 17 samples of *Austropeplea lessoni* were sequenced for the 16S gene and the ITS-2 region (Table 3.2). One individual from each sample was sequenced, except for one population (WA 1) in which two individuals were sequenced. In addition, one individual of *A. viridis, Bullastra cumingiana, 'Lymnaea' brevispina*, *Radix quadrasi* and *R. rubiginosa* were also sequenced (Table 3.2). Sequences for other taxa were obtained from Genbank, *Radix*

auricularia 16S: AF485646, ITS-2: AJ319628, *R. peregra* 16S: U82074, ITS-2: AJ319633, *Stagnicola elodes* 16S: AF485625, ITS-2: AF013138, *S. emarginata* 16S: U82081, ITS-2: AF013142, *S. caperata* 16S: U82080, ITS-2: AF013140 and also16S for *B. cumingiana* U82068, *R. quadrasi* U82075, and *R. rubiginosa* U82080.

3.2.2.2 DNA extraction, PCR amplification and sequencing

Lymnaeids are intermediate hosts to a number of parasitic trematodes (Brown 1978). Development and multiplication of the parasites takes place inside the body cavity of the snail, and usually within the digestive gland (which is situated in the upper spirals of the shell). In order to ensure that no parasite DNA was extracted from the snails, only a small piece of foot tissue was used for the extraction of DNA. A CTAB method was employed for the extraction of the DNA. A small piece of foot tissue was sliced from the animal and this tissue placed in a solution of 200 µl of 2% CTAB and 100 µg proteinase K. The tissue was then broken up by grinding with a plastic pestle and digested for two hours at 55°C, with inversion every 30 minutes. Polymuccosaccarides were extracted from the sample in four steps; (1) 200 µl of chloroform/ isoamylalcohol (24:1) was added to the solution; (2) mixing was carried out by repeated inversion for two minutes; (3) separation of the phases by centrifuging for four minutes at 13 200 rpm; and (4) r the upper phase (containing the DNA) was carefully removed. The lower phase is distinguished by the white polymucosacharide layer that forms above the polymucosacharide-containing supernatant. These extraction steps were repeated three times to ensure all polymuccosaccarides had been removed from the sample. Genomic DNA was precipitated by the addition of two volumes of absolute ethanol and incubated at -20ºC for 20 minutes. The DNA pellet was then centrifuged for 15 minutes at 13 200 rpm, the supernatant removed and the pellet washed with 70% ethanol at -20ºC. The genomic DNA was redissolved in 50 µl of 1 mM Tris-HCl (pH 8) and stored at 4°C. This genomic DNA solution was used directly in the PCR reaction.

Table 3.2 Summary of taxa and voucher numbers for material used in the systematic study of the *Austropeplea lessoni* complex.

Code	Australian Museum Accession No.	Latitude	Longitude	Locality	Shells measured	Anatomical examination	16S sequenced	ITS-2 sequenced
Austropeplea lessoni complex								
NSW-1	C.431243	29° 27.870' S	151° 37.180' E	Glenn Innes, NSW, AUS	Yes	Yes	Yes	Yes
NSW-2	C.449005	34° 11.650' S	150° 42.700' E	Nepean River, Sydney, NSW, AUS	No	Yes	Yes	Yes
NSW-3	EBU.35505	35° 30.133' S	149° 42.633' E	Braidwood, NSW, AUS	No	No	Yes	Yes
NSW-4	EBU.35595	35° 58.800' S	148° 43.300' E	Adaminaby, NSW, AUS	No	No	Yes	No
QLD-1	C.451980	19° 24.000' S	146° 44.000' E	Ross River, Townsville, QLD, AUS	Yes	Yes	Yes	Yes
QLD-2	C.423243	23° 16.750' S	145° 24.100' E	Barcaldine, QLD, AUS	No	No	Yes	Yes
QLD-2 (2)	C.407248	20° 50.667' S	144° 11.900' E	Hugenden, QLD, AUS	Yes	Yes	No	No
QLD-3	C.431244	25° 40.000' S	151° 56.000' E	Stanthorpe, QLD, AUS	Yes	Yes	Yes	Yes
QLD-4	C.428189	17° 29.120' S	140° 50.380' E	Karumba, QLD	Yes	Yes	No	No
WA-1	C.426640	15° 32.960' S	128° 15.580' E	Parry's lagoon, WA, AUS	Yes	Yes	Yes	Yes
WA-2	C.439182	16° 58.460' S	122° 40.070' E	Beagle Bay, Broome, WA, AUS	Yes	Yes	Yes	Yes
WA-3	C.436051	17° 10.960' S	125° 15.340' E	Lennard River, WA, AUS	No	No	Yes	Yes
WA-4	C.431120	17° 44.380' S	123° 34.430' E	Cockatoo Creek, Derby, NT, AUS	Yes	Yes	Yes	Yes

WA-5	C.451978	22° 27.000' S	118° 18.000' E	Karijini National Park, WA, AUS	No	No	Yes	Yes
WA-5 (2)	C. 377262	20° 46.000' S	117° 7.000' E	Roeburne, WA, AUS	Yes	Yes	No	No
NT-1	C.436053	12° 33.940' S	131° 18.380' E	Humpty Doo, NT, AUS	Yes	Yes	Yes	Yes
NT-2	C.443999	12° 39.740' S	132° 31.570' E	South Alligator River, NT, AUS	No	No	Yes	Yes
NT-2 (2)	C.439475	12° 35.000' S	132° 27.000' E	South Alligator River, NT, AUS	Yes	Yes	No	No
NT-3	C.436052	13° 15.050' S	132° 31.570' E	Adelaide River, NT, AUS	No	No	Yes	No
NT-4	C.439184	16° 02.900' S	130° 23.150' E	Gregory National Park, NT, AUS	No	No	Yes	Yes
NT-5	C.451979	23° 42.000' S	133° 53.000' E	Alice Springs, NT, AUS	No	No	Yes	Yes
Austropeplea viridis	C. 449003	31º 56.000' S	115º 50.000' E	Perth, WA, AUS	n/a	Yes	Yes	Yes
Bullastra cumingiana	C.416760	14º 05.000' S	121º 11.000' E	Luzon, Philippines	n/a	Yes	Yes	Yes
Radix auricularia	C.449004	50º 0 8.000' S	167º 44.000' E	North Island, NZ	n/a	Yes	Yes	Yes
Radix brevispina	Loan Material	02º 34.900' S	98º 49.400' E	Sumatra	n/a	Yes	No	Yes
Radix peregra	C.428190	60º 10.000' N	24º 27.000' E	Finland	n/a	Yes	Yes	Yes
Radix quadrasi	C.416769	14° 13.000' S	121º 11.000' E	Luzon, Philippines	n/a	Yes	Yes	Yes
Radix rubiginosa	Loan Material	06º 49.240' S	107º 12.730' E	West Java	n/a	Yes	No	No

AUS= Australia, NSW= New South Wales, Australia, NT= Northern Territory, NZ= New Zealand, QLD= Queensland, Australia, WA= Western Australia.

The primers used to amplify 16S were 5'-CCG GTC TGA ACT CAG ATC ACG T-3' and 5'-CGC CTG TTT AAC AAA AAC AT-3' (Simon *et al.*, 1994). Reactions were performed in a total volume of 20 μl. Reactions contained 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 1.25 mM MgCl$_2$, 25 μM of each dNTP, 60 nM of each primer, 0.75 U of *Taq* DNA polymerase (Promega) and 0.5 μl of DNA template. A negative control was also prepared with the above constituents but without DNA, so that if contamination was present, it could be detected. Amplifications were performed in a gradient thermocycler with an initial denaturation step of 94°C for 2 min; then 35 cycles of 94°C for 30 sec, 45-65°C for 30 sec,72°C for 1 min; one cycle at 72°C for 5 min, and one cycle at 30°C for 1 min. Reaction conditions for each taxon were optimised with respect to MgCl$_2$ concentration and annealing temperature. The same procedure was followed for the amplification of the ITS-2 rDNA region using primers LT1 5' TCG TCT GTG TGA GGG TCG 3' (Bargues *et al.* 2001) and BD2 5' TAT GCT TAA ATT CAG CGG GT 3' (Remigio and Blair 1997b).

Amplification products were purified using a polylene glycol precipitation method, whereby 66 μl of PEG (30% w/v in 1.5 M NaCl) was added to 110 μl of PCR product. This mixture was incubated at room temperature for one hour, centrifuged for 15 minutes at 13 200 rpm and the supernatant removed. The pellet was washed with 70% ethanol at -20ºC and centrifuged for 10 minutes at 13 200 rpm. The supernatant was removed and the pellet again washed and recentrifuged for 5 minutes at 13 200 rpm. The supernatant was then removed and the pellet resuspended in 15 μl TE buffer (1 mM Tris-HCl pH 8, 0.1 mM EDTA). The final concentration required for subsequent sequencing reactions was determined by visualizing 1 μl of the purified product on a 1% agarose gel.

Direct sequencing of PCR products from a portion of the 16S rDNA and ITS-2 rDNA was performed using the Big Dye® Terminator v.3.1 cycle sequencing kit as described by the manufacturer. Sequencing reactions were made up to 12 μl with 40 nM of primer, 4 μl of Dye Terminator and 1 μl of template DNA. Conditions for cycling were 30 cycles of: 96°C for 30 sec; 50°C for 15 sec; 60°C for 4 min.

Sequencing products were purified and precipitated by adding 8 μL of nuclease free water, 2 μL of 125 mM EDTA (pH=8), 2 μL of 3M sodium acetate (pH 4.5), 50 μL of absolute ethanol and leaving for 15 minutes at room temperature. The products were centrifuged for 15 minutes at 13 200 rpm and the supernatant removed. The pellet was washed with 70% ethanol at -20ºC, allowed to air dry, and stored at -20°C until analysis was. Both DNA strands were sequenced.

Sequence electropherograms were edited manually by comparing both strands for all taxa using Bioedit v.3.0.9 (Hall, 1999). Prior to alignment a blast search was carried out on Genbank, to ensure that the all sequences were free of parasite contamination. Sequences were aligned using CLUSTAL W (Thompson *et al.* 1994), as distributed with the Bioedit program (Hall 1999). Multiple alignments were further improved by manual adjustment. Gene sequence length, base frequencies, and genetic distances were calculated in PAUP* 4.08b (Swofford 1998).

3.2.2.3 Phylogenetic analyses

Phylogenetic analyses were performed on the individual datasets, 16S and ITS-2, to assess congruence of the phylogenetic trees produced. The data were then combined for the final analysis. Taxa for which all datasets were not complete were deleted, creating a dataset of 24 samples (15 *Austropeplea lessoni*, and one each of *A. viridis*, *Bullastra cumingiana*, *Radix auricularia, R. peregra, R. quadrasi,* and *R. peregra* and *Stagnicola caperata, S. elodes* and *S. emarginata*). To determine whether significant incongruence existed between the 16S and ITS-2 datasets, incongruence length difference (ILD) tests (Farris *et al*. 1995) were conducted by excluding all invariant sites and using the partition homogeneity test in PAUP*4.08b (Swofford 2002) with 100 iterations.

To reconstruct phylogenies, I used both maximum parsimony (MP) as implemented in PAUP* 4.08b (Swofford 2002) and Bayesian inference using Markov chain Monte Carlo in the program MrBayes 3.1 (Huelsenbeck *et al.* 2001; Ronquist and Huelsenbeck 2003). MP searches were heuristic with 100 random-taxon-addition replicates, TBR branch swapping, and no maxtrees restrictions. All characters were treated as equal and unordered, with gaps treated as missing data. Clade support was

assessed with 1000 bootstrap replicates, each with 100 random-addition heuristic searches (Felsenstein 1985). *Stagnicola elodes*, *S. emarginata* and *S. caperata* were selected as outgroup taxa in the MP analyses based on Remigio (2002).

For Bayesian inference, hierarchical likelihood ratio tests were performed as implemented in the program MrModelTest, in order to determine which nucleotide substitution model best fit each data set, (Nylander *et al.* 2004). A general time reversal model of molecular evolution (nst=6), with the rates across sites being subject to a gamma distribution (rates=gamma), was selected for the 16S dataset. The alignment of the ITS-2 region resulted in large indel regions. Due to the variable nature of these regions, they were excluded from the phylogenetic analysis (see Appendix 3.4). For this dataset, the best fit model was a HKY model (nst=2) with the rates across sites being subject to a gamma distribution (rates=gamma). I ran three independent simulations of four simultaneous chains of Markov Chain Monte Carlo for 3 million generations for the single datasets (16S, ITS-2) sampling every 100 generations. Each independent simulation was started from different randomly chosen trees. Stationarity was defined as when the standard deviation reached 0.01, which was reached at 300 000 and 200 000 generations in the 16S and ITS-2 datasets, respectively. Therefore, burnin discarded the first 2000 and 3000 for the 16S and ITS-2 datasets, respectively.

The combined dataset (16S + ITS-2) was partitioned for the Bayesian analysis so that the best fit models were applied separately to the 16S and ITS-2 data (unlink command). The best fit models for the 16S and ITS-2 data were the same as those used in the single dataset analyses. For the combined dataset, I ran three independent simulations of four simultaneous chains of Markov Chain Monte Carlo for 5 million generations. Sampling occurred every 100 generations and each independent simulation was started from different randomly chosen trees. Stationarity was defined as when the standard deviation reached 0.01, which was reached at 400 000 generations. Therefore the first 4000 trees were discarded as the burn-in for the combined molecular dataset.

3.2.3 Anatomical morphology

I examined formalin and/or ethanol preserved material of 14 samples of the *Austropeplea lessoni* complex*,* and one sample each of *A. brevispina*, *A. viridis*, *Bullastra cumingiana*, *Radix auricularia*, *R. quadrasi*, *R. peregra*, and *R. rubiginosa* (Table 3.2). *Stagnicola elodes* and *S. catascopium* were coded using images and descriptions from Paraense (1994b) and Walter (1969), respectively. For each population, at least three adult and parasite free specimens were examined. I examined the internal body and dissected all animals under a Wild M3C Leica dissecting microscope. All morphological features that were identified as differing between samples were coded.

For *Austropeplea lessoni*, *A. viridis*, *Radix auricularia* and *R. quadrasi* a total of three radulae from each sample were examined using a scanning electron microscope. The extracted radulae were cleaned by heating to 60-80ºC in 5% NaOH solution overnight. Each radula was then rinsed in distilled water, and subject to ultrasound to remove any debris. Radulae were mounted on specimen stubs using a dry method, whereby radulae were allowed to dry at room temperature on a glass cover slip that was attached to the stub with double sided tape. The radulae were then coated with gold for examination with the scanning electron microscope. Radula for other taxa were coded using images from the relevant literature (Hubendick 1951; Walter 1969; Monzon *et al.* 1993; Paraense 1994b).

A total of 63 characters from the shell, outer body, pallial cavity, nervous system, reproductive systems and the radula were identified as variable between samples and were employed in the phylogenetic analysis (Table 3.3). A full description of these characters and their respective states are in Appendix 2.1. The full dataset for the 27 taxa is presented in Appendix 3.1. Maximum parsimony analyses of the data were performed using PAUP* 4.0b8 (Swofford 2002). A heuristic search was performed with 100 random addition sequence replicates, whereby all characters were treated as equal and unordered. To estimate tree support, bootstrap analysis was performed, with 1000 replicates. The distribution of character states on the trees was examined using McClade 4.0 (Maddison and Maddison 2000)**.**

Table 3.3 Characters and character states used in anatomical analysis of the *Austropeplea lessoni* complex.

Character number	Character	Character state and codification
1	Shell umbilicus	Closed (1); half open (2); open (3)
2	Shell thickness	Thin (1); thick (2)
3	Number of whorls	Three (1); four (2); five (3); 3.5 (4); 2.5 (5); 4.5 (6)
4	Columella fold	Absent (1); slight (2); distinct (3)
5	Shell sculpture	Absent (1); present (2)
6	Periostracum ornamentation	Absent (1); hairy (2)
7	Broadest area of foot	Anterior end of foot (1); same width along length (2)
8	Foot shape at posterior end	Tapering to a point (1); rounded (2)
9	Foot width to length ratio	2:1 (1); less than 2:1 (2); greater than 2:1 (3)
11	Eye lobe	Absent (1); well developed (2); undeveloped (3)
11	Tentacle shape	Wider than long (1); width equal to length (2); longer than wide (3); twice as long as wide (4)
12	Lateral sides of snout	Developed (1); undeveloped (2)
13	Pallial roof pigmentation	Mottled black and white (1); black (2)
14	Visceral coil pigmentation	Absent (1); present (2)
15	Mantle expansion	Absent (1); just outside of shell (2); covering some parts of the shell (3); covering large parts of the shell (4)
16	Expanded mantle pigmentation	Absent (1); present (2)
17	Number of pneumostomal ridges	One (1); two (2)
18	Outer lobe	Absent (1); present (2)
19	Upper plate of pneumostome	Thin (1); thick (2)
20	Broadest area of kidney	Anterior end (1); same width along length (2); posterior end (3); middle (4)
21	Kidney width to length ratio	3:1 (1); 2:1 (2); greater than 3:1 (3)
22	Right lobe of kidney	Absent (1); present (2)
23	Position of pulmonary vein	To the right of kidney (1); inside right lobe (2)
24	Pulmonary vein length	One third the length of the kidney (1); less than one third the length of the kidney (2); greater than one third the length of the kidney (3)
25	Ureter	Absent (1); present (2)
26	Opening of kidney	Inside pnuemostome (1); anterior to the pnuemostome (2)
27	Buccal mass shape	Longer than wide (1); width equal to length (2)
28	Cerebral commissure length	Half as long as distance between cerebral ganglion (1); one third the distance between cerebral ganglion (2); less than a third the distance between cerebral ganglion (3)
29	Pedal commissure	Absent (1); short (2)

30	Pedal commissure extra lobe	Normal (1); enlarged(2)
31	Statocysts	Absent (1); present (2)
32	Radula sac	Equal in length to buccal mass (1); longer than buccal mass (2); shorter than buccal mass (3)
33	Salivary glands relative size	Equal size (1); right longest(2); left longest (3)
34	Uterus/ vagina length relative to oothecal gland length	greater than half the length (1); less than half the length (2); equal or longer (3)
35	Spermathecal duct length	Shorter than uterus/ vagina (1); equal to uterus/ vagina (2); longer than uterus/ vagina (3)
36	Spermathecal duct width	Equal to uterus/ vagina (1); thinner than uterus/ vagina (2)
37	Uterus shape	Parallel (1); tapering distally (2)
38	Oviducal caecum size relative to oothecal gland	¼ width (1); ½ width (2); between ½ and one width (3); wider (4); absent (5)
39	Oothecal gland shape	Globular (1); pyriform (2); rectangular (3); square (4)
40	Oviduct 1	With brain like convolutions (1); with radial ridges (2); bosselated wall (3)
41	Position of uterus relative to oothecal gland	At right angles (1); greater than right angles (2); less than right angles (3)
42	Velum shape	Horse-shoe shaped (1); circular (2); absent (3)
43	Penis sheath length relative to praeputium	Less than half the length (1); Greater than half the length (2); Equal in length (3); Half the length (4); longer than praeputium (5)
44	Penis in penis sheath head	Looped (1); straight (2)
45	Seminal vesicle	Pockets present (1); low blisters (2)
46	Seminal vesicle shape	Short and wide (1); long and narrow (2)
47	Seminal vesicle form	U shaped (1); convoluted (2); straight (3); looped (4)
48	Junction of vas deferens and prostate	Simple (1); small sac (2)
49	Prostate ventral wall	Large fold present (1); slightly concave (2)
50	Upper prostate	Thin (1); wide (2)
51	Length of prostate relative to female reproductive system	Equal in length (1) longer (2); much longer (3); shorter (4)
52	Shape of lower prostate	Straight (1); bent to left (2)
53	Central tooth	Bicuspid (1); unicuspid (2); tricuspid (3); multicuspid (4)
54	Position of small cusp on central tooth	Left (1); right (2)
55	Radula teeth shape	Blunt (1); sharp (2)
56	Lateral teeth	Bicuspid (1); tricupsid (2); unicuspid (3); multicuspid (4)
57	Marginal teeth	Bicuspid (1); tricuspid (2); tetracuspid (3), 5 cups (4), greater than 5 cusps (5)
58	Ureter length	Short (1); medium (2); long (3)
59	Pedal ganglion shape	As long as wide (1); wider than long (2)

60	Insemination pocket	Absent (1); present (2)
61	Vaginal bulb	Absent (1); present (2)
62	Penal knot	Absent (1); present (2)
63	Prostate pouch	Absent (1); present (2)

3.2.4 Combined anatomical and molecular analyses

Phylogenetic analyses were performed on a combined dataset consisting of the molecular and anatomical data. Taxa for which all datasets were not complete were deleted, creating a dataset of 18 samples (11 attributed to *Austropeplea lessoni*, and one each of *A. viridis*, *Bullastra cumingiana*, *Radix auricularia, R. peregra, R. quadrasi,* and *R. peregra* and *Stagnicola elodes*). To determine whether significant incongruence existed between the molecular and anatomical datasets, incongruence length difference (ILD) tests (Farris *et al.* 1995) were conducted by excluding all invariant sites and using the partition homogeneity test in PAUP*4.08b (Swofford 2002) with 100 iterations. A maximum parsimony analysis was performed as described in Section 3.2.4.3.

The combined dataset (16S + ITS-2) was partitioned for the Bayesian analysis so that the best fit models were applied separately to the 16S, ITS-2 and anatomical data (unlink command). The best fit models for the 16S and ITS-2 data were the same as used in previous analyses. The anatomical data was subject to a gamma distribution. For the combined molecular and anatomical dataset, I ran three independent simulations of four simultaneous chains of Markov Chain Monte Carlo for 5 million generations. Sampling occurred every 100 generations and each independent simulation was started from different randomly chosen trees. Stationarity was defined as when the standard deviation reached 0.01, which was reached at 100 000 generations. Therefore the first 1000 trees were discarded as the burn-in for the combined molecular and anatomical dataset.

3.2.5 Shell morphometrics

Up to ten shells from ten samples (representing 82 individuals) of the *Austropeplea lessoni* complex were measured for a shell morphometrical analysis (Table 3.4). Up

to ten adult shells of each sample were measured. Prior to measurement, each specimen was checked to ensure that it was parasite-free and was a reproductively mature adult. Each specimen was drawn with the aid of a camera lucida, with shell measurements taken from these drawings. These measurements were: shell length, shell width, last whorl length, aperture length, aperture width and spire height (Figure 3.2). Each population was assigned to one of the geographic regions, as shown in Table 3.4.
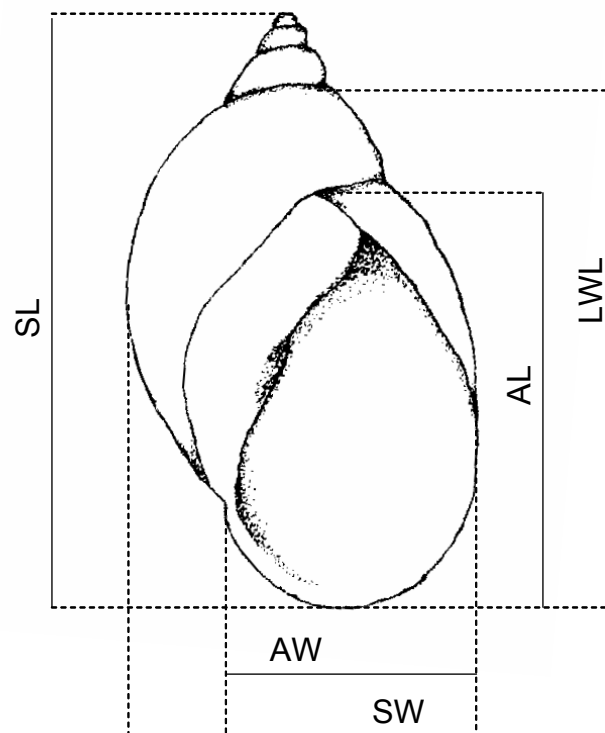
Figure 3.2 Five shell measurements taken in the shell morphometrics analysis of the *Austropeplea lessoni* complex. AL= aperture length, AW= aperture width, LWL= last whorl length, SL= shell length, SW= shell width.

A discriminant function analysis (DFA) was used to assess whether populations of *Austropeplea lessoni* from different geographic regions had significantly different shell morphologies, based on the five variables measured. This was performed using the discriminant function platform in SPSS 11.5. All assumptions required for the DFA to be performed were tested. These assumptions are that no two morphometric variables were highly correlated, and therefore measured essentially the same trait; that there was no significant deviation from multivariate

normality; and that there was equality in group covariance matrices (Klecka 1980; Hair *et al.* 1998). *A priori* groups for the DFA were the geographic regions as shown in Table 3.4.

Table 3.4 Australian samples of the *Austropeplea lessoni* complex used for shell morphometrics study, showing number of shells measured for each samples and the *a priori* geographic region assigned to each sample.

Population code	Australian Museum Accession No.	Latitude	Longitude	No. of shells measured	Geographic Region
Austropeplea lessoni					
NSW-1	C.431243	29° 27.870' S	151° 37.180' E	5	NSW
QLD-1	C.451980	19° 24.000' S	146° 44.000' E	5	QLD
QLD-2 (2)	C.407248	20° 50.667' S	144° 11.900' E	10	QLD
QLD-3	C.431244	25° 40.000' S	151° 56.000' E	6	QLD
QLD-4	C.428189	17° 29.120' S	140° 50.380' E	6	QLD
WA-1	C.426640	15° 32.960' S	128° 15.580' E	10	WA
WA-2	C.439182	16° 58.460' S	122° 40.070' E	10	WA
WA-4	C.431120	17° 44.380' S	123° 34.430' E	10	WA
WA-5 (2)	C. 377262	20° 46.000' S	117° 07.000' E	10	WA
NT-1	C.436053	12° 33.940' S	131° 18.380' E	7	NT
NT-2 (2)	C.439475	12° 35.000' S	132° 27.000' E	9	NT

NSW= New South Wales, Australia, NT= Northern Territory, Australia, QLD= Queensland, Australia, WA= Western Australia.

3.3 Results

In this section I will first present the molecular analyses including sequence variation and phylogenies. This will be followed by the anatomical analyses, the combined molecular and morphology analyses and lastly the shell moprhometrics study will be presented.

3.3.1 Sequence variation

The 16S and ITS-2 regions resulted in aligned data matrices of 446 bp and 579 bp including indels, respectively (Table 3.5; Appendix 3.2, 3.3). The combined 16S and ITS-2 data matrix was 1025 bp long. The alignment of the ITS-2 sequences

resulted in large indel regions. These regions were excluded from all phylogenetic analyses, although pilot analyses including these regions produced trees largely congruent with those excluding such regions. Characteristics of the three molecular datasets are shown in Table 3.5. The ITS-2 alignment (excluding variable regions) had the largest number of parsimony informative characters, although the combined molecular analyses had the least number of equally parsimonious trees (Table 3.5).

Table 3.5 Descriptive statistics for molecular data sets and indices for the trees analysed

Data set	16S	ITS-2	16S + ITS-2
Number of characters	447bp	579bp	1025bp
Number of variable sites (% of data partition)	159 (36)	449 (76)	702 (68)
Number parsimony informative sites (% of data partition)	106 (24)	319 (55)	435 (42)
% A	36	17	27
% C	17	29	23
% G	13	28	20
% T	34	26	30
Test of homogeneity	n.s.	n.s.	n.s.
Sequence divergence (%)	0-22	0.2-59	0-59
Tree L	234	249	984
CI*	0.67	0.70	0.71
RI*	0.75	0.82	0.81
RC*	0.55	0.61	0.61
Number of MP trees	1082	9	6

The 16S gene sequence length varied between 405 and 438 base pairs between the 26 samples (Table 3.6). There was, however, little variation in the length of the 16S gene amongst the *Austropeplea lessoni* samples. The ITS-2 sequences showed a much greater level of variation in length than the 16S gene, with sequence length ranging from 358 to 500 base pairs. *Austropeplea lessoni* had the longest sequence for ITS-2. Moreover, variation between the *A. lessoni* samples was observed between gene sequences (Table 3.6). The Northern Territory and Western Australian samples of *A. lessoni* generally had longer ITS-2 sequences than the New South Wales and Queensland samples of *A. lessoni* (Table 3.6).

Table 3.6 16S and ITS-2 sequence length measured in number of base pairs.

Taxa	16S gene length (base pairs)	ITS-2 region length (base pairs)
Austropeplea lessoni		
New South Wales	426-431	472-479
Queensland	428-432	474-480
Northern Territory	427-430	485-500
Western Australia	429-431	477-495
Austropeplea brevispina	n/a	358
Austropeplea viridis	431	397
Bullastra cumingiana	431	454
Radix auricularia	438	401
Radix peregra	422	395
Radix quadrasi	419	419
Radix rubiginosa	405	431
Stagnicola elodes	396	435
Stagnicola emarginata	365	448
Stagnicola caperata	365	448

Sequence divergence between the 26 samples varied from zero to 22% difference in the 16S dataset (Appendix 3.4). Within the samples of *Austropeplea lessoni,* sequence divergence ranged between 0 and 1.4%. New South Wales and Queensland samples had between 0.69 and 0.71% divergence, and the Western Australian and Northern Territory samples had between 0.23 and 0.9% divergence. The divergence between the New South Wales and Queensland samples compared to the Northern Territory and Western Australia samples ranged from 0.69 to 1.4%. Samples of the *A. lessoni* complex compared to *Bullastra cumingiana* had sequence divergences ranging from 8.2 to 9.4%. Sequence divergence between the *A. lessoni* complex and *A. viridis* ranged from 12.0 to14.7%. The *Austropeplea lessoni* complex with *Radix* samples had sequence divergences ranging from 12.4 to 15.2% divergence, while the sequence divergence between the *A. lessoni* complex and *Stagnicola* samples ranged from 14.3 to 21.0%.

Sequence divergence in the ITS-2 dataset was greater than that observed in the 16S dataset (Appendix 3.5). Within the samples of the *Austropeplea lessoni* complex

sequence divergence ranged between 0.2 and 12.1%. New South Wales and Queensland samples had between 0.2 and 2.1% divergence, and the Western Australian and Northern Territory samples had between 0.4 and 6.6% divergence. The divergence between the New South Wales and Queensland samples compared to the Northern Territory and Western Australia samples ranged from 4.2 to 12.1%. Samples of *A. lessoni* compared to *Bullastra cumingiana* had sequence divergences ranging from 32.8 to 36.8%. Sequence divergence between *A. lessoni* and *A. viridis* ranged from 30.8 to 33.0%. *Austropeplea lessoni* with *Radix* had sequence divergences ranging from 11.9 to 15.7% divergence, while the sequence divergence between *A. lessoni* and *Stagnicola* samples ranged from 14.2 to 21.0%.

3.3.2 Molecular phylogenies

3.3.2.1 16S

Both the Bayesian and MP analyses of the 16S dataset resulted in trees with very similar topologies (Figs 3.3, 3.4). The 16S phylogenies divide the samples of the *Austropeplea lessoni* complex into a number of clades. The New South Wales and Queensland samples form a well supported clade, and within this clade the New South Wales and Queensland samples form smaller separate clades (Figs3.3, 3.4). The distinction between the Northern Territory and Western Australian samples is less clear. Two Northern Territory samples (NT 5, NT 4) of *A. lessoni* are placed basally to New South Wales and Queensland samples of *A. lessoni* (Figs 3.3, 3.4), while all other Northern Territory and Western Australian samples of *A. lessoni* form a large polytomy which is basal to the New South Wales, Queensland and NT 5 and NT 4 clade. Three Western Australian samples (WA 3, WA, 2, WA 5) form a clade within the polytomy (Figs 3.3, 3.4). The divergence between the New South Wales and Queensland samples and the Northern Territory and Western Australian samples is only small as indicated by the short branch lengths (Fig 3.4).

Bullastra cumingiana forms a well supported sister taxon to the monophyletic the *Austropeplea lessoni* complex in both the MP and Bayesian phylogenies (Figs 3.3, 3.4). *Radix auricularia*, *R. peregra* and *A. viridis* form a sister group to *B. cumingiana* in the MP analysis, although there is less than 50% bootstrap support for *A. viridis* to

be included in the *R. auricularia* and *R. peregra* clade (Fig 3.3). The sister taxon to the *B. cumingiana* and *A. lessoni* clade remains unresolved in the Bayesian analysis, as both *A. viridis* and the *R. auricularia* and *R. peregra* clade are shown to be equally likely as sister taxa (Fig 3.4). In both the MP and Bayesian analyses *R. quadrasi* and *R. rubiginosa* form a well supported clade and are placed as sister to the *Austropeplea, Bullastra, R. auricularia* and *R. peregra* clade. Both *Austropeplea* and *Radix* are recovered as non monophyletic groups in the 16S phylogenies.

3.3.2.2 ITS-2

Bayesian and MP analyses of the ITS-2 dataset resulted in similar tree topologies as shown in Figures 3.5, 3.6. The samples of the *Austropeplea lessoni* complex are divided into two well supported lineages (Figs 3.5, 3.6). The New South Wales and Queensland samples are recovered as sister to the Northern Territory and Western Australian samples. The branch lengths between these two clades are however quite short (Fig 3.6), as was observed in the 16S phylogenies. Within each of the clades, other smaller clades formed with varying levels of support. These smaller clades however do not represent any geographic structuring (Figs 3.5, 3.6).
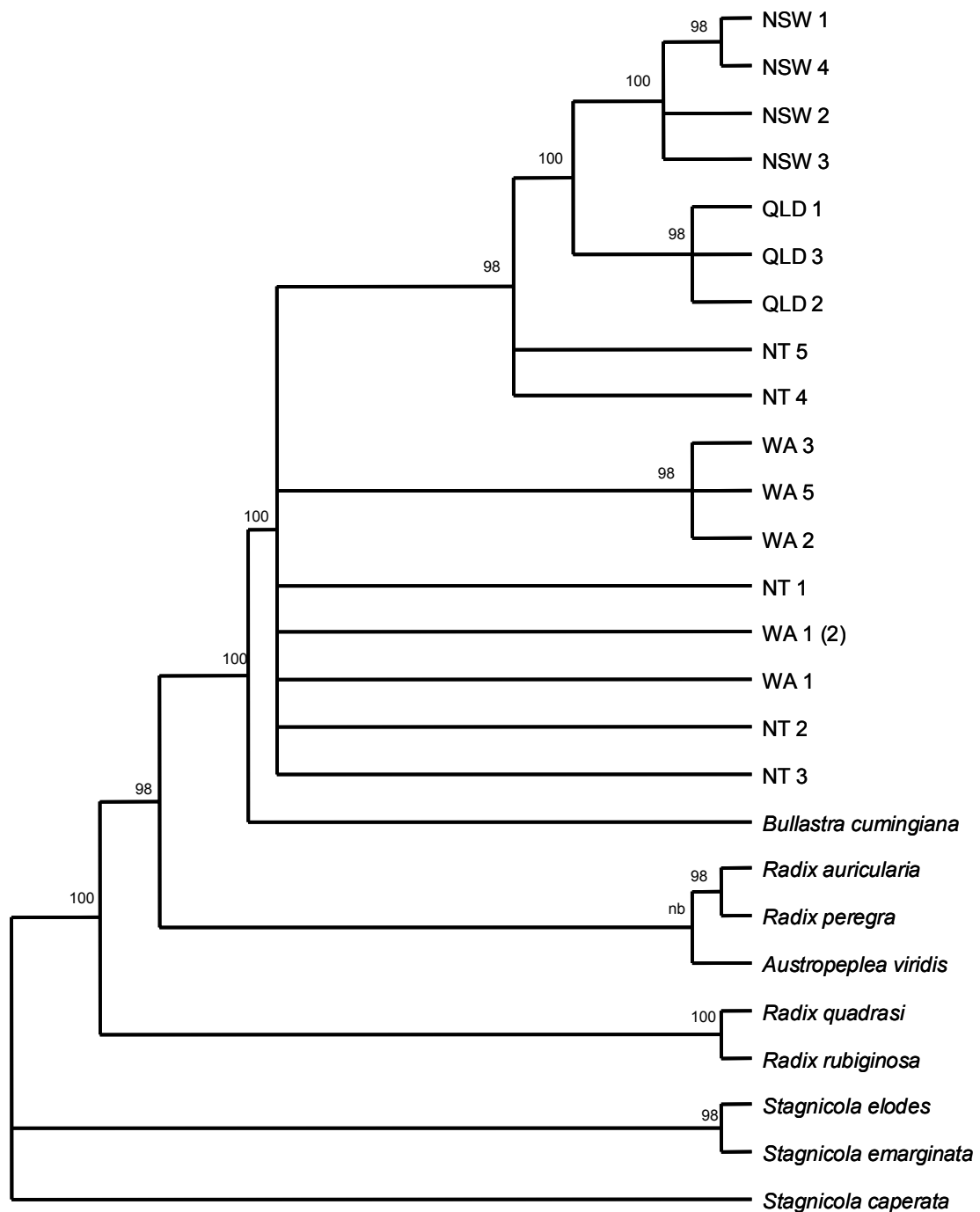
Figure 3.3 Phylogeny of the *Austropeplea lessoni* complex based on 16S rRNA sequences. Strict consensus tree of 1082 maximum parsimony trees, with tree length 234. Numbers above the branches are bootstrap scores, with only bootstrap >50% given. nb= no bootstrap support, NSW= New South Wales, Australia, NT= Northern Territory, Australia, QLD= Queensland, Australia, WA=Western Australia. Taxa without names are currently recognised as *A. lessoni*.
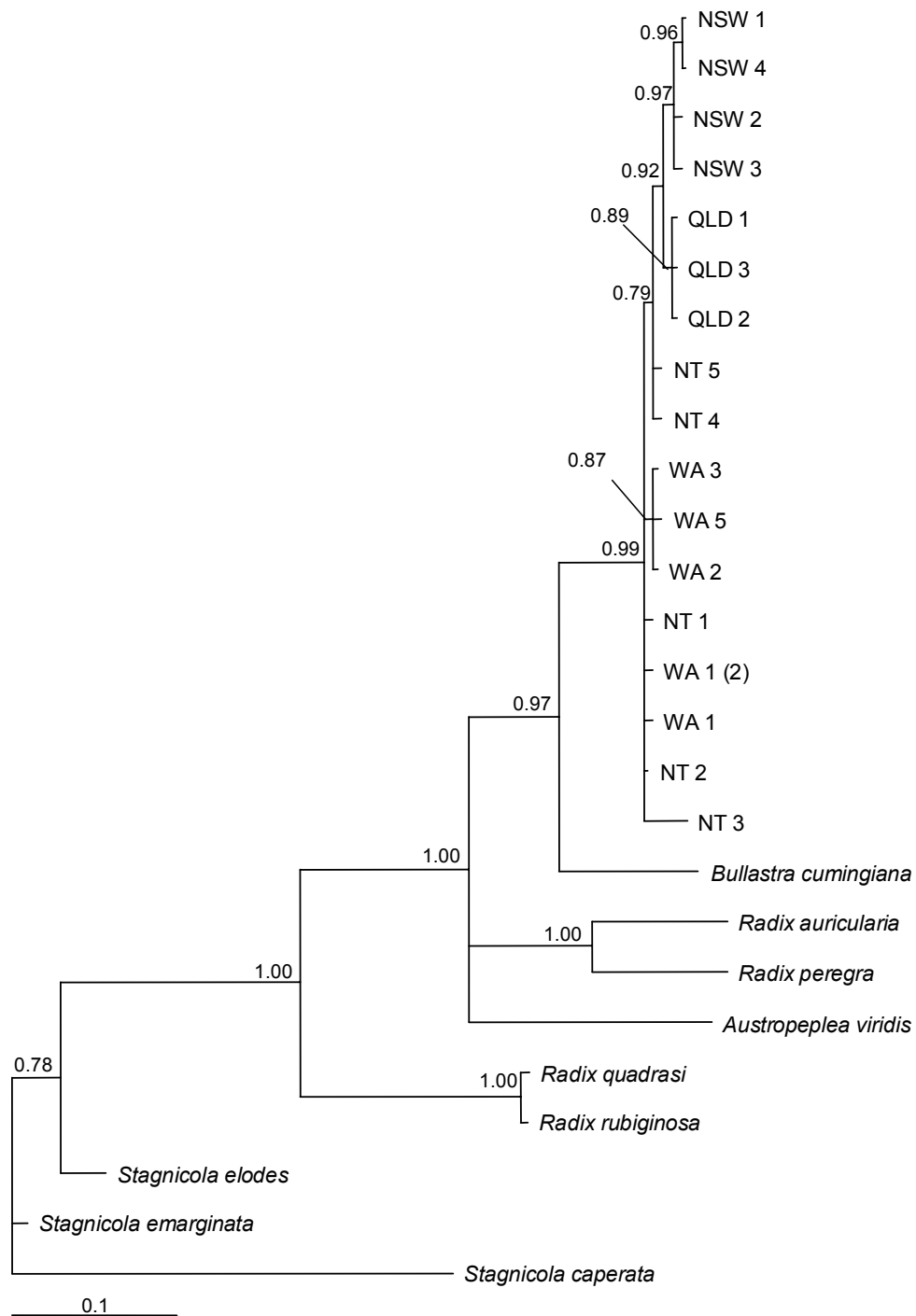
Figure 3.4 Phylogeny of the *Austropeplea lessoni* complex based on 16S rRNA sequences. Majority consensus tree based on Bayesian inference, with maximum likelihood setting under a general time reversal model for DNA substitution. Bayesian analysis based on 3 000 000 generation replicates, with posterior possibilities indicated above the branch, only >50% posterior probability given. gb= gene bank, NSW= New South Wales, Australia, NT= Northern Territory, Australia, QLD= Queensland, WA= Western Australia. Taxa without names are currently recognised as *A. lessoni*.

Bullastra cumingiana is shown as sister to the monophyletic *Austropeplea lessoni* in the MP analysis (Fig 3.5). However, in the Bayesian analysis, *B. cumingiana* plus *'Lymnaea' brevispina* are shown as sister to the *A. lessoni* clade (Fig 3.6). In the MP analysis *'L.' brevispina* is placed as sister to the *Austropeplea, Bullastra* and *Radix* clade, although support for this relationship is very weak (bp=52; Fig 3.5). In the Bayesian analysis, *Radix quadrasi, R. rubiginosa* and *R. auricularia* form a well supported clade. This *Radix* clade, *R. peregra* and *A. viridis* are shown to be equally likely as sister taxa to the *A. lessoni, A. brevispina, B. cumingiana* clade (Fig 3.6). In the MP analysis, a *Radix* clade plus *A. viridis* are recovered as sister taxa to the *A. lessoni* and *B. cumingiana* clade. However, some branches within the *Radix* and *A. viridis* have less than 50% bootstrap support (Fig 3.5). As in the 16S phylogenies, both *Radix* and *Austropeplea* are polyphyletic.

3.3.2.3 Combined 16S and ITS-2 phylogenies

The ILD test indicated significant incongruence between the 16S and ITS-2 datasets (p= 0.01). The datasets were still combined, as the topology of the 16S and ITS-2 was similar. The only major differences in the topologies were the position of some of the members of Radix. It is possible that this might be influencing the significant result obtained here.

Both the MP and Bayesian analyses produced trees with similar topologies (Figs 3.7, 3.8). Moreover, these trees showed a similar pattern of relationships to the previous single gene analyses. The *Austropeplea lessoni* complex is divided into two well support clades. The New South Wales and Queensland samples of *A. lessoni* are shown as sister to the Northern Territory and Western Australian samples of *A. lessoni* (Figs 3.7, 3.8). The New South Wales and Queensland clade has higher support and greater geographic structuring than the Northern Territory and Western Australian clade (Figs 3.7, 3.8). In both analyses, the New South Wales and Queensland samples of *A. lessoni* form smaller geographic clades. The branch lengths between the two clades, as in previous analyses, are only short (Fig 3.8).
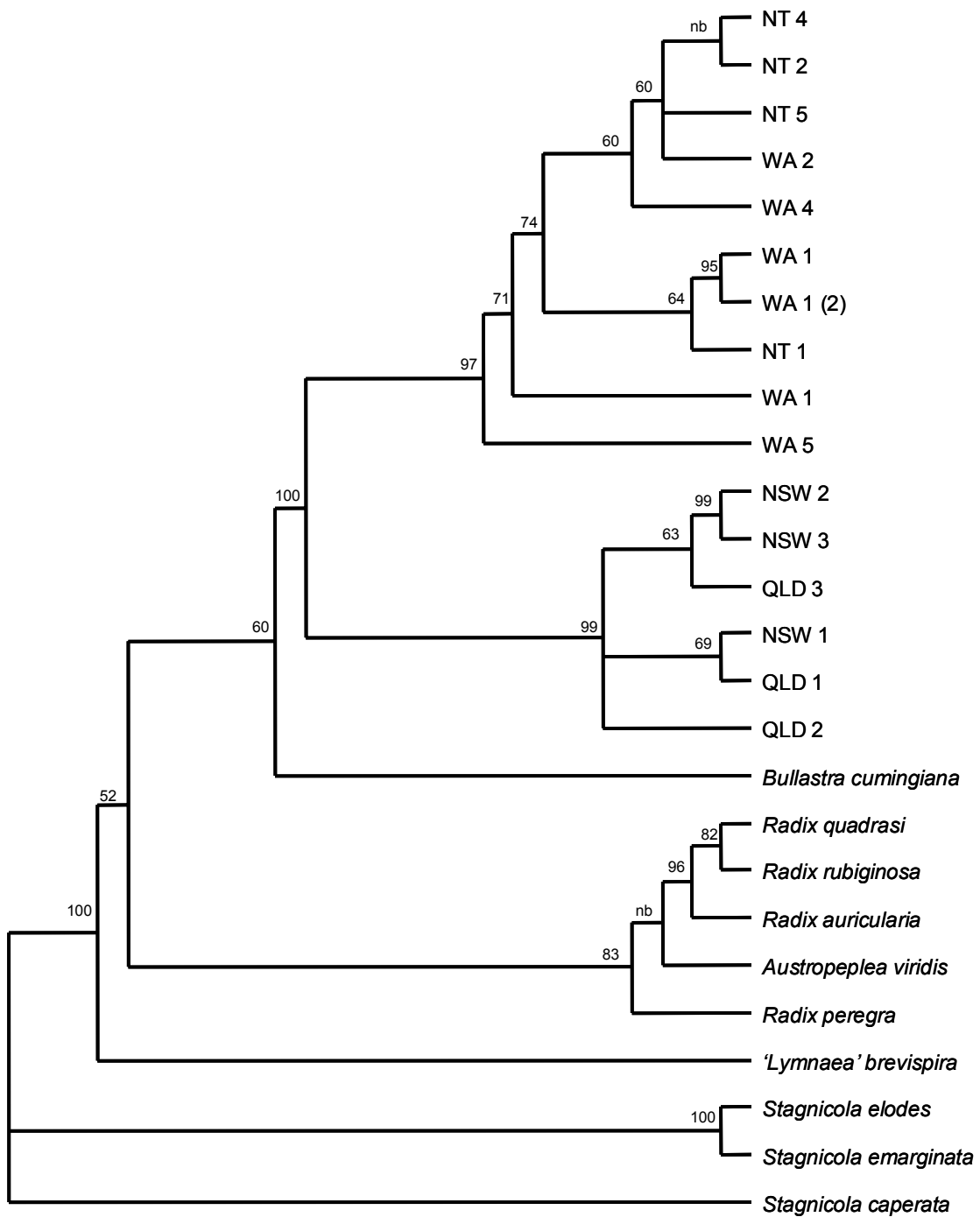
Figure 3.5 Phylogeny of the *Austropeplea lessoni* complex based on ITS-2 rRNA sequences. Strict consensus tree of 9 maximum parsimony trees, with a tree length of 249. Numbers above the branches are bootstrap scores, with only bootstrap >50% given. nb= no bootstrap support, NSW= New South Wales, Australia, NT= Northern Territory, Australia, QLD= Queensland, Australia, WA= Western Australia. Taxa without names are currently recognised as *A. lessoni*.
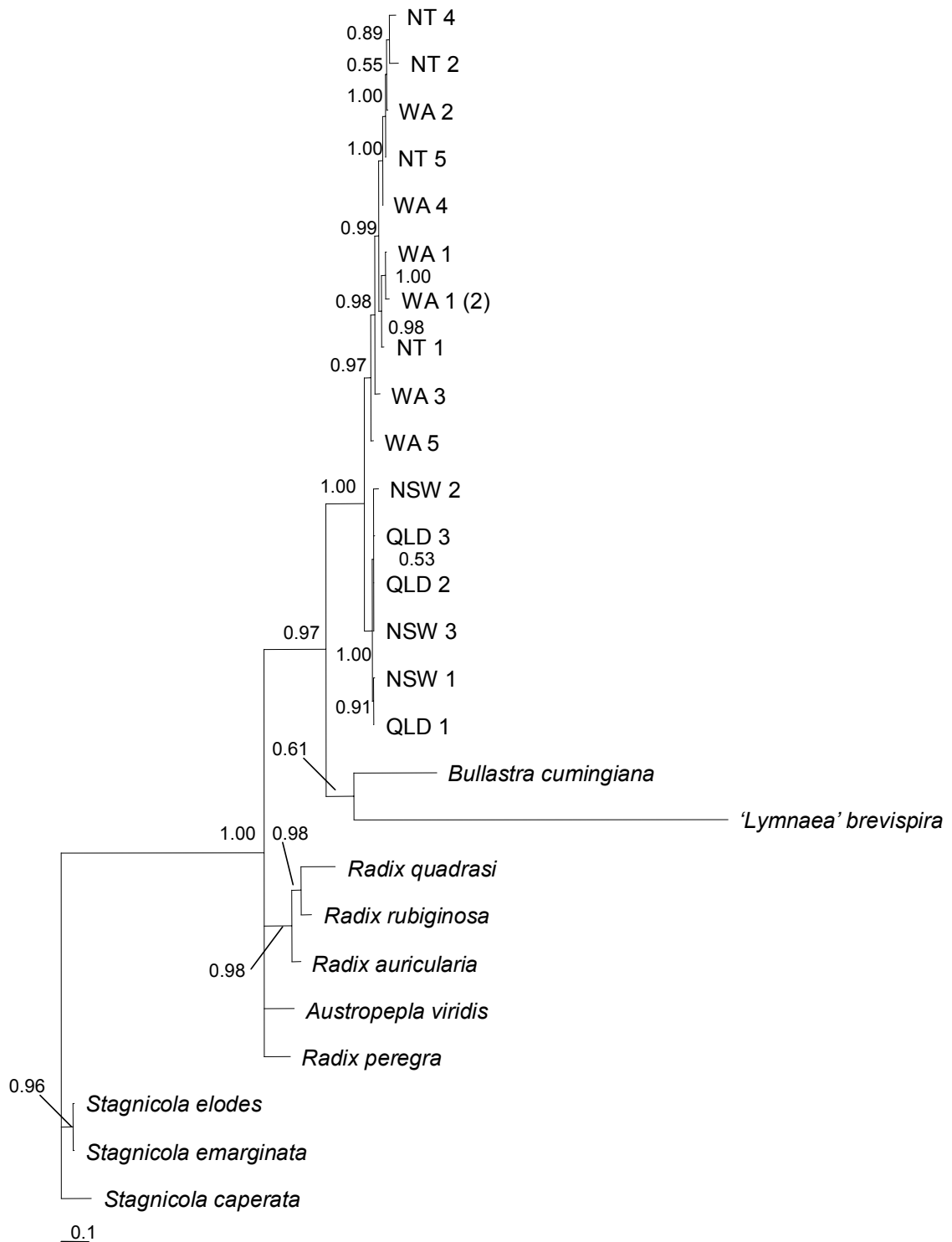
Figure 3.6 Phylogeny of the *Austropeplea lessoni* complex based on ITS-2 rRNA sequences.
Majority consensus tree based on Bayesian inference, with maximum likelihood setting under
HKY for DNA substitution. Bayesian analysis based on 3 000 000 generation replicates, with
posterior possibilities indicated above the branch, only >50% posterior probability given. NSW=
New South Wales, Australia, NT= Northern Territory, Australia, QLD= Queensland, Australia,
WA= Western Australia. Taxa without names are currently recognised as *A. lessoni*.

Bullastra cumingiana is shown as sister to the monophyletic the *Austropeplea lessoni* complex, a relationship that has high bootstrap and posterior probability support. All of the members of *Radix* in the study form a monophyletic clade in both the MP and Bayesian analyses, although the basal posterior probability is low (0.52) in the Bayesian analysis (Fig 3.8) and some branches in the MP analysis have less than 50% bootstrap support (Fig 3.7). In the MP analysis, the *Radix* clade and *A. viridis* are shown as sister to the *A. lessoni* and *B. cumingiana* clade (Figure 3.7). However, the Bayesian analysis shows the *Radix* clade and *A. viridis* as equally likely as sister taxa to *A. lessoni* and *B. cumingiana* (Figure 3.8). In the combined analysis, as in previous single gene analyses, *Austropeplea* is polyphyletic.

3.3.3 Anatomical phylogeny and variation

Of the 63 characters used in the MP analyses, 42 were parsimony informative, resulting in 92 equally parsimonious trees with a tree length of 138 (CI=0.46, RI=0.64, RC=0.32). A strict consensus of these 92 trees is shown in Figure 3.9. A list of the character statistics for the anatomical analysis can be found in Appendix 3.6.

The anatomical phylogeny is similar to the molecular phylogenies in terms of topology and the relationships shown. The *Austropeplea lessoni* complex forms a weakly supported (bp=57) monophyletic group, which diverges into two distinct clades in the bootstrap tree (Fig 3.10). One clade being comprised of the New South Wales and Queensland samples of the *A. lessoni* complex and the other clade represented Northern Territory and Western Australia samples of the *A. lessoni* complex from the Northern Territory and Western Australia (Fig 3.10). Support for the New South Wales and Queensland clade is reasonable (bp=72); however the support for the Northern Territory and Western Australian clade is lower (bp=61; Figure 3.10). In the strict consensus tree, some West Australian samples form small clades (Fig 3.9), however, these relationships have less than 50% bootstrap support (Fig 3.10).
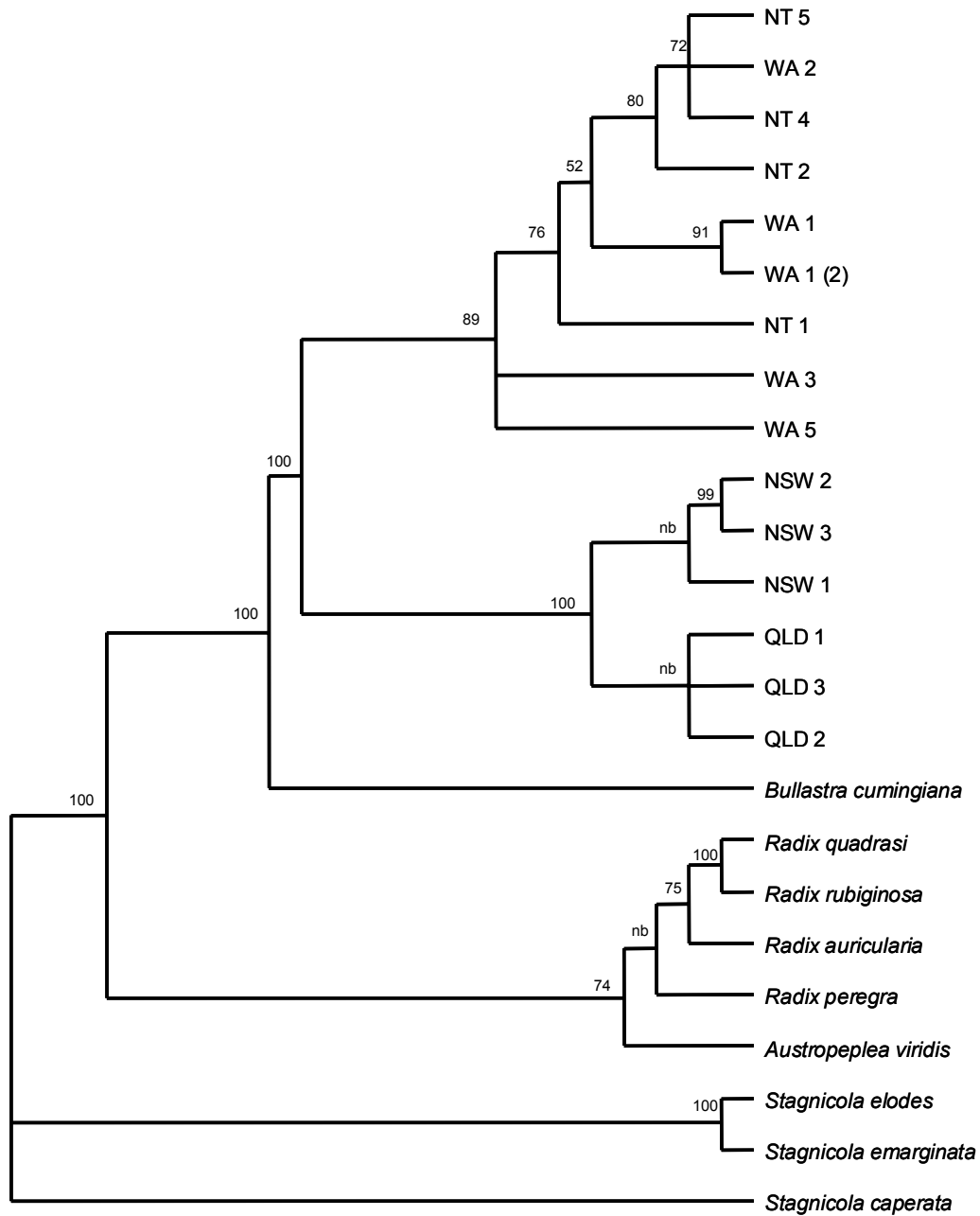
Figure 3.7 Phylogeny of the *Austropeplea lessoni* complex based on combined 16S and ITS-2 sequences. Strict consensus tree of 6 maximum parsimony trees, with a tree length of 984. Numbers above the branches are bootstrap scores, with only bootstrap >50% given. nb= no bootstrap support, NSW= New South Wales, Australia, NT= Northern Territory, Australia, QLD= Queensland, Australia, WA= Western Australia. Taxa without names are currently recognised as *A. lessoni.*
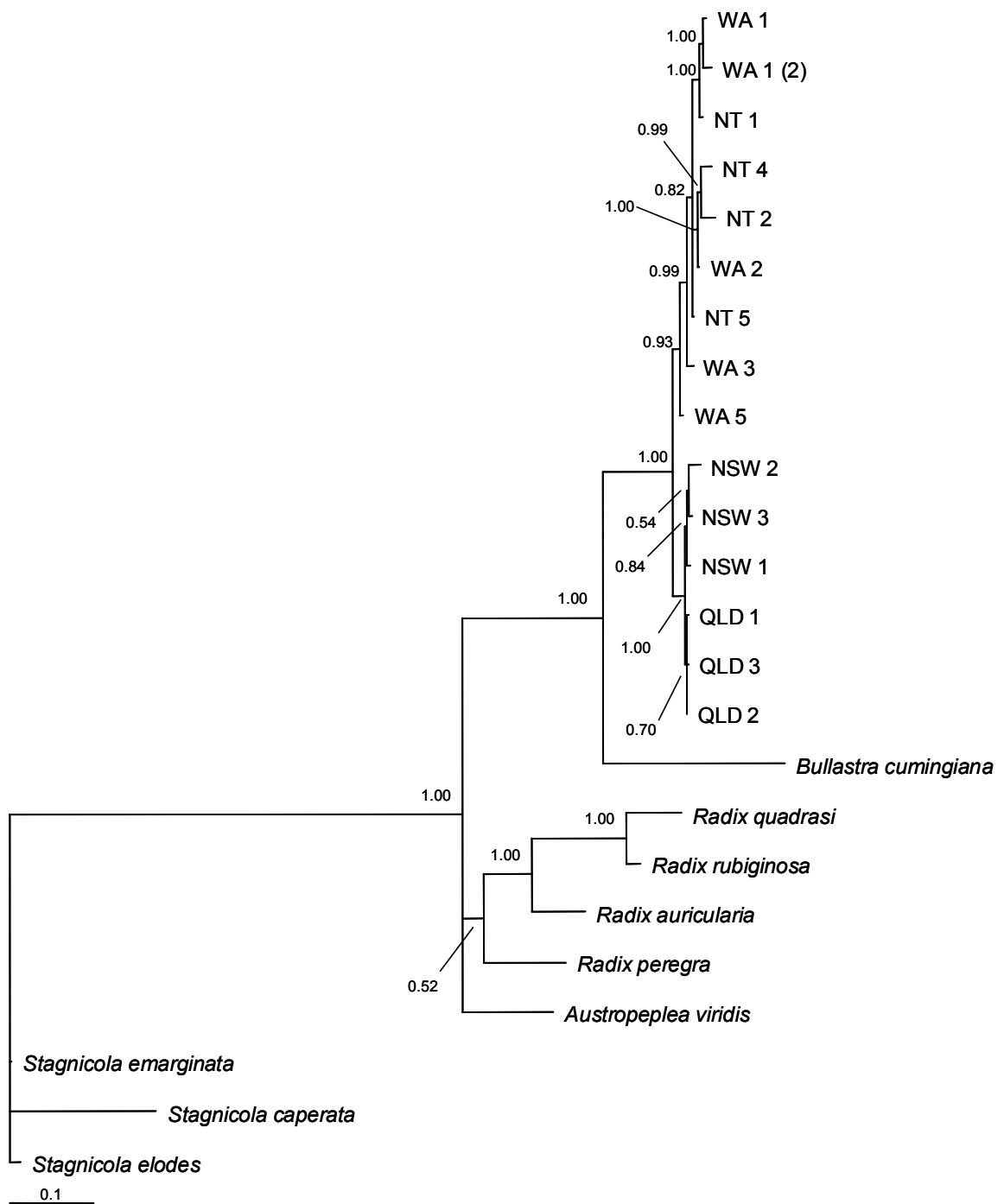
Figure 3.8 Phylogeny of the *Austropeplea lessoni* complex based on combined 16S and ITS-2 sequences. Majority rules consensus tree based on Bayesian inference with maximum likelihood setting under a partitioned model for DNA substitution. Bayesian analysis is based on the 5 000 000 generations replicates, with the posterior possibilities indicated above the branch, only posterior probabilities >50% are given. NSW= New South Wales, Australia, NT= Northern Territory, Australia, QLD= Queensland, Australia, WA= Western Australia. Taxa without names are currently recognised as *A. lessoni*.

One Queensland sample (QLD 4) of the *A. lessoni* complex is shown to be more closely related to samples from the Northern Territory and Western Australia, than with other samples of *A. lessoni* from Queensland and New South Wales (Fig3.10). In the strict consensus tree this sample is recovered between the two groups (Fig 3.9).

In previous molecular analyses, *Bullastra cumingiana* was consistently placed as sister to the *Austropeplea lessoni* complex. The anatomical phylogeny, however, does not show this relationship. In the strict consensus tree, all samples of *Radix*, *A. brevispina* and *Bullastra cumingiana* form a sister clade to the *A. lessoni* complex (Fig 3.9). However this relationship has less than 50% bootstrap support, with the sister to *A. lessoni* unresolved (Fig 3.10). *Austropeplea viridis* is weakly supported (bp=64) as sister to the *Radix*, *A. brevispina*, and *Bullastra* clade (Fig 3.10).

Examination of the parsimony informative anatomical characters revealed that all but one of the female reproductive characters (oviduct 1 shape, character number 40) was homoplastic when traced on the tree topology. Some male reproductive characters formed autapomoprhies (absence or presence of penal knot and upper prostate width, character numbers 63 and 50, respectively), however, only one male reproductive character was useful in defining phylogenetic relationships (the absence or presence of a prostate pouch, character 64). Moreover, several characters were polymorphic within samples from the same geographic area, indicating that they are not useful for distinguishing between samples of *A. lessoni* (character numbers 3, 10, 11, 14, 15, 21, 27, 28, 29, 37, 38, 44, and 47). Characters from the shell, outer body, kidney, nervous system, and radula were useful in defining phylogenetic relationships. Of the 64 parsimony informative characters used in the anatomical analysis, 10 formed unique synapomorphies on the strict consensus tree (Fig 3.9). One unique synapomorphy supports the monophyly of *Austropeplea lessoni*, an enlarged area of the ganglion surrounding the statocysts (Fig 3.9). No synapomorphies supported the separation of *A. lessoni* into two distinct groups. However there were consistent differences in several characters (character numbers 8, 15, 25, 33, 34, 41, 43, 48) between the New South Wales and Queensland samples of *A. lessoni* and the Northern Territory and Western Australian samples of *A. lessoni*. These characters and their states will be described and discussed in more detail in Chapter 5.

The *Radix auricularia*, *R. peregra* and *Austropeplea brevispina* clade was supported by one unique synapomorphy, oviduct 1 having brain-like convolutions (Fig 3.9). The *Austropeplea lessoni*, *A. brevispina*, *Bullastra*, and *Radix* clade was supported by three unique synapomorhies, a wide foot, the presence of a right kidney lobe and the pulmonary vein running through the right lobe of the kidney (Fig 3.9). The ingroup was supported by three unique synapomorphies, tricuspid laterals, tetracuspid marginals, and the absence of a prostate pouch (Fig 3.9).

3.3.4 Combined molecular and anatomical phylogenies

The ILD test indicated no significant incongruence between the molecular (16S + ITS-2) and anatomical datasets (p= 0.25). The combined molecular and anatomical dataset contained 304 parsimony informative characters and produced 6 equally parsimonious trees with tree length 992 (CI=0.66, RI=0.74, RC=0.55). A strict consensus of these 6 trees is shown in Figure 3.11. Bootstrap analysis and Bayesian inference produced trees with similar topologies to the strict consensus tree (Figs 3.11, 3.12). There are a smaller number of parsimony informative characters in this dataset than the combined molecular dataset due to smaller number of taxa included in these analyses

Both analyses show the *Austropeplea lessoni* complex as a monophyletic group that is divided into two distinct clades, each with strong support. The New South Wales and Queensland samples of *A. lessoni* form a sister clade to the Northern Territory and Western Australian samples of *A. lessoni*. This relationship is the same as that observed in all previous phylogenetic analyses, although the branch lengths in the combined anatomical and molecular analyses are longer than in previous molecular analysis (Fig 3.12). The New South Wales samples form a separate clade to the Queensland samples of *A. lessoni*, although bootstrap support is low (bp=64) and posterior probability high (0.97).
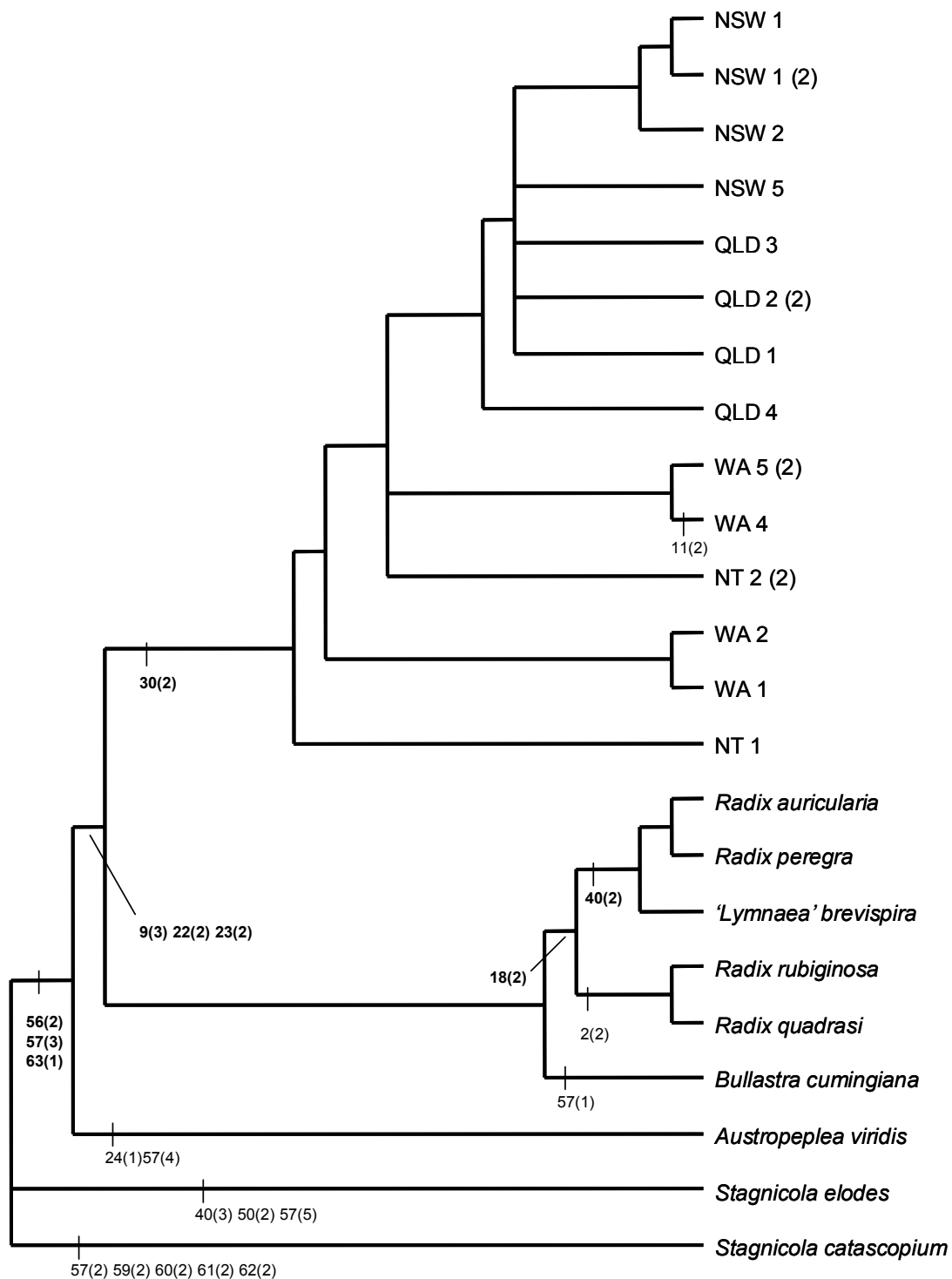
Figure 3.9 Phylogeny of the *Austropeplea lessoni* complex based on 64 anatomical characters. Strict consensus tree of 92 maximum parsimony trees, with a tree length of 138. Below branches are autapomorphies and synapomorphies (in bold), numbers corresponding with characters and character states as listed in Table 2.3. NSW= New South Wales, Australia, NT= Northern Territory, Australia, QLD= Queensland, Australia, WA= Western Australia. Taxa without names are currently recognised as *A. lessoni*.
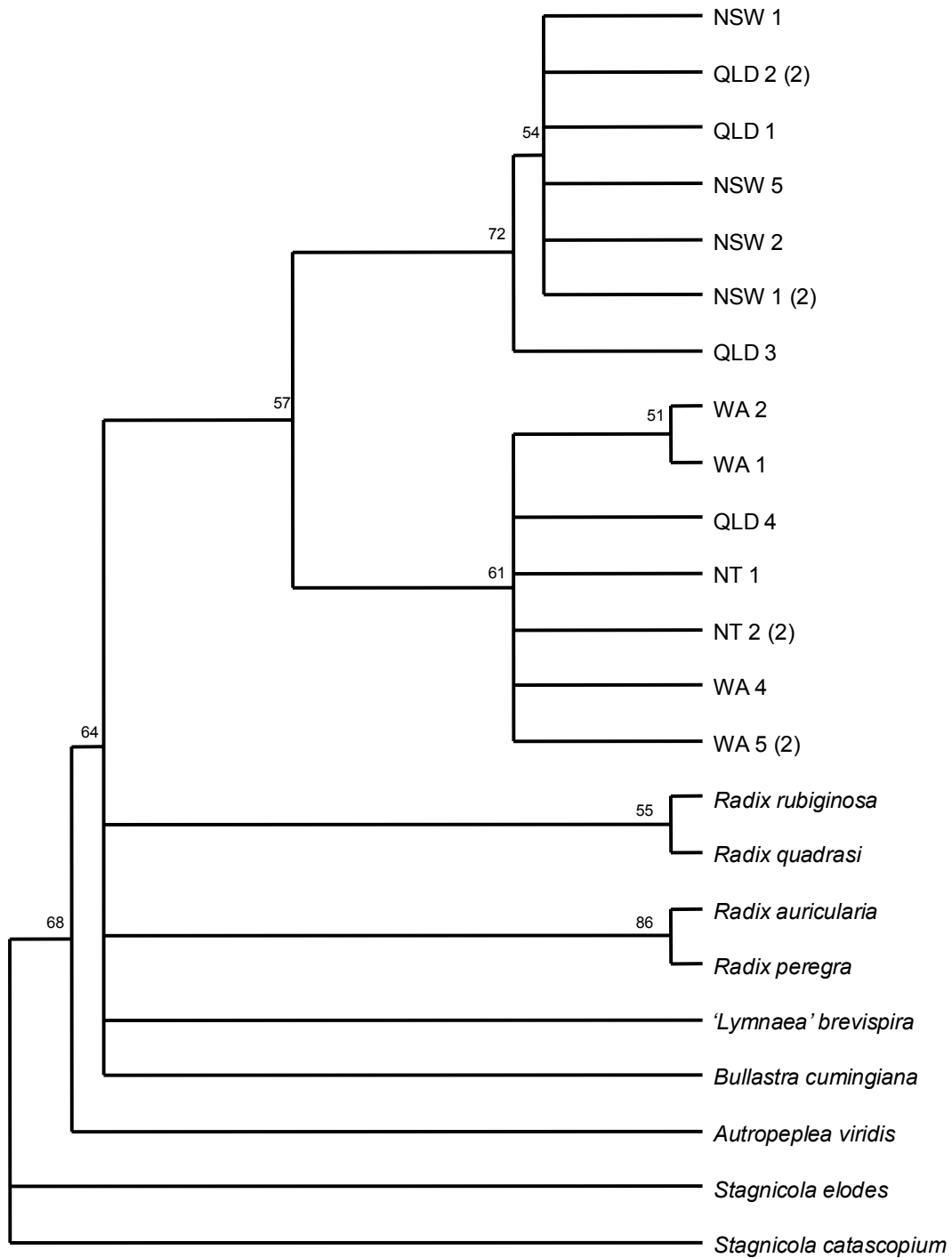
Figure 3.10 Phylogeny of the *Austropeplea lessoni* complex based on 64 anatomical characters. Majority rule bootstrap tree, with only >50% bootstrap shown. Numbers above the branches are bootstrap scores. NSW= New South Wales, Australia, NT= Northern Territory, Australia, QLD= Queensland, Australia, WA= Western Australia. Taxa without names are currently recognised as *A. lessoni*.

There is little geographic separation of the Northern Territory and Western Australian samples of *A. lessoni*, although the sample of *A. lessoni* from the Pilbara region in Western Australia (WA 5) is the most divergent of the Northern Territory and Western Australian samples.

Bullastra cumingiana, as in previous molecular analyses, is sister to the *Austropeplea lessoni* clade, with high bootstrap and posterior probability support (Figs 3.11, 3.12). The sister group to the *Bullastra* and *A. lessoni* clade remains unresolved. The *Radix* clade, *A. viridis* and *Stagnicola elodes* are all shown as equally likely as sister taxa in both analyses (Figs 3.11, 3.12). *Radix* forms a monophyletic clade in the Bayesian analysis, although Bayesian posterior probability is quite low (0.59). The MP analysis shows a *Radix* clade with *A. viridis* as sister, although there is less than 50% bootstrap support for some of the branches within this clade (Fig 3.11).

3.3.5 Shell morphometrics

Shell morphology is a character that is widely used to identify and distinguish molluscan species. Therefore I examined whether this technique could reliably be used to distinguish the phylogenetic groups within the *Austropeplea lessoni* complex as identified above.

The DFA showed a significant difference in the shell morphology between the four *a priori* geographic regions of *Austropeplea lessoni*. (Wilk's Lambda= 0.361, χ^2=82.571, df =20, p<0.0001). While the New South Wales and Queensland samples of *A. lessoni* were not significantly different from one another, both were significantly different from the Northern Territory and Western Australian samples of *A. lessoni*.
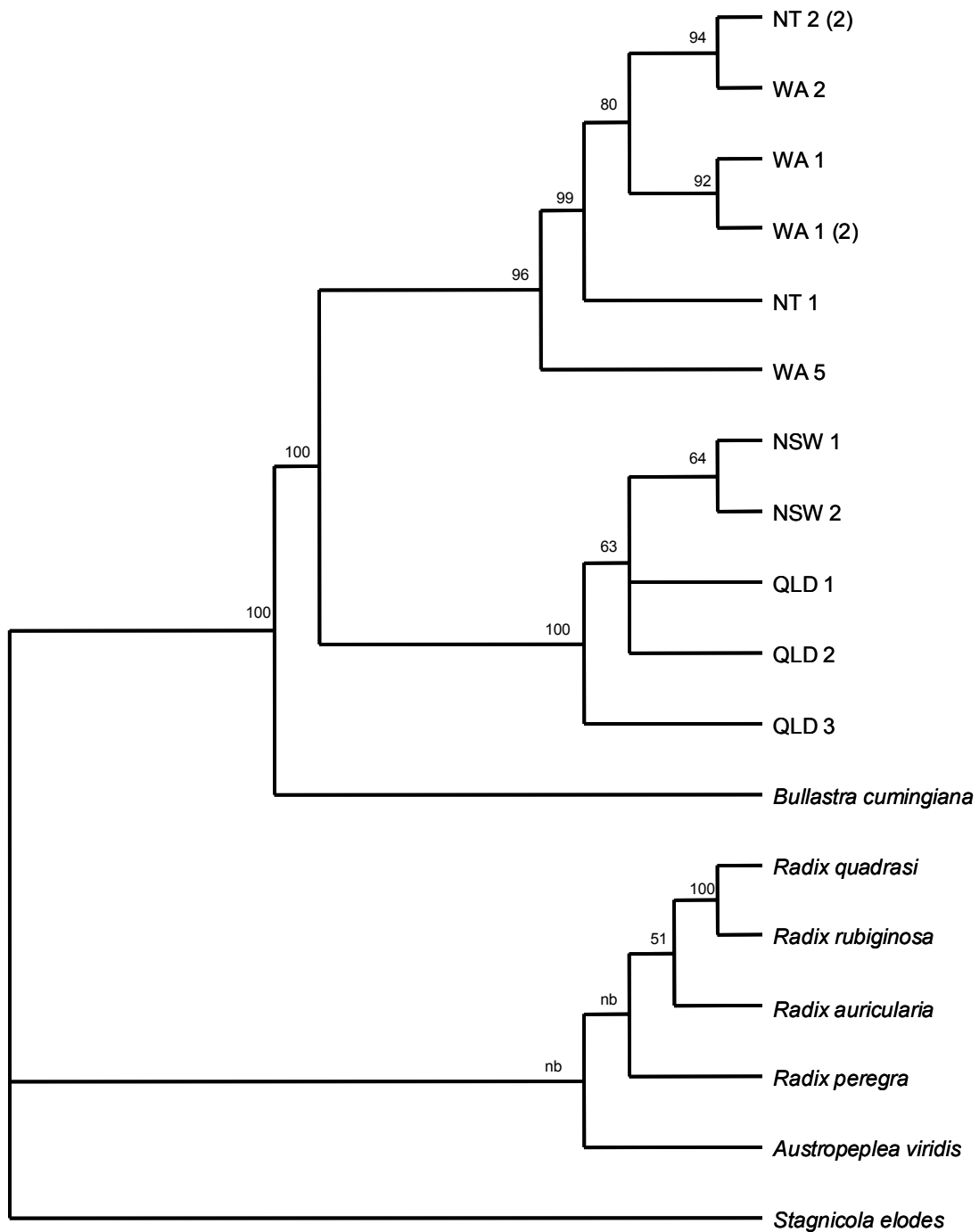
Figure 3.11 Phylogeny of the *Austropeplea lessoni* complex, based on anatomical and molecular characters. Strict consensus tree of 6 maximum parsimony trees with a tree length of 992. Numbers above the branches are bootstrap scores, with only bootstrap >50% given. nb= no bootstrap support, NSW= New South Wales, Australia, NT= Northern Territory, Australia, QLD= Queensland, Australia, WA= Western Australia. Taxa without names are currently recognised as *A. lessoni.*

Figure 3.12 Phylogeny of the *Austropeplea lessoni* complex, based on anatomical and molecular characters. Majority rules consensus tree based on Bayesian inference, maximum likelihood setting under a partitioned model for DNA substitution, 5 000 000 generations. Posterior probabilities indicated above the branch, only posterior probabilities >50% given. NSW= New South Wales, Australia, NT= Northern Territory, Australia, QLD= Queensland, Australia, WA= Western Australia. Taxa without names are currently recognised as *A. lessoni*.

The Northern Territory and Western Australian samples of *A. lessoni* were not significantly different from one another (Fig 3.13). The shell morphology of the QLD 4 individuals was not significant different from Northern Territory and Western Australia samples of *A. lessoni*, but they were significantly different from the New South Wales and other Queensland samples of *A. lessoni*. The DFA based on shell morphology distinguished two groups of *A. lessoni*, representing the same phylogenetic groups identified in the molecular and anatomical analyses.

The DFA classified only 48.3% into their correct groups, and 41.4% of cross-validated grouped cases were correctly identified. Of the five variables measured, only the aperture width did not contribute significantly to the DFA analysis. Last whorl length and aperture width contributed the most to canonical function 1 and shell length and last whorl length contributed the most to canonical function 2. The first and second canonical function account for 94.1% of the total variation, with respective eigen values of 1.335 and 0.088.

The results of the DFA, however, need to be interpreted with caution, as two assumptions of the DFA were violated. All five variables were very highly correlated with one another (variance inflation factors greater than 10; Hair *et al.* 1998). Therefore, the assumption of the DFA that no two variables should be highly correlated was violated. The assumption of homogeneity of covariance matrices was also violated (Box's M= 100.897, approx. $F_{30, 7664}$=2.964, p=0.000).
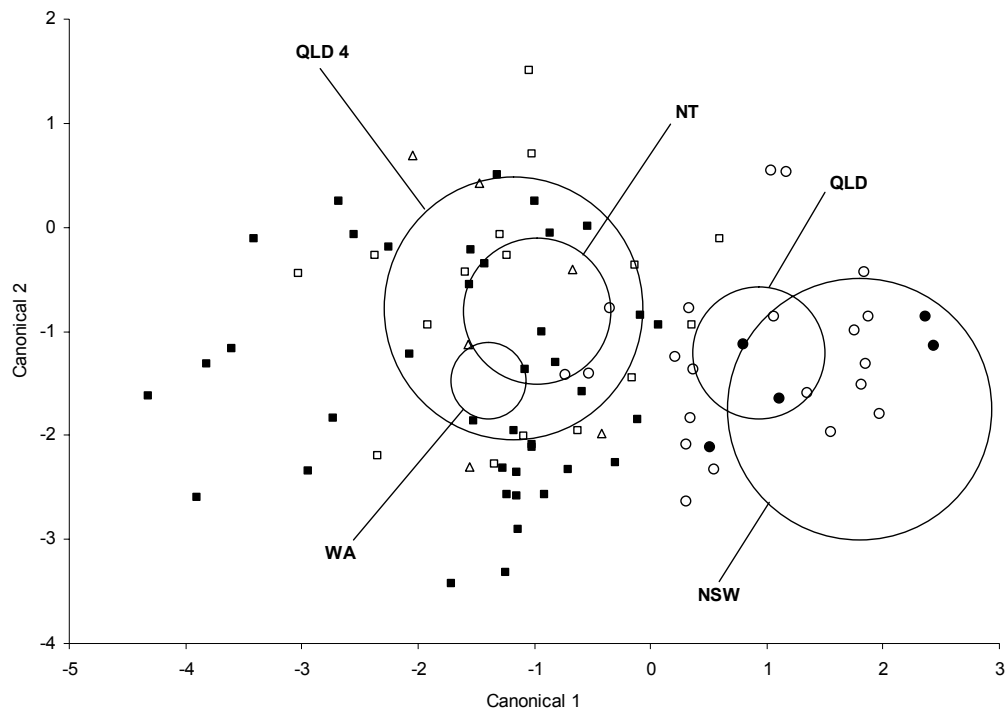
Figure 3.13 Canonical plot showing the point and multivariate means of DFA for shell measurements of samples of the *Austropeplea lessoni* complex. Circles correspond to multivariate mean of each group with a 95% confidence limit. NSW= New South Wales, Australia (●), NT= Northern Territory, Australia (□), QLD= Queensland, Australia (○), QLD 4= Queensland sample 4, Australia (△), WA= Western Australia (■).

3.4 Discussion

3.4.1 Species within the *Austropeplea lessoni* complex

The western (NT and WA) and eastern (NSW and QLD) Australian samples of the *Austropeplea lessoni* complex appear to represent separate species, a finding that is in contrast to the currently accepted taxonomy (Hubendick, 1951;Boray and McMichael 1961). The two divergent clades represented in the ITS-2 phylogeny, combined molecular and the combined molecular and anatomical phylogenetic analyses clearly indicate the presence of two independently evolving lineages. ITS-2 sequence divergence between the western Australian and eastern Australian samples of *A. lessoni* was as high as between other lymnaeid species (Bargues *et al.* 2001; Bargues *et al.* 2003). Moreover, based on shell morphologies, two distinct groups, which correlate with the western and eastern groups, can be identified.

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Anatomical characteristics and shell morphology of individuals from the northern most Queensland sample (QLD 4) included in the analysis suggest that this sample is part of the western species of the *A. lessoni* complex. This is perhaps surprising considering this sample is geographically closer to other Queensland samples of the *A. lessoni* complex, but the QLD 4 sample, like the majority of the western samples occurs in a monsoonal environment, and drainage from this area would also indicate that this sample is part of the western species. Therefore, the western species of the *A. lessoni* complex may extend throughout northern monsoonal Australia, and indeed as far east as the coastal drainages of the Gulf of Carpentaria, although this end of the distribution remains to be tested with molecular data.

The limited amount of 16S gene sequence divergence and the small branch lengths separating the lineages, indicate a relatively recent divergence between the eastern and western species of the *Austropeplea lessoni* complex. Mitochondrial DNA divergence for freshwater molluscs is thought to range between 1-5% per million years (Pfenninger *et al.* 2003). Under a conservative assumption of 1%, the estimated age of separation between the western and eastern species of the *A. lessoni* complex would be in the Pleistocene (0.69 - 1.4 Mya, based on a 0.69 to 1.4% sequence divergence). This separation time would accord with a period in Australia's climate history that was dominated by glacial and interglacial phases. Periods of very dry weather alternated with high increased rainfall dominated Australia until approximately 12 000 years ago (Kershaw *et al.* 2003). Such climatic oscillations deeply changed the hydrological flood regimes within Australia, and particularly in northern Australia (Kershaw *et al.* 2003), potentially modifying the levels of connectivity between populations of the *A. lessoni* complex.

Moreover, assuming a less conservative 5% level of divergence per million years, separation between the western and eastern species of the *A. lessoni* complex would be between 138 000 to 280 000 years ago. This time frame coincides with the estimated period of peak wetness during the last interglacial, which is suggested to have occurred between 120 000 and 130 000 years ago (Croke *et al.* 1999). While separation between the eastern and western species of the *A. lessoni* complex, may have already been taking place, this maximum period of peak wetness may represent

the last period of significant gene flow between the two groups. Since the late Quaternary, there has been a trend throughout Australia towards drier climate conditions, with the degree of lake filling and fluvial activity becoming reduced (Kershaw *et al.* 2003). It is likely that this reduction in flood activity coupled with ongoing aridity progressively isolated the eastern and western species of the *A. lessoni* complex.

The Northern Territory and Western Australian samples of the *Austropeplea lessoni* complex display a larger amount of sequence variation compared to the New South Wales and Queensland samples of the *A. lessoni* complex. Variation in ITS-2 length was greatest in the Western Australian samples of *A. lessoni*. Moreover, sequence divergence of both the 16S gene and the ITS-2 region was greater within the Northern Territory and Western Australian samples than within the New South Wales and Queensland samples. Greater gene sequence variation may be reflective of greater levels of isolation between populations within the Northern Territory and Western Australia. This would accord with the ephemeral rivers, high level of aridity and greater drainage divide that characterise northern, northwestern and central Australia.

3.4.2 Sister taxon to *Austropeplea lessoni*

Based on chromosome number, anatomical features and gene sequences, previous workers have suggested a close relationship between *Austropeplea lessoni* and *Bullastra cumingiana* (Burch 1980; Remigio and Pagulayan 1986; Ponder and Waterhouse 1997; Remigio and Blair 1997b; Remigio 2002). The results in Chapter 2 also clearly show that the *A. lessoni* complex belongs in a different clade from the type species of *Austropeplea*.

Results of this study, in all but the anatomical phylogeny, resolve *B. cumingiana* as sister to *A. lessoni*, thus corroborating previous works. Given the evidence of such a close phylogenetic relationship between the Australian *A. lessoni* complex and *B. cumingiana*, their placement in the same genus has been suggested to be more suitable than the current taxonomy (Remigio 2002). Levels of 16S gene divergence greater than 10% are thought indicate separate genera within the Lymnaeidae (Remigio 2002); 16S gene divergence between the Australian *A. lessoni* and *B. cumingiana* ranged between 8.2 to 9.4%, close to the cut off point. Thus, the *lessoni* complex

could be considered a separate genus from *Bullastra*, as suggested by anatomical data. Furthermore, there are a large number of anatomical differences between *Bullastra* and the *lessoni* complex that would suggest placement in separate genera. The taxonomy of the "*Austropeplea*" *lessoni* complex will be further discussed in Chapter 5.

'Lymnaea' brevispina is endemic to the Indonesian island of Sumatra. The relationship of this species to the "*Austropeplea*" *lessoni* complex is thought to be close due its geographical proximity to Australia and similarities in shell shape (Inaba 1969; Ponder and Waterhouse 1997). Results of the Bayesian ITS-2 analysis suggest that *'L'. brevispina* is closely related to *Bullastra cumingiana*. However, this relationship had low posterior probability support (0.61) and was not corroborated by the MP analysis. Moreover, the anatomical phylogeny proved to be of little use in understanding the phylogenetic relationships of *L. brevispina*. Individuals of *L. brevispina* used in this study provided poor quality genomic DNA. Therefore, I was unable to amplify the 16S gene and not all of the ITS-2 was completely amplified. The relationship of *L. brevispina* to the other lymnaeids of the region remains inconclusive.

The generic concepts of *Radix* and "*Austropeplea*" are questionable, as both groups were polyphyletic in these analyses. Previous studies have identified *Radix* as polyphyletic, composed of two distinct groups, European *Radix* and Asian *Radix* (Remigio 2002). However, both the current analyses and Remigio (2002) did not include all members of the *Radix* and "*Austropeplea*" genus. In order to gain a better understanding of the relationships within this group, more extensive sampling is needed. Further analysis of these relationships will be carried out in Chapter 4.

In none of the phylogenetic analysis was *Austropeplea viridis* shown to be closely related to *A. lessoni*. The 16S Bayesian analysis placed *A. viridis* as more closely related to the European *Radix*, which would support suggestions by other workers (Ponder and Waterhouse 1997). However, previous molecular analyses of the *A. tomentosa* complex (Chapter 2), showed *A. viridis* as sister taxon to *A. tomentosa*, while others have shown *A. viridis* to be the most derived species within the genus

(Remigio 2002. The relationship of *A. viridis* to other members of *Austropeplea* will be explored further in Chapter 4.

3.4.3 Utility of the separate datasets

The correspondence of relationships of *Austropeplea lessoni* within the molecular, anatomical, and shell morphometrics studies was surprising considering the lack of congruence between the datasets in the examination of the systematics of the *Austropeplea tomentosa* complex (Chapter 2). Comparison between these two sets of results indicates that anatomical characters should not be routinely excluded from phylogenetic analyses based on a lack of utility in other groups.

The small amount of divergence and differentiation observed in the 16S phylogenies, as well as the incongruence between the two molecular datasets, is not unusual in closely related species. Mitochondrial DNA has been shown to obscure species boundaries in recent species radiations (Avise 1994; Shaw 2002). This lack of divergence in the 16S gene within the *A. lessoni* complex accords with the proposed recent divergence between the eastern and western species of the *A. lessoni* complex. Moreover, lack of divergence in the 16S gene could be the result of Intraspecific hybridisation between the eastern and western species. The proximity of the two species in northern Queensland could facilitate such hybridisation events.

The anatomical study produced a tree with a similar topology to the molecular datasets, indicating that *Austropeplea lessoni* can be divided into two distinct species, eastern (NSW and QLD, excluding QLD 4) and western (NT , WA and QLD 4). However, the bootstrap support for these groups and on the anatomical tree was in general, low. While there are consistent morphological differences between the eastern and western species of the *A. lessoni* complex, only one of these characters formed a unique synapomorphy. Moreover, the *A. lessoni* complex is supported by just one unique synapomorphy. This level of homoplasy is thought to be common in closely related species (Fukuda and Ponder 2005), indicating that anatomical characters should be used in conjunction with molecular methods when trying to understand the relationships of closely related species. Moreover, the anatomical

characters did not resolve Bullastra cumingiana as sister to the *A. lessoni* complex, suggesting that the two may not be congeneric.

The shell morphology study grouped samples of *Austropeplea lessoni* into an eastern species (NSW and QLD, excluding QLD 4) and western species (NT, WA and QLD 4). Thus this technique distinguished the phylogenetic groups of *Austropeplea lessoni* identified in the molecular and anatomical analyses, indicating that the shells of these two species are different.

3.4.4 Widely dispersed freshwater molluscs

The Australian freshwater molluscan fauna has previously been thought to be composed of a small number of widely distributed species. The results of this study indicate widely dispersed Australian freshwater molluscs previously thought to be represented by one species may need further examination.

Chapter 4　　Phylogenetic relationships of Australasian *Lymnaeidae*

4.1 Introduction

The Lymnaeidae Rafinesque, 1815 are one of the most widespread freshwater snail groups, occurring on every continent except Antarctica (Bargues *et al.* 2001). Many species within the family are of applied interest because of their role as intermediate hosts to numerous digenean trematode species. Lymnaeids are involved in the life cycle of a number of medical and vetinary important trematodes, including fasciolids, schistosomatids and echinostomatids. The role of lymnaeids in transmitting the larvae of these economically important parasites has been the main impetus for recent phylogenetic studies of the group (Bargues *et al.* 2001; Remigio 2002; Bargues *et al.* 2003).

The Lymnaeidae have a rather long and confusing systematic history, with about 1800 species group and 34 genera group names being listed by Hubendick (1951). The systematic confusion in which the family is immersed is largely due to the reliance on shell and to a lesser extent, anatomical, features, to determine phylogenetic relationships. Intraspecific variation in shell shape and homoplasy of morphological characters has been demonstrated within the family (Hubendick 1951; Arthur 1982; Lam and Calow 1988; Evans 1989; Oviedo *et al.* 1995; Ward *et al.* 1997; Remigio and Blair 1997a; Wullschleger and Jokela 2002). In addition, previous workers have failed to agree on the importance of different morphological characters (Hubendick 1951; Walter 1968; Inaba 1969; Jackiewicz 1993a), resulting in different authors placing different emphasis and interpretation on morphological features. For example, Hubendick (1951) places heavy weight on the penal morphology, whilst Jackiewicz (1993a) places emphasis on the female reproductive anatomy in addition to the male.

Owing to these problems, recent workers have addressed the issue of lymnaeid systematics using DNA sequences (Bargues *et al.* 2001; Remigio 2002; Bargues *et al.* 2003). Despite the use of new technologies for the development of a robust hypothesis

for the relationships within the Lymnaeidae, a large number of species within the family have yet to be sufficiently sampled. The Australian and New Zealand lymnaeids are one of the groups that have been underrepresented in previous systematic studies of the family. The recent discovery of new lymnaeid species in Australia and New Zealand (Ponder and Waterhouse 1997; see chapters 2 and 3) warrants a re-examination of the relationships of this group in relation to other members of the family. Moreover, additional sampling of under represented groups within the Lymnaeidae has proven useful in understanding the phylogenetic relationships within the family (Remigio 2002; Bargues *et al.* 2003).

As outlined in previous chapters, the Australian and New Zealand Lymnaeidae are currently represented by two genera, *Austropeplea* Cotton, 1942 and *Kutikina* Ponder and Waterhouse 1997. *Austropeplea* is currently represented by three groups, the *A. lessoni* (Deshayes, 1830) complex, the *A. tomentosa* (Pfeiffer, 1855) complex and *A. viridis* (Quoy and Gaimard, 1833). Results of systematic studies of the *A. tomentosa* complex and the *A. lessoni* complex (Chapter 2 and 3, respectively) suggest that the genus is in fact represented by five species. A*ustropeplea*, as it is currently recognised, is characterised as having tricuspid lateral radula teeth, a bicuspid central (the smaller cusp on the left) and 16 pairs of chromosomes (Inaba 1969). However, the relationship of the members currently attributed to *Austropeplea* with other members of the family is not clear.

4.1.1 Phylogenetic relationships of the Australasian Lymnaeidae within the family

Studies of the Australian lymnaeids and their phylogenetic relationships with other members of the family have produced conflicting results. Lymnaeidae have 16 to 19 chromosome pairs, with *Austropeplea* characterised by 16 (Inaba 1969). With the smallest number of chromosomes within the family, Burch and co-workers (1965; 1967; Inaba 1969) suggested that *Austropeplea* represented the most primitive of the recent lymnaeids, who in turn gave rise to lymnaeids with 17 pairs of chromosomes. Lymnaeids with 18 pairs of chromosomes are thought to represent the most advanced groups within the family (1965; 1967; Inaba 1969) Some morphological support for

this hypothesis came with *Austropeplea viridis* (*ollula*) considered one of the primitive lymnaeids (Walter 1968).

However, outgroup comparison with Lacinae, Chilinidae and Latiidae suggests that 18 pairs of chromosomes is more likely to be the pleisomorphic state (Ponder and Waterhouse 1997). This was supported by molecular analyses (Remigio and Blair 1997a; Remigio 2002), with lymnaeids having 18 pairs of chromosomes being resolved as the most basal groups, and those with 16 pairs the most derived (Remigio and Blair 1997a; Remigio 2002). To date, however, the Australian lymnaeids have been poorly represented in molecular analyses, with only one individual of the *A. lessoni* and *A. tomentosa* complexes included. Moreover, outgroup selection was limited in the molecular analyses to date, this being significant because outgroup choice can influence branch order, branch length, clade monophyly and divergence rates (Lyons-Weiler *et al.* 1998).

Australian lymnaeids are thought to be closely related to lymnaeid species from the South East Asian region, with several others suggesting a close relationship between the *Austropeplea lessoni* complex and lymnaeids from the Asian region, based on chromosome number, shell shape and other anatomical features (Inaba 1965; Burch 1967; Ponder and Waterhouse 1997). Current understanding of the systematics of South East Asian lymnaeids (Burch 1967) suggests they are largely represented by the genus *Radix* Monfort, 1810 (17 pairs of chromosomes), with the exception of *A. viridis* and *Bullastra cumingniana* (Pfeiffer, 1839; 16 pairs of chromosomes). Burch (1967) suggested that A. *viridis* (*ollula*) is an archaic lymnaeid that gave rise to *'Lymnaea' brevispina* (Sumatra), *B. cumingiana* (Philippines) and the *A. lessoni* complex (Australia). Moreover, *Austropeplea* and *Radix* are thought to be closely related genera within the Lymnaeidae, with *Austropeplea* being thought of as an archaic form, from which the *Radix* was the first group to diverge (Inaba 1969).

The close geographic distances between the many islands of the region and the collision between the Australia and Asia tectonic plates 20 Mya (Hall 1998) could have facilitated movement of ancestral stock throughout the region. Indeed, the placement of *Bullastra cumingiana* as sister to the *Austropeplea lessoni* complex (see Chapter 3) suggests a close shared history between the regions. Despite this, one

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molecular analysis (Remigio 2002) using 16S indicated that the Australian lymnaeids are more closely related to the European members of *Radix* than to the Asian members of that genus, with *Radix* resolved as paraphyletic. These relationships are tested below.

4.1.2 Phylogenetic relationships of taxa included in *Austropeplea*

The phylogenetic relationships of the *Austropeplea lessoni* complex, the *A. tomentosa* complex and *A. viridis,* was not tested using molecular data until recently (Remigio 2002). Based on 16S gene sequences the *A. tomentosa* complex is thought to have been the source of the ancestral lymnaeid from which the *A. lessoni* complex and *Bullastra cumingiana* derived (Remigio 2002). However, results presented in Chapter 2 conflict with this theory and the *A. lessoni* complex was placed as sister to the *A. tomentosa* complex in combined molecular analyses of 16S and ITS-2 using members of *Radix* as the outgroup. This relationship is further tested below using additional members of the Lymnaeidae and several outgroup taxa.

The phylogenetic relationships of *Austropeplea viridis* with other members attributed to *Austropeplea* are unclear, with several studies producing conflicting results. Based on chromosome numbers, radula dentition and geography, *A. viridis* was thought to have been the archaic form that gave rise to the South East Asian lymnaeids and also to the *A. lessoni* complex (Burch 1967). The first molecular phylogeny (Remigio and Blair 1997a) resolved *A. viridis* as a derived group rather than a basal. Increased taxon sampling revealed that *A. viridis* was more closely related to the *A. tomentosa* complex and the *A. lessoni* complex than to other South East Asian lymnaeids (Remigio 2002). Despite this relationship, the inclusion of *A. viridis* in *Austropeplea* was questioned due to the large amount of sequence divergence, shell differences and anatomical differences, between this species and other members of *Austropeplea* (Ponder and Waterhouse 1997; Remigio 2002).

Results of the systematic study of the *Austroepeplea tomentosa* complex however, suggest that *A. viridis* may be sister to the *A. tomentosa* complex. This relationship is also supported by the current anatomical examinations of this species

and other members currently attributed to *Austropeplea* (see Chapter 2). In this chapter, this matter is re-examined using a large number of taxa.

Molecular studies (Remigio and Blair 1997a; Remigio 2002) as well as the data presented on Chapters 2 and 3 strongly suggest that *Austropeplea* is not monophyletic. Sequence analysis of a combined 16S and ITS-2 dataset placed *Bullastra cumingiana,* the type species of *Bullastra*, as sister to the *A. lessoni* complex. Based on the phylogeny and gene sequence divergence their placement in the same genus, *Bullastra*, was recommended. With the inclusion of the *A. lessoni* complex, the existing concept of *Austropeplea* is therefore no longer monophyletic group. Here this issue is re-examined using a larger number of taxa.

4.1.3 Anatomical characters and their phylogenetic utility

Anatomical studies of the soft bodied parts of snails have proved useful in the past for identifying and separating species. However, internal anatomical characteristics are thought to be problematic in their application to lymnaeid systematics. Some authors have proposed that anatomical characters are too variable and should be avoided in phylogenetic studies, being more prone to selective processes and hence more homoplastic than other characters (Hubendick 1951; Bargues *et al.* 2001; Remigio 2002). Although interestingly, more recent descriptions of lymnaeids have identified numerous morphological characteristics that can be used to distinguish between species (Paraense 1976, 1982, 1984, 1994a, 1995; Ponder and Waterhouse 1997; Samadi *et al.* 2000).

The phylogenetic utility of anatomical characters in lymnaeid systematics has never been formally examined with only one cladistic analysis using such characters having been performed within the Lymnaeidae (Jackiewicz 1993a). This included only European lymnaeids and was based on 11 reproductive characters. The dismissal of anatomical characters could result in the loss of important information for reconstructing lymnaeid phylogenies. Moreover, comparison between the previous systematic studies of the *A. lessoni* and *A. tomentosa* complexes (Chapter 3 and 2, respectively) indicate that characters should not be routinely excluded from phylogenetic analyses based on a lack of utility in other groups. Homologous and

nonhomologous similarity of lymnaeid anatomical characters have to be recognised in light of an inferred phylogeny.

4.1.4 Summary of Aims

The primary objectives of this study were to

> 1. determine whether taxa previously attributed to *Austropeplea* represent a basal lymnaeid lineage,
>
> 2. examine the phylogenetic relationships between the Australian lymnaeids, and the South East Asian lymnaeids, and
>
> 3. to examine the phylogenetic relationships within members currently attributed to *Austropeplea*.

These objectives were met by using partial mitochondral 16S gene sequences and the sequences of the ITS-2 region in conjunction with anatomical studies.

4.2 Methods

4.2.1 DNA Methods

4.2.1.1 Material examined

The species of lymnaeids used in this study are listed in Table 4.1. All of these samples were either sequenced by the author or sequences were obtained from GenBank.

Table 4.1 Summary of taxa and voucher numbers for material used in the molecular systematic study of the Lymnaeidae. Samples marked with n/a were sequenced by the author.

Taxa	Australian Museum Accession No.	Locality	16S Accession Number	ITS-2 Accession number
Lymnaeidae				
Austropeplea lessoni NSW	EBU.35505	Braidwood, AUSTRALIA	n/a	n/a
Austropeplea lessoni NT	C.436053	Humpty Doo, AUSTRALIA	n/a	n/a
Austropeplea lessoni QLD	C.451980	Townsville, AUSTRALIA	n/a	n/a
Austropeplea lessoni WA	C.439182	Broome, AUSTRALIA	n/a	n/a

Austropeplea ollula	-	PHILIPPINES	U82067	-
Austropeplea sp. Hawaii	-	Kauai Island, HAWAII	AF485644	-
Austropeplea sp. China	-	Wuhan, CHINA	AF485643	-
Austropeplea tomentosa NSW	C.431874	Penrith, AUSTRALIA	n/a	n/a
Austropeplea tomentosa NZn	C.422731	North of Napier, NEW ZEALAND	n/a	n/a
Austropeplea tomentosa NZs	C.433513	Little River, NEW ZEALAND	n/a	n/a
Austropeplea tomentosa SA	C.427947	Penola, AUSTRALIA	n/a	n/a
Austropeplea tomentosa TAS	C.422098	Launceston, AUSTRALIA	n/a	n/a
Austropeplea viridis 1	C.449003	Perth, AUSTRALIA	n/a	n/a
Austropeplea viridis 2	-	Queensland, AUSTRALIA	AF485642	-
Bulimnaea megasoma	-	USA	U82068	-
Bullastra cumingiana	C.416760	Luzon, PHILIPPINES	n/a	n/a
Fossaria truncatula	C.451976	South Island, NEW ZEALAND	n/a	n/a
Fossaria bulmoides	-	Oklahoma, USA	AF485657	-
Fossaria obrussa	-	Ontario, CANADA	AF485658	-
Kutikina hispida	C.422107	Franklin River, AUSTRALIA	n/a	n/a
Lymnaea stagnalis 1	-	GERMANY	U82071	-
Lymnaea stagnalis 2	-	ITALY	U82072	-
Lymnaea stagnalis 3	-	Manitoba, CANADA	AF485659	-
Lymnaea stagnalis 4	-	Manitoba, CANADA	AF485660	-
Lymnaea stagnalis 5	-	DENAMRK	AY577461	-
Omphiscola glabra	-	DENMARK	AY577463	-
Psuedosuccinea columella 1	-	USA	U82073	AY186751
Psuedosuccinea columella 2	-	REF AT HOME	AY651244	-
Radix auricularia	C.449004	North Island, NEW ZEALAND	AF485646	AJ319628
Radix rubiginosa	-	West Java, INDONESIA	U82076	n/a
Radix luteola	-	SRI LANKA	AF485648	-
Radix natalensis	W.923	SOUTH AFRICA	n/a	n/a
Radix ovata	-	Tubingen, GERMANY	AF485647	AJ319640
Radix peregra	C.429190	FINLAND	U82074	AJ319633
Radix quadrasi	C.416769	Luzon, PHILIPPINES	U82075	n/a
Radix sp. Canada	-	CANADA	AF485650	-
Radix sp. Romania	-	ROMANIA	AF485651	-
Radix sp. Philippines	-	PHILIPPINES	AF485649	-
Stagnicola bonnevillensis	-	Utah, USA	AF485655	-

Stagnicola caperata	-	CANADA	U82077	-
Stagnicola catascopium	-	USA	U82078	-
Stagnicola corvus	-	BULGARIA	U82079	AJ319625
Stagnicola elodes 1	-	USA	U82080	-
Stagnicola elodes 2	-	CANADA	AF485652	-
Stagnicola elrodi	-	Flathead Lake, USA	AF485656	-
Stagnicola emarginata	-	USA	U82081	-
Stagnicola palustris	-	GERMANY	U82082	AJ319620
Stagnicola sp.	-	Flathead Lake, USA	AF485654	-
Stagnicola sp.	-	Manitoba, CANADA	AF485653	-
Stagnicola sp. Ukraine	-	Sasyk Lake, UKRAINE	AF485662	-
Outgroups				
Ancyloplanorbidae				
Ameriana carinata	-	AUSTRALIA	U82065	-
Ancylus fluviatilis	-	DENMARK	AY577466	-
Aplexa hypnorum	-	DENMARK	AY577464	-
Biomphalaria peregrina	-	Nova Lima, BRASIL	AY030232	-
Biomphalaria schrammi	-	Minas Gerars, BRASIL	AY030233	-
Bulinus bavyayi	-	MADAGASCAR	AY029544	-
Bulinus globosus	-	Zanzibar, TANZANIA	AY029546	-
Burnupia kempi	-	UGANDA	AY577468	-
Burnupia stuhlmanni	-	UGANDA	AY577467	-
Ferrissia fragilis	-	DENMARK	AY577462	-
Planorbis planorbis	-	DENMARK	AY577476	-
Pettancylus sp.	-	Ilolio, PHILIPPINES	AF485663	-
Physidae				
Physa acuta	-	Missouri, USA	AY651226	-
Physa fontinalis	-	DENMARK	AY577465	-
Physa heterostropha	-	Philadelphia, Pennsylvania, USA	AY651231	-
Physella johnsoni	-	CANADA	AF346750	-
Physella wrighti	-	CANADA	AF419322	-

NSW= New South Wales, Australia, NT= Northern Territory, NZn= North Island, New Zealand, NZs= South Island, New Zealand QLD= Queensland, Australia, SA= South Australia TAS= Tasmania, Australia, WA= Western Australia, VIC= Victoria, Australia

4.2.1.2 DNA extraction, PCR amplification and sequencing

Lymnaeids are intermediate hosts to a number of parasitic trematodes (Brown 1978).

Development and multiplication of the parasites takes place inside the body cavity of

the snail, and usually within the digestive gland (which is situated in the upper spirals of the shell). In order to ensure that no parasite DNA was extracted from the snails, only a small piece of foot tissue was used for the extraction of DNA. A CTAB method was employed for the extraction of the DNA. A small piece of foot tissue was sliced from the animal and this tissue placed in a solution of 200 μl of 2% CTAB and 100 μg proteinase K. The tissue was then broken up by grinding with a plastic pestle and digested for two hours at 55°C, with inversion every 30 minutes. Polymuccosaccarides were extracted from the sample in four steps; (1) 200 μl of chloroform/ isoamylalcohol (24:1) was added to the solution; (2) mixing was carried out by repeated inversion for two minutes; (3) separation of the phases by centrifuging for four minutes at 13 200 rpm; and (4) the upper phase (containing the DNA) was carefully removed. The lower phase is distinguished by the white polymucosacharide layer that forms above the polymucosacharide-containing supernatant. These extraction steps were repeated three times to ensure all polymuccosaccarides had been removed from the sample. Genomic DNA was precipitated by the addition of two volumes of absolute ethanol and incubated at -20ºC for 20 minutes. The DNA pellet was then centrifuged for 15 minutes at 13 200 rpm, the supernatant removed and the pellet washed with 70% ethanol at -20ºC. The genomic DNA was redissolved in 50 μl of 1 mM Tris-HCl (pH 8) and stored at 4°C. This genomic DNA solution was used directly in the PCR reaction.

4.2.1.3 Phylogenetic analysis of 16S

To reconstruct 16S phylogenies, I used both maximum parsimony (MP) as implemented in PAUP* 4.08b (Swofford 2002) and Bayesian inference using Markov chain Monte Carlo in the program MrBayes 3.1 (Huelsenbeck *et al.* 2001; Ronquist and Huelsenbeck 2003). MP searches were heuristic with 500 random-taxon-addition replicates, TBR branch swapping, and no maxtrees restrictions. All characters were treated as equal and unordered, with gaps treated as missing data. Clade support was assessed with 1000 bootstrap replicates, each with 100 random-addition heuristic searches (Felsenstein 1985). The sister taxa to the Lymnaeidae, and indeed relationships within the Hygrophila, are currently unclear. Therefore a number of outgroup taxa were used in this analysis, as listed in Table 4.1.

For Bayesian inference, hierarchical likelihood ratio tests were performed as implemented in the program MrModelTest, in order to determine which nucleotide substitution model best fit the 16S data set (Nylander *et al.* 2004). A general time reversal model of molecular evolution (nst=6), with the rates across sites being subject to a variable gamma distribution (rates= invgamma) was selected for the 16S dataset. Variable regions were excluded from the phylogenetic analysis (see Appendix 4.2). I ran three independent simulations of four simultaneous chains of Markov Chain Monte Carlo for 5 million generations, sampling every 100 generations. Each independent simulation was started from different randomly chosen trees. Stationarity was defined as when the standard deviation reached 0.01, which was reached at 1 000 000 generations. Therefore, burnin discarded the first 10 000 trees.

4.2.1.4 Phylogenetic analyses of combined molecular dataset

A second set of analyses were undertaken to assess the relationship between *Austropeplea* and *Radix*, using a combined dataset of 16S and ITS-2 (Appendix 4.3, 4.4). Only members of *Austropeplea*, *Radix*, *Kutikina*, and *Bullastra* were included in the ingroup.

To reconstruct phylogenies, I used both maximum parsimony (MP) as implemented in PAUP* 4.08b (Swofford 2002) and Bayesian inference using Markov chain Monte Carlo in the program MrBayes 3.1 (Huelsenbeck *et al.* 2001; Ronquist and Huelsenbeck 2003). MP searches were performed as for the 16S dataset (see section 4.2.1.3). Outgroup taxa were *Lymnaea stagnalis* (Linneaus, 1758), *Psuedosuccinea columella* (Say, 1817), *Stagnicola corvus* (Gmelin, 1788) and *S. palustris* (Müller, 1774).

For Bayesian inference, hierarchical likelihood ratio tests were performed as implemented in the program MrModelTest, in order to determine which nucleotide substitution model best fit each data set (Nylander *et al.* 2004). A general time reversal model of molecular evolution (nst=6), with the rates across sites being subject to a gamma distribution (rates=gamma), was selected for both the 16S and ITS-2 dataset. The alignment of the ITS-2 region resulted in regions of large

insertions. Due to the variable nature of these inserts, they were excluded from the phylogenetic analysis (see Appendix 4.4). The combined dataset (16S + ITS-2) was partitioned for the Bayesian analysis so that the best fit models were applied separately to the 16S and ITS-2 data (unlink command). The Bayesian analysis was performed as in the 16S dataset (see section 4.2.1.3). Stationarity was reached at 200 000 generations. Therefore the first 2000 trees were discarded as the burn-in for the combined molecular dataset.

4.2.2 Anatomical methods

The species of lymnaeids used in for the anatomical study are listed in Table 4.2. I examined formalin and/or ethanol preserved material of the following species; *Austropeplea lessoni, A. tomentosa, A. viridis, Bullastra cumingiana, 'Lymnaea' brevispina* (Martin, 1897), *Radix auricularia* (Linneus, 1758), *R. quadrasi* (Möllendorf, 1898) , *R. peregra* Müller, 1774), and *R. rubiginosa* ((Michelin, 1831;Table 4.2). For each population at least three adult and parasite free specimens were examined. Dissections were done under a Wild M3C Leica dissecting microscope. All other species were coded using images and descriptions from the relevant literature (Table 4.2). All morphological features that were identified as differing between samples were coded.

For *Austropeplea lessoni, A. tomentosa, A. viridis, Radix auricularia* and *R. quadrasi* three radulae from each sample were examined using a scanning electron microscope. The extracted radulae were cleaned by heating to 60-80ºC in 5% NaOH solution overnight. Each radula was then rinsed in distilled water, and subject to ultrasound to remove any debris. Radulae were mounted on specimen stubs using a dry method, whereby radulae were allowed to dry at room temperature on a glass cover slip that was attached to the stub with double sided tape. The radulae were then coated with gold for examination with the scanning electron microscope. Radula for other species were coded using images from the relevant literature (Table 4.2)

Table 4.2 Summary of taxa, voucher numbers and relevant literature used in the anatomical systematic study of the Lymnaeidae. Samples marked with n/a were dissected by the author.

Taxa	Australian Museum Accession No.	Locality	Reference
Austropeplea lessoni NSW	EBU.35505	Braidwood, AUSTRALIA	n/a
Austropeplea lessoni NT	C.436053	Darwin, AUSTRALIA	n/a
Austropeplea lessoni QLD	C.451890	Townsville, AUSTRALIA	n/a
Austropeplea lessoni WA	C.439182	Broome, AUSTRALIA	n/a
Austropeplea ollula	-	JAPAN	Itagaki and Itagaki (1955)
Austropeplea tomentosa NSW	C.431874	Penrith, AUSTRALIA	n/a
Austropeplea tomentosa TAS	C.422098	Launceston, AUSTRALIA	n/a
Austropeplea tomentosa SA	C.427947	Penola, AUSTRALIA	n/a
Austropeplea tomentosa NZn	C.422731	North of Napier, NEW ZEALAND	n/a
Austropeplea tomentosa NZs	C.433513	Little River, NEW ZEALAND	n/a
Austropeplea viridis	C. 449003	Perth, AUSTRALIA	n/a
Bullastra cumingiana	C.416760	Luzon, PHILIPPINES	Hubendick (1951)
Fossaria truncatula	-	JAPAN	Itagaki (1956)
Kessinaria papillosa	-	Northern Territory, AUSTRALIA	Walker and Ponder (2001)
Kutikina hispida	C.422107	Franklin River, TAS, AUSTRALIA	Ponder and Waterhouse (1997)
'Lymnaea' brevispina	Loan Material	Sumatra, INDONESIA	n/a
'Lymnaea' cousini	-	ECUDAOR	Paraense (1995); Pointier *et al.* (2004)
'Lymnaea' rupestris	-	BRASIL	Paraense (1982)
Lymnaea stagnalis	-	POLAND	Jackiewicz (1986; 1988a; 1993a; 1993b; 1998; 1998)
'Lymnaea' viatrix	-	ARGENTINA, CHILE	Paraense (1976)
Omphiscola glabra	-	SWEDEN, GERMANY	Jackiewicz (1986; 1988a; 1993a; 1993b; 1998; 1998)
Physa acuta	-	FRANCE	Paraense and Pointier (2003)
Psuedosuccinea columella	-	AUSTRALIA	n/a
Radix auricularia	C.449004	North Island, NEW ZEALAND	n/a

Radix luteola	-	SRI LANKA	(Annandale and Rao 1925; Hubendick 1951)
Radix natalensis	C.341942	AFRICA	Pretorius and van Eden 1969
Radix ovata	-	Tubingen, GERMANY	Jackiewicz (1986; 1988a; 1993a; 1993b; 1998; 1998)
Radix peregra	C.999999	FINLAND	Jackiewicz (1986; 1988a; 1993a; 1993b; 1998; 1998)
Radix quadrasi	C.416769	Luzon, PHILIPPINES	n/a
Radix rubiginosa	-	West Java, INDONESIA	n/a
Stagnicola elodes	-	MEXICO	Paraense (1994b)
Stagnicola catascopium	-	USA	Walter (1969)
Stagnicola corvus	-	BULGARIA	Jackiewicz (1986; 1988a; 1993a; 1993b; 1998; 1998)
Stagnicola palustris	-	GERMANY	Jackiewicz (1986; 1988a; 1993a; 1993b; 1998; 1998)

AUS= Australia, NSW= New South Wales, Australia, NT= Northern Territory, NZn= North Island, New Zealand, NZs= South Island, New Zealand QLD= Queensland, Australia, SA= South Australia, TAS= Tasmania, Australia, WA= Western Australia, Australia, VIC= Victoria, Australia

A total of 80 characters from the shell, outer body, pallial cavity, nervous system, reproductive systems and the radula were identified as variable between species. A full description of these characters and their respective states are in Appendix 2.1. However, only 60 characters were included in the analysis. Character numbers 2, 7, 9, 14, 27 to 33, 40, 44 to 49, and 59 were excluded from the analysis, due to large gaps in the dataset for these characters (Table 4.3). The full dataset for the 35 taxa is presented in Appendix 4.1. Maximum parsimony analyses of the data of the data were performed using PAUP* 4.0b8 (Swofford 2002). A heuristic search was performed with 100 random addition sequence replicates, whereby all characters were treated as equal and unordered. *Physa acuta* and *Kessinaria papillosa* were designated outgroup taxa. To estimate tree support, bootstrap analysis was performed, with 1000 replicates. The distribution of character states on the trees was examined using McClade 4.0 (Maddison and Maddison 2000).

Table 4.3 Character and character states used in the anatomical analysis of the Lymnaeidae

Character number	Character	Character state and codification
1	Shell umbilicus	Closed (1); half open (2); open (3)
3	Number of whorls	Three (1); four (2); five (3); 3.5 (4); 2.5 (5); 4.5 (6)
4	Columella fold	Absent (1); slight (2); distinct (3)
5	Shell sculpture	Absent (1); present (2)
6	Periostracum ornamentation	Absent (1); hairy (2)
8	Foot shape at posterior end	Tapering to a point (1); rounded (2)
10	Eye lobe	Absent (1); well developed (2); undeveloped (3)
11	Tentacle shape	Wider than long (1); width equal to length (2); longer than wide (3); twice as long as wide (4)
12	Lateral sides of snout	Developed (1); undeveloped (2)
13	Pallial roof pigmentation	Mottled black and white (1); black (2)
15	Mantle expansion	Absent (1); just outside of shell (2); covering some parts of the shell (3); covering large parts of the shell (4)
16	Expanded mantle pigmentation	Absent (1); present (2)
17	Number of pneumostomal ridges	One (1); two (2)
18	Outer lobe	Absent (1); present (2)
19	Upper plate of pneumostome	Thin (1); thick (2)
20	Broadest area of kidney	Anterior end (1); same width along length (2); posterior end (3); middle (4)
21	Kidney width to length ratio	3:1 (1); 2:1 (2); greater than 3:1 (3)
22	Right lobe of kidney	Absent (1); present (2)
23	Position of pulmonary vein	To the right of kidney (1); inside right lobe (2)
24	Pulmonary vein length	One third the length of the kidney (1); less than one third the length of the kidney (2); greater than one third the length of the kidney (3)
25	Ureter	Absent (1); present (2)
26	Opening of kidney	Inside pnuemostome (1); anterior to the pnuemostome (2)
33	Salivary glands relative size	Equal size (1); right longest(2); left longest (3)
34	Uterus/ vagina length relative to oothecal gland length	greater than half the length (1); less than half the length (2); equal or longer (3)
35	Spermathecal duct length	Shorter than uterus/ vagina (1); equal to uterus/ vagina (2); longer than uterus/ vagina (3)
36	Spermathecal duct width	Equal to uterus/ vagina (1); thinner than uterus/ vagina (2)
37	Uterus shape	Parallel (1); tapering distally (2)
38	Oviducal caecum size relative to	¼ width (1); ½ width (2); between ½ and one width (3); wider

	oothecal gland	(4); absent (5)
39	Oothecal gland shape	Globular (1); pyriform (2); rectangular (3); square (4)
41	Position of uterus relative to oothecal gland	At right angles (1); greater than right angles (2); less than right angles (3)
42	Velum shape	Horse-shoe shaped (1); circular (2); absent (3)
43	Penis sheath length relative to praeputium	Less than half the length (1); Greater than half the length (2); Equal in length (3); Half the length (4); longer than praeputium (5)
50	Upper prostate	Thin (1); wide (2)
51	Length of prostate relative to female reproductive system	Equal in length (1) longer (2); much longer (3); shorter (4)
52	Shape of lower prostate	Straight (1); bent to left (2)
53	Central tooth	Bicuspid (1); unicuspid (2); tricuspid (3); multicuspid (4)
54	Position of small cusp on central tooth	Left (1); right (2)
55	Radula teeth shape	Blunt (1); sharp (2)
56	Lateral teeth	Bicuspid (1); tricupsid (2); unicuspid (3); multicuspid (4)
57	Marginal teeth	Bicuspid (1); tricuspid (2); tetracuspid (3) , 5 cups (4), greater than 5 cusps (5)
58	Ureter length	Short (1); medium (2); long (3)
60	Insemination pocket	Absent (1); present (2)
61	Vaginal bulb	Absent (1); present (2)
62	Penal knot	Absent (1); present (2)
63	Prostate pouch	Absent (1); present (2)
64	Shell orientation	Dextral (1); sinistral (2)
65	Spire height	Absent or largely reduced (1); short (2); medium (3); high (4)
66	Tentacle form	Triangular and flat (1); fillaform and circular (2)
67	Columella digitations of the mantle	Absent (1); present (2)
68	Number of flexures in ureter	Zero (1); Two (2)
69	Ureter shape	Bent to left (1); straight (2)
70	Spermatheca shape	Round (1); egg shaped (2)
71	Penis shape	Short and wide (1); long and thin (2)
72	Penis sheath head	Well developed (1); poorly developed (2)
73	Width of penis sheath relative to praeputium width	One third as wide (1); half as wide (2); equal (3)
74	Praeputium retractor insertion point	Laterally (1); at base of praeputium head (2)
75	Number of internal folds of the praeputium	One (1); two (2)
76	Preputial gland	Absent (1); present (2)
77	Prostate structure	Series of small lobes (1); one large structure (2)
78	Prostate shape	Long and thin (1); wider anterior thin posterior (2); wide

		anterior and posterior (3); wide anterior and posterior, thin in the middle (4)
79	Internal prostate fold	Absent (1); One (2); numerous (3)
80	Number of chromosome pairs	16 (1); 17 (2); 18(3)

4.2.3 Combined anatomical and molecular analyses

Phylogenetic analyses were performed on a combined dataset consisting of the 16S molecular and anatomical datasets. Taxa for which all datasets were not complete were deleted, creating a dataset of 29 taxa. To determine whether significant incongruence existed between the molecular and anatomical datasets, incongruence length difference (ILD) tests (Farris *et al.* 1995) were conducted by excluding all invariant sites and using the partition homogeneity test in PAUP*4.08b (Swofford 2002) with 100 iterations. A maximum parsimony analysis was performed as described in Section 4.2.2.

The combined dataset (16S + ITS-2) was partitioned for the Bayesian analysis so that the best fit models were applied separately to the 16S and anatomical data (unlink command). For Bayesian inference, hierarchical likelihood ratio tests were performed as implemented in the program MrModelTest, in order to determine which nucleotide substitution model best fit for the 16S data set, (Nylander *et al.* 2004). A general time reversal model of molecular evolution (nst=6), with the rates across sites being subject to a gamma distribution (rates=gamma), was selected. The anatomical data was subject to a gamma distribution. For the combined molecular and anatomical dataset, I ran three independent simulations of four simultaneous chains of Markov Chain Monte Carlo for 5 million generations. Sampling occurred every 100 generations and each independent simulation was started from different randomly chosen trees. Stationarity was defined as when the standard deviation reached 0.01, which was reached at 200 000 generations. Therefore the first 2000 trees were discarded as the burn-in for the combined molecular and anatomical dataset.

4.3 Results

4.3.1 Molecular phylogeny of the Lymnaeidae

The 16S sequences resulted in aligned data matrices of 547 base pairs including indels (Appendix 4.2). *Stagnicola caperata* and *S. emarginata* had the shortest sequence length with 365 base pairs, whilst *Radix luteola* had the longest with 436 base pairs (Table 4.4)

Table 4.4 16S gene sequence length measured in number of base pairs.

Species	Number of Base Pairs
Austropeplea lessoni	429 - 431
Austropeplea tomentosa	427 – 429
Austropeplea viridis	429 – 431
Bullastra cumingiana	431
Bulimnea megasoma	414
Fossaria bulmoides	427
Fossaria obrussa	422
Fossaria truncatula	426
Radix auricularia	438
Radix luteola	436
Radix natalensis	430
Radix ovata	432
Radix peregra	422
Radix quadrasi	419
Radix rubiginosa	405
Radix sp. Philippines	433
Radix sp. Canada	432
Radix sp. Romania	434
Kutikina hispida	427
Austropeplea sp. Hawaii	429
Lymnaea stagnalis	419
Austropeplea sp. China	431
Pseudosuccinea columella	422
Omphiscola glabra	421
Stagnicola elrodi	431
Stagnicola bonnevillensis	431
Stagnicola sp. USA	429
Stagnicola sp. Canada	434
Stagnicola sp. Ukraine	434
Stagnicola elodes	396- 429
Stagnicola corvus	410
Stagnicola emarginata	365
Stagnicola catascopium	371
Stagnicola caperata	365
Stagnicola palustris	421

Sequence divergence between the lymnaeid samples varied from 0.04 to 21% difference in the 16S dataset. Sequence divergence between *Austropeplea viridis* and *A. lessoni* ranged from 12.2 to 13.1%, whilst divergence between *A. viridis* and *A. tomentosa* ranged from 10.5 to 14.8%. Sequence divergence between *A. lessoni* and *A. tomentosa* ranged from 10.6 to 12.2%. Within *Radix*, sequence divergence was quite large, with *R. natalensis* diverging from 11.3 to 13.8% from the European *Radix* members (*R. auricularia*, *R. ovata* and *R. peregra*). Sequence divergence between *R. natalensis* and members of the Asian *Radix* (*R. luteola*, *R. rubiginosa*, *R. quadrasi*, and *Radix* spp.) group ranged from 14.3 to 16.0%. Sequence divergence between the Asian *Radix* group and the European *Radix* group ranged from 15.2 to 18.4%.

Of the 474 characters used in the MP analyses, 277 were parsimony informative, resulting in two equally parsimonious trees with a tree length of 1648 (CI=0.33, RI=0.69, RC=0.23). One of these trees is Figure 4.1. Pilot analyses including variable regions also produced trees with the same topology as the tree excluding variable regions. Results of the Bayesian analyses are shown in Figure 4.2.

The Lymnaeidae form a well supported monophyletic group in both analyses. *Austropeplea*, as it is currently recognised, is resolved as one of the most derived groups within the Lymnaeidae in both the MP and Bayesian analyses (Figs 4.1, 4.2). The North American and European lymnaeids occupy the basal positions of the lymnaeid phylogeny. *Omphiscola glabra* is placed as sister to all other lymnaeid taxa in the analyses. This relationship has less than 50% bootstrap support (Fig 4.1) but high posterior probability support (Fig 4.2).

The currently accepted concepts of *Austropeplea* and *Radix* are polyphyletic in both the MP and Bayesian analyses. The Asian *Radix* and *R. natalensis* clade is placed as a well supported sister group to *"Austropeplea"* and the European *Radix* in the Bayesian analysis (Fig 4.2). In the MP analysis, the Asian *Radix* and *R. natalensis* clade is shown in the same position, however, there is less than 50% bootstrap support for this relationship (Fig 4.1).

The relationship between "*Austropeplea*" and the European *Radix* differs between MP and Bayesian analyses. In the MP analyses, the *Austropeplea viridis* clade is shown as sister to the European *Radix*, the *A. tomentosa* complex, *Bullastra* and the *A. lessoni* complex clade, although bootstrap support is low (bp= 59; Fig 4.1). The European *Radix* is placed as sister to the *A. lessoni* and *A. tomentosa* complexes and *Bullastra* clade, although these three clades form a polytomy in the bootstrap analysis (Fig 4.1). In the Bayesian analysis, the *A. lessoni* complex and *Bullastra* clade is placed as sister to a polytomy of the *A. tomentosa* complex, *A. viridis* and the European *Radix*, although support for this relationship is low (0.59; Fig 4.2).

The North American lymnaeids in the MP analysis are placed as sister to the *Austropeplea*, *Kutikina*, *Bullastra* and *Radix* clade, although there is less than 50% bootstrap support for this relationship (Fig 4.1). The North American lymnaeids form two separate clades in the MP analysis. The first clade is well supported and composed of the North American *Stagnicola* species, with the exception of *S. caperata*. Sister to the North American Stagnicola is a clade composed of the other North American lymnaeids, *Fossaria*, *Pseudosuccinea*, *Bulimnea*, and *S. caperata* (Fig 4.1). There is however less than 50% bootstrap support for this sister relationship and indeed for some relationships within the *Fossaria*, *Pseudosuccinea*, *Bulimnea*, and *S. caperata* clade (Fig 4.1).

In the Bayesian analysis, only the North American Stagnicoline clade (except *S. caperata*) are resolved as sister to the *Austropeplea*, *Kutikina*, *Bullastra* and *Radix* clade, although support is low (0.50; Fig 4.2). The *Fossaria*, *Pseudosuccinea*, *Bulimnea*, and *S. caperata* clade is more closely related to the European *Stagnicola* and Holarctic *Lymnaea stagnalis* (Fig 4.2). *Stagnicola* is polyphyletic in both the MP and Bayesian analyses, with the European *Stagnicola* being more closely resolved to *Lymnaea stagnalis*.
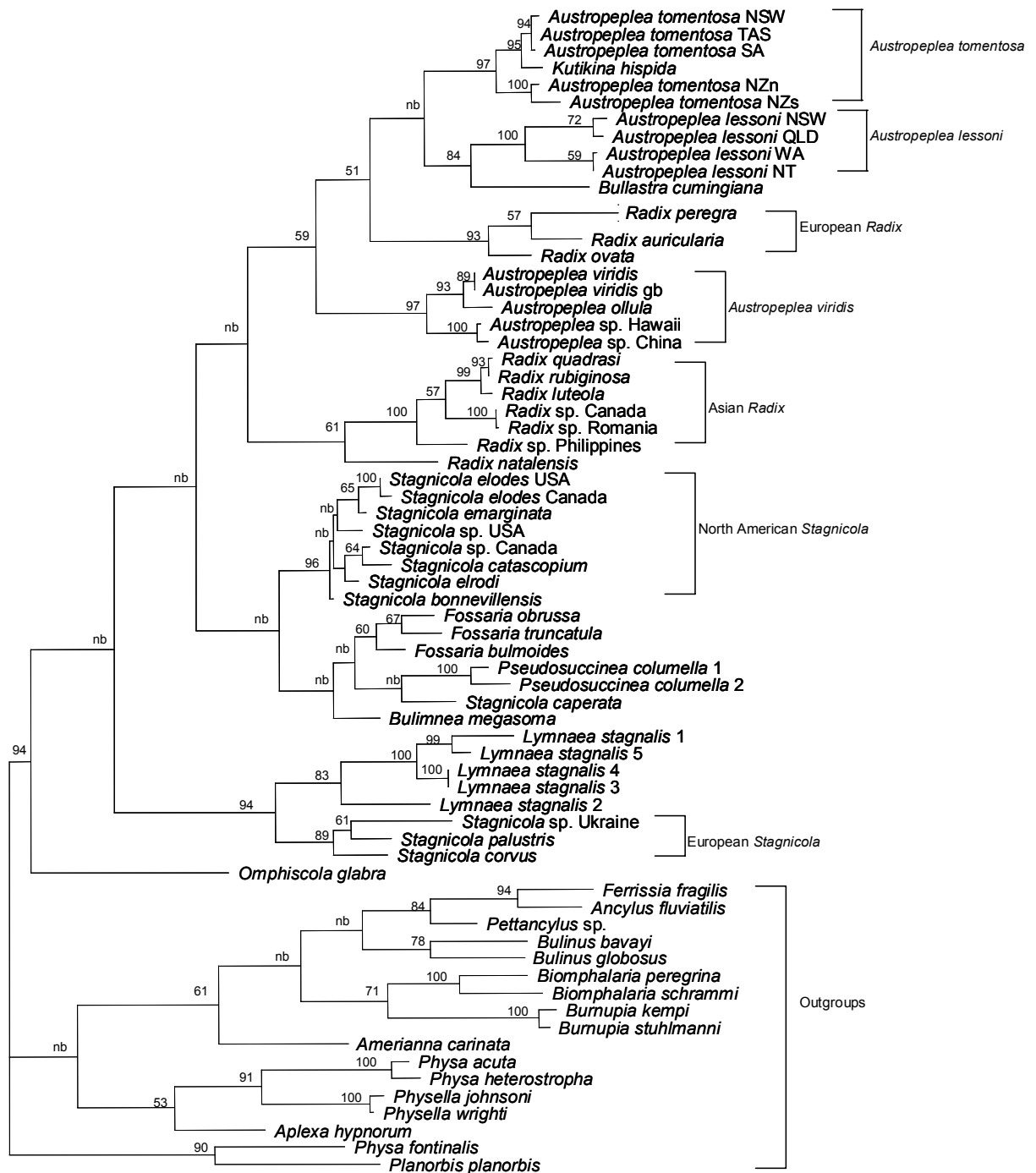
Figure 4.1 Molecular phylogeny of the Lymnaeidae based on 16S rRNA sequences. One of the two most parsimonious trees, with a tree length of 234. Numbers above branches are bootstrap scores, only bootstrap > 50% given. nb= no bootstrap, NSW= New South Wales, Australia, NT= Northern Territory, Australia, NZn= North Island, New Zealand, NZs= South Island, New Zealand, QLD= Queensland, Australia, SA= South Australia, TAS= Tasmania, Australia, WA=Western Australia.

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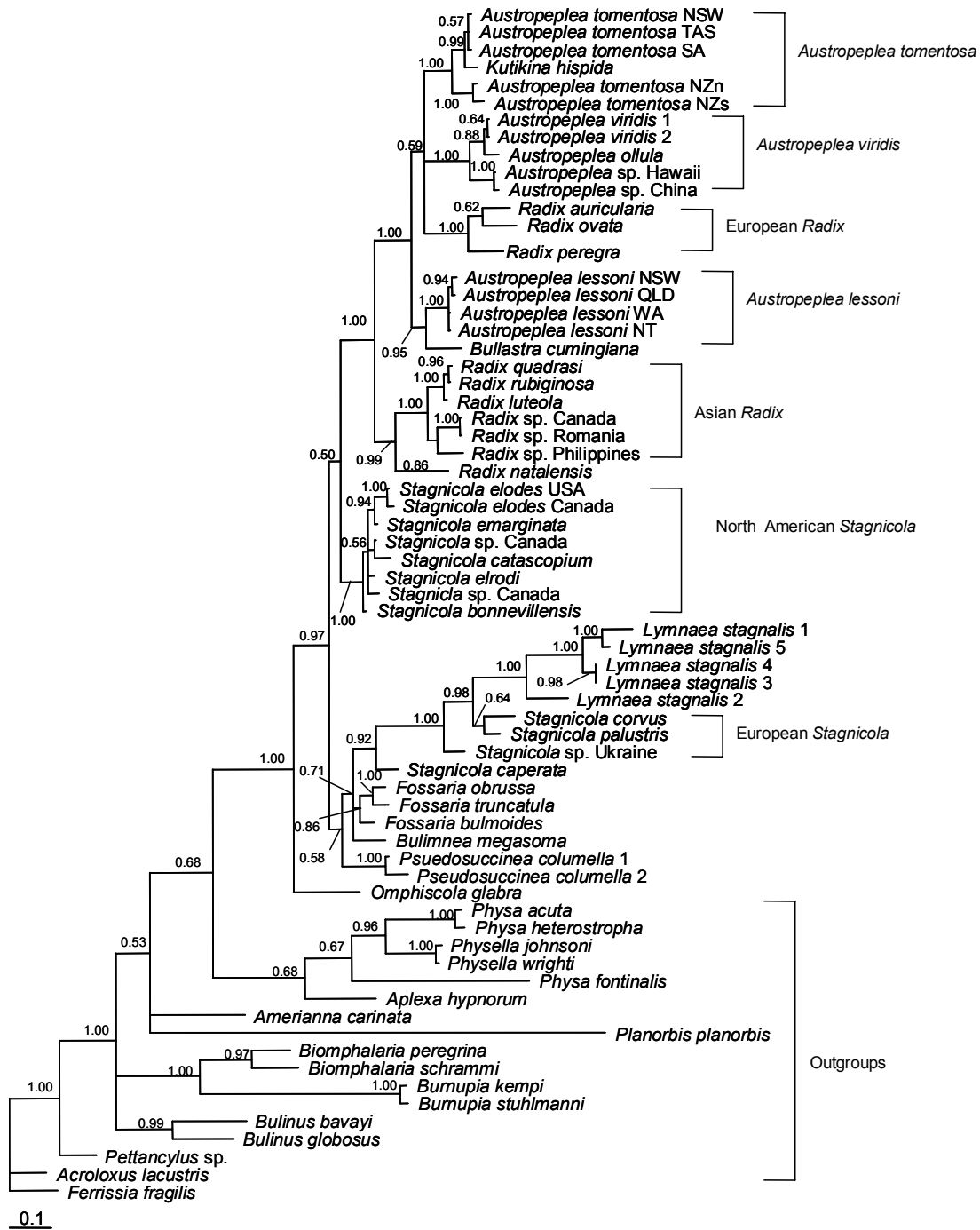
Figure 4.2 Molecular phylogeny of the Lymnaeidae based on 16S rRNA sequences. Majority consensus tree based on Bayesian inference, with maximum likelihood setting under a general time reversal model for DNA substitution. Bayesian analysis based on 5 000 000 generation replicates, with posterior possibilities indicated above the branch, only >50% posterior probability given. NSW= New South Wales, Australia, NT= Northern Territory, Australia, NZn= North Island, New Zealand, NZs= South Island, New Zealand, QLD= Queensland, Australia, SA= South Australia, TAS= Tasmania, Australia, WA=Western Australia.

4.3.2 Combined molecular phylogeny of Australasian Lymnaeidae

The aligned combined 16S and ITS-2 sequnces resulted in matrix of 1019 bases.

Table 4.5 ITS-2 region sequence length measured in number of base pairs.

Species	Number of Base Pairs
Austropeplea lessoni	412 – 428
Austropeplea tomentosa	379 – 409
Austropeplea viridis	390
Bullastra cumingiana	384
Radix auricularia	384
Radix natalensis	373
Radix ovata	371
Radix peregra	379
Radix quadrasi	404
Radix rubiginosa	342
Kutikina hispida	408
Lymnaea stagnalis	427
Pseudosuccinea columella	353
Stagnicola corvus	428
Stagnicola palustris	423

Sequence divergence between the lymnaeid samples varied from 1.4 to 50.7% difference in the ITS-2 dataset. Sequence divergence between *Austropeplea viridis* and *A. lessoni* ranged from 22.1 to 24.4%, whilst divergence between *A. viridis* and *A. tomentosa* ranged from 9.0 to 10.3%. Sequence divergence between *A. lessoni* and *A. tomentosa* ranged from 23.4 to 27.2%. Within *Radix*, sequence divergence was quite large, with *R. natalensis* diverging from 8.8 to 13.9% from the European *Radix* members (*R. auricularia*, *R. ovata* and *R. peregra*). Sequence divergence between *R. natalensis* and members of the Asian *Radix* (*R. luteola*, *R. rubiginosa*, *R. quadrasi*, and *Radix* spp.) group ranged from 7.3 to 16.6%. Sequence divergence between the Asian *Radix* group and the European *Radix* group ranged from 4.2 to 21.4%.

Of the 908 characters used in the MP analyses, 408 were parsimony informative, resulting in one most parsimonious tree with a tree length of 1187 (CI=0.62, RI=0.76, RC=0.49; Fig 4.3). Pilot analyses including variable regions also produced just one tree with the same topology as the tree excluding variable regions. The tree topology of the Bayesian analysis was very similar to the most parsimonious tree, as shown in Figure 4.4.

The *Austropeplea lessoni* complex and *Bullastra cumingiana* are shown as the sister clade to the *A. tomentosa* complex, *A. viridis*, *Kutikina hispida* and all species of *Radix* included in the analysis. This relationship has both high posterior probability and bootstrap support. *Bullastra cumingiana* is also shown as sister to the *A. lessoni* complex, with reasonable levels of support (Figs 4.3, 4.4). In the MP analysis, the *Austropeplea tomentosa* complex and *Kutikina hispida* are shown as sister to the *Radix* and *A. viridis* clade (Fig 4.3). However, the lack of support for *A. viridis* as sister to the *Radix* clade resulted in a polytomy of the *A. tomentosa* complex, *Radix* and *A. viridis* in the bootstrap tree. In the Bayesian analysis, the *Radix* clade is shown as sister to clade containing the *A. tomentosa* complex, *A. viridis* and *Kutikina hispida*. However, posterior probability support for this relationship is low (Fig 4.4; 0.56).

Unlike the 16S molecular analyses of the Lymnaeidae, members of *Radix* in this analysis form a monophyletic group with high posterior probability support (0.99) and lower bootstrap support (bp=69). In the MP and Bayesian analyses *R. quadrasi*, *R. rubiginosa* and *R. natalensis* form a well supported clade within the *Radix* group (Figs 4.3, 4.4). The position of *R. auricularia* as sister to this Asian group has both high bootstrap and posterior probability support (0.94). *Radix ovata* and *R. peregra* as sister all other members of *Radix* has high posterior probability support (0.94), but low bootstrap support (bp=57; Fig 4.3, 4.4).
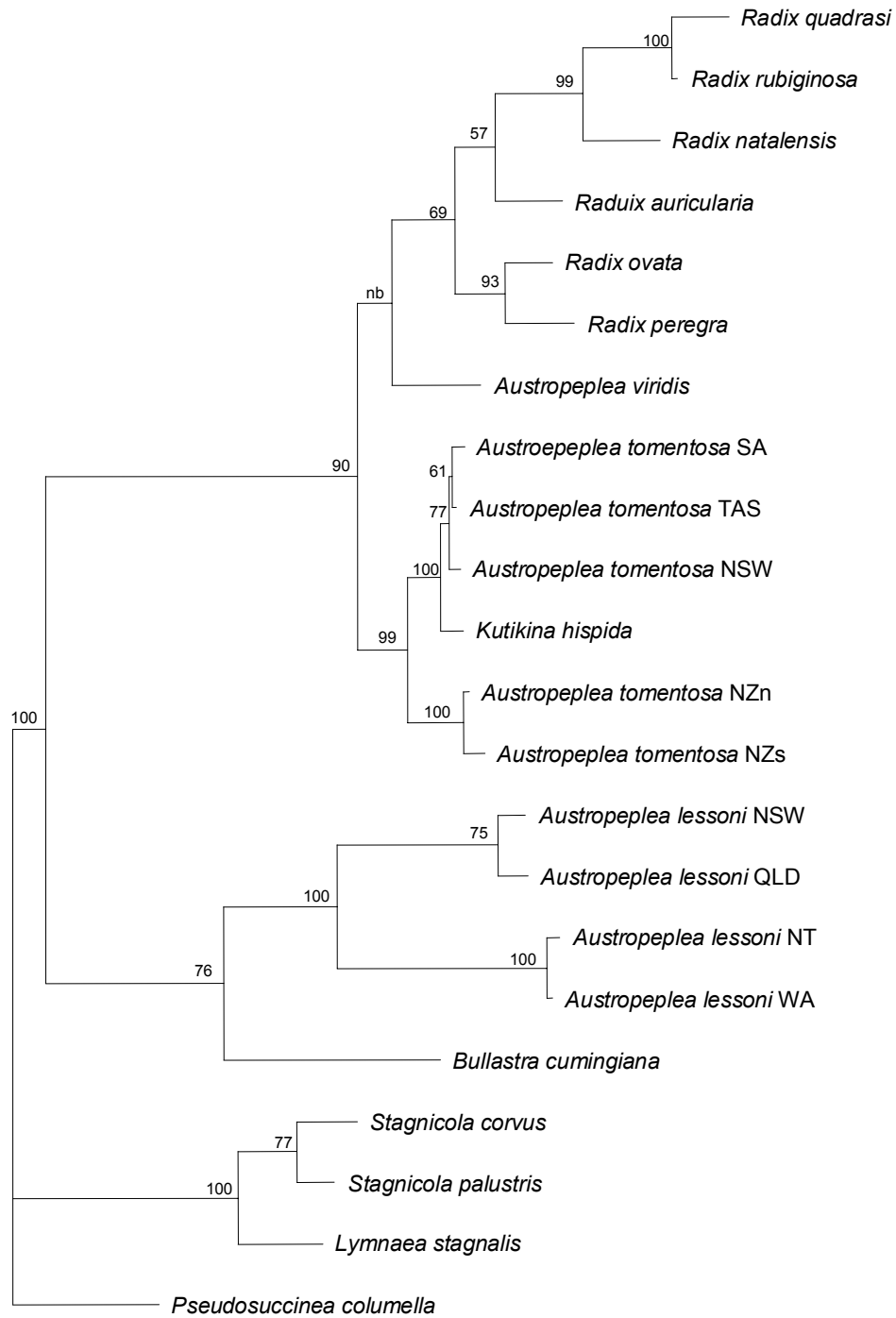
Figure 4.3 Molecular phylogeny of the Lymnaeidae based on 16S and ITS-2 rRNA sequences. Most parsimonious tree, with tree length 234. Numbers above branches are bootstrap scores, with only bootstrap >50% given. nb= no bootstrap, NSW= New South Wales, Australia, NT= Northern Territory, Australia, NZn= North Island, New Zealand, NZs= South Island, New Zealand, QLD= Queensland, Australia, SA= South Australia, TAS= Tasmania, Australia WA=Western Australia.
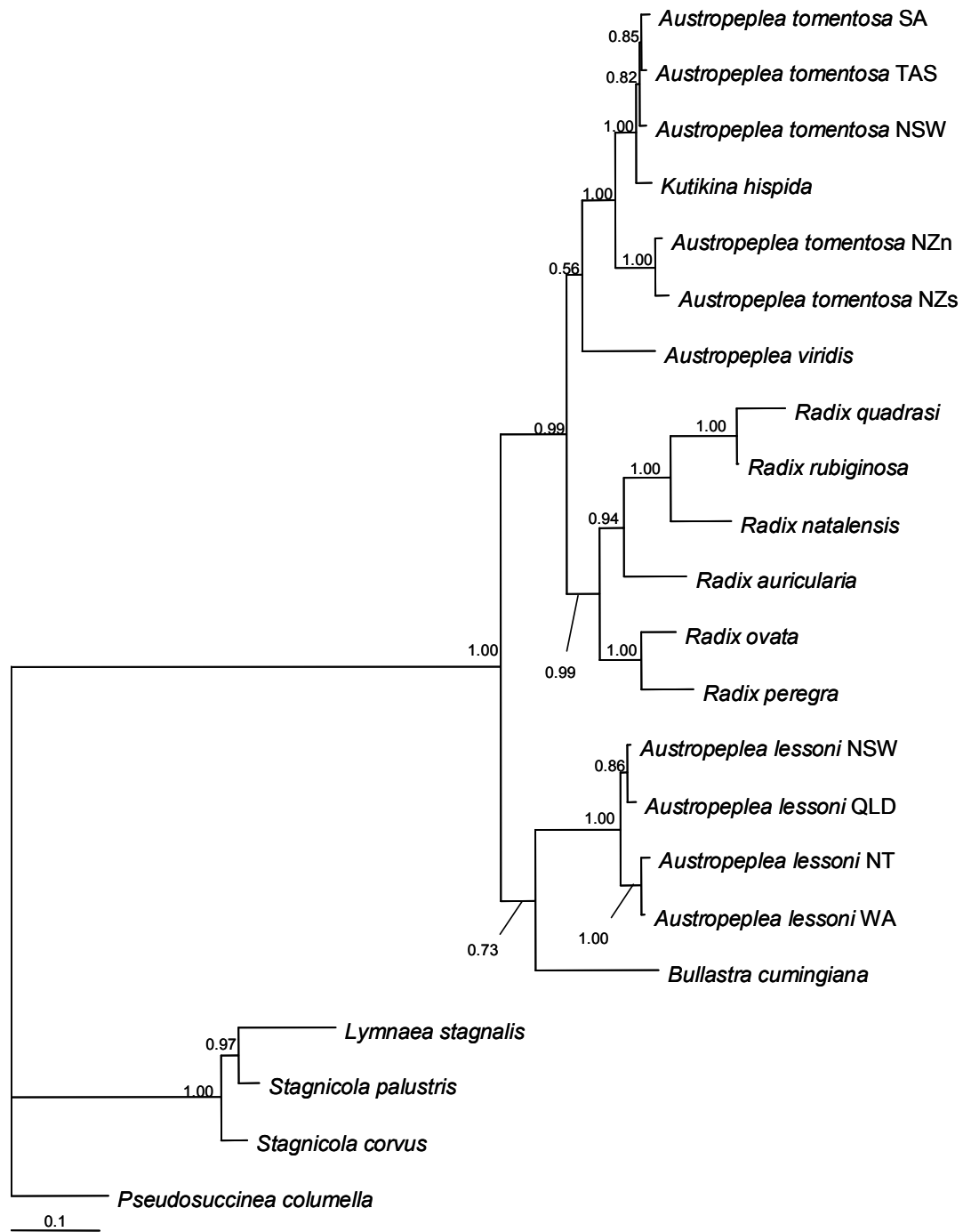
Figure 4.4 Molecular phylogeny of the Lymnaeidae based on 16S and ITS-2 rRNA sequences. Majority consensus tree based on Bayesian inference, with maximum likelihood setting under a partitioned model for DNA substitution. Bayesian analysis based on 5 000 000 generation replicates, with posterior possibilities indicated above the branch, only >50% posterior probability given. NSW= New South Wales, Australia, NT= Northern Territory, Australia, NZn= North Island, New Zealand, NZs= South Island, New Zealand, QLD= Queensland, Australia, SA= South Australia, TAS= Tasmania, Australia WA=Western Australia.

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4.3.3 Anatomical phylogeny of the Lymnaeidae

Of the 60 characters used in the MP analyses, 50 were parsimony informative, resulting in three equally parsimonious trees, with tree a length of 268 (CI=0.36, RI=0.58, RC=0.23; Fig 4.5). The topology of the anatomical tree differs from the 16S phylogeny. The Lymnaeidae form a well supported monophyletic group (bp=85), however, resolution within the group is very poor due to only a few branches within the ingroup having greater than 50% bootstrap support (Fig 4.5). The relationships of the strict consensus will therefore be discussed largely without reference to branch support.

The anatomical phylogeny, as in the molecular phylogenies, resolves taxa *Austropeplea* as one of the more recently derived groups within the Lymnaeidae (Fig 4.5). The most basal branch was *Fossaria truncatula* and the second a clade composed of several European, North American and South American taxa (*'Lymnaea' rupestris*, *'L'. viatrix*, *L. stagnalis*, *Stagnicola elodes*, *S. catascopium* and *S. corvus*; Fig 4.5). *Omphiscola glabra,* which was resolved as sister to the other lymnaeids in the molecular analysis, was resolved at a more intermediate position in the tree and is sister to *S. palustris* (Fig 4.5).

Austropeplea, as it is currently recognised, does not form a monophyletic group due to the inclusion of *Kutikina* (Fig 4.5). The *Austropeplea lessoni* complex is resolved as sister to the European *Radix*, Asian *Radix*, *Bullastra*, *'Lymnaea' brevispina* and *Myxas* clade in the anatomical phylogeny. This result is in contrast to the previous combined molecular analyses, where the *A. tomentosa* complex and *A. viridis* are more closely related to *Radix*. *Austropeplea viridis* is resolved as more closely related to the *A. tomentosa* complex in the anatomical phylogeny (Fig 4.5), a relationship is also supported by the16S Bayesian and combined 16S and ITS-2 analyses. *Kutikina hispida* is resolved as sister to *A. viridis* and *A. ollula* in the anatomical phylogeny (Fig 4.5), whereas the molecular analyses resolved *K. hispida* as sister to the Australian *A. tomentosa*.

Radix does not form a monophyletic clade, as *Radix natalensis* (an African species) is placed as sister to *Pseudosuccinea columella* (a North and South American

species; Fig 4.5). In the molecular phylogenies *R. natalensis* was resolved as sister to the Asian *Radix* group. Moreover, *'Lymnaea' brevispina*, *Bullastra* and *Myxas*, are resolved between the Asian and European *Radix* groups (Fig 4.5). The European lymnaeids form a monophyletic group.

Interestingly, the two European lymnaeids, *Stagnicola palustris* and *S. corvus*, are not resolved as sister taxa in the anatomical phylogeny. This conflicts with the molecular phylogenies were they were strongly supported as sister species. The South American lymnaeids are also polyphyletic, as *'Lymnaea' cousini* is resolved as being more closely related to *Radix natalensis* than to other South American species (*'L'. rupestris* and *'L'. viatrix*). In the anatomical phylogeny the North American *Stagnicola catascopium* and *S. elodes* are closely related to one another and also to the European *S. corvus*. While the North American *Stagnicola* were closely related in the molecular phylogeny, they are not in the same clade as *S. corvus*.

Examination of the parsimony informative anatomical characters revealed that only five characters formed synapomorphies (character numbers 24, 64, 66, 67, 77; Fig 4.5). Four of these five characters, shell direction (64), tentacle form (66), columella digitations (67) and prostate structure (77), distinguished the ingroup from the outgroup (Fig 4.5). The only synapmorphy within the ingroup was the pulmonary vein length, which distinguished the New Zealand, South Australian and Tasmanian samples of *A. tomentosa* from other members of the ingroup (Fig 4.5). Two characters (22 and 23) could possibly form a synapomorphy for the European and Asian *Radix*, *'Lymnaea' brevispina*, *Bullastra*, *Myxas* clade. However, the states of these two characters were not scored in *Radix luteola* and *Myxas glutinosa* due to inadequate data.
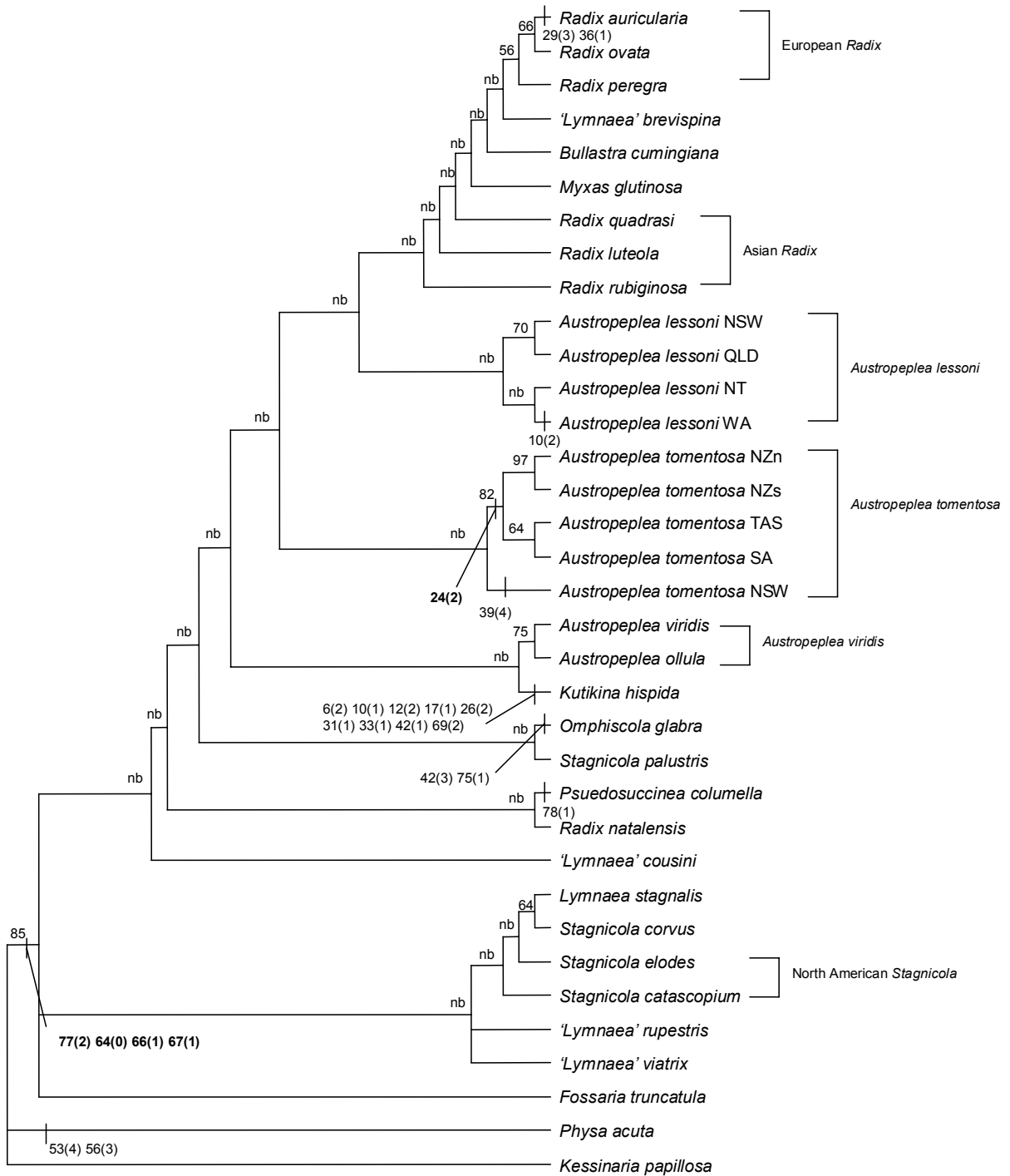
Figure 4.5 Phylogeny of the Lymnaeidae based on 60 anatomical characters. Strict consensus tree of three equally parsimonious trees, with a tree length of 268. Numbers above the branches are bootstrap scores, with only bootstrap >50% given. Below branches are autapomorphies and synapomorphies (in bold), numbers corresponding with characters and character states as listed in Table 4.3. nb= no bootstrap support, NSW= New South Wales, Australia, NT= Northern Territory, Australia, NZn= North Island, New Zealand, NZs= South Island, New Zealand, QLD= Queensland, Australia, SA= South Australia, TAS= Tasmania, Australia WA=Western Australia.

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4.3.4 Combined 16S and anatomical phylogenies of the Lymnaeidae

The ILD test indicated significant incongruence between the molecular and anatomical datasets (p= 0.02). The ILD test has been demonstrated to be a conservative test of compatility and may have been futher weakened by the smaller number of anatomical characters in the analyse (Collin 2003). However, the incongruence between the 16S and anatomical datasets can be observed by the differences in tree topologies of the single datasets.

Of the 588 characters used in the MP analyses, 198 were parsimony informative, resulting in two equally parsimonious trees, with tree length of 1021 (CI=0.41, RI=0.55, RC=0.26; Fig 4.6). The topology of the MP and Bayesian analyses were similar, although some phylogenetic relationships were resolved differently between methods (Figs 4.6, 4.7).

Austropeplea, as in other previous phylogenies, is one of the more derived groups within the Lymnaeidae, with the European and North American lymnaeids occupying the basal positions in the tree (Figs 4.6, 4.7). Resolution of the basal groups in both the MP and Bayesian phylogenies is quite poor. In the MP analysis, *Fossaria truncatula* and *Psuedosuccinea* forms a small clade, as does North American *Stagnicola* and the European *Stagnicola* also. The relationship between these three clades is unresolved, forming a basal polytomy (Fig 4.6). *Omphiscola glabra* in the MP analysis is placed outside the ingroup with the outgroup *Physa acuta*, although this relationship has less than 50% bootstrap support (Fig 4.6). In the Bayesian analysis, a basal polytomy is formed comprising the North American *Stagnicola* clade, *O. glabra*, *F. truncatula* and *P. acuta*, although support is low (Fig 4.7). The European *Stagnicola* and *Lymnaea stagnalis* clade form a polytomy with *Psuedosuccinea*, which is basal to the *Radix*, *Austropeplea*, *Bullastra*, and *Kutikina* clade (Fig 4.7). *Omphiscola glabra* was resolved as sister to the other lymnaeids in the molecular analyses.

As in previous analyses, *Austropeplea,* as it is currently recognised, does not form a monophyletic group (Figs 4.6, 4.7). In the MP analysis, *A. viridis* is resolved as

sister to the *A. tomentosa* complex, the *A. lessoni* complex, *Bullastra*, *Kutikina* and the European *Radix* clade, although there is less than 50% bootstrap support for this relationship (Fig 4.6). In the Bayesian analysis, *A. viridis*, the *A. tomentosa* complex and *Kutikina* form a sister group to a clade including *Radix*, *Bullastra* and the *A. lessoni* complex. The support for this relationship is high (0.81), although the support for *A. viridis* as sister to the *A. tomentosa* complex and *Kutikina* is low (0.61). *Kutikina hispida* is resolved in the combined molecular and anatomical phylogeny as more closely related to the New South Wales *A. tomentosa* (Fig 4.6, 4.7). In previous molecular analyses *K. hispida* is shown as well supported sister species to the Australian samples of *Austropeplea tomentosa*.

Members of *Radix* used in this analyses form a polyphyletic group, as in the 16S phylogeny. The Asian *Radix* and *R. natalensis* clade are sister to the *Austropeplea*, *Bullastra*, *Kutikina* and European *Radix* clade in both analyses. Bootstrap support for this relationship is fairly low (bp=65; Fig 4.6), while posterior probability is moderate (0.79; Fig 4.7). *Radix natalensis*, as in previous molecular phylogenies, is resolved as sister to the Asian *Radix*, with high posterior probability support (0.99; Fig 4.7), but lower bootstrap support (bp=68; Fig 4.6). The European *Radix* form a well supported monophyletic clade and is resolved as more closely related to the *A. lessoni* complex, in both the MP and Bayesian analyses (Figs 4.6, 4.7). The MP analysis places the European *Radix* as sister to the *Bullastra* and the *A. lessoni* complex clade, with moderate support (bp=71). The Bayesian analysis does not resolve the relationship between *Bullastra*, the *A. lessoni* complex and the European *Radix* (Fig 4.7). This relationship differs to the 16S Bayesian analysis and the combined molecular analyses, where the *A. tomentosa* complex is resolved as more closely related to the European *Radix*.

As in previous molecular analyses, *Stagnicola* is polyphyletic, being divided into well supported North American and European clades. *Lymnaea stagnalis* is resolved as sister to the European *Stagnicola* (Figs 4.6, 4.7).
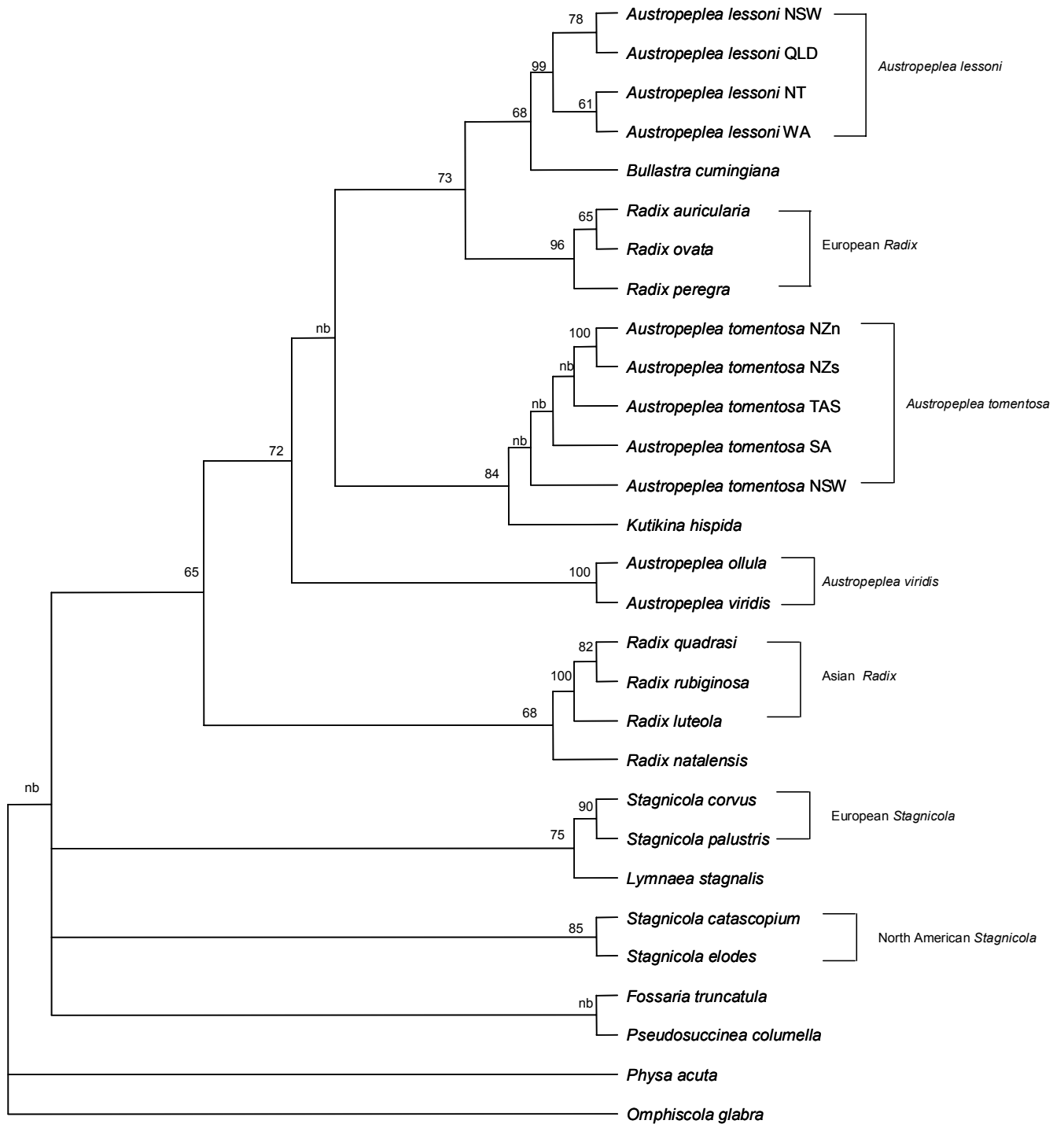
Figure 4.6 Phylogeny of the Lymnaeidae based on anatomical and molecular characters. Strict consensus tree of two maximum parsimony trees, with tree length 1021. Numbers above the branches are bootstrap scores, with only bootstrap >50% given. nb= no bootstrap support, NSW= New South Wales, Australia, NT= Northern Territory, Australia, NZn= North Island, New Zealand, NZs= South Island, New Zealand, QLD= Queensland, Australia, SA= South Australia, TAS= Tasmania, Australia WA=Western Australia.
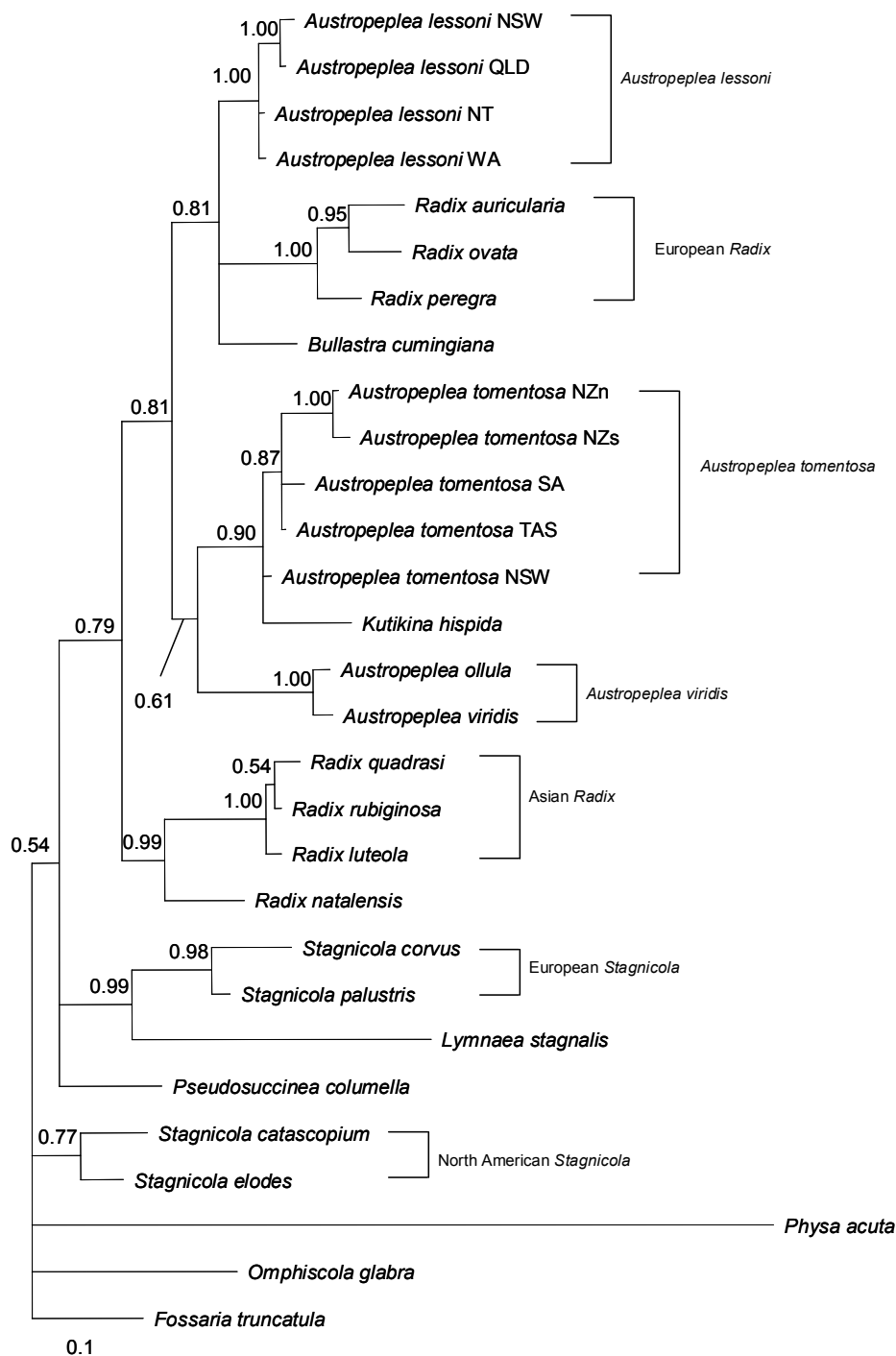
Figure 4.7 Phylogeny of the Lymnaeidae based on anatomical and molecular characters. Majority rules consensus tree based on Bayesian inference, maximum likelihood setting under a partitioned model, 5 000 000 generations. Posterior probabilities indicated above the branch, only posterior probabilities >50% given. NSW= New South Wales, Australia, NT= Northern Territory, Australia, NZn= North Island, New Zealand, NZs= South Island, New Zealand, QLD= Queensland, Australia, SA= South Australia, TAS= Tasmania, Australia WA=Western Australia.

4.4 Discussion

4.4.1 Relationship of *"Austropeplea"* within the Lymnaeidae

The taxa previously included in *Austropeplea* represent some of the most derived groups within the Lymnaeidae, as indicated by the 16S phylogenies, anatomical phylogenies, and combined molecular and anatomical phylogenies. These results are in disagreement with several previous taxonomic schemes and theories of Lymnaeidae evolution, whereby *Austropeplea* was considered the most archaic extant lymnaeid group, and an increase in chromosome number occurred during lymnaeid evolution (Walter 1968; Inaba 1969; Patterson and Burch 1978). Results of the current study suggest a reduction in chromosome number occurred, as suggested by Ponder and Waterhouse (1997). These results also agree with previous molecular studies (Remigio and Blair 1997a; Remigio 2002). Furthermore, the anatomical phylogeny, although poorly resolved, also resolved the lymnaeids with 18 pairs of chromosomes as basal. *Stagnicola catascopium*, *S. corvus* and *L. stagnalis* have previously been described as the most advanced lymnaeids (Walter 1968). However, these species were consistently resolved as basal lymnaeids in all the analyses.

Molecular studies of the ITS-2 region have placed *Radix* as a monophyletic sister group to other European and North American lymnaeids including the genera, *Stagnicola*, *Omphiscola*, and *Fossaria* (Bargues *et al.* 2001; Bargues *et al.* 2003). Furthermore, other studies based on anatomy and numbers of chromosome pairs suggested that *Radix* alone gave rise to all other lymnaeids, except *Austropeplea* (Walter 1968; Inaba 1969; Patterson and Burch 1978). The results of the current study are more in agreement with Remigio (2002), whereby *Radix* represents a more derived group within the Lymnaeidae, with *Stagnicola*, *Omphiscola* and *Fossaria* representing the more basal groups within the family. Molecular studies based on the ITS-2 region (Bargues *et al.* 2001; Bargues *et al.* 2003) did not sample any members of *Radix* from outside Europe, utilising only European and North American lymnaeids. Conclusions drawn from such limited sampling of the family should therefore be treated with caution.

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4.4.2 Phylogenetic relationships of taxa previously included in *Radix*

The relationship between the Australian lymnaeids, *Austropeplea* and members of *Radix* has not been unambiguously resolved in this study. The inability to establish a robust phylogeny between these groups is largely due to the conflicting results surrounding the monophyly of *Radix*. The 16S phylogeny resolves *Radix* as polyphyletic, with members from the Asian region being placed as a well supported sister to the *Austropeplea*, *Kutikina*, *Bullastra* and European *Radix* clade. The separation of *Radix* into two clades is supported by gene divergences, whereby the Asian species included in *Radix* members and those from Europe have 16S sequence divergences greater than 10%, a level suggested by previous authors to represent distinct genera (Remigio and Blair 1997a).

Previous studies of 16S gene sequences resolved *Radix* as paraphyletic (Remigio 2002). In the 16S Bayesian analysis, support for the *A. lessoni* complex as sister to the *A. tomentosa* complex, *A. viridis*, *Kutikina* and European *Radix* clade is only 0.59. It is possible that that if this branch was collapsed, the European *Radix* may be the most basal clade. *Radix* would then form a paraphyletic group as indicated by Remigio (2002). However, results of the combined 16S and ITS-2 analyses resolved a monophyletic *Radix*, with the European taxa being resolved as sister to those from Asia. The incongruence between the 16S and the combined molecular dataset could be due either to the addition of the ITS-2 data to the 16S dataset or alternatively it may be the result of inadequate sampling within the combined molecular dataset.

Studies based on the number of chromosome pairs and anatomical characters suggested that *Austropeplea* was an archaic group from which *Radix* diverged (Inaba 1969). Results of the 16S phylogeny suggest that Asian and African *Radix* (*R. natalensis*) is the earlier evolved group, as they are resolved as a well supported sister group to *Austropeplea*, *Bullastra*, *Kutikina* and the European *Radix*. The phylogeny of the combined 16S and ITS-2 dataset, however, produced conflicting results. The *Austropeplea lessoni* complex plus *Bullastra* were placed as sister to the *A. tomentosa* complex, *Kutikina*, *A. viridis* and *Radix* clade, thereby supporting the suggestion that *Radix* diverged from *Austropeplea*.

Radix natalensis has a large distribution throughout Africa and Madagascar, and displays a considerable amount of conchological variation (Hubendick 1951; Stothard et al. 2000). This species is shown as a well supported sister taxon to the Asian Radix taxa, although not closely related as indicated by the branch lengths of the 16S phylogeny and a 16S gene divergence of between 11 and 16% with both the Asian and European Radix. Such divergence accords with the geographic distance between these members of Radix. However, further investigation of this species is needed, as Radix natalensis may represent a distinct genus.

Interestingly, Radix sp. from Romania is resolved as more closely related to other Radix members from the Asian region than to other European Radix species. Presumably the distribution of the unidentified Radix species from Romania would overlap with the concept of Radix auricularia of Hubendick (1951) which has a large distribution throughout Europe, north east Asia and the near East. It is possible that this species from Romania is in fact an introduced unidentified species from the Asian region. Alternatively, Hubendick's broad concept may need to be refined as already (although not convincingly) indicated by Kruglov and Starobogatov (1989). The Canadian unidentified Radix species represents an introduction, as no Radix species are known to occur in North or South America (Remigio 2002). The unidentified Radix species from the Philippines could either represent a recent introduction to the Philippines or an additional unknown species from the area. Three lymnaeid species are known to occur within the Philippines, Bullastra cumingiana, Radix quadrasi and R. rubiginosa, the first two endemic to the Philippines. The presence of a third endemic lymnaeid species would not be too unexpected taking into account that the Philippines are considered one of the worlds 25 hot spots for biodiversity (Myers et al. 2000). More intensive sampling of the Radix throughout Europe, eastern Europe, and Asia is needed to accurately assess specific status of groups and also to determine whether Radix as it is currently recognised is a monophyletic group.

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4.4.3 Relationships of taxa previously included within *Austropeplea*

Austropeplea, as currently recognised, does not represent a monophyletic group. *Austropeplea* was thought to be characterised by tricuspid lateral teeth, a bicuspid central and 16 pairs of chromosomes (Inaba 1969). However, in the molecular analyses, *Kutikina hispida* is resolved as sister to the Australian *A. tomentosa* in addition to *Bullastra cumingiana* being resolved as sister to the *A. lessoni* complex indicate that the concept of the genus requires revision (see Chapter 5).

Previous studies of taxa within the *Austropeplea tomentosa* and *A. lessoni* complexes have suggested a sister relationship (Burch 1967; Inaba 1969), however, results of the current study suggest this may not be an accurate description of the relationship between these two groups. Results of the current study indicate that the *A. lessoni* complex is more closely related to the South East Asian lymnaeids, as in the 16S phylogeny the *A. lessoni* complex is resolved more closely to the Asian *Radix* and *R. natalensis* clade. *Bullastra cumingiana* has consistently been resolved as sister to the *A. lessoni* complex. Furthermore, the *A. lessoni* complex and *Bullastra* clade are clearly placed as distinct from the *A. tomentosa* complex in the combined 16S and ITS-2 phylogeny. These findings confirm previous suggestions that the *A. lessoni* complex is not appropriately placed in *Austropeplea* or *Bullastra*. This will be discussed further in Chapter 5.

Austropeplea viridis is clearly closely related to *A. tomentosa* and members of *Radix*, as indicated by the Bayesian 16S phylogeny and the combined 16S and ITS-2 phylogenies. Based on anatomical differences, it has previously been thought that there was little close relationship between *A. viridis* and either *A. lessoni* and *A. tomentosa* (Ponder and Waterhouse 1997). The current more extensive anatomical studies would suggest that *A. viridis* is more closely related to *A. tomentosa* then to any members of *Radix* sampled. The anatomical similarities between *A. viridis* and *A. tomentosa* plus the combined 16S and ITS-2 Bayesian phylogeny indicate that *A. viridis* is sister to *A. tomentosa* complex. The placement of *Austropeplea viridis* into the *Austropeplea* has previously been questioned due to divergent molecular

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sequences and shell morphology (Remigio 2002). Molecular divergence between *A. tomentosa* and *A. viridis* is higher than 10%. Based on this and significant differences in shell and anatomy, it is recommended that *A. viridis* be placed in a separate genus. This will be discussed further in Chapter 5.

Samples of *Austropeplea* sp. from China and Hawaii raise the question of the specific status of *A. viridis*, as suggested by Remigio (2002). These two samples represent distinct lineages from the *A. viridis* and *A. ollula* samples, moreover sequence divergence between these two groups are as high as between other lymnaeid species (Ponder and Waterhouse 1997; Remigio and Blair 1997a; Remigio 2002). *Austropeplea viridis* has a large distribution throughout South East Asia, from China and Japan to Burma and the Philippines (Ponder and Waterhouse 1997). It is plausible that the current concept of *A. viridis* (following Hubendick 1951) may actually represent a number of distinct taxa. It has been suggested that the Hawaiian sample may represent one of the most recently diverged lineages within the family, which would accord with the young age of the Hawaiian Islands (Remigio 2002). However, considering the geographic distance between the Chinese and Hawaiian samples, it is more probable the Hawaiian sample has been introduced from China.

4.4.4 Utility of anatomical characters

The utility of shell and anatomical characters in the recovery of relationships within the lymnaeids has been thought to be questionable, as previous studies of the Lymnaeidae have suggested that anatomical characters in the family are too variable, exhibiting high levels of homoplasy (Hubendick 1951; Bargues *et al.* 2001; Remigio 2002). Results of the current study support these findings. The anatomical phylogeny was characterised by a lack of bootstrap support for any of the major clades within the family. Moreover, there were limited unique synapmorphies supporting the major genera or clades within the ingroup.

Whilst the anatomical phylogeny should be treated with some caution, it should be taken into account that only a small number of anatomical characters (61) were available relative to the molecular datasets (277: 16S, 408: 16S + ITS-2). Additional problems associated with using anatomical characters in systematic studies

of the Lymnaeidae, is the lack on congruence between morphological descriptions of species. In total 19 characters were excluded from the anatomical analysis, due to a high number of characters that could not be coded for a large number of the taxa. It is therefore difficult to determine whether the inability to obtain a robust anatomical phylogeny is the result of character homoplasy or the result of a limited anatomical character set. This problem can only be solved by undertaking a critical comparative anatomical study across the family.

Interestingly, no internal fold of the prostate and the absence of a velum were thought to be pleisomorphic states within the Lymnaeidae (Hubendick 1951; Jackiewicz 1993a). *Omphiscola glabra* is the only known lymnaeid to possess both these traits (Jackiewicz 1993a) and this species was in all the molecular analyses, resolved as sister to all other lymnaeids in the analysis, suggesting that some anatomical characters may be useful in helping to resolve phylogenetic relationships within the family.

4.4.5 Biogeography implications

The most recent theory of lymnaeid biogeography suggested that the group arose in the late Jurassic to early Cretaceous, implying a Laurasian origin (Remigio and Blair 1997a). The separation of Laurasia into the Paleartic and Neartic regions approximately 65 million years ago (Mya) could have resulted in the split of the ancestral lymnaeids between Europe and North America (Remigio and Blair 1997a). The basal positions occupied by the North American and European lymnaeids in the molecular phylogenies support a northern hemisphere origin with their close relationship supporting a Lurasian origin. If *Stagnicola* were extant at the time, a Lurasian origin would also explain their polyphyletic position (Remigio and Blair 1997a).

Contact between the North America and north east Asia in the late Cretaceous (65 Mya) is thought to have resulted in the dispersal of lymnaeids into Asia with this stock probably giving rise to *Radix* and *Austropeplea* (as they are currently recognised) in Eurasia, followed by a rapid expansion into Asia, Europe, Africa and Australia (Remigio and Blair 1997a). The basal position of the Asian *Radix* and *R.*

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natalensis relative to the European *Radix* in the 16S phylogeny supports the theory that *Radix* occurred in Asia initially, dispersing later into Europe via Eurasia. *Radix natalensis*, is sister to the Asian *Radix* clade, which indicates that lymnaeids may have radiated through Africa, then moved into the Middle East and finally through into Asia. The distribution of *R. natelensis* throughout Africa and the Middle East would support this theory. Dispersal of lymnaeids into Africa may have occurred in the Eocene (50 Mya) from the *Stagnicola* ancestral stock in Europe. The only problem with this scenario would be if the North American lymnaeids are as more closely related to the *Radix*, *Austropeplea* groups than the European *Stagnicola* and *Lymnaea*. Current molecular analysis resolved the North American *Stagnicola* as sister to the *Radix Austropeplea* clade, however there was less than 50% bootstrap support and posterior probability was only 0.50. Further examination of these relationships is needed.

Collision of the New Guinea margin with parts of South East Asia 20 Mya (Hall 1998) could have facilitated the dispersal of the ancestors of *A. lessoni* and *Bullastra cumingiana* into Australia and the Philippines, respectively (Remigio and Blair 1997a; Remigio 2002). If *Radix* is in fact polyphyletic, as indicated by the Bayesian 16S phylogeny, and the European *Radix* are more closely related to *A. tomentosa* and *A. viridis*, it is possible that ancestors of the Asian *Radix* group could have moved up into eastern Asia and through into Eurasia and Europe, thereby giving rise to the second group of lymnaeids in Europe. The distribution of *R. auricularia* would support this theory, as it occurs throughout Europe, north east Asia and the near east (Hubendick 1951; Bargues *et al.* 2001). The Eurasian stock could have then given rise to *A. viridis*. *Austropeplea viridis* then spread throughout China, Japan, South East Asia and the Philippines. *Austropeplea tomentosa* could have arisen from this stock and possibly crossed into Australia at a similar time to *A. lessoni*. This theory would suggest a separate derivation of the 16 chromosome number, as suggested by Ponder and Waterhouse (1997).

Expansion of *Austropeplea lessoni* and *A. tomentosa* throughout Australia then occurred, with *A. lessoni* having a more northern Australian distribution, whilst *A. tomentosa* radiated into southern Australia, Tasmania and eventually New Zealand. *Kutikina hispida* later diverged from the populations of *A. tomentosa* in Tasmania

possibly during the last ice age (See Chapter 2 and 3 for a more thorough examination of the biogeography of the Australian lymnaeids).

Ponder and Waterhouse (1997) have suggested an alternative theory for the biogeography of the Australasian lymnaeids. *Austropeplea tomentosa* is suggested to have had a Gondwanan origin, whilst *A. lessoni* is thought to be derived from South East Asia ancestors. The recent discovery of a Lymnaeidae Pliocene fossil from Antarctica (Ashworth and Preece 2003) supports a Gondwanan radiation. Australia and Antarctica separated 50 Mya, therefore lymnaeids wools have had to have entered Antarctica prior to this separation. The basal position of the New Zealand *A. tomentosa* relative to the Australian *A. tomentosa* accords with this theory. Presumably, the *A. tomentosa* groups would have radiated after India and Africa had separated from Gondwana, considering neither of these two species show a close affinity with *A. tomentosa* in the 16S phylogeny or the combined molecular phylogeny. The recently derived position at which *A. tomentosa* is resolved in the Lymnaeidae phylogeny however does not support a Gondwanan origin. While it seems clear that the *A. lessoni* complex and the *A. tomentosa* complex have had separate derivations, the current lymnaeid phylogeny does not advocate any one of these theories more than the other.

4.4.6 Gaps of knowledge in Lymnaeidae systematics

The phylogenetic relationships of a number of lymnaeid taxa still remain unknown. One of the largest groups are the South American taxa, which are thought to be represented by at least six species (Hubendick 1951; Paraense 1976, 1982, 1984, 1995). The origin of this group has been suggested to be North American (Remigio and Blair 1997a), although others have suggested a close relationship to the Australian lymnaeids based on anatomical characteristics (Ponder and Waterhouse 1997). Results of the anatomical phylogeny suggest the South American lymnaeids may be more closely related to the North American taxa, but taking into account the lack of support in the anatomical tree, molecular investigations of the group are needed to establish the phylogenetic relationships of this group. It has been suggested that the *Austropeplea tomentosa* complex may have been derived from a Gondwanan ancestor (Ponder and Waterhouse 1997). The position of *R. natalensis* as sister to the Asian

Radix groups does not support this theory. However, resolving the phylogenetic relationships of the South American lymnaeids would help to test this theory more rigorously.

Both the Japan and Hawaii have a number of endemic lymnaeids, some with quite divergent morphologies. Understanding the phylogenetic relationships of these groups in relation to other members of the family could be useful in further determining the utility of anatomical characters in Lymnaeidae systematics. The origin of the Hawaiian lymnaeids is also of interest. The Hawaiian fauna are thought to be closely related to the North American lymnaeids, having 18 pairs of chromosomes. However, considering the young age of the Hawaiian Islands (0.5 to 5 million years old), it is possible that colonisation may have been from the Western Pacific and not the Eastern Pacific.

Further examination of the phylogenetic relationships of the European *Myxas glutinosa* is needed. In the anatomical phylogeny, *M. glutinosa* was resolved as more closely related to the Asian and European *Radix*. Previous cladistic analyses have placed *M. glutinosa* as sister to *R. peregra* and *R. auricularia* (Jackiewicz 1993a). It is possible that this species may have evolved from the *Radix* stock that entered Europe from Asia. Molecular data for this species is required to test this hypothesis.

Chapter 5   Taxonomy of the Australasian Lymnaeidae

5.1 Introduction

Within Australian and New Zealand, under the current classification there are two lymnaeid species, *Austropeplea lessoni* (Deshayes, 1830) and *A. tomentosa* (Pfeiffer, 1855). However, the proceeding chapters have identified that the *A. lessoni* complex represents two distinct species, as does the *A. tomentosa* complex. In this chapter I describe the four taxa discriminated in the proceeding chapters. Furthermore the previous chapters have also identified that not all of the taxa previously attributed to *Austropeplea* Cotton, 1842 should be placed within this genus. Therefore the generic status of the taxa previously attributed to *Austropeplea* will also be addressed in this chapter.

5.2 Taxonomic descriptions

5.2.1 Species previously attributed to *Austropeplea lessoni* (Deshayes, 1830)

Peplimnea Iredale, 1943
Peplimnea Iredale 1943, type species *Limnea lessoni* Deshayes 1830
Austropeplea Inaba, 1969: 162, Fig77 (in part)

Diagnosis

Shell ovate, with up to 5 whorls; columella fold distinct; foot broad anteriorly, anal lobe and upper plate absent; expanded mantle either absent or expanded just outside of shell; kidney broad posteriorly, right lobe present; oviduct 1 characterised by radial ridges; oothecal gland pyriform in shape, spermatheca egg-shaped; seminal vesicle longer than wide; penis sheath greater than half the length of the praeputium; penis sheath head poorly developed; prostate longer than female reproductive system.

Peplimnea lessoni (Deshayes, 1830)

Limnaea lessoni Deshayes, 1830: pl.16, Figs1-2 (Australia; type status and whereabouts unknown, presumed lost).

Limnaea perlevis Conrad, 1850: 80, pl i, Figs 5-6 (Salamanca and Balonne Rivers, NSW, holotype, ANSP 59027a).

Amphipeplea strangei Pfeiffer, 1854: 6, plii, Figs 5-6 (Moreton Bay, QLD, syntypes probable BMNH 1983087).

Amphipeplea melbournensis Pfeiffer, 1856: 70, pl xix, Figs 14-15 (Melbourne, VIC, syntypes BMNH 1983088).

Amphipeplea iuvoluta Schmeltz, 1869: 69 (*nom. nud.*, see Hubendick 1951)

Limnaea cumingii Sowerby, 1872: 38, pl.vi (Australia; type status and whereabouts unknown, presumed lost).

Limnaea globosa Sowerby, 1872: sp.84, pl.12, Fig 84 (Austrlia; syntypes BMNH 1841.4.2.28.98-99).

Amphipeplea queenslandica Clessin, 1886:405, pl.53, Fig 2 (nom. Nov. for *Amphipeplea iuvoluta* Schmeltz, 1869)

Peplimnea lilimera Iredale, 1943: 212 (Burdekin River, QLD, synypes AM C.100607, C.118660).

Peplimnea lessoni thema Iredale, 1944: 212, 118 (Cobar, NSW, syntypes AM C.30983).

Peplimnea opima Iredale 1944: 118, Fig 5 (Hornsby, NSW, syntypes probable AM C.100761).

Peplimnea spiringer Iredale 1944: 118, Fig 5 (Glenn Innes, NSW, syntypes AM C.100609).

Lymnaea lessoni; Hubendick 1951: 48, Figs 41, 44, 283 (in part).

Lymnaea lessoni; Boray and McMichael 1961: 150 (in part).

Austropeplea lessoni; Ponder and Waterhouse 1997: 442, Smith 1992: 256, Smith *et al*. 2003 (in part).

Material Examined

Glenn Innes, NSW (AM C.431243, 29° 27.870'S, 151° 37.180' E), Nepean River (AM C.449005, 34° 11.650' S,150° 42.700'), Braidwood, NSW (AM EBU.35505, 35° 30.133' S, 149° 42.633' E), Adaminaby, NSW (AM EBU.35595, 35° 58.800' S, 148° 43.300' E), Ross River, QLD (AM C.451980, 19° 24.000' S, 146°

44.000' E), Barcaldine, QLD (AM C.423243, 23° 16.750' S, 145° 24.100' E), Hugenden, QLD (AM C.407248, 20° 50.667' S, 144° 11.900' E), Stanthorpe, QLD (AM C.431244, 25° 40.000' S, 151° 56.000' E).

Diagnosis

Shell ovate, thin and semitransparent, up to 5 whorls, umbilicus closed; columella fold distinct; tentacles, usually longer than wide but can be equal in length and width; foot broad anteriorly, rounded posteriorly; visceral coil pigmentation absent; expanded mantle absent or either just outside of shell; kidney width between one third and half the length; ureter short; buccal mass longer than wide or width equal to length; longest lobe of salivary gland on right; cerebral commissure half as long as distance between ganglion; pedal commissure absent to short, ganglion surrounding conspicuous statocysts enlarged; seminal vesicle longer than wide, straight or looped; oviducal caecum large, oothecal gland pyriform; uterus/ vagina short and at right angles to oothecal gland; spermathecal duct longer than uterus; penis sheath equal in length to praeputium; penis sheath head not well developed; penis straight in penis sheath head; junction of prostate and vas deferens simple; prostate longer than female system; radula bicuspid central, small cusp on left, 9-10 tricuspid laterals and 15 tetracuspid marginals.

Description
Shell

Shell ovate, up to 5 whorls, last whorl and aperture enlarged relative to spire (Fig 5.1). Shell thin, fragile, and semi-transparent in live animals. Shell lacking any distinct sculpture; periostracum ornamentation absent. Aperture large, thin outer lip. Shell umbilicus closed, columella fold distinct (Fig 5.1).
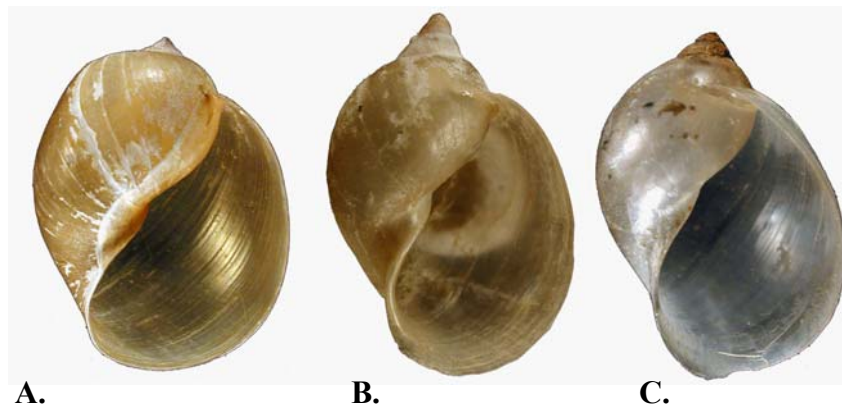
Figure 5.1 Shell variation in *Peplimnea lessoni*, A. Townsville, Queensland, C.451980, shell length 10 mm B. Hugendon, Queensland, C.407248, shell length 10.08 mm, C. Glenn Innes, New South Wales, C.431244, shell length 12.16 mm.

Table 5.1 Shell variables of *Peplimnea lessoni*. Measurements in mm, n=26.

	SL	SW	AL	AW	LWL
range	7.04 - 12.64	5.92 - 9.92	5.76 - 11.2	3.68 - 7.12	6.24 - 11.84
mean	10.86	7.91	8.86	5.67	9.88
sd	1.63	1.58	1.77	1.09	1.73

AL= aperture length, AW= aperture width, LWL= last whorl length, sd= standard deviation, SL= shell length, SW= shell width.

Radula

Radula has asymmetrical central teeth bearing two cusps, smaller on left (Fig 5.2B). Nine to 10 pairs of tricuspid lateral teeth in each row, 11th pair transitional with the marginal teeth, having three large cusps and one small cusp on outer side (Fig 5.2C). The remainder of marginal teeth have 4 large cusps and 1-3 minute denticles on the outer side. The outer most row with four small cusps; 15 pairs of marginals (Fig 5.2A,D).

Head-foot and visceral mass

Head-foot is typical of the Lymnaeidae, with short, bluntly triangular cephalic tentacles, usually longer than wide in live specimens (Fig 5.3A), but tentacle width can also equal length. Eyes located inside the tentacle proximal inner edge on distinct eye lobe (Fig 5.3A). Snout is short and broad, the mouth antero-ventral, prominent thick jaw. Lateral sides of snout form a distinct junction with the side of the foot. Thick mantle collar is present, sometimes extending just outside of the shell (Fig

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5.3A). Pallial roof mottled with black pigmentation, mantle covering the visceral coil unpigmented (Fig 5.3A). Foot is widest anteriorly, with the width being greater than half the length (Fig 5.3B).
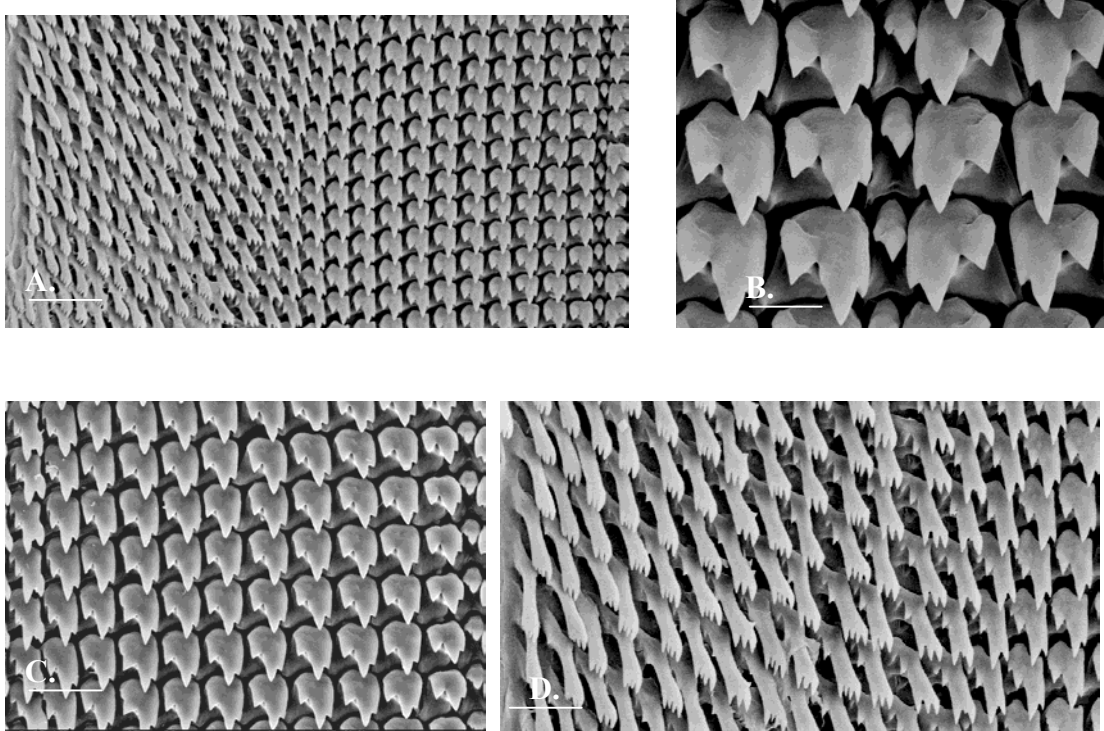
Figure 5.2 *Peplimnea lessoni* **C.431244. Radula teeth, dorsal views A. Half rows, B. Detail of central and inner laterals, C. Detail of lateral and inner most marginal, D. Detail of marginal. Scales; A 55 μm, B 20 μm, C 400 μm, D 45 μm.**

Pallial cavity

The pneumostome like many other lymnaeids has two internal ridges, but lacks an anal flap and upper plate (Fig 5.4). Pulmonary roof has the heart and kidney in their typical position. Kidney is thin walled and broad, the width being one half to a third the length of the kidney. Posterior region of kidney the broadest, right lobe present (Fig 5.4A). The right lobe is situated to the right of the pulmonary vein, extending from the anterior region of the kidney all the way to the renopericardium cavity, being enclosed by the thick pulmonary muscle (Fig 5.4). Pulomanry vein enclosed in right lobe (Fig 5.4A). A short ureter opens inside pneumostome (Fig 5.4A). As in other lymnaeids the anus is situated at the posterior edge of the pneumostome.
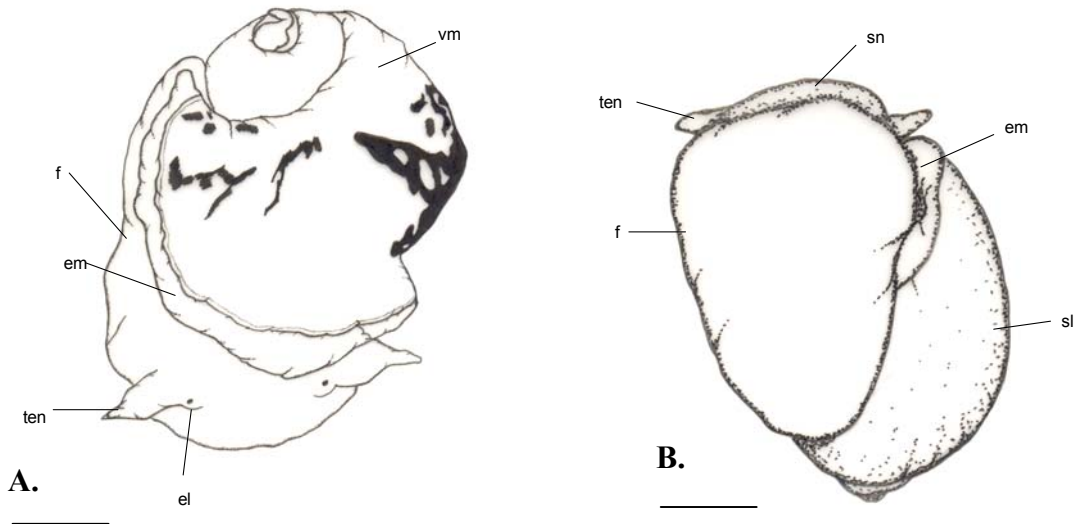
Figure 5.3 *Peplimnea lessoni*, C.431243. External view of animal showing external features and pigmentation patterns. A. Dorsal view of animal showing expanded mantle edge, B. Ventral view of foot. Abbreviations: el= eye lobe, em= expanded mantle edge, f= foot, sl= shell, sn= snout, ten= tentacle, vm= visceral mass. Scale: 2.25 mm.
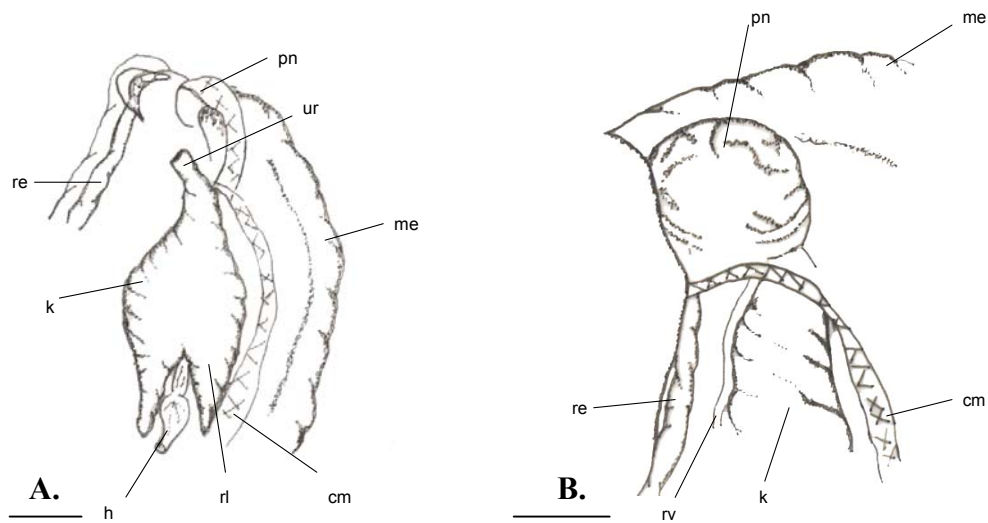
Figure 5.4 *Peplimnea lessoni* C.431243. Pallial cavity viewed from the ventral side. A. Kidney shape, B. Pnuemostome. Abbreviations: cm= cut muscle, h= heart, k= kidney, me=mantle edge, pn= pneumostome, re=rectum, rl= right lobe, rv= renal vien, ur=ureter. Scales: A 3 mm ; B 2 mm.

Digestive systems

Buccal mass large and is either longer than wide or equal in width to length (Fig 5.5A). Radula sac shorter than the buccal mass (Fig 5.5B). Salivary ducts lie alongside the oesophagus and beneath the cerebral commissure. Salivary glands joined dorsally and laterally, the right hand lobe the longest (Fig 5.5A). Stomach similar to other lymnaeids.
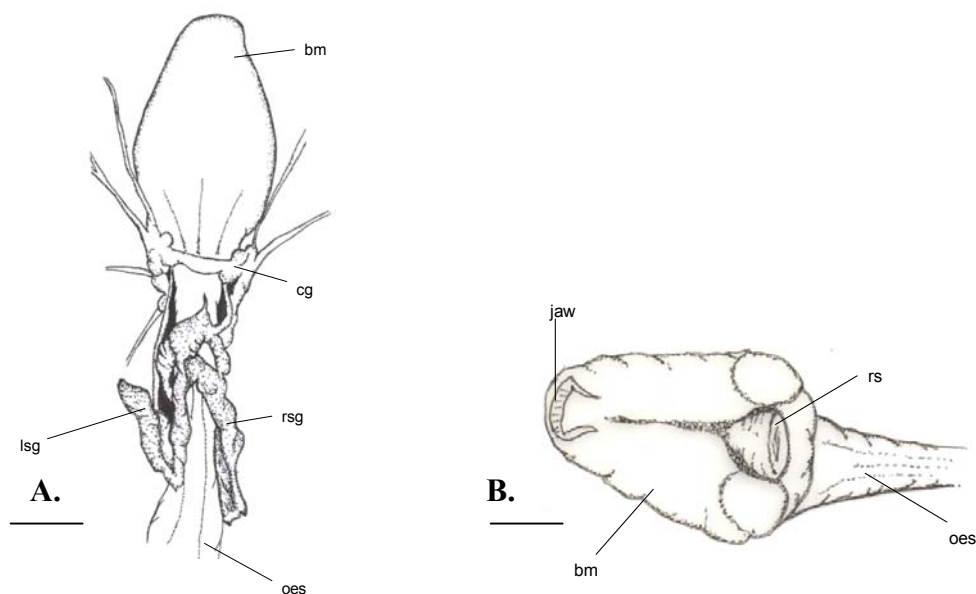
Figure 5.5 Buccal mass and salivary glands of *Peplimnea lessoni* C.431243. A. Dorsal view of the buccal mass, salivary glands and anterior gut. B. Ventral view of the buccal mass and position of the radula sac. Abbreviations: bm= buccal mass, cg= cerebral ganglion, jaw= jaw of radua, lsg= left salivary gland, oes= oesophagus, rs= radula sac, rsg= right salivary gland. Scales: A 2.5 mm; B 1.25 mm.

Nervous system

Parietal nerve ring is pentaganglionic, the ganglion either separated by short connectives or just abutting one another (Fig 5.6A). Cerebral ganglion widely separated, cerebral commissure measures half the length between the cerebral ganglion (Fig 5.6B). Pedal ganglion either abutting or separated by a short commissure (Fig 5.6B). The pedal ganglion enlarged in area surrounding the conspicuous statocysts. Pedal ganglion equal in length to width (Fig 5.6B).
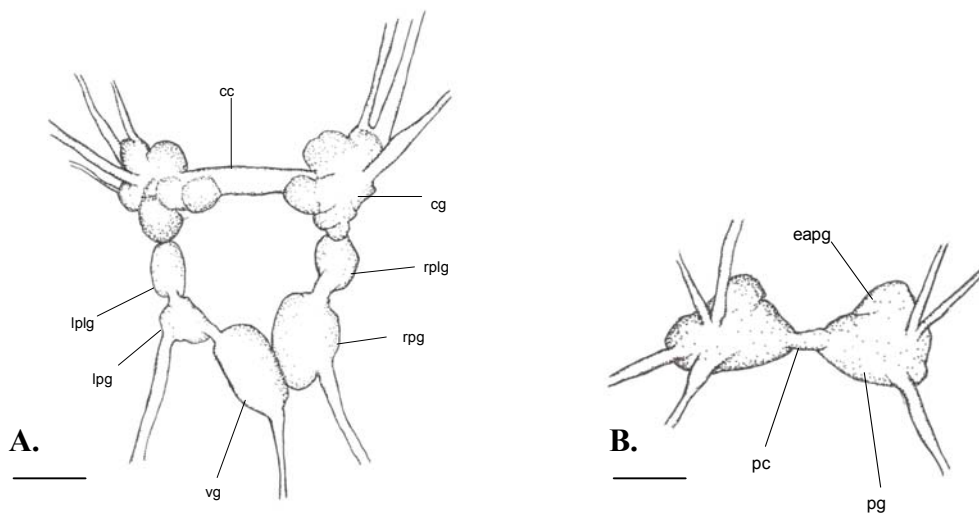
Figure 5.6 Nervous system of *Peplimnea lessoni* C.407248. A. Dorsal view of the ganglionic ring, B. Posterior view of pedal ganglion. Abbreviations: eapg= expanded area of pedal ganglion, cc= cerebral commissure, cg= cerebral ganglion, lpg= left parietal ganglion, lplg= left pleural ganglion, pc= pedal commissure, pg= pedal ganglion, rpg= right parietal ganglion, rplg= right pleural ganglion, vg= visceral ganglion. Scales: A 2 mm; B 1.5 mm.

Reproductive system

Reproductive system comprises most of the body volume, as in other lymnaeids. Ovotestis embedded in the middle of the columella side of digestive gland. Seminal vesicle long and narrow, either straight or looped, with distinct pockets (Fig 5.7A). Spermoviduct opens to a smooth, egg-shaped carrefour. The spermduct departs adjacent to the junction of the spermoviduct. Albumen gland opens laterally to the carrefour from a broad duct. Oviduct joins carrefour to the oothecal gland, and is divided into two distinct regions. Oviduct 1 opens from the carrefour, and is short, forming a radial fan around the carrefour (Fig 5.7B). Oviduct 2, the distal portion of the oviduct, has thinner, undulating walls then oviduct 1, and is much longer (Fig 5.7C). Oviduct 2 joins the oothecal gland. Prior to this junction is the oviducal caecum. Oviducal caecum is pouch-like, with smooth, transverse folds, oval in shape, and situated under the oviduct. Oviducal caecum is large, being greater than one half the width of the oothecal gland (Fig 5.7C). Oothecal gland, the largest part of the female system, is pyriform in shape, and characterised by a smooth surface with distinct transverse folds (Fig 5.7C). Anteriorly it opens to a narrow uterus, which goes onto form the vagina. Uterus/ vagina is parallel, short, being less than half the length

of the oothecal gland, and placed at right angles to the oothecal gland (Fig 5.8C). Spermathecal duct joins the uterus/ vagina just prior to opening of female gonophore (Fig 5.7C). Spermathecal duct is narrow, thinner than uterus/ vagina and shorter than the uterus/ vagina. The spermatheca forms an egg-shape at the distal end of spermathecal duct (Fig 5.7C).
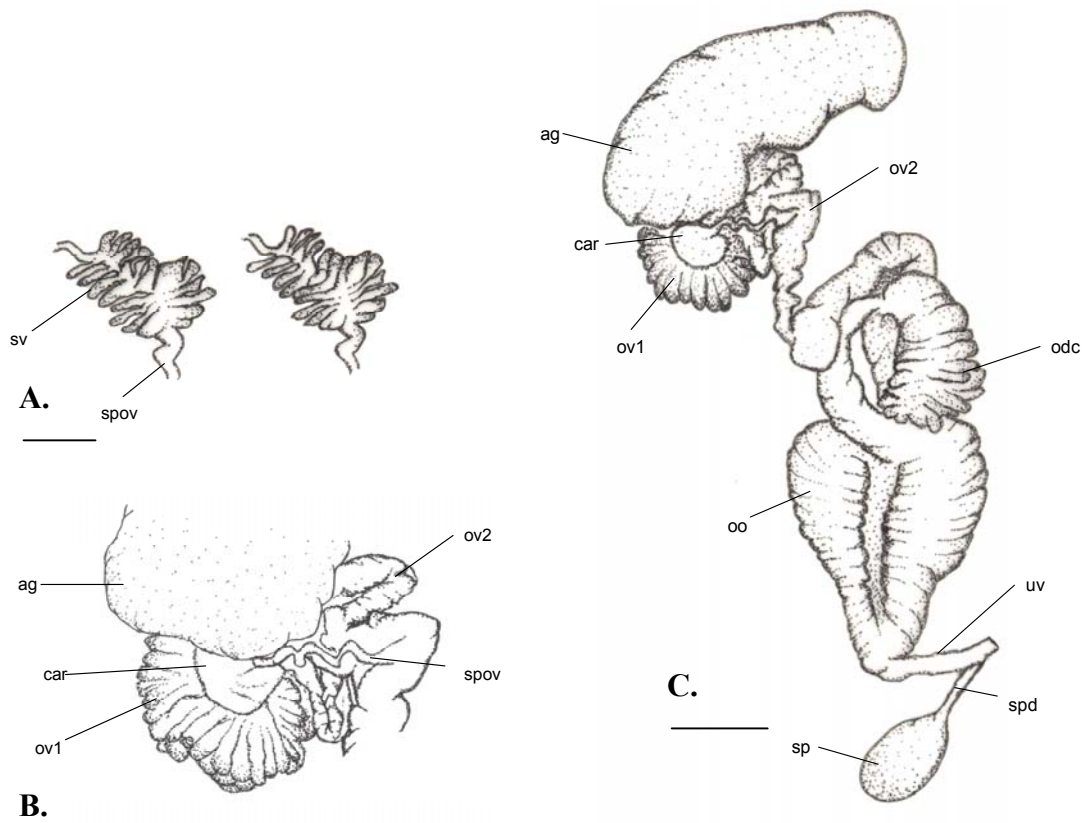
Figure 5.7 Reproductive organs of *Peplimnea lessoni*, excluding male copulatory organs and prostate C.431244. A. Dorsal view of seminal vesicle, B. Ventral view of oviduct 1, C. Ventral view of female reproductive system. Abbreviations: ag= albumen gland, car= carrefour, odc= oviducal caecum, oo= oothecal gland, ov1= oviduct 1, ov2= oviduct 2, sp= spermatheca, spd= spermathecal duct, spov= spermoviduct, sv= seminal vesical uv= uterus/ vagina. Scales: A 2.6mm; B 1 mm; C 2 mm.

Upper prostate is initially narrow and broadens posteriorly, with upper prostate being flattened against oothecal gland. Prostate longer than female system, large fold in the ventral wall (Fig 5.8A). Internally, prostate has one large crescent fold (Fig 5.8B). Posterior end of prostate makes a simple junction with vas deferens (Fig 5.8A). Vas deferens joins base of penis sheath. Penal sheath cylindrical, and head structure not very well developed. Penis sheath equal in length and a third the width of praeputium in its retracted state (Fig 5.8C). Simple penis is about as long as the penis

sheath, running straight in penis sheath head, duct opening at its pointed tip (Fig 5.8E). Praeputium has two large internal folds of equal size, a circular velum surrounding the sacrobelum (Fig 5.8 C, E). Praeputium retractor muscle attaches at head of praeputium and penis sheath retractor muscle attaches to penis sheath head. A branch of the penal nerve enters the penis sheath from the right cerebral ganglion (Fig 5.8C).

Distribution

Peplimnea lessoni occurs throughout New South Wales, possibly as far south as Victoria (Fig 5.9). This species extends into eastern Queensland, at least as far north as Townsville.

Remarks

The original description of *Peplimnea lessoni* was based only on shell characteristics (Deshayes 1830). Furthermore, previous anatomical examinations of *Peplimnea lessoni* have only been based on limited samples (Hubendick 1951; Ponder and Waterhouse 1997). The description of *P. lessoni* produced here is in agreement with both previous examinations. Hubendick (1951) suggested that *Lymnaea lessoni* occurred in New Guinea, Australia and New Zealand. The New Zealand taxa attributable to *L. lessoni* was synonymised with *Simlimnea (=Austropeplea) tomentosa* (Dell 1956). Examination of the New Zealand lymnaeids by the author found no reason to suggest that *Peplimnea lessoni* occurs in New Zealand. The status of the New Guinea taxa has yet to be tested.

Smith (1992) has placed *Limnaea spirulata* as a synonymy of *Austropeplea lessoni*, however examination of the illustration of *L. spiruata* has revealed that this species is a synonymy of *Austropeplea viridis*.
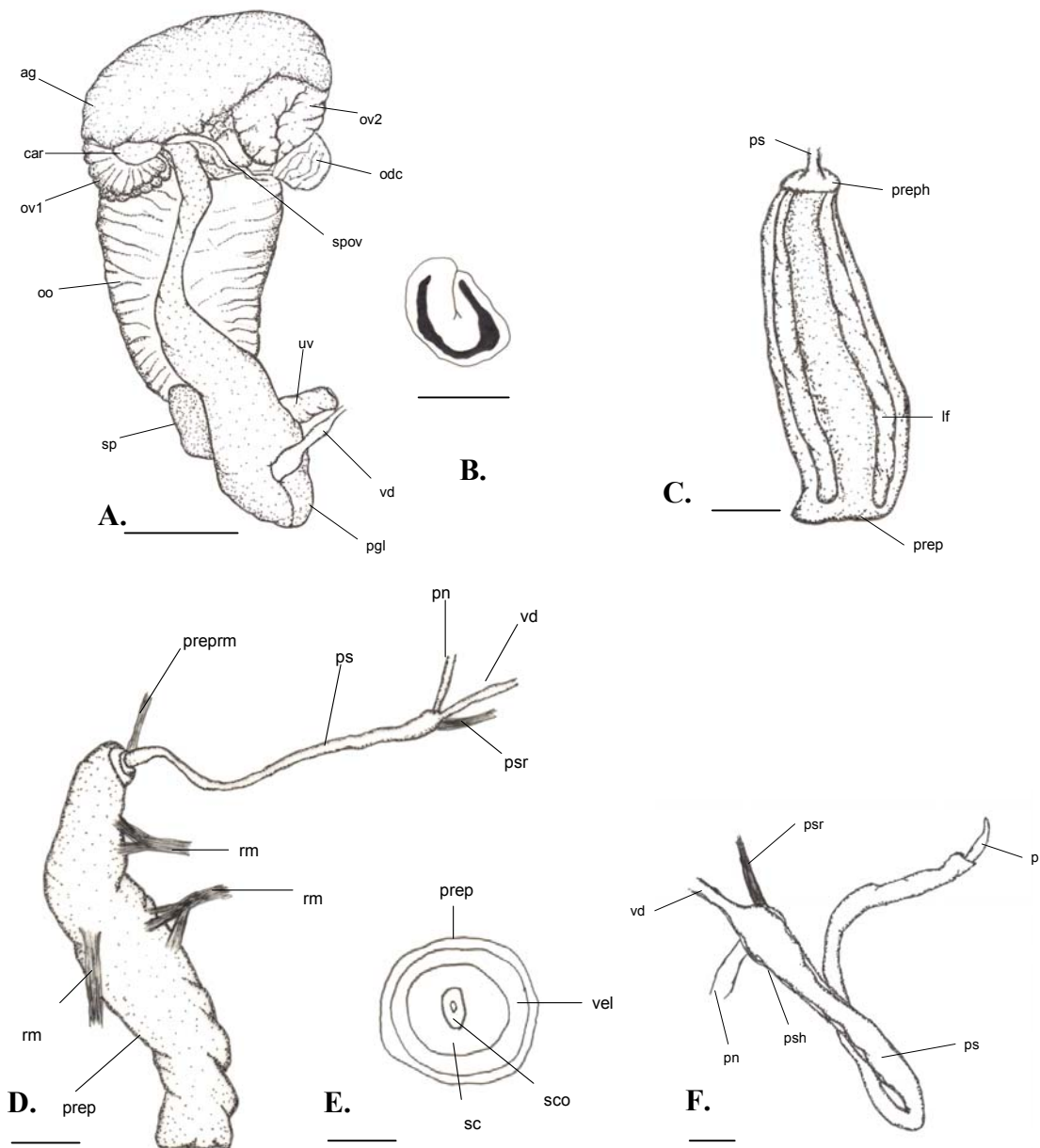
Figure 5.8 Male reproductive organs of *Peplimnea lessoni* C.431244. A. Ventral view of the prostate in its natural position, B. cross section of prostate, C. Internal longitudinal folds of the praeputium, D. Dorsal view of praeputium and penis sheath, E. Cross section of praeputium head, F. Dorsal view of penis in penis sheath head. Abbreviations: ag= albumen gland, car= carrefour, lf= longitidunal fold, odc= oviducal caecum, oo=oothecal gland, ov1= oviduct 1, ov2= oviduct 2, p= penis, pgl= prostate gland, pn= penal nerve, prep= praeputium, preph= praeputium head, preprm= praeputium retractor muscle, ps= penis sheath, psh= penis sheath head, psr= penis sheath retractor, rm= retractor muscle, sc= sacrobelum, sco= sacrobelum opening, sp= spermatheca, spov= spermoviduct, uv= uterus/ vagina, vd= vas deferens, vel= velum. Scales: A 3.75 mm; B 2.0 mm; C 2 mm; D; 2 mm E 0.25 mm; F 0.5 mm .
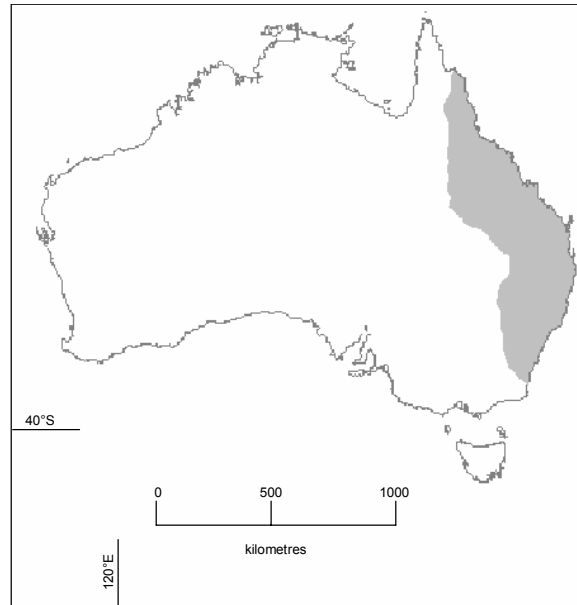
Figure 5.9 Distribution of *Peplimnea lessoni*, based on results of molecular and anatomical phylogenies.

Peplimnea affins (Küster, 1862)

Limnaeus affinis Kuster, 1862:55, pl.12, Figs 5-6 (Australia, status and whereabouts unknown presumed lost).

Amphipeplea vinosa Adams and Adams, 1864: 415 (tributary of Adelaide River, Arnhem Land, NT; syntypes BMNH 1870.10.26.182).

Amphipepela phillipsi Adams and Adams, 1864: 416 (Arnhem Land, NT; syntypes BMNH 1870.10.26.181).

Limnaea angasi Sowerby, 1872, pl.2, Fig 11 (Port Darwin, NT; syntypes BMNH 1872.5.28.9).

Limnaea deshayesii Sowerby, 1872, 272, pl. 14, Fig 95 (Coronet Creek and Ropers Lake, NT, syntypes BMNH 1846.10.7.19-21).

Peplimnea vinolenta Iredale, 1943: 213 (Palm Creek, Darwent River, NT, syntypes AM C.2161).

Peplimnea caurina Iredale, 1943: 213, (Lennard River, WA, syntypes AM C.79556).

Lymnaea lessoni Hubendick 1951: 48, Figs 41, 44, 283 (in part).

Lymnaea lessoni; Boray and McMichael 1961: 150 (in part).

Austropeplea vinosa Blair and Finlayson 1981: 758

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Austropeplea lessoni; Ponder and Waterhouse 1997: 442, Smith 1992: 256, Smith *et al*. 2003 (in part).

Material examined

Karumba, QLD-4 (AMS C.428189, 17° 29.120' S, 140° 50.380' E), Parry's Lagoon, WA (AMS C.426640, C.426641, 15° 32.960' S, 128° 15.580' E), Broome, WA (AMS C.439182, 16° 58.460' S, 122° 40.070' E), Lennard River, WA (AMS C.436051, 17° 10.960', S, 125° 15.340' E), Cockatoo Creek, Derby, WA (AMS C.431120, 17° 44.380' S, 123° 34.430' E), Karijini National Park, WA (AMS C.451978, 22° 27.000' S, 118° 18.000' E), Roeburne, WA (AMS C. 377262, 20° 46.000' S, 117° 7.000' E), Humpty Doo, NT (AMS C.436053, 12° 33.940' S, 131° 18.380' E), South Alligator River, NT (AMS C.443999, 12° 39.740' S, 132° 31.570' E, AMS C.439475, 12° 35.000' S, 132° 27.000' E), Adelaide River, NT (AMS C.436052, 13° 15.050' S, 132° 31.570' E), Gregory National Park, NT(AMS C.439184, 16° 02.900' S, 130° 23.150' E), Alice Springs, NT (AMS C.451979, 23° 42.000' S, 133° 53.000' E).

Diagnosis

Only characters that differ between *Peplimnea lessoni* and *P. affinis* have been included in the following diagnosis. Tentacles longer than wide; visceral coil pigmentation sometimes present; no mantle expansion; ureter absent; buccal mass longer than wide; salivary glands equal or with a longer right lobe; cerebral commissure one third to half as long as distance between ganglion; seminal vesicle straight, looped or convoluted; oviducal caecum either large or extra large; uterus /vagina long and at greater than right angles to oothecal gland; spermathecal duct shorter than uterus; penis sheath less than the length of the praeputium; penis looped in penis sheath head; junction of prostate and vas deferens makes a small sac; prostate longer than female system.

Description

Shell

The shells of *Peplimnea affinis* can be larger than the *P. lessoni*, and generally have a larger spire (Fig 5.10; Table 5.2).

Table 5.2 Shell variables of *Peplimnea affinis*. Measurements in mm, n=61.

	SL	SW	AL	AW	LWL
Range	7.06-23.49	5.42-16.19	5.73-17.46	3.47-11.7	6.93-21.27
Mean	15.32	10.11	11.32	7.00	13.66
Sd	4.71	3.23	3.58	2.30	4.36

AL= aperture length, AW= aperture width, LWL= last whorl length, sd= standard deviation, SL= shell length, SW= shell width.
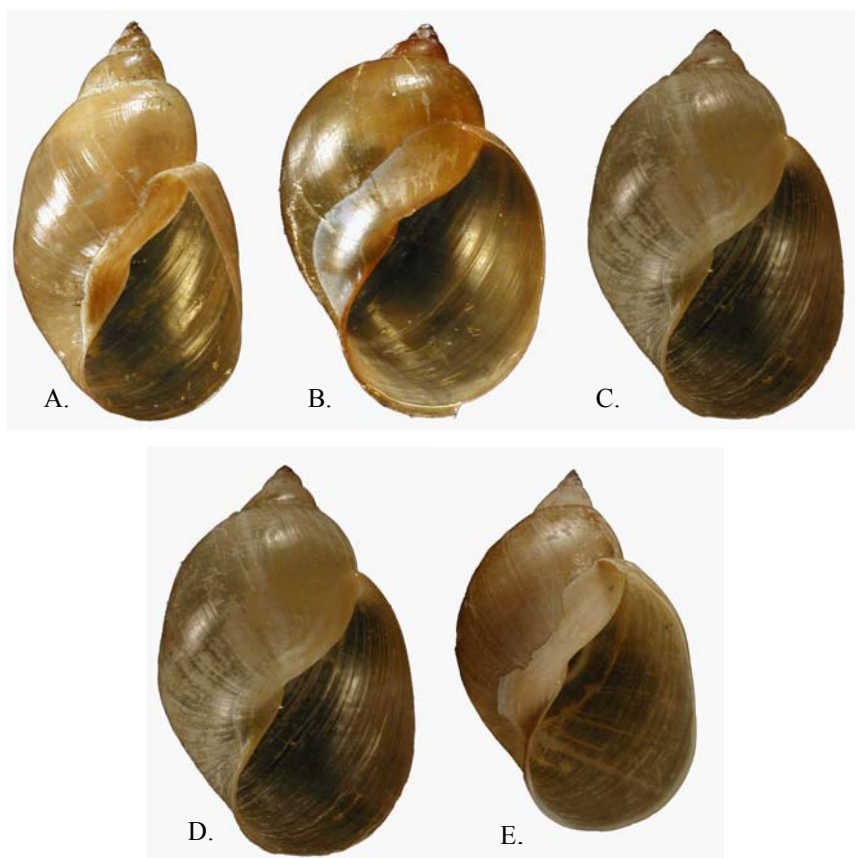
Figure 5.10 Shell variation of *Peplimnea affinis*, A. Pilbara region, Western Australia, C.377262, shell length 9.36 mm, B. Broome, Western Australia, C.431982, shell lenth 18.25 mm, C. Derby, Western Australia, C.431120, shell length 14.0 mm, D. Darwin, Northern Territory, C.436053, shell length 18.25 mm, E. Karumba, Queensland, C.428189, shell length 15 mm.

Radula

As in *Peplimnea lessoni.*

Head-foot and visceral mass

Tentacles always longer than wide in live specimens. Eyes located inside the tentacle proximal inner edge on an eye lobe; eye lobe distinctive or undeveloped (Fig 5.11A). Thick mantle collar is present, but never extending outside of shell (Fig 5.11B). Black-gray pigmentation sometimes present on mantle covering visceral coil (Fig 5.11A).
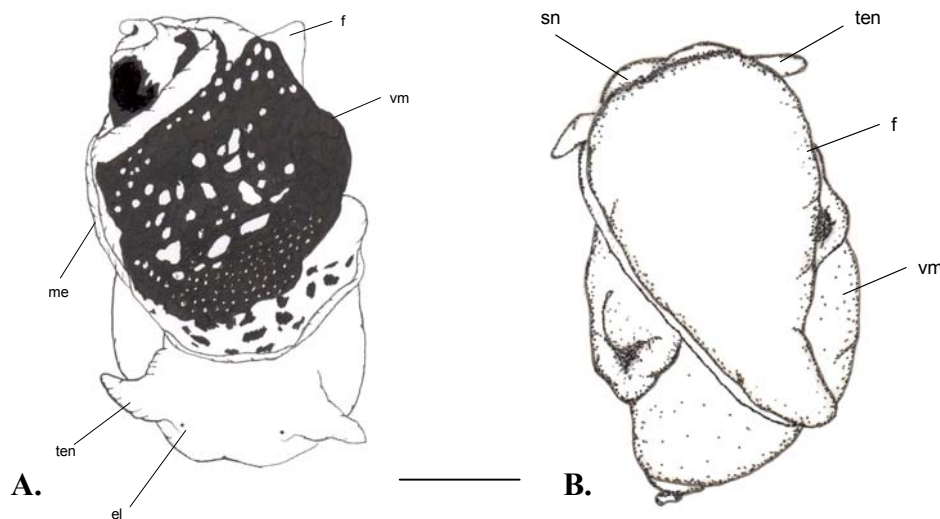
Figure 5.11 *Peplimnea affinis* C.436053. External view of animal showing external features and pigmentation patterns. A. Dorsal view of animal, B. Ventral view of foot. Abbreviations: el= eye lobe, f= foot, me=mantle edge, sn= snout, ten= tentacle, vm= visceral mass. Scale: 3 mm.

Pallial cavity

The pallial cavity of *Peplimnea affinis* is the same as *P. lessoni* except that a ureter is absent (Fig 5.12).
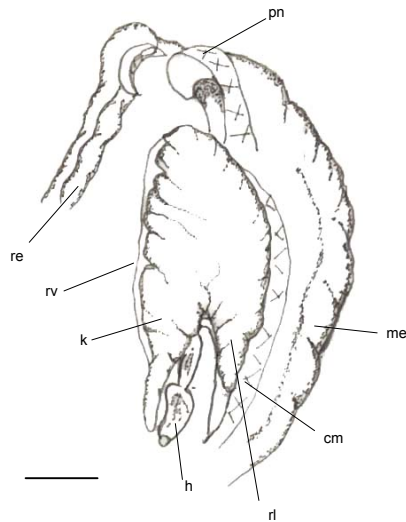
Figure 5.12 Ventral view of pallial cavity of *Peplimnea affinis* C.439182. Abbreviations: cm= cut muscle, h= heart, k= kidney, me=mantle edge, pn= pneumostome, re=rectum, rl= right lobe rv= renal vein. Scale: 2.1 mm.

Digestive system

The digestive system of *Peplimnea affinis* is the same as *P. lessoni*.

Nervous system

The nervous system of *Peplimnea affinis* is the same as *P. lessoni*, except the cerebral commissure measures between one third and half the length between the cerebral ganglion.

Reproductive system

The reproductive system of *Peplimnea affinis* is the same as *P. lessoni* except for the following. Seminal vesicle is straight, convoluted or looped (Fig 5.13A). Oviducal caecum is large to extra large, being either greater than one half the width or equal in width to oothecal gland (Fig 5.13B). Uterus/ vagina is either parallel or tapers distally, and is long, being equal in length to the oothecal gland. The uterus/ vagina is greater than right angles to the oothecal gland (Fig 5.13B). A small simple sac is formed at the junction of posterior end of prostate and vas deferens (Fig 5.13C). Penis sheath is less than equal in length to praeputium (Fig 5.14A). Penis is looped in penis sheath head (Fig 5.14B).
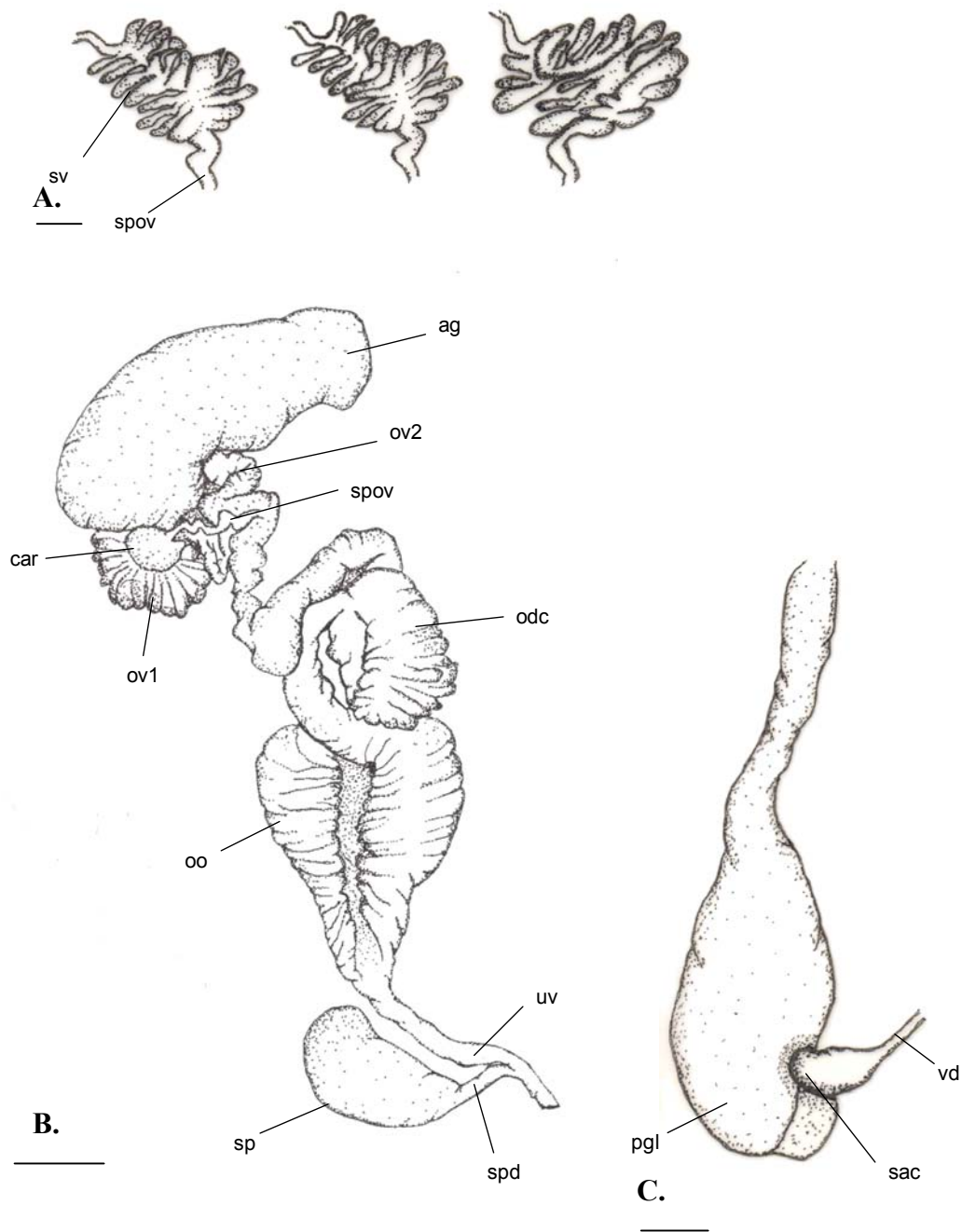
Figure 5.13 Reproductive organs of *Peplimnea affinis* C.439182. A. Dorsal view of seminal vesicle, B. Ventral view of female reproductive system, C. Ventral view of prostrate. Abbreviations: ag= albumen gland, car= carrefour, odc= oviducal caecum, oo=oothecal gland, ov1= oviduct 1, ov2= oviduct 2, pgl= prostate gland, sac= small sac at junction prostate and vas deferens, sp= spermatheca, spd= spermathecal duct, spov= spermoviduct, sv= seminal vesical uv= uterus/ vagina, vd= vas deferens. Scales: A 1.25mm; B 2.1mm; C 1.25mm.
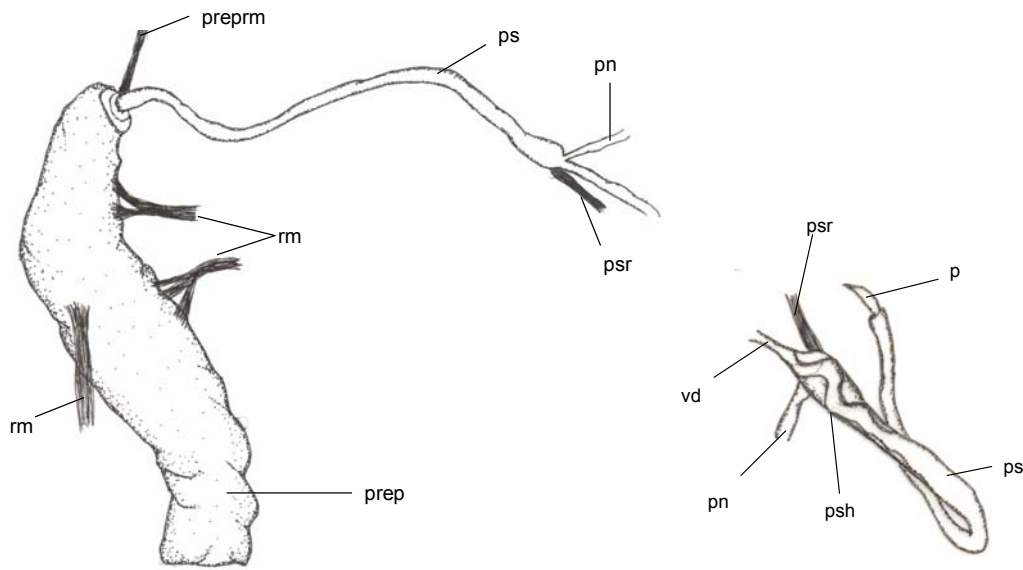
A.
B.
Figure 5.14 Male copulatory organs of *Peplimnea affinis* C.436053. A. Dorsal view of praeputium and penis sheath, B. Dorsal view of the penis in penis sheath. Abbreviations:, p= penis, pn= penal nerve, prep= preaputium, preprm= preputium retractor muscle, ps= penis sheath, psh= penis sheath head, psr= penis sheath retractor, rm= retractor muscle, vd= vas deferens. Scales: A 2 mm; B 1.1mm.

Distribution

     Peplimnea affinis occurs throughout Northern Australia, from the Pilbara region, into Broome and across the Kimberly region in Western Australia. This species is recorded from Alice Springs, and is likely to occur throughout the Finke River system. *Peplimnea affinis* also occurs throughout the northern area of the Northern Territory, including Arhem Land (Fig 5.15). It is possible that this species may occur as far east as the Gulf of Carpentaria, although further investigations need to be undertaken to assess the easterly range of this species.
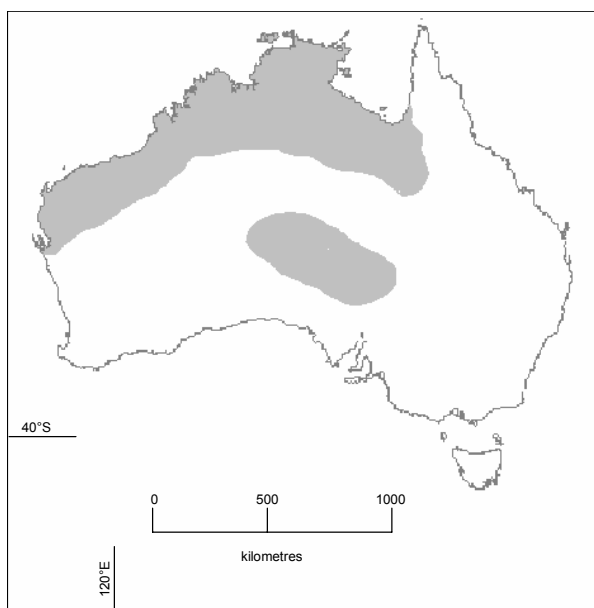
Figure 5.15 Distribution of *Peplimnea affinis*, based on results of molecular and anatomical phylogenies

Remarks

Peplimnea affinis was not identified by previous taxonomic studies (Hubendick 1951), as samples from this region were not studied. The original description of this species (Küster 1862) was based only on shell characters. There have been no previously published anatomical descriptions of this species from this region, although this description does agree with some unpublished anatomical data (Ponder, pers. comm.). Iredale (1943; 1944) identified a number of distinct taxa from the north and north west of Australia based on shells, but only one species is present throughout this entire region.

5.2.2 Species previously attributed to *Austropeplea tomentosa* (Peiffer, 1855)

Austropeplea Cotton, 1942

Austropeplea Cotton, 1942 Type species: *Limnea papyracea* Tate, 1880.

Glacilimnea Iredale, 1943 Type species: *Glacilimnea gelida* Iredale, 1943.

Simlimnea Iredale, 1943 Type species: *Limnaea brazieri* Smith, 1883.

Kutikina Ponder and Waterhouse, 1997 Type spcies: *Kutikina hispida* Ponder and Waterhouse, 1997.

Diagnosis

Shell with up to four whorls, columella fold slight or absent; right lobe of kidney absent; oviduct 1 forms brain like convolutions around carrefour; spermatheca spherical shape; penis sheath length either half the length of praeputium, but never equal. Penis sheath head well developed.

Austropeplea tomentosa (Pfeiffer, 1855)

Succinea tomentosa Pfeiffer, 1855: 297 (Auckland, New Zealand Type data; syntypes BMNH 1950.5.16.11-12)

Limnaea leptosoma Hutton, 1885: pl.l2, Fig 11 (Wellington, New Zealand).

Myxas arguta Hutton, 1885: 54, pl.24, fig 15 (Avon River, New Zealand

Myxas ampulla Hutton, 1885: pl.24, Fig 14 (Lake at Arthurs Pass, New Zealand).

Myxas ampulla globosa Suter, 1890: 93, pl.18, Figs 12a-c (Tasman Valley, New Zealand).

Limnaea venustula Cherry, 1896: 183 (*nom. nud.,* see Hubendick, 1951).

Lymnaea tomentosa; Hubendick 1951: 106, Figs 279, 291 (in part).

Lymnaea tomentosa; Boray and McMichael 1961 (in part).

Austropeplea tomentosa; Ponder and Waterhouse 1997: 458, Figs 4b,d,10a-c, Smith 1992: 257, Smith *et al*. 2003 (in part).

Material examined

East Cape, North Island (C.422732, 37º 39.400' S, 178º 29.610' E), North of Napier, North Island (C.422731, 39º 13.040' S, 176º 53.380' E), Arthur's Pass, South Island, (C.433250, 42º 54.340' S, 171º 33.618' E), Avon River, Christchurch, South Island (C.433525, 43º 32.000' S, 172º 38.000' E), Little River, South Island (C.433513, 43º 44.929' S, 172º 49.450' E), Mole Lake, South Island (C.433524, 45º 00.437' S, 168º 34.384' E).

Diagnosis

Shell thin, umbilicus closed, up to 4 whorls; foot maximum width at mid foot; foot width about half as long; tentacles wider than longer, or sometimes tentacle width is equal to length; expanded mantle covering some parts of the shell; kidney width half the length; pulmonary vein less than one third kidney length; ureter absent; pedal commissure absent or short; radula sac longer than buccal mass; salivary gland longest lobe on right or left; uterus/ vagina long; spermathecal duct longer than uterus; uterus parallel; oothecal gland globular; oviduct 1 forms radial ridges around the carrefour; uterus/ vagina at less then right angles to oothecal gland; penis sheath greater than half the length of praeputium; penis in penis sheath head looped; seminal vesicle low blisters and u-shaped; upper prostate wide; prostate as long as female system; bicuspid asymmetrical, smaller cusp on left hand side; radula teeth shape blunt; marginal teeth with five cusps.

Shell

Shell shape thin, fragile, up to 4 whorls, short spire, lacking any distinct sculpture; aperture large; thin outer lip; inner lip slightly reflected; shell umbilicus closed, columella fold slight (Fig 5.16).

Table 5.3 Shell variables of *Austropeplea tomentosa*. Measurements in mm, n=18.

	SL	SW	AL	AW	LWL
range	4.68 - 7.94	3.44 - 4.94	4.13 – 6.06	2.34 - 3.81	4.38 - 7.19
mean	5.95	4.01	4.87	3.01	5.51
sd	0.84	0.54	0.60	0.47	0.73

AL= aperture length, AW= aperture width, LWL= last whorl length, sd= standard deviation, SL= shell length, SW= shell width.
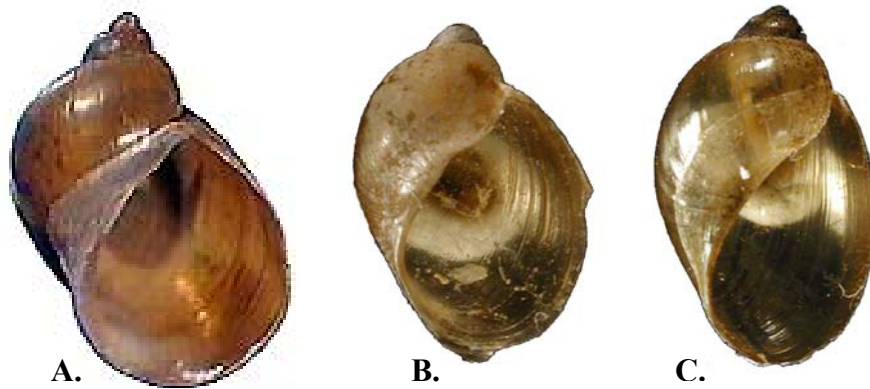
Figure 5.16 Shell variation in *Austropeplea tomentosa*. A. North Island New Zealand, BMNH 1950.5.16.11-12, shell height unknown, B. North Island New Zealand, C. 422731, shell height, 5.5 mm, C. South Island New Zealand, C.433542, shell height 5.2 mm.

Radula

Radula has asymmetrical central teeth bearing two cusps, smaller on left (Fig 5.17B). Seven pairs of tricuspid lateral teeth in each row, 8th pair transitional with the marginal teeth, having three large cusps and one small cusp on outer side (Fig 5.17B,C). The remainder of marginal teeth have 5 large cusps and 1-3 minute denticles on the outer side. The outer most row of marginal teeth consists of four small cusps. There are up to 13 pairs of marginals (Fig 5.17A,D).

Head-foot and visceral mass

Head-foot is typical of the Lymnaeidae, with short, bluntly triangular cephalic tentacles, usually wider than long in live specimens, but can also be equal in length to width (Fig 5.18A). Eyes located inside the tentacle proximal inner edge on distinct eye lobe (Fig 5.18A). Foot maximum width at mid foot, foot width about half as long (Fig 5.18B). Snout is short and broad, the mouth antero-ventral, prominent thick jaw. Lateral sides of snout form a distinct junction with the side of the foot (Fig 5.18A). Thick mantle collar is present, extending to cover some parts of the shell (Fig 5.18A). The pallial roof mottled with black pigmentation, mantle covering the visceral coil unpigmented (Fig 5.18A).
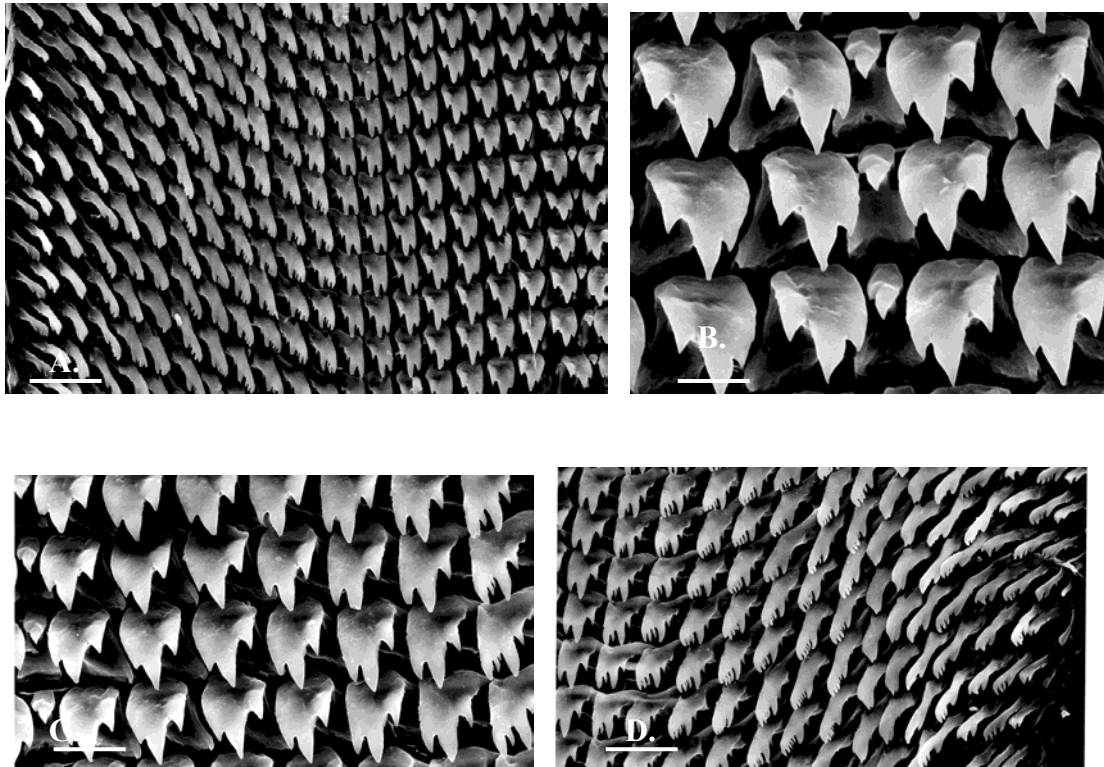
Figure 5.17 Radula teeth of *Austropeplea tomentosa* C.433524. Radula teeth, dorsal views A. Half rows, B. Detail of central and inner laterals, C. Detail of lateral and inner most marginal, D. Detail of marginal. Scales; A 25 μm, B 7 μm, C 10 μm, D 20 μm.

Pallial cavity

The pneumostome, like many other lymnaeids, has two internal ridges, but lacks an anal flap and upper plate (Fig 5.19A, B). Pulmonary roof has the heart and kidney in their typical position. Kidney is thin walled and broad anteriorly, width half the length of kidney. Pulmonary vein short, running less than one third the right hand side of kidney (Fig 5.19A). Ureter is absent, and as in other lymnaeids, the anus is situated at the posterior edge of the pneumostome (Fig 5.19A).
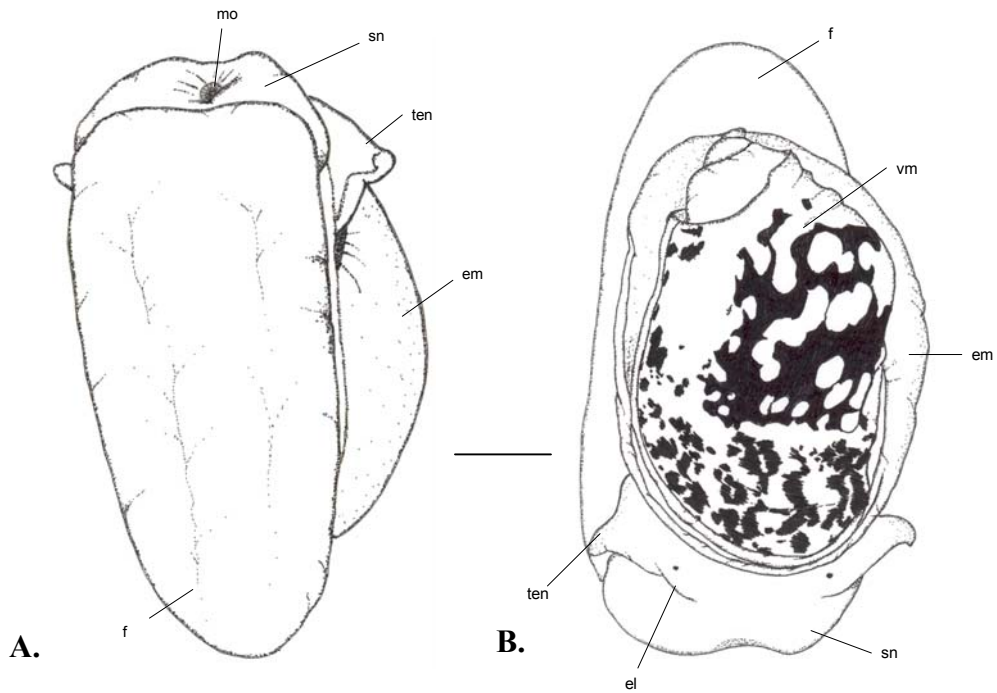
Figure 5.18 *Austropeplea tomentosa* **C.422731. External view of animal showing external features and pigmentation patterns. A. Dorsal view of animal showing expanded mantle edge, B. Ventral view of foot. Abbreviations; el= eye lobe, em= expanded mantle edge, f= foot, mo= mouth, sn= snout, ten= tentacle, vm= visceral mass. Scale: 3.25 mm**
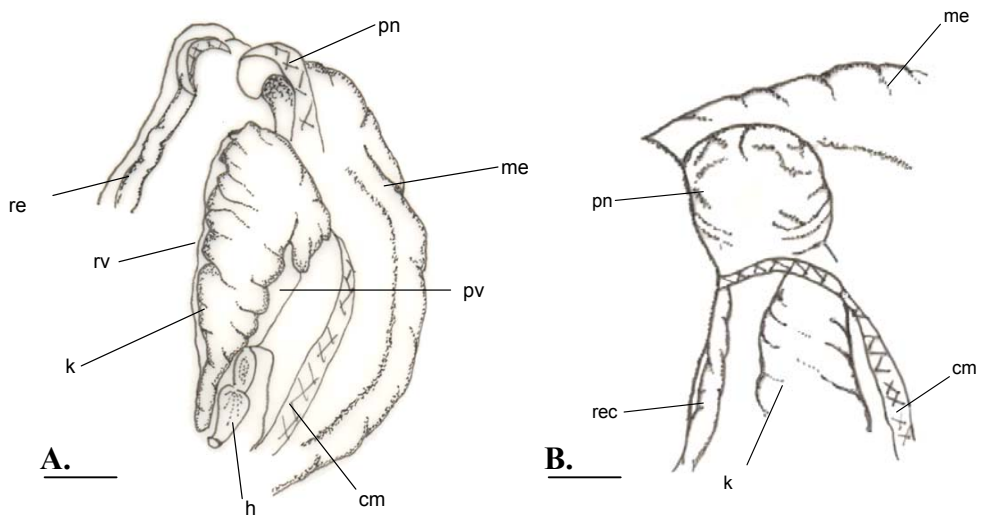
Figure 5.19 Ventral view of pallial cavity of *Austropeplea tomentosa* C.433524. A. Kidney shape and size, B. Pnuemostome. Abbreviations: cm= cut muscle, h= heart, k= kidney, me=mantle edge, pn= pneumostome, pv= pulmonary vein, re=rectum, rv=renal vein. Scales: A 4 mm; B 2 mm.

Digestive system

Buccal mass is large, being longer than wide or equal in width to length (Fig 5.20A). Radula sac longer than the buccal mass (Fig 5.20B). Salivary ducts lie

alongside the oesophagus and beneath the cerebral commissure. Salivary glands joined dorsally and laterally, either the right or left hand lobe being longer (Fig 5.20A). Stomach similar to other lymnaeids, gizzard formed from the two bulbous, lateral muscle pads. Behind the gizzard a thin walled area opens to a caecum and an opening to the digestive gland.

Nervous system

Parietal nerve ring is pentaganglionic, the ganglion either separated by short connectives or just abutting one another (Fig 5.21A). Cerebral ganglion of medium length, being of equal length to the cerebral ganglion (Fig 5.21A). Pedal ganglion either abutting or separated by a short commissure, pedal ganglion length equal to width (Fig 5.21B).
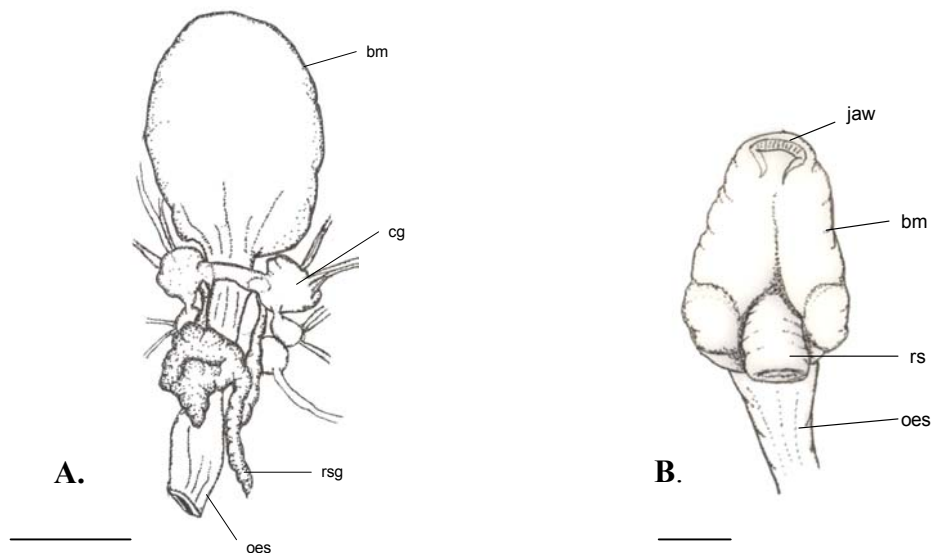
Figure 5.20 Buccal mass and salivary glands of *Austropeplea tomentosa* C.422731. A. Dorsal view of the buccal mass, salivary glands and anterior gut. B. Ventral view of the buccal mass and position of the radula sac. Abbreviations: bm= buccal mass, cg= cerebral ganglion, jaw= jaw of radula, , oes= oesophagus, rs= radula sac, rsg= right salivary gland. Scales: A 3 mm; B 1.5 mm.

Reproductive system

The reproductive system comprises most of the body volume, as in other lymnaeids. Ovotestis embedded in the middle of the columella side of digestive gland. Seminal vesicle long, narrow, and u-shaped, with low blisters (Fig 5.22B).

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Spermoviduct opens to a smooth, egg-shaped carrefour. The spermduct departs adjacent to the junction of the spermoviduct. Albumen gland opens laterally to the carrefour from a broad duct. Oviduct joins carrefour to the oothecal gland, and is divided into two distinct regions. The reproductive system comprises most of the body volume, as in other lymnaeids. Ovotestis embedded in the middle of the columella side of digestive gland.
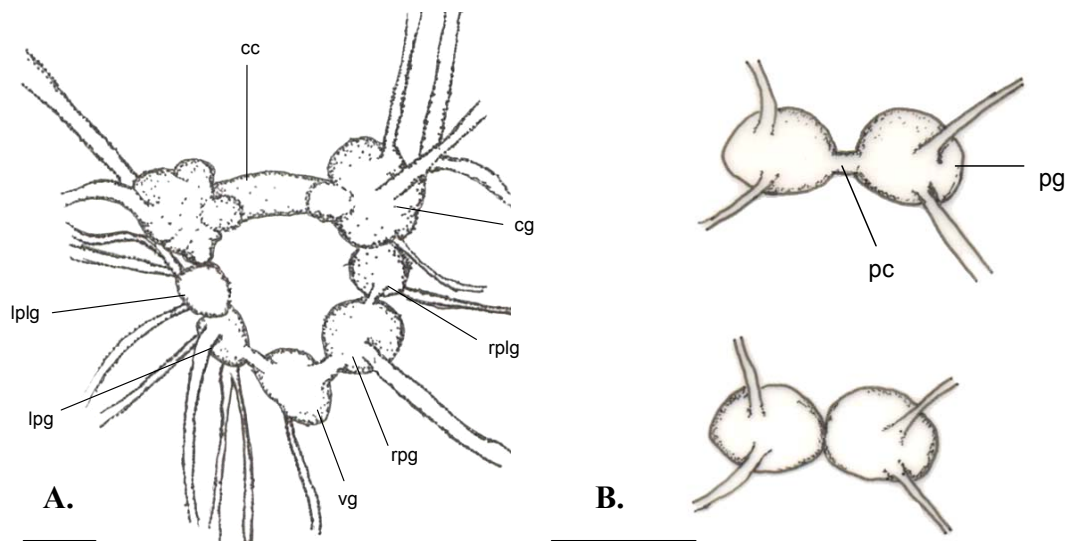
Figure 5.21 Nervous system of *Austropeplea tomentosa* C.422731. A. Dorsal view of the ganglionic ring, B. Posterior view of pedal ganglion. Abbreviations: cc= cerebral commissure, cg= cerebral ganglion, lpg= left parietal ganglion, lplg= left pleural ganglion, pc= pedal commissure, pg= pedal ganglion, rpg= right parietal ganglion, rplg= right pleural ganglion, vg= visceral ganglion. Scales: A 1 mm; B 1.5 mm.

Seminal vesicle long, narrow, and u-shaped, with low blisters (Fig 5.22B). Spermoviduct opens to a smooth, egg-shaped carrefour. The spermduct departs adjacent to the junction of the spermoviduct. Albumen gland opens laterally to the carrefour from a broad duct. Oviduct joins carrefour to the oothecal gland, and is divided into two distinct regions. Oviduct 1 opens from the carrefour, and is short, forming a radial fan around the carrefour (Fig 5.22B). Oviduct 2, the distal portion of the oviduct, has thinner, undulating walls than oviduct 1, and is much longer than oviduct 1 (Fig 5.22C). Oviduct 2 joins the oothecal gland. Prior to this junction is the oviducal caecum. Oviducal caecum is pouch-like, with smooth, transverse folds, oval in shape, and situated under the oviduct. Oviducal caecum is large, being either half or greater than one half the width of oothecal gland (Fig 5.22C). Oothecal gland, the

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largest part of the female system, is globular in shape, and characterised by a smooth surface with distinct transverse folds (Fig 5.22C). Anteriorly it opens to a narrow uterus, which goes on to form the vagina. Uterus/ vagina is parallel, long, being just shorter than the length of the oothecal gland, and placed at less than right angles to the oothecal gland. Spermathecal duct joins the uterus/ vagina just prior to opening of female opening (Fig 5.22C). Spermathecal duct is narrow, thinner than uterus/ vagina and longer than the uterus/ vagina. The spermatheca forms a round-shape at the distal end of spermathecal duct (Fig 5.22C).

Upper prostate, while initially thin from the carrefour, widens rapidly, and continues to widen posteriorly; upper prostate being flattened against oothecal gland. Prostate equal in length to female system, large fold in the ventral wall (Fig 5.23A). Internally, prostate has one large crescent fold (Fig 5.23B). Posterior end of prostate makes a small sac like junction with vas deferens (Fig 5.23B). Vas deferens joins base of penis sheath. Penal sheath cylindrical, and head structure well developed. Penis sheath greater than half the length of the praeputium in its retracted state, but never equal (Fig 5.23C). Simple penis is about as long as the penis sheath, looped in penis sheath head, duct opening as its pointed tip. Praeputium has two large internal folds of unequal size, a circular velum surrounding the sacrobelum (Fig 5.23D, E). Praeputium retractor muscle attaches at head of praeputium and penis sheath retractor muscle attaches to penis sheath head. A branch of the penal nerve enters the penis sheath from the right cerebral ganglion (Fig 5.23C).

Distribution

Austropeplea tomentosa is restricted to New Zealand, and occurs throughout both the North and South Islands.

Remarks

Anatomical examinations of New Zealand populations of *Austropeplea tomentosa* were based on only a small number of populations, due to scarcity of *A. tomentosa* on the North Island and also the lack of reproductively mature individuals sampled. However, the anatomical studies of the New Zealand populations of *A. tomentosa* agree with that of Hubendick (1951). Other anatomical examinations of *A. tomentosa* from New Zealand have found the spermathecal duct to be short (Pullan

and Climo 1972) or of varying length (Boray and McMichael 1961), while this study and Hubendick (1951) identified the spermathecal duct to be long. Boray and McMichael (1961) provide no illustrations of the variation in spermathecal duct length therefore one cannot be totally confident of such statements of variation. Pullan and Climo (1972) sampled 19 populations from the North Island and 10 from the South Island, a much larger sample size than either this study or previous studies, however they only provide one small illustration of the spermatheca and spermathecal duct. A more detailed examination of this character needs be carried out.

There were only a few differences between the North and South Island samples of *Austropeplea tomentosa*. The North Island samples had tentacles that were in width to length, the buccal mass was longer than wide, the right hand lobe of the salivary gland was the longest, the oviducal caecum was greater than half the width of the oothecal gland and there was a short pedal commissure. These differences may be attributable to variation within the species, or may represent discrete differences between the North and South Island samples. This cannot be adequately determined on the limited material examined here.

Material was not examined from Auckland, the type locality of *Austropeplea tomentosa*. It is possible that I may not apply to the species described here. Further investigation of lymnaeids from this region is needed to varify that the name *Austropeplea tomentosa* is applicable to the species described here.
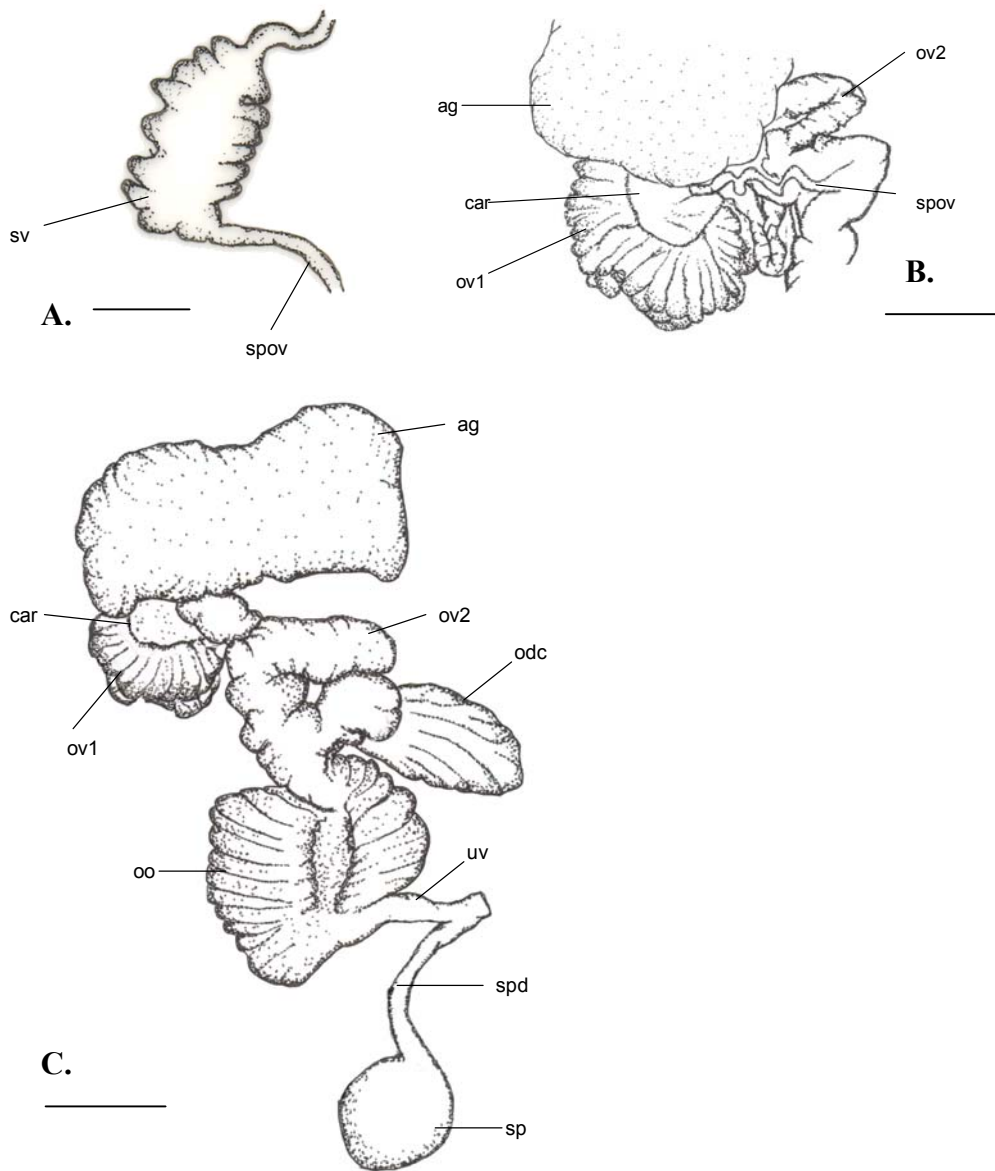
Figure 5.22 Reproductive organs of *Austropeplea tomentosa*, excluding male copulatory organs and prostate. A. Dorsal view of seminal vesicle C.433513, B. Ventral view of oviduct 1 C.433513, C. Ventral view of female reproductive system C.422731. ag= albumen gland, car= carrefour, odc= oviducal caecum, oo=oothecal gland, ov1= oviduct 1, ov2= oviduct 2, sp= spermatheca, spd= spermathecal duct, spov= spermoviduct, sv= seminal vesical uv= uterus/ vagina. Scale: A 1.50mm ; B; 1.25mm C 1.65mm.
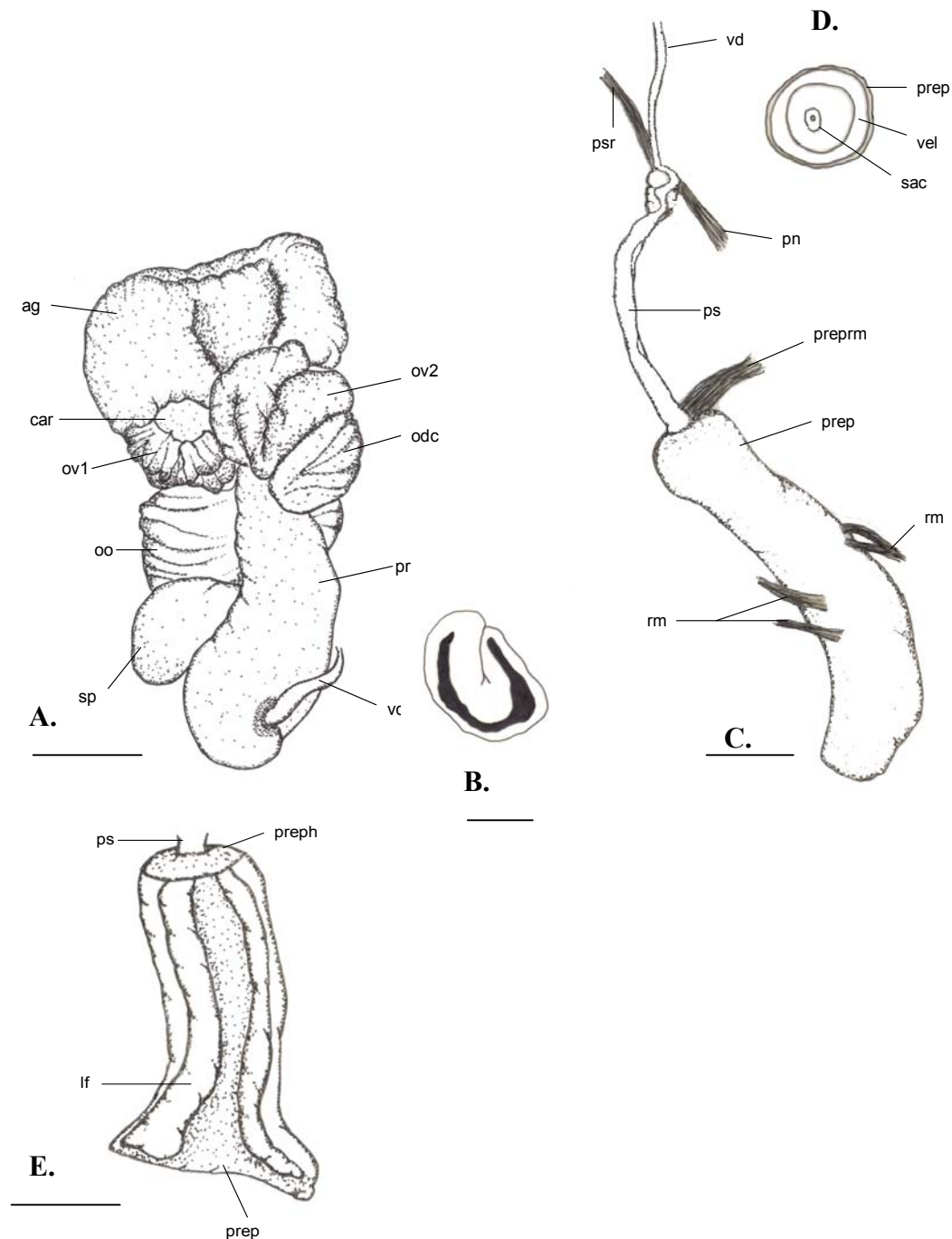
Figure 5.23 Male reproductive organs of *Austropeplea tomentosa* C.422731. A. Ventral view of the prostate in its natural position, B. cross section of prostate, C. Dorsal view of praeputium and penis sheath, D. cross section of praeputium head, E. internal longitudinal folds of praeputium. Abbreviations: ag= albumen gland, car= carrefour, lf= longitudinal fold of praeputium, odc= oviducal caecum, oo=oothecal gland, ov1= oviduct 1, ov2= oviduct 2, pn= penal nerve, ps= penis sheath, prep= praeputium, preph= praeputium head, preprm= praeputium retractor muscle, psr= penis sheath retractor, rm= retractor muscle, sac= sacrobelum, sp= spermatheca, spov= spermoviduct, uv= uterus/ vagina, vd= vas deferens, vel= velum. Scale: A 1.6mm; B 1.25mm; C, D, E 1.7mm.

Austropeplea huonensis (Tenison-Woods, 1876)

Limnaea huonensis Tenison-Woods, 1876: 71 (River Huon, TAS; TMH E865/8206, paratypes SAMA D15686, QVM 9:115).

Limnaea launcestonensis Tenison-Woods, 1876: 71 (creek near Launceston, TAS; syntypes (probable) QVM 9:125)

Limnaea papyracea Tate, 1880: 103, pl. 4, figs 5a-c (Penola, SA; holotype SAMA D13591, paratypes SAMA D13591, SAMA D5342, AM C510).

Limnaea subaquatilis Tate, 1880: 103, pl. 4, figs 6a & b (River Torrens, Adelaide, SA ; holotype SAMA D5352, paratypes SAMA D15677, AM C506)

Limnaea brazieri Smith, 1882: 274 (Glebe Point, Sydney, NSW ; holotype BMNH 1875.5.21.601, paratypes BMNH 1875.5.21.602-6, AM C42272).

Limnaea victoriae Smith, 1882: 274, pl. 5, Fig 16 (Bairnsdale, VIC; syntypes BMNH 1983066).

Limnaea viridula Tate, 1882: 76 (*nom. nud.,* see Hubendick, 1951)

Limnaea gunnii Petterd, 1889: 60-82 pls 1-4 (South Esk River, near Launceston, TAS syntypes AM C27761, SAMA D15684).

Limnaea lutosa Petterd, 1889: 67, pl. 2, Fig 14 (Brighton, River Jordon, TAS; holotype QVM 9:114, paratypes AM C27762, SAMA D15685, TMH E15183).

Limnaea subaquatilis neglecta Petterd, 1889: 60-82 pls 1-4 (Launceston, TAS; syntypes AM C27763, SAMA D15687, QVM 9:113).

Lymnaea aruntalis Cotton, and Godfrey, 1938: 36 (*nom. nov.* for *Limnea papyracea* Tate, 1880).

Glacilimnea gelida Iredale, 1943: 214 (Blue Lake, Mt Kosciusko, NSW; syntypes AM C22791).

Simlimnea aegrifer Iredale, 1944: 119, fig 5 (Bombala, NSW; holotype AM C51721, paratypes AM C170828).

Simlimnea morbida Iredale, 1944; 119, Fig 5 (Walcha, NSW; holotype AM C51687, paratypes AM C51686 35).

Lymnaea tomentosa; Hubendick, 1951: 84, Fig189

Lymnaea tomentosa; Boray and McMicahel 1961 (in part)

Austropeplea tomentosa; Ponder and Waterhouse 1997: 458, Figs 4b,d,10a-c, Smith 1992: 257, Smith *et al*. 2003 (in part).

Material examined

Guyra, NSW (AM C.442100, 30º 13.330' S, 151º 40.170' E, AM C.431236, 30º 27.649' S, 151º 21.392' E), Walcha, NSW (AM C.431248, 30º 54.586' S, 151º 17.306' E), Penrith, NSW (AM C.431874, 33º 38.500' S, 150º 41.500' E, AM C.407263, 33º 46.230' S, 150º 45.660' E), Windsor, NSW (AM C.431876, 33º 37.000' S, 150º 49.000' E, AM C.309424, 33º 38.500' S, 150º 45.660' E), Braidwood, NSW (AM C.442102, 35º 31.483' S, 149º 31.700' E), Kosciuszko Plateau, NSW (AM C.436026, AM C.22791, 36° 24.368' S, 148° 19.064' E), Bombala, NSW (AM EBU.35591, 37º 08.783' S, 149º 28.087' E) Bemboka, NSW (AM EBU.35582, 36º 34.500' S, 149º 41.467' E), Penola, SA (AM C.427947, 37º 15.299' S, 140º 26.114' E), Mt. Gambier, SA (AM C.428299, 37º 22.301' S, 140º 12.624' E, AM C.427946, 37º 27.051' S, 140º 14.651' E), Millicent, SA (AM C.427948, 37º 09.688' S, 140º 06.075' E, AM C.427949, 37º 04.927'S, 140º 04.927' E), Launceston, TAS (AM C.422098, 41º 26.873' S, 147º 07.286' E, South Esk River, TAS (AM C.422104, 42º 16.800' S, 147º 35.400' E), Lake Augusta, TAS (AM C.422096, 41º 52.340' S, 146º 30.779' E), Clyde River, TAS (AM C.422102, 42º 21.385' S, 147º 01.395' E), Lemont, TAS (AM C.422101, 42º 16.807' S, 147º 35.411' E), Castlemaine, VIC (AM C.422092, 37º 19.277' S, 144º 21.777' E).

Diagnosis

Only characters that differ between *Austropeplea tomentosa* and *A. huonensis* have been included in the following diagnosis.Radula cusps pointed; oviduct 1 forms brain like convolutions around the carrefour, seminal vesicle with distinct pockets.

Description
Shell

Austropeplea huonensis shells are characterised by a large amount of variation, as shown in Figure 5.24. Shell is usually thin, up to four whorls, with a large aperture and a short spire (Fig 5.24A-C). The samples of *A. huonensis* from the Kosciuszko region however have a thicker shell, with only three whorls and a much reduced spire (Fig 5.24D). Samples of *A. huonensis* from the South Australian region have a much larger aperture with up to 4.5 whorls.
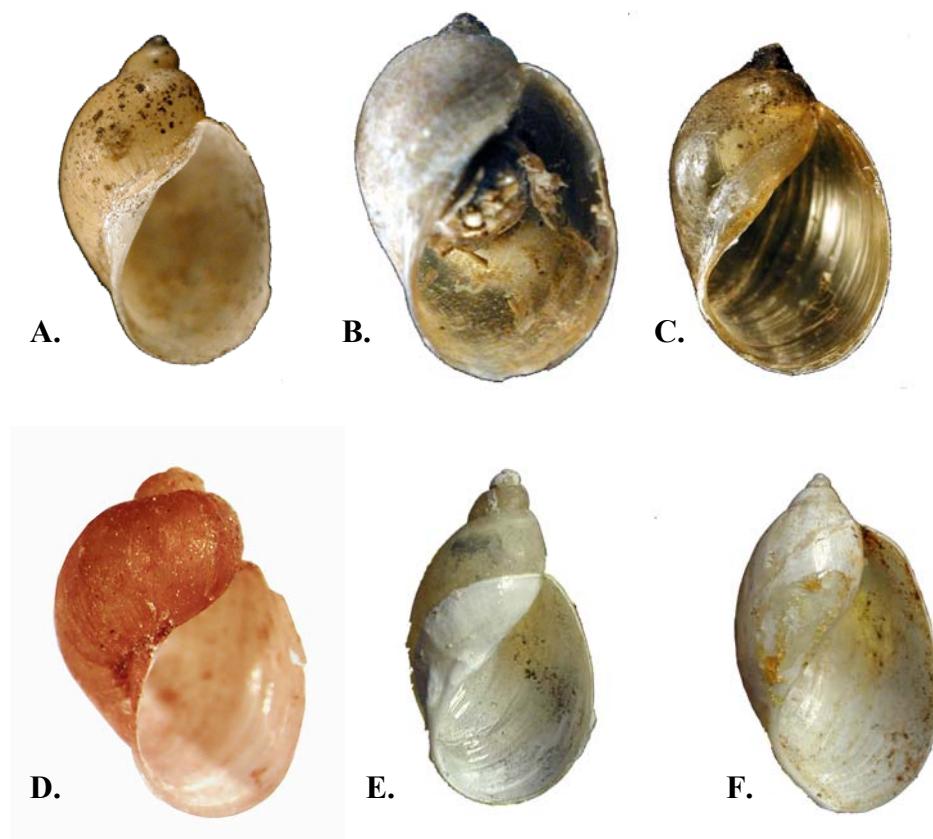
Figure 5.24 Shell variation within *Austropeplea huonensis*. A. Northern New South Wales, C.431236, Shell height 6.0 mm, B. Southern New South Wales, C.44102, shell height 7.3 mm, C. Tasmania, C.422104, shell height 7.8 mm, D. Kosciuszko, C.22791, shell height 9.3 mm, E. South Australia, D.5342, shell height 9.3 mm, F. South Australia, D.13591, shell height 12.1 mm.

Radula

Radula as in *A. tomentosa*, except the radula teeth are more pointed and the number of laterals can range from 7 to 10 and the marginals from 13 to 19. Marginals are usually tetracuspid, although the Tasmanian and South Australian samples have marginals with five cusps (Fig 5.25).

Table 5.4 Shell variables of *Austropeplea huonensis*.

New South Wales and Victorian samples. Measurements in mm, n=62.

	SL	SW	AL	AW	LWL
range	4.64 – 8.44	3.23 - 5.94	3.23 - 6.94	2.36 - 5.00	4.18 - 7.81
mean	6.30	4.20	4.76	3.08	5.66
sd	1.06	0.97	1.01	0.80	1.07

Kosciuszko samples. Measurements in mm, n=8

	SL	SW	AL	AW	LWL
range	6.80 - 10.40	5.20 - 7.60	6.20 - 8.80	4.20 - 6.00	6.60 - 10.0
mean	8.03	6.33	6.87	4.86	7.73
sd	1.34	1.01	1.08	0.74	1.26

South Australian samples. Measurements in mm, n=17

	SL	SW	AL	AW	LWL
range	8.10 - 14.00	4.60 - 8.40	6.30 - 10.60	3.50 – 6.70	7.30 - 11.80
mean	10.65	6.12	8.33	4.75	9.38
sd	1.68	1.08	1.40	0.99	1.36

Tasmanian samples. Measurements in mm, n=30.

	SL	SW	AL	AW	LWL
range	5.73 - 8.00	3.33 - 4.53	4.67 - 6.13	2.47 - 3.67	5.13 - 7.13
mean	7.00	4.43	5.46	3.40	6.38
sd	0.64	0.60	0.58	0.51	0.63

AL= aperture length, AW= aperture width, LWL= last whorl length, sd= standard deviation, SL= shell length, SW= shell width.
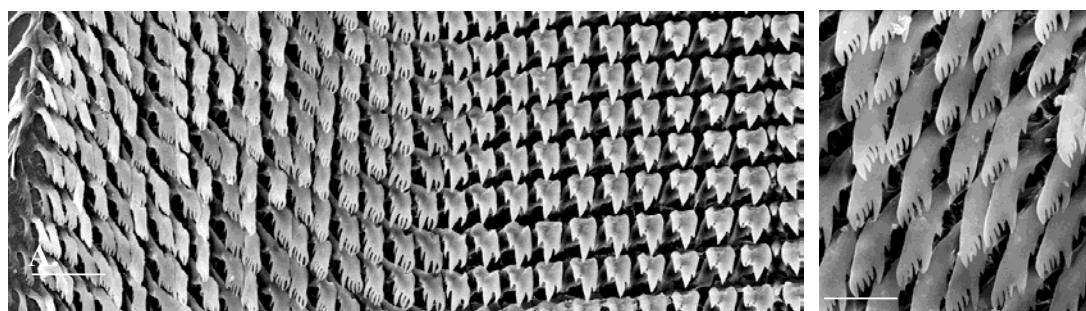
Figure 5.25 Radula teeth of *Austropeplea huonensis*. A. half row C.427948, B. details of the marginals C.422104. Scales, A 30 μm; B 15 μm.

Head-foot and visceral mass

Tentacle shape varies with Tasmania, southern New South Wales and Victorian samples having tentacles longer than wide (Fig 5.26A). Tentacle width is equal to length in the northern New South Wales samples and the Kosciuszko samples (Fig 5.26B), while the South Australian samples have tentacles much longer than wide (Fig 5.26C). A thick mantle collar is present, with the expansion of the mantle

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edge varying between geographic regions within the distribution of the species. The New South Wales samples have no extended mantle edge (Fig 5.26A). The Tasmania, Victorian and Kosciuszko samples have some extension of the mantle edge outside of the shell (Fig 5.26B), and the South Australian populations have a large expansion of the mantle edge, covering the majority of the shell (Fig 5.26C). Pigmentation of the mantle roof covering the visceral coil can be either absent or present.
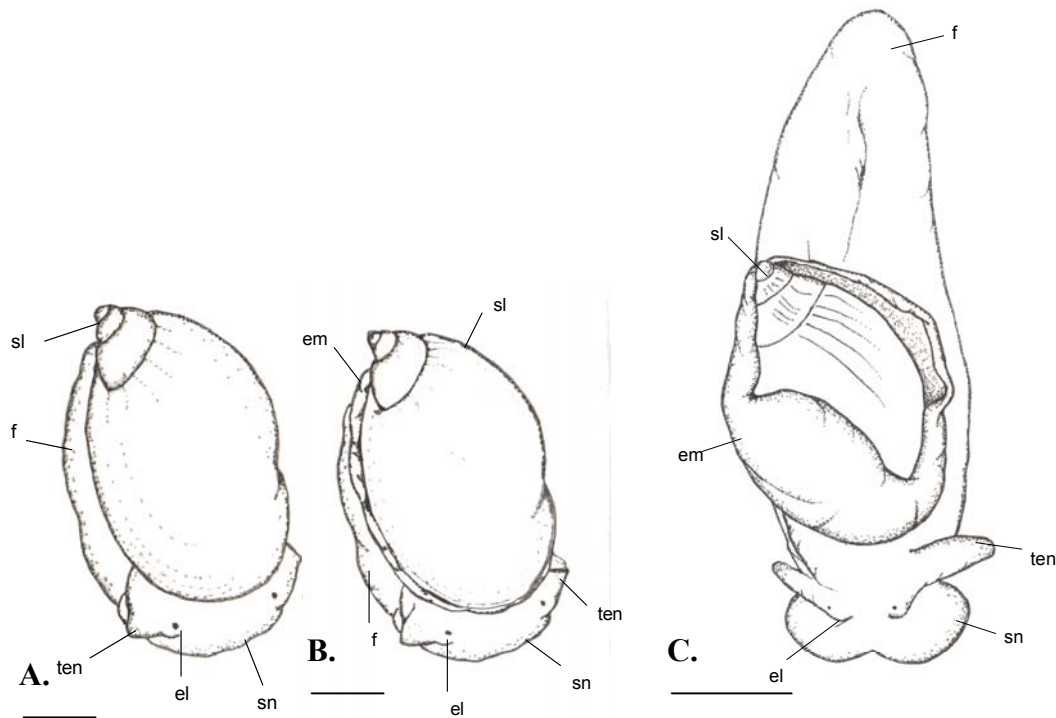
Figure 5.26 *Austropeplea huonensis*. Dorsal view of animal showing external features. A. Absent expanded mantle edge C.431248, B. Mantle edge expanded to outside of shell C.407248, C. Large expansion of mantle edge C.427947. Abbreviations; el= eye lobe, em= expanded mantle edge, f= foot, sl= shell, sn= snout, ten= tentacle. Scales: A 2.3 mm, B 2 mm; C 5.3 mm.

Foot width is generally broad anteriorly (Fig 5.27A), with the exception of samples from South Australia, where the maximum foot width occurs in the middle of the foot (Fig 5.27B). Foot width is greater than half the length (Fig 5.27A), except for in the South Australian samples where the foot is very long and width much less than half the length (Fig 5.27B).
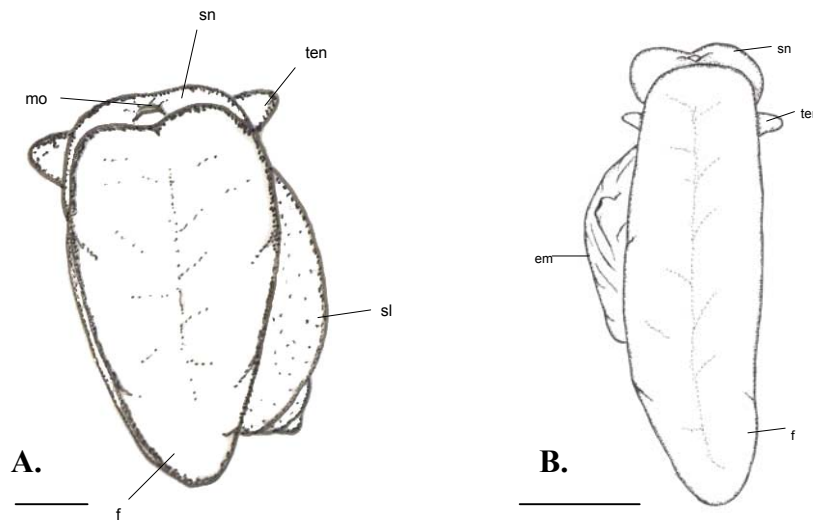
Figure 5.27 *Austropeplea huonensis*, **ventral view of foot. A. Samples from New South Wales, Victoria and Tasmania C.422092. B. Samples from South Australia C.427947. Abbreviations: em= expanded mantle edge, f= foot, mo= mouth, sl= shell, sn= snout, ten= tentacle. Scales: A 2 mm; B 5.3 mm.**

Pallial cavity

Pallial cavity, as in *Austropeplea tomentosa,* except for the following. Kidney shape, pulmonary vein length and the absence or presence of the ureter varies throughout the distribution of *A. huonensis*. The kidney can be narrow, width about one third the length, as in the New South Wales and Victorian samples (Fig 5.28A). The kidney can also be more broad, width greater than one third the length, in the Kosciuszko, Tasmanian and South Australian samples (Fig 5.28B). Pulmonary vein can be long, where it is greater than one third the length of the kidney, as in the New South Wales and Victorian populations (Fig 5.28A), or it can be short, where it is less than one third the length of the kidney, as in the Kosciuszko, Tasmanian and South Australian samples (Fig 5.28B). The absence or presence of the ureter differs between samples, with the Kosciuszko and northern New South Wales samples lacking a ureter (Fig 5.28A) and all other samples having a short ureter.
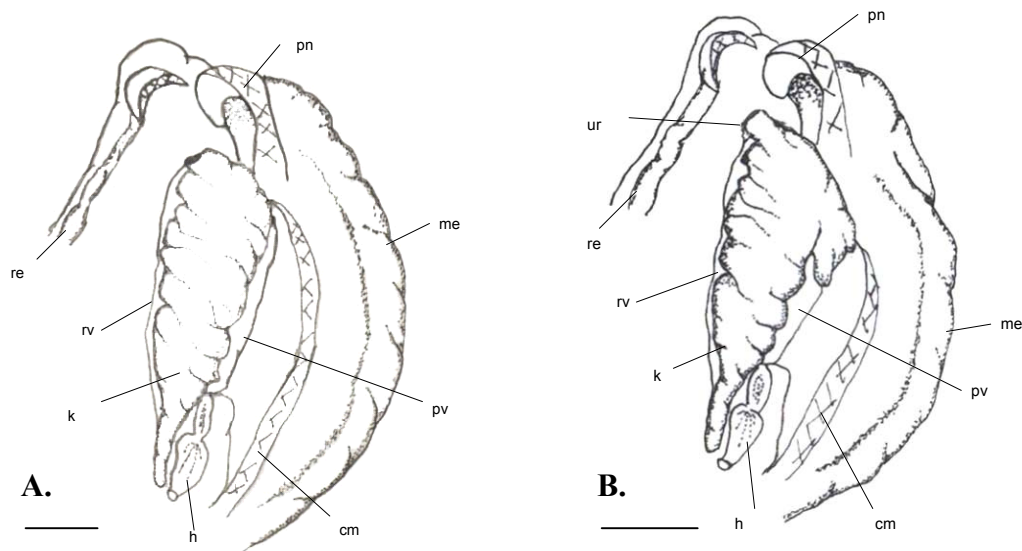
Figure 5.28 Ventral view of pallial cavity of *Austropeplea huonensis*. A. New South Wales and Victorian samples C.442102, B. South Australia, Tasmania and Kosciuszko samples C.422104. Abbreviations: cm= cut muscle, h= heart, k= kidney, me=mantle edge, pn= pneumostome, pv= pulmonary vein, re=rectum, rv=renal vein. Scales: A. 4 mm; B 3.25 mm.

Digestive system

Buccal mass is longer than wide, with the radula sac being equal in length to the buccal mass (Fig 5.29). Relative size of salivary glands varies between geographic regions of *A. huonensis* (Fig 5.30).
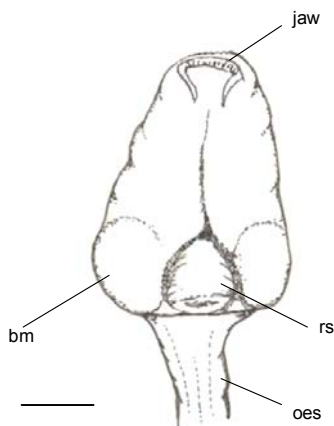
Figure 5.29 Ventral view of buccal mass of *Austropeplea huonensis*, radula sac equal in length to buccal mass C.4231248. Abbreviations: bm= buccal mass, jaw= jaw of radula, oes= oesophagus, rs= radula sac. Scale: 2.5 mm.

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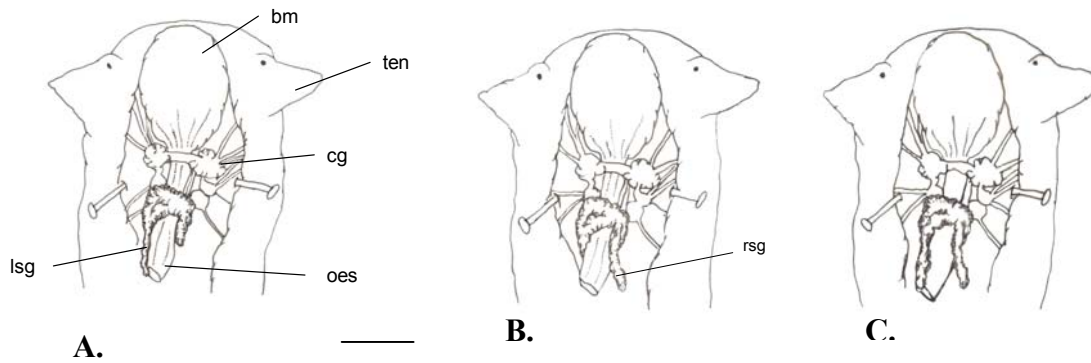
Figure 5.30 Variation in salivary glands of *Austropeplea huonensis*. A. Left lobe of salivary gland larger C.428299, B. Right lobe of salivary gland larger C.428299, C. Lobes of salivary glands equal in size C.422092. Abbreviations: bm= buccal mass, cg= cerebral ganglion, lsg= left salivary glands, oes= oesophagus rsg= right salivary gland, ten= tentacle. Scale: 3 mm.

Nervous system

      The nervous system of *Austropeplea huonensis* is the same as that in *A. tomentosa*.

Reproductive system

      The reproductive system is the same as *Austropeplea tomentosa* except for the following. Seminal vesicle is long, narrow with distinct pockets. The seminal vesicle can be straight, u-shaped or convoluted (Fig 5.31A). Oviduct 1 forms brain like convolutions around the carrefour (Fig 5.31B). Oviducal caecum varies between being half as wide as the oothecal gland to being greater than half, although it is never equal in width to the oothecal gland.
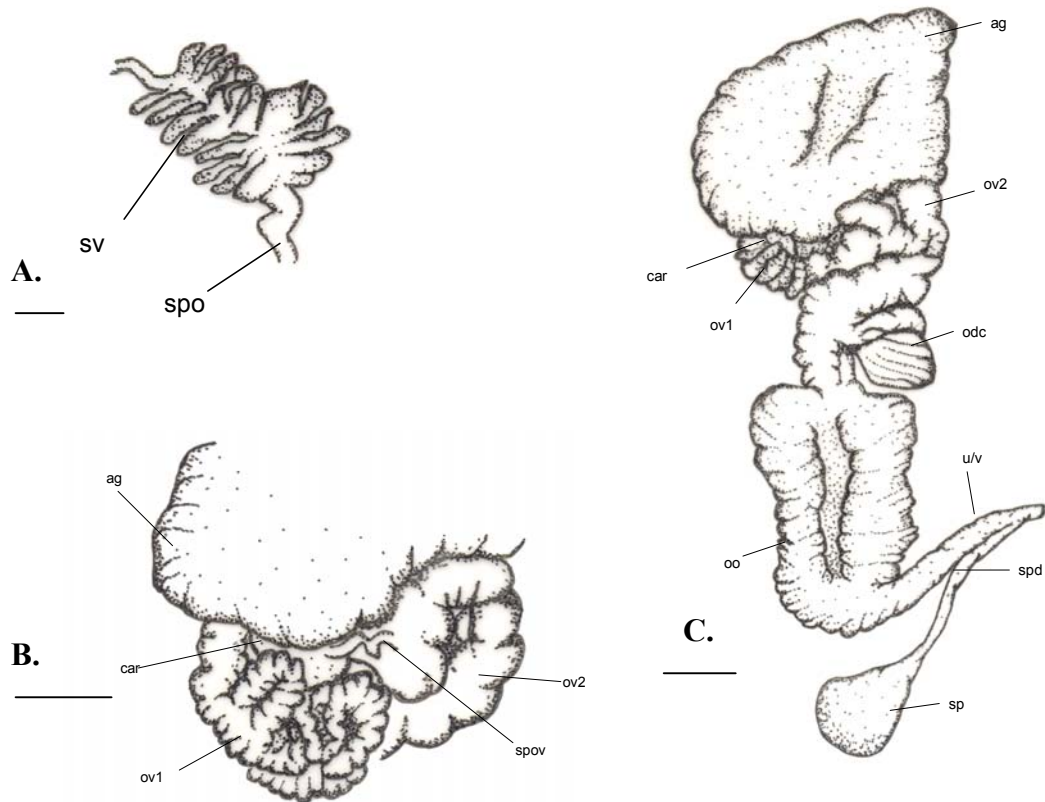
Figure 5.31 Female reproductive organs of *Austropeplea huonensis* C.422104. A. Seminal vesicle, B. Oviduct 1 brain like convolutions around the carrefour, C. Generalised female reproductive system. Abbreviations: ag= albumen gland, car= carrefour, odc= oviducal caecum, oo= oothecal gland, ov1= oviduct 1, ov2= oviduct2, sp= spermatheca, spd= spermathecal duct, spov= spermoviduct, sv= seminal vesicle, uv=uterus/ vagina. Scales: A 0.5mm B 1mm; C 1.9mm.

Oothecal gland varies in shape between the geographic regions of *A. huonensis*. A square oothecal gland characterises the New South Wales samples (Fig 5.32A), a pyriform shape in the Victorian samples (Fig 5.32B), a globular oothecal gland in the Kosciuszko samples (Fig 5.32C) and a rectangular oothecal gland in the Tasmanian and South Australian specimens (Fig 5.32D). Uterus/ vagina is either parallel or tapers distally, and is at right angles to the oothecal gland (Fig 5.32). The exception is the northern New South Wales samples where the uterus/vagina is at less than right angles to the oothecal gland (Fig 5.32A). Uterus/ vagina length also varies within *A. huonensis*, with the northern New South Wales samples having a short uterus/ vagina, only half the length of the oothecal gland (Fig 5.32A). The South Australian samples have a uterus/ vagina that is longer than the oothecal gland, and all other samples have a uterus/ vagina that is nearly equal in length to the oothecal gland (Fig 5.32D). Spermathecal duct is usually thinner than the uterus/ vagina, except in the northern New South Wales samples, where the spermathecal duct is equal in width

to the oothecal gland (Fig 5.32B). Spermathecal duct varies within *A. huonensis*, the Kosciuszko sample spermathecal duct shorter than the uterus/ vagina (Fig 5.32C), the New South Wales and Victorian samples equal in length to the uterus/ vagina (Fig 5.32B). The South Australian and Tasmanian samples have spermathecal ducts that are longer than the uterus/ vagina (Fig 5.32D).
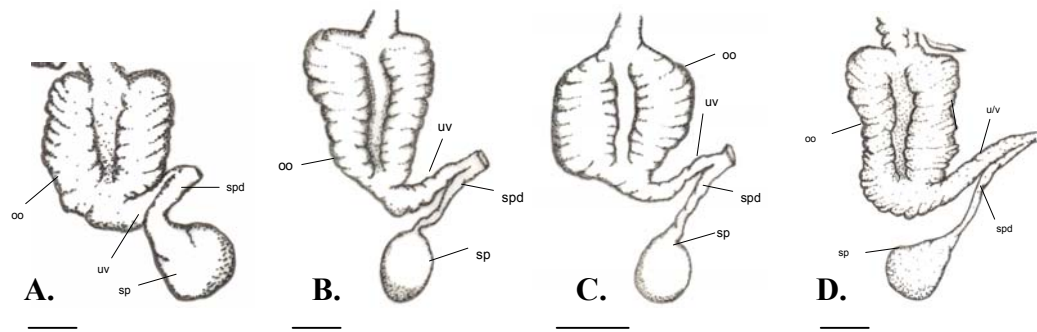
Figure 5.32 Variation in shape of oothecal gland of *Austropeplea huonensis*. A. Square shaped oothecal gland C.431236, B. Pyriform shaped oothecal gland C.422092, C. Globular oothecal gland C.436026, D. Rectangular oothecal gland C.428299. Abbreviations: oo= oothecal gland, 2, sp= spermatheca, spd= spermathecal duct, uv= uterus/ vagina. Scales: A 1mm; B 4mm; C 4mm; D 5mm.

Upper prostate is either wide or thin. A wide upper prostate is exhibited in the South Australian, Tasmanian and Kosciuszko samples (Fig 5.33C), and thin in New South Wales and Victorian samples (Fig 5.33A). Prostate length varies in *A. huonensis*, the prostate being equal in length to the female reproductive system in the northern New South Wales samples (Fig 5.33A), longer in the southern New South Wales, Victorian, Tasmanian and Kosciuszko samples (Fig 5.33B), and much longer in the South Australian samples (Fig 5.33C).
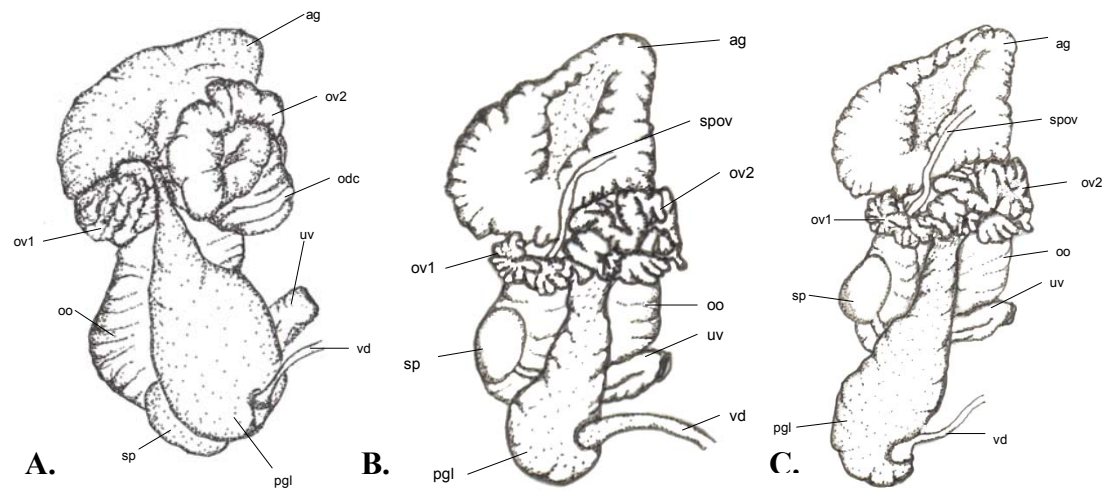
Figure 5.33 Variation in the length of the prostate of *Austropeplea huonensis*. A. Prostate equal in length to the female reproductive system C.431236, B. Prostate longer than female reproductive system C.422104, C. Prostate much longer than female system C.427947. Abbreviations: ag= albumen gland, odc= oviducal caecum, oo=oothecal gland, ov1= oviduct 1, ov2= oviduct 2, pgl= prostate gland, sp= spermatheca, spov= spermoviduct, uv= uterus/ vagina, vd= vas deferens. Scales: A2.5mm; B 1,5mm; C1.8mm.

Penis in penis sheath head usually is looped, although it runs straight through the penis sheath head in the Tasmanian samples. Length of the penis sheath relative to the praeputium is usually greater than half the length of the praeputium, but never equal (Fig 5.34A). The Kosciuszko and South Australian samples however have a penis sheath that is only half the length of the praeputium (Fig 5.34B).

Remarks

Austropeplea huonensis is characterised by a large amount of geographical morphological variation. However, these geographical morphs of *A. huonensis* are not supported by the molecular phylogeny (see Chapter 2). Previous examinations of this species by Ponder and Waterhouse (1997) agree with the material examined here from southern New South Wales and Tasmania. Samples of *A. huonensis* from Northern New South Wales, South Australia or Kosciuszko have been examined in any anatomical detail.
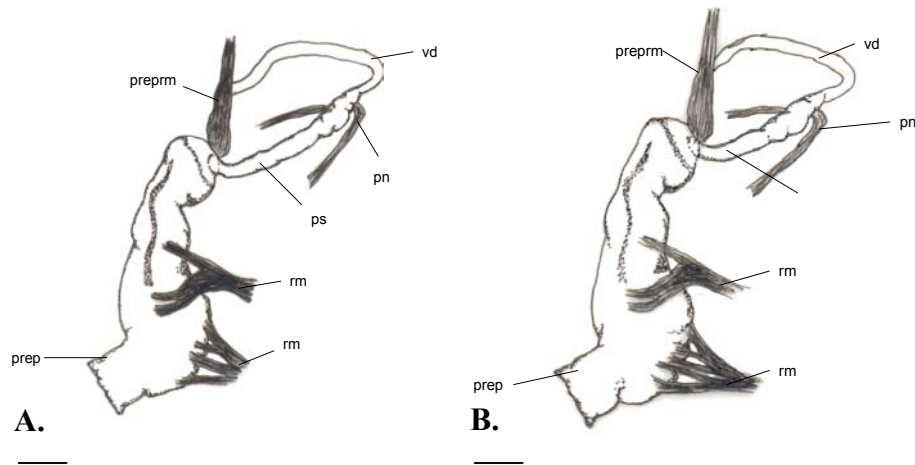
Figure 5.34 Variation in the penis sheath length of *Austropeplea huonensis*. A. Penis sheath greater than half the length of the praeputiumC.422104 , B. Penis sheath equal to half the length of the praeputium C.427947. Abbreviations: pn= penal nerve, prep= praeputium, preprm= preputium retractor muscle, ps= penis sheath, psr= penis sheath retractor, rm= retractor muscle, vd= vas deferens. Scales: A 2mm; B 3.4mm.

Distribution

   Austropeplea huonensis has a large distribution throughout out eastern

Australia, including Tasmania (Fig 5.35).
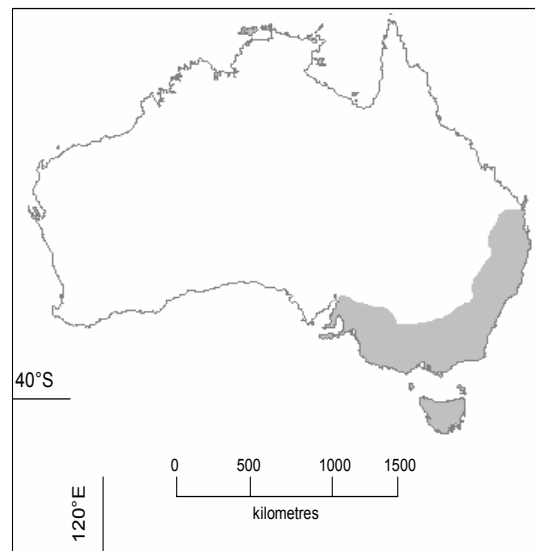
Figure 5.35 Distribution of *Austropeplea huonensis*,

based on results of molecular and anatomical phylogenies

5.2.3 Species previously attributed to *Austropeplea viridis* (Quoy and Gaimard, 1833)

Viridigalba Kruglov and Starobogatov, 1985

Viridigalba Kruglov and Starobogatov, 1985 Type species: *Limnaea viridis* Quoy and Gaimard, 1833.

Diagnosis

Shell umbilicus half open; up to 4.5 whorls; right lobe absent; simple junction of the prostae and vas deferens; prostate equal in length to female reproductive system; penis sheath head well developed; penis sheath equal in length to praeputium, oviducal caecum as wide as oothecal gland.

Chapter 6   Summary of major findings and directions for future research

The main aims of this study was to understand the systematics of the Australiasian Lymnaeidae Rafinesque, 1815. Initially the taxonomy of the Australiasian lymnaeids was determined, followed by an investigation of the phylogenetic relationships of the Australiasian lymnaeids within the family. The major findings of these aspects of the study will be outlined below.

The *Austropeplea tomentosa* (Pfeiffer, 1855) complex has a wide distribution throughout both Australia and New Zealand. Based on the molecular analyses of the 16S gene and the ITS-2 region, this complex is represented by two distinct species, *A. tomentosa* and *Austropeplea huonensis* (Tenison-Woods, 1876). *Austropeplea tomentosa* occurs in both the North and South Island, while *A. huonensis* occurs in southern Australia including Tasmania. Anatomically, *A. huonensis* and *A. tomentosa*, are very similar; the anatomical phylogeny was not able to resolve *A. tomentosa* as a distinct group from *A. huonensis*. Furthermore, the shells of the *A. tomentosa* were not distinguishable in a discriminant function analysis from samples of *A. huonensis* from the east coast of Australia. The occurrence of only one species within Australia was surprising considering the large geographical barriers that separate populations of *A. huonensis*. *Austropeplea huonensis*, under this new classification exhibits a large amount of shell and anatomical variation. Further investigation of this group may result in the recognition of more Australian lymnaeid species.

Kutikina hispida (Ponder and Waterhouse, 1997), a recently discovered Australian lymnaeid, was thought to be closley related to the *Austropeplea tomentosa* complex. Analyses of the 16S gene and the ITS-2 region resolved *Kutikina hispida* as sister to *Austropeplea huonensis* (Australian *Austropeplea*), with *A. tomentosa* being resolved as sister to the *A. huonensis* + *Kutkina* clade. Therefore *Kutikina* was syonymised into *Austropeplea* based on the molecular phylogenies. The small amount of sequence divergence between *A. huonensis* and *A. hispida* suggests that these two species have only recently diverged.

The *Austropeplea lessoni* (Deshayes, 1830) complex within Australia was previously thought be closely related to the *Austropeplea tomentosa* complex. Results of both molecular and anatomical phylogenies clearly show that the *A. lessoni* complex belongs in a different clade from the type species of *Austropeplea*. *Bullastra cumingiana* was resolved as sister to the *A. lessoni* complex, suggesting that the *A. lessoni* complex may be part of *Bullastra*. However, based on a large amount of sequence and anatomical divergence, the *Austropeplea lessoni* complex was considered as a separate genus from *Bullastra*, and appropriately placed in *Peplimnea* (Iredale, 1943).

The *Austropeplea lessoni* complex has a wide distribution within Australia, and prior to this study was though to be represented by one widespread species, that varied between regions. Based on the sequences of the 16S gene and ITS-2 region in addition to anatomical and shell data, *Austropeplea lessoni* is now recognised as two distinct species, *Peplimnea lessoni* (Deshayes, 1830) and *Peplimnea affinis* (Küster, 1862). Unlike the *Austropeplea tomentosa* complex, there was congruence between the molecular and anatomical phylogenies, with each species being distinguished by a shell morphometrics study. Anatomically a number of characters of the reproductive system, in addition to shell shape can be used to distinguish the two species of *Peplimnea*.

The Asian *Austropeplea viridis* (Quoy and Gaimard, 1832) was previously placed in the "*Austropeplea*" genus based on chromosome numbers and radula dentition. However the molecular phylogenies indicate there is a large degree of divergence between *A. viridis* and the other members that were previously assigned to "*Austropeplea*". In addition, there are a number of anatomical divergences that suggest this species should not be placed in *Austropeplea*. *Austropeplea viridis* is therefore placed in *Viridigalba* Kruglov and Starobogatov, 1985.

Based on anatomical features and chromosomes, the Australasian Lymnaeidae have previously been thought to represent one of the most basal groups of the family (Walter 1968; Inaba 1969). However, based on 16S, anatomical and combined molecular and anatomical phylogenies, the Australasian Lymnaeidae were resolved as

some of the most derived groups within the Lymnaeidae. The results of this study are in marked contrast to earlier ideas that proposed *Austropeplea* as the most archaic extant lymnaeid group, and that a reduction in chromosome number occurred during lymnaeid evolution. The results of this study agree with previous molecular studies (Remigio and Blair 1997a; Remigio 2002), in addition to suggestions by Ponder and Waterhouse (1997)

Previous workers have suggested that the *Austropeplea tomentosa* and *A. lessoni* complexes were sister taxa (Burch 1967; Inaba 1969), however, molecular results of this study suggest that two groups are not sister taxa, and that the two complexes may have had separate derivations and that 16 pairs of chromosomes within the Lymnaeidae may have arisen twice. The *Austropeplea lessoni* complex is more closely related to the *Bullastra cumingiana* (a Philippine endemic) and the Asian *Radix* group. Such a close relationship of the Australian *A. lessoni* complex with Asian lymnaeids indicates that the *A. lessoni* complex may have had ancestors in the South East Asian region. Previous studies have suggested the collision of the New Guinea margin with parts of South East Asia 20 million years ago could have resulted in the dispersal of ancestors of the *A. lessoni* complex and *B. cumingiana* (Remigio and Blair 1997a, Remigio 2002).

Kutikina hispida (= *Austropeplea hispida*, as renamed above) is placed as sister to the Australian *A. tomentosa*, and the *A. tomentosa* complex is more closely related to *A. viridis* and European *Radix* than to the *A. lessoni* complex. The relationship of the *A. tomentosa-Kutikina*, complex with the European *Radix* and *A. viridis* was not resolved in the 16S phylogeny. However, the combined 16S and ITS-2 Bayesian analysis placed *A. viridis* as sister to the *A. tomentosa-Kutikina complex*. Although support for this relationship was not strong (0.56), anatomically these two groups are more similar than either is to the European *Radix* group.

The utility of anatomical characters in understanding the phylogenetic relationships within the Lymnaeidae have been previously questioned (Hubendick 1951; Remigio and Blair 1997; Bargues *et al.* 2001; Remigio 2002). The results of this study however suggest that anatomical characters should not be routinely excluded from phylogenetic analyses, as in some cases they can provide a useful

understanding of relationships. The anatomical phylogenies overall had lower consistency values than the molecular trees, suggesting higher levels of homoplasy are occurring within the anatomical tree.

Previous studies of Lymnaeidae evolution have suggested two different scenarios for the biogeography of Australasian group. The first theory, based on a 16S phylogeny, has suggested a South East Asian origin (Remigio and Blair 1997a, Remigio 2002), whilst the second theory suggested a possible separate derivation of the Australian lymnaeids, with the *Austropeplea tomentosa* complex having a Gondwanan origin and the *A. lessoni* complex having a South East Asian origin (Ponder and Waterhouse 1997). The 16S and combined 16S and ITS-2 phylogeny indicates that *A. lessoni* and *A. tomentosa* had separate derivations, and that *A. lessoni* was more than likely derived from a South East Asian ancestor. However, the derived position of the *A. tomentosa* complex on the tree would indicate that the group are only recently derived and not an older Gondwanan group. Given the significant unknowns in the current phylogeny of the Lymnaeidae neither of the two theories can be advocated. There are significant gaps in our knowledge of Lymnaeidae systematics. The South American taxa, represents the largest group of lymnaeids that has yet sampled for molecular techniques. Understanding the relationship of these taxa is essential to test the two current theories of Lymnaeidae evolution.

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Shell characters (1-6, 64, 65)

Lymnaieds in general have a simple shell, with very little ornamentation, colouring or sculpture. Shell characters can sometimes display intraspecific variability and are therefore sometimes unsuitable for phylogenetic analysis. However, some shell characters within the family have proved useful in taxonomic discrimination. No characters of the protoconch have been included in the analysis. Colouration of the shell is incredibly variable within the Lymnaeidae and has not been coded.

1. *Shell umbilicus*; Closed (1); half open (2); open (3)

2. *Shell thickness*; Thin (1); thick (2)

3. *Number of whorls*; Three (1); four (2); five (3); 3.5 (4); 2.5 (5); 4.5 (6)

The columella fold in most lymnaeid species is generally distinct, although some species can be coded as having an absent columella fold when there is little to no trace of the fold (e.g. *Kutikina hispida*, Fig 2A; Ponder and Waterhouse 1997), or as slight when there is a small fold present or as distinct (e.g. *Austropeplea tomentosa*, Fig 5.16), when the fold is easily distinguishable (e.g. *A. lessoni*, Fig 5.10). Most lymnaeid shells are generally very simple lacking any shell sculpture or pattern. Some species however, do exhibit shell sculpture and variation, for e.g. *Psuedosuccinea columella*. In most members of the Lymnaeidae the perisostracum is thin and closely adherent. It is however more conspicuous in *K. hispida* (Fig 2C,F; Ponder and Waterhouse 1997). The Lymnaeidae are generally characterised by dextral shell, with only species, *Pseudoisidora producta*, in the family displaying a sinistral form. Other members of the Lymnaeoidae are characterised by sinistral shells. Shell spire height is very diverse throughout the Lymnaeidae. Variation can range from a largely reduced spire to high. The spire height was coded as absent or largely reduced if the last whorl and aperture were large compared to the spire (e.g. *K. hispida* fig 2A; Ponder and Waterhouse 1997). A short spire was coded as being less than one quarter the height of the aperture (e.g. *A. tomentosa*, Fig 5.16). A medium spire height was coded as being less than half the height of the aperture (e.g. *A. lessoni*, Fig 5.10), and high was coded as the spire being half the height of the aperture (e.g. *Lymnaea stagnicola*, pl. II, fig 6, Jackiewicz 1993a)

4. *Columella fold*; Absent (1); slight (2); distinct (3)

5. *Shell sculpture*; absent (1); present (2)

6. *Periostracum ornamentation*; absent (1); hairy (2)

64. *Shell orientation*; dextral (1); sinistral (2)

65. *Spire height*; Absent or largely reduced (1); short (2); medium (3); high (4)

Head- Foot and visceral mass characters (7-16, 66, 67)

The broadest area of the foot is defined as the region of the foot where the foot is the widest, either at the anterior end of the animal (Fig 5.27A) or with the same width along the animal (Fig 5.27B). Ponder and Waterhouse (1997) identified some variation within the Australian lymnaeids in relation to posterior foot shape, whereby species could be identified by their posterior foot shape (Figs 5.3B, 5.11B). Specimens need to be properly relaxed and preserved to obtain accurate measurements for this character. Foot width was measured at the maximum width at any point along the foot. Foot length was measured from the anterior end of the foot to the posterior end of the foot.

7. *Broadest area of foot*; anterior end of foot (1); same width along length (2)

8. *Foot shape at posterior end*; tapering to a point (1); rounded (2)

9. *Foot width to length ratio*; 2:1 (1); less than 2:1 (2); greater than 2:1 (3)

Lymnaeids have a small lobe at the base of the tentacle in which the eye is usually found. The eye lobe is coded as absent if the lobe is missing and the eye is located at the inner base of the tentacle (e.g. *Kutikina hispida*, fig 4C; Ponder and Waterhouse 1997), undeveloped, if the lobe is but is reduced in size (Fig 5.11), or developed, where the lobe is very distinct (Fig 5.3). The Lymnaeidae are characterised by cephalic tentacles that are flattened, short and broadly triangular. Both Ponder and Waterhouse (1997) and Jackiewicz and Buksalewicz (1998) have used tentacle shape to distinguish between lymnaeid taxa. Jackiewicz and Buksalewicz (1998) could distinguish taxa at a genus level based on tentacle shape, while Ponder and Waterhouse (1997) could distinguish species using tentacle shape. These previous findings suggest that the variation within tentacle shape may represent phylogenetically important information. While improper or poor preservation of animals could interfere with the coding of this character, some general trends in the distribution of tentacle shape have been noted. Tentacle width was measured at the base of the tentacle from the posterior to anterior side. Tentacle length was measured

from the base to the tip of the tentacle. Posterior lateral sides of the snout are usually quite distinct in most lymnaeid species. The lateral sides of the snout were coded as developed if the posterior lateral sides of the snout formed a distinct fold with the foot and undeveloped if the posterior lateral sides of the snout merged into the side of the foot (Fig 4C,D, Ponder and Waterhouse 1997). Pallial roof pigmentation in the Lymnaeidae is common, with most species displaying a mottled black and white pigmentation pattern. Some species such as *Psuedosuccinea columella* have variable pigmentation of the mantle roof. Pigmentation of the mantle roof covering the visceral coil can also occur, and was coded as such if found present. Expansion of the mantle edge occurs in a number of lymnaeid taxa throughout the world, for e.g. *Bullastra cumingiana*, *Myxas glutinosa* and *Austropeplea tomentosa*. The degree of the expansion of the mantle edge also differs between groups of lymnaeids. The mantle expansion was coded as absent if there was no expansion of the mantle edge (Fig 5.11), as just outside of the shell if there was small amount of mantle expansion (Fig 5.26B), as covering some parts of the shell if the mantle expanded outside of the shell and covered a small portion of the shell of the animal (Fig 5.18B), or as covering large of parts of the shell if the majority of the shell was covered by the expanded mantle edge (Fig 5.26C). Pigmentation of the expanded mantle can also occur and was either recorded as absent or present. Columella digitations of the mantle are not found in the Lymnaeidae, but occur in the Planorbidae and Physidae.

10. *Eye lobe*; absent (1); well developed (2); undeveloped (3)

11. *Tentacle shape*; Wider than long (1); width equal to length (2); longer than wide (3); twice as long as wide (4).

12. *Lateral sides of snout*; Developed (1); undeveloped (2)

13. *Pallial roof pigmentation*; Mottled black and white (1); black (2)

14. *Visceral coil pigmentation*; absent (1); present (2)

15. *Mantle expansion*; absent (1); just outside of shell (2); covering some parts of the shell (3); covering large parts of the shell (4)

16. *Expanded mantle pigmentation*; absent (1); present (2)

66. *Tentacle form*; triangular and flat (1); fillaform and circular (2)

67. *Columella digitations of the mantle*; absent (1); present (2)

Pallial cavity characters (17-26, 68, and 69)

The pallial cavity has never been used in any formal cladistic analysis of the Lymnaeidae. Paraense (1976; 1982; 1984; 1994a; 1994b; 1995) has used pallial characters to distinguish South American lymnaeid taxa, as does Ponder and Waterhouse (1997) with the Australian lymnaeids. Ponder and Waterhouse (1997) and Jackiewicz and Dudzien(1998) distinguished lymnaeid species and genera based on differences in the pneumosotme. The ventral wall of the pnuemostome in expanded outwards to form a 'siphon' in most lymnaeids, with a prominent internal ridge on both of the short lateral walls of the pneumostome which subdivides the opening into upper and lower apertures. Some species are however lacking these ridges. The number of pneumostomal ridges was coded as one if the pneumostome was lacking internal ridges and not forming an obvious siphon (e.g. *Kutikina hispida*, fig 4a,b; Ponder and Waterhouse 1997). Jackiewicz and Dudzien(1998) identifies the presence of an outer lobe and upper plate in the European *Radix*. The outer lobe forms a sinus into which the rectum opens into. In species where the outer lobe is not present (e.g. *R. peregra*, fig 9; Jackiewicz and Dudzien 1998) if not formed and the faeces are excreted directly outside through the anus (e.g. *Omphiscola glabra*, fig 1; Jackiewicz and Dudzien 1998) The upper plate of the pneumostome in some species is thick, forming a distinct near border on the outer edge of the mantle (e.g. *R. peregra*, fig 9; Jackiewicz and Dudzien 1998). A thin upper plate does not have a distinct near border on the mantle edge (e.g. *Omphiscola glabra*, fig 1Jackiewicz and Dudzien 1998).

17. *Number of pneumostomal ridges*; one (1); two (2)

18. *Outer lobe*; absent (1); present (2)

19. *Upper plate of pneumostome*; thin (1); thick (2)

Shape of kidney has been identified by Ponder and Waterhouse (1997) and Paraense (1976; 1982; 1984; 1994a; 1994b; 1995) as an important character in distinguishing lymnaeid species, although the utility of these characters have never formally been tested. The kidney in the Lymnaeidae is generally characterised by internal ridges dividing the lumen into irregular, transverse compartments. Some species however have an additional lobe situated on the right hand side of the kidney (Fig 5.4), that is composed of a more granular spongy tissue. The pulmonary vein runs from the pericardium cavity along the right hand side of the kidney (Fig 5.19), and generally if the right lobe is present the pulmonary vein runs through the right lobe (Fig 5.4), and is not visible from the ventral view. The pulmonary vein can vary in

size relative to the kidney, either being long and greater than one third the length of the kidney, medium and being equal to one third the length of the kidney or short being less than one third the length of the kidney. The ureter is located at the distal end of the kidney and if the distal end of the kidney was unmodified the ureter was coded as absent (Fig 5.19). If there was some modification of the distal end of the kidney the ureter was coded as present (Fig 5.4). If the ureter is present it generally opens inside the pnuemostome, although it is know in *Kutikina hispida* to open anteriorally to the pneumostome (fig 6; Ponder and Waterhouse 1997). Ureter length varies within in the Lymnaeidae, a short ureter was coded if the ureter was just extending beyond the margin of the kidney (eg. Fig 5.28), while a medium ureter was coded if the ureter length was longer than the width of the anterior width of the kidney (e.g. *'Lymnaea' rupestris*; fig 2; Paraense 1982), and a long ureter was coded if the ureter was considerably longer than anterior width of the kidney (e.g. *Psuedosuccinea columella*, fig 7; Paraense 1994a). The ureter in most lymnaeid species bends to the left with no flexures, although two lymnaeid species (*P. columella* and *'L.' cousini*) are known to have a ureter that has two flexures (fig 3; Paraense 1995), and one species (*K. hispida*) has ureter that is straight (fig 6; Ponder and Waterhouse, 1997).

20. *Broadest area of kidney*; Anterior end (1); same width along length (2); posterior end (3); middle (4)

21. *Kidney width to length ratio*; 3:1 (1); 2:1 (2); greater than 3:1 (3)

22. *Right lobe of kidney*; absent (1); present (2)

23. *Position of pulmonary vein*; to the right of kidney (1); inside right lobe (2)

24. *Pulmonary vein length*; one third the length of the kidney (1); less than one third the length of the kidney (2); greater than one third the length of the kidney (3)

25. *Ureter*; absent (1); present (2)

26. *Opening of kidney*; inside pnuemostome (1); anterior to the pnuemostome (2)

58. *Ureter length*; short (1); medium (2); long (3)

68. *Number of flexures in ureter*; zero (1); two (2)

69. *Ureter shape*; bent to left (1); straight (2)

Digestive and Nervous system characters (27-33)

The buccal mass of the Lymnaeidae is largely composed of muscle and is generally quite large. Differences in the shape of the buccal mass and the size of the radula sac relative to the buccal mass were useful in discriminating the Australian

lymnaeids (Ponder and Waterhouse 1997). The radula sac was coded from the ventral view. The radula sac was coded as equal if the radula sac ended at the posterior end of the buccal mass (Fig 5.30B); longer than buccal mass, if the radula sac extended over the posterior end of the buccal mass (Fig 5.20B); and shorter if the posterior end of the buccal mass could been seen beyond the radula sac (Fig 5.5B). The salivary glands of the Lymnaeidae are generally irregular in shape and composed of a left and right lobe. The relative size of the salivary gland lobes have proved useful in discriminating between some Australian lymnaeids (Ponder and Waterhouse1997). From the dorsal view, the left hand lobe is situated on the left hand side of the investigator.

27. *Buccal mass shape*; longer than wide (1); width equal to length (2)

32. *Radula sac*; equal in length to buccal mass (1); longer than buccal mass (2); shorter than buccal mass (3)

33. *Salivary glands relative size*; equal size (1); right lobe longer (2); left lobe longer (3)

Nervous system characters (28-59)

The nervous system of the Lymnaeidae is pentaganglionic. Ponder and Waterhouse (1997) identified differences in the Australian lymnaeids in the pentaganglionic ring and the pedal ganglion. The cerebral commissure length was measured from the dorsal view, and distance between the cerebral ganglion was measured from the outer edge of one cerebral ganglion to another. Pedal ganglion observed from the posterior view, and if pedal commissure was absent, then pedal ganglion would be abutting (Fig 5.21B). The expansion of the pedal commissure around the statocysts of the pedal ganglion was only observed in the *A. lessoni* complex in this study (Fig 5.6B). Statocysts have been identified as absent in *Kutikina hispida* by Ponder and Waterhouse (1997). Pedal ganglion are usually equal in length and width (Fig 5.21B), although *K. hispida* has pedal ganglion that are wider than long (fig 4.7B; Ponder and Waterhouse 1997).

28. *Cerebral commissure length*; half as long as distance between cerebral ganglion (1); one third the distance between cerebral ganglion (2); less than a third the distance between cerebral ganglion (3)

29. *Pedal commissure*; absent (1); short (2)

30. *Expansion of the pedal ganglion*; normal (1); enlarged(2)

31. *Statocysts*; absent (1); present (2)

59. *Pedal ganglion shape*; as long as wide (1); wider than long (2)

Female reproductive system (34-41, 60, 61, 70)

The female reproductive system has been largely used by Jackiewicz and co-workers (1959; 1974; 1988b; 1989; 1990b; 1991; 1993a) to distinguish between European lymnaeids. Hubendick (1951) however only identified the distal end of the female reproductive system as useful in discrimination between species. However anatomical examinations of the Australian (Ponder and Waterhouse 1997) and South American lymnaeids (Paraense (1976; 1982; 1984; 1994a; 1994b; 1995) clearly show that lymnaeid species can be distinguished by attributes of the female reproductive system. All female reproductive characters were measured from the ventral view and follow the terminology of Ponder and Waterhouse (1997). The uterus/ vagina was measured from the opening of the female pore to the base of the oothecal gland, and oothecal gland length was measured from the posterior end where oviduct 2 joins to the base where the uterus joins the oothecal gland (Fig 5.32). Spermathecal duct was measured from where it joins the uterus/ vagina to the base of the spermatheca (Fig 5.32). Spermathecal duct and uterus/ vagina width were measured at the mid point of both the spermathecal duct and the uterus/ vagina. The spermathecal duct width in most lymnaeids is usually thinner than uterus (Fig 5.32D), although in some samples it was equal in width (Fig 5.32A). The uterus was coded as parallel if the sides of the uterus ran parallel to one another (Fig 5.32C), and as tapering distally if the uterus/ vagina at the base of the oothecal gland is wide and the tapers to the female pore (Fig 5.32D). Oviducal caecum size was measured in terms of width relative to the width of the oothecal gland. Width was determined as the maximal lateral width across both the oviducal caecum and oothecal gland (Fig 5.22). Oothecal gland shape is as shown in Figure 5.32. Oviduct 1 wraps around the carrefour in the Lymnaeidae, and either forms a radial fan around the carrefour (Fig 5.7B) or forms brain like convolutions around and on top of the carrefour (Fig 5.31B). The position of the uterus/ vagina relative to the oothecal gland refers to the angle made between the uterus/ vagina at the junction of the oothecal gland (Fig 5.32). The insemination pocket and vagina bulb were considered by Walter (1968) as important characters in lymnaeid evolution, although only present in a small number of North American lymnaeids (fig 67, 66).

Spermathecal shape can vary from round (e.g. *Austropeplea tomentosa*, Fig 5.22A) to egg shaped (Fig 5.13A).

34. *Uterus/ vagina length relative to oothecal gland length*; greater than half the length (1); less than half the length (2); equal or longer (3)

35. *Spermathecal duct length*; shorter than uterus/ vagina (1); equal to uterus/ vagina (2); longer than uterus/ vagina (3)

36. *Spermathecal duct width*; equal to uterus/ vagina (1); thinner than uterus/ vagina (2)

37. *Uterus shape*; parallel (1); tapering distally (2)

38. *Oviducal caecum size relative to oothecal gland*; one quarter the width (1); half width (2); greater than half the width (3); wider (4); absent (5)

39. *Oothecal gland shape*; globular (1); pyriform (2); rectangular (3); square (4)

40. *Oviduct 1*; *w*ith brain like convolutions (1); with radial ridges (2); bosselated wall (3)

41. *Position of uterus relative to oothecal gland*; at right angles (1); greater than right angles (2); less than right angles (3)

60. *Insemination pocket*; absent (1); present (2)

61. *Vaginal bulb*; absent (1); present (2)

70. *Spermatheca shape*; round (1); egg shaped (2)

Male reproductive system characters (42-52, 62, 63, 71-79)

Characters of the male reproductive system are considered by some workers to be the most important in lymnaeid evolution and in the discrimination of the species (Hubendick 1951; Walter 1968; Jackiewicz 1993a). Jackiewicz (1984) and Hubendick (1951) use a number of internal characters to classify, including the shape of the velum. A horse-shoe shape velum is exhibited by only one species *Kutikina hispida* (fig 9D; Ponder and Waterhouse, 1997). The majority of lymnaeid species exhibit a circular velum (Fig 4.23D), although *Omphiscola glabra* has no velum (fig 14; Jackiewicz 1993a). The penis sheath is measured from the where the vas deferens joins the penis sheath to where the penis sheath joins the praeputium. The position of the penis in the penis sheath head can vary within the Lymnaeidae and even within species, sometimes be looped in the penis sheath (Fig 5.23C0 or it can be straight (Fig 5.8F). The seminal vesicle in the Lymnaeidae is generally has distinct pockets (fig 5.7), although some species exhibit only very small pockets that resemble small

blisters (fig 9B, Ponder and Waterhouse, 1997). The shape of the seminal vesicle can vary with some members of *Radix* have a short and wide seminal vesicle, where the length and width of the seminal vesicle are equal, while Austropeplea have a long thin seminal vesicle, where the length of the vesicle is much greater than the width (Fig 5.13A). The form of the seminal vesicle can also vary from being straight, through to being looped, u-shaped and convoluted (Fig 5.13A).The junction of the vas deferens and the prostate is usually a simple junction (Fig 5.8), however some species have a small sac like formation at the junction (Fig 5.13C). Characters of the prostate where considered by Walter (1968) and Hubendick (1951) as some of the most important in lymnaeid classification. Usually there is a large fold present on the ventral wall (Fig 5.13C), although in *K. hispida* the wall is just slightly concave. The upper prostate can vary in size, either being thin (Fig 5.13C) or wide (Fig 5.23A). The length of the prostate is measured relatively to the female reproductive system, with some lymnaeids exhibiting large differences in the size of the prostate (Fig 5.33). The prostate in some members of *Radix* at the anterior end bends towards the left when looking from the ventral view, whereas most species have a straight prostate (fig 5.13C). The penal knot and prostate pouch as identified by Walter (1968) are only present in some North American and European lymnaeid species (fig 91, 60,). Penis shape can vary in the Lymnaeidae, with a number of European lymnaeids identified with short and wide penis, while the majority of lymnaeids have long and thin penis (Jackiewicz 1993a). The penis sheath head is coded as well developed when apical chambers of the penis sheath head are clearly present (Fig 5.34), and coded as poorly developed when the apical chambers are not visible (Fig 5.8F). Width of the penis sheath head and praeputium retractor insertion point are as described by Jackiewicz (1993a). The praeputium in the Lymnaeidae is characterised by internal longitudinal folds. The relative size and number of these folds were used extensively by Hubendick (1951) to classify species, with most species have two internal longitudinal folds. *Omphiscola glabra* has only one longitudinal internal fold. The presence of a the preputial gland is characteristic of the Physidae, and a prostate that is one large structure compared to a series of small lobes is characteristic of the Lymnaeidae. Prostate shape can vary considerably within the Lymnaeidae, with *Psuedosuccinea columella* having a long and thin prostate (Fig4(Paraense 1994a), *A. lessoni* having a wide anterior thin posterior prostate (Fig 5.13C), A. tomentosa with a wide anterior and posterior prostate (Fig 5.23A) and *'Lymnaea viatrix'* having wide

anterior and posterior, thin in the middle prostate (fig10; Paraense 1976). The internal fold of the prostate was considered an important character by Walter (1978), although most species have one fold (Fig 5.23B), Omphiscola glabra has no folds (fig 13; Jackiewicz 1993a) and *Lymnaea stagnalis* has numerous folds (fig 28; Jackiewicz 1993a).

42. *Velum shape*; horse-shoe shaped (1); circular (2); absent (3)

43. *Penis sheath length relative to praeputium*; less than half the length (1); Greater than half the length (2); Equal in length (3); Half the length (4); longer than praeputium (5)

44. *Penis in penis sheath head*; looped (1); straight (2)

45. *Seminal vesicle*; pockets present (1); low blisters (2)

46. *Seminal vesicle shape*; short and wide (1); long and narrow (2)

47. *Seminal vesicle form*; U shaped (1); convoluted (2); straight (3); looped (4)

48. *Junction of vas deferens and prostate*; simple (1); small sac (2)

49. *Prostate ventral wall*; large fold present (1); slightly concave (2)

50. *Upper prostate*; thin (1); wide (2)

51. *Length of prostate relative to female reproductive system*; equal in length (1) longer (2); much longer (3); shorter (4)

52. *Shape of lower prostate*; straight (1); bent to left (2)

62. *Penal knot*; absent (1); present (2)

63. *Prostate pouch*; absent (1); present (2)

71. *Penis shape*; short and wide (1); long and thin (2)

72. *Penis sheath head*; well developed (1); poorly developed (2)

73. *Width of penis sheath relative to praeputium width*; one third as wide (1); half as wide (2); equal (3)

74. *Praeputium retractor insertion point*; laterally (1); at base of praeputium head (2)

75. *Number of internal folds of the praeputium*; one (1); two (2)

76. *Preputial gland*; absent (1); present (2)

77. *Prostate structure*; series of small lobes (1); one large structure (2)

78. *Prostate shape*; long and thin (1); wider anterior thin posterior (2); wide anterior and posterior (3); wide anterior and posterior, thin in the middle (4)

79. *Internal prostate fold*; absent (1); one (2); numerous (3)

Radula characters (53-57)

Radula characters tend to be rather uniform across the family, although some species have quite divergent radula teeth (e.g. *Kutikina hispida*). The central in the lymnaeids is usually unicuspid, although some species have bicuspid centrals (Fig 5.17B). The position of the smaller cusp can vary from being on the left (e.g. Austropeplea tomentosa, Fig 5.17B) or on the right (e.g. *Omphiscola glabra*). Lateral teeth in the Lymnaeidae are usually either bicuspid, (e.g. *Stagnicola*) or tricuspid (e.g. *Austropeplea*; Fig 5.17B). Marginal teeth are usually characterised by a number of cusps, usually four or five (Fig 5.17D).

53. *Central tooth*; bicuspid (1); unicuspid (2); tricuspid (3); multicuspid (4)

54. *Position of small cusp on central tooth*; left (1); right (2)

55. *Radula teeth shape*; blunt (1); sharp (2)

56. *Lateral teeth*; bicuspid (1); tricupsid (2); unicuspid (3); multicuspid (4)

57. *Marginal teeth*; bicuspid (1); tricuspid (2); tetracuspid (3) , 5 cups (4), greater than 5 cusps (5)

80. *Number of chromosome pairs*; 16 (1); 17 (2); 18(3)

Appendix 2.2. Matrix of anatomical characters used for phylogenetic analysis of the *Austropeplea tomentosa* complex. Taxa without names are currently recognised as *A. tomentosa*. See Appendix 2.1 for a description of each character and state.

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NSW 1                    1 1 4 2 1 1 1 1 3 2   2 1 1 1 1 - 2 1 1 1 1 1 1 3 1 1   1 2   2 1 2 1   1   2 2 1 2   3 4 1 3 2 2   2 1 2   3 2 1 1 1 1 1 1 1 2 3
NSW 2                    1 1 4 2 1 1 1 1 3 2   2 1 1 1 1 - 2 1 1 1 1 1 1 3 1 1   1 2   2 1 2 1   1   2 2 1 2   3 4 1 3 2 2   2 1 2   3 2 1 1 1 1 1 1 1 2 3
NSW 2(2)                 1 1 4 2 1 1 1 1 3 2   2 1 1 1 1 - 2 1 1 1 1 1 1 3 1 1   1 2   2 1 2 1   1   2 2 1 2   3 4 1 3 2 2   2 1 2   3 2 1 1 1 1 1 1 1 2 3
NSW 3                    1 1 4 2 1 1 1 1 3 2   3 1 1 1 1 - 2 1 1 1 1 1 1 3 2 1   1 2   1 1 2 1   1   1 2 2 2   3 4 1 1 2 2   1 1 2   2 2 1 1 2 1 1 1 1 2 3
NSW 4                    1 1 4 2 1 1 1 1 3 2   3 1 1 1 1 - 2 1 1 1 1 1 1 3 2 1   1 2   1 1 2 1   1   1 2 2 2   3 4 1 1 2 2   1 1 2   2 2 1 1 2 1 1 1 1 2 3
NSW 5                    1 1 4 2 1 1 1 1 3 2   3 1 1 2 1 - 2 1 1 1 1 1 1 3 2 1   1 2   1 1 2 1   1   1 2 2 2   3 4 1 1 2 2   1 1 2   2 2 1 1 2 1 1 1 1 2 3
KOS 1                    1 2 2 2 1 1 1 1 1 2   2 1 1 1 2 1 2 1 1 1 1 2 1 1 1 1   1 2   2 1 2 3   2   1 1 2 1   2 1 1 1 1 2 1 1&2 1 2   2 2 1 1 2 1 1 1 1 2 3
NSW 8                    1 1 4 2 1 1 1 1 3 2   3 1 1 2 1 - 2 1 1 1 1 1 1 3 2 1   1 2   1 1 2 1   1   2 2 1 2   3 4 1 1 2 2   1 1 2   2 2 1 1 2 1 1 1 1 2 4
NZn 2                    1 1 4 2 1 1 2 1 1 2 1&2 1 1 1 3 1 2 1 1 1 1 2 1 1 2 1 1   1 2   2 1 2 2   2   1 3 2 1   3 1 2 3 2 2   1 2 2   1 2 1 2 2 1 1 1 2 2 4
NZs 3                    1 1 4 2 1 1 2 1 3 2   1 1 1 1 3 1 2 1 1 1 2 1 1 2 1 1   1 2   1 1 2 2   2   1 3 2 1   2 1 2 3 2 2   1 2 2   1 2 1 2 2 1 1 1 2 2 4
NZs 4                    1 1 4 2 1 1 2 1 3 2   1 1 1 1 3 1 2 1 1 1 2 1 1 2 1 1 1&2 2   1 1 2 2   3   1 3 2 1   2 1 2 3 2 2   1 2 2   1 2 1 2 2 1 1 1 2 2 4
SA 1                     1 1 5 2 1 1 2 1 2 2   4 1 1 2 4 1 2 1 1 1 2 1 1 2 2 1   1 2 1&2 1 2 1   2   3 3 2 2   2 3 1 1 2 4   1 1 2 2&3 2 1 2 3 1 1 1 1 2 4
SA 2                     1 1 5 2 1 1 2 1 2 2   4 1 1 2 4 1 2 1 1 1 2 1 1 2 2 1   1 2 1&2 1 2 1 2&3 3 3 2 2   3 3 1 1 2 4   1 1 2   2 2 1 2 3 1 1 1 1 2 4
SA 3                     1 1 5 2 1 1 2 1 2 2   4 1 1 2 4 1 2 1 1 1 2 1 1 2 2 1   1 2 1&2 1 2 1   2   3 3 2 2   3 3 1 1 2 4   1 1 2   3 2 1 2 3 1 1 1 1 2 4
SA 3(2)                  1 1 5 2 1 1 2 1 2 2   4 1 1 2 4 1 2 1 1 1 2 1 1 2 2 1   1 ? 1&2 1 2 1   2   3 3 2 2   3 3 1 1 2 4   1 1 2   2 2 1 2 3 1 1 1 1 2 4
SA 2(2)                  1 1 5 2 1 1 2 1 2 2   4 1 1 2 4 1 2 1 1 1 2 1 1 2 2 1   1 2   1 1 2 1   2   3 3 2 2   2 3 1 1 2 4   1 1 2   3 2 1 2 3 1 1 1 1 2 4
TAS 1                    1 1 2 2 1 1 1 1 1 2   3 1 1 1 3 1 2 1 1 1 2 1 1 2 2 1   1 2   1 1 2 1   1   1 3 2 1   3 3 1 1 2 2   2 1 2   2 2 1 2 2 1 1 1 2 4
TAS 2                    1 1 4 2 1 1 1 1 1 2   3 1 1 2 3 1 2 1 1 1 2 1 1 2 2 1   1 2   1 1 2 1   2   1 3 2 1   3 3 1 1 2 2   2 1 2   2 2 1 2 2 1 1 1 2 4
TAS 3                    1 1 4 2 1 1 1 1 1 2   3 1 1 2 3 1 2 1 1 1 2 1 1 2 2 1   1 2   1 1 2 1   3   1 3 2 1   3 3 1 1 2 2   2 1 2   2 2 1 2 2 1 1 1 2 4
TAS 5                    1 1 4 2 1 1 1 1 1 2   3 1 1 2 3 2 2 1 1 1 2 1 1 2 2 1   1 2   1 1 2 1   3   1 3 2 1   3 3 1 1 2 2   2 1 2   2 2 1 2 2 1 1 1 2 4
VIC 1                    1 1 4 2 1 1 1 1 1 2   2 1 1 1 2 1 2 1 1 1 1 1 1 2 1 1   1 2   2 1 2 1   1   1 2 2 1   3 2 1 1 2 2   1 1 2   2 2 1 1 2 1 1 1 1 2 4
Kutikina hispida         2 2 1 1 2 2 2 2 3 1   1 2 1 1 1 - 1 1 1 2 3 1 1 3 3 2   2 1   2 1 1 2   3 1&2 2 2 1   1 1 1 1 1 3   2 2 2   1 1 2 1 1 1 1 2 - 2 3 1
Austropeplea viridis     2 1 5 2 1 1 1 1 2 2   3 1 1 1 1 - 2 1 1 2 3 1 1 1 1 1   1 1   2 1 2 1   1   1 1 2 2   4 3 2 1 2 3   1 1 1   2 1 1 1 1 1 1 1 1 2 4
Austropeplea lessoni NSW 1 1 4 3 1 1 2 1 2 2   3 1 1 1 2 1 2 1 1 3 3 2 2 - 1 1   1 1   2 2 2 1   2   3 2 2 2   3 2 2 1 2 3   2 1 2   4 1 1 1 2 1 1 1 1 2 3
Austropeplea lessoni NT  1 1 6 3 1 1 1 1 3 3   3 1 1 1 2 1 2 1 1 3 3 2 2 - 1 1   1 1 1&2 2 2 1   2   3 1 2 2   3 2 2 1 2 3   1 1 2   4 2 1 1 2 1 1 1 1 2 3
Austropeplea lessoni QLD 1 1 6 3 1 1 1 2 1 2   3 1 1 1 2 1 2 1 1 3 3 2 2 - 1 1   1 1   2 2 2 1   2   3 2 2 2   4 2 2 1 2 3   2 1 2   4 1 1 1 2 1 1 1 1 2 3
Austropeplea lessoni WA  1 1 6 3 1 1 1 1 3 3   3 1 1 1 2 1 2 1 1 3 3 2 2 - 1 1   1 1   1 2 2 3   2   3 1 2 2 3&4 2 2 1 2 3   1 1 2   4 2 1 1 2 1 1 1 1 2 3
Bullastra cumingiana     1 1 4 1 1 1 2 2 3 2   3 1 1 1 4 2 2 1 1 3 2 2 2 - 1 1   1 1   2 1 2 3   1   3 3 2 2   3 3 2 2 2 1   2 1 2   3 1 1 1 1 1 1 1 1 2 1
Radix peregra            1 1 4 3 1 1 2 2 3 2   3 1 1 1 1 - 2 2 2 3 1 2 2 - 1 1   1 ?   ? 1 2 3   ?   2 1 2 2   4 3 1 2 2 3   1 1 1   3 1 1 1 1 2 2 - 1 2 3
Radix auricularia        2 1 4 3 1 1 2 2 3 2   3 1 1 1 1 - 2 2 2 3 1 2 2 - 3 1   1 3   1 1 2 3   1   3 3 1 2   4 3 1 2 2 3   2 1 1   3 1 1 1 1 2 2 - 1 2 3
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Appendix 2.3 Alignment of 16S rRNA used for phylogenetic analysis of the *Austropeplea tomentosa* complex. Taxa without names are currently recognised as *A. tomentosa*.

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                     ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                           10         20         30         40         50         60         70         80         90        100
NSW  1               AGGAAA-TTT TGTTCGAACA GAACATTCTA TTATGACGGT TAGTCTCTTA GCTCCTAGTC CAACATCGAG GTCACAAACT AAATAATTCA TTATGCTGTT
NSW  2               AGGAAA-TTT TGTTCGAACA GAACATTCTA TTATGACGGT TAGTCTCTTA GCTCCTAGTC CAACATCGAG GTCACAAACT AAATAATTCA TTATGCTGTT
NSW  3               AGGAAA-TTT TGTTCGAACA GAACATTCTA TTATGACGGT TAGTCTTCTA GCTCCTAGTC CAACATCGAG GTCACAAACT AAATAATTCA TTATGCTGTT
NSW  4               AGGAAA-TTT TGTTCGAACA GAACATTCTA TTATGACGGT TAGTCTTCTA GCTCCTAGTC CAACATCGAG GTCACAAACT AAATAATTCA TTATGCTGTT
NSW  5               AGGAAA-TTT TGTTCGAACA GAACATTCTA TTATGACGGT TAGTCTTTTA GCTCCTAGTC CAACATCGAG GTCACAAACT AAATAATTCA TTATGCTGTT
NSW  6               AGGAAA-TTT TGTTCGAACA GAACATTCTA TTATGACGGT TAGTCTTTTA GCTCCTAGTC CAACATCGAG GTCATAAACT AAATAATTCA TTATGCTGTT
NSW  6(2)            AGGAAA-TTT TGTTCGAACA GAACATTCTA TTATGACGGT TAGTCTTTTA GCTCCTAGTC CAACATCGAG GTCATAAACT AAATAATTCA TTATGCTGTT
NSW  7               AGGAAA-TTT TGTTCGAACA GAACATTCTA TTATGACGGT TAGTCTTTTA GCTCCTAGTC CAACATCGAG GTCACAAACT AAATAATTCA TTATGCTGTT
NSW  8               AGGAAA-TTT TGTTCGAACA GAACATTCTA TTAAGACGGT TAGTCTTCTA GCTCCTAGTC CAACATCGAG GTCACAAACT AAATAATTCA TTATGCTGTT
NZn  1               AGGAAA-TTT TGTTCGAACA GAACATTCTA TTATGACTGT TAGTCTTTTA GTTCCTAGTC CAACATCGAG GTCACAAACT AAATTATTCA TTATGCTGTT
NZn  2               AGGAAA-TTT TGTTCCAACA GAACATTCTA TTATGACTGT TAGTCTTTTA GTTCCTAGTC CAACATCGAG GTCACAAACT AAATTATTCA TTATGCTGTT
NZs  1               AGGAAA-TTT TGTTCGAACA GAACATTCTA TTATGACTGT TAGTCTTTTA GTTCCTAGTC CAACATCGAG GTCACAAACT AAATTATTCA TTATGCTGTT
NZs  2               AGGAAA-TTT TGTTCGAACA GAACATTCTA TTATGACTGT TAGTCTTTTA GTTCCTAGTC CAACATCGAG GTCACAAACT AAATTATTCA TTATGCTGTT
NZs  3               AGGAAA-TTT TGTTCGAACA GAACATTCTA TTATGACTGT TAGTCTTTTA GTTCCTAGTC CAACATCGAG GTCACAAACT AAATTATTCA TTATGCTGTT
NZs  4               AGGAAA-TTT TGTTCGAACA GAACATTCTA TTATGACTGT TAGTCTTTTA GTTCCTAGTC CAACATCGAG GTCACAAACT AAATTATTCA TTATGCTGTT
SA  1                AGGAAA-TTT TGTTCGAACA GAACATTCTA TTATGACGGT TAGTCTTTTA GCTCCTAGTC CAACATCGAG GTCACAAACT AAATAATTCA TTATGCTGTT
SA  2                AGGAAA-TTT TGTTCGAACA GAACATTCTA TTATGACGGT TAGTCTTTTA GCTCCTAGTC CAACATCGAG GTCACAAACT AAATAATTCA TTATGCTGTT
SA  3                AGGAAA-TTT TGTTCGAACA GAACATTCTA TTATGACGGT TAGTCTTTTA GCTCCTAGTC CAACATCGAG GTCACAAACT AAATAATTCA TTATGCTGTT
TAS  1               AGGAAA-TTT TGTTCGAACA GAACATTCTA TTATGACGGT TAGTCTTTTA GCTCCTAGTC CAACATCGAG GTCACAAACT AAATAATTCA TTATGCTGTT
TAS  2               AGGAAA-TTT TGTTCGAACA GAACATTCTA TTATGACGGT TAGTCTTTTA GCTCCTAGTC CAACATCGAG GTCACAAACT AAATAATTCA TTATGCTGTT
TAS  3               AGGAAA-TTT TGTTCGAACA GAACATTCTA TTATGACGGT TAGTCTTTTA GCTCCTAGTC CAACATCGAG GTCACAAACT AAATAATTCA TTATGCTGTT
TAS  4               AGGAAA-TTT TGTTCGAACA GAACATTCTA TTATGACGGT TAGTCTTTTA GCTCCTAGTC CAACATCGAG GTCACAAACT AAATAATTCA TTATGCTGTT
TAS  5               AGGAAA-TTT TGTTCGAACA GAACATTCTA TTATGACGGT TAGTCTTTTA GCTCCTAGTC CAACATCGAG GTCACAAACT AAATAATTCA TTATGCTGTT
VIC  1               AGGAAA-TTT TGTTCGAACA GAACATTCTA TTATGACGGT TAGTCTTTTA GCTCCTAGTC CAACATCGAG GTCACAAACT AAATAATTCA TTATGCTGTT
VIC 1(2)             AGGAAA-TTT TGTTCGAACA GAACATTCTA TTATGACGGT TAGTCTTTTA GCTCCTAGTC CAACATCGAG GTCACAAACT AAATAATTCA TTATGCTGTT
Kutikina hispida     AGGAAA-TTT TGTTCGAACA GAACATTCTA TTATGACGGT TAGTCTTTTA GCTCCTAGTC CAACATCGAG GTCATAAACT AAATAATTCA TTATGCTGTT
Kutikina hispida(2)  AGGAAA-TTT TGTTCGAACA GAACATTCTA TTATGACGGT TAGTCTTTTA GCTCCTAGTC CAACATCGAG GTCATAAACT AAATAATTCA TTATGCTGTT
Austropeplea lessoni  NSW   AGGAAATTTT TGTTCGAACA GAACATTCTA TTTTGACGGT TAGTCTTCTA GTTCCTAGTC CAACATCGAG GTCACAAGCT AAAAATTTTA TTATGCTGTT
Austropeplea lessoni  NT    AGGAAATTTT TGTTCGAACA GAACATTCTA TTTTGACTGT TAGTCTTCTA GTTCCTAGTC CAACATCGAG GTCACAAGCT AAAAATTTTA TTATGCTGTT
Austropeplea lessoni  QLD   AGGAAGTTTT TGTTCGAACA GAACATTCTA TTTTGACTGT TAGTCTTCTA GTTCCTAGTC CAACATCGAG GTCACAAGCT AAAAATTTTA TTATGCTGTT
Austropeplea lessoni  WA    AGGAAATTTT TGTTGCAACA GAACATTCTA TTTTGACTGT TAGTCTTCTA GTTCCTAGTC CAACATCGAG GTCACAAGCT AAAAATTTTA TTATGCTGTT
Austropeplea viridis  AGGAAACTGT TGTTCGAACA GAACAATCTA TTTTGACGGT TAATCTTTTA GTTCCTAGTC CAACATCGAG GTCATAAGCT ATATTAATCA TTATGCTGTT
Bullastra cumingiana  AGGAAAAATC TGTTCGAACA GAACATTCTA TTTTGACTGT TAGTCTTCTA GTTCCTAGTC CAACATCGAG GTCACAAGCT AAAAATTTTA TTATGCTGTT
Radix auricularia     AGGAGA-AAT TGTTCGAACA GAACACTCTA TTTTGACTGT TAGTCCTTTA GTTCCTAGTC CAACATCGAG GTCATAAGCT AATAAATTCA TTATGCTGTT
Radix peregra         ??????????? ????????CA GAACACTCTA TTTTGACGGT TAGTCTTTTA GTTCCTAGTC CAACATCGAG GTCACAAGCT AATAATTTCA TTATGCTGTT
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                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                           110        120        130        140        150        160        170        180        190        200

NSW   1                    ATCCCTAAGG TAATTTAATC TTAATAGAAA AA-TGA-CTT GTTTTTA-TT GAAAGTTTAA AT----GTTT CAATGTCGCC CCAACAAAAA TATATAATTT
NSW   2                    ATCCCTAAGG TAATTTAATC TTAATAGAAA AA-TGA-CTT GTTTTTA-TT GAAAGTTTAA AT----GTTT CAATGTCGCC CCAACAAAAA TATATAATTT
NSW   3                    ATCCCTAAGG TAATTTAATC TTAATAGGAA AA-TGA-CTT GTTTTTA-TT GAAAGTTTAA AT----GTTT CAATGTCGCC CCAACAAAAA TATATAATTT
NSW   4                    ATCCCTAAGG TAATTTAATC TTAATAGAAA AA-TGA-CTT GTTTTTA-TT GAAAGTTTAA AT----GTTT CAATGTCGCC CCAACAAAAA TATATAATTT
NSW   5                    ATCCCTAAGG TAATTTAATC TTAATAGAAA AA-TGA-CTT GTTTTTA-TT GAAAGTTTAA AT----GTTT CAATGTCGCC CCAACAAAAA TATATAATTT
NSW   6                    ATCCCTAAGG TAATTTAATC TTAATAGAAA AA-TGA-CTT GTTTTTA-TT GAAAGTTTAA AT----GTTT CAATGTCGCC CCAACAAAAA TATATAATTT
NSW   6(2)                 ATCCCTAAGG TAATTTAATC TTAATAGAAA AA-TGA-CTT GTTTTTA-TT GAAAGTTTAA AT----GTTT CAATGTCGCC CCAACAAAAA TATATAATTT
NSW   7                    ATCCCTAAGG TAATTTAATC TTAATAGAAA AA-TGA-CTT GTTTTTAATT GAAAGTTTAA AT----GTTT CAATGTCGCC CCAACAAAAA TATATAATTT
NSW   8                    ATCCCTAAGG TAATTTAATC TTA-TAGAAA AA-TGA-CTT GTTTTTA-TT GAAAGTTTAA AT----GTTT CAATGTCGCC CCAACAAAAA TATATAATTT
NZn   1                    ATCCCTAAGG TAATTTAATC TTTAAAAAAA A--TGA-CTT GTTTTAATT GAAAGTT-AA TT----GTTT CATTGTCGCC CCAACAAAAA TATATAATTT
NZn   2                    ATCCCTAAGG TAATTTAATC TTTAAAAAAA A--TGA-CTT GTTTTTAATT GAAAGTT-AA CT----GTTT CATTGTCGCC CCAACAAAAA TATATAATTT
NZs   1                    ATCCCTAAGG TAATTTAATC TTTTAAAAAA A--TGA-TTT GTTTTTAATT GAAAGTT-GA TT----GTTT CATTGTCGCC CCAACAAAAA TATATAATTT
NZs   2                    ATCCCTAAGG TAATTTAATC TTTTAAAAAA AA-TGA-TTT GTTTTTAATT GAAAGTT-GA TT----GTTT CATTGTCGCC CCAACAAAAA TATGTAATTT
NZs   3                    ATCCCTAAGG TAATTTAATC TTTTAAAAAA AA-TGA-TTT GTTTTTAATT GAAAGTT-GA TT----GTTT CATTGTCGCC CCAACAAAAA TATGTAATTT
NZs   4                    ATCCCTAAGG TAATTTAATC TTTTAAAAAA AA-TGA-TTT GTTTTTAATT GAAAGTT-GA TT----GTTT CATTGTCGCC CCAACAAAAA TATATAATTT
SA  1                      ATCCCTAAGG TAATTTAATC TTAATAGAAA AA-TGA-CTT GTTTTTA-TT GAAAGTTTAA AT----GTTT CAATGTCGCC CCAACAAAAA TATATAATTT
SA  2                      ATCCCTAAGG TAATTTAATC TTAATAGAAA AA-TGA-CTT GTTTTTA-TT GAAAGTTTAA AT----GTTT CAATGTCGCC CCAACAAAAA TATATAATTT
SA  3                      ATCCCTAAGG TAATTTAATC TTAATAGAAA AA-TGA-CTT GTTTTTA-TT GAAAGTTTAA AT----GTTT CAATGTCGCC CCAACAAAAA TATATAATTT
TAS   1                    ATCCCTAAGG TAATTTAATC TTAATAGAAA A--TGA-CTT GTTTTTA-TT GAAAGTTTAA AT----GTTT CAATGTCGCC CCAACAAAAA TATATAATTT
TAS   2                    ATCCCTAAGG TAATTTAATC TTAATAGAAA A--TGA-CTT GTTTTTA-TT GAAAGTTTAA AT----GTTT CAATGTCGCC CCAACAAAAA TATATAATTT
TAS   3                    ATCCCTAAGG TAATTTAATC TTAATAGAAA A--TGA-CTT GTTTTTAATT GAAAGTTTAA AT----GTTT CAATGTCGCC CCAACAAAAA TATATAATTT
TAS   4                    ATCCCTAAGG TAATTTAATC TTAATAGAAA A--TGA-CTT GTTTTTA-TT GAAAGTTTAA AT----GTTT CAATGTCGCC CCAACAAAAA TATATAATTT
TAS   5                    ATCCCTAAGG TAATTTAATC TTAATAGAAA A--TGA-CTT GTTTTTA-TT GAAAGTTTAA AT----GTTT CAATGTCGCC CCAACAAAAA TATATAATTT
VIC   1                    ATCCCTAAGG TAATTTAATC TTAATAGAAA AA-TGA-CTT GTTTTTA-CT GAAAGTTTAA AT----GTTT CAATGTCGCC CCAACAAAAA TATATAATTT
VIC 1(2)                   ATCCCTAAGG TAATTTAATC TTAATAGAAA AA-TGA-CTT GTTTTTA-CT GAAAGTTTAA AT----GTTT CAATGTCGCC CCAACAAAAA TATATAATTT
Kutikina hispida          ATCCCTAAGG TAATTTAAAC TTAATAAAAA A--TGA-CTT GTTTTAA-TT GAAAGTTTAA AT----GTTT CATTGTCGCC CCAACAAAAA TATATAATTT
Kutikina hispida(2)       ATCCCTAAGG TAATTTAAAC TTAATAAAAA A--TGA-CTT GTTTTAA-TT GAAAGTTTAA AT----GTTT CATTGTCGCC CCAACAAAAA TATATAATTT
Austropeplea lessoni  NSW ATCCCTAAGG TAATTTAATC TAACAAAAAA A--TGA-CTT GTAATTA-TT AAAAGTTTAA AAT---GTTT TATTGTCGCC CCAACAAAAA TAAAAAGATT
Austropeplea lessoni  NT  ATCCCTAAGG TAATTTAATC TAACAAAAAA AA-TGA-CTT GTAATTA-TT AAAAGTTTAA AAT---GTTT TAATGTCGCC CCAACAAAAA TAAAAAAATT
Austropeplea lessoni  QLD ATCCCTAAGG TAATTTAATC TAACAAAAAA AA-TGA-CTT GTAATTA-TT AAAAGTTTAA AAT---GTTT TATTGTCGCC CCAACAAAAA TAAAAAAATT
Austropeplea lessoni  WA  ATCCCTAAGG TAATTTAATC TAACAAAAAA AA-TGA-CTT GTAATTA-TT AAAAGTTTAA AAT---GTTT TAATGTCGCC CCAACAAAAA TAAAAAAATT
Austropeplea viridis      ATCCCTAAGG TAATTTTACC ATACAAAAAA AA-TGA-CAT GTTTTTATAT GAAAGTTTAA AT----GTTT CATTGTCGCC CCAACAAAAA TAAGAGATTT
Bullastra cumingiana      ATCCCTAAGG TAATTTATTC TTACAAATAA A--TGA-CTT GTTTAAT-TT GAAAGTTTTA AT----GTTT CAATGTCGCC CCAACAAAAA TAAATAAATT
Radix auricularia         ATCCCTAAGG TAATTTGATC GTGCAAAGAA AATTGTTGTG TAAAAATCTT GAAAGTTTAA TAT---GTTT CAATGTCGCC CCAACAAAAA TAAATCTTAA
Radix peregra             ATCCCTAAGG TAATTTGATC ATTCAAAATA AA-TGTCTCG TAAAAATTGT GAAAGTTCAA AATTTTGTTT CAATGTCGCC CCAACAAAAA TAAGTTTACA
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                              ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  210        220        230        240        250        260        270        280        290        300

NSW  1                        ATG--AAATT AATTAATAAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTT AAAAAAAGTA TTTTCACTTT TTAAATAAAT TCAATAAAAT TTTTAA-GAG
NSW  2                        ATG--AAATT AATTAATAAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTT CGGAAAAGTA TTTTCACTTT TTAAATAAAT TCGATAAAAT TTTTAA-GAG
NSW  3                        ATG--AAATT AATTAATAAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTT CGAAAAAGTA TTTTCACTTT TTAAATAAAT TCAATAAAAT TTTTAA-GAG
NSW  4                        ATG--AAATT AATTAATAAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTT CGAAAAAGTA TTTTCACTTT TTAAATAAAT TCAACAAAAT TTTTAA-GAG
NSW  5                        ATG--AAATT AATTAATAAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTT CGAAAAAGTA TTTTCACTTT TTAAATAAAT TCAATAAAAT TTTTAA-GAG
NSW  6                        ATG--AAATT AATTAATAAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTT CGAAAAAGTA TTTTCACTTT TTAAATAAAT TCAATAAAAT TTTTAA-GAG
NSW  6(2)                     ATG--AAATT AATTAATAAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTT CGAAAAAGTA TTTTCACTTT TTAAATAAAT TCAATAAAAT TTTTAA-GAG
NSW  7                        ATG--AAATT AATTAATAAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTT CGAAAAAGTA TTTTCACTTT TTAAATAAAT TCAATAAAAT TTTTAA-GAG
NSW  8                        ATG--AAATT AATTAATAAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTT CGAAAAAGTA TTTTCACTTT TTAAATAAAT TCAATAAAAT TTTTAA-GAG
NZn  1                        AAAT-AAATT AATTAATTAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTA TAAAAAAGTA TTTTCACTTT TTAAATAAGT TCATTAAAAT TTTAAA-GAG
NZn  2                        AAAT-AAATT AATTAATTAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTA TAAAAAAGTA TTTTCACTTT TTAAATAAGT TCATTAAAAT TTTAAA-GAG
NZs  1                        AAAT-AAATT AATTAATTAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTA TAAAAAAGTA TTTTCACTTT TTAAATAAAT TCATTAAAAT TTTAAA-GAG
NZs  2                        AAAT-AAATT AATTAATTAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTA TAAAAAGGTA TTTTCACTTT TTAAATAAAT TCATTAAAAT TTTAAA-GAG
NZs  3                        AAAT-AAATT AATTAATTAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTA TAAAAAGGTA TTTTCACTTT TTAAATAAAT TCATTAAAAT TTTAAA-GAG
NZs  4                        AAAT-AAATT AATTAATTAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTA TAAAAAAGTA TTTTCACTTT TTAAATAAAT TCATTAAAAT TTTAAA-GAG
SA  1                         ATG--AAATT AATTAATAAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTT CGGAAAAGTA TTTTCACTTT TTAAATAAAT TCAATAAAAT TTTTAA-GAG
SA  2                         ATG--AAATT AATTAATAAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTT CGAAAAAGTA TTTTCACTTT TTAAATAAAT TCAATAAAAT TTTTAA-GAG
SA  3                         ATG--AAATT AATTAATAAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTT CGAAAAAGTA TTTTCACTTT TTAAATAAAT TCAATAAAAT TTTTAA-GAG
TAS  1                        ATG--AAATT AATTAATAAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTT CGAAAAAGTA TTTTCACTTT TTAAATAAAT TCAATAAAAT TTTTAA-GAG
TAS  2                        ATG--AAATT AATTAATAAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTT CGAAAAAGTA TTTTCACTTT TTAAATAAAT TCAATAAAAT TTTTGA-GAG
TAS  3                        ATG--AAATT AATTAATAAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTT CGAAAAAGTA TTTTCACTTT TTAAATAAAT TCAATAAAAT TTTTAA-GAG
TAS  4                        ATG--AAATT AATTAATAAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTT CGAAAAAGTA TTTTCACTTT TTAAATAAAT TCAATAAAAT TTTTAA-GAG
TAS  5                        ATG--AAATT AATTAATAAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTT CGAAAAAGTA TTTTCACTTT TTAAATAAAT TCAATAAAAT TTTTAA-GAG
VIC  1                        ATG--AAATT AATTAATAAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTT TTTAAAAGTA TTTTCACTTT TTAAATAAAT TCAATAAAAT TTTTAA-GAG
VIC  1(2)                     ATG--AAATT AATTAATAAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTT TTTAAAAGTA TTTTCACTTT TTAAATAAAT TCAATAAAAT TTTTAA-GAG
Kutikina hispida              ATG--AAATT AATTAATAAA AATTCTAAGG GTCTTCTCGT CTTTTTTGTT CAAAAAAGTA TTTTCACTTT TTAAATAAAT TCAATAATAT TTTTAA-GAG
Kutikina hispida  (2)         ATG--AAATT AATTAATAAA AATTCTAAGG GTCTTCTCGT CTTTTTTGTT CAAAAAAGTA TTTTCACTTT TTAAATAAAT TCAATAATAT TTTTAA-GAG
Austropeplea lessoni  NSW     ATAAA-CCTT AATTAATGAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTT AGTAGAAGTA TTTTCACTTC ATAAATAAAT TCAAATAAAT TTAAAA-GAG
Austropeplea lessoni  NT      ATAAA-CCTT AATTAATGAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTT AGTAGAAGTA TTTTCACTTC ATAAATAAAT TCAAATAAAT TTAAAA-GAG
Austropeplea lessoni  QLD     ATAAA-CCTT AATTAATGAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTT AGTAGAAGTA TTTTCACTTC ATAAATAAAT TCAAATAAAT TTAAAA-GAG
Austropeplea lessoni  WA      ATAAA-CTTT AATTAATGAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTT AGCAGAAGTA TTTTCACTTC ATAAATAAAT TCAAATAAAT TTAAAA-GAG
Austropeplea viridis          AAAAAACCTT --TTAATAAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTA ATAAAAAGTA TTTTCACTTT ATAAATAAAT TCATATAAGC TAAAAA-GAG
Bullastra cumingiana          ATAA-AATTT AATTAATTAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTA AATAAAAGTA TTTTCACTTT TTAAATAAAT TCAAAAAAAT TTTAAA-GAG
Radix auricularia             ATAATAAAAT AA--AATTAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTA GATAAAAGTA TTTTCACTTT ATAAATAAAT TCACAAAAAT TAAAAA-GAG
Radix peregra                 ATAAAGTAAA A-GAAATTAA AATTCTAAGG GTCTTCTCGT CTTTTTTCTT TAAAAAAGTA TTTTCACTTA ATAAATAAAT TCATAGAAAT TAAAAAAGAG
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                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         310        320        330        340        350        360        370        380        390        400

NSW   1             ACAGTTAATT CTTTATAAAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAATA--T
NSW   2             ACAGTTAATT CTTTATAAAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAATA--T
NSW   3             ACAGTTAATT CTTTATAAAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAATA--T
NSW   4             ACAGTTAATT CTTTATAAAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAATA--T
NSW   5             ACAGTTAATT CTTTATAAAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAATA--T
NSW   6             ACAGTTAATT CTTTATAAAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAATA--T
NSW   6(2)          ACAGTTAATT CTTTATAAAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAATA--T
NSW   7             ACAGTTAATT CTTTATAAAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAATA--T
NSW   8             ACAGTTAATT CTTTATAATA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAATA--T
NZn   1             ACAGTTAATT CTTTATAAAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAATA--T
NZn   2             ACAGTTAATT CTTTATAAAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAATA--T
NZs   1             ACAGTTAATT CTTTATAAAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAATA--T
NZs   2             ACAGTTAATT CTTTATAAAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAGTA--T
NZs   3             ACAGTTAATT CTTTATAAAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAGTA--T
NZs   4             ACAGTTAATT CTTTATAAAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAATA--T
SA  1               ACAGTTAATT CTTTATAAAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAATA--T
SA  2               ACAGTTAATT CTTTATAAAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAATA--T
SA  3               ACAATTAATT CTTTATAAAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAATA--T
TAS  1              ACAGTTAATT CTTTATAAAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAATA--T
TAS  2              ACAGTTAATT CTTTATAAAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAATA--T
TAS  3              ACAGTTAATT CTTTATAAAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAATA--T
TAS  4              ACAGTTAATT CTTTATAAAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAATA--T
TAS  5              ACAGTTAATT CTTTATAAAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAATA--T
VIC  1              ACAGTTAATT CTTTATAAAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAATA--T
VIC 1(2)            ACAGTTAATT CTTTATAAAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAATA--T
Kutikina hispida    ACAGTTAATT CTTTATAAAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAATA--T
Kutikina hispida(2) ACAGTTAATT CTTTATAAAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAATA--T
Austropeplea lessoni  NSW  ACAGCTAATT CTTTATTTAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAATA--A
Austropeplea lessoni  NT   ACAGCTAATT CTTTATTTAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAAG TACTGCGGCC GCTAATA--A
Austropeplea lessoni  QLD  ACAGCTAATT CTTTATTTAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAATA--A
Austropeplea lessoni  WA   ACAGCTAATT CTTTATTTAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAATA--A
Austropeplea viridis ACAGAAAATT CTTTAACAAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAATA-CA
Bullastra cumingiana ACAGCTTATT CTTTATTAAA CCTTTCATTC CAGACTCCAA TTAAAAGCCA ACTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTGATGTGT
Radix auricularia    ACAGTAAATT CTTTATTTAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAATTTTT
Radix peregra        ACAGTTAATT TTTTATTTAA CCTTTCATTC CAGACTTCAA TTAAAAGCCA ATTGATTATG CTACCTTAGC ACAGTCAAGG TACTGCGGCC GCTAATAATT
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                          ....|....| ....|....| ....|....| ....|....| ....|.
                             410        420        430        440
NSW   1                   AAAC----GC TGGGCAGAAA TTACTTAAAA TATATT-CTA TAAGCT
NSW   2                   AAAC----GC TGGGCAGAAA TTACTTAAAA TATATT-CTA TAAGCT
NSW   3                   AAAC----GC TGGGCAGAAG TTACTTAAAA TATATT-CTA TAAGCT
NSW   4                   AAAC----GC TGGGCAGAAG TTACTTAAAA TATATT-CTA TAAGCT
NSW   5                   AAAC----GC TGGGCAGAAG TTACTTAAAA TATATT-CTA TAAGCT
NSW   6                   AAAC----GC TGGGCAGAAG TTACTTAAAA TATATT-CTA TAAGCT
NSW   6(2)                AAAC----GC TGGGCAGAAG TTACTTAAAA TATATT-CTA TAAGCT
NSW   7                   AAAC----GC TGGGCAGAAG TTACTTAAAA TATATT-CTA TAAGCT
NSW   8                   AAAC----GC TGGGCAGAAG TTACTTAAAA TATGTT-CTA TAAGCT
NZn   1                   AAAC----GC TGGGCAGAAG TAACTTAAAA TATATT-CTT AAAGCT
NZn   2                   AAAC----GC TGGGCAGAAG TAACTTAAAA TATATT-CTT AAAGCT
NZs   1                   AAAC----GC TGGGCAGAAG TAACTTAAAA TATGTT-CTT AAAGCT
NZs   2                   AAAC----GC TGGGCAGAAG TAACTTAAAA TATGTT-CTT AAAGCT
NZs   3                   AAAC----GC TGGGCAGAAG TAACTTAAAA TATGTT-CTT AAAGCT
NZs   4                   AAAC----GC TGGGCAGAAG TAACTTAAAA TATGTT-CTT AAAGCT
SA   1                    AAAC----GC TGGGCAGAAG TTACTTAAAA TATATT-CTA TAAGCT
SA   2                    AAAC----GC TGGGCAGAAG TTACTTAAAA TATATT-CTA TAAGCT
SA   3                    AAAC----GC TGGGCAGAAG TTACTTAAAA TATATT-CTA TAAGCT
TAS   1                   AAAC----GC TGGGCAGAAG TTACTTAAAA TATATT-CTA TAAGCT
TAS   2                   AAAC----GC TGGGCAGAAG TTACTTAAAA TATATT-CTA TAAGCT
TAS   3                   AAAC----GC TGGGCAGAAG TTACTTAAAA TATATT-CTA TAAGCT
TAS   4                   AAAC----GC TGGGCAGAAG TTACTTAAAA TATATT-CTA TAAGCT
TAS   5                   AAAC----GC TGGGCAGAAG TTACTTAAAA TATATT-CTA TAAGCT
VIC   1                   AAAC----GC TGGGCAGAAG TTACTTAAAA TATATC-CTT TAAGCT
VIC 1(2)                  AAAC----GC TGGGCAGAAG TTACTTAAAA TATATC-CTT TAAGCT
Kutikina hispida          AAAC----GC TGGGCAGAAG TTACTTACAA TATATT-CTA TAAGCT
Kutikina hispida(2)       AAAC----GC TGGGCAGAAG TTACTTACAA TATATT-CTA TAAGCT
Austropeplea lessoni  NSW T-AC----GC TGGGCAGAAT TCACCTAAAA TAAGTT-CTT CAGACT
Austropeplea lessoni  NT  T-AC----GC TGGGCAGAAT TCACCTAAAA TAAGTT-CTT CAGGCT
Austropeplea lessoni  QLD T-AC----GC TGGGCAGAAT TCACCTAAAA TAAGTT-CTT CAGGCT
Austropeplea lessoni  WA  T-AC----GC TGGGCAGAAT TCACCTAAAA TAAGTT-CTT CAGGCT
Austropeplea viridis      TAAC----GC TGGGCAGAAT TCACTTAAAA TGTATC-CTT CAAGCT
Bullastra cumingiana      TTAC----GC TGGGCAGAAT TCACCTAAAA TATGTC-CTC TAGGCT
Radix auricularia         TAACCAACGC TGGGCAGAAT TCACTTAAAA TATAAT-CTT TAAGCT
Radix peregra             TTAC----GC TGGGCAGAAC TTACTTAAAA TAAATTTCTT TAAGCT
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Appendix 2.4 Alignment of ITS-2 rRNA used for phylogenetic analysis of the *Austropeplea tomentosa* complex. Taxa without names are currently recognised as *A. tomentosa*. All characters with an astertix above them where excluded from all phylogenetic analyses

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                                         ** *    ***                          **** ********      ***** *          *
                              ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                  10         20         30         40         50         60         70         80         90        100

NSW  1                        GCTAGTGTCA AAACAATCGC GTCGCTC--- -GCTCG---T GCGACGCGCT CTGGACCTTC GCGGCCA-TT AAATCCGGCG CTCACGGCA- TCATCGCTC-
NSW  2                        GCTAGTGTCA AAACAATCGC GTCGCTC--- -GCTCG---T GCGACGCGCT CTGGACCTTC GCGGCCA-TT AAATCCGGCG CTCACGGCA- TCATCGCTC-
NSW  3                        GCTAGTGTCA AAACAATCGC GTCGCTC--- -GCTCG---T GCGACGCGCT CTGGACCTTC GCGGCCA-TT AAATCCGGCG CTCACGGCA- TCATCGCTC-
NSW  4                        GCTAGTGTCA AAACAATCGC GTCGCTC--- -GCTCG---T GCGACGCGCT CTGGACCTTC GCGGCCA-TT AAATCCGGCG CTCACGGCA- TCATCGCTC-
NSW  5                        GCTAGTGTCA AAACAATCGC GTCGCTC--- -GCTCG---T GCGACGCGCT CTGGACCTTC GCGGCCA-TT AAATCCGGCG CTCACGGCA- TCATCGCTC-
NSW  6                        GCTAGTGTCA AAACAATCGC GTCGCTC--- -GCTCG---T GCGACGCGCT CTGGACCTTC GCGGCCA-TT AAATCCGGCG CTCACGGCA- TCATCGCTC-
NSW  6(2)                     GCTAGTGTCA AAACAATCGC GTCGCTC--- -GCTCG---T GCGACGCGCT CTGGACCTTC GCGGCCA-TT AAATCCGGCG CTCACGGCA- TCATCGCTC-
NSW  7                        GCTAGTGTCA AAACAATCGC GTCTCTC--- -GCTCG---T GAGACGCGCT CTGGACCTTC GCGGCCA-TT AAATCCGGCG CTCCCGGCA- TCATCGCTC-
NSW  8                        GCTAGTGTCA AAACAATCGT GTCGCTC--- -GCTCG---T GCGACGCGCT CTGGACCTTC GCGGCCA-TT AAATCCGGCG CTCACGGCA- TCATCGCTC-
NZn  1                        GCTAGTGTCA AAACAATCGC GTCGCTC--- -GCTCG---T GCGACGCGCT CTGGACCTTC GCGGCCA-TT AAATCCGGCG CTCAC----- -CACCGCTC-
NZn  2                        GCTAGTGTCA AAACAATCGC GTCGCTC--- -GCTCG---T GCGACGCGCT CTGGACCTTC GCGGCCA-TT AAATCCGGCG CTCAC----- -CACCGCTC-
NZs  1                        GCTAGTGTCA AAACAATCGC GTCGCTC--- -GCTCG---T GCGACGCGCT CTGGACCTTC GCGGCCA-TT AAATCCGGCG CTCAC----- -CACCGCTC-
NZs  2                        GCTAGTGTCA AAACAATCGC GTCGCTC--- -GCTCG---T GCGACGCGCT CTGGACCTTC GCGGCCA-TT AAATCCGGCG CTCAC----- -CACCGCTC-
NZs  3                        GCTAGTGTCA AAACAATCGC GTCGCTC--- -GCTCG---T GCGACGCGCT CTGGACCTTC GCGGCCA-TT AAATCCGGCG CTCAC----- -CACCGCTC-
NZs  4                        GCTAGTGTCA AAACAATCGC GTCGCTC--- -GCTCG---T GCGACGCGCT CTGGACCTTC GCGGCCA-TT AAATCCGGCG CTCAC----- -CACCACTC-
SA  1                         GCTAGTGTCA AAACAATCGC GTCGCTC--- -GCTCG---T GCGACGCGCT CTGGACCTTC GCGGCCA-TT AAATCCGGCG CTCACAGCA- TCATCGCTC-
SA  2                         GCTAGTGTCA AAACAATCGC GTCGCTC--- -GCTCG---T GCGACGTGCT CTGGACCTTC GCAGCCA-TA AAATCCGGCT CTCACTGCA- TCATCGCTC-
SA  3                         GCTAGTGTCA AAACAATCGC GTCGCTC--- -GCTCG---T GCGACGCGCT CTGGACCTTC GCGGCCA-TT AAATCCGGCG CTCACTGCA- TCATCGCTC-
TAS  1                        GCTAGTGTCA AAACAATCGC GTCGCTC--- -GCTCG---T GCGACGCGCT CTGGACCTTC GCGGCCA-TT AAATCCGGCG CTCACAGCA- TCATCGCTC-
TAS  2                        GCTAGTGTCA AAACAATCGC GTCGCTC--- -GCTCG---T GCGACGCGCT CTGGACCTTC GCGGCCA-TT AAATCCGGCG CTCACAGCA- TCATCGCTC-
TAS  3                        GCTAGTGTCA AAACAATCGC GTCGTTC--- -GCTCG---T GCGACGCGCA CTGGACCTTC GCGGCCA-TT AAATCCGGCG CTCACAGCA- TCATCGCTC-
TAS  4                        GCTAGTGTCA AAACAATCGC GTCGCTC--- -GCTCG---T TCGACGCGCT CTGGACCTTC GCGGCCA-TT AAATCCGGCG CTCACAGCA- TCATCGCTC-
TAS  5                        GCTAGTGTCA AAACAATCGC GTCGCTC--- -GCTCG---T GCGACGCGCT CTGGACCTTC GCGGCCA-TT AAATCCGGCG CTCACAGCA- TCATCGCTC-
VIC  1                        GCTAGTGTCA AAACAATCGC GTCGCTC--- -GCTCG---T GCGACGCGCT CTGGACCTTC GCGGCCA-TT AAATCCGGCG CTCACGGCA- TCATCGCTC-
VIC  2                        GCTAGTGTCA AAACAATCGC GTCGCTC--- -GCTCG---T GCGACGCGCT CTGGACCTTC GCGGCCA-TT AAATCCGGCG CTCACGGCA- TCATCGCTC-
Kutikina  hispida            GCTAGTGTCA AAACAATCGC GTCGATC--- -GCTCG---T ACGACGCGCT CTGGACCTTC GCGGCCA-TT AAATCCGGCG CTCACAGCA- TCATCGCTC-
Kutikina hispida 2)          GCTAGTGTCA AAACAATCGC GTCGATC--- -GCTCG---T ACGACGCGCT CTGGACCTTC GCGGCCA-TT AAATCCGGCG CTCACAGCA- TCATCGCTC-
Austropeplea  lessoni  NSW   GCTAGTGTTA AAACAATCGG GTCGCTT--- -GCTCTCGTA GCGACGCGCA CTGGACCATC GCGGCC---- ---------G CTCACCGAA- TCTT-CCTTC
Austropeplea lessoni  NT     GCTAGTGTTA AAACAATCGT GTCGCTT--- -GCTCTCGTT GCGACGCGCC CTGGACCATC GCGGCC---- ---------G CTCACCGAA- ACTTTCCTTC
Austropeplea lessoni  QLD    GCTAGTGTTA AAACAATCGC GTCGCTT--- -GCTCTCGTA GCGACGCGCA CTGGACCATC GCGGCC---- ---------G CTCACCGAA- TCTT-CCTTC
Austropeplea lessoni  WA     ??????GTTA AAACAATCGT GTCGCTT--- -GCTCTCGTT GCGACGCGCC CTGGACCATC GCGGCC---- ---------G CTCACCGAA- ACTTTCCTTC
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Austropeplea viridis      GCTAGTGTCA AAACAATCGC GTCGCTT--- -GCTCG---C GCGACGCGCC CTGGACCTTC GCGGCCCGTT AAATCCGGCG CTCACCGAA- TCCCGCTTT-
Bullastra cumingiana      ????GTGTTA AAACAATCGC CGTCGCCCGT TGCTCTCGTG GCGACGCGCC CTGGACCGTC GCGGTCGC-A AAATCCGGCG GCGGCTCTGA CCGTAGCATC
Radix auricularia         GCTAGTGTCA AA-CAATCGT GTCGCTTT-- -GCTCG---T GCGACGCGCT CTGGTCCGTC GCGGCCA-TA AAATCCAGCG TTCACCGCCC TCATCGCTTT
Radix peregra             GCTAGTGTCA AA-CAATCGC GTCGCTT--- -GCTCT---T GCGACGCGCT CTGGACCTTC GCGGCC-GTA AAATCCGGCG CTCACCGAA- TCGCTC----


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                          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                               110        120        130        140        150        160        170        180        190        200
NSW  1                    GCTCGGCGGT GTTGCACG-- GTGTT--GCC CGGT------ ---------- ---------- ---------- ---------- GGCCCCGTGG TCTCAAGCAC
NSW  2                    GCTCGGCGGT GTTGCACG-- GTGTT--GCC CGGT------ ---------- ---------- ---------- ---------- GGCCCCGTGG TCTCAAGCAC
NSW  3                    GCTCGGCGGT GTTGCACG-- GTGTT--GCC CGGT------ ---------- ---------- ---------- ---------- GGCCCCGTGG TCTCAAGCAC
NSW  4                    GCTCGGCGGT GTTGCACG-- GTGTT--GCC CGGT------ ---------- ---------- ---------- ---------- GGCCCCGTGG TCTCAAGCAC
NSW  5                    GCTCGGCGGT GTTGCACG-- GTGTT--GCC CGGT------ ---------- ---------- ---------- ---------- GGCCCCGTGG TCTCAAGCAC
NSW  6                    GCTCGGCGCT GTTGCACG-- GTGTT--GCC CGGT------ ---------- ---------- ---------- ---------- GGCCCCGTGG TCTCAAGCAC
NSW  6(2)                 GCTCGGCGGT GTTGCACG-- GTGTT--GCC CGGT------ ---------- ---------- ---------- ---------- GGCCCCGTGG TCTCAAGCAC
NSW  7                    GCTCGGCGGT GTTGCACG-- GTGTT--GCC CGGT------ ---------- ---------- ---------- ---------- GGCCCCGTGG TCTCAAGCAC
NSW  8                    GCTCGGCGGT GTTGCACG-- GTGTT--GCC CGGT------ ---------- ---------- ---------- ---------- GGCCCCGTGG TCTCAAGCAC
NZn  1                    GCTCG----- -CTCGGCG-- GTGTT--GCC CGGT------ ---------- ---------- ---------- ---------- GGCCCCGTGG TCTCAAGCAC
NZn  2                    GCTCG--CTC GCTCGGCG-- GTGTT--GCC CGGT------ ---------- ---------- ---------- ---------- GGCCCCGTGG TCTCAAGCAC
NZs  1                    GCTCG----- -CTCGGCG-- GTGTC--GCC CGGT------ ---------- ---------- ---------- ---------- GGCCCCGTGG TCTCAAGCAC
NZs  2                    GCTCG----- -----GCG-- GTGTC--GCC CGGT------ ---------- ---------- ---------- ---------- GGCCCCGTGG TCTCAAGCAC
NZs  3                    GCTCG----- -----GCG-- GTGTC--GCC CGGT------ ---------- ---------- ---------- ---------- GGCCCCGTGG TCTCAAGCAC
NZs  4                    GCTCG----- -----GCG-- GTGTT--GCC CGGT------ ---------- ---------- ---------- ---------- GGCCCCGTGG TCTCAAGCAC
SA  1                     GCTCGGCGGT GTTGCACG-- GTGTT--GCC CGGT------ ---------- ---------- ---------- ---------- GGCCCCGTGG TCTCAAGCAC
SA  2                     GCTCGGCGGT GTTGCACG-- GTGGT--GCC CGGT------ ---------- ---------- ---------- ---------- GGCCCCGTGG TCTCAAGCAC
SA  3                     GCTCGGCGGT GTTGCACG-- GTGTT--GCC CGGT------ ---------- ---------- ---------- ---------- GGCCCCGTGG TCTCAAGCAC
TAS  1                    GCTCGGCGGT GTTGCACG-- GTGTT--GCC CGGT------ ---------- ---------- ---------- ---------- GGCCCCGTGG TCTCAAGCAC
TAS  2                    GCTCGGCGGT GTTGCACG-- GTGTT--GCC CGGT------ ---------- ---------- ---------- ---------- GGCCCCGTGG TCTCAAGCAC
TAS  3                    GCTCGGCGGT GTTGCACG-- GTGTT--GCC CGGT------ ---------- ---------- ---------- ---------- GGCCCCGTGG TCTCAAGCAC
TAS  4                    GCTCGGCGGT GTTGCACG-- GTGTT--GCC CGGT------ ---------- ---------- ---------- ---------- GGCCCCGTGG TCTCAAGCAC
TAS  5                    GCTCGGCGGT GTTGCACG-- GTGTT--GCC CGGT------ ---------- ---------- ---------- ---------- GGCCCCGTGG TCTCAAGCAC
VIC  1                    GCTCGGCGGT GTTGCACG-- GTGTT--GCC CGGT------ ---------- ---------- ---------- ---------- GGCCCCGTGG TCTCAAGCAC
VIC  2                    GCTCGGCGGT GTTGCACG-- GTGTT--GCC CGGT------ ---------- ---------- ---------- ---------- GGCCCCGTGG TCTCAAGCAC
Kutikina  hispida         GCTCGGCGGT GTTGCACG-- GTGTT--GCC CGGT------ ---------- ---------- ---------- ---------- GGCCCCGTGG TCTCAAGCAC
Kutikina hispida(2)       GCTCGGCGGT GTTGCACG-- GTGTT--GCC CGGT------ ---------- ---------- ---------- ---------- GGCCCCGTGG TCTCAAGCAC
Austropeplea  lessoni  NSW  CTTCCATCC- ---------- -TTGCTCTCA CGGATGGATT GGGGAT---- ---------- -------TGT TTCATTGAGT GGCCCCGTGG TCTTAAGCAC
Austropeplea lessoni  NT    GTTCCTTCCT TCCTTCC--- -TTGCTCTCG CGGATGGAT- GGGGATAGGG ATAGGGATAG GGATAGG-GA TAGGTTGAGT GGCCCCGTGG TCTTAAGCAC
Austropeplea lessoni  QLD   CTTCCATCC- ---------- -TTGCTCTCA CGGATGGATT GGGGAT---- ---------- -------TGT TTCATTGAGT GGCCCCGTGG TCTTAAGCAC
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Austropeplea lessoni  WA       GTTCCTTCCT TCCTTCC--- -TTGCTCTCG CGGATGGAT- GGGGATAGGG ATAGGGATAG GGATAGG-GA TAGGTTGAGT GGCCCCGTGG TCTTAAGCAC
Austropeplea viridis           GCTCGGCGGT GTTG------ GTGTTGCGCC CGGT------ ---------- ---------- ---------- ---------G GGCCCCGTGG TCTTAAGCAC
Bullastra cumingiana           GCTCTCCGCT TCGGTTTGCC GTCGGTGGCC CCGTGGTCTC AAGCACATGC CGCGCCGTTG TCCGTGTTCG TCTCGGAAAC GACCCCGCCT CGCTCTCGGC
Radix auricularia              GCTCGGCGAT GTCGTGTGT- GTGTTGTGCC TGGT------ ---------- ---------- ---------- ---------- GGCCCCGTGG ACTTAAGCAC
Radix peregra                  GCTCGGCGGT -----TTGC- GTGTTGCGCC CGGT------ ---------- ---------- ---------- ---------- GGGCCCCGTGG TCTTAAGCAC


                                        *                        *******                            ******* ********** ******
                               ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                        210        220        230        240        250        260        270        280        290        300
NSW  1                         AAGCCGCGCC GTT-GTCCGT GTTCGTCTCG GGACGTCCGC GACGCCGCCT TGCTCTCGGC GGCGGCCAAA T--------- ---------- -----TTTCC
NSW  2                         AAGCCGCGCC GTT-GTCCGT GTTCGTCTCG GGACGTCCGC GACGCCGCCT TGCTCTCGGC GGCGGCCAAA T--------- ---------- -----TTTCC
NSW  3                         AAGCCGCGCC GTT-GTCCGT GTTCGTCTCG GGACGTCCGC GACGCCGCCT TGCTCTCGGC GGCGGCCAAA T--------- ---------- -----TTTCC
NSW  4                         AAGCCGCGCC GTT-GTCCGT GTTCGTCTCG GGACGTCCGC GACGCCGCCT TGCTGTCGGC GGCGGCCAAA T--------- ---------- -----TTTCC
NSW  5                         AAGCCGCGCC GTT-GTCCGT GTTCGTCTCG GGACGTCCGC GACGCCGCCT TGCTCTCGGC GGCGGCCAAA T--------- ---------- -----TTTCC
NSW  6                         AAGCCGCGCC GTT-GTCCGT GTTCGTCTCG GGACGTCCGC GACGCCGCCT TGCTCTCGGC GGCGGCCAAA T--------- ---------- -----TTTCC
NSW  6(2)                      AAGCCGCGCC GTT-GTCCGT GTTCGTCTCG GGACGTCCGC GACGCCGCCT TGCTCTCGGC GGCGGCCAAA T--------- ---------- -----TTTCC
NSW  7                         AAGCCGCGCC GTT-GTCCGT GTTCGTCTCG GGACGTCCGC GACGCCACCT TGCTCTCGGC GGCGGCCAAA T--------- ---------- -----TTTCC
NSW  8                         AAGCCGCGCC GTT-GTCCGT GTTCGTCTCG GGACGTCCGC GACGCCGCCT TGCTCTCGGC GGCGGCCAAA T--------- ---------- -----TTTCC
NZn  1                         AAGCCGCGCC GTT-GTCCGT GTTCGTCTCG GGACGTCCGC GACGCCGCCT TGCTCTCGGC GGCGGCCAAA T--------- ---------- -----TTTCC
NZn  2                         AAGCCGCGCC GTT-GTCCGT GTTCGTCTCG GGACGTCCGC GACGCCGCCT TGCTCTCGGC GGCGGCCAAA T--------- ---------- -----TTTCC
NZs  1                         AAGCCGCGCC GTT-GTCCGT GTTCGTCTCG GGACGTCCGC GACGCCGCCT TGCTCTCGGC GGCGGCCAAA T--------- ---------- -----TTTCC
NZs  2                         AAGCCGCGCC GTT-GTCCGT GTTCGTCTCG GGACGTCCGC GACGCCGCCT TGCTCTCGGC GGCGGCCAAA T--------- ---------- -----TTTCC
NZs  3                         AAGCCGCGCC GTT-GTCCGT GTTCGTCTCG GGACGTCCGC GACGCCGCCT TGCTCTCGGC GGCGGCCAAA T--------- ---------- -----TTTCC
NZs  4                         AAGCCGCGCC ATT-GTCCGT GTTCGTCTCG GGACGTCCGC GACGCCGCCT TGCTCTCGGC GGCGGCCAAA T--------- ---------- -----TTTCC
SA  1                          AAGCCGCGCC GTT-GTCCGT GTTCGTCTCG GGACGTCCGC GACGCCGCCT CGCTCTCGGC GGCGGCCAAA T--------- ---------- -----TTTCC
SA  2                          AAGCCGCGCC GTT-GTCCGT GTTCGTCTCG GGACGTCCGC GACGCCGCCT TGCTCTCGGC GGCGGCCAAA T--------- ---------- -----TTTCC
SA  3                          AAGCCGCGCC GTT-GTCCGT GTTCGTCTCG GGACGTCCGC GACGCCGCCT TGCTCTCGGC GGCGGCCAAA T--------- ---------- -----TTTCC
TAS  1                         AAGCCGCGCC GTT-GTCCGT GTTCGTCTTG GGACGTCCGC GACGCCGCCT TGCTCTCGGC GGTGGCCAAA T--------- ---------- -----TTTCC
TAS  2                         AAGCCGCGCC GTT-GTCCGT GTTCGTCTTG GGACGTCCGC GACGCCGCCT TGCTCTCGGC GGTGGCCAAA T--------- ---------- -----TTTCC
TAS  3                         AAGCCGCGCC GTT-GTCCGT GTTCGTCTTG GGACGTCCGC GACGCCGCCT TGCTCTCGGC GGTGGCCAAA T--------- ---------- -----TTTCC
TAS  4                         AAGCCGCGCC GTT-GTCCGT GTTCGTCTCG GGACGTCCGC GACGCCGCCT TGCTCTCGGC GGCGGCCAAA T--------- ---------- -----TTTCC
TAS  5                         AAGCCGCGCC GTT-GTCCGT GTTCGTCTTG GGACGTCCGC GACGCCGCCT TGCTCTCGGC GGTGGCCAAA T--------- ---------- -----TTTCC
VIC  1                         AAGCCGCGCC GTT-GTCCGT GTTCGTCTCG GGACGTCCGC GACGCCGCCT TGCTCTCGGC GGCGGCCAAA T--------- ---------- -----TTTCC
VIC  2                         AAGCCGCGCC GTT-GTCCGT GTTCGTCTCG GGACGTCCGC GACGCCGCCT TGCTCTCGGC GGCGGCCAAA T--------- ---------- -----TTTCC
Kutikina  hispida             AAGCCGCGCC GTT-GTCCGT GTTCGTCTCG GGACGTCCGC GACGCCGCCT TGCTCTCGGC GGCGGCCAAA T--------- ---------- -----TTTCC
Kutikina hispida(2)           AAGCCGCGCC GTT-GTCCGT GTTCGTCTCG GGACGTCCGC GACGCCGCCT TGCTCTCGGC GGCGGCCAAA T--------- ---------- -----TTTCC
Austropeplea  lessoni  NSW    ATGCCGCGCC GTTTGTCCGT GCTCGTCTCG GGCCGTCCGC -------CTT TGCTCTCGGC GGCGTCCGCC CACACTACTG TGTGATTCTT TTTTTTTTCC
Austropeplea lessoni  NT      ATGCCGCGCC GTTTGTCCGT GCTCGTCTCG GGCCGTCCGC -------CTT TGCTCTCGGC GGCATCCGCC CACACTACGG TGTGAAT--- TTTTTTTTCC
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Austropeplea lessoni  QLD   ATGCCGCGCC GTTTGTCCGT GCTCGTCTCG GGCCGTCCGC -------CTT TGCTCTCGGC GGCGTCCGCC CACACTACTG TGTGATTCTT TTTTTTTTCC
Austropeplea lessoni  WA    ATGCCGCGCC GTTTGTCCGT GCTCGTCTCG GGCCGTCCGC -------CTT TGCTCTCGGC GGCATCCGCC CACACTACGG TGTGAAT--- TTTTTTTTCC
Austropeplea viridis        AAGCCGCGCC GTT-GTCCGT GTTCGTCTCG GGACGTCCGC GACGCCGCCT TGCTCTCGGC GGCGGCCAAA T--------- ---------- ------TTCC
Bullastra cumingiana        GGAGCCCGCC -T----CGCT CTCGGCGGCG GTA-G-CC-- AACG---TTT TCGAA--GGT GTAA------ ---------- --------TT TTTTTCTTCC
Radix auricularia           AAGCCGCGCC GTT-GTCCGT GTTCGTCTCG GGACGTCCGC GACGCCGCCT TGCTCTCGGC GGCGGCCAAA T--------- ---------- ------TTCC
Radix peregra               AAGCCGCGCC GTT-GTCCGT GTTCGTCTCG GGACGTCCGC GACGCCGCCT TGCTCTCGGC GGCGGCCAAA T--------- ---------- ------TTCC


                            *       *                                   *           **                                   ****

                            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                               310        320        330        340        350        360        370        380        390        400
NSW  1                      -TCCTCG-TC ACCGCTATGC GGGACCCGGC TCGCTCTCG- CTAACGGGCC CGCTCGTAAC AAGCTCCAGG GTGATTGCGG AGGAGAGAAG ----CTTACG
NSW  2                      -TCCTCG-TC ACCGCTATGC GGGACCCGGC TCGCTCTCG- CTAACGGGCC CGCTCGTAAC AAGCTCCAGG GTGATTGCGG AGGAGAGAAG ----CTTACG
NSW  3                      -TCCTCG-TC ACCGCTATGC GGGACCCGGC TCGCTCTCG- CTAACGGGCC CGCTCGTAAC AAGCTCCAGG GTGATTGCGG AGGAGAGAAG ----CTTACG
NSW  4                      -TCCTCG-TC ACCGCTATGC GGGACCCGGC TCGCTCTCG- CTAACGGGCC CGGTCGTAAC AAGCTCCAGG GTGATTGCGG AGGAGAGAAG ----CTTACG
NSW  5                      -TCCTCG-TC ACCGCTATGC GGGACCCGGC TCGCTCTCG- CTAACGGGCC CGCTCGTAAC AAGCTCCAGG GTGATTGCGG AGGAAAGAAG ----CTTACG
NSW  6                      -TCCTCG-TC ACCGCTATGC GGGACCCGGC TCGCTCTCG- CTAACGGGCC CGCTCGTAAC AAGCTCCAGG GTGATTGCGG AGGAGAGAAG ----CTTACG
NSW  6(2)                   -TCCTCG-TC ACCGCTATGC GGGACCCGGC TCGCTCTCG- CTAACGGGCC CGCTCGTAAC AAGCTCCAGG GTGATTGCGG AGGAGAGAAG ----CTTACG
NSW  7                      -TCCTCG-TC ACCGCTATGC GGGACCCGGC TCGCTGTT-- CTAACGGGCC CGCTCGTAAC AAGCTCCAGG GTGATTGCGG AGGAGAGAAG ----CTTACG
NSW  8                      -TCCTCG-TC ACCGCTATGC GGGACCCGGC TCGCTCTCG- CTAACGGGCC CGCTCGTAAC AAGCTCCAGG GTGATTGCGG AGGAGAGAAG ----CTTACG
NZn  1                      -TCCTCG-TC ACCGCCGTGC GGGACCCGGC TCGCTCTCG- CTAACGGGCC CGCTCGTAAC AAGCTCCAGG GTGATTGCGG AGGAGAGAAG ----CTTACG
NZn  2                      -TCCTCG-TC ACCGCCGTGC GGGACCCGGC TCGCTCTCG- CTAACGGGCC CGCTCGTAAC AAGCTCCAGG GTGATTGCGG AGGAGAGAAG ----CTTACG
NZs  1                      -TCCTCG-TC ACCGCCGTGC GGGACCCGGC TCGCTCTCG- CTATCGGGCC CGCTCGTAAC AAGCTCCAGG GTGATTGCGG AGGAGAGAAG ----CTTACG
NZs  2                      -TCCTCG-TC ACCGCCGTGC GGGACCCGGC TCGCTCTCG- CTATCGGGCC TGCTCGTAAC AAGCTCCAGG GTGATTGCGG AGGAGAGAAG ----CTTACG
NZs  3                      -TCCTCG-TC ACCGCCGTGC GGGACCCGGC TCGCTCTCG- CTATCGGGCC TGCTCGTAAC AAGCTCCAGG GTGATTGCGG AGGAGAGAAG ----CTTACG
NZs  4                      -TCCTCG-TC ACCGCCGTGC GGGACCCGGC TCGCTCTCG- CTATCGGGCC TGCTCGTAAC AAGCTCCAGG GTGATTGCGG AGGAGAGAAG ----CTTACG
SA  1                       -TCCTCG-TC ACCGCTATGC GGGACCCGGC TCGCTCTCG- CTAACGGGCC CGCTCGTAAC AAGCTCCAGG GTGATTGCGG AGGAGAGAAG ----CTTACG
SA  2                       -TCCTCG-TC ACCGCTATGC GGGACCCGGC TCGCTCTCG- CTAACGGGCC CGCTCGTAAC AAGCTCCAGG GTGATTGCGG AGGAGAGAAG ----CTTACG
SA  3                       -TCCTCG-TC ACCGCTATGC GGGACCCGGC TCGCTCTCG- CTAACGGGCC CGCTCGTAAC AAGCTCCAGG GTGATTGCGG AGGAGAGAAG ----CTTACG
TAS  1                      -TCCTCG-TC ACCGCTATGC GGGACCCGGC TCGCTCTCG- CTAACGGGCC CGCTCGTAAC AAGCTCCAGG GTGATTGCGG AGGAGAGAAG ----CTTACG
TAS  2                      -TCCTCG-TC ACCGCTATGC GGGACCCGGC TCGCTCTCG- CTAACGGGCC CGCTCGTAAC AAGCTCCAGG GTGATTGCGG AGGAGAGAAG ----CTTACG
TAS  3                      -TCCTCG-TC ACCGCTATGC GGGACCCGGC TCGCTCTCG- CTAACGGGCC CGCTCGTAAC AAGCTCCAGG GTGATTGCGG AGGAGAGAAG ----CTTACG
TAS  4                      -TCCTCG-TC ACCGCTATGC GGGACCCGGC TCGCTCTCG- CTAACGGCC CGCTCGTAAC AAGCTCCAGG GTGATTGCGG AGGAGAGAAG ----CTTACG
TAS  5                      -TCCTCG-TC ACCGCTATGC GGGACCCGGC TCGCTCTCG- CTAACGGGCC CGCTCGTAAC AAGCTCCAGG GTGATTGCGG AGGAGAGAAG ----CTTACG
VIC  1                      -ACCTCG-TC ACCGCTATGC GGGACCCGGC TCGCTCTCG- CTAACGGGCC CGCTCGTAAC AAGCTCCAGG GTGATTGCGG AGGAGAGAAG ----CTTACG
VIC  2                      -TCCTCG-TC ACCGCTATGC GGGACCCGGC TCGCTCTCG- CTAACGGGCC CGCTCGTAAC AAGCTCCAGG GTGATTGCGG AGGAGAGAAG ----CTTACG
Kutikina  hispida           -TCCTCG-TC ACCGCTATGC GGGACCCGGC TCGCTCTCG- CTAACGGGCC CGCTCGTAAC AAGCTCCAGG GTGATTGCGG AGGAGAGAAG ----CTTACG
Kutikina hispida(2)         -TCCTCG-TC ACCGCTATGC GGGACCCGGC TCGCTCTCG- CTAACGGGCC CGCTCGTAAC AAGCTCCAGG GTGATTGCGG AGGAGAGAAG ----CTTACG
Austropeplea  lessoni NSW   -TCTGCGGTC ACCGCCATGC GGGACCCGGC TCGCTCTCGC CTCACGGGCT CGC--ATTAA AAGCTCCAGG GTGATCGCGG AGGAGAGAAA ---GGTCGCA
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Austropeplea lessoni  NT     -TCTGCGGTC ACCGCCATGC GGGACCCGGC TCGCTCTCGC CTCACGGGCT CGC--ATTAA AAGCTCCAGG GTGATCGCGG AGGAGAGAAA ---GGTCGCA
Austropeplea lessoni  QLD    -TCTGCGGTC ACCGCCATGC GGGACCCGGC TCGCTCTCGC CTCACGGGCT CGC--ATTAA AAGCTCCAGG GTGATCGCGG AGGAGAGAAA ---GGTCGCA
Austropeplea lessoni  WA     -TCTGCGGTC ACCGCCATGC GGGACCCGGC TCGCTCTCGC CTCACGGGCT CGC--ATTAA AAGCTCCAGG GTGATCGCGG AGGAGAGAAA ---GGTCGCA
Austropeplea viridis         -TCTCCG-TC ACCGCCATGC GGGACCCGGC TCGCTCTCG- CTAACGGGCC CGCTTGTAAC CAGCTCGAGG GTGATTGCGG AGGAGAGAAG ----CTTACG
Bullastra cumingiana         -TCTGCG-TC ACCGCAATGC GGGACCCGGC TCGCTCTCGC CAAACGGGCC CGCACAAAAC A-GCTCGAGG GTGATCGCGG AGGAGGAGAA GAAGAAA---
Radix auricularia            ATCTGCG-TC ACCGCTAAGC GGGACCCGGC TCGCTCTCG- CTAACGGGCC CGCTTATATC AAGCTCAAGG GTGATTGCGG AGGGGGAAAA AAAGCTTACG
Radix peregra                -TCTCCG-TC ACCGCTATGC GGGACCCGGC TCGCTCTCG- CTAACGGGCC CGCTTATAAC CAGCTTCAGG GTGACGGGCG GAGGAGAGAA ---GCTTACG


                                 **             * ********** *******               * *    *****       ** ********** ********** **********
                             ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                410        420        430        440        450        460        470        480        490        500

NSW  1                       CTGACGC--T CGCTTGACA- ---------- -------AAT CGGCGCCCGT ACGAA----- TTGAAGAAGA AGAAGA---- ---------- ----------
NSW  2                       CTGACGC--T CGCTTGACA- ---------- -------AAT CGGCGCCCGT ACGAA----- TTGAAGAAGA AGAAGA---- ---------- ----------
NSW  3                       CTGACGC--T CGCTTGACA- ---------- -------AAT CGGCGCCCGT ACGAA----- TTGAAGAAGG AGAAGA---- ---------- ----------
NSW  4                       CTGACGC--T CGCTTGACA- ---------- -------AAT CGGCGCCCGT ACGAA----- TTGAAGAAGA AGAAGA---- ---------- ----------
NSW  5                       CATGCGC--T CGCTTGACA- ---------- -------AAT CGGCGCCTGT ACGAA----- TTGAAGAAGG AGAAGA---- ---------- ----------
NSW  6                       CTGACGC--T CGCTTGACA- ---------- -------AAT CGGCGCCTGT ACGAA----- TTGAAGAAGG AGAAGA---- ---------- ----------
NSW  6(2)                    CTGACGC--T CGCTTGACA- ---------- -------AAT CGGCGCCTGT ACGAA----- TTGAAGAAGG AGAAGA---- ---------- ----------
NSW  7                       CTGACGC--T CGCTTGACA- ---------- -------AAT CGGCGCCTGT ACGAA----- TTGAAGAAGG AGAAGA---- ---------- ----------
NSW  8                       CTGACGC--T CGCTTGACA- ---------- -------AAT CGGCGCCCGT ACGAA----- TTGAAGAAGG AGAAGA---- ---------- ----------
NZn  1                       CTGACGC--T CGCTTGACA- ---------- -------GAT CGGCGCCCGT ACGAAAA--- TTGAAGAA-- ---------- ---------- ----------
NZn  2                       CTGACGC--T CGCTTGACA- ---------- -------GAT CGGCGCCCGT ACGAAAA--- TTGAAGAA-- ---------- ---------- ----------
NZs  1                       CTGACGC--T CGCTTGACA- ---------- -------GAT CGGCGCCCGT ACGAAAA--- TTGAAGAA-- ---------- ---------- ----------
NZs  2                       CTGGCGC--T CGCTTGACA- ---------- -------GAT CGGCGCCCGT ACGAA----- TTGAAGAA-- ---------- ---------- ----------
NZs  3                       CTGACGC--T CGCTTGACA- ---------- -------GAT CGGCGCCCGT ACGAA----- TTGAAGAA-- ---------- ---------- ----------
NZs  4                       CTGACGC--T CGCTTGACA- ---------- -------GAT CGGCGCCCGT ACGAA----- TTGAAGAA-- ---------- ---------- ----------
SA  1                        CTGACGC--T CGTTTGACA- ---------- -------AAT CGGCGCCCGT ACGAA----- CTGAAGAAAA AGA------- ---------- ----------
SA  2                        CTGACGC--T CGCTTGACA- ---------- -------AAT CGGCGCCCGT ACGAA----- TTGAAGAAGA AGA------- ---------- ----------
SA  3                        CTGACGC--T CGCTTGACA- ---------- -------AAT CGGCGCCCGT ACGAA----- TTGAAGAAGA AGA------- ---------- ----------
TAS  1                       CTGACGC--T CGCTTGACA- ---------- -------AAT CGGCGCCCGT ACGAA----- TTGAAGAAGA AGAAGA---- ---------- ----------
TAS  2                       CTGACGC--T CGCTTGACA- ---------- -------AAT CGGCGCCCGT ACGAA----- TTGAAGAAGA AGAAGA---- ---------- ----------
TAS  3                       CTGACGC--T CGCTTGACA- ---------- ------AAT CGGCGCCCGT ACGAA----- TTGAAGAAGA AGAAGA---- ---------- ----------
TAS  4                       CTGACGC--T CGCTTGACA- ---------- -------AAT CGGCGCCCGT ACGAA----- TTGAAGAAGA AGAAGA---- ---------- ----------
TAS  5                       CTGACGC--T CGCTTGACA- ---------- -------AAT CGGCGCCCGT ACGAA----- TTGAAGAAGA AGAAGA---- ---------- ----------
VIC  1                       CTGACGC--T CGCTTGACA- ---------- -------AAT CGGCGCCTGT ACGAA----- TTGAAGAAGG AGAAGA---- ---------- ----------
VIC  2                       CTGACGC--T CGCTTGACA- ---------- -------AAT CGGCGCCTGT ACGAA----- TTGAAGAAGG AGAAGA---- ---------- ----------
Kutikina  hispida            CTGACGC--T CGCTTGACA- ---------- -------AAT CGGCGCCCGT ACGAA----- TTGAAGAAGA AGAAGAAGAA GAAGAAGACT ATGCT-----
Kutikina hispida(2)          CTGACGC--T CGCTTGACA- ---------- -------AAT CGGCGCCCGT ACGAA----- TTGAAGAAGA AGAAGAAGAA GAAGAAGACT ATGCT-----
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Austropeplea  lessoni  NSW    CGCTCGC--T CGCTCGCTCG CTAGTGTTTG ATGACGAAAT CGGCGCCAC- -CGAA----- ATGAAGAAGA TGAAGAAAGG AGGAGATTGA GA--TTGATT
Austropeplea lessoni  NT     CGCTCGC--T CGCTCGC--- -TAGTGTTT- --GACGAAAT CGGCGCCAC- -CGAA----- ATGAAGAAGA AGAAAAAA-- AGGAGATTGA GAGATTGTGA
Austropeplea lessoni  QLD    CGCTCGC--T CGCTCGCTCG CTAGTGTTTG ATGACGAAAT CGGCGCCAC- -CGAA----- ATGAAGAAGA TGAAGAAAGG AGGAGATTGA GA--TTGATT
Austropeplea lessoni  WA     CGCTCGC--T CGCTCGCTCG TCAGTGTTT- --GACGAAAT CGGCGCCAC- -CGAA----- ATGAA-AAGA A--------A AGGAGATTGA GAGA----GA
Austropeplea viridis          CAGACGCGCT CGCTTGA--C G--------- -------TAT CGGCGCCCGT ACGAA----- --GAAAAAAA AA-------- ---------- ----------
Bullastra cumingiana          ---------- ---TTGAGAC G--------- ------AAAT CGGCGCCCCA GATCGCAAGA GAGGGAATGA GATGGTGGGA GGGAGATTCT TCTGTCTCTC
Radix auricularia             CGGACGA--T CGCTTGACA- ---------- ---AC-AAAT CGGCGCCCGT ACGAA----- TTGAAGTGAA AAAAATA--- ---------- ----------
Radix peregra                 CTGACGC--T CGATCGA--C G--------- -------AGT CGGCGCCCGT ACGAAAAAAA TTGGAAAAAA AATAACCGTG CGTTGCGCGT TA--------


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                              ....|....| ....|....| ....|....| ....|....| ....|....| ....|....
                                  510        520        530        540        550

NSW  1                        ---TTCTTTT T---TTTTTC TTCTTTC-AC AAATTTCCGA CCTCAGATCG GACGAGATT
NSW  2                        ---TTCTTTT TT--TTTTTC TTCTTTC-AC AAATTTCCGA CCTCAAATCG GACGAGATT
NSW  3                        ---TTCCATT TTTCTTCTTC TTCTTTC-AC AAATTTCCGA CCTCAGATCG GACGAGATT
NSW  4                        ---TTCCATT TTTCTTCTTC TTCTTTC-AC AAATTTCCGA CCTCAGATCG GACGAGATT
NSW  5                        ---TTCCATT TTTCTTCTTC TTCTTTC-AC AAATTTCCGA CCTCAGATCG GACGAGATT
NSW  6                        ---TTCTTTT T---TTCTTC TTCTTTC-AC AAATTTCCGA CCTCAGATCG GACGAGATT
NSW  6(2)                     ---TT-TTTT TCT-TTCTTC TTCTTTC-AC AAATTTCCGA CCTCAGATCG GACGAGATT
NSW  7                        ---TTCCATT TTTCTTCTTC TTCTTTC-AC AAATTTCCGA CCTCAGATCG GACGAGATT
NSW  8                        ---TTCTTTT T---TTCTTC TTCTTTC-AC AAATTTCCGA CCTCAGATCG GACGAGATT
NZn  1                        ---TT----- ----GTCTTC TTCTTTCCAC AAATTTCCGA CCTCAGATCG GACGAGATT
NZn  2                        ---TT----- ----GTCTTC TTCTTTCCAC AAATTTCCGA CCTCAAATCG GACGAGATT
NZs  1                        ---TT----- ----GTCTTC TTCTTTCCAC AAATTTCCGA CCTCAGATCG GACGAGATT
NZs  2                        ---TT----- ----GTCTTC TTCTTTC-AC AAATTTCCGA CCTCAGATCG GACGAGATT
NZs  3                        ---TT----- ----GTCTTC TTCTTTC-AC AAATTTCCGA CGTCAGATCG GACGAGATT
NZs  4                        ---TT----- ----GTCTTC TTCTTTC-AC AAATTTCCGA CCTCAGATCG GACGAGATT
SA  1                         ---TTCT--- ----GTCTTC TTCTTTC-AC AAATTTCCGA CCTCAGATCG GACGAGATT
SA  2                         ---TTCT--- ----GTCTTC TTCTTTC-AC AAATTTCCGA CCTCAGATCG GACGAGATT
SA  3                         ---TTCT--- ----GTCTTC TTCTTTC-AC AAATTTCCGA CCTCAGATCG GACGAGATT
TAS  1                        ---TTCTTTT TTT-TCTTTC TTCTTTC-AC AAATTTCCGA CCTCAGATCG GACGAGATT
TAS  2                        ---TTCTTTT TTTTTTTTTC TTCTTTC-AC AAATTTCCGA CCTCAGATCG GACGAGATT
TAS  3                        ---TTCT--- ----GCTTTC TTCTTTC-AC AAATTTCCGA CCTCAGATCG GACGAGATT
TAS  4                        ---TTCTTTT T---TCTTTC TTCTTTC-AC AAATTTCCGA CCTCAGATCG GACGAGATT
TAS  5                        ---TTCTTTT TTT-TCTTTC TTCTTTC-AC AAATTTCCGA CCTCAGATCG GACGAGATT
VIC  1                        ---TT-TTTT TTT-TTCTTC TTCTTTC-AC AAATTTCCGA CCTCAGATCG GACGAGATT
VIC  2                        ---TT-TTTT TCT-TTCTTC TTCTTTC-AC AAATTTCCGA CCTCAGATCG GACGAGATT
Kutikina  hispida            ---TTCTTAG A---CTCTTC TTCTTTC-AC AAATTTCCGA CCTCAGATCG GACGAGATT
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Kutikina hispida(2)          ---TTCTTAG A---CTCTTC TTCTTTC-AC AAATTTCCGA CCTCAGATCG GACGAGATT
Austropeplea  lessoni  NSW   T-ATTTCTCT TTCCTTTTTT TT-CTC--TC TTTTCTCCGA CCTCAGATCG GACGAGATT
Austropeplea lessoni  NT     TGATTTCTCT TTCCTTGTTT TC------TC TTTTCTCCGA CCTCAGATCG GACGAGATT
Austropeplea lessoni  QLD    T-ATTTCTCT TTCCTTTTTT TTTCTC--TC TTTTCTCCGA CCTCAGATCG GACGAGATT
Austropeplea lessoni  WA     TGATTTCTCT TTCCTTGTTT TC------TC TTTTCTCCGA CC---GATCG GA???????
Austropeplea viridis         ---TTTTTTT TTTTT----- --------AC CAATTTCCGA CCTCAGATCG GACGAGATT
Bullastra cumingiana         TCTCTCGCTT TTCGTTCGTT CCCCTTC--- ---TTTC?????????????????????????
Radix auricularia            ---TTCTTTT TTTTTTTTCA T-TTT---CA TATCTC?????????????????????????
Radix peregra                -TTTTTTTTT TTCCTTTCTT TC??????????????????????????????????????
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Appendix 2.5. p distances of 16S rRNA sequences, as calculated in PAUP for the analysis of the *Austropeplea tomentosa* complex. Taxa without names are currently recognised as *A. tomentosa*.

		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	29	30	31
NSW 1	1	-																														
NSW 2	2	0.9	-																													
NSW 3	3	1.4	1.4	-																												
NSW 4	4	1.4	1.4	0.5	-																											
NSW 5	5	0.9	0.9	0.5	0.5	-																										
NSW 6	6	1.2	1.2	0.7	0.7	0.2	-																									
NSW 7	7	0.9	0.9	0.5	0.5	0.0	0.2	-																								
NSW 8	8	1.9	1.9	0.9	0.9	0.9	1.2	0.9	-																							
NZn 1	9	5.2	5.9	5.4	5.4	4.9	5.2	4.9	5.9	-																						
NZn 2	10	5.2	5.9	5.4	5.4	4.9	5.2	4.9	5.9	0.7	-																					
NZs 1	11	5.6	6.3	5.9	5.9	5.4	5.6	5.4	5.7	1.4	1.6	-																				
NZs 2	12	6.3	7.0	6.6	6.6	6.1	6.3	6.1	6.3	2.1	2.3	0.7	-																			
NZs 3	13	6.3	7.0	6.6	6.6	6.1	6.3	6.1	6.3	2.1	2.3	0.7	0.0	-																		
NZs 4	14	5.6	6.3	5.9	5.9	5.4	5.6	5.4	5.6	1.4	1.6	0.0	0.7	0.7	-																	
SA 1	15	1.2	0.7	0.7	0.7	0.2	0.5	0.2	1.2	5.2	5.2	5.6	6.3	6.3	5.6	-																
SA 2	16	0.9	0.9	0.5	0.5	0.0	0.2	0.0	0.9	4.9	4.9	5.4	6.1	6.1	5.4	0.2	-															
SA 3	17	1.2	1.2	0.7	0.7	0.2	0.5	0.2	1.2	5.2	5.2	5.6	6.3	6.3	5.6	0.5	0.2	-														
TAS 1	18	0.9	0.9	0.5	0.5	0.0	0.2	0.0	0.9	4.9	4.9	5.4	6.1	6.1	5.4	0.2	0.0	0.2	-													
TAS 2	19	1.2	1.2	0.7	0.7	0.2	0.5	0.2	1.2	5.2	5.2	5.6	6.3	6.3	5.6	0.5	0.2	0.5	0.2	-												
TAS 3	20	0.9	0.9	0.5	0.5	0.0	0.2	0.0	0.9	4.9	4.9	5.4	6.1	6.1	5.4	0.2	0.0	0.2	0.0	0.2	-											
TAS 4	21	0.9	0.9	0.5	0.5	0.0	0.2	0.0	0.9	4.9	4.9	5.4	6.1	6.1	5.4	0.2	0.0	0.2	0.0	0.2	0.0	-										
TAS 5	22	0.9	0.9	0.5	0.5	0.0	0.2	0.0	0.9	4.9	4.9	5.4	6.1	6.1	5.4	0.2	0.0	0.2	0.0	0.2	0.0	0.0	-									
VIC 1	23	1.9	2.1	1.9	1.9	1.4	1.6	1.4	2.3	5.2	5.2	5.6	6.3	6.3	5.6	1.4	1.4	1.6	1.4	1.6	1.4	1.4	1.4	-								
Kutikina hispida	24	2.6	3.0	2.6	2.6	2.1	1.9	2.1	3.1	5.2	5.6	6.1	6.8	6.8	6.1	2.3	2.1	2.3	2.1	2.3	2.1	2.1	2.1	3.3	-							
Austropeplea lessoni NSW	25	11.5	11.8	11.3	11.1	11.3	11.5	11.3	11.1	12.2	12.2	12.0	12.7	12.7	12.0	11.3	11.3	11.5	11.3	11.5	11.3	11.3	11.3	11.5	12.5	-						
Austropeplea lessoni NT	26	11.3	11.5	11.0	10.8	11.0	11.3	11.0	10.8	12.0	12.0	11.8	12.4	12.4	11.7	11.0	11.0	11.3	11.1	11.3	11.1	11.1	11.1	11.3	12.7	1.2	-					
Austropeplea lessoni QLD	27	11.5	11.7	11.3	11.0	11.3	11.5	11.3	11.1	11.8	11.8	11.5	12.2	12.2	11.5	11.3	11.3	11.5	11.3	11.5	11.3	11.3	11.3	11.5	12.5	0.9	0.7	-				
Austropeplea lessoni WA	28	11.5	11.7	11.3	11.0	11.3	11.5	11.3	11.1	12.2	11.8	12.0	12.6	12.6	11.9	11.3	11.3	11.5	11.3	11.5	11.3	11.3	11.3	11.7	12.9	1.9	1.2	1.4	-			
Austropeplea viridis	29	12.0	12.7	12.4	12.2	12.0	11.7	12.2	12.7	11.3	11.3	11.5	11.7	11.7	11.5	12.2	12.0	12.2	12.0	12.3	12.2	12.0	12.0	11.5	12.3	11.7	11.9	11.7	12.4	-		
Bullastra cumingiana	30	10.5	11.2	10.5	10.5	10.8	11.0	10.8	10.6	10.5	11.0	10.5	11.2	11.2	10.5	10.8	10.8	11.0	10.8	11.0	10.8	10.8	10.8	10.5	11.5	9.6	8.9	9.1	9.1	14.5	-	
Radix auricularia	31	13.6	14.1	13.6	13.8	13.6	13.4	13.8	14.3	12.4	13.1	12.9	13.6	13.6	12.9	13.6	13.6	13.8	13.6	13.9	13.9	13.6	13.6	13.9	13.6	13.1	13.3	13.6	14.7	12.4	-	
Radix peregra	32	14.9	15.7	15.4	15.2	14.9	15.2	15.1	15.7	14.4	14.6	14.6	14.9	14.9	14.6	15.2	14.9	15.2	15.0	15.2	15.2	15.0	15.0	14.9	15.7	13.2	13.2	13.2	12.9	15.7	15.0	9.2

Appendix 2.6. p distances of ITS-2 rRNA sequences, as calculated in PAUP for the analysis of the *Austropeplea tomentosa* complex. Taxa without names are currently recognised as *A. tomentosa*.

		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	29	30	31	32	33	34
NSW 1	1	-																																	
NSW 2	2	0.2	-																																
NSW 3	3	1.0	1.2	-																															
NSW 4	4	1.2	1.5	0.7	-																														
NSW 5	5	2.2	2.4	1.2	1.9	-																													
NSW 6	6	1.0	1.2	1.0	1.7	1.7	-																												
NSW 6 (2)	7	0.7	1.2	1.0	1.7	1.7	0.2	-																											
NSW 7	8	2.7	2.9	1.7	2.4	2.4	2.2	2.2	-																										
NSW 8	9	0.7	1.0	0.7	1.5	1.9	0.7	0.5	2.4	-																									
NZn 1	10	2.6	2.9	2.3	2.9	3.7	2.6	2.6	4.2	2.6	-																								
NZn 2	11	3.6	3.4	3.4	3.9	4.7	3.6	3.6	5.2	3.6	0.3	-																							
NZs 1	12	3.1	3.4	2.9	3.4	4.2	3.1	3.1	4.7	3.1	0.5	0.8	-																						
NZs 2	13	2.9	3.2	2.6	3.2	3.4	2.9	2.9	4.5	2.9	1.1	1.3	0.5	-																					
NZs 3	14	2.9	3.2	2.6	3.2	4.0	2.9	2.9	4.5	2.9	1.1	1.3	0.5	0.5	-																				
NZs 4	15	2.9	3.2	2.6	3.2	4.0	2.9	2.9	4.5	2.9	1.0	1.3	1.0	1.0	1.0	-																			
SA 1	16	1.8	2.0	2.0	2.2	3.2	2.2	2.0	3.7	2.0	2.8	3.8	3.4	3.2	3.2	3.2	-																		
SA 2	17	2.0	2.3	2.2	2.5	3.5	2.5	2.2	4.0	2.2	3.4	4.4	3.9	3.7	3.7	3.7	2.5	-																	
SA 3	18	0.8	1.0	1.0	1.2	2.2	1.2	1.0	2.7	1.0	2.1	3.1	2.6	2.4	2.4	2.4	1.2	1.2	-																
TAS 1	19	1.0	1.2	1.9	2.2	3.1	2.0	1.9	3.6	1.7	3.4	4.4	3.9	3.7	3.7	3.7	2.3	2.8	1.5	-															
TAS 2	20	0.7	1.0	1.9	2.2	3.1	1.7	1.7	3.6	1.5	3.2	4.2	3.7	3.5	3.5	3.5	2.0	2.5	1.3	0.2															
TAS 3	21	1.7	2.0	2.5	2.7	3.7	2.7	2.5	4.2	2.5	3.6	4.6	4.1	4.0	3.9	4.0	2.5	3.0	1.7	0.8	1.0														
TAS 4	22	0.7	1.0	1.7	1.9	2.9	1.7	1.5	3.4	1.5	3.1	4.1	3.7	3.5	3.4	3.5	2.0	2.5	1.3	0.7	1.0	1.5													
TAS 5	23	1.0	1.2	1.9	2.2	3.1	2.0	1.9	3.6	1.7	3.4	4.4	3.9	3.7	3.7	3.7	2.3	2.8	1.5	0.0	0.2	0.8	0.7												
VIC 1	24	1.0	1.2	1.0	1.7	1.7	0.5	0.5	2.2	0.7	2.9	3.9	3.4	3.2	3.2	3.2	2.3	2.5	1.3	1.9	1.7	2.7	1.7	1.9											
VIC 2	25	0.7	1.2	1.0	1.7	1.7	0.2	0.0	2.2	0.5	2.6	3.6	3.1	2.9	2.9	2.9	2.0	2.2	1.0	1.9	1.7	2.5	1.5	1.9	0.5										
Kutikina hispida	26	2.0	2.3	2.5	2.7	3.7	2.5	2.3	4.2	2.3	3.0	4.0	3.5	3.3	3.3	3.3	1.8	2.3	1.1	2.5	2.3	2.0	2.0	2.5	2.5	2.3									
Kutikina hispida (2)	27	2.0	2.3	2.5	2.7	3.7	2.5	2.3	4.2	2.3	3.0	4.0	3.5	3.3	3.3	3.3	1.8	2.3	1.1	2.5	2.3	2.0	2.0	2.5	2.5	2.3	0.0								
Austropeplea lessoni NSW	28	19.1	19.3	19.8	20.0	20.0	19.4	19.8	21.1	19.7	19.0	19.5	19.1	19.4	19.6	19.9	20.7	20.4	19.9	19.9	19.8	20.2	19.6	19.9	19.8	19.8	23.5	23.5							
Austropeplea lessoni NT	29	19.8	20.1	20.2	20.4	20.5	19.9	20.3	21.5	19.9	19.0	19.9	19.1	18.7	19.0	19.3	20.9	20.6	20.1	20.6	20.5	21.2	20.4	20.6	20.3	20.3	23.8	23.8	4.6						
Austropeplea	30	19.0	19.2	19.7	19.9	20.0	19.4	19.8	21.0	19.9	19.0	19.4	19.1	19.3	19.6	19.9	20.7	20.4	19.9	19.9	19.8	20.1	19.6	19.9	19.8	19.8	23.5	23.5	0.2	4.6					

lessoni QLD

Austropeplea lessoni WA	31	21.2	21.5	21.7	21.8	22.0	21.3	21.8	23.1	21.3	20.4	21.3	20.6	20.2	20.4	20.8	22.4	22.2	21.6	22.1	22.0	22.7	21.8	22.1	21.8	21.8	25.3	25.3	5.2	0.2	5.2			
Austropeplea viridis	32	6.7	7.0	7.7	7.9	8.5	7.5	7.2	9.5	7.3	6.3	7.4	6.9	7.0	7.0	6.7	7.4	8.5	7.1	7.2	7.2	7.9	7.0	7.2	7.2	7.2	8.4	8.4	19.5	19.5	19.3	20.5		
Bullastra cumingiana	33	35.8	35.7	36.1	36.1	36.7	35.9	36.6	37.4	36.1	35.0	35.9	35.3	35.0	35.0	35.3	36.5	35.7	35.4	36.5	36.3	37.0	36.5	36.5	36.6	36.6	38.4	38.4	40.4	41.8	40.3	40.9	34.9	
Radix auricularia	34	12.3	12.3	13.4	13.7	14.5	13.1	13.2	15.3	12.5	13.4	14.1	14.0	13.6	13.3	13.6	13.2	13.4	12.6	13.0	12.7	14.1	12.8	13.0	13.3	13.2	14.2	14.2	24.2	23.8	24.1	24.6	12.6	37.6	-
Radix peregra	35	12.5	12.5	13.8	14.0	14.6	13.6	13.6	15.8	13.3	12.3	13.0	12.9	13.6	13.3	13.4	13.1	13.7	12.8	13.6	13.5	14.4	13.1	13.6	13.6	13.6	17.3	17.3	25.1	24.5	24.9	24.5	8.9	37.0	14.0

Appendix 2.7. Statistics for the anatomical analysis of the *Austropeplea tomentosa* complex dataset.

```
                                       Min   Tree  Max                                   G-
Character                       Range  steps steps steps    CI    RI    RC    HI   fit
-------------------------------------------------------------------------------------------
 1 shell umbilicus                1     1     3     3    0.333 0.000 0.000 0.667 0.600
 2 shell thickness                1     1     2     2    0.500 0.000 0.000 0.500 0.750
 3 number of whorls               4     4     7    12    0.571 0.625 0.357 0.429 0.500
 4 columella fold                 2     2     3     8    0.667 0.833 0.556 0.333 0.750
 5 Shell sculpture                1     1     1     1    1.000   0/0   0/0 0.000 1.000
 6 periostracum ornamentation     1     1     1     1    1.000   0/0   0/0 0.000 1.000
 7 broadest area of foot          1     1     4    12    0.250 0.727 0.182 0.750 0.500
 8 foot shape posterior end       1     1     3     6    0.333 0.600 0.200 0.667 0.600
 9 foot shape                     2     2     6    15    0.333 0.692 0.231 0.667 0.429
10 eye lobe                       2     2     2     3    1.000 1.000 1.000 0.000 1.000
11 tentacle shape                 3     3     6    14    0.500 0.727 0.364 0.500 0.500
12 lateral sides of snout         1     1     1     1    1.000   0/0   0/0 0.000 1.000
14 visceral coil pigmentation     1     1     4    10    0.250 0.667 0.167 0.750 0.500
15 mantle expnasion               3     3     5    19    0.600 0.875 0.525 0.400 0.600
16 exapanded mantle pigmentation  1     1     2     2    0.500 0.000 0.000 0.500 0.750
17 number of pnuemostomal ridges  1     1     1     1    1.000   0/0   0/0 0.000 1.000
18 anal flap                      1     1     1     2    1.000 1.000 1.000 0.000 1.000
19 upper plate of pnuemostome     1     1     1     2    1.000 1.000 1.000 0.000 1.000
20 broadest area of kidney        2     2     2     9    1.000 1.000 1.000 0.000 1.000
21 kidney shape                   2     2     4    16    0.500 0.857 0.429 0.500 0.600
22 right lobe of kidney           1     1     1     7    1.000 1.000 1.000 0.000 1.000
23 position of pulmonary vein     1     1     1     7    1.000 1.000 1.000 0.000 1.000
24 pulmonary vein length          2     2     3    11    0.667 0.889 0.593 0.333 0.750
25 ureter                         2     2     6    16    0.333 0.714 0.238 0.667 0.429
26 opening of kidney              1     1     1     1    1.000   0/0   0/0 0.000 1.000
27 buccal mass shape              1     1     1     1    1.000   0/0   0/0 0.000 1.000
28 cerebral commissure length     2     2     2     8    1.000 1.000 1.000 0.000 1.000
29 pedal commissure               1     1     5    11    0.200 0.600 0.120 0.800 0.429
30 pedal commissure extra lobe    1     1     1     4    1.000 1.000 1.000 0.000 1.000
31 statocysts                     1     1     1     1    1.000   0/0   0/0 0.000 1.000
32 radula sac                     2     2     5     9    0.400 0.571 0.229 0.600 0.500
33 salivary gland                 2     2     7    16    0.286 0.643 0.184 0.714 0.375
34 uterus/ vagina length          2     2     4    15    0.500 0.846 0.423 0.500 0.600
35 spermathecal duct length       2     2     6    16    0.333 0.714 0.238 0.667 0.429
36 spermathecal duct width        1     1     2     4    0.500 0.667 0.333 0.500 0.750
37 uterus shape                   1     1     3    10    0.333 0.778 0.259 0.667 0.600
38 oviducal caecum                3     3     7    10    0.429 0.429 0.184 0.571 0.429
39 oothecal gland shape           3     3     7    17    0.429 0.714 0.306 0.571 0.429
40 oviduct 1                      1     1     4     9    0.250 0.625 0.156 0.750 0.500
41 position of uterus/ vagina     2     2     3     9    0.667 0.857 0.571 0.333 0.750
42 velum shape                    1     1     1     1    1.000   0/0   0/0 0.000 1.000
43 penis sheath length            3     3     4    15    0.750 0.917 0.688 0.250 0.750
44 penis in penis sheath head     1     1     7    12    0.143 0.455 0.065 0.857 0.333
45 seminal vesicle                1     1     2     4    0.500 0.667 0.333 0.500 0.750
46 seminal vesicle shape          1     1     2     3    0.500 0.500 0.250 0.500 0.750
47 seminal vesicle shape          3     3     6    16    0.500 0.769 0.385 0.500 0.500
48 junction of vas deferens       1     1     2     7    0.500 0.833 0.417 0.500 0.750
49 prostate ventral wall          1     1     1     1    1.000   0/0   0/0 0.000 1.000
50 upper prostate                 1     1     1    12    1.000 1.000 1.000 0.000 1.000
51 length of prostate             2     2     4    13    0.500 0.818 0.409 0.500 0.600
52 shape of lower prostate        1     1     1     2    1.000 1.000 1.000 0.000 1.000
53 central tooth                  1     1     2     3    0.500 0.500 0.250 0.500 0.750
55 radula teeth shape             1     1     2     4    0.500 0.667 0.333 0.500 0.750
56 lateral teeth                  1     1     1     1    1.000   0/0   0/0 0.000 1.000
57 marginal teeth                 2     2     6    15    0.333 0.692 0.231 0.667 0.429

2 constant characters not shown
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245

Appendix 3.1. Matrix of anatomical characters used for phylogenetic analysis of the *Austropeplea lessoni* complex. Taxa without names are currently recognised as *A. lessoni*. See Appendix 2.1 for a description of each character and state.

```
NSW 1                    1 1 2 2 1 1 1 2 3 2   3 1 1 1 2 1 2 1 1 3   4 2 2 - 2 1   1    1      1 2 2 3     2 2 1 2 1   3 2 2 1 2 3   2 1 2   3 1 1 1 2 1 1 1 1 2   3 2 1     1 1 1 1 1
NSW 1 (2)                1 1 6 2 1 1 1 2 3 2 2&3 1 1 1 2 1 2 1 1 3   4 2 2 - 2 1 1&2    1  1&2 2 2 3     2 2 1 2 1   3 2 2 1 2 3   2 1 2   3 1 1 1 2 1 1 1 1 2   3 2 1     2 1 1 1 1
NSW 2                    1 1 6 2 1 1 1 2 3 2 2&3 1 1 2 2 1 2 1 1 3   4 2 2 - 2 1 1&2    1      1 2 2 3     2 2 1 2 1   3 2 2 1 2 3   2 1 2   4 1 1 1 2 1 1 1 1 2   3 2 1     1 1 1 1 1
NSW 5                    1 1 3 2 1 1 1 2 3 2 2&3 1 1 1 2 1 2 1 1 3   4 2 2 - 2 1   1    1  1&2 2 2 3     2 2 1 2 1   3 2 2 1 2 3   2 1 2   4 1 1 1 2 1 1 1 1 2   3 2 1     1 1 1 1 1
NT 1                     1 1 3 2 1 1 1 1 3 2   3 1 1 1 1 - 2 1 1 3   2 2 2 - 1 1   1    1  1&2 2 2 3     3 3 1 2 2   4 2 2 2 2 2   2 1 2 2&4 2 1 1 2 1 1 1 1 2   3 - 1     1 1 1 1 1
NT 2 (2)                 1 1 3 2 1 1 1 1 3 2   3 1 1 ? 1 - 2 1 1 3   4 2 2 - 1 1   1  1&2    1 2 2 3     3 3 1 2 1   3 2 2 2 2 2   2 1 2 2&4 2 1 1 2 1 1 1 1 2   3 - 1     3 1 1 1 1
QLD 1                    1 1 3 2 1 1 1 2 3 2 2&3 1 1 1 2 1 2 1 1 3   4 2 2 - 2 1   1    1      1 2 2 3     2 1 1 2 1   3 2 2 1 2 3   2 1 2   4 1 1 1 2 1 1 1 1 2   3 2 1     1 1 1 1 1
QLD 2 (2)                1 1 3 2 1 1 1 2 3 2   3 1 1 1 2 1 2 1 1 3   4 2 2 - 2 1   1    1  1&2 2 2 3     2 1 1 2 1   3 2 2 1 2 3   2 1 2   3 1 1 1 2 1 1 1 1 2   3 2 1     1 1 1 1 1
QLD 3                    1 1 3 2 1 1 1 2 3 2   3 1 1 1 1 - 2 1 1 3 1&2 2 2 - 2 1 1&2    1  1&2 2 2 3     2 1 1 2 1   3 2 2 1 2 3   2 1 2   3 1 1 1 2 1 1 1 1 2   3 2 1 1&3 1 1 1
QLD 4                    1 1 3 2 1 1 1 1 3 2   3 1 1 1 1 - 2 1 1 3   4 2 2 - 1 1   1    1  1&2 2 2 3     2 3 1 2 1   3 2 2 2 2 2   2 1 2   4 2 1 1 2 1 1 1 1 2   3 - 1     1 1 1 1 1
WA 1                     1 1 3 2 1 1 1 1 3 2   3 1 1 2 1 - 2 1 1 3   2 2 2 - 1 1   1    2  1&2 2 2 3     3 3 1 2 1   3 2 2 2 2 2   2 ? 2   4 2 1 1 2 1 1 1 1 2   3 - 1     1 1 1 1 1
WA 2                     1 1 3 2 1 1 1 1 3 3   ? 1 1 2 1 - 2 1 1 3 1&2 2 2 - 1 1   1    1      2 2 2 3 2&3 3 1 2 1 3&4 2 2 2 2 2   1 1 2   4 2 1 1 2 1 1 1 1 2   3 - 1     1 1 1 1 1
WA 4                     1 1 3 2 1 1 1 1 3 3   2 1 1 1 1 - 2 1 1 3   4 2 2 - 1 1   1    1  1&2 2 2 3     3 3 1 2 1   3 2 2 2 2 2   2 1 2   4 2 1 1 2 1 1 1 1 2   3 - 1     1 1 1 1 1
WA 5 (2)                 1 1 3 2 1 1 1 1 3 3   3 1 1 1 1 - 2 1 1 3   4 2 2 - 1 1   1    1      2 2 2 3     3 3 1 2 2   3 2 2 2 2 2   2 1 2   3 2 1 1 2 1 1 1 1 2   3 - 1     1 1 1 1 1
Austropeplea viridis     2 1 6 2 1 1 1 1 2 2   3 1 1 1 1 - 2 1 1 2   3 1 1 1 1 1   1    1      2 1 2 1     3 1 1 2 2   4 3 2 1 2 3   1 1 1   2 1 1 1 1 1 1 1 1 2   4 - 1     1 1 1 1 1
Radix auricularia        2 1 2 2 1 2 2 3 2   3 1 1 1 1 - 2 2 2 3     1 2 2 - 2 1   1    3      1 1 2 3     3 3 3 1 2   4 3 1 2 2 3   2 1 1   3 1 1 1 1 2 2 - 1 2     3 1 1     3 1 1 1 1
Radix peregra            1 1 2 2 1 2 2 3 2   3 1 1 1 1 - 2 2 2 3     1 2 2 - 1 1   1    ?      ? 1 2 3     ? 2 1 2 2   4 3 1 2 2 3   1 1 1   3 1 1 1 1 2 2 - 1 2     3 - 1     ? 1 1 1 1
Bullastra cumingiana     1 1 2 1 1 2 2 3 2   3 1 1 1 4 2 2 1 1 3     2 2 2 - 1 1   1    1      2 1 2 3     3 3 3 2 2   3 3 2 2 2 1   2 1 2   3 1 1 1 1 1 2 - 1 2     1 - 1     2 1 1 1 1
Austropeplea brevispira  1 1 3 1 1 1 2 2 3 2   3 1 1 1 2 1 2 2 2 3   4 2 2 - 1 1   1    2      1 1 2 3     2 1 3 2 2   3 1 1 2 2 2   2 1 1   4 1 1 1 1 1 1 ? 1 2     ? - 1     2 1 1 1 1
Radix rubiginosa         1 2 6 2 1 1 1 2 3 2   3 1 1 1 1 - 2 2 2 3     3 2 2 - 1 1   1    2      2 1 2 1     2 2 3 2 1   2 1 2 1 2 2   1 1 2   3 1 1 1 1 1 2 - 1 2 3&4 - 1     1 1 1 1 1
Radix quadrasi           1 2 3 2 1 1 1 2 3 2   3 1 1 2 2 2 2 2 2 3   2 2 2 - 1 1   1    2  1&2 1 2 1     2 3 2 2 2   4 1 2 1 2 4   2 1 2   3 1 1 1 2 1 1 1 1 2     3 - 1     1 1 1 1 1
Stagnicola elodes        1 ? 8 2 1 1 ? 2 ? 2   3 1 1 ? 1 - 2 1 2 2   3 1 1 3 2 1   ?    ?      ? 1 2 ?     ? 1 1 2 2   2 2 3 1 2 4   ? 1 2   2 1 1 2 2 2 2 - 1 1     5 2 1     ? 1 1 1 2
Stagnicola catascopium   ? 1 7 2 1 1 1 2 2 2   3 1 1 2 1 - 2 1 2 3   3 1 1 3 2 1   1    1      2 1 2 3     3 1 3 2 2   2 3 2 1 2 2 1&2 1 2 3 1 1 1 2 ? 1 1 1 1     2 1 2     2 2 2 2 2
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Appendix 3.2 Alignment of 16S rRNA used for phylogenetic analysis of the *Austropeplea lessoni* complex. Taxa without names are currently recognised as *A. lessoni*.

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                       ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                            10         20         30         40         50         60         70         80         90        100
NSW 1                  AGGAAATTTT TGTTCGAACA GAACATTCTA TTTTGACTGT TAGTCTTCTA GTTCCTAGTC CAACATCGAG GTCACAAGCT AAAAATTTTA TTATGCTGTT
NSW 2                  AGGAAATTTT TGTTCGAACA GAACATT--A TTTTGACTGT TAGTCTTCTA GTTCCTAGTC CAACATCGAG GTCACAAGCT AAAAATTTTA TTATGCTGTT
NSW 3                  AGGAAATTTT TGTTCGAACA GAACATTCTA TTTTGACTGT TAGTCTTCTA GTTCCTAGTC CAACATCGAG GTCACAAGCT AAAAATTTTA TTATGCTGTT
NSW 4                  AGGAAATTTT TGTTCGAACA GAACATTCTA TTTTGACTGT TAGTCTTCTA GTTCCTAGTC CAACATCGAG GTCACAAGCT AAAAATTTTA TTATGCTGTT
NT 1                   AGGAAATTTT TGTTCGAACA GAACATTCTA TTTTGACTGT TAGTCTTCCA GTTCCTAGTC CAACATCGAG GTCACAAGCT AAAAATTTTA TTATGCTGTT
NT 2                   AGGAAATTTT TGTTCGAACA GAACATTCTA TTTTGACTGT TAGTCTTCTA GTTCCTAGTC CAACATCGAG GTCACAAGCT AAAAATTTTA TTATGCTGTT
NT 3                   AGGAAATTTT TGTTCGAACA GAACATTCTA GGTTGACTGT TAGTCTTCTA GTTCCTAGTG CAACATCGAG GTCACAAGCT AAAAATTTTA TTATGCTGTT
NT 4                   AGGAAATTTT TGTTCGAACA GAACATTCTA TTTTGACTGT TAGTCTTCTA GTTCCTAGTC CAACATCGAG GTCACAAGCT AAAAATTTTA TTATGCTGTT
NT 5                   AGGAAATTTT TGTTCGAACA GAACATTCTA TTTTGACTGT TAGTCTTCTA GTTCCTAGTC CAACATCGAG GTCACAAGCT AAAAATTTTA TTATGCTGTT
QLD 1                  AGGAAGTTTT TGTTCGAACA GAACATTCTA TTTTGACTGT TAGTCTTCTA GTTCCTAGTC CAACATCGAG GTCACAAGCT AAAAATTTTA TTATGCTGTT
QLD 2                  AGGAAGTTTT TGTTCGAACA GAACATTCTA TTTTGACTGT TAGTCTTCTA GUTCCTAGTC CAACATCGAG GTCACAAGCT AAAAATTTTA TTATGCTGTT
QLD 3                  AGGAAGTTTT TGTTCGAACA GAACATTCTA TTTTGACTGT TAGTCTTCTA GTTCCTAGTC CAACATCGAG GTCACAAGCT AAAAATTTTA TTATGCTGTT
WA 1                   AGGAAATTTT TGTTCGAACA GAACATTCTA TTTTGACTGT TAGTCTTCTA GTTCCTAGTC CAACATCGAG GTCACAAGCT AAAAATTTTA TTATGCTGTT
WA 1 2                 AGGAAATTTT TGTTCGAACA GAACATTCTA TTTTGACTGT TAGTCTTCTA GTTCCTAGTC CAACATCGAG GTCACAAGCT AAAAATTTTA TTATGCTGTT
WA 2                   AGGAAATTTT TGTTCGAACA GAACATTCTA TTTTGACTGT TAGTCTTCTA GTTCCTAGTC CAACATCGAG GTCACAAGCT AAAAATTTTA TTATGCTGTT
WA 3                   AGGAAATTTT TGTTCGAACA GAACATTCTA TTTTGACTGT TAGTCTTCTA GTTCCTAGTC CAACATCGAG GTCACAAGCT AAAAATTTTA TTATGCTGTT
WA 5                   AGGAAATTTT TGTTCGAACA GAACATTCTA TTTTGACTGT TAGTCTTCTA GTTCCTAGTC CAACATCGAG GTCACAAGCT AAAAATTTTA TTATGCTGTT
Austropeplea viridis   AGGAAACTGT TGTTCGAACA GAACAATCTA TTTTGACGGT TAATCTTTTA GTTCCTAGTC CAACATCGAG GTCATAAGCT ATATTAATCA TTATGCTGTT
Bullastra cumingiana   AGGAAAAATC TGTTCGAACA GAACATTCTA TTTTGACTGT TAGTCTTCTA GTTCCTAGTC CAACATCGAG GTCACAAGCT AAAAATTTTA TTATGCTGTT
Radix auricularia      AGGAGAAATT -GTTCGAACA GAACACTCTA TTTTGACTGT TAGTCCTTTA GTTCCTAGTC CAACATCGAG GTCATAAGCT AATAAATTCA TTATGCTGTT
Radix peregra          ?????????? ????????CA GAACACTCTA TTTTGACGGT TAGTCTTTTA GTTCCTAGTC CAACATCGAG GTCACAAGCT AATAATTTCA TTATGCTGTT
Radix quadrasi         AGGAGTTAAA TGTTCGAACA GAACAATCTA GTTTGACGGT TAGTCTAATA GTTCCTAGTC CAACATCGAG GTCACAAACT ATAATAATCA TTATGCTGTT
Radix rubiginosa       ?????????? ????CGAACA GAACAATCTA ATTTGACGGT TAGTCTAATA GTTCCTAGTC CAACATCGAG GTCACAAACT ATAATAATCA TTATGCTGTT
Stagnicola elodes      ?????????? ????????CA GAACAAACTA TTTTGACGGT TAGTCTTTTA GTTCCTAGTC CAACATCGAG GTCACAAACT AAAAAATTAA TTATGCTGTT
Stagnicola emarginata  ?????????? ?????????? ?????????? ?TTTGACGGT TAGTCTTCTA GTTCCTAGTC CAACATCGAG GTCACAAACT AAAAAATTAA TTATGCTGTT
Stagnicola caperata    ?????????? ?????????? ????CAATCT ATTTGACGGT TAGTCAACTA GTTCCTAGTC CAACATCGAG GTCACAAACT AAATAATTAA TTATGCTGTT


                       ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                           110        120        130        140        150        160        170        180        190        200
NSW 1                  ATCCCTAAGG TAATTTAATC TAACAAAAAA A--TGACTTG TAATTATT-- AAAAGTTTAA AA---TGTTT TATTGTCGCC CCAACAAAAA TAAAAAG--A
NSW 2                  ATCCCTA-GG TAATTTAATC TAACAAAAAA A--TGACTTG TAATTATT-- AAAAGTTTAA AA---TGTTT TATTGTCGCC CCAACAAAAA TAAAAAA--A
NSW 3                  ATCCCTAAGG TAATTTAATC TAACAAAAAA AAATGACTTG TAATTATT-- AAAAGTTTAA AA---TGTTT TATTGTCGCC CCAACAAAAA TAAAAAA--A
NSW 4                  ATCCCTAAGG TAATTTAATC TAACAAAAAA A--TGACTTG TAATTATT-- AAAAGTTTAA AA---TGTTT TATTGTCGCC CCAACAAAAA TAAAAAG--A
NT 1                   ATCCCTAAGG TAATTTAATC TAACAAAAAA A--TGACTTG TAATTATT-- AAAAGTTTAA AA---TGTTT TAATGTCGCC CCAACAAAAA TAAAAAA--A
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NT 2                    ATCCCTAAGG TAATTTAATC TAACAAAAAA A--TGACTTG TAATTATT-- AAAAGTTTAA AAA--TGTTT TAATGTCGCC CCAACAAAAA TAAAAAA--A
NT 3                    ATCCCTAAGG TAATTTAATC TAACAAAAAA A--TGGCTTG TAATTATT-- AAAAGTTGAA AA---TGTTT TAATGTCGCC CCAACAAAAA TAAAAAA--A
NT 4                    ATCCCTAAGG TAATTTAATC TAACAAAAAA A--TGACTTG TAATTATT-- AAAAGTTTAA AA---TGTTT TAATGTCGCC CCAACAAAAA TAAAAAA--A
NT 5                    ATCCCTAAGG TAATTTAATC TAACAAAAAA AA-TGACTTG TAATTATT-- AAAAGTTTAA AA---TGTTT TAATGTCGCC CCAACAAAAA TAAAAAA--A
QLD 1                   ATCCCTAAGG TAATTTAATC TAACAAAAAA AA-TGACTTG TAATTATT-- AAAAGTTTAA AA---TGTTT TATTGTCGCC CCAACAAAAA TAAAAAA--A
QLD 2                   ATCCCTAAGG TAATTTAATC TAACAAAAAA A--TGACT-G TAATTATT-- AAAAGTTTAA AA---TGTTT TATTGTCGCC CCAACAAAAA TAAAAAA--A
QLD 3                   ATCCCTAAGG TAATTTAATC TAACAAAAAA AAATGACTTG TAATTATT-- AAAAGTTTAA AAA--TGTTT TATTGTCGCC CCAACAAAAA TAAAAAA--A
WA 1                    ATCCCTAAGG TAATTTAATC TAACAAAAAA AA-TGACTTG TAATTATT-- AAAAGTTTAA AA---TGTTT TAATGTCGCC CCAACAAAAA TAAAAAA--A
WA 1 2                  ATCCCTAAGG TAATTTAATC TAACAAAAAA A--TGACTTG TAATTATT-- AAAAGTTTAA AA---TGTTT TAATGTCGCC CCAACAAAAA TAAAAAA--A
WA 2                    ATCCCTAAGG TAATTTAATC TAACAAAAAA A--TGACTTG TAATTATT-- AAAAGTTTAA AA---TGTTT TAATGTCGCC CCAACAAAAA TAAAAAA--A
WA 3                    ATCCCTAAGG TAATTTAATC TAACAAAAAA AAATGACTTG TAATTATT-- AAAAGTTTAA AA---TGTTT TAATGTCGCC CCAACAAAAA TAAAAAA--A
WA 5                    ATCCCTAAGG TAATTTAATC TAACAAAAAA AAATGACTTG TAATTATT-- AAAAGTTCAA AA---TGTTT TAATGTCGCC CCAACAAAAA TAAAAAA--A
Austropeplea viridis   ATCCCTAAGG TAATTTTACC ATACAAAAAA AA-TGACATG TTTTTATAT- GAAAGTTTAA A----TGTTT CATTGTCGCC CCAACAAAAA TAAGAGAT-T
Bullastra cumingiana   ATCCCTAAGG TAATTTATTC TTACAAATAA A--TGACTTG TTTAATTT-- GAAAGTTTTA A----TGTTT CAATGTCGCC CCAACAAAAA TAAATAA--A
Radix auricularia      ATCCCTAAGG TAATTTGATC GTGCAAAGAA AATTGTTGTG TAAAAATCTT GAAAGTTTAA TA---TGTTT CAATGTCGCC CCAACAAAAA TAAATCTT-A
Radix peregra          ATCCCTAAGG TAATTTGATC ATTCAAAATA AA-TGTCTCG TAAAAATTGT GAAAGTTCAA AATTTTGTTT CAATGTCGCC CCAACAAAAA TAAGTTTA-C
Radix quadrasi         ATCCCTAAGG TAATTTTATC TTACAAAACT TAATGTCCAG TAGAATATT- TAAAGTTTAA A----TGTTT AAATGTCGCC CCAACAAAAA AATAAACT-T
Radix rubiginosa       ATCCCTAAGG TAATTTTATC TTACAAAACT TAATGTCCAG TAGAATATT- TAAAGTTTAA A----TGTTT AAATGTCGCC CCAACAAAAA AATAAACT-T
Stagnicola elodes      ATCCCTAAGG TAATTTAACC TTTCAAATCT TAATGTCATG TAAAAAAA-- GAAAGTTTAA A----TGTTT CAATGTCGCC CCAACAAAAA AATAATAT-T
Stagnicola emarginata  ATCCCTAAGG TAATTTAATC TTTCAAATCT TAATGTCATG TGAAAAAA-- GAAAGTTTAA A----TGTTT CAATGTCGCC CCAACAAAAA AATAAAAA-T
Stagnicola caperata    ATCCCTAAGG TAATTTACTC TTTCAAACCT TAATGTCGTG AAGAAGA--- GAAAGTTTAA G----TGTTT CAATGTCGCC CCAACAAAAA AATAAT-A-T


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                             210        220        230        240        250        260        270        280        290        300
NSW 1                  TTATAAACCT TAATTAATGA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TAGTAGAAGT ATTTTCACTT CATAAATAAA TTCAAATAAA TTTAAAA-GA
NSW 2                  TTATAAACCT TAATTAATGA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TAGTAGAAGT ATTTTCACTT CATAAATAAA TTCAAATAAA TTTAAAA-GA
NSW 3                  TTATAAACCT TAATTAATAA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TAGTAGAAGT ATTTTCACTT CATAAATAAA TTCAAATAAA TTTAAAA-GA
NSW 4                  TTATAAACCT TAATTAATGA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TAGTAGAAGT ATTTTCACTT CATAAATAAA TTCAAATAAA TTTAAAA-GA
NT 1                   TTATAAACTT TAATTAATGA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TAGTAGAAGT ATTTTCACTT CATAAATAAA TTCAAATAAA TTTAAAA-GA
NT 2                   TTATAAACTT TAATTAATGA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TAGTAGAAGT ATTTTCACTT CATAAATAAA TTCAAATAAA TTTAAAA-GA
NT 3                   TTATAAACTT TAATTAATGA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TAGTAGAAGT ATTTTCACTT CATAAATAAA TTCAAATAAA TTTAAAA-GA
NT 4                   TTATAAACCT TAATTAATGA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TAGAAGAAGT ATTTTCACTT CATAAATAAA TTCAAATAAA TTTAAAA-GA
NT 5                   TTATAAACCT TAATTAATGA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TAGTAGAAGT ATTTTCACTT CATAAATAAA TTCAAATAAA TTTAAAA-GA
QLD 1                  TTATAAACCT TAATTAATGA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TAGTAGAAGT ATTTTCACTT CATAAATAAA TTCAAATAAA TTTAAAA-GA
QLD 2                  TTATAAACCT TAATTAATGA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TAGTAGAAGT ATTTTCACTT CATAAATAAA TTCAAATAAA TTTAAAA-GA
QLD 3                  TTATAAACCT TAATTAATGA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TAGTAGAAGT ATTTTCACTT CATAAATAAA TTCAAATAAA TTTAAAA-GA
WA 1                   TTATAAACTT TAATTAATGA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TAGTAGAAGT ATTTTCACTT CATAAATAAA TTCAAATAAA TTTAAAA-GA
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WA 1 2                TTATAAACTT TAATTAATGA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TAGTAGAAGT ATTTTCACTT CATAAATAAA TTCAAATAAA TTTAAAA-GA
WA 2                  TTATAAACTT TAATTAATGA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TAGCAGAAGT ATTTTCACTT CATAAATAAA TTCAAATAAA TTTAAAA-GA
WA 3                  TTATAAACTT TAATTAATGA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TAGCAGAAGT ATTTTCACTT CATAAATAAA TTCAAATAAA TTTAAAA-GA
WA 5                  TTATAAACTT TAATTAATGA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TAGCAGAAGT ATTTTCACTT CATAAATAAA TTCAAATAAA TTTAAAA-GA
Austropeplea viridis  TAAAAAACCT T--TTAATAA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT AATAAAAAGT ATTTTCACTT TATAAATAAA TTCATATAAG CTAAAAA-GA
Bullastra cumingiana  TTATAAAATT TAATTAATTA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT AAATAAAAGT ATTTTCACTT TTTAAATAAA TTCAAAAAAA TTTTAAA-GA
Radix auricularia     AATAATAAAA T--AAAATTA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT AGATAAAAGT ATTTTCACTT TATAAATAAA TTCACAAAAA TTAAAAA-GA
Radix peregra         AATAAAGTAA AAG-AAATTA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TTAAAAAAGT ATTTTCACTT AATAAATAAA TTCATAGAAA TTAAAAAAGA
Radix quadrasi        TAAAAAA-GT T-GTTATTAA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TTAATTAAGT ATTTTCACTT ACTAAATAAA TTCAATAAAA TTTAAAG-AA
Radix rubiginosa      TAAAAAA-GT T-GTTATTAA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TTAATTAAGT ATTTTCACTT ACTAAATAAA TTCAATAAAA TTTAAAG-AA
Stagnicola elodes     AAAAATA--GT T---TATTAA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT ACGTATAAGC ATTTTCACTT AATAAATAAA TTCAAAAAAA TATTATA-AA
Stagnicola emarginata AAAAATAT-TT T--TTATTAA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT ACGTGTAAGT ATTTTCACTT AATAAATAAA TTCAAAAAAA TAACATA-AA
Stagnicola caperata   AAATGTATT- ---TTATTAA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TAATGTAAGT ATTTTCACTT ACTAAATAAC TTCAAAAAAA TTCCCCT-GA


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                          310        320        330        340        350        360        370        380        390        400
NSW 1                 GACAGCTAAT TCTTTATTTA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATAAT
NSW 2                 GACTGCTAAT TCTTTATTTA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATAAT
NSW 3                 GACAGCTAAT TCTTTATTTA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATAAT
NSW 4                 GACAGCTAAT TCTTTATTTA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATAAT
NT 1                  GACAGCTAAT TCTTTATTTA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATAAT
NT 2                  GACAGCTAAT TCTTTATTTA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATAAT
NT 3                  GACAGCTAAT TCTTTATTTA ACCTTTCATT CCAGGCTTCA ATTAAAAGCC AATTGATTAT GCT-CCTTAG -ACAGTCAAG GTACTGCGGC CGCTAAAAAT
NT 4                  GACAGCTAAT TCTTTATTTA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATAAT
NT 5                  GACAGCTAAT TCTTTATTTA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAA GTACTGCGGC CGCTAATAAT
QLD 1                 GACAGCTAAT TCTTTATTTA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATAAT
QLD 2                 GACAGCTAAT TCTTTATTTA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATAAT
QLD 3                 GACAGCTAAT TCTTTATTTA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATAAT
WA 1                  GACAGCTAAT TCTTTATTTA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAGTAAT
WA 1 2                GACAGCTAAT TCTTTATTTA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATGAT
WA 2                  GACAGCTAAT TCTTTATTTA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATAAT
WA 3                  GACAGCTAAT TCTTTATTTA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATAAT
WA 5                  GACAGCTAAT TCTTTATTTA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATAAT
Austropeplea viridis  GACAGAAAAT TCTTTAACAA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATACA
Bullastra cumingiana  GACAGCTTAT TCTTTATTAA ACCTTTCATT CCAGACTCCA ATTAAAAGCC AACTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTGATGTG
Radix auricularia     GACAGTAAAT TCTTTATTTA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATTTT
Radix peregra         GACAGTTAAT TTTTTATTTA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATAAT
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Radix quadrasi        GACAGAAAAT TCTTTATTAA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGTTAACAAA
Radix rubiginosa      GACAGAAAAT TCTTTATTAA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGTTAACAAA
Stagnicola elodes     GACAGTGAAT TCCCATTAAA CCCCTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATATT
Stagnicola emarginata GACAGTAAAT TCCCATTAA- CCCGTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATATT
Stagnicola caperata   GACAGCTACT CCCCATCAGT -CCGTTCATT CCAGACTTCA ATTAAAAGCC AACTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGTAATATTA


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                           410        420        430        440
NSW 1                 -------ACG CTGGGCAGAA TTCACCTAAA ATAAGTT-CT TCAGACT
NSW 2                 -------ACG CTGGGCAGAA TTCACCTAAA ATAAGTT-CT TCAGACT
NSW 3                 -------ACG CTGGGCAGAA TTCACCTAAA ATAAGTT-CT TCAGACT
NSW 4                 -------ACG CTGGGCAGAA TTCACCTAAA ATAAGTT-CT TCAGACT
NT 1                  -------ACG CTGGGCAGAA TTCACCTAAA ATAAGTT-CT TCAGGCT
NT 2                  -------ACG CTGGGCAGAA TTCACCTAAA ATAAGTT-CT TCAGGCT
NT 3                  -------ACG CTGGGCAGAA TTCACCTAAA ATAAGTT-CT TCAGGCT
NT 4                  -------ACG CTGGGCAGAA TTCACCTAAA ATAAGTT-CT TCAGGCT
NT 5                  -------ACG CTGGGCAGAA TTCACCTAAA ATAAGTT-CT TCAGGCT
QLD 1                 -------ACG CTGGGCAGAA TTCACCTAAA ATAAGTT-CT TCAGGCT
QLD 2                 -------ACG CTGGGCAGAA TTCACCTAAA ATAAGTT-CT TCAGGCT
QLD 3                 -------ACG CTGGGCAGAA TTCACCTAAA ATAAGTT-CT TCAGGCT
WA 1                  -------ACG CTGGGCAGAA TTCACCTAAA ATAAGTT-CT TCAGGCT
WA 1 2                -------ACG CTGGGCAGAA TTCACCTAAA ATAAGTT-CT TCAGGCT
WA 2                  -------ACG CTGGGCAGAA TTCACCTAAA ATAAGTT-CT TCAGGCT
WA 3                  -------ACG CTGGGCAGAA TTCACCTAAA ATAAGTT-CT TCAGGCT
WA 5                  -------ACG CTGGGCAGAA TTCACCTAAA ATAAGTT-CT TCAGGCT
Austropeplea viridis  TA-----ACG CTGGGCAGAA TTCACTTAAA ATGTATC-CT TCAAGCT
Bullastra cumingiana  TTT----ACG CTGGGCAGAA TTCACCTAAA ATATGTC-CT CTAGGCT
Radix auricularia     TTAACCAACG CTGGGCAGAA TTCACTTAAA ATATAAT-CT TTAAGCT
Radix peregra         TTT----ACG CTGGGCAGAA CTTACTTAAA ATAAATTTCT TTAAGCT
Radix quadrasi        TTATT--GCG CTGGGCAGAA TTTACCTAAA ?????????? ???????
Radix rubiginosa      TTATT--GCG CTGGGCAGAA TTTACCTAAA ?????????? ???????
Stagnicola elodes     ATTT---ACG CTGGGCAGA- TT-ACCAGTG GT???????? ???????
Stagnicola emarginata ATTTT--ACG ?????????? ?????????? ?????????? ???????
Stagnicola caperata   CGCT?????? ?????????? ?????????? ?????????? ???????
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Appendix 3.3. Alignment of ITS-2 rRNA used for phylogenetic analysis of the *Austropeplea lessoni* complex. Taxa without names are currently recognised as *A. lessoni*. All characters with an astertix above them where excluded from all phylogenetic analyses

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                              *       ***         **** ********** *******                     *
                         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                             10        20        30        40        50        60        70        80        90       100

NSW_1                    GCTAGTGTTA AAACAATCGG -GTCGC---T TGCTCTCGTA GCGACG---- ---------- -------CGC ACTGGACCAT CGCGGCC-GC TCACCGAATC
NSW_2                    GCTAGTGGTT AA-CAATCGC -GTCGC---T TGCTCTCG-A GCGACG---- ---------- -------CGC CCTGGACCAT CGCGGCC-GC TCACTGAATC
NSW_3                    GCTAGTGTTA AAACAATCGC -GTCGC---T TGCTCTCGTA GCGACG---- ---------- -------CGC CCTGGACCAT CGCGGCC-GC TCACCGAATC
QLD_1                    GCTAGTGTTA AAACAATCGC -GTCGC---T TGCTCTCGTA GCGACG---- ---------- -------CGC ACTGGACCAT CGCGGCC-GC TCACCGAATC
QLD_2                    GCTAGTGTTA AAACAATCGC -GTCGC---T TGCTCTCGTA GCGACG---- ---------- -------CGC CCTGGACCAT CGCGGCC-GC TCACCGAATC
QLD_3                    GCTAGTGTTA AAACAATCGC -GTCGC---T TGCTCTCG-A GCGACG---- ---------- -------CGC CCTGGACCAT CGCGGCC-GC TCACCGAATC
NT_1                     GCTAGTGTTA AAACAATCGT -GTCGC---T TGCTCTCGTT GCGACG---- ---------- -------CGC CCTGGACCAT CGCGGCC-GC TCACCGAAAC
NT_2                     GCTAGTGTTA AAACAATAGT -GTCGC---T TGCTCTCGTT GCGACG---- ---------- -------CGC CCTGGACCAT CGAGGCC-GC TCACCGAAAC
NT_3                     GCTAGTGTTA AAACAATCGT -GTCGC---T TGCTCTCGTT GCGACG---- ---------- -------CGC CCTGGACCAT CGCGGCC-GC TCACCGAAAC
NT_5                     GCTAGTGTTA AAACAATCGT -GTCGC---T TGCTCTCGTT GCGACG---- ---------- -------CGC CCTGGACCAT CGCGGCC-GC TCACCGAAAC
WA_1                     GCTAGTGTTA AAACAATCGT -GTCGC---T TGCTCTCGTT GCGACG---- ---------- -------CGC CCTGGACCAT CGCGGCC-GC TCCCCGAATC
WA_2                     GCTAGTGTTA AAACAATCGT -GTCGC---T TGCTCTCGTT GCGACG---- ---------- -------CGC CCTGGACCAT CGCGGCC-GC TCACCGAAAC
WA_3                     GCTAGTGTTA AAACAAATCG -GTCGC---T TGCTCTCGTT GCGACG---- ---------- -------CGC CCTGGACCAT CGCGGCC-GC TCACCGAAAC
WA_4                     GCTAGTGTTA AAACAAATCG -GTCGC---T TGCTCTCGTT GCGACG---- ---------- -------CGC CC-GGACCAT CGCGGCC-GC TCACCGAAAC
WA_5                     GCTAGTGTTA AAACAATCGG -GTCGC---T TGCTCTCGTT GCGACG---- ---------- -------CGC CCTGGACCAT CGCGGCC-GC TCACCGGATC
WA_6                     GCTAGTGTTA AAACAATCGT -GTCGC---T TGCTCTCGTT GCGACG---- ---------- -------CGC CCTGGACCAT CGCGGCC-GC TCACCGAAAC
Austropeplea viridis     GCTAGTGTCA AAACAATCGC -GTCGC---T TGCTCGCG-- -CGACG---- ---------- -------CGC CCTGGACCTT CGCGGCCCGT TAAATCCGGC
Bullastra cumingiana     ????GTGTTA AAACAATCGC CGTCGCCCGT TGCTCTCGTG GCGACG---- ---------- -------CGC CCTGGACCGT CGCGGTC-GC AAAATCCGGC
'Lymnaea' brevispina     GCTAGTGTCA AA-CAATTGG -GTCGC---T CGTTCGTG-- -GGACC---- ---------- -------CGG TATGGACCTT CGCAGCC-AA TAAATCGGGC
Radix auricularia        GCTAGTGT-C AAACAATCGT -GTCGCT--T TGCTCGTG-- -CGACG---- ---------- -------CGC TCTGGTCCGT CGCGGCC-AT AAAATCCAGC
Radix peregra            GCTAGTGTCA AA-CAATCGC -GTCGC---T TGCTCTTG-- -CGACG---- ---------- -------CGC TCTGGACCTT CGCGGCC-GT AAAATCCGGC
Radix rubiginosa         GCTAGAGTCA AAACAATCGT -GTCGCT--T TGCTCGTG-- -CGACG---- ---------- -------CGC TCTGGTCCGT CGCGGCC-AT AAAATCCAGC
Radix quadrasi           ?????????A AAACAATCGT -GTCGCT--C TGTTCTTG-- -CGACG---- ---------- -------CGC TCTGGTCCGT CGCTGCC-AT AAAATCCAGC
Stagnicola caperata      ??TAGTCACA AAGCAATCGT -GTCCT--GT AGCTCTCGCA AAACTGGAGC CGTCTCCC-- ----CCTGGC ACACACCGTC TCCGACTTGC TCGTTGGAG-
Stagnicola elodes        ??TAGTCACA AAGCAATCGT -GTCCTT-GC AGCTCTCGCA GGACCGGAGC CTTCCGCCGT GGACTCTCAT TCACAGCGCC TCCGACTTGC TCGTCGGGGT
Stagnicola emarginat     ??TAGTCACA AAGCAATCGT -GTCCTT-GC AGCTCTCGCA GGACCGGAGC CTTCCGCCGT GGACTCTCAT TCACAGCGCC TCCGACTTGC TCGTCGGGGT
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                             *******             *********    **** *                 ***** ********** ********** **********
                         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                            110       120       130       140       150       160       170       180       190       200

NSW_1                    TT-------- CCTTCCTTCC ATCCTTGCTC TCA----CGG ATGGAT---- -TGGGGATTG TTTCA----- ---------- ---------- ----------
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NSW_2               TT-------- CCTTCTTTCC ATCCTTGCTC TCA----CGG ATGGAT---- -TGGGGATTG TTTCA----- ---------- ---------- ----------
NSW_3               TT-------- CCTTCCTTCC ATCCTTGCTC TCA----CGG ATGGAT---- -TGGGGATTG TTTCA----- ---------- ---------- ----------
QLD_1               TT-------- CCTTCCTTCC ATCCTTGCTC TCA----CGG ATGGAT---- -TGGGGATTG TTTCA----- ---------- ---------- ----------
QLD_2               TT-------- CCTTCCTTCC ATCCTTGCTC TCA----CGG ATGGAT---- -TGGGGATTG TTTCA----- ---------- ---------- ----------
QLD_3               TT-------- CCTTCCTTCC ATCCTTGCTC TCA----CGG ATGGAT---- -TGGGGATTG TTTCA----- ---------- ---------- ----------
NT_1                TT---TCCTT CGTTCCTTCC TTCCTTCCTT GCTCTCGCGG ATGGATGGGG ATAGGGATAG GGATAGGGAT ---------- ---------- ---AGGGATA
NT_2                CT---TCGTT CCTTCCTTCC TTCCTTGCTC TC----GCGG ATGGATGGGG ATAGGGATAG GGATAGGGAT ---------- ---------- ---AGGG---
NT_3                TT---TCCTT CGTTCCTTCC TTCCTTCCTT GCTCTCGCGG ATGGATGGGG ATAGGGATAG GGATAGGGAT ---------- ---------- ---AGGGATA
NT_5                CT---TCCTT CGTTCCTTCC TTCCTTCCTT CCTTGCTCGG ATGGATGGGG ATAGGGATAG GGATAGGGAT ---------- ---------- ---AGGGATA
WA_1                TTCCTTCCTT CCTTCCTTCC CTCCTTCCTT GCTCTCGCGG ATGGATGGGG ATAGGGATAG GGATAGGGAT ---------- ---------- ---AGGGATA
WA_2                TT---TCCTT CGTTCCTTCC TTCCTTCCTT GCTCTCGCGG ATGGATGGGG ATAGGGATAG GGATAGGGAT ---------- ---------- ---AGGGATA
WA_3                CT---TCCTT CGTTCCTTCC TTCCTTGCTC TCG----CGG ATGGATGGGG ATAGGGATAG GGATAGGG-- ---------- ---------- ----------
WA_4                CT---TCCTT CGTTCCTTCC TTCCTTGCTC TCG----CGG ATGGATGGGG ATAGGGATAG GGATAGGGAT ---------- ---------- ---AGGGATA
WA_5                TT----CCTT CCTTCCTTCC TTCCTTGCTC TCG----CGG ATGGATGGGG ATGGGGATGG GGATAGGGAT ---------- ---------- ---AGGG---
WA_6                TT---TCCTT CGTTCCTTCC TTCCTTCCTT GCTCTCGCGG ATGGATGGGG ATAGGGATAG GGATAGGGAT ---------- ---------- ---AGGGATA
Austropeplea viridis  GC-------- ----TCACCG AATCCCGCTT T--------G CTCGGCGGTG -TTGGTGTTG CGCC------ ---------- ---------- ----------
Bullastra cumingiana  GG-----CGG CTCTGACCGT AGCATCGCTC TCCGCTTCGG TTTGCCGTCG GTGGCCCGTG GTCTCAAGC- ---------- ---------- ----------
'Lymnaea' brevispina  GC-------- --------TC ACCCTCGCTC ---------G CTGGGCG--- ---------G TGTCG----- ---------- ---------- ----------
Radix auricularia     GT-------- --TCACCGCC CTCATCGCTT T--------G CTCGGCGATG -TCGTGTGTG TGTTGTG--- ---------- ---------- ----------
Radix peregra         GC-------- ------TCAC CGAATCGCTC ---------G CTCGGC---- --GGTTTGCG TGTTGCG--- ---------- ---------- ----------
Radix rubiginosa      GT-------- --TCACCGCC G-CATCGCTT T--------G CTCGGCGATG -TCGTGTGTG TGATGTG--- ---------- ---------- ----------
Radix quadrasi        CT-------- --TCACCGCC G-CATTTCAT T--------G CTGGGCGACG -TTGCTTGTG GGATTTG--- ---------- ---------- ----------
Stagnicola caperata   ACGCGACGCT GGGGGGAGTA GGCACCGGTT GGACACGC-C CTGGACCCTC GCGGCCTATA CCGTCGTCGC CGCCGCCTTC CCTGGTGGTT GGTGGCGGTG
Stagnicola elodes     GCTTGGGGGA CCCGTCGTTC GGCACCGGTC GGACACGC-C CTGGACCCTC GCGGCCTACA CCGTCGCTGC ---------- ---------- --TTGCGGTG
Stagnicola emarginat  GCTTGGGGGA CCCGTCGTTC GGCACCGGTC GGACACGC-C CTGGACCCTC GCGGCCTACA CCGTCGCTGC ---------- ---------- --TTGCGGTG


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                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                       210        220        230        240        250        260        270        280        290        300
NSW_1               --TTGAGTGG ----CCCCGT GGTCTTAAGC ACATGCCGCG CCGTTTGTCC GTGCTCG-TC TCGGGCCGTC CGCCTTTGCT CTCGGCGGCG TCCGCCCACA
NSW_2               --TTGAGTGG ----CCCCGT GGTCTTAAGC ACATGCCGCG CCGTTTGTCC GTGCTCG-TC TCGGGCCGTC CGCCTTTGCT CTCGGCGGCG TCCGCCCACA
NSW_3               --TTGAGTGG ----CCCCGT GGTCTTAAGC ACATGCCGCG CCGTTTGTCC GTGCTCG-TC TCGGGCCGTC CGCCTTTGCT CTCGGCGGCG TCCGCCCACA
QLD_1               --TTGAGTGG ----CCCCGT GGTCTTAAGC ACATGCCGCG CCGTTTGTCC GTGCTCG-TC TCGGGCCGTC CGCCTTTGCT CTCGGCGGCG TCCGCCCACA
QLD_2               --TTGAGTGG ----CCCCGT GGTCTTAAGC ACATGCCGCG CCGTTTGTCC GTGCTCG-TC TCGGGCCGTC CGCCTTTGCT CTCGGCGGCG TCCGCCCACA
QLD_3               --TTGAGTGG ----CCCCGT GGTCTTAAGC ACATGCCGCG CCGTTTGTCC GTGCTCG-TC TCGGGCCGTC CGCCTTTGCT CTCGGCGGCG TCCGCCCACA
NT_1                GGTTGAGTGG ----CCCCGT GGTCTTAAGC ACATGCCGCG CCGTTTGTCC GTGCTCG-TC TCGGGCCGTC CGCCTTTGCT CTCGGCGGCA TCCGCCCACA
NT_2                ---TGAGCGG ----CCCCGT GGTCTTAAGC ACATGCCGCG CCGTTTGTCC GTGCTCG-TC TCGGGCCGTC CGCCTTTGCT CTCGGCGGCA TCCGCCCACA
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NT_3                 GGTTGAGTGT ----CCCCGT GGTCTTAAGC ACATGCCGCG CCGTTTGTCC GTGCTCG-TC TCGGGCCGTC CGCCTTTGCT CTCGGCGGCA TCCGCCTACA
NT_5                 GGGTGAGCGG ----CCCCGT GGTCTTAAGC ACATGCCGGG CCGTTTGTCC GTGCTCG-TC TCGGGCCGTC CGCCTTTGCT CTCGGCGGCA TCCGCCCACA
WA_1                 GGGTGAGCGG ----CCCCGT GGTCTTAAGC ACATGCCGCG CCGTTTGTCC GTGCTCG-TC TCGGGCCGTC CGCCTTTGCT CTCGGCGGCG TCCGCCCACA
WA_2                 GGTTGAGTGG ----CCCCGT GGTCTTAAGC ACATGCCGCG CCGTTTGTCC GTGCTCG-TC TCGGGCCGTC CGCCTTTGCT CTCGGCGGCA TCCGCCCACA
WA_3                 ---TGAGCGG ----CCCCGT GGTCTTAAGC ACATGCCGCG CCGTTTGTCC GTGCTCG-TC TCGGGCCGTC CGCCTTTGCT CTCGGCGGCA TCCGCCCACA
WA_4                 GGGTGAGCGG ----CCCCGT GGTCTTAAGC ACATGCCGCG CCGTTTGTCC GTGCTCG-TC TCGGGCCGTC CGCCTTTGCT CTCGGCGGCA TCCGCCCACA
WA_5                 ---TGAGCGG ----CCCCGT GGTCTTAAGC ACATGCCGCG CCGTTTGTCC GTGCTCG-TC TCGGGCCGTC CGCCTTTGCT CTCGGCGGCG TCCGCCCACA
WA_6                 GGTTGAGTGG ----CCCCGT GGTCTTAAGC ACATGCCGCG CCGTTTGTCC GTGCTCG-TC TCGGGCCGTC CGCCTTTGCT CTCGGCGGCA TCCGCCCACA
Austropeplea viridis ---CGGTGGG ----CCCCGT GGTCTTAAGC ACAAGCCGCG CCG-TTGTCC GTGTTCG-TC TCGGGACGTC CGCGACGCCG CCTTGCTCTC GGCGGCGGC-
Bullastra cumingiana --ACATGCCG ----CGCCGT TGTCC---GT GTTCGTCTCG GAAACGACCC CGCCTCGCTC TCGGCGGAGC CCGCCTCGCT CTCGGCGGCG GTAGCCAACG
'Lymnaea' brevispina --CCCGGTGG ----CCCCGT GGTCTCAAGC ACAAGCCGCG CCG-TTGTCC GTGTTTG-TC TAGGGACGTG CGGGACGCCG CCTTGCTGTG GGCGGCGAC-
Radix auricularia    --CCTGGTGG ----CCCCGT GGACTTAAGC ACAAGCCGCG CCG-TTGTCC GTGTTCG-TC TCGGGACGTC CGCGACGCCG CCTTGCTCTC GGCGGCGGC-
Radix peregra        --CCCGGTGG ----GCCCGT GGTCTTAAGC ACAAGCCGCG CCG-TTGTCC GTGTTCG-TC TCGGGACGTC CGCGACGCCG CCTTGCTCTC GGCGGCGGC-
Radix rubiginosa     --TCTGGTGG ----CCCCGT GGTCTTAAGC ACAAGCCGCG CCG-TTGTCC GGGTTCG-TC TCGGGACGTC CGCGACGCCG CCTTGCTCTC GGCGGCGGC-
Radix quadrasi       --TGTGGTGG ----CCCCGT GGTGTTAACC CCAAGCCGCG CCG-TTGTCC GTGTTCG-TC TCGGGACGTC CGCGACGCCG CCTTGCTATG GGCGCGCGGC-
Stagnicola caperata  ATAGTGGTGG GTGGCCCCGT GGTCTTAAGC GCAAGCCGCG CCG-TTGTCC GT--TCA-TC TCGTAACGTC TTCGACGCTG CCCTGCTCTT GGCGGTGGT-
Stagnicola elodes    ACAGTGGTGG ----CCCCGT GGTCTTAAGC GCAAGCCGCG CCG-TTGTCC GT--TCA-TC TCGTAACGTC TTCGACGCTG CCCTGCTCTT GGCGGC----
Stagnicola emarginat ACAGTGGTGG ----CCCCGT GGTCTTAAGC GCAAGCCGCG CCG-TTGTCC GT--TCA-TC TCGTAACGTC TTCGACGCTG CCCTGCTCTT GGCGGC----


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                     ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         310        320        330        340        350        360        370        380        390        400
NSW_1                CT----ACTG TGTGATTCTT TTTTTTTTCC TCTGCGGTCA CCGCCATGCG GGACCCGGCT CGCTCTCGCC TCACGGGCTC GCATT--AAA -AGCTCCAGG
NSW_2                CT----ACTG TGTGATTCTT TTTTTTT-CC TCTGCGGTCA CCGCCATGCG GGACCCGGCT CGCTCTCGCC TCACGGGCTC GCATT--AAA -AGCTCCAGG
NSW_3                CT----ACTG TGTGATTCTT TTTTTTT-CC TCTGCGGTCA CCGCCATGCG GGACCCGGCT CGCTCTCGCC TCACGGGCTC GCATT--AAA -AGCTCCAGG
QLD_1                CT----ACTG TGTGATTCTT TTTTTTTTCC TCTGCGGTCA CCGCCATGCG GGACCCGGCT CGCTCTCGCC TCACGGGCTC GCATT--AAA -AGCTCCAGG
QLD_2                CT----ACTG TGTGATTCTT TTTTTTT-CC TCTGCGGTCA CCGCCATGCG GGACCCGGCT CGCTCTCGCC TCACGGGCTC GCATT--AAA -AGCTCCAGG
QLD_3                CT----ACTG TGTGATTCTT TTTTTTT-CC TCTGCGGTCA CCGCCATGCG GGACCCGGCT CGCTCTCGCC TCACGGGCTC GCATT--AAA -AGCTCCAGG
NT_1                 CT----ACGG TGTGAATTTT TTTTT---CC TCTGCGGTCA CCGCCATGCG GGACCCGGCT CGCTCTCGCC TCACGGGCTC GCATT--AAA -AGCTCCAGG
NT_2                 AT----ACGG TGTGAATTTT TTTTT---CC TCTGCGGTCG CCGCCATGCG GGACCCGGCT CGCTCTCGCC TCACGGGCTC GCATT--AAA -AGCTCCAGG
NT_3                 CT----ACCG TGTGAATTTT TTTTT---CC TTTGCGGTCG CCGCCATGCG GGACCCGGAT CGCTCTCGCC TCACGGGCTC GCATT--AAA -AGCTCCAGG
NT_5                 CT----ACTG TGTGAATTTT TTTTT---CC TCTGCGGTCG CCGCCCTGCG GGACCCGGCT CGCTCTCGCC TCACGGGCTC GCCTT--AAA -AGCTCCAGG
WA_1                 CT----GCTG TGTGATTTTT TTTTC----C TCTGCGGTCA CCGCCATGCG GGACCCGGCT CGCTCTCGCC TCACGGGCTC GCATT--AAT -AGCTCCAGG
WA_2                 CT----ACGG TGTGAATTTT TTTTT---CC TCTGCGGTCA CCGCCATGCG GGACCCGGCT CGCTCTCGCC TCACGGGCTC GCATT--AAA -AGCTCCAGG
WA_3                 CT----ACGG TGTGAATTTT TTTTTT--CC TCTGCGGTCG CCGCCATGCG GGACCCGGCT CGCTCTCGCC TCACGGGCTC GCATT--AAA -AGCTCCAGG
WA_4                 CT----ACGG TGTGAATTTT TTTTTT--CC TCTGCGGTCG CCGCCATGCG GGACCCGGCT CGCTCTCGCC TCACGGGCTC GCATT--AAA -AGCTCCAGG
WA_5                 CT----GCTG TGTGATTTTT TTTTT---CC TCTGCGGTCA CCGCCATGCG GGACCCGGCT CGCTCTCGCC TCACGGGCTC GCATT--AAA -AGCTCCAGG
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WA_6                  CT----ACGG TGTGAATTTT TTTTT---CC TCTGCGGTCA CCGCCATGCG GGACCCGGCT CGCTCTCGCC TCACGGGCTC GCATT--AAA -AGCTCCAGG
Austropeplea viridis  ---------- --CAAATTT- --------CC TCTCCG-TCA CCGCCATGCG GGACCCGGCT CGCTCTCGC- TAACGGGCCC GCTTG-TAAC CAGCTCGAGG
Bullastra cumingiana  TTTTCGAAGG TGTAATTTTT TTCTT---CC TCTGCG-TCA CCGCAATGCG GGACCCGGCT CGCTCTCGCC AAACGGGCCC GCACA--AAA CAGCTCGAGG
'Lymnaea' brevispina  ---------- --CAAATTT- --------CC TCTTAG-TCA CCGCCGTGCG GGACCCGGCT CGCTCTCGC- TATCGGGCCT GCTAG-TAAC AAGATCCAGG
Radix auricularia     ---------- --CAAATTT- -------CCA TCTGCG-TCA CCGCTAAGCG GGACCCGGCT CGCTCTCGC- TAACGGGCCC GCTTA-TATC AAGCTCAAGG
Radix peregra         ---------- --CAAATTT- --------CC TCTCCG-TCA CCGCTATGCG GGACCCGGCT CGCTCTCGC- TAACGGGCCC GCTTA-TAAC CAGCTTCAGG
Radix rubiginosa      ---------- --CAAATTTT TTTTT--CCA TCTGCG-TCC CCGCTAAGCG GGACCCGGCT CGCTCTCGC- TAAGGGGCCC GTTTA-TACA AAGCTCAAGG
Radix quadrasi        ---------- --CAAATTTT TTT----CCA TATGCG-TCA CCGATAAGCG GGACCCGGCT CGCTCTCCC- TAACGGGCCC GCTTAATACG AAGCTCAAGG
Stagnicola caperata   ---------C TGTCCATTTT ---------C TCT------A CCGCCAGGCA GGACCCGGCT CGCTACTC-- ---------- ---GCGGTCT CGGGCCTGCA
Stagnicola elodes     ---------C TGTCCATTTT ---------C TCT------A CCGCCAGGCA GGACCCGGCT CGCTACTTTA T--------- GGTGCGATCA CGGGCCTGCA
Stagnicola emarginat  ---------C TGTCCATTTT ---------C TCT------A CCGCCAGGCA GGACCCGGCT CGCTACTTTA T--------- GGTGCGATCA CGGGCCTGCA


                                            ********** *****                  ****    **        ****
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                          410        420        430        440        450        460        470        480        490        500
NSW_1                 GTGATCG-CG GAGGAGAGAA AGGTCGCACG CTCGCTCGCT CGCTCGCTAG TGTTTGAT-G ACGA--AATC GGCGCCACCG AAATGAAGAA GATGAAGAAA
NSW_2                 GTGATCG-CG GAGGAGAGAA AGGTCGCACG CTCGCTCGCT C----GCTAG TGTTTGAT-G A-GA--AATC GGCGCCACCG AAATGAAGAA GATGAAGAAA
NSW_3                 GTGATCG-CG GAGGAGAGAA AGGTCGCACG CTCGCTCGCT C----GCTAG TGTTTGAT-G ACGA--AATC GGCGCCACCG AAATGAAGAA GATGAAGAAA
QLD_1                 GTGATCG-CG GAGGAGAGAA AGGTCGCACG CTCGCTCGCT CGCTCGCTAG TGTTTGAT-G ACGA--AATC GGCGCCACCG AAATGAAGAA GATGAAGAAA
QLD_2                 GTGATCG-CG GAGGAGAGAA AGGTCGCACG CTCGCTCGCT C----GCTAG TGTTTGAT-G ACGA--AATC GGCGCCACCG AAATGAAGAA GATGAAGAAA
QLD_3                 GTGATCG-CG GAGGAGAGAC AGGTCGCACG CTCGCTCGCT C----GCTAG TGTTTGAT-G ACGA--AATC GGCGCCACCG AAATGAAGAA GATGAAGAAA
NT_1                  GTGATCG-CG GAGGAGAGAA AGGTCGCACG CTCGCTCGCT C----GCT-- --AGTGTTTG ACGA--AATC GGCGCCACCG AAATGAAGAA GAAGAAAAA-
NT_2                  GTGATCG-CG GAGGAGAGAA AGGTCGCACG TTCGCTCGCT C----GCTAG TGTTTG---- ACGA--AATC GGCGCCACCG AAATGAAGAA GAAGAAAAA-
NT_3                  GTGATCG-CG GAGGAGAGAA AGGTCGCACG CTCGCTCGCT C----GCTCG CTAGTGTTTG ACGA--AGTC GGCGCCACCG AAATGAAGAA GAAGAAGAA-
NT_5                  GTGATCG-CG GAGGAGAGAA AGGTCGCACG TTCGCTCGCT C----GCTCG CTAGTGTTTG ACGA--AATC GGAGCCACCG AAATGAAGAA GAAGAAAAAT
WA_1                  GTGATCG-CG GAGGAGAGAA AGGTCGC--- -TCGCTCGCT C----GCTAG TGTTTG---- ACGA--AATC GGCGCCACCG AAATGAAG-- ----AAGAA-
WA_2                  GTGATCG-CG GAGGAGAGAA AGGTCGCACG CTCGCTCGCT C----GCTCG TCAGTGTTTG ACGA--AATC GGCGCCACCG AAATGAA--- ----AAGAA-
WA_3                  GTGATCG-CG GAGGAGAGAA AGGTCGCACG TTCGCTCGCT C----GCTCG TGTTTGTTTG ACGA--AATC GGCGCCACCG AAATGAAGAA GAAGAAAAAA
WA_4                  GTGATCC-CG GAGGAGAAAA AGGTCCCACG TTCCCTCGCT C----GCTAG TGTTTG---- ACGA--AATC GGCGCCACCG AAATGAAGAA GAAAAAAAAA
WA_5                  GTGATCG-CG GAGGAGAGAA AGGTCGCA-- --CGCTCGCT C----GCTAG TGTTTG---- ACGA--AATC GGCGCCACCG AAATGAAGAA G---AAAAA-
WA_6                  GTGATCG-CG GAGGAGAGAA AGGTCGCACG CTCGCTCGCT C----GCTAG TGTTTG---- ACGA--AATC GGCGCCACCG AAATGAAGAA GAAGAAAAA-
Austropeplea viridis  GTGATTG-CG GAGGAGAGAA GCTTACG--- ---------- ----CAGACG CGCTCGCTTG ACGT---ATC GGCGCC---C GTACGAAGAA AAAAAAAT--
Bullastra cumingiana  GTGATCG-CG GAGGAGGAGA AGA------- ---------- --------AG AAATTGA--G ACGA--AATC GGCGCC-CCA GATCGCAAGA GAGGGAATGA
'Lymnaea' brevispina  GTGATTG-CG GAGGAGAGAA GCTTAC---- ---------- -----GCTGA CGCTCGATTG ACAG---ATC GGCGCC---C GTAAGAAAGA AGAATG----
Radix auricularia     GTGATTG-CG GAGGGGGAAA AAAAGCTTA- ---------- ----CGCGGA CGATCGCTTG ACAACAAATC GGCGCC---C GTACGAATT- GAAGTGAAAA
Radix peregra         GTGACGGGCG GAGGAGAGAA GCTTACGCTG ---------- ---ACGCTCG ------ATCG ACGA---GTC GGCGCC---C GTACGAAAAA AATTGGAAAA
Radix rubiginosa      GTGATTG-CG GAGGGGGGAA AAAAAA---- ---------- ----CGCCGA CGCTCGCTTG ACAA---ATC GGCCCC---C GTACGAATTT GAAATGAAAA
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Radix quadrasi          GTGATTG-CG GAGGGGGGAA AAAAA----- ---------- ----CGCCGA CGCTCGCGCG ACAA---ATG GGCGCC---C GTACGAATTT GAAATGAAAA
Stagnicola caperata     GTCC--A-TG GC--GTTAT- ---TGCTCTA -------GGG TGGAGTTTGA GGGCTTTCTA TCGA-GGAC- GATACCT--G ATCGGCGCCA GCCTTTCACT
Stagnicola elodes       GTCC--A-TG GC--ATCGC- ---TGCTCTA -------GGG CGGAGAATCG GGGCT--CTA TCGA-GGACC GATACCT--G ATCGGCGCCC GTCTGTCACT
Stagnicola emarginat    GTCC--A-TG GC--ATCGC- ---TGCTCTA -------GGG TGGAGAATCG GGGCT--CTA TCGA-GGACC GATACCT--G ATCGGCGCCC GTCTGTCACT


                                      ***                       ****
                        ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....
                          510        520        530        540        550        560        570
NSW_1                   GGAGGAGATT GAGA---TTG ATTTATTTCT CTTTCCTTTT TTTT-CTCTC TTTTCTCCGA CCTCAGATCG GACGAGATT
NSW_2                   GGAGGAGATT GAGA---TTG ATTTATTTCT CTTTCCTTTT TTTTTCTCTC TTTTCTCCGA CCTCGATCG? ?????????
NSW_3                   GGAGGAGATT GAGA---TTG ATTTATTTCT CTTTCCTTTT TTTTTCTCTC TTT??????? ?????????? ?????????
QLD_1                   GGAGGAGATT GAGA---TTG ATTTATTTCT CTTTCCTTTT TTTTTCTCTC TTTTCTCCGA CCTCAGATCG GACGAGATT
QLD_2                   GGAGGAGATT GAGA---TTG ATTTATTTCT CTT-CCTTTT TTTTTCTCT- ?????????? ?????????? ?????????
QLD_3                   GGAGGAGATT GAGA---TTG ATTTATTTCT CTTTCCTTTT TTTTTCTCTC TTTTCTCCGA CCTCAAATCG GACGAGATT
NT_1                    -AAGGAGATT GAGAGATTGT GATGATTTCT CTTTCCTTGT TTT----CTC TTTTCTCCGA CCTCAGATCG GACGAGATT
NT_2                    -AAGGAGATT GAGAGATTGT GATGATTTCT CTTTCCTTGT TTT----CTC TTTTCTCCGA CCTCAGATCG GACGAGATT
NT_3                    -AAGGAGATT GAGAGATTGA GATGATTTCT CTTTCCTTGT TTT----CTC TTTTCTCCGA CCTCAAATCG GACGAGATT
NT_5                    TA-GGAGATT GAGAAATTGT GATGATTTCT CTTTCCTTGT TTT----CTC TTTCCCCCGA CCTCAGATCG GAGGAGATT
WA_1                    --AGGAGATT GAGAGATTGT GATGATTTCT CTTTCCTTGT TTT----CTC TTTTCTCCGA CCTCAGATCG GACGAGATT
WA_2                    -AAGGAGATT GAGAGA---- GATGATTTCT CTTTCCTTGT TTT----CTC TTTTCTCCGA CC---GATCG GA???????
WA_3                    AAAGGAGATT GAGAGATTGT GATGATTTCT CTTTCCTTGT TTT----CTC TTTTCTCCGA CCTC-GATC? ?????????
WA_4                    AAAGGAGATT GAGAGATTGG GAAGATTTCT CTTTCCTTGT TTT----CTC TTTTCTCCGA CCTC-GATCG GACGAGATT
WA_5                    -AAGGAGATT GAGAGATT-- GATTATTTCT CTTTCCTTGT TTTT--TCTC TTTTCTCCGA CCTCAGATCG GACGAGATT
WA_6                    -AAGGAGATT GAGAGATTGT GATGATTTCT CTTTCCTTGT TTT----CTC TTTTCTCCGA CCTCAGATCG GACGAGATT
Austropeplea viridis    ---------- ------TTTT TTTTTTTACC AATTTCCGAC CTCAGATCGG ACGAGATT?? ?????????? ?????????
Bullastra cumingiana    GATGGTGGGA GGGAGATTCT TCTG-TCTCT CTCTCTCGCT TTTC--GTTC GTTCCCCTTC TTTC?????? ?????????
'Lymnaea' brevispina    ---------- -------TCT TCTCTTCACA AATTCCGACT ?????????? ?????????? ?????????? ?????????
Radix auricularia       AAAT---ATT ------CTTT TTTTTTTTTC ATTTCATAT CTC??????? ?????????? ?????????? ?????????
Radix peregra           AAAATAACCG TGCGTTGCGC GTTATTTTTT TTTTTCCTTT CTTTC????? ?????????? ?????????? ?????????
Radix rubiginosa        AAACGCTGTT ------TTTT CTCTCTTTCC TATCCCCCAC CTCAGATCGG ACGAAATTAC CCGCTGATT? ?????????
Radix quadrasi          AAAC-CAGTT ------TTTT GTTTGTGTTT TTTCCCCAA TTCATATCCC CAACCTCTAA CCGCAGATT? ?????????
Stagnicola caperata     TATTTATTAT GT??????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????
Stagnicola elodes       -ACCGGATAT ATTCATTCAT ATATATATAT ATCTTCTTAC CT???????? ?????????? ?????????? ?????????
Stagnicola emarginat    -ACCGGATAT ATTCATTCAT ATATATATAT ATCTTCTTAC CT???????? ?????????? ?????????? ?????????
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Appendix 3.4. p distances of 16S rRNA sequences, as calculated in PAUP for the analysis of the *Austropeplea lessoni* complex. Taxa without names are currently recognised as *A. lessoni*.

		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
NSW 1	1	-																								
NSW 2	2	0.5	-																							
NSW 3	3	0.5	0.5	-																						
NSW 4	4	0.0	0.5	0.5	-																					
NT 1	5	1.2	1.2	1.2	1.2	-																				
NT 2	6	0.9	0.9	0.9	0.9	0.2	-																			
NT 3	7	1.4	1.4	1.4	1.4	0.7	0.5	-																		
NT 4	8	0.9	0.9	0.9	0.9	0.7	0.5	0.9	-																	
NT 5	9	0.9	0.9	0.9	0.9	0.7	0.5	0.9	0.5	-																
QLD 1	10	0.7	0.7	0.7	0.7	0.9	0.7	1.2	0.7	0.7	-															
QLD 2	11	0.7	0.7	0.7	0.7	0.9	0.7	1.2	0.7	0.7	0.0	-														
QLD 3	12	0.7	0.7	0.7	0.7	0.9	0.7	1.2	0.7	0.7	0.0	0.0	-													
WA 1	13	1.2	1.2	1.2	1.2	0.5	0.2	0.7	0.7	0.7	0.9	0.9	0.9	-												
WA 1 2	14	1.2	1.2	1.2	1.2	0.5	0.2	0.7	0.7	0.7	0.9	0.9	0.9	0.5	-											
WA 2	15	1.2	1.2	1.2	1.2	0.5	0.2	0.7	0.5	0.7	0.9	0.9	0.9	0.5	0.5	-										
WA 3	16	1.2	1.2	1.2	1.2	0.5	0.2	0.7	0.5	0.7	0.9	0.9	0.9	0.5	0.5	0.0	-									
WA 5	17	1.4	1.4	1.4	1.4	0.7	0.5	0.7	0.7	0.9	1.2	1.2	1.2	0.7	0.7	0.2	0.2	-								
Austropeplea viridis	20	12.4	12.5	11.9	12.4	12.7	12.4	12.9	12.0	12.4	12.2	12.2	12.2	12.7	12.7	12.4	12.4	12.6	14.7	12.4	-					
Bullastra cumingiana	18	9.4	9.4	9.1	9.4	8.7	8.4	8.9	8.9	8.9	9.1	9.2	9.1	8.7	8.2	8.7	8.7	8.9	-							
Radix auricularia	19	13.2	13.5	13.3	13.2	12.9	12.7	13.2	12.9	12.9	13.1	13.2	13.3	12.9	12.7	12.9	13.1	13.3	13.8	-						
Radix peregra	21	12.7	13.0	12.7	12.7	12.5	12.4	12.5	12.0	12.4	12.4	12.2	12.6	12.5	12.5	12.2	12.2	11.9	14.6	14.6	9.4	-				
Radix quadrasi	22	14.9	15.2	14.6	14.9	14.9	14.6	15.1	14.4	14.8	14.8	14.7	14.8	14.9	14.9	14.6	14.5	14.8	15.0	16.2	17.3	15.7	-			
Radix rubiginosa	23	14.2	14.5	13.8	14.2	14.2	13.9	14.4	13.7	14.1	14.1	13.9	14.1	14.2	14.2	13.9	13.8	14.1	14.0	15.5	16.9	15.7	0.3	-		
Stagnicola	24	15.7	15.8	15.1	15.7	15.4	15.2	15.7	15.4	15.4	15.3	15.4	15.3	15.4	15.4	15.4	15.3	15.6	16.7	16.0	16.4	16.6	14.0	14.0	-	

elodes

Stagnicola emarginata	25	15.1	15.1	14.4	15.1	14.5	14.2	14.8	14.8	14.7	14.7	14.8	14.6	14.5	14.5	14.5	14.4	14.7	16.8	15.1	16.2	15.8	13.6	13.6	3.3	-
Stagnicola caperata	26	21.1	21.0	20.7	21.1	20.8	20.5	21.1	21.1	21.0	21.0	21.1	20.9	20.5	20.5	20.8	20.7	21.0	23.8	20.4	21.9	22.3	17.4	17.0	14.7	12.0

Appendix 3.5. p distances of ITS-2 rRNA sequences, as calculated in PAUP for the analysis of the *Austropeplea lessoni* complex. Taxa without names are currently recognised as *A. lessoni.*

		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
NSW 1	1	-																								
NSW 2	2	2.3	-																							
NSW 3	3	1.5	0.8	-																						
QLD 1	4	0.2	2.1	1.3	-																					
QLD 2	5	0.4	1.9	1.1	0.2	-																				
QLD 3	6	0.8	1.9	1.1	0.6	0.4	-																			
NT 1	7	7.0	8.8	7.9	7.0	6.8	7.3	-																		
NT 2	8	10.3	12.2	11.2	10.3	10.2	10.6	4.7	-																	
NT 4	9	8.9	10.2	9.3	8.9	8.7	8.7	5.4	5.0	-																
NT 5	10	7.0	8.8	7.9	7.0	6.8	7.3	3.1	3.6	2.0	-															
WA 1	11	7.5	9.3	8.4	7.7	7.6	8.0	1.9	4.7	5.0	2.9	-														
WA 1 2)	12	8.5	10.3	9.4	8.7	8.5	9.0	3.1	6.3	6.6	4.6	1.7	-													
WA 2	13	6.7	8.3	7.3	6.7	6.5	6.9	3.8	4.3	2.1	0.2	3.4	4.8	-												
WA 3	14	6.0	7.9	6.9	6.0	5.8	6.3	4.1	6.1	5.2	3.2	4.8	5.8	3.4	-											
WA 4	15	6.3	8.1	7.2	6.4	6.2	6.6	2.7	4.6	3.0	0.4	2.7	4.1	1.0	2.7	-										
WA 5	16	4.2	6.2	5.3	4.4	4.2	4.7	3.4	6.1	6.3	4.1	3.4	4.3	4.7	3.0	3.6	-									
Austropeplea viridis	17	31.4	31.8	31.1	31.4	31.2	31.4	31.7	32.2	33.0	31.1	31.6	32.6	31.8	32.0	31.1	30.8	-								
Bullastra cumingiana	18	33.3	33.3	32.8	33.1	32.9	33.2	34.0	35.8	36.8	34.6	34.1	34.9	35.1	35.6	34.6	33.3	37.8	-							
Lymnaea' brevispina	19	58.8	59.0	58.8	59.0	58.7	58.7	60.2	60.9	61.0	60.5	59.8	60.2	61.8	61.5	60.5	58.8	57.6	60.5	-						
Radix auricularia	20	31.4	31.8	31.3	31.4	31.4	31.6	31.9	32.8	32.9	30.6	31.9	32.8	31.4	32.0	31.1	31.5	18.9	38.6	57.8	-					
Radix peregra	21	31.0	31.2	30.9	31.0	31.0	31.2	32.2	33.4	32.7	31.8	32.1	32.9	31.0	32.3	31.7	31.0	16.6	37.8	56.5	18.0	-				
Radix quadrasi	22	35.3	36.5	36.1	35.2	34.9	35.6	34.2	35.6	35.6	33.4	34.8	35.4	34.2	34.2	33.1	33.7	26.8	42.6	60.3	13.8	24.9	-			
Radix rubiginosa	23	34.8	36.3	35.6	35.0	34.7	35.4	34.5	35.6	35.5	33.5	34.3	35.2	33.9	35.5	33.7	34.2	19.0	41.8	55.6	8.1	22.2	13.4	-		
Stagnicola caperata	24	51.8	51.5	51.8	51.9	51.8	51.6	52.9	53.0	52.9	51.0	53.0	53.2	51.2	51.8	51.4	51.9	44.9	58.7	67.1	45.0	43.7	48.3	45.8	-	
Stagnicola elodes	25	49.2	49.5	48.9	49.2	49.1	48.8	51.2	52.2	52.4	50.9	51.7	52.3	50.3	51.1	50.8	49.9	44.4	58.4	66.3	46.7	43.1	49.6	47.1	14.6	-
Stagnicola emarginata	26	49.5	49.8	49.1	49.5	49.3	49.0	51.4	52.4	52.6	51.1	51.9	52.6	50.6	51.4	51.1	50.2	44.4	58.4	66.3	46.7	43.1	49.6	47.1	14.4	0.2

Appendix 3.5. Statistics for the anatomical analysis of the *Austropeplea lessoni* complex dataset.

Character	Min Range	Tree steps	Max steps	steps	CI	RI	G-RC	HI	fit
1 shell umbilicus	1	1	2	2	0.500	0.000	0.000	0.500	0.750
2 shell thickness	1	1	1	2	1.000	1.000	1.000	0.000	1.000
3 number of whorls	4	4	8	10	0.500	0.333	0.167	0.500	0.429
4 colmella fold	1	1	2	2	0.500	0.000	0.000	0.500	0.750
7 broadest area of foot	1	1	2	4	0.500	0.667	0.333	0.500	0.750
8 foot shape posterior end	1	1	3	8	0.333	0.714	0.238	0.667	0.600
9 foot shape	1	1	1	2	1.000	1.000	1.000	0.000	1.000
10 eye lobe	1	1	2	3	0.500	0.500	0.250	0.500	0.750
11 tentacle shape	1	1	1	1	1.000	0/0	0/0	0.000	1.000
14 visceral coil pigmentation	1	1	4	5	0.250	0.250	0.063	0.750	0.500
15 mantle expansion	2	2	5	9	0.400	0.571	0.229	0.600	0.500
16 exapanded mantle pigmentation	1	1	2	2	0.500	0.000	0.000	0.500	0.750
18 anal flap	1	1	1	5	1.000	1.000	1.000	0.000	1.000
19 upper plate of pnuemostome	1	1	2	7	0.500	0.833	0.417	0.500	0.750
20 broadest area of kidney	1	1	2	2	0.500	0.000	0.000	0.500	0.750
21 kidney shape	3	3	6	12	0.500	0.667	0.333	0.500	0.500
22 right lobe of kidney	1	1	1	3	1.000	1.000	1.000	0.000	1.000
23 position of pulmonary vein	1	1	1	3	1.000	1.000	1.000	0.000	1.000
24 pulmonary vein length	1	1	1	1	1.000	0/0	0/0	0.000	1.000
25 ureter	1	1	3	10	0.333	0.778	0.259	0.667	0.600
28 cerebral commissure length	2	2	3	5	0.667	0.667	0.444	0.333	0.750
29 pedal commissure	1	1	2	5	0.500	0.750	0.375	0.500	0.750
30 pedal commissure extra lobe	1	1	1	9	1.000	1.000	1.000	0.000	1.000
32 radula sac	1	1	2	3	0.500	0.500	0.250	0.500	0.750
33 salivary gland	1	1	3	9	0.333	0.750	0.250	0.667	0.600
34 uterus/ vagina length	2	2	7	13	0.286	0.545	0.156	0.714	0.375
35 spermathecal duct length	2	2	4	6	0.500	0.500	0.250	0.500	0.600
36 spermathecal duct width	1	1	1	1	1.000	0/0	0/0	0.000	1.000
37 uterus shape	1	1	3	10	0.333	0.778	0.259	0.667	0.600
38 oviducal caecum	2	2	5	8	0.400	0.500	0.200	0.600	0.500
39 oothecal gland shape	2	2	4	8	0.500	0.667	0.333	0.500	0.600
40 oviduct 1	2	2	2	4	1.000	1.000	1.000	0.000	1.000
41 position of uterus/ vagina	1	1	3	11	0.333	0.800	0.267	0.667	0.600
43 penis sheath length	3	3	6	13	0.500	0.700	0.350	0.500	0.500
44 penis in penis sheath head	1	1	4	4	0.250	0.000	0.000	0.750	0.500
46 seminal vesicle shape	1	1	2	4	0.500	0.667	0.333	0.500	0.750
47 seminal vesicle form	2	2	8	12	0.250	0.400	0.100	0.750	0.333
48 junction of vas deferens	1	1	2	7	0.500	0.833	0.417	0.500	0.750
50 upper prostate	1	1	1	1	1.000	0/0	0/0	0.000	1.000
51 length of prostate	1	1	3	6	0.333	0.600	0.200	0.667	0.600
52 shape of lower prostate	1	1	2	3	0.500	0.500	0.250	0.500	0.750
53 central tooth	1	1	4	5	0.250	0.250	0.063	0.750	0.500
56 lateral teeth	1	1	1	2	1.000	1.000	1.000	0.000	1.000
57 marginal teeth	4	4	4	4	1.000	0/0	0/0	0.000	1.000
58 ureter length	1	1	2	2	0.500	0.000	0.000	0.500	0.750
59 pedal ganglion shape	1	1	1	1	1.000	0/0	0/0	0.000	1.000
60 cerebral commissure shape	2	2	6	6	0.333	0.000	0.000	0.667	0.429
61 insemination pocket	1	1	1	1	1.000	0/0	0/0	0.000	1.000
62 vaginal bulb	1	1	1	1	1.000	0/0	0/0	0.000	1.000
63 penal knot	1	1	1	1	1.000	0/0	0/0	0.000	1.000
64 prostate pouch	1	1	1	2	1.000	1.000	1.000	0.000	1.000

13 constant characters not shown

Appendix 4.1. Matrix of anatomical characters used for phylogenetic analysis of the Lymnaeidae. See Appendix 2.1 for a description of each character and state.

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Austropeplea tomentosa NSW    1 1 2 3 1 1 1 1 3 2  3 1  1 1 1 - 2 1 1 1 1 1 1 3 2 1  1  2  1 1 2 1  3  1 2 2 2  3 4 1 1 2 2  1  1 2  2 2 1 1 2 1 1 1 1 2  3 2 1 1 1 1 1 1 3 1 1 1 1 1 ? 1 1 2  2 1 2 2 2 1
Austropeplea tomentosa NZN    1 1 2 3 1 1 2 1 1 2  1&2 1  1 1 3 1 2 1 1 1 2 1 1 2 1 1  1  2  2 1 2 2  2  1 3 2 1  3 1 2 3 2 2  1  2 2  1 2 1 2 2 1 1 1 2 2  4 - 1 1 1 1 1 1 3 1 1 - - 1 ? 1 1 2  2 1 2 3 2 ?
Austropeplea tomentosa NZS    1 1 2 3 1 1 2 1 3 2  1 1  1 1 3 1 2 1 1 1 2 1 1 2 1 1  1  2  1 1 2 2  2  1 3 2 1  2 1 2 3 2 2  1  2 2  1 2 1 2 2 1 1 1 2 2  4 - 1 1 1 1 1 3 1 1 - - 1 ? 1 1 2  2 1 2 3 2 ?
Austropeplea tomentosa TAS    1 1 1 3 1 1 1 1 1 2  3 1  1 1 3 1 2 1 1 1 1 2 1 1 2 2 1  1  2  1 1 2 1  3  1 3 2 1  3 3 1 1 2 2  2  1 2  2 2 1 2 2 1 1 1 1 2  4 2 1 1 1 1 1 1 3 1 1 1 1 1 ? 1 1 2  2 1 2 3 2 1
Austropeplea tomentosa SA     1 1 6 3 1 1 2 1 2 2  4 1  1 2 4 1 2 1 1 1 2 1 1 2 2 1  1  2 1&2 1 2 1  2  3 3 2 2  2 3 1 1 2 4  1  1 2 2&3 2 1 2 3 1 1 1 1 2  4 2 1 1 1 1 1 3 1 1 1 1 1 ? 1 1 2  2 1 2 3 2 1
Kutikina hispida              2 2 5 1 2 2 2 2 3 1  1 2  1 1 1 - 1 1 1 2 3 1 1 3 2 2  2  1  2 1 1 2  1 1&2 2 2 1  1 1 1 1 1 3  2  2 2  1 1 2 1 1 1 1 1 1 1  1 1 2 1 1 1 1 1 3 1 1 1 2 1 ? 1 1 2  2 1 2 ? 2 ?
Austropeplea lessoni NSW      1 1 2 2 1 1 1 2 3 2  3 1  1 1 2 1 2 1 1 1 3 2 2 2 - 1  1  1  1 1 2 3  2  2 2 2 1  3 2 2 1 2 3  2  1 2  3 1 1 1 2 1 1 1 1 2  3 2 1 1 1 1 1 1 2 1 1 1 1 2 ? 2 1 2  2 1 2 2 2 1
Austropeplea lessoni WA       1 1 3 2 1 1 1 1 3 3  ? 1  1 2 1 - 2 1 1 3 2 2 2 - 1 1  1  1  2 2 2 3 2&3 3 1 2 1 3&4 2 2 2 2 2  1  1 2  4 2 1 1 2 1 1 1 1 2  3 - 1 1 1 1 1 1 3 1 1 - - 2 2 1 2  2 1 2 2 2 1
Austropeplea lessoni QLD      1 1 3 2 1 1 2 3 2  3 1  1 1 1 - 2 1 1 3 2 2 2 - 2 1 1&2  1 1&2 2 2 3  2  1 1 2 1  3 2 2 1 2 3  2  1 2  3 1 1 1 2 1 1 1 1 2  3 2 1 1 1 1 1 1 2 1 1 1 1 2 ? 2 1 2  2 1 2 2 2 1
Austropeplea lessoni NT       1 1 6 2 1 1 1 3 2  3 1  1 1 1 - 2 1 1 3 2 2 2 - 1 1  1 1&2 2 2 3  3  3 1 2 2  4 2 2 2 2 2  2  1 2 2&4 2 1 1 2 1 1 1 1 2  3 - 1 1 1 1 1 3 1 1 - - 2 1 1 2  2 1 2 2 2 1
Austropeplea ollula           2 1 6 3 1 1 1 2 2  3 1  1 ? 1 - 2 ? ? ? ? ? ? ? ? 1  1 1&2 1 2 1  3  1 1 2 2  4 3 ? 1 2 3  ?  1 1  2 1 1 1 1 1 1 1 1 2  4 ? 1 1 1 1 1 3 1 1 ? ? ? 1 ? 2  2 1 2 2 2 1
Austropeplea viridis          2 1 6 2 1 1 1 2 2  3 1  1 1 1 - 2 1 1 2 3 1 1 1 1 1  1  2  1 2 1  3  1 1 2 2  4 3 2 1 2 3  ?  1 1  2 1 1 1 1 1 1 1 1 2  4 - 1 1 1 1 1 3 1 1 - - - 2 1 1 2  2 1 2 2 2 1
Bullastra cumingiana          1 1 2 1 1 1 2 2 3 2  1 1  4 2 2 1 1 3 2 2 2 - 1 1  1  2  2 2 3  3  3 3 2 2  3 3 2 2 2 1  2  1 2  3 1 1 1 1 1 2 1 1 1  1 - 1 1 1 1 1 1 1 1 - - 2 2 2 1 2  2 1 2 2 2 1
Radix auricularia             2 1 2 2 1 2 2 3 2  2 1  1 1 1 - 2 2 2 3 1 2 2 - 2 1  1  3  1 1 2 3  3  3 3 1 2  4 3 1 2 2 3  2  1 1  3 1 1 1 2 2 - 2 2  3 1 1 1 1 1 1 2 1 1 1 1 1 2 2 2 2  2 1 2 2 2 2
Radix peregra                 1 1 2 2 1 2 2 3 2  2 1  1 1 1 - 2 2 2 3 1 2 2 - 1 1  1  ?  1 1 2 3  ?  2 1 2 2  4 3 1 2 2 3  1  1 1  3 1 1 1 2 2 - 1 2  3 - 1 1 1 1 1 1 2 1 1 - - 2 2 1 2  2 1 2 2 2 2
Radix quadrasi                1 2 2 2 1 1 2 3 2  3 1  1 2 2 2 2 2 3 2 2 2 - 1 1 1&2  2  1  2 1 1 ?  2  3 2 2 2  4 1 2 1 2 4  2  1 2  3 1 1 2 1 1 1 1 2  3 - 1 1 1 1 1 3 1 1 - - 2 2 1 2  2 1 2 2 2 2
Radix rubiginosa              1 2 3 2 1 1 1 1 2  3 1  1 1 1 - 2 2 2 3 1 2 2 - 2 1  1  ?  1 1 2 1  ?  1 1 2 1  2 1 1 1 2 2  1  1 1  3 1 1 2 1 1 1 1 2 3&4 2 1 1 1 1 1 3 1 1 1 1 1 2 1 1 2  2 1 2 2 2 2
Fossaria truncatula           2 2 7 3 1 1 1 2 2  3 1  1 1 1 - 2 1 2 ? ? ? ? ? ? ? 1  1  1  2 1 2 ?  ?  2 3 2 1  3 1 ? 1 2 1  ?  2 2  3 1 1 1 4 1 3 - 1 2  5 ? 1 2 1 1 1 3 1 1 ? ? 2 1 1 2 1  2 1 2 2 2 2
Lymnaea brevispina            1 1 3 2 1 1 2 3 2  ? 1  1 1 1 - 2 2 2 3 1 2 2 - 1 1 1&2  1  1 2 ?  ?  3 3 2 2  4 2 1 2 2 2  2  1 1  4 ? 1 1 1 1 2 1 2  4 - 1 1 1 1 1 2 1 1 - - - - 1 1 2  2 1 2 2 2 ?
Lymnaea cousini               1 ? 3 3 1 1 1 ? ? 2  ? 1  1 1 1 - 2 ? ? 1 3 1 1 3 2 1  ?  ?  ? 1 2 ?  ?  1 3 2 2  3 2 3 2 2 5  ?  1 2  4 1 1 2 1 1 1 2 1 2  5 1 ? 1 2 1 1 1 3 1 1 2 1 2 2 1 1 2  2 1 2 4 2 ?
Lymneae rupestris             3 ? 2 3 1 1 1 ? 2  ? 1  1 ? 1 - 2 ? ? 3 3 1 1 3 2 1  ?  ?  ? 1 2 ?  ?  1 3 2 1  1 3 3 3 2 2  ?  1 ?  4 ? 1 1 2 1 1 1 1 1  4&5 1 ? 1 1 1 1 1 3 1 1 1 1 2 1 1 2  2 1 2 2 2 2 2
Lymnaea stagnalis             1 1 9 3 2 1 1 2 2  3 1  1 2 1 - 2 1 2 3 3 1 1 3 2 1  ?  ?  ? 1 2 ?  ?  2 3 2 2  2 2 ? 2 2 2  1  ? ?  ? ? 1 1 2 1 2 - 1 1  5 ? ? 1 2 1 1 4 1 1 1 2 1 ? 1 1 2  2 1 2 2 3 3
Myxas glutinosa               1 1 2 2 1 1 2 2 1 2  2 1  1 1 4 1 2 2 2 3 ? ? ? ? ? ? 1  2  ?  ? 1 2 ?  ?  3 1 2 2  3 1 ? 1 2 3  ?  ? ?  ? ? 1 1 2 1 2 - 1 2  4 ? ? 1 1 1 1 1 1 1 ? ? ? 2 2 1 2  2 1 2 ? 2 ?
Lymnaea viatrix               1 ? 7 1 1 1 1 ? ? 2  ? 1  1 ? 1 - 2 ? ? 3 3 1 1 3 2 1  ?  2  ? 1 2 ?  ?  2 3 2 2  3 3 1 1 2 1  ? 1&2 2  3 1 1 1 4 1 1 1 1 1  5 3 ? 1 1 1 1 3 1 1 1 1 1 2 1 1 2  2 1 2 4 2 ?
Psuedosuccinea columella      1 2 3 2 2 1 1 1 ? 2  3 1 1&2 2 1 - 2 ? ? 2 3 1 1 3 2 1  1  1  1 1 2 1  3  2 2 2 2  3 1 ? 2 2 2  1  2 2  3 1 1 1 4 1 2 - 2 2  4 3 1 1 1 1 2 1 4 1 1 2 1 1 1 ? 1 3 ? 1&2 1 2 1 1 3
Omphiscola glabra             1 ? 9 1 1 1 ? ? ? 2  3 1  1 2 1 - 2 1 2 ? ? ? ? ? ? 1  ?  ?  ? 1 2 ?  ?  3 2 2 1  3 2 ? 2 2 3  3  ? ?  ? ? 1 2 2 1 1 2 1 2  3 ? ? 1 1 1 1 1 4 1 1 ? ? 1 1 2 1  1 1 2 3 1 ?
Stagnicola corvus             ? 2 8 2 2 1 1 1 2 2  3 1 1&2 2 1 - 2 1 2 ? ? ? ? ? ? 1  ?  ?  ? 1 2 ?  ?  2 3 2 2  3 2 ? 1 2 1  ? ? ?  ? ? 1 1 2 1 1 2 - 1 2  ? ? ? 1 1 2 1 1 4 1 1 1 ? ? 2 1 1 2  2 1 2 2 3 3
Stagnicola elodes             1 2 8 2 1 1 1 2 2  3 1  1 ? 1 - 2 ? ? 2 3 1 1 3 2 1  ?  ?  ? 1 2 ?  ?  1 1 2 2  2 2 3 1 2 4  ?  1 2  2 1 2 2 2 2 - 1 1  5 2 ? 1 1 2 1 4 1 1 1 1 1 2 1 1 2  2 1 2 3 2 3
Stagnicola catascopium        1 2 7 2 1 1 1 1 2 2  3 1  1 ? 1 - 2 ? ? 2 3 3 1 1 3 2 1  1  1  2 1 2 3  3  1 3 2 2  2 3 2 1 2 2 1&2  1 ?  3 1 1 1 2 ? 1 1 1 1  2 1 2 2 2 2 2 1 4 1 1 1 1 1 1 1  1 2 ? 1 2 2 2 2 3
Stagnicola palustris          1 2 8 3 2 1 1 2 2  3 1 1&2 2 1 - 2 1 2 ? ? ? ? ? ? 1  ?  ?  ? 1 2 ?  ?  3 3 2 1  3 1 ? 2 2 3  ? ? ?  ? ? 1 1 3 1 2 - 1 1  2 ? ? 1 1 1 4 1 1 ? 1 1 1 1 2  2 1 2 4 2 ?
Radix ovata                   2 1 3 2 1 1 ? ? 2  ? 1  1 ? 1 - 2 2 2 3 1 2 2 - 2 1  1  ?  ? 1 2 ?  ?  ? ? ? 2  ? ? ? ? 2 2  ? ? ?  ? ? 1 ? ? ? 2 - 1 2  ? ? ? 1 1 1 1 2 1 1 ? 1 ? ? ? ? 2  2 1 2 ? 2 2
Radix luteola                 1 1 3 3 1 1 1 1 2  ? 1  1 ? ? 2 ? ? ? 3 ? ? ? ? ? 1  ?  ?  ? 1 2 ?  ?  3 1 2 2  ? 1 ? 1 2 1  ?  1 1  2 1 1 2 1 1 1 1 1 2  3 ? ? 1 1 1 1 3 1 1 1 1 1 2 1 1 2  2 1 2 2 3 2
Radix natalensis              1 1 3 2 1 1 1 1 2  3 1  1 2 1 - 2 ? 4 3 1 1 3 2 1  1  1  1  2 1 2 ?  ?  2 1 2 2  3 3 3 2 2 4  1  1 1  4 1 1 1 4 1 2 - 1 2  3 1 - 1 1 1 1 3 1 1 1 1 1 1 2 1 1 2  2 1 2 2 3 2
Physa acuta                   1 1 3 2 1 1 2 1 2 2  3 1  1 1 1 - 2 - - 4 3 1 1 3 2 1  1  ?  ? 1 2 ?  ?  2 3 2 1  5 3 ? 1 2 3  ?  1 2  3 - - - - - 4 - 2 4  5 1 ? 1 1 1 - 2 3 2 2 1 1 2 ? 1 2 2  ? 2 1 - - 3
Kessinaria papillosa          1 1 8 2 1 1 ? 2 ? 2  3 1  1 1 1 - 2 - - 3 3 1 1 3 2 1  ?  ?  ? 1 2 ?  ?  1 3 2 1  5 1 ? 2 2 1  ?  1 2  3 - - - - - 1 2 1 2  4 1 ? 1 1 1 - 2 3 2 2 1 1 2 1 1 3 2  2 2 1 - - 3
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Appendix 4.2 Alignment of 16S rRNA used for phylogenetic analysis of the Lymnaeidae. All characters with an astertix above them where excluded from all phylogenetic analyses.

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                              ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       10         20         30         40         50         60         70         80         90        100
Austropeplea lessoni NSW      ??AGGAAATT TTTGTTCGAA CAGAACATTC TATTTT-GAC TGTTAGTC-T TCTAGTTCCT AGTCCAACAT CGAGGTCACA AGCTAAA--- -AATTTTA-T
Austropeplea lessoni WA       ??AGGAAATT TTTGTTCGAA CAGAACATTC TATTTT-GAC TGTTAGTC-T TCTAGTTCCT AGTCCAACAT CGAGGTCACA AGCTAAA--- -AATTTTA-T
Austropeplea lessoni NT       ??AGGAAATT TTTGTTCGAA CAGAACATTC TATTTT-GAC TGTTAGTC-T TCTAGTTCCT AGTCCAACAT CGAGGTCACA AGCTAAA--- -AATTTTA-T
Austropeplea lessoni QLD      ??AGGAAGTT TTTGTTCGAA CAGAACATTC TATTTT-GAC TGTTAGTC-T TCTAGTTCCT AGTCCAACAT CGAGGTCACA AGCTAAA--- -AATTTTA-T
Austropeplea tomentosa NSW    ??AGGAAATT TT-GTTCGAA CAGAACATTC TATTAT-GAC GGTTAGTC-T TCTAGCTCCT AGTCCAACAT CGAGGTCACA AACTAAA--- -TAATTCA-T
Austropeplea tomentosa NZN    ??AGGAAATT TT-GTTCCAA CAGAACATTC TATTAT-GAC TGTTAGTC-T TTTAGTTCCT AGTCCAACAT CGAGGTCACA AACTAAA--- -TTATTCA-T
Austropeplea tomentosa NZS    ??AGGAAATT TT-GTTCGAA CAGAACATTC TATTAT-GAC TGTTAGTC-T TTTAGTTCCT AGTCCAACAT CGAGGTCACA AACTAAA--- -TTATTCA-T
Austropeplea tomentosa SA     ??AGGAAATT TT-GTTCGAA CAGAACATTC TATTAT-GAC GGTTAGTC-T TTTAGCTCCT AGTCCAACAT CGAGGTCACA AACTAAA--- -TAATTCA-T
Austropeplea tomentosa TAS    ??AGGAAATT TT-GTTCGAA CAGAACATTC TATTAT-GAC GGTTAGTC-T TTTAGCTCCT AGTCCAACAT CGAGGTCACA AACTAAA--- -TAATTCA-T
Austropeplea ollula          ??AGGAAATT TT-GTTCGAA CAGACCAATC TATTTT-GAC GGTTAATC-T TTTAGTTCCT AGTCCAACAT CGAGGTCATA AGCTATA--- -TTAATCA-T
Austropeplea viridis 1        ??AGGAAACT GTTGTTCGAA CAGAACAATC TATTTT-GAC GGTTAATC-T TTTAGTTCCT AGTCCAACAT CGAGGTCATA AGCTATA--- -TTAATCA-T
Austropeplea viridis 2        ??AGGAAACT GTTGTTCGAA CAGAACAATC TATTTT-GAC GGTTAATC-T TTTAGTTCCT AGTCCAACAT CGAGGTCATA AGCTATA--- -TTAATCA-T
Austropeplea sp. China        ??AGGAATAA A-TGTTCGAA CAGAACAATC TAATTT-GAC GGTTAATC-T TCTAGTTCCT AGTCCAACAT CGAGGTCATA AGCTATA--- -TAAATTA-T
Austropeplea sp. Hawaii       ??AGGAATAA AATGTTCGAA CAGAACAATC TAATTT-GAC GGTTAATC-T TCTAGTTCCT AGTCCAACAT CGAGGTCATA AGCTATA--- -TAAATCA-T
Bullastra cumingiana          ??AGGAAAAA TCTGTTCGAA CAGAACATTC TATTTT-GAC TGTTAGTC-T TCTAGTTCCT AGTCCAACAT CGAGGTCACA AGCTAAA--- -AATTTTA-T
Bulimnea megasoma             ?????????? ??TGTTCGAA CAGAACAATC TATTTT-GAC GGTTAGTC-A ACTAGTTCCT AGTCCAACAT CGAGGTCACA AACTAAA--- -TAGTTAA-T
Fossaria bulmoides            ??AGGAATTT CT-GTTCGAA CAGAACAATC TATTTT-GAC TGTTAGTC-A ACTAGTTCCT AGTCCAACAT CGAGGTCACA AACTAAA--- -TAATTAA-T
Fossaria obrussa              ?????ATGTT CT-GTTCGAA CAGAACAATC TATTTT-GAC TGTTAGTC-A ACTAGTTCCT AGTCCAACAT CGAGGTCACA AACTAAT--- -TTATTAA-T
Fossaria truncatula           ??AGGAATTT CT-GTTCGAA CAGAACAATC TATTTT-GAC TGTTAGTC-A ACTAGTTCCT AGTCCAACAT CGAGGTCACA AACTAAT--- -TTATTAA-T
Kutikina hispida              ??AGGAAATT TT-GTTCGAA CAGAACATTC TATTAT-GAC GGTTAGTC-T TTTAGCTCCT AGTCCAACAT CGAGGTCATA AACTAAA--- -TAATTCA-T
Lymnaea stagnalis 1           ?????????? ???????GAA CAGAACAGTC GATGTT-AAC GGTTAGTT-A TTTAGTTCCT AGTCCAACAT CGAGGTCACA AACTTTA--- -TAAGAAA-T
Lymnaea stagnalis 2           ??????GATA TTTGTTCGAA CAGAACAATC TATATT-GAC GGTTAGTC-A TCTAGTTCCT AGTCCAACAT CGAGGTCACA AACTTTA--- -TAAAAAA-T
Lymnaea stagnalis 3           ??AGGAATAA A-TGTTCGAA CAGAACAATC TATGTT-AAC GGTTAGTT-A TTTAGTTCCT AGTCCAACAT CGAGGTCACA AACTTTA--- -TAAAAAA-T
Lymnaea stagnalis 4           ??AGGAATAA A-TGTTCGAA CAGAACAATC TATGTT-AAC GGTTAGTT-A TTTAGTTCCT AGTCCAACAT CGAGGTCACA AACTTTA--- -TAAAAAAT
Lymnaea stagnalis 5           ?????????? ?????????? ??GCACAGTC TATGTT-AAC GGTTAGTT-A TTTAGTTCCT AGTCCAACAT CGAGGTCACA AACTTTA--- TTAAAAA-T
Omphiscola glabra             ?????ATTTC CT-GTTCGAA CAGA-CAATC TATTTT-GAC GGCTAGTC-T ATTAGTTCCT AGTCCAACAT CGAGGTCACA AACTAAA--- -TAATTAA-T
Pseudosuccinea columella 1    ????GAATTT CT-GANCGAA CAGAACAATC TATTTA-GAC GGTTAATC-T TCTAGTTCCT AGTCCAACAT CGAGGTCACA AACTAAA--- -TAATTAA-T
Pseudosuccinea columella 2    ?????????? ?????????? ?????????? ?????????? ?????GTT-T ACT-GTTTCT AGAGCAACAT CGAGGTCACA AACTAAA--- -TTATTAA-T
Radix auricularia             ??AGGAGAAA TT-GTTCGAA CAGAACACTC TATTTT-GAC TGTTAGTC-C TTTAGTTCCT AGTCCAACAT CGAGGTCATA AGCTAAT--- -AAATTCA-T
Radix balthica                ?????????? ?????????? CAGAACACTC TATTTT-GAC GGTTAGTC-T TTTAGTTCCT AGTCCAACAT CGAGGTCACA AGCTAAT--- -AATTTCA-T
Radix luteola                 ??AGGAGTTA AATGTTCGAA CAGAACAATC TAATTT-GAC GGTTAGTC-T TATAGTTCCT AGTCCAACAT CGAGGTCACA AACTATA--- -ATAATCA-T
Radix natalensis              ??AGGAGTAT TATGTTCGAA CAGAACAATC TATTTT-GAC GGTTAGTC-T CTTAGTTCCT AGTCCAACAT CGAGGTCATA AGCTATT--- -TTAATTA-T
Radix ovata                   ??AGGAGAAA ATTGTTCGAA CAGAACATTC TATTTT-GAC TGTTAGTC-T TTTAGTTCCT AGTCCAACAT CGAGGTCATA AACTAAA--- -TAATTCA-T
Radix peregra                 ?????????? ?????????? CAGAACACTC TATTTT-GAC GGTTAGTC-T TTTAGTTCCT AGTCCAACAT CGAGGTCACA AGCTAAT--- -AATTTCA-T
Radix quadrasi                ??AGGAGTTA AATGTTCGAA CAGAACAATC TAGTTT-GAC GGTTAGTC-T AATAGTTCCT AGTCCAACAT CGAGGTCACA AACTATA--- -ATAATCA-T
Radix rubiginosa              ?????????? ??????CGAA CAGAACAATC TAATTT-GAC GGTTAGTC-T AATAGTTCCT AGTCCAACAT CGAGGTCACA AACTATA--- -ATAATCA-T
Radix sp. Philippines         ??A-GAATTT AATGTTCGAA C-GAACAATC TATTTT-GAC GGTTAGTC-T TTTAGTTCCT AGTCCAACAT CGAGGTCACA AGCTATA--- -TAAATCA-T
Radix sp. Canada              ????GAGTTA AATGTTCGAA CAGAACAATC TATTTT-GAC GGTTAGTC-T TTTAGTTCCT AGTCCAACAT CGAGGTCACA AACTATG--- -AAAATCA-T
Radix sp. Romania             ??AGGAGTTA AATGTTCGAA CAGAACAATC TATTTT-GAC GGTTAGTC-T TCTAGTTCCT AGTCCAACAT CGAGGTCACA AACTATG--- -AAAATCA-T
Stagnicola elrodi             ??AGGAATTT CT-GTTCGAA CAGAACAATC TATTTT-GAC GGTTAGTC-T TCTAGTTCCT AGTCCAACAT CGAGGTCACA AACTAAA--- -AAATTAA-T
Stagnicola bonnevillensis     ??AGGAATTT CT-GTTCGAA CAGAACAATC TATTTT-GAC GGTTAGTC-T TCTAGTTCCT AGTCCAACAT CGAGGTCACA AACTAAA--- -AAATTAA-T
Stagnicola sp. USA            ????GAATTT CT-GTTCGAA CAGAACAAAT CTATTTGAC GGTTAGTC-T TCTAGTTCCT AGTCCAACAT CGAGGTCACA AACTAAA--- -AAATTAA-T
Stagnicola sp. Canada         ??AAGAATTT CT-GTTCGAA CAGAACAATC TATTTT-GAC GGTTAGTC-T TTTAGTTCCT AGTCCAACAT CGAGGTCACA AACTAAA--- AAAATTAA-T
Stagnicola sp. Urkraine       ??AGAG-TTT CT-GTTCGAA CAGAACAATC TATATT-GAC GGTTAGTC-A TTAAGTTCCT AGTCCAACAT CGAGGTCACA AACTTTA--- -TAAAAAA-T
Stagnicola elodes USA         ??AGGAATTT CT-GTTCGAA CAGAACAAAC TATTTT-GAC GGTTAGTC-T TTTAGTTCCT AGTCCAACAT CGAGGTCACA AACTAAA--- AAAATTAA-T
Stagnicola elodes Canada      ?????????? ?????????? CAGAACAAAC TATTTT-GAC GGTTAGTC-T TTTAGTTCCT AGTCCAACAT CGAGGTCACA AACTAAA--- -AAATTAA-T
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Stagnicola corvus       ??AGGAATTT CT-GTTCGAA CAGAACAATC TATATT-AAC GGTTAGTC-A TTTAGTTCCT AGTCCAACAT CGAGGTCACA AACTTTA--- -AACAAAA-T
Stagnicola emarginata   ?????????? ?????????? ?????????? ???TTT-GAC GGTTAGTC-T TCTAGTTCCT AGTCCAACAT CGAGGTCACA AACTAAA--- -AAATTAA-T
Stagnicola catascopium  ---------- ---------- ---------- ---TTT-GAC GGTTAGTC-T TTTAGTTCCT AGTCCAACAT CGAGGTCACA AACTAAA--- -AAATTAA-T
Stagnicola caperata     ---------- ---------- -----CAATC TAT-TT-GAC GGTTAGTC-A ACTAGTTCCT AGTCCAACAT CGAGGTCACA AACTAA---- -TAATTAA-T
Stagnicola palustris    --AGGAATTT CT-GTTCGAA CAGAACAATC TATATT-GAC GGTTAGTC-T TTTAGTTCCT AGTCCAACAT CGAGGTCACA AACTTTA--- -TTAAAAA-T
Acroloxus lacustris     ---------- ATTGTTCGAA CAGAACAATC TCTTTT-AAC TGCTAGTT-A TAAAGTTCCT AGTCCAACAT CGAGGTCACA AACTTTA--- --AAAAA--T
Amerianna carinata      ---------- ---------- ---AACAAAT CTATTTTGAC TGCTAGCC-A TAAAGTTCCT AGTCCAACAT CGAGGTCACA AACTTAA--- --AAAAAA-T
Aplexa hypnorum         ---------- ATTGTTCGAA CAGAACATTT CTTTTA-AAC TGCTAGTT-T AAAGATTCCT AGTCCAACAT CGAGGTCACA AACTAAT--- -TTATATCAT
Ancylus fluviatilis     ---------- --TGTTCGAA CAGAACAA-T CTTTTTTAAC TGCTAGCT-A TAAAGTTCCT AGTCCAACAT CGAGGTCACA AACTTTT--- -TTAAA---T
Biomphalaria peregrina  GTAGGATATA ATTGTTCGAA CAGAACAAAT CTTTTTTGAC GGCTAGCC-A TAAAGTTCCT AGTCCAACAT CGAGGTCACA ATCATTT--- -TTAAAAA-T
Biomphalaria schrammi   GTAGGATAAT ATTGTTCGAA CAGAACAAAT CTTATTTGAC GGTTAGCC-A TAAAGTTCCT AGTCCAACAT CGAGGTCACA ATCATTT--- -TAAAAAA-T
Bulinus bavayi          --AGGATAGT ATTATTCGAA CAGAACAAAT CTATTTTGAC TGCTAGCC-A TAAAGTTCCT AGTCCAACAT CGAGGTCATA AACATTA--- -AAAAAAA-T
Bulinus globosus        --AGGATAAA ATTATTCGAA CAGAATAAAT CTTTTCTGAC TGCTAGCC-A TAAAGTTCCT AGTCCAACAT CGAGGTCACA AACATTA--- -AACATA--T
Burupia kempi           ---------- ------CGAA CAGAACAAAA TTTGNTTG-- TGCTAGCCAC ANATTTTCCT AGTCCAACAT CGAGGTCACA ATCAAAAA-- -TTTTTAT-T
Burnapia stuhlmanni     ---------- ---------- ---------- TTTGCTTC-- TGCTAGCCGC AAATTTTCCT AGTCCAACAT CGAGGTCACA ATCAAAAA-- -TTTTTAT-T
Pettancylus sp.         --AGGAAATT ATTGTTCGAA CAGAACAA-T CTTTTATGAC TGCTAGCC-A TAAAGTTCCT AGTCCAACAT CGAGGTCACA AACTTTT--- -AAAA---T
Physa acuta             ---GGGGTTT ATTGTTCGAA CAGAACAA-G CTTTTATCAC GGTTAGTG-T ACAAGTCCCT AGTCCAACAT CGAGGTCACA AACTAAT--- ATTTATCA-T
Physella johnsoni       --AGGAAATT ATTGTTCGAA CAGAACA-TT CTTTTTACAC TGTTAGTGG- TAAAGTTCCT AGTCCAACAT CGAGGTCACA AACTAAATAA AATTATCA-T
Physa heterostropha     ---GGGGTTT ATTGTTCGAA CAGAACAA-G CTTTTATCAC GGTTAGTG-T ACAAGTCCCT AGTCCAACAT CGAGGTCACA AACTAAT--- ATTTATCA-T
Physella wrighti        --AGGAAATT ATTGTTCGAA CAGAACA-TT CTTTTTACAC TGTTAGTGG- TAAAGTTCCT AGTCCAACAT CGAGGTCACA AACTAAATAA AATTATCA-T
Physa fontinalis        -------TTA AATGTTCGAA CAGAACAATC TTTTAACACT GGTTAGTGGT TAAAGTTCCT AGTCCAACAT CGAGGTCACA AACTAAT--- ATTTATCA-T
Planorbis planorbis     ---------- --TGTTCGAA CAGAACAAAT CTAATTAGAC GGTTAGCC-A TATAGTTCCT AGTCCAACAT CGAGGTCACA AACTAAA--- -TAATTAA-T


                                              **        ****** ******       ****** ***                ****
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                           110        120        130        140        150        160        170        180        190        200
Austropeplea lessoni NSW    TATGCTGTTA TCCCTAAGGT AATTTAAT-C TAACAAAAAA AA--ATGACT TGT------- ------AATT ATT------- ----AAAAGT TTAAAA----
Austropeplea lessoni WA     TATGCTGTTA TCCCTAAGGT AATTTAAT-C TATATAACCA A----TGACT TGT------- ------AATT ATT------- ----AAAAGT TTAAAA----
Austropeplea lessoni NT     TATGCTGTTA TCCCTAAGGT AATTTAAT-C TAACAAAAA A----TGACT TGT------- ------AATT ATT------- ----AAAAGT TTAAAAA---
Austropeplea lessoni QLD    TATGCTGTTA TCCCTAAGGT AATTTAAT-C TAACAAAAAA AA---TGACT TGT------- ------AATT ATT------- ----AAAAGT TTAAAA----
Austropeplea tomentosa NSW  TATGCTGTTA TCCCTAAGGT AATTTAAT-C TTAATAGGAA AA---TGACT TGT------- ------TTTT ATT------- ----GAAAGT TTAAA-----
Austropeplea tomentosa NZN  TATGCTGTTA TCCCTAAGGT AATTTAAT-C TTTTAAAAAA A----TGACT TGT------- ------TTTT AATT------ ----GAAAGT T-AAC-----
Austropeplea tomentosa NZS  TATGCTGTTA TCCCTAAGGT AATTTAAT-C TTTTAAAAAA AA---TGATT TGT------- ------TTTT AATT------ ----GAAAGT T-GAT-----
Austropeplea tomentosa SA   TATGCTGTTA TCCCTAAGGT AATTTAAT-C TTAATAGAAA AA---TGACT TGT------- ------TTTT ATT------- ----GAAAGT TTAAA-----
Austropeplea tomentosa TAS  TATGCTGTTA TCCCTAAGGT AATTTAAT-C TTAATAGAAA A----TGACT TGT------- ------TTTT ATT------- ----GAAAGT TTAAA-----
Austropeplea ollula         TATGCTGTTA TCCCTAAGGT AATTTTAT-C ATACAAAAAA A----TGACA TGT------- ------TTTT ATAT------ ----AAAAGT TTAAA-----
Austropeplea viridis 1      TATGCTGTTA TCCCTAAGGT AATTTTAC-C ATACAAAAAA AA---TGACA TGT------- ------TTTT ATAT------ ----GAAAGT TTAAA-----
Austropeplea viridis 2      TATGCTGTTA TCCCTAAGGT AATTTTAC-C ATACAAAAAA AA---TGACA TGT------- ------TTTT ATAT------ ----GAAAGT TTAAA-----
Austropeplea sp. China      TATGCTGTTA TCCCTAAGGT AATTTTAC-C ACACAAAAAA A----TGACT TGT------- ------TTTT TTTT------ ---GAAA-GT TTAAAT----
Austropeplea sp. Hawaii     TATGCTGTTA TCCCTAAGGT AATTTTAT-C ACACAAAAAG A----TGACT TGT------- ------TTTT TTT------- ----GAAAGT TTAAAT----
Bullastra cumingiana        TATGCTGTTA TCCCTAAGGT AATTTATT-C TTACAAATAA A----TGACT TGT------- ------TTAA TTT------- ----GAAAGT TTTAA-----
Bulimnea megasoma           TATGCTGTTA TCCCTAAGGT AATTTATT-C TTTCAAATAT AA--ATGTCA TGA------- ------ATTA AT-------- ----GAAAGT TTAAA-----
Fossaria bulmoides          TATGCTGTTA TCCCTAAGGT AATTTATT-C TTTCAAATCT TA--ATGTCA TGA------- ------AATA TA-------- ----GAAAGT TTTAT-----
Fossaria obrussa            TATGCTGTTA TCCCTAAGGT AATTTATT-C TTTCAAATCT TA--ATGTCA TGA------- ------AATA AT-------- ----GAAAGT TTAAA-----
Fossaria truncatula         TATGCTGTTA TCCCTAAGGT AATTTATT-C TTTCAAGTCT TA--ATGTCA TGA------- ------AATA AA-------- ----GAAAGT TTAAT-----
Kutikina hispida            TATGCTGTTA TCCCTAAGGT AATTTAAA-C TTAATAAAAA A----TGACT TGT------- ------TTTA ATT------- ----GAAAGT TTAAA-----
Lymnaea stagnalis 1         TATGCTGTTA TCCCTAAGGT AATTATACTC TTATAAATCT AA--TTGTCA TGT------- ------AAAC TTT------- ---TAAATGT TTAATG----
Lymnaea stagnalis 2         TATGCTGTTA TCCCTAAGGT AATTTCTT-C TATATAACCA AA--TTGTTA TGT------- ------ATAT AAA------- ----TAAATGT TTA-------
Lymnaea stagnalis 3         TATGCTGTTA TCCCTAAGGT AATTATACTC TTATAAATCT AA--TTGTCG TGT------- ------AAAC TTG------- ---TAAATGT TTAAAG----
Lymnaea stagnalis 4         TATGCTGTTA TCCCTAAGGT AATTATACTC TTATAAATCT AA--TTGTCG TGT------- ------AAAC TTG------- ---TAAATGT TTAAAG----
Lymnaea stagnalis 5         TATGCTGTTA TCCCTAAGGT AATTATACTC TTATAAATCT AA--TTGTCA TGT------- ------AAAC TTT------- ----AAATGT TTAATG----
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Omphiscola glabra           TATGCTGTTA TCCCTAAGGT AATTTATT-C TTTCAATAGT TA---TGTCT TGT------- ------AATT AT-------- ---GAAAGT TTCTAA----
Pseudosuccinea columella 1  TATGCTGTTA TCCCTAAGGT AATTTATT-C TTTCAAACCT AA--ATGTCT TGA------- ------TTGT TAA------- ---TGAAAGT TTAAT-----
Pseudosuccinea columella 2  TATGCTGTTA TCCCTAAGGT AATTTACTCT TTCAAACCTA AA---TGTCT TGA------- ------TTGT TAA------- ---TGAAAGT TTAAT-----
Radix auricularia           TATGCTGTTA TCCCTAAGGT AATTTGAT-C GTGCAAAGAA AA--TTGTTG TGT------- ------AAAA ATCT------ ---TGAAAGT TTAATA----
Radix balthica              TATGCTGTTA TCCCTAAGGT AATTTGAT-C ATTCAAAATA AA--T-GTCT CGT------- ------AAAA ATTG------ ---TGAAAGT TCAAAA----
Radix luteola               TATGCTGTTA TCCCTAAGGT AATTTTAC-C TTGCAAAACT TA--ATGTC- CAG------- ------TAAA GTAT------ ---TTAAAGT TTAAA-----
Radix natalensis            TATGCTGTTA TCCCTAAGGT AATTTAAT-C TTACAAACTT TA--ATGTCA TGT------- ------ATAA AAA------- ----TAAAGT TTAAA-----
Radix ovata                 TATGCTGTTA TCCCTTAGGT AATTTGAT-C TTTTAAAAAA AT--TTGTCA TGT------- ------AAAT CTT------- ----GAAAGT TAAATA----
Radix peregra               TATGCTGTTA TCCCTAAGGT AATTTGAT-C ATTCAAAATA AA--T-GTCT CGT------- ------AAAA ATTG------ ---TGAAAGT TCAAAA----
Radix quadrasi              TATGCTGTTA TCCCTAAGGT AATTTTAT-C TTACAAAACT TA--ATGTC- CAG------- ------TAGA ATAT------ ---TTAAAGT TTAAA-----
Radix rubiginosa            TATGCTGTTA TCCCTAAGGT AATTTTAT-C TTACAAAACT TA--ATGTC- CAG------- ------TAGA ATAT------ ---TTAAAGT TTAAA-----
Radix sp. Philippines       TATGCTGTTA TCCCTAAGGT AATTTTAT-C GTGCAAAACT TA--ATGTC- TAG------- ------TAAA AAAC------ ---GTTAAAG TTAAG-----
Radix sp. Canada            TATGCTGTTA TCCCTAAGGT AATTTTGT-C TTACAAAACT TA--ATGTC- TAG------- ------TAAA TTA------- ----TTAAAG TTTAAA----
Radix sp. Romania           TATGCTGTTA TCCCTAAGGT AATTTTGT-C TTACAAAACT TA--ATGTC- TAG------- ------TAAA TTA------- ----TTAAAG TTTAAA----
Stagnicola elrodi           TATGCTGTTA TCCCTAAGGT AATTTAAT-C TTCCAAATCT TA--ATGTCA TAT------- ------GTAA AAAA------ ---GAAA-GT TTAAAA----
Stagnicola bonnevillensis   TATGCTGTTA TCCCTAAGGT AATTTAAT-C TTTCAAATCT TA--ATGTCA TGT------- ------GAAA AA-------- ---AGAAAGT TTAAA-----
Stagnicola sp. USA          TATGCTGTTA TCCCTAAGGT AATTTAAT-C TTTCAAATCT TA--ATGTCA TGT------- -----GAAA AA-------- ----GAAAGT TTAAA-----
Stagnicola sp. Canada       TATGCTGTTA TCCCTAAGGT AATTTAAT-C TTTCAAATCT TA--ATGTCA TGT------- -----GAGA AA-------- ----GAAAGT TTAAAA----
Stagnicola sp. Urkraine     TATGCTGTTA TCCCTAAGGT AATTTGCT-C CTTTAAATCT TA--ATGTTG AGT------- ------ATTT TAA------- ---CAAACGT TTAAA-----
Stagnicola elodes USA       TATGCTGTTA TCCCTAAGGT AATTTAAC-C TTTCAAATCT TA--ATGTCA TGT------- ------AAA AA-------- ---AGAAAGT TTAAA-----
Stagnicola elodes Canada    TATGCTGTTA TCCCTAAGGT AATTTAAC-C TTTCAAATCT TA--ATGTCA TGT------- ------AAA AAA------- ---AGAAAGT TTAAA-----
Stagnicola corvus           TATGCTGTTA TCCCTAAGGT AATTTGCT-C TTTTAAATCT AA--GTGTTT AGT------- ------TGTT GTAA------ ---TAAATGT TCAAA-----
Stagnicola emarginata       TATGCTGTTA TCCCTAAGGT AATTTAAT-C TTTCAAATCT TA--ATGTCA TGT------- ------GAAA AA-------- ---AGAAAGT TTAAA-----
Stagnicola catascopium      TATGCTGTTA TCCCTAAGGT AATTTAAT-C TTTCAAATCT TA--ATGTCA TGT------- ------GAGA AA-------- ---GAAAGTT TAAAA-----
Stagnicola caperata         TATGCTGTTA TCCCTAAGGT AATTTACT-C TTTCAAACCT TA--ATGTCG TGA------- ------AGAA G--------- ---AGAAAGT TTAAG-----
Stagnicola palustris        TATGCTGTTA TCCCTAAGGT AATTTGCT-C TTTTAAATCT AA--GTGTTT AGT------- ------ATTA CTAA------ ---TAAATGT TTGTAA----
Acroloxus lacustris         TATGCTGTTA TCCCTAAGGT AATTTAATTT TTACTGAAAT AA--CCGATT AAAT------ ---------A AATA------ ---ATTAAGG TTAATA----
Amerianna carinata          TATGCTGTTA TCCCTAAGGT AATTTATTCT TATATAACAA TT--TCGTTT TGAAAA---- ---------A AAA------- ---TTAAAGT TTA-------
Aplexa hypnorum             TATGCTGTTA TCCCTAAGGT AATTTAGTTA CTCATAAAAA TT--TCGTCG TATT-----T G-----ATAT ATAC-TAC-- ATAAAAAAG- TTA-------
Ancylus fluviatilis         TATGCTGTTA TCCCTAAGGT AATTTATTTT TTACTGAAAA AA--CCGATT AAA------- ---------A AATA------ ---ATTAAAG TT--------
Biomphalaria peregrina      AATGCTGTTA TCCCTAAGGT AGTTTAATTA CTCTTATAAA AAAATCGATA TATTCCA--- -----AATTA ATTA------ ---CTAAAGT TT--------
Biomphalaria schrammi       AATGCTGTTA TCCCTAAGGT AGTTTAATTA TTTTTATAAT AA--TCGATG TATT------ --------TA ATTT------ ---ATAAAGT TA--------
Bulinus bavayi              TATGCTGTTA TCCCTAAAGT AGTTTTTCTT TACTTAAAAA AT--TCGCCT TATT----- ---------A AGAA------ ---TTTAGGT TA--------
Bulinus globosus            TATGCTGTTA TCCCTAAGGT AATTTTTTAT TCCTTAAAAA AT--TCGTCT GTTT------ ---------A AAAA------ ---ATTAGGT TT--------
Burupia kempi               AAG-CTGTTA TCCCTAAGGT AGTTNATTTT TGATTAATAC AA--TCGTTA TATTT----- -------TTA AA-------- ---AGAAGAT TT--------
Burnapia stuhlmanni         AAGGCTGTTA TCCTTAAGGT AGTTTATTTT TTATCAATAT AA--TCGTTA TATTT----- -------TTA AA-------- ---AGAAGAT TT--------
Pettancylus sp.             TATGCTGTTA TCCCTAAGGT AATTTAATTT TTATTAATAT AA--TCGTTT TATT----- ---------A AAAA------ ---AT-AAAG TT--------
Physa acuta                 TATGCTGTTA TCCCTCAGGT AGTTTAGTCA TTCATAATAA TA--TCGTCA GAT-----CT AATATCACTT TTAG-TGTCA AT-AGAAAGG TTA-------
Physella johnsoni           TATGCTGTTA TCCCTAAGGT AATTTAGTTA TTCATAAAAA TT--TCGTCA CATAATTATC ATAAATTTTT TTAA-TAAAA TT-AGAAAAG TTT--CTTTT
Physa heterostropha         TATGCTGTTA TCCCTCAGGT AGTTTAGTCA TTCATAATAA AT--TCGTCA GAT-----CT AATGCCACTT TTAG-TGTCA AT-AGAAAGG TTA-------
Physella wrighti            TATGCTGTTA TCCCTAAGGT AATTTAGTTA TTCATAAAAA TT--TCGTCA CATAATTCTC ATAAAAATTT TTAAATAAAA TTTAGAAAAG TTATACTTTT
Physa fontinalis            TATGCTGTTA TCCCTAAGGT AGTTTACTTA TACATAAAAA AT--TCGTCA TAT-----TT G----TATTT ATAA-C---- -T-AGAAAGT TTT-------
Planorbis planorbis         TATGCTGTTA TCCCTAAGGT AATTTAATTT CTATTAAAAA AA--TCGTTA TAT------- ---------- ATAC------ ---AATAAAT TTAAGA----


                            *******                                     *** ********** ****        ********* *******
                            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                              210        220        230        240        250        260        270        280        290        300
Austropeplea lessoni NSW    -------TGT TTTAT-TG-T CGCCCCAACA AAAATA---- ---------- ----AAAAAA T--------- -------TAT AAACCTTAA- -TTAATAAAA
Austropeplea lessoni WA     -------TGT TTTAA-TG-T CGCCCCAACA AAAATA---- ---------- ----AAAAAA T--------- -------TAT AAACTTTAA- -TTAATGAAA
Austropeplea lessoni NT     -------TGT TTTAA-TG-T CGCCCCAACA AAAATA---- ---------- ----AAAAAA T--------- -------TAT AAACTTTAA- -TTAATGAAA
Austropeplea lessoni QLD    -------TGT TTTAT-TG-T CGCCCCAACA AAAATA---- ---------- ----AAAAAA T--------- -------TAT AAACCTTAA- -TTAATGAAA
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Austropeplea tomentosa NSW      -------TGT TTCAA-TG-T CGCCCCAACA AAAATA---- ---------- ----TATAAT T--------- -------TAT G-AAATTAA- -TTAATAAAA
Austropeplea tomentosa NZN      -------TGT TTCAT-TG-T CGCCCCAACA AAAATA---- ---------- ----TATAAT T--------- -------TAA ATAAATTAA- -TTAATTAAA
Austropeplea tomentosa NZS      -------TGT TTCAT-TG-T CGCCCCAACA AAAATA---- ---------- ----TGTAAT T--------- -------TAA ATAAATTAA- -TTAATTAAA
Austropeplea tomentosa SA       -------TGT TTCAA-TG-T CGCCCCAACA AAAATA---- ---------- ----TATAAT T--------- -------TAT G-AAATTAA- -TTAATAAAA
Austropeplea tomentosa TAS      -------TGT TTCAA-TG-T CGCCCCAACA AAAATA---- ---------- ----TATAAT T--------- -------TAT G-AAATTAA- -TTAATAAAA
Austropeplea ollula             -------TGT TTCAT-TG-T CGCCCCAACA AAAATA---- ---------- ----AGAGAT T--------- -------TA- AAAAACCTT- -TTAATAAAA
Austropeplea viridis 1          -------TGT TTCAT-TG-T CGCCCCAACA AAAATA---- ---------- ----AGAGAT T--------- -------TA- AAAAACCTT- -TTAATAAAA
Austropeplea viridis 2          -------TGT TTCAT-TG-T CGCCCCAACA AAAATA---- ---------- ----AGAGAT T--------- -------TA- AAAAACCTT- -TTAATAAAA
Austropeplea sp. China          -------TGT TTCAT-TG-T CGCCCCAACA AAAATA---- ---------- ----AAAGAT T--------- -------TAT AAAAAGCTT- -TTAATAAAA
Austropeplea sp. Hawaii         -------TGT TTCAT-TG-T CGCCCCAACA AAAATA---- ---------- ----AAAGAT T--------- -------TAT AAAAAGCTT- -TTAATAAAA
Bullastra cumingiana            -------TGT TTCAA-TG-T CGCCCCAACA AAAATA---- ---------- ----AATAAA T--------- -------TAT AAAATTTAA- -TTAATTAAA
Bulimnea megasoma               -------TGT TTCAA-TG-T CGCCCCAACA AAAAAA---- ---------- ----TAA--T A--------- -------CTA AAATATT--- -TTATTAAAA
Fossaria bulmoides              -------TGT TTCAA-TG-T CGCCCCAACA AAAAAA---- ---------- ----TAA--T A--------- -------TAA AAATGTATAT TTTATTAAAA
Fossaria obrussa                -------TGT TTCTT-TG-T CGCCCCAACA AAAAAA---- ---------- ----TAA--T A--------- -------TTA A--TCTATAT TTTATTAAAA
Fossaria truncatula             -------TGT TTCAT-TGTA CGCCCCAACA AAAAAA---- ---------- ----TAA--T A--------- -------TTA A--CTAATAT TTTATTAAAA
Kutikina hispida                -------TGT TTCAT-TG-T CGCCCCAACA AAAATA---- ---------- ----TATAAT T--------- -------TAT G-AAATTAA- -TTAATAAAA
Lymnaea stagnalis 1             -------TGA TTTA--CT-G TCGCCCCAAC AAAA------ ---------- ----TTTATG G--------- -------TTT TAGTTTACAT TTTAATAAAA
Lymnaea stagnalis 2             ------CTGA TTTAT-TG-T CGCCCCAACA AAAACA---- ---------- ----TGATT- ---------- ---------- -AAAATAATC ATTTATAAAA
Lymnaea stagnalis 3             -------TGA TTTAT-TG-T CGCCCCAACA AAAA------ ---------- ----TTAA-G G--------- ---------T TTTAGTCACC ATTCATAAAA
Lymnaea stagnalis 4             -------TGA TTTAT-TG-T CGCCCCAACA AAAA------ ---------- ----TTAA-G G--------- ---------T TTTAGTCACC ATTCATAAAA
Lymnaea stagnalis 5             -------TGA TTTAT-TG-T CGCCCCAACA AAAA------ ---------- ----TTTATG G--------- ---------- TATAGTA-CA TTTTATAAAA
Omphiscola glabra               -------TGT TTTCA-TG-T CGCCCCAACA AAAAAA---- ---------- ----TATAA- ---------- --------AA AAATACTTTA ATTATTAAAA
Pseudosuccinea columella 1      -------TGT TTCAA-TG-T CGCCCCAACA AAAAAA---- ---------- ----AAT--T A--------- -------TTA A-----ATAT TTTATTAAAA
Pseudosuccinea columella 2      -------TGT TTCAA-TG-T CGCCCCAACA AAAAAA---- ---------- ----AATTA- ---------- -------TTA A-----ATAT TTTATTAAAA
Radix auricularia               -------TGT TTCAA-TG-T CGCCCCAACA AAAATA---- ---------- ----AATCTT A--------- -------AAT AATA-AAAT- -AAAATTAAA
Radix balthica                  ----TTTTGT TTCAA-TG-T CGCCCCAACA AAAATA---- ---------- ----AGTTTA C--------- -------AAT AAAGTAAAA- -GAAATTAAA
Radix luteola                   -------TGT TTAAA-TG-T CGCCCCAACA AAAAAA---- ---------- ----TAAACT T--------- -------TAA AAAAAGTTG- -TTATTAAAA
Radix natalensis                -------TGT TTAAA-TG-T CGCCCCAACA AAAAAA---- ---------- ----TAAATT T--------- -------TAT ATAAA-TTA- -TCATTAAAA
Radix ovata                     -------TGT TTCAG-TG-T CGCCCCAACA AAAAAA---- ---------- ----TATTTT T--------- -------AAT AAAA-AAAC- -TAAGTAAAA
Radix peregra                   ----TTTTGT TTCAA-TG-T CGCCCCAACA AAAATA---- ---------- ----AGTTTA C--------- -------AAT AAAGTAAAA- -GAAATTAAA
Radix quadrasi                  -------TGT TTAAA-TG-T CGCCCCAACA AAAAAA---- ---------- ----TAAACT T--------- -------TAA AAAA-GTTG- -TTATTAAAA
Radix rubiginosa                -------TGT TTAAA-TG-T CGCCCCAACA AAAAAA---- ---------- ----TAAACT T--------- -------TAA AAAA-GTTG- -TTATTAAAA
Radix sp. Philippines           -------TGT TTAAA-TG-T CGCCCCAACA AAAAAA---- ---------- ----TAAAAA T--------- -------TAT AAAAAGTTG- -TTATTCAAA
Radix sp. Canada                -------TGT TTAAA-TG-T CGCCCCAACA AAAAAA---- ---------- ----TAAA-- ---------- -------TA- AAGTTTATTT TTTATTAAAA
Radix sp. Romania               -------TGT TTAAA-TG-T CGCCCCAACA AAAAAA---- ---------- ----TAAA-- ---------- -------TA- AAGTTTATTT TTTATTAAAA
Stagnicola elrodi               -------TGT TTCAA-TG-T CGCCCCAACA AAAAAA---- ---------- ----TAA-AA ---------- -------TAA AATGA--TTT TTTATTAAAA
Stagnicola bonnevillensis       -------TGT TTCAA-TG-T CGCCCCAACA AAAAAA---- ---------- ----TAA-AA A--------- -------TAA AATAA--TTT TTTATTAAAA
Stagnicola sp. USA              -------TGT TTCAA-TG-T CGCCCCAACA AAAAAA---- ---------- ----TAAA-- ---------- -------TAA AAATAA-TTT TTTATTAAAA
Stagnicola sp. Canada           -------TGT TTCAA-TG-T CGCCCCAACA AAAAAA---- ---------- ----TAAAAA ---------- -------TAA AATAA-TTTT TTTATTAAAA
Stagnicola sp. Urkraine         -------TGG TTCAA-AG-T CGCCCCAACT AAAATA---- ---------- ----TAAAAC T--------- -----TATAT AATTAAATTT ATTAATAAAA
Stagnicola elodes USA           -------TGT TTCAA-TG-T CGCCCCAACA AAAAAA---- ---------- ----TAATAA T--------- -------TAA AATAG----- TTTATTAAAA
Stagnicola elodes Canada        -------TGT TTCAA-TG-T CGCCCCAACA AAAAAA---- ---------- ----TAATA- T--------- -------TAA AATAG----- TTTATTAAAA
Stagnicola corvus               -------TGA TTCAA-TG-T CGCCCCAACA AAAATA---- ---------- ----AAGATT T--------- -------TAG GATAAAAACA ATTAATAAAA
Stagnicola emarginata           -------TGT TTCAA-TG-T CGCCCCAACA AAAAAA---- ---------- ----TAA-AA A--------- -------TAA AATAT---TT TTTATTAAAA
Stagnicola catascopium          -------TGT TTCAA-TG-T CGCCCCAACA AAAAAA---- ---------- ----TAA-AA A--------- -------TAA AATAA-TTTT TTTATTAAAA
Stagnicola caperata             -------TGT TTCAA-TG-T CGCCCCAACA AAAAAA---- ---------- ----TAA--T A--------- -------TAA A----TGTAT TTTATTAAAA
Stagnicola palustris            ------ATGA TTCAA-TG-T CGCCCCAACT AAAATA---- ---------- ----AAAATT ---------- --------AA AAATAAAATT ATTAATAAAA
Acroloxus lacustris             ----TTATTG CTTAATTG-T CGCCCCAACA AAAAGA---- ---------- ----AAAAAA A--------- ---------- TTATT-ATAA TTTTCTAAAA
Amerianna carinata              -----ATATG TTTAATTG-T CGCCCCAACA AAAATA---- ---------- ----TATTAA A--------- ---------- TAATATTTAA ATTTATGAAA
Aplexa hypnorum                 ----TA-TGT TTTTT-TG-T CGCCCCAACA AAAAA----- ---------- ---TTAATAA T--------- --------TT TAAAATTATT TTTTTTAAAA
Ancylus fluviatilis             ----TAATTG TTTAATGG-T CGCCCCAACA AAAAGA---- ---------- ----AATAAT T--------- ---------- GTATA-AATA TATTCTAAAA
Biomphalaria peregrina          -----AATTG TTTACTTG-T CGCCCCAACA AAAACT---- ---------- ----AA-AA- ---------- ---------- ---------- -TTAGTTAAA
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Biomphalaria schrammi     -----TAT-G TTTATTTG-T CGCCCCAACA AAAAAT---- ---------- ----AATAAT A--------- ---------- ---------A ATTTATAAAA
Bulinus bavayi            -----TTTTA CTAAATTG-T CGCCCCAACA AAAAGA---- ---------- ----TATT-T A--------- ---------- ---------- ACATCTAAAA
Bulinus globosus          -----AAATT CTAAATTG-T CGCCCCAACA AAAAAT---- ---------- ----TAAA-T A--------- ---------- ATAAA--AAT TTAATTAAAA
Burupia kempi             -----AATT- TTCCTATG-T CGCCCCAACA AGAACA---- ---------- ----CAAAT A--------- ---------- GTAAA----- -TATGTAAAA
Burnapia stuhlmanni       -----AATT- TTCCTATG-T CGCCCCAACA AAAACA---- ---------- ----CAAAT A--------- ---------- GTAAA----- -TATGTAAAA
Pettancylus sp.           ----CAATTG TTTATTTG-T CGCCCCAACA AAAAGA---- ---------- ----AATAA A--------- ---------- ATAAATATTT TATTCTAAAA
Physa acuta               ----TATTGC TTTCT-TG-T CGCCCCAACA AAAAAGTGTT TTGTATAATA ACTCTAATAG TAGA--TAAA TTAAAGTCAT AAACAATCTT TATTTCAAAA
Physella johnsoni         AAGTTTTTGT TTTCT-TG-T CGCCCCAACA AAAAATTAGG ATGTAA---- ---TCTATTT TTTTTCTATT TAAAAAA-TG TTATACACTT TTCTTTAAAA
Physa heterostropha       ----TATTGC TTTCT-TG-C CGCCCCAACA AAAAAGTGTA TTGTATAATT TCTTTAAGAG TAGTATCTAA TTAAAGTCTT AAACAATCTT TATTTCAAAA
Physella wrighti          TAAGTTTTGT TTTCT-TG-T CGCCCCAACA AAAAATTAAG ATGTAA---- ---TCAAATT TTTTTCTAATT TAAAAAAATG TTATACACTT TTCTTTAAAA
Physa fontinalis          ----TAATGT TTTCT-TG-T CGCCCCAACA AAAAA----- ---------- ----TAAGAA ---------- --------AT AAAATATTCC TCTCTTTAAA
Planorbis planorbis       ---TTATATT TTAAACTG-T CGCCCCAACA AAAAAA---- ---------- ---------- ---------- ---------- ---------- ---TATAAAA
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                                  ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                       310        320        330        340        350        360        370        380        390        400
Austropeplea lessoni NSW          ATTCTAAGGG TCTTCTCGTC TTTTTTCTTA GTA-GAAGTA TTTTCACTTC ATAAATAAAT TCAAATAAAT TTAAAA-GAG ACAGCTAATT CTTTATTTAA
Austropeplea lessoni WA           ATTCTAAGGG TCTTCTCGTC TTTTTTCTTA GCA-GAAGTA TTTTCACTTC ATAAATAAAT TCAAATAAAT TTAAAA-GAG ACAGCTAATT CTTTATTTAA
Austropeplea lessoni NT           ATTCTAAGGG TCTTCTCGTC TTTTTTCTTA GTA-GAAGTA TTTTCACTTC ATAAATAAAT TCAAATAAAT TTAAAA-GAG ACAGCTAATT CTTTATTTAA
Austropeplea lessoni QLD          ATTCTAAGGG TCTTCTCGTC TTTTTTCTTA GTA-GAAGTA TTTTCACTTC ATAAATAAAT TCAAATAAAT TTAAAA-GAG ACAGCTAATT CTTTATTTAA
Austropeplea tomentosa NSW        ATTCTAAGGG TCTTCTCGTC TTTTTTCTTC GAA-AAAGTA TTTTCACTTT TTAAATAAAT TCAATAAAAT TTTTAA-GAG ACAGTTAATT CTTTATAAAA
Austropeplea tomentosa NZN        ATTCTAAGGG TCTTCTCGTC TTTTTTCTAT AAA-AAAGTA TTTTCACTTT TTAAATAAGT TCATTAAAAT TTTAAA-GAG ACAGTTAATT CTTTATAAAA
Austropeplea tomentosa NZS        ATTCTAAGGG TCTTCTCGTC TTTTTTCTAT AAA-AAGGTA TTTTCACTTT TTAAATAAAT TCATTAAAAT TTTAAA-GAG ACAGTTAATT CTTTATAAAA
Austropeplea tomentosa SA         ATTCTAAGGG TCTTCTCGTC TTTTTTCTTC GGA-AAAGTA TTTTCACTTT TTAAATAAAT TCAATAAAAT TTTTAA-GAG ACAGTTAATT CTTTATAAAA
Austropeplea tomentosa TAS        ATTCTAAGGG TCTTCTCGTC TTTTTTCTTC GAA-AAAGTA TTTTCACTTT TTAAATAAAT TCAATAAAAT TTTTAA-GAG ACAGTTAATT CTTTATAAAA
Austropeplea ollula               ATTCTAAGGG TCTTCTCGTC TTTTTTCTAA TAA-AAAGTA TTTTCACTTT ATAAATAAAT TCATATAAGC TAAAAA-GAG ACAGAAAATT CTTTAACAAA
Austropeplea viridis 1            ATTCTAAGGG TCTTCTCGTC TTTTTTCTAA TAA-AAAGTA TTTTCACTTT ATAAATAAAT TCATATAAGC TAAAAA-GAG ACAGAAAATT CTTTAACAAA
Austropeplea viridis 2            ATTCTAAGGG TCTTCTCGTC TTTTTTCTAA TAA-AAAGTA TTTTCACTTT ATAAATAAAT TCATATAAGC TAAAAA-GAG ACAGAAAATT CTTTAACAAA
Austropeplea sp. China            ATTCTAAGGG TCTTCTCGTC TTTTTTCTAA TAT-AAAGTA TTTTCACTTA ATAAATAAAT TCATAAAAAC TAAAAA-GAG ACAGAAAATT CTTTAATAAA
Austropeplea sp. Hawaii           ATTCTAAGGG TCTTCTCGTC TTTTTTCTAA TAT-AAAGTA TTTTCACTTA ATAAATAAAT TCATAAAAAC TAAAAA-GAG ACAGAAAATT CTTTAATAAA
Bullastra cumingiana              ATTCTAAGGG TCTTCTCGTC TTTTTTCTAA ATA-AAAGTA TTTTCACTTT TTAAATAAAT TCAAAAAAT TTTAAA-GAG ACAGCTTATT CTTTATAAAA
Bulimnea megasoma                 ATTCTAAGGG TCTTCTCGTC TTTTTTCTAA TAA-TAAGTA TTTTCACTTA ATAAATAAAT TCAATAAATT TAAATA-AAG ACAGTAAACC CCC-ATTTAA
Fossaria bulmoides                ATTCTAAGGG TCTTCTCGTC TTTTTTCTAA AAA-TAAGTA TTTTCACTTA ATAAATAATT TCAAATAAT TATTAA-GAG ACAGCAAGTC CCC-ATTAAC
Fossaria obrussa                  ATTCTAAGGG TCTTCTCGTC TTTTTTCTAA TTA-TAAGTA TTTTCACTTA ATAAATAATT TCAAATAAAT TATTTA-GAG ACAGCAAGTC CTC-ATTAAT
Fossaria truncatula               ATTCTAAGGG TCTTCTCGTC TTTTTTCTAA CTA-TAAGTA TTTTCACTTA ATAAATAGAT TCAAAAAAT TATATA-GAG ACAGCAAGTC CTC-ATTAAC
Kutikina hispida                  ATTCTAAGGG TCTTCTCGTC TTTTTTGTTC AAA-AAAGTA TTTTCACTTT TTAAATAAAT TCAATAATAT TTTTAA-GAG ACAGTTAATT CTTTATAAAA
Lymnaea stagnalis 1               ATTCTAAGGG TCTTCTCGTC TTTTATCTAA ATA-TAAGTA TTTTCACTTA ATAAAAAATT TCAATAAAAT TAAAAA-GAG ACAGTTCTTC CCT-ATTAAT
Lymnaea stagnalis 2               ATTCTTAGGG TCTTCTCGTC TTTTATCAAT TTA-TAAGTA TTTTCACTTA ATAAATAAAT TCAATAAATT TAATA--GAG ACAGTTCTCT CCT-ATTATC
Lymnaea stagnalis 3               ATTCTAAGGG TCTTCTCGTC TTTTATCTAA ATA-TAAGTA TTTTCACTTA ATAAAAAATT TCAATAATT TAAAAA-GAG ACAGTTCCTT CCT-ATTAAT
Lymnaea stagnalis 4               ATTCTAAGGG TCTTCTCGTC TTTTATCTAA ATA-TAAGTA TTTTCACTTA ATAAAAAATT TCAAATAATT TAAAAA-GAG ACAGTTCCTT CCT-ATTAAT
Lymnaea stagnalis 5               ATTCTAAGGG TCTTCTCGTC TTTTATCTAG ATA-TAAGTA TTTTCACTTA ATAAAAAATT TCAATATATT TAAAAA-GAG ACAGTTCTTC CCT-ATTAAT
Omphiscola glabra                 ATTCTAAGGG TCTTCTCGTC TTTTTTCTGT GTA-AAAGTA TTTTCACTCT ATAAATAAAT TCAATAAATT AAAACA-GAG ACAGCTTACT TCC-ATTTTC
Pseudosuccinea columella 1        -TTCTAAGGG TCTTCTCGTC TTTTTTCTTT GTA-TAAGTA TTTTCACTTA ATAAATAAAT TCAAAATAAT AGTATA-GAG ACAGCTAACC CCT-ATTAGT
Pseudosuccinea columella 2        -TTCTAAGGG TCTTCTCGTC TTTTTTCTTT GTA-TAAGTA TTTTCACTTA ATAAATAAAT TCAAAAAAT AGTATA-GAG ACAGCTAACC CCT-ATTAGT
Radix auricularia                 ATTCTAAGGG TCTTCTCGTC TTTTTTCTAG ATA-AAAGTA TTTTCACTTT ATAAATAAAT TCACAAAAT TAAAAA-GAG ACAGTAAATT CTTTATTTAA
Radix balthica                    ATTCTAAGGG TCTTCTCGTC TTTTTTCTTT AAA-AAAGTA TTTTCACTTA ATAAATAAAT TCATAGAAAT TAAAAAGAG ACAGTTAATT TTTTATTTAA
Radix luteola                     ATTCTAAGGG TCTTCTCGTC TTTTTTCTTT AAT-TAAGTA TTTTCACTTA CTAAATAAAT TCAATAAAAT TTAAAG-AAG ACAGAAAATT CTTTATTAAA
Radix natalensis                  ATTCTTAGGG TCTTCTCGTC TTTTTTCTAA GTT-AAAGTA TTTTCACTTA ATAAATAAGT TCAATAAAAT CCAAAA-GAG ACAGAAAATT CTTTATTATA
Radix ovata                       ATTCTAAGGG TCTTCTCGTC TTTTTTCTTT AAA-AAAGTA TTTTCACTTT ATAAATAAAT TCAAAAAAT CAAAAA-GAG ACAGTAAATT CTTTATTTAA
Radix peregra                     ATTCTAAGGG TCTTCTCGTC TTTTTTCTTT AAA-AAAGTA TTTTCACTTA ATAAATAAAT TCATAGAAAT TAAAAAGAG ACAGTTAATT TTTTATTTAA
Radix quadrasi                    ATTCTAAGGG TCTTCTCGTC TTTTTTCTTT AAT-TAAGTA TTTTCACTTA CTAAATAAAT TCAATAAAAT TTAAAG-AAG ACAGAAAATT CTTTATTAAA
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Radix rubiginosa          ATTCTAAGGG TCTTCTCGTC TTTTTTCTTT AAT-TAAGTA TTTTCACTTA CTAAATAAAT TCAATAAAAT TTAAAG-AAG ACAGAAAATT CTTTATTAAA
Radix sp. Philippines     ATTCTAAGGG TCTTCTCGTC TTTTTTCTTT AAT-TAAGTA TTTTCACTTA TTAAATAAAT TCAATAAAAT TTAAGG-GAG ACAGAAAATT CTTTATTGGA
Radix sp. Canada          ATTCTAAGGG TCTTCTCGTC TTTTTTCTTT GAT-TAAGTA TTTTCACTTA TTAAATAAAT TCAATAAAAT TTAAAG-AAG ACAGAAAATT CTTTATTAAA
Radix sp. Romania         ATTCTAAGGG TCTTCTCGTC TTTTTTCTTT GAT-TAAGTA TTTTCACTTA TTAAATAAAT TCAATAAAT TTAAAG-AAG ACAGAAAATT CTTTATTAAA
Stagnicola elrodi         ATTCTAAGGG TCTTCTCGTC TTTTTTCTAC GTG-TAAGTA TTTTCACTTA ATAAATAAAT TCAAAAAAT AACATA-AAG ACAGTAAATT CCC-ATTAAC
Stagnicola bonnevillensis ATTCTAAGGG TCTTCTCGTC TTTTTTCTGC GTAATAAGTA TTTTCACTTA ATAAATAAAT TCAAAAAAT AACATA-AAG ACAGTAAATT CCC-ATTAAC
Stagnicola sp. USA        ATTCTAAGGG TCTTCTCGTC TTTTTTCTAC GTA-TAAGTA TTTTCACTTA ATAAATAAAT TCAAAAAAT AACATAAAAG ACAGTAAATT CCC-ATTAAC
Stagnicola sp. Canada     ATTCTAAGGG TCTTCTCGTC TTTTTTCTAC GTG-TAAGTA TTTTCACTTA ATAAATAAAT TCAAAAAAT ATCATA-AAG ACAGTAAATT CCC-ATTAAC
Stagnicola sp. Urkraine   ATTCTAAGGG TCTTCTCGTC TTTTTTCTGA GTG-TAAGTA TTTTCACTTA CTAAATAATT TCAATAATT TCTTCT-GAG ACAGTTTTTC CCCTATTTTC
Stagnicola elodes USA     ATTCTAAGGG TCTTCTCGTC TTTTTTCTAC GTA-TAAGTA TTTTCACTTA ATAAATAAAT TCAAAAAAT ATTATA-AAG ACAGTGAATT CCC-ATTAAC
Stagnicola elodes Canada  ATTCTAAGGG TCTTCTCGTC TTTTTTCTAC GTA-TAAGCA TTTTCACTTA ATAAATAAAT TCAAAAAAT ATTATA-AAG ACAGTGAATT CCC-ATTAAA
Stagnicola corvus         ATTCTTAGGG TCTTCTCGTC TTTTTTCTTT TTA-TAAGTA TTTTCACTTA TTAAATAAAT TCAATTAATT TTATTT-GAG ACAGTTTTTT CTT-ATTTTC
Stagnicola emarginata     ATTCTAAGGG TCTTCTCGTC TTTTTTCTAC GTG-TAAGTA TTTTCACTTA ATAAATAAAT TCAAAAAAT AACATA-AAG ACAGTAAATT CCC-ATTAAC
Stagnicola catascopium    ATTCTAAGGG TCTTCTCGTC TTTTTTCTAC GTG-TAAGTA TTTTCACTTA ATAAATAAAT TCAAAAAAT ATCATA-AAG ACAGTAAATT CCC-ATTAAC
Stagnicola caperata       ATTCTAAGGG TCTTCTCGTC TTTTTTCTTA ATG-TAAGTA TTTTCACTTA CTAAATAACT TCAAAAAAT TCCCCT-GAG ACAGCTACTC CCC-ATCAGT
Stagnicola palustris      ATTCTAAGGG TCTTCTCGTC TTTTTTCTTA TTA-TAAGTA TTTTCACTTA TTAAATAAAT TCAATTAATT TTTTTGAGAG ACAGTTTTTT CCT-ATTTTC
Acroloxus lacustris       ATTCTTAGGG TCTTGTCGTC TTTTTTCTAT AAAA-AAGTA TTTTCACTTT TTAAATAAAT TTAATACTAT ATACTT-GAA ACAGCGTTTC CCC-ATAAAT
Amerianna carinata        ATTCTAAGGG TCTTCTCGTC TTTTTTCTTA AAAT-AGGTA TTTTCACCTA ATAATTAAAT TCAAATAAAT AGTTTT-AAG ACAGTATTTC TCC-AAAACT
Aplexa hypnorum           ATTCTAAGGG TCTTCTCGTC TTTTTTATTA AT-ATAGGTA TTTTCACCTA ATGAATAAGT TTATTATAAT -TTTTTTAAT AAAGCTAATA TCC-AATATT
Ancylus fluviatilis       ATTCTTAGGG TCTTCTCGTC TTTTTTAAAA ACAA-AAGTA TTTTCACTTT TTTAATAAAT TCAATATTAT ACATTT-GAA ACAGATAAATC CCC-ATAAAT
Biomphalaria peregrina    ACTCTAAGGG TCTTCTCGTC TTTTTTCTAA AAAAATGATA TTTTCATCAT TTAAATAAAT TCAAAAAATT TCTTTT-AAG ACAGAATTAT TTC-ATGCTT
Biomphalaria schrammi     ACTCTAAGGG TCTTCTCGTC TTTTTTCTAA GAAA-TAGTA TTTTCACTAT TTAAATAAAT TAAGAAAATC TCTATT-AAG ACAGAATTAT TTC-ATTTTT
Bulinus bavayi            ACTTTTAGGG TCTTCTCGTC TTTTATTTTT AAAA-AAGTA TTTTCACTTT TTAACTAAAT TAAAATTAAT ATGAAT-AAG ACAGCTTAAC CCC-ATTAAT
Bulinus globosus          ATTCTTAGGG TCTTCTCGTC TTTTTTTTAA AAAA-AAGTA TTTTCACCTT TTCAATAAAT TAAATCAGT ATTTTT-AAG ACAGCATTTC TTC-ATTATT
Burupia kempi             ACTCTTAGGG TCTTCTCGTC TTTATTTTAA AATT-TAGTA TTTTCACTAA ATAAATAAAT TAAAATTAAT ATTTTA-AAT AAAGTAAGCC CTA-ATTATC
Burnapia stuhlmanni       ACTCTTAGGG TCTTCTCGTC TTTATTTTAA AATT-TAGTA TTTTCACTAA ATAAATAAAT TAAAATTAAT ATTTTA-AAT AAAGTAAGCC CTA-ATTATT
Pettancylus sp.           ATTCTTAGGG TCTTCTCGTC TTTTATAATT ATAA-AAGTA TTTTCACTTT TTAAATAAGT TAAAAATAAT ATTTTT-GAA ACAGCTTATC CCC-ATAAAT
Physa acuta               ACTCTAAGGG TCTTCTCGTC TTTTTTCTAC AT-ACTGGTA TTTTCACCAG TTCATTAAAT TAAA-ATAAT ATTTTTTATT ATAGGTACTA TTC-ATTACT
Physella johnsoni         ATTCTAAGGG TCTTCTCGTC TTTTTTCTAA ATTACTGGTA TTTTCACCAG ATAAACAAAT TAAA-AAAAC ACTAATTATT ATAGCTACTA TTC-ATTACT
Physa heterostropha       ACTCTAAGGG TCTTCTCGTC TTTTTTCTAT AT-ATTGGAA TTTTCACCAG TTCATTAAAT TAAA-ATAAT ATTTTTTATT ATAGCTACTA TTC-ATTACT
Physella wrighti          ATTCTAAGGG TCTTCTCGTC TTTTTTCTAA ATTACTGGTA TTTTCACCAG ATAAACAAAT TAAA-AAAAC ACTAATTATT ATAGCTACTA TTC-ATTACT
Physa fontinalis          ACTCTAAGGG TCTTCTCGTC TTTTTTCTTA AT-ATAGGTA TTTTCACCTA ATAAATAAAT TAAA-ATAAT ATTATTTGTT ATAGTCCCTA TTC-ATTCTT
Planorbis planorbis       ATTCTAAGGG TCTTCTCGTC TTTATTTTTA AAA-AAGGTA TTTTCACCTT TTAAATAAAT TTTAATAAAA GCAACT-AAG ACAGATTAAC TTC-ATTATC
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                              410        420        430        440        450        460        470        480        490        500
Austropeplea lessoni NSW    CCT-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATAA- ------TACG CTGGGCAGAA
Austropeplea lessoni WA     CCT-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATAA- ------TACG CTGGGCAGAA
Austropeplea lessoni NT     CCT-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATAA- ------TACG CTGGGCAGAA
Austropeplea lessoni QLD    CCT-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATAA- ------TACG CTGGGCAGAA
Austropeplea tomentosa NSW  CCT-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATATA ------AACG CTGGGCAGAA
Austropeplea tomentosa NZN  CCT-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATATA ------AACG CTGGGCAGAA
Austropeplea tomentosa NZS  CCT-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAGTATA ------AACG CTGGGCAGAA
Austropeplea tomentosa SA   CCT-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATATA ------AACG CTGGGCAGAA
Austropeplea tomentosa TAS  CCT-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATATA ------AACG CTGGGCAGAA
Austropeplea ollula        CCT-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATACA T-----AACG CTGGGCAGAA
Austropeplea viridis 1     CCT-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATACA T-----AACG CTGGGCAGAA
Austropeplea viridis 2     CCT-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATACA T-----AACG CTGGGCAGAA
Austropeplea sp. China     CCT-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATAAA A-----CACG CTGGGCAGAA
Austropeplea sp. Hawaii    CCT-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATAA- ------TACG CTGGGCAGAA
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Bullastra cumingiana         CCT-TTCATT CCAGACTCCA ATTAAAAGCC AACTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTGATGTG TT----TACG CTGGGCAGAA
Bulimnea megasoma            CCA-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATATA ------TACG CTGGGCAGAA
Fossaria bulmoides           CCG-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATTTT ------TACG CTGGGCAGAA
Fossaria obrussa             CCG-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATATT ------TACG CTGGGCAGAA
Fossaria truncatula          CCG-TTCATT CCAGACTCCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATATC ------TACG CTGGGCAGAA
Kutikina hispida             CCT-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATATA ------AACG CTGGGCAGAA
Lymnaea stagnalis 1          CCG-TTCATT CCAGACTATA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATAAA T-----TACG CTGGGCAGAA
Lymnaea stagnalis 2          CCG-TTCATT CCAGACTACA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATAAA T-----TACG CTGGGCAGAA
Lymnaea stagnalis 3          CCA-TTCATT CCAGACTACA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATAAA T-----TACG CTGGGCAGAA
Lymnaea stagnalis 4          CCA-TTCATT CCAGACTACA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATAAA T-----TACG CTGGGCAGAA
Lymnaea stagnalis 5          CCG-TTCATT CCAGACTACA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAA GTACTGCGGC CGCTAATAAA ------CACG CTGGGCAGAA
Omphiscola glabra            CCA-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAGA GTACTGCGGC CGTTTATAAA T------TACA CTGGGCAGAA
Pseudosuccinea columella 1   CCT-TTCATT CCAGACTACA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATAAA T-----TACG CTGGGCAGAA
Pseudosuccinea columella 2   CCT-TTCATT CCAGACTACA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAA GTACTGCGGC CGCTAATAAA T-----TACG CTGGGCAGAA
Radix auricularia            CCT-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATTTT TTAACCAACG CTGGGCAGAA
Radix balthica               CCT-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATAAT TT----TACG CTGGGCAGAA
Radix luteola                CCT-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGTTAACAAA TTA---TGCG CTGGGCAGAA
Radix natalensis             CCT-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTAATGCGGC CGCTAATTTT T------ACG CTGGGCAGAA
Radix ovata                  CCT-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATAAA T-----AACG CTGGGCAGAA
Radix peregra                CCT-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATAAT TT----TACG CTGGGCAGAA
Radix quadrasi               CCT-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGTTAACAAA TTAT--TGCG CTGGGCAGAA
Radix rubiginosa             CCT-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGTTAACAAA TTAT--TGCG CTGGGCAGAA
Radix sp. Philippines        CCT-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAACGGA TCA---TGCG CTGGGCAGAA
Radix sp. Canada             CCT-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAACAAA TTA---TGCG CTGGGCAGAA
Radix sp. Romania            CCT-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAACAAA TTA---TGCG CTGGGCAGAA
Stagnicola elrodi            CCG-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATTAT TT----TACG CTGGGCAGAA
Stagnicola bonnevillensis    CCG-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATTAT TT----TACG CTGGGCAGAA
Stagnicola sp. USA           CCG-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATTAT TT----TACG CTGGGCAGAA
Stagnicola sp. Canada        CCG-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATTAT TTT---TACG CTGGGCAGAA
Stagnicola sp. Urkraine      CCG-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATATT A-----TACG CTGGGCAGAA
Stagnicola elodes USA        CCG-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATATT ATT---TACG CTGGGCAGAA
Stagnicola elodes Canada     CCCCTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATATT ATT---TACG CTGGGCAGA?
Stagnicola corvus            CCA-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATATA T-----TACG CTAGGCAGAA
Stagnicola emarginata        CCG-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATATT ATTT--TACG ??????????
Stagnicola catascopium       CCT-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATTAT TTT---TACG TGGG??????
Stagnicola caperata          CCG-TTCATT CCAGACTTCA ATTAAAAGCC AACTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CG-TAATAT- ------TACG CT????????
Stagnicola palustris         CCA-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATAAT TA----TACG CTGGGCAGAA
Acroloxus lacustris          CCT-TTCATT CCAGACTCAA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAA GTACTGCGGC CATTTATAAA TT----TACA CTGGGCAGGA
Amerianna carinata           CCA-TTCATT CCAGACTTTA ATTAAAAGCC AATTGATTAT GCTACCTTTG CACAGTCAGG GTACTGCGGC CATTTATAAA A-----AACA CTGGGCAGGA
Aplexa hypnorum              CCT-TTCATT CTAGACTACA ATTAATAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGTTAATAAT ------TACA CTGGGCAGAA
Ancylus fluviatilis          CCT-TTCATT CCAGACTCAA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAA GTACTGCGGC CGTTAATATT ATA---TACA CTGGGCAGAA
Biomphalaria peregrina       CCT-TTCATT CTAGACTCTA ATTAGAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CATTTAAAAA AAAA--ATCA GTGGGCAGAA
Biomphalaria schrammi        CCA-TTCATT CCAGACTCAA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CATTAAAAAA ------TTCA TTGGGCAGAA
Bulinus bavayi               TCT-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAGG GTACTGCGGC CCTTTATATT ------AACA CTGGGCAGAA
Bulinus globosus             TCT-TTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAGG GTACTGCGGC CATTTATATA TTAG--TACA CTGGGCAGAA
Burupia kempi                CCA-TTCATT CCAGACTCTA ATTAGAAGTC AATTTATTAT GCTACCTTAG CACAGTCAAT GTACTGCGGC CGTTAAAAAT AA----ATCA CTGGGCAGAA
Burnapia stuhlmanni          CCA-TTCATT CCAGACTCTA ATTAGAAGTC AATTTATTAT GCTACCTTAG CACAGTCAAT GTACTGCGGC CGTTAAAAAT AA----ATCA CTGGGCAGAA
Pettancylus sp.              CCT-TTCATT CCAGACTCTA ATTAAGAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CATTTATATA ------AACA CTGGGCAGGA
Physa acuta                  TCA-TTCATA CTAGACTACA ATTAATAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGTTTATATA ------AACA CCGGGCAGAA
Physella johnsoni            TCT-TTCATT CCAGACTACA ATTAATAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGTTAATAAA GT----TACA CCGGGCAGAA
Physa heterostropha          TCA-TTCATA CTAGACTACA ATTAATAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGTTTATATA ------AACA CCGGGCAGAA
Physella wrighti             TCT-TTCATT CCAGACTACA ATTAATAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGTTAATAAA ATTT--TACA CCGGGCAGAA
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Physa fontinalis        CCT-TTCATT CCAGACTACA ATTAATAGCC AACTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGTTAATACA ------AACA CTGGGCAGAA
Planorbis planorbis     CCA-TTCATT CTAGACCTTA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CATTGATTTT TTTAA-AACA TT????????


                             ....|....| ....|....| ....|....| ....|....| ....|..
                                 510        520        530        540
Austropeplea lessoni NSW        TTCACCTAAA ATAAGTT-CT TCAGACT??? ?????????? ???????
Austropeplea lessoni WA         TTCACCTAAA ATAAGTT-CT TCAGGCT??? ?????????? ???????
Austropeplea lessoni NT         TTCACCTAAA ATAAGTT-CT TCAGGCT??? ?????????? ???????
Austropeplea lessoni QLD        TTCACCTAAA ATAAGTT-CT TCAGGCT??? ?????????? ???????
Austropeplea tomentosa NSW      GTTACTTAAA ATATATT-CT ATAAGCT??? ?????????? ???????
Austropeplea tomentosa NZN      GTAACTTAAA ATATATT-CT TAAAGCT??? ?????????? ???????
Austropeplea tomentosa NZS      GTAACTTAAA ATATGTT-CT TAAAGCT??? ?????????? ???????
Austropeplea tomentosa SA       GTTACTTAAA ATATATT-CT ATAAGCT??? ?????????? ???????
Austropeplea tomentosa TAS      GTTACTTAAA ATATATT-CT ATAAGCT??? ?????????? ???????
Austropeplea ollula             GTAACTTAAA ATATGTT-CT TAAAGCT??? ?????????? ???????
Austropeplea viridis 1          TTCACTTAAA ATGTATC-CT TCAAGCT??? ?????????? ???????
Austropeplea viridis 2          TTCACTTAAA ATGTATC-CT TCAAGCT??? ?????????? ???????
Austropeplea sp. China          CTCACTTAAA ATGTATC-CT TCAAGCT??? ?????????? ???????
Austropeplea sp. Hawaii         CTCACTTAAA ATGTATC-CT TCAAGCT??? ?????????? ???????
Bullastra cumingiana            TTCACCTAAA ATATGTC-CT CTAGGCT??? ?????????? ???????
Bulimnea megasoma               TTTACCTAAA ATATATC--T TCAGGCT??? ?????????? ???????
Fossaria bulmoides              TTTACCTGAA ATAAATC--T TCAGGCT??? ?????????? ???????
Fossaria obrussa                TTTACCTAAA ATAAAAC--T TCAAGCT??? ?????????? ???????
Fossaria truncatula             TTTACCTAAA ATAAATC--T TTAAGCT??? ?????????? ???????
Kutikina hispida                GTTACTTACA ATATATT-CT ATAAGCT??? ?????????? ???????
Lymnaea stagnalis 1             ATTACTTAAA ATATAGA-CT TAAAGCT??? ?????????? ???????
Lymnaea stagnalis 2             ATTACTTGCA ATAAAAC--T ACAAGCT??? ?????????? ???????
Lymnaea stagnalis 3             ATTACTTAAA ATATAAA-CT TCAAGCT??? ?????????? ???????
Lymnaea stagnalis 4             ATTACTTAAA ATATAAA-CT TCAAGCT??? ?????????? ???????
Lymnaea stagnalis 5             ATTACTTAAA ?????????? ?????????? ?????????? ???????
Omphiscola glabra               ATTATCTATA ATATTTTTGC TA??????? ?????????? ???????
Pseudosuccinea columella 1      TTTACCCAAA ATAAATC--T TTGGGCT??? ?????????? ???????
Pseudosuccinea columella 2      TTTACCCAAA ATAAATC--T TTGGGCT??? ?????????? ???????
Radix auricularia               TTCACTTAAA ATATAAT-CT TTAAGCT??? ?????????? ???????
Radix balthica                  CTTACTTAAA ATAAATTCT TTAAGCT??? ?????????? ???????
Radix luteola                   TTTACCTAAA ATGAATTCT TTAGGCT??? ?????????? ???????
Radix natalensis                CTTACCTAAA ATATATT-CT TCAAGCT??? ?????????? ???????
Radix ovata                     TTTACTTAAA ATATAAT-CT TTAAGCT??? ?????????? ???????
Radix peregra                   CTTACTTAAA ATAAATTCT TTAAGCT??? ?????????? ???????
Radix quadrasi                  TTTACCTAAA ?????????? ?????????? ?????????? ???????
Radix rubiginosa                TTTACCTAAA ?????????? ?????????? ?????????? ???????
Radix sp. Philippines           TTTACCTAAA ATGATTT-CT TTAAGCT??? ?????????? ???????
Radix sp. Canada                TTTACCTAAA ATAAACTTCT TTAAGCT??? ?????????? ???????
Radix sp. Romania               TTTACCTAAA ATAAACTTCT TTAGGCT??? ?????????? ???????
Stagnicola elrodi               TTTACCTAAG ATGGTTA-CT TTAGGCT??? ?????????? ???????
Stagnicola bonnevillensis       TTTACCTAAA ATGGTTA-CT TTAGGCT??? ?????????? ???????
Stagnicola sp. USA              TTTACCTAAG ATGGGTA-CT TTAGGCT??? ?????????? ???????
Stagnicola sp. Canada           TTTACCTAAG ATGGTTTACT TTAGGCT??? ?????????? ???????
Stagnicola sp. Urkraine         GTTACTTAAA ATATAA--CT TCAAGCT??? ?????????? ???????
Stagnicola elodes USA           TTTACCCAAG ATGGTTA-CT TTAGGCT??? ?????????? ???????
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Stagnicola elodes Canada    -TTACC--AG -TGGT????? ?????????? ?????????? ??????
Stagnicola corvus           GT???????? ?????????? ?????????? ?????????? ??????
Stagnicola emarginata       ?????????? ?????????? ?????????? ?????????? ??????
Stagnicola catascopium      ?????????? ?????????? ?????????? ?????????? ??????
Stagnicola caperata         ?????????? ?????????? ?????????? ?????????? ??????
Stagnicola palustris        GTTACTCAAA A????????? ?????????? ?????????? ??????
Acroloxus lacustris         ATAATT???? ?????????? ?????????? ?????????? ??????
Amerianna carinata          TTTATTTAAA ATAATTACTT CAAACT???? ?????????? ??????
Aplexa hypnorum             TTAACCTAGT ATAAAT???? ?????????? ?????????? ??????
Ancylus fluviatilis         ATAATT-AAA AATTTATACT TTTAACTATG TTTTTGATAA ACTGGCG
Biomphalaria peregrina      ATCATTTAAG ATAATT--TC CTTAAACT?? ?????????? ??????
Biomphalaria schrammi       TACATTCAAA ATAAATAATC TTTGAACT?? ?????????? ??????
Bulinus bavayi              TA???????? ?????????? ?????????? ?????????? ??????
Bulinus globosus            TA???????? ?????????? ?????????? ?????????? ??????
Burupia kempi               TT???????? ?????????? ?????????? ?????????? ??????
Burnapia stuhlmanni         ?????????? ?????????? ?????????? ?????????? ??????
Pettancylus sp.             AAAAATTCAA AATATTTTCT TTGAACT??? ?????????? ??????
Physa acuta                 GATATCGACA ATAAGAAT-- TTAGATCACC TGCCGACT?? ??????
Physella johnsoni           GATATCAATA ATCTTTT--- AAAAATTTTC TACTGACT?? ??????
Physa heterostropha         GATATCGACA ATAAAAAT-- TTATTTCATC TGCCGACT?? ??????
Physella wrighti            GATATCAATA ATCTTTTTAA AAAAATTTTC TACTGACT?? ??????
Physa_fontinalis            ACCACCGATA ATAT?????? ?????????? ?????????? ??????
Planorbis_planorbis         ?????????? ?????????? ?????????? ?????????? ??????
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Appendix 4.3 Alignment of 16S rRNA used for phylogenetic analysis *Austropeplea* and *Radix*.

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                             ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                     10         20         30         40         50         60         70         80         90        100
Austropeplea lessoni NSW     AGGAAATTTT TGTTCGAACA GAACATTCTA TTTTGACTGT TAGTCTTCTA GTTCCTAGTC CAACATCGAG GTCACAAGCT AAAAATTTTA TTATGCTGTT
Autropeplea lessoni WA       AGGAAATTTT TGTTCGAACA GAACATTCTA TTTTGACTGT TAGTCTTCTA GTTCCTAGTC CAACATCGAG GTCACAAGCT AAAAATTTTA TTATGCTGTT
Austropeplea lessoni NT      AGGAAATTTT TGTTCGAACA GAACATTCTA TTTTGACTGT TAGTCTTCTA GTTCCTAGTC CAACATCGAG GTCACAAGCT AAAAATTTTA TTATGCTGTT
Austropeplea lessoni QLD     AGGAAGTTTT TGTTCGAACA GAACATTCTA TTTTGACTGT TAGTCTTCTA GTTCCTAGTC CAACATCGAG GTCACAAGCT AAAAATTTTA TTATGCTGTT
Austropeplea tomentosa NSW   AGGAAATT-T TGTTCGAACA GAACATTCTA TTATGACGGT TAGTCTTCTA GCTCCTAGTC CAACATCGAG GTCACAAACT AAATAATTCA TTATGCTGTT
Austropeplea tomentosa NZN   AGGAAATT-T TGTTCCAACA GAACATTCTA TTATGACTGT TAGTCTTTTA GTTCCTAGTC CAACATCGAG GTCACAAACT AAATTATTCA TTATGCTGTT
Austropeplea tomentosa NZS   AGGAAATT-T TGTTCGAACA GAACATTCTA TTATGACTGT TAGTCTTTTA GTTCCTAGTC CAACATCGAG GTCACAAACT AAATTATTCA TTATGCTGTT
Austropeplea tomentosa SA    AGGAAATT-T TGTTCGAACA GAACATTCTA TTATGACGGT TAGTCTTTTA GCTCCTAGTC CAACATCGAG GTCACAAACT AAATAATTCA TTATGCTGTT
Austropeplea tomentosa TAS   AGGAAATT-T TGTTCGAACA GAACATTCTA TTATGACGGT TAGTCTTTTA GCTCCTAGTC CAACATCGAG GTCACAAACT AAATAATTCA TTATGCTGTT
Austropeplea viridis         AGGAAACTGT TGTTCGAACA GAACAATCTA TTTTGACGGT TAATCTTTTA GTTCCTAGTC CAACATCGAG GTCATAAGCT ATATTAATCA TTATGCTGTT
Bullastra cumingiana         AGGAAAAATC TGTTCGAACA GAACATTCTA TTTTGACTGT TAGTCTTCTA GTTCCTAGTC CAACATCGAG GTCACAAGCT AAAAATTTTA TTATGCTGTT
Kutikina hispida             AGGAAATT-T TGTTCGAACA GAACATTCTA TTATGACGGT TAGTCTTTTA GCTCCTAGTC CAACATCGAG GTCACAAACT AAATAATTCA TTATGCTGTT
Lymnaea stagnalis 1          ????GATATT TGTTCGAACA GAACAATCTA TATTGACGGT TAGTCATCTA GTTCCTAGTC CAACATCGAG GTCACAAACT TTATAAAAAA TTATGCTGTT
Pseudosuccinea columella 1   ???GAATTTC TGANCGAACA GAACAATCTA TTTAGACGGT TAATCTTCTA GTTCCTAGTC CAACATCGAG GTCACAAACT AAATAATTAA TTATGCTGTT
Radix auricularia            AGGAGAAA-T TGTTCGAACA GAACACTCTA TTTTGACTGT TAGTCCTTTA GTTCCTAGTC CAACATCGAG GTCATAAGCT AATAAATTCA TTATGCTGTT
Radix luteola                AGGAGTTAAA TGTTCGAACA GAACAATCTA ATTTGACGGT TAGTCTTATA GTTCCTAGTC CAACATCGAG GTCACAAACT ATAATAATCA TTATGCTGTT
Radix natalensis             AGGAGTATTA TGTTCGAACA GAACAATCTA TTTTGACGGT TAGTCTCTTA GTTCCTAGTC CAACATCGAG GTCACAAACT ATTTTAATTA TTATGCTGTT
Radix ovata                  AGGAGAAAAT TGTTCGAACA GAACATTCTA TTTTGACTGT TAGTCTTTTA GTTCCTAGTC CAACATCGAG GTCATAAACT AAATAATTCA TTATGCTGTT
Radix peregra                ?????????? ????????CA GAACACTCTA TTTTGACGGT TAGTCTTTTA GTTCCTAGTC CAACATCGAG GTCACAAGCT AATAATTTCA TTATGCTGTT
Radix quadrasi               AGGAGTTAAA TGTTCGAACA GAACAATCTA GTTTGACGGT TAGTCTAATA GTTCCTAGTC CAACATCGAG GTCACAAACT ATAATAATCA TTATGCTGTT
Radix rubiginosa             ?????????? ????GCAACA GAACAATCTA ATTTGACGGT TAGTCTAATA GTTCCTAGTC CAACATCGAG GTCACAAACT ATAATAATCA TTATGCTGTT
Stagnicola corvus            ?AGGAATTTC TGTTCGAACA GAACAATCTA TATTAACGGT TAGTCATTTA GTTCCTAGTC CAACATCGAG GTCACAAACT TTAAACAAAA TTATGCTGTT
Stagnicola palustris         AGGAATTTC TGTTCGAACA GAACAATCTA TATTGACGGT TAGTCTTTTA GTTCCTAGTC CAACATCGAG GTCACAAACT TTATTAAAAA TTATGCTGTT


                             ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                                    110        120        130        140        150        160        170        180        190        200
Austropeplea lessoni NSW     ATCCCTAAGG TAATTTAATC TAACAAAAAA AAATGACTTG TAATTAT--T AAAAGTTTAA AA---TGTTT TATTGTCGCC CCAACAAAAA TAAAAAAAT-
Autropeplea lessoni WA       ATCCCTAAGG TAATTTAATC TAACAAAAAA A--TGACTTG TAATTAT--T AAAAGTTTAA AA---TGTTT TAATGTCGCC CCAACAAAAA TAAAAAAAT-
Austropeplea lessoni NT      ATCCCTAAGG TAATTTAATC TAACAAAAAA A--TGACTTG TAATTAT--T AAAAGTTTAA AAA--TGTTT TAATGTCGCC CCAACAAAAA TAAAAAAAT-
Austropeplea lessoni QLD     ATCCCTAAGG TAATTTAATC TAACAAAAAA AA-TGACTTG TAATTAT--T AAAAGTTTAA AA---TGTTT TATTGTCGCC CCAACAAAAA TAAAAAAAT-
Austropeplea tomentosa NSW   ATCCCTAAGG TAATTTAATC TTAATAGGAA AA-TGACTTG TTTTTAT--T GAAAGTTTAA A----TGTTT CAATGTCGCC CCAACAAAAA TATATAATT-
Austropeplea tomentosa NZN   ATCCCTAAGG TAATTTAATC TTT-AAAAAA AA-TGACTTG TTTTTAA--T TGAAAGTTAA C----TGTTT CATTGTCGCC CCAACAAAAA TATATAATT-
Austropeplea tomentosa NZS   ATCCCTAAGG TAATTTAATC TTTTAAAAAA AA-TGATTTG TTTTTAA--T TGAAAGTTGA T----TGTTT CATTGTCGCC CCAACAAAAA TATGTAATT-
Austropeplea tomentosa SA    ATCCCTAAGG TAATTTAATC TTAATAGAAA AA-TGACTTG TTTTTAT--T GAAAGTTTAA A----TGTTT CAATGTCGCC CCAACAAAAA TATATAATT-
Austropeplea tomentosa TAS   ATCCCTAAGG TAATTTAATC TTAATAGAAA A--TGACTTG TTTTTAT--T GAAAGTTTAA A----TGTTT CAATGTCGCC CCAACAAAAA TATATAATT-
Austropeplea viridis         ATCCCTAAGG TAATTTTACC ATACAAAAAA AA-TGACATG TTTTTATA-T GAAAGTTTAA A----TGTTT CATTGTCGCC CCAACAAAAA TAAGAGATT-
Bullastra cumingiana         ATCCCTAAGG TAATTTATTC TTACAAATAA A--TGACTTG TTTAATT--T GAAAGTTTTA A----TGTTT CAATGTCGCC CCAACAAAAA TAAATAAAT-
Kutikina hispida             ATCCCTAAGG TAATTTAAAC TTAATAAAAA A--TGACTTG TTTTAAT--T GAAAGTTTAA A----TGTTT CATTGTCGCC CCAACAAAAA TATATAATT-
Lymnaea stagnalis 1          ATCCCTAAGG TAATTTCTTC TATATAACCA AATTGTTATG TA-TATAAAT AAATGTTTAC -----TGATT TATTGTCGCC CCAACAAAAA CATGATT---
Pseudosuccinea columella 1   ATCCCTAAGG TAATTTATTC TTTCAAACCT AAATGTCTTG ATTGTTAA-T GAAAGTTTAA T----TGTTT CAATGTCGCC CCAACAAAAA AAAATTATT-
Radix auricularia            ATCCCTAAGG TAATTTGATC GTGCAAAGAA AATTGTTGTG TAAAAATCTT GAAAGTTTAA TA---TGTTT CAATGTCGCC CCAACAAAAA TAAATCTTA-
Radix luteola                ATCCCTAAGG TAATTTTACC TTGCAAAACT TAATGTCCAG TAAAGTAT-T TAAAGTTTAA A----TGTTT AAATGTCGCC CCAACAAAAA AATAAACTT-
Radix natalensis             ATCCCTAAGG TAATTTAATC TTACAAACTT TAATGTCATG TATAAAAA-T -AAAGTTTAA A----TGTTT AAATGTCGCC CCAACAAAAA AATAAATTT-
Radix ovata                  ATCCCTTAGG TAATTTGATC TTTTAAAAAA ATTTGTCATG TAAA--TCTT GAAAGTTAAA TA---TGTTT CAGTGTCGCC CCAACAAAAA AATATTTTT-
Radix peregra                ATCCCTAAGG TAATTTGATC ATTCAAAATA AA-TGTCTCG TAAAAATTGT GAAAGTTCAA AATTTTGTTT CAATGTCGCC CCAACAAAAA TAAGTTTAC-
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Radix quadrasi              ATCCCTAAGG TAATTTTATC TTACAAAACT TAATGTCCAG TAGAATAT-T TAAAGTTTAA A----TGTTT AAATGTCGCC CCAACAAAAA AATAAACTT-
Radix rubiginosa            ATCCCTAAGG TAATTTTATC TTACAAAACT TAATGTCCAG TAGAATAT-T TAAAGTTTAA A----TGTTT AAATGTCGCC CCAACAAAAA AATAAACTT-
Stagnicola corvus           ATCCCTAAGG TAATTTGCTC TTTTAAATCT AAGTGTTTAG TTGTTGTAAT AAATGTTCAA A----TGATT CAATGTCGCC CCAACAAAAA TAAAGATTTT
Stagnicola palustris        ATCCCTAAGG TAATTTGCTC TTTTAAATCT AAGTGTTTAG TATTACTAAT AAATGTTTGT AAA--TGATT CAATGTCGCC CCAACTAAAA TAAAAATT--


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                                210        220        230        240        250        260        270        280        290        300
Austropeplea lessoni NSW    -TATAAACCT TAATTAATAA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TAGTAGAAGT ATTTTCACTT CATAAATAAA TTCAAATAAA TTTAAAA-GA
Autropeplea lessoni WA      -TATAAACTT TAATTAATGA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TAGCAGAAGT ATTTTCACTT CATAAATAAA TTCAAATAAA TTTAAAA-GA
Austropeplea lessoni NT     -TATAAACTT TAATTAATGA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TAGTAGAAGT ATTTTCACTT CATAAATAAA TTCAAATAAA TTTAAAA-GA
Austropeplea lessoni QLD    -TATAAACCT TAATTAATGA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TAGTAGAAGT ATTTTCACTT CATAAATAAA TTCAAATAAA TTTAAAA-GA
Austropeplea tomentosa NSW  -TATG-AAAT TAATTAATAA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TCGAAAAAGT ATTTTCACTT TTTAAATAAA TTCAATAAAA TTTTTAA-GA
Austropeplea tomentosa NZN  -TAAATAAAT TAATTAATTA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT ATAAAAAAGT ATTTTCACTT TTTAAATAAG TTCATTAAAA TTTTTAA-GA
Austropeplea tomentosa NZS  -TAAATAAAT TAATTAATTA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT ATAAAAAGGT ATTTTCACTT TTTAAATAAA TTCATTAAAA TTTTTAA-GA
Austropeplea tomentosa SA   -TATG-AAAT TAATTAATAA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TCGGAAAAGT ATTTTCACTT TTTAAATAAA TTCAATAAAA TTTTTAA-GA
Austropeplea tomentosa TAS  -TATG-AAAT TAATTAATAA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TCGAAAAAGT ATTTTCACTT TTTAAATAAA TTCAATAAAA TTTTTAA-GA
Austropeplea viridis        -TAAA-AAAC CTTTTAATAA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT AATAAAAAGT ATTTTCACTT TATAAATAAA TTCATATAAG CTAAAAA-GA
Bullastra cumingiana        -TATAAAATT TAATTAATTA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT AAATAAAAGT ATTTTCACTT TTTAAATAAA TTCAAAAAAA TTTTAAA-GA
Kutikina hispida            -TATG-AAAT TAATTAATAA AAATTCTAAG GGTCTTCTCG TCTTTTTTGT TCAAAAAAGT ATTTTCACTT TTTAAATAAA TTCAAATAATA TTTTTAA-GA
Lymnaea stagnalis 1         ---AAAATAA TCATTTATAA AAATTCTTAG GGTCTTCTCG TCTTTTATCA ATTTATAAGT ATTTTCACTT AATAAATAAA TTCAATAAAT TTAATA--GA
Pseudosuccinea columella 1  -AA------A TATTTTATTA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TTGTATAAGT ATTTTCACTT AATAAATAAA TTCAAAATAA TAGTATA-GA
Radix auricularia           -AATAATA-A AATAAAATTA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT AGATAAAAGT ATTTTCACTT TATAAATAAA TTCACAAAAA TTAAAAA-GA
Radix luteola               -TAAAAAAAG TTGTTAATAA GGTCTTCTCG TCTTTTTTCT TTAATTAAGT ATTTTCACTT ACTAAATAAA TTCAATAAAA TTTAAAG-AA
Radix natalensis            -TATATAA-A TTATCATTAA AAATTCTTAG GGTCTTCTCG TCTTTTTTCT AAGTTAAAGT ATTTTCACTT AATAAATAAG TTCAATAAAA TCCAAAA-GA
Radix ovata                 -AATAAAA-A AACTAAGTAA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TTAAAAAAGT ATTTTCACTT TATAAATAAA TTCAAAAAAA TCAAAAA-GA
Radix peregra               -AATAAAGTA AAAGAAATTA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TTAAAAAAGT ATTTTCACTT AATAAATAAA TTCATAGAAA TTAAAAAAGA
Radix quadrasi              -TAAAAAA-G TTGTTATTAA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TTAATTAAGT ATTTTCACTT ACTAAATAAA TTCAATAAAA TTTAAAG-AA
Radix rubiginosa            -TAAAAAA-G TTGTTATTAA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TTAATTAAGT ATTTTCACTT ACTAAATAAA TTCAATAAAA TTTAAAG-AA
Stagnicola corvus           AGGATAAAAA CAATTAATAA AAATTCTTAG GGTCTTCTCG TCTTTTTTCT TTTTATAAGT ATTTTCACTT ATTAAATAAA TTCAATTAAT TTTATTT-GA
Stagnicola palustris        AAAAATAAAA TTATTAATAA AAATTCTAAG GGTCTTCTCG TCTTTTTTCT TATTATAAGT ATTTTCACTT ATTAAATAAA TTCAATTAAT TTTTTTGAGA


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                                310        320        330        340        350        360        370        380        390        400
Austropeplea lessoni NSW    GACAGCTAAT TCTTTATTTA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATA--
Autropeplea lessoni WA      GACAGCTAAT TCTTTATTTA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATA--
Austropeplea lessoni NT     GACAGCTAAT TCTTTATTTA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATA--
Austropeplea lessoni QLD    GACAGCTAAT TCTTTATTTA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATA--
Austropeplea tomentosa NSW  GACAGTTAAT TCTTTATAAA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATA--
Austropeplea tomentosa NZN  GACAGTTAAT TCTTTATAAA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATA--
Austropeplea tomentosa NZS  GACAGTTAAT TCTTTATAAA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAGTA--
Austropeplea tomentosa SA   GACAGTTAAT TCTTTATAAA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATA--
Austropeplea tomentosa TAS  GACAGTTAAT TCTTTATAAA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATA--
Austropeplea viridis        GACAGAAAAT TCTTTAACAA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATA--
Bullastra cumingiana        GACAGCTTAT TCTTTATTAA ACCTTTCATT CCAGACTCCA ATTAAAAGCC AACTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTGATG--
Kutikina hispida            GACAGTTAAT TCTTTATAAA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATG--
Lymnaea stagnalis 1         GACAGTTC-T CTCCTATTAT CCCGTTCATT CCAGACTACA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATA--
Pseudosuccinea columella 1  GACAGCTAAC -CCCTATTAG TCCTTTCATT CCAGACTACA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATA--
Radix auricularia           GACAGTAAAT TCTTTATTTA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATTTT
Radix luteola               GACAGAAAAT TCTTTATTAA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGTTAACAA-
Radix natalensis            GACAGAAAAT TCTTTATTAT ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTAATGCGGC CGCTAA----
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Radix ovata              GACAGTAAAT TCTTTATTTA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAAT---
Radix peregra            GACAGTTAAT TTTTTATTTA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATA--
Radix quadrasi           GACAGAAAAT TCTTTATTAA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGTTAACAA-
Radix rubiginosa         GACAGAAAAT TCTTTATTAA ACCTTTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGTTAACAA-
Stagnicola corvus        GACAGTTT-T TTCTTATTTT CCCATTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATA--
Stagnicola palustris     GACAGTTT-T TTCCTATTTT CCCATTCATT CCAGACTTCA ATTAAAAGCC AATTGATTAT GCTACCTTAG CACAGTCAAG GTACTGCGGC CGCTAATA--


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                              410        420        430        440
Austropeplea lessoni NSW   -----ATACG CTGGGCAGAA TTCACCTAAA ATAAGTT-CT TCAGACT
Autropeplea lessoni WA     -----ATACG CTGGGCAGAA TTCACCTAAA ATAAGTT-CT TCAGGCT
Austropeplea lessoni NT    -----ATACG CTGGGCAGAA TTCACCTAAA ATAAGTT-CT TCAGGCT
Austropeplea lessoni QLD   -----ATACG CTGGGCAGAA TTCACCTAAA ATAAGTT-CT TCAGGCT
Austropeplea tomentosa NSW ----TAAACG CTGGGCAGAA GTTACTTAAA ATATATT-CT ATAAGCT
Austropeplea tomentosa NZN ----TAAACG CTGGGCAGAA GTAACTTAAA ATATATT-CT TAAAGCT
Austropeplea tomentosa NZS ----TAAACG CTGGGCAGAA GTAACTTAAA ATATGTT-CT TAAAGCT
Austropeplea tomentosa SA  ----TAAACG CTGGGCAGAA GTTACTTAAA ATATATT-CT ATAAGCT
Austropeplea tomentosa TAS ----TAAACG CTGGGCAGAA GTTACTTAAA ATATATT-CT ATAAGCT
Austropeplea viridis       --CAT-AACG CTGGGCAGAA TTCACTAAA  ATGTATC-CT TCAAGCT
Bullastra cumingiana       --TGTTTACG CTGGGCAGAA TTCACCTAAA ATATGTC-CT CTAGGCT
Kutikina hispida           ----TAAACG CTGGGCAGAA GTTACTTACA ATATATT-CT ATAAGCT
Lymnaea stagnalis 1        ---AGTTACG CTGGGCAGAA ATTACTTGCA ATAAAAC--T ACAAGCT
Pseudosuccinea columella 1 ---AATTACG CTGGGCAGAA TTTACCCAAA ATAAATC--T TTGGGCT
Radix auricularia          TTAACCAACG CTGGGCAGAA TTCACTTAAA ATATAAT-CT TTAAGCT
Radix luteola              --ATTATGCG CTGGGCAGAA TTTACCTAAA ATGAATTTCT TTAGGCT
Radix natalensis           --TTTTTACG CTGGGCAGAA CTTACCTAAA ATATATT-CT TCAAGCT
Radix ovata                --AAATAACG CTGGGCAGAA TTTACTTAAA ATATAAT-CT TTAAGCT
Radix peregra              --ATTTTACG CTGGGCAGAA CTTACTTAAA ATAAATTTCT TTAAGCT
Radix quadrasi             -ATTATTGCG CTGGGCAGAA TTTACCTAAA ?????????? ???????
Radix rubiginosa           -ATTATTGCG CTGGGCAGAA TTTACCTAAA ?????????? ???????
Stagnicola corvus          ---TATTACG CTAGGCAGAA GT???????? ?????????? ???????
Stagnicola palustris       --ATTATACG CTGGGCAGAA GTTACTCAAA A????????? ???????
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Appendix 4.4 Alignment of ITS-2 rRNA used for phylogenetic analysis *Austropeplea* and *Radix*. All characters with an astertix above them where excluded from all phylogenetic analyses.

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                              10         20         30         40         50         60         70         80         90        100
Austropeplea lessoni NSW  GCTAGTGTTA AAACAATCGC -GTCGC---T TGCTCTCGTA GCGAC--GCG CCCTGGACCA -TCGCGG--- ------CCGC TCACCGAA-- ---TCTT-CC
Austropeplea lessoni NT   ????????TA AAACAATAGT -GTCGC---T TGCTCTCGTT GCGAC--GCG CCCTGGACCA -TCGAGG--- ------CCGC TCACCGAAA- ---CCTT-CG
Austropeplea lessoni QLD  GCTAGTGTTA AAACAATCGC -GTCGC---T TGCTCTCGTA GCGAC--GCG CACTGGACCA -TCGCGG--- ------CCGC TCACCGAA-- ---TCTT-CC
Austropeplea lessoni WA   ??????GTTA AAACAATCGT -GTCGC---T TGCTCTCGTT GCGAC--GCG CCCTGGACCA -TCGCGG--- ------CCGC TCACCGAAAC TTTCCTT-CG
Austropepela tomentosa NSW GCTAGTGTCA AAACAATCGC -GTCGC---T CGCTCGTT-- GCGAC--GCG CTCTGGACCT -TCGCGG--- ------CC-- --ATTAAA-- ---TCCGGCG
Austropeplea tomentosa NZN GCTAGTGTCA AAACAATCGC -GTCGC---T CGCTCGT--- GCGAC--GCG CTCTGGACCT -TCGCGG--- ------CC-- --ATTAAA-- ---TCCGGCG
Austropeplea tomentosa NZS GCTAGTGTCA AAACAATCGC -GTCGC---T CGCTCGT--- GCGAC--GCG CTCTGGACCT -TCGCGG--- ------CC-- --ATTAAA-- ---TCCGGCG
Austropeplea tomentosa SA GCTAGTGTCA AAACAATCGC -GTCGC---T CGCTCGT--- GCGAC--GCG CTCTGGACCT -TCGCGG--- ------CC-- --ATTAAA-- ---TCCGGCG
Austropeplea tomentosa TAS GCTAGTGTCA AAACAATCGC -GTCGC---T CGCTCGT--- GCGAC--GCG CTCTGGACCT -TCGCGG--- ------CC-- --ATTAAA-- ---TCCGGCG
Austropeplea viridis     GCTAGTGTCA AAACAATCGC -GTCGC---T TGCTCGC--- GCGAC--GCG CCCTGGACCT -TCGCGG--- ------CCC- --GTTAAA-- ---TCCGGCG
Bullastra cumingiana     ????GTGTTA AAACAATCGC CGTCGCCCGT TGCTCTCGTG GCGAC--GCG CCCTGGACCG -TCGCGG--- ------TCGC A----AAA-- ---TCCGGCG
Kutikina hispida         GCTAGTGTCA AAACAATCGC -GTCGA---T CGCTCGT--- ACGAC--GCG CTCTGGACCT -TCGCGG--- ------CC-- --ATTAAA-- ---TCCGGCG
Lymnaea stagnalis 1      GCTAGTCACA AAGCAATCGT -GTCCCG-TT TGCTCTCACG AAA-CCGGAG CCTTTCTCGG TTCCCCCCAT CAACCACCGC TCGTTGGGTG GAAGGGGGGG
Pseudosuccinea columella 1 -CTAGCCACA AAGCAATCGT -GTCCGC-GT AGCTCTCACG AAA-CCGGAG CCGGCAGCC- -CCGCCGCAC TCTC--TTGC TCTCGAGAAG GCGTGTTGGG
Radix auricularia        GCTAGTGTCA AA-CAATCGT -GTCGC--TT TGCTCGT--- GCGAC--GCG CTCTGGTCCA -TCGCGG--- ------CC-- --ATAAA-- ---TCCAGCG
Radix natalensis         GCTAGTGTCA AAACAATCGT -GTCGCGTTT TGCTCGT--- GCGAC--GCG CTCTGGACCG -TCGCGG--- ------CC-- --ATAAAA-- ---TCCAGCG
Radix ovata              GCTAGTGTCA AA-CAATCGC -GTCGC-T- TGCTCTT--- GCGAC--GCG CTCTGGACCT -TCGCGG--- ------CC-- --ATAAA-- ---TCCGGCG
Radix peregra            GCTAGTGTCA AA-CAATCGC -GTCGC--T- TGCTCTT--- GCGAC--GCG CTCTGGACCT -TCGCGG--- ------CC-- --GTAAA-- ---TCCGGCG
Radix quadrasi           ????????AA AA-CAATCGT -GTCGC--TC TGTTCTT--- GCGAC--GCG CTCTGGTCCG -TCGCTG--- ------CC-- --ATAAAA-- ---TCCAGCC
Radix rubiginosa         ?????????? ?????????? ?????????? ?GCTCGT--- GCGAC--GCG CTCTGGTCCG -TCGCGG--- ------CC-- --ATAAA--- ---TCCAGCG
Stagnicola corvus        GCTAGTCACA AA-CAATCGT -GTCCCG-TT TGCTCTCACG AAAGCCGGAG CCTTTCTCGG ATCGCCC-AC CA-CCTCTGC TCGTTTAG-- GTCGGTTGGG
Stagnicola palustris     GCTAGTCACA AAGCAATCGT -GTCCCG-TT TGCTCTCACG AAAGCCGGAG CCTTTCTCGG ATCCCCC-AC C----TCTGC TCGTTT---- ---GGTTGGG
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                             110        120        130        140        150        160        170        180        190        200
Austropeplea lessoni NSW  TTCCTTCCAT CCTT------ ------GCTC TCA---CGGA TGGAT----- TGGGGATTGT TTCAT----- ---------T GAGTGG-CCC CGTGGTCTTA
Austropeplea lessoni NT   TTCCTTCCTT CCTTCCTT-- ------GCTC TCG---CGGA TGGATGGGGA TAGGGATAGG GATAGGGG-- GGG------T GAGCGG-CCC CGTGGTCTTA
Austropeplea lessoni QLD  TTCCTTCCAT CCTT------ ------GCTC TCA---CGGA TGGAT----- TGGGGATTGT TTCAT----- ---------T GAGTGG-CCC CGTGGTCTTA
Austropeplea lessoni WA   TTCCTTCCTT CCTTCCTT-- ------GCTC TCG---CGGA TGGATGGGGA TAGGGATAGG GATAGGGATA GGGATAGGTT GAGTGG-CCC CGTGGTCTTA
Austropepela tomentosa NSW CTCACGGCAT -CAT------ ------CGC- TCG---CTCG GCGGT----- GTTGCACGGT GTTGC----- ---------C CGGTGG-CCC CGTGGTCTCA
Austropeplea tomentosa NZN CTCACCACCG -C-T------ ------CGC- TCG---CTCG CTCGG----- ------CGGT GTTGC----- ---------C CGGTGG-CCC CGTGGTCTCA
Austropeplea tomentosa NZS CTCACCACCG -C-T------ ------CGC- TCG------- ----G----- ------CGGT GTCGC----- ---------C CGGTGG-CCC CGTGGTCTCA
Austropeplea tomentosa SA CTCACAGCAT -CAT------ ------CGC- TCG---CTCG GCGGT----- GTTGCACGGT GTTGC----- ---------C CGGTGG-CCC CGTGGTCTCA
Austropeplea tomentosa TAS CTCACAGCAT -CAT------ ------CGC- TCG---CTCG GCGGT----- GTTGCACGGT GTTGC----- ---------C CGGTGG-CCC CGTGGTCTCA
Austropeplea viridis     CTCACCGAAT -CCC------ ------GCT- TTG---CTCG GCGGT----- GTTGGT--GT TGCGC----- ---------C CGGTGGGCCC CGTGGTCTCA
Bullastra cumingiana     GCGGCTCTGA CCGT------ ------AGCA TCG---CTCT CCGCT----- ------TCGG TTTGCCG--- ---------T CGGTGG-CCC CGTGGTCTCA
Kutikina hispida         CTCACAGCAT -CAT------ ------CGC- TCG---CTCG GCGGT----- GTTGCACGG GTTGC----- ---------C CGGTGG-CCC CGTGGTCTCA
Lymnaea stagnalis 1      GGGACTGAAT CGGCACCGGC TGGACACGCT CTGGACCTTC GCGGTCTGCG CTGTCGCTGC GCCATTCGGT GCGGTGATGG CAACGG-CCC CGTGGTCTTA
Pseudosuccinea columella 1 CGTGCTGG-C AAGC---GGT TGGACACGCT CTGGACCCTC GCGGGCTACC TAAACCGAC- ---------A GTGTTGGTGG TGGTGG-CCC CGTGGTCTTA
Radix auricularia        TTCACCGCCG TCAT------ ------CGCT TTG---CTCG GCGATGTCGT GTGTGTGTGT TGTGC----- ---------C TGGTGG-CCC CGTGGACTTA
Radix natalensis         CCCGC----- ---T------ ------CGC- CCG---CTCG CT-------- --------GA GCGGC----- ---------C TGGTGG-CCC CGTGGTCTTA
Radix ovata              CTCACCGA-- --AT------ ------CGC- TCG---CTCG GCG------- GTTTGCGTGT TGTGC----- ---------C CGGTGG-CCC CGTGGTCTTA
Radix peregra            CTCACCGA-- --AT------ ------CGC- TCG---CTCG GCG------- GTTTGCGTGT TGCGC----- ---------C CGGTGG-GCC CGTGGTCTTA
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Radix quadrasi              TTCACCGCCG -CAT------ ------TTCA TTG---CTGG GCGACGT--T GCTTGTGGGA TTTGT----- ---------G TGGTGG-CCC CGTGGTGTTA
Radix rubiginosa            TTCACCGCCG -CAT------ ------CGCT TTG---CTCG GCGATGT--C GTGTGTGTGA TGTGT----- ---------C TGGTGG-CCC CGTGGTCTTA
Stagnicola corvus           GGGACTGAAT CGGCACCGGT TGGACACGCC CTGGACCTTC GCGGTCTGCG CCGTCGCTGC ACTACCCG-T GCGGTGATGG CAACGG-CCC CGTGGTCTTA
Stagnicola palustris        GGGACTGAAT AGGCACCGGT TGGACACGCC CTGGACCTTC GCGGTCTGAG CCGTCGCTGC ACTACCCG-T GCGGTGATGG CAACGG-CCC CGTGGTCTTA


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                                210        220        230        240        250        260        270        280        290        300
Austropeplea lessoni NSW    AGCACATGCC GCGCCGTTTG TCCGTGCTCG TCTCGG---- ---------- -GCCGTCCGC --------CT TTGCTCTCGG CGGCGTCCGC CCACACTACT
Austropeplea lessoni NT     AGCACATGCC GCGCCGTTTG TCCGTGCTCG TCTCGG---- ---------- -GCCGTCCGC --------CT TTGCTCTCGG CGGCATCCGC CCACAATACG
Austropeplea lessoni QLD    AGCACATGCC GCGCCGTTTG TCCGTGCTCG TCTCGG---- ---------- -GCCGTCCGC --------CT TTGCTCTCGG CGGCGTCCGC CCACACTACT
Austropeplea lessoni WA     AGCACATGCC GCGCCGTTTG TCCGTGCTCG TCTCGG---- ---------- -GCCGTCCGC --------CT TTGCTCTCGG CGGCATCCGC CCACACTACG
Austropepela tomentosa NSW  AGCACAAGCC GCGCCGTT-G TCCGTGTTCG TCTCGG---- ---------- -GACGTCCGC GACGCC-GCC TTGCTCTCGG CGGCG---GC CAAA------
Austropeplea tomentosa NZN  AGCACAAGCC GCGCCGTT-G TCCGTGTTCG TCTCGG---- ---------- -GACGTCCGC GACGCC-GCC TTGCTCTCGG CGGCG---GC CAAA------
Austropeplea tomentosa NZS  AGCACAAGCC GCGCCGTT-G TCCGTGTTCG TCTCGG---- ---------- -GACGTCCGC GACGCC-GCC TTGCTCTCGG CGGCG---GC CAAA------
Austropeplea tomentosa SA   AGCACAAGCC GCGCCGTT-G TCCGTGTTCG TCTCGG---- ---------- -GACGTCCGC GACGCC-GCC TCGCTCTCGG CGGCG---GC CAAA------
Austropeplea tomentosa TAS  AGCACAAGCC GCGCCGTT-G TCCGTGTTCG TCTTGG---- ---------- -GACGTCCGC GACGCC-GCC TTGCTCTCGG CGGTG---GC CAAA------
Austropeplea viridis        AGCACAAGCC GCGCCGTT-G TCCGTGTTCG TCTCGG---- ---------- -GACGTCCGC GACGCC-GCC TTGCTCTCGG CGGCG---GC CAAA------
Bullastra cumingiana        AGCACATGCC GCGCCGTT-G TCCGTGTTCG TCTCGGAAAC GACCCCGCCT CGCTCTCGGC GGAGCCCGCC TCGCTCTCGG CGGCGGTAGC CAACGTTTTC
Kutikina hispida            AGCACAAGCC GCGCCGTT-G TCCGTGTTCG TCTCGG---- ---------- -GACGTCCGC GACGCC-GCC TTGCTCTCGG CGGCG---GC CAAA------
Lymnaea stagnalis 1         AGTACAAGCC GCGCCGTT-G TCCGTT--CA TCTCGT---- ---------- -AACGTCTTC GACGCT-GCC CTGCTCATGG CGGCCT--GT CCGT------
Pseudosuccinea columella 1  AGCGCAAGCC GCGCCGTT-G TCCGTTTACA TCTCGT---- ---------- -AACGTCTTC GACGCT-GCC CTGCTCTCGG CGGCCT--GT CCGT------
Radix auricularia           AGCACAAGCC GCGCCGTT-G TCCGTGTTCG TCTCGG---- ---------- -GACGTCCGC GACGCC-GCC TTGCTCTCGG CGGCG---GC CAAA------
Radix natalensis            AGCACAAGCC GCGCCGTT-G TCCGTGTTCG TCTCGT---- ---------- -GACGTCCGC GACGCC-GCC TTGCTCTCGG CGGCG---GC CAAA------
Radix ovata                 AGCACAAGCC GCGCCGTT-G TCCGTGTTCG TCTCGG---- ---------- -GACGTCCGC GACGCC-GCC TTGCTCTCGG CGGCG---GC CAAA------
Radix peregra               AGCACAAGCC GCGCCGTT-G TCCGTGTTCG TCTCGG---- ---------- -GACGTCCGC GACGCC-GCC TTGCTCTCGG CGGCG---GC CAAA------
Radix quadrasi              ACCCCAAGCC GCGCCGTT-G TCCGTGTTCG TCTCGG---- ---------- -GACGTCCGC GACGCC-GCC TTGCTATGGG CCGCG---GC CAAA------
Radix rubiginosa            AGCACAAGCC GCGCCGTT-G TCCGTGTTCG TCTCGG---- ---------- -GACGTCCGC GACGCC-GCC TTGCTCTCGG CGGCG---GC CAAA------
Stagnicola corvus           AGTACAAGCC GCGCCGTT-G TCCGTT--CA TCTCGT---- ---------- -AACGTCTTC GACGCT-GCC CTGCTCTCGG CGGCCT--GT CCGT------
Stagnicola palustris        AGTACAAGCC GCGCCGTT-G TCCGTT--CA TCTCGT---- ---------- -AACGTCCTC GACGCT-GCC CTGCTCATGG CGGCCT--GT CCCT------


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                                310        320        330        340        350        360        370        380        390        400
Austropeplea lessoni NSW    GTGTGATTC- -TTTTTTTTT CC-TCTGCGG TCACCGCCAT GCGGGACCCG GCTCGCTCTC GCCTCACGGG CTCGCATT-- AA----AAGC TCCAGGGTGA
Austropeplea lessoni NT     GTGTGAA--- -TTTTTTTTT CC-TCTGCGG TCGCCGCCAT GCGGGACCCG GCTCGCTCTC GCCTCACGGG CTCGCATT-- AA----AAGC TCCAGGGTGA
Austropeplea lessoni QLD    GTGTG-ATTC TTTTTTTTTT CC-TCTGCGG TCACCGCCAT GCGGGACCCG GCTCGCTCTC GCCTCACGGG CTCGCATT-- AA----AAGC TCCAGGGTGA
Austropeplea lessoni WA     GTGTGAA--- -TTTTTTTTT CC-TCTGCGG TCACCGCCAT GCGGGACCCG GCTCGCTCTC GCCTCACGGG CTCGCATT-- AA----AAGC TCCAGGGTGA
Austropepela tomentosa NSW  ---------- ------TTTT CC-TCCTCGT C-ACCGCTAT GCGGGACCCG GCTCGCTCTC GCTA-ACGGG CCCGCTCG-T AAC---AAGC TCCAGGGTGA
Austropeplea tomentosa NZN  ---------- ------TTTT CC-TCCTCGT C-ACCGCCGT GCGGGACCCG GCTCGCTCTC GCTA-ACGGG CCCGCTCG-T AAC---AAGC TCCAGGGTGA
Austropeplea tomentosa NZS  ---------- ------TTTT CC-TCCTCGT C-ACCGCCGT GCGGGACCCG GCTCGCTCTC GCTA-TCGGG CCTGCTCG-T AAC---AAGC TCCAGGGTGA
Austropeplea tomentosa SA   ---------- ------TTTT CC-TCCTCGT C-ACCGCTAT GCGGGACCCG GCTCGCTCTC GCTA-ACGGG CCCGCTCG-T AAC---AAGC TCCAGGGTGA
Austropeplea tomentosa TAS  ---------- ------TTTT CC-TCCTCGT C-ACCGCTAT GCGGGACCCG GCTCGCTCTC GCTA-ACGGG CCCGCTCG-T AAC---AAGC TCCAGGGTGA
Austropeplea viridis        ---------- -------TTT CC-TCTCCGT C-ACCGCCAT GCGGGACCCG GCTCGCTCTC GCTA-ACGGG CCCGCTTG-T AAC---CAGC TCGAGGGTGA
Bullastra cumingiana        GAAGGTGTAA TTTTTTTCTT CC-TCTGCGT C-ACCGCAAT GCGGGACCCG GCTCGCTCTC GCCAAACGGG CCCGCACAAA A-----CAGC TCGAGGGTGA
Kutikina hispida            ---------- ------TTTT CC-TCCTCGT C-ACCGCTAT GCGGGACCCG GCTCGCTCTC GCTA-ACGGG CCCGCTCG-T AAC---AAGC TCCAGGGTGA
Lymnaea stagnalis 1         ---------- --------TT ---TCTCT-- --ACCGCCAG GCAGGACCCG GCTCGCTACT TGATTAATTT CTGGAGGCGA TAA---CGGG CCTGCAGTCC
Pseudosuccinea columella 1  ---------- -------TTT ---TCTCT-- --ACCGCCAG GCAGGATCCG GCTCGCTCAC TT-------- ---GAAGCGT CCA---CGGG CCTGCCGTCC
Radix auricularia           ---------- -------TTT CCATCTGCGT C-ACCGCTAA GCGGGACCCG GCTCGCTCTC GCTA-ACGGG CCCGCTTA-T ATC---AAGC TCAAGGGTGA
Radix natalensis            ---------- -------TTT CC-TCTGCGT C-ACCGCTAA GCGGGACCCG GCTCGCTCTC GCTA-ACGGG CCCGCTTA-T CATCAAAAGC TCGAGGGTGA
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Radix ovata              ---------- -------TTT CC-TCTCCGT C-ACCGCTAT GCGGGACCCG GCTCGCTCTC GCTA-ACGGG CCCGCTTA-T AAC---GAGC TTCAGGGTGG
Radix peregra            ---------- -------TTT CC-TCTCCGT C-ACCGCTAT GCGGGACCCG GCTCGCTCTC GCTA-ACGGG CCCGCTTA-T AAC---CAGC TTCAGGGTGA
Radix quadrasi           ---------- ---TTTTTTT CCATATGCGT C-ACCGATAA GCGGGACCCG GCTCGCTCTC CCTA-ACGGG CCCGCTTAAT ACG---AAGC TCAAGGGTGA
Radix rubiginosa         ---------- -TTTTTTTTT CCATCTGCGT C-ACCGCTAA GCGGGACCCG GCTCGCTCTC GCTA-ACGGG CCCGCTTA-T ACG---AAGC TCAAGGGTGA
Stagnicola corvus        ---------- --------TT ---TCTCT-- --ACCGCCAG GCAGGACCCG GCTCGCTACT TGATTAATTT CTGGAGGCGG TCT---CGGG CCTGCAGTCC
Stagnicola palustris     ---------- --------TT ---TCTCT-- --ACCGCCAG GCAGGACCCG GCTCGCTACT TGATTAATTT CTGGAGGCGG TCT---CGGG CCTGCAGTCC


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                         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                              410        420        430        440        450        460        470        480        490        500
Austropeplea lessoni NSW    TCGCGGAGGA GAGAAAGGTC GCACGCTCGC TCGCTCGCT- ---AGTGTTT GATGACGAAA TCGGCGCCAC CGAAATGAAG AAGATGAAG- AAAGGAGGAG
Austropeplea lessoni NT     TCGCGGAGGA GAGAAAGGTC GCACGTTCGC TCGCTCGCT- ---AGTGTTT ---GACGAAA TCGGCGCCAC CGAAATGAAG AAGA-AGAAA AAAAGGAGAT
Austropeplea lessoni QLD    TCGCGGAGGA GAGAAAGGTC GCACGCTCGC TCGCTCGCTC GCTAGTGTTT GATGACGAAA TCGGCGCCAC CGAAATGAAG AAGAT-GAAG AAAGGAGGAG
Austropeplea lessoni WA     TCGCGGAGGA GAGAAAGGTC GCACGCTCGC TCGCTCGCTC GTCAGTGTTT ---GACGAAA TCGGCGCCAC CGAAATGAA- AAGA------ ---AAAGGAG
Austropepela tomentosa NSW  TTGCGGAGGA GAGAA----- --------GC TTACGCTGAC GC--TCGCTT GACAA----A TCGGCGCCCG -TACGAATTG AAGAAGGAGA AGA-------
Austropeplea tomentosa NZN  TTGCGGAGGA GAGAA----- --------GC TTACGCTGAC GC--TCGCTT GACAG----A TCGGCGCCCG -TACGAA--- -AATTGAAGA A---------
Austropeplea tomentosa NZS  TTGCGGAGGA GAGAA----- --------GC TTACGCTGAC GC--TCGCTT GACAG----A TCGGCGCCCG -TACGAA--- ---TTGAAGA A---------
Austropeplea tomentosa SA   TTGCGGAGGA GAGAA----- --------GC TTACGCTGAC GC--TCGTTT GACAA----A TCGGCGCCCG -TACGAACTG AAGAAAAAGA ----------
Austropeplea tomentosa TAS  TTGCGGAGGA GAGAA----- --------GC TTACGCTGAC GC--TCGCTT GACAA----A TCGGCGCCCG -TACGAATTG AAGAAGAAGA AGA-------
Austropeplea viridis       TTGCGGAGGA GAGAA----- --------GC TTACGCAGAC GCGCTCGCTT GACGT----A TCGGCGCCCG -TACGAA--- GAAAAAAAAA TT--------
Bullastra cumingiana       TCGCGGAGGA GGAGAAGA-- ---------- ---------- ---AGAAATT GA-GACGAAA TCGGCGCCCC AGATCGCAAG AGAGGGAATG AGATGGTGGG
Kutikina hispida           TTGCGGAGGA GAGAA----- --------GC TTACGCTGAC GC--TCGCTT GACAA----A TCGGCGCCCG -TACGAATTG AAGAAGAAGA AGAAGAAGAA
Lymnaea stagnalis 1        CCATGGCCCA TCGTCCTTTG CC-------T CTGCCAGCTC GTTCATCGGT GGTAG----G CGGGGGACGG TGGAGAGAAA GGGACGGAAG AGACGCTCA-
Pseudosuccinea columella 1 --ACGGCGA- ---------- ---------- -CACTTGCTC ---------- --TAG---G GTGGA----- --GAGAGACC ATCTCTTAGA AGACGGTTA-
Radix auricularia          TTGCGGAGGG GGAAAAAAA- --------GC TTACGCGGAC GA--TCGCTT GACAAC-AAA TCGGCGCCCG -TACGAATT- GAAGTGAAAA AAATA-----
Radix natalensis           TTGCGGAGGA GAGAAA---- --------GC TTACGCCGAC GC--TCGCTT GACAA----A TCGGCGCCCG GTACGAATC- ---TTGAAGA ----------
Radix ovata                ---CGG-CGG AGGAGAGAA- --------GC TTACGCTGAC GC--TCGCTC GACGA----G TCGGCGCCCG -TACGAAAA- AA--TAAATA AAAAA-----
Radix peregra              ---CGGGCGG AGGAGAGAA- --------GC TTACGCTGAC GC--TCGATC GACGA----G TCGGCGCCCG -TACGAAAA- AAATTGGAAA AAAAA-----
Radix quadrasi             TTGCGGAGGG GGGAAAAAA- ---------- --ACGCCGAC GC--TCGCGC GACAA----A TGGGCGCCCG -TACGAATTT GAAATGAAAA AAAC------
Radix rubiginosa           TTGCGGAGGG GGGAAAAAA- ---------- --ACGCCGAC GC--TCGCTT GACAA----A TCGGCGCCCG -TACGAATTT GAAATGAAGA AAACG-----
Stagnicola corvus          CCACGGCACA TCGTCCCTCA CC-------T CCGCTAGCTC G------AGT GGTAG----G CGGGAGACGG TGGAGAGATA GGGTCGGAAG AGACGTTCG-
Stagnicola palustris       GCATGGCACA TCGTCCCTAA CC-------T CCGCTAGCTC G------AGT GGTAG----G CGGGGGACGA TGGAGAGATA GGGGCGGAAG AGACGTTCG-


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                         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ..
                              510        520        530        540        550        560        570
Austropeplea lessoni NSW    ATTGAGATTG ATTT-ATTTC TCTTTCCTTT TTTTTTCTCT CTTTTCTCCG ACCTCAGATC GGACGAGATT ??
Austropeplea lessoni NT     TGAGAGATTG TGATGATTTC TCTTTCCTTG TTTT----CT CTTTTCTCCG ACCTCAGATC GGACGAGATT ??
Austropeplea lessoni QLD    ATTGAGATTG -ATTTATTTC TCTTTCCTTT TTTTTTCTCC CTTTTCCCCG ACCTCGAATC GGACGAGATT ??
Austropepela tomentosa NSW  ---------- -TTCCATTTT TC-TTCTTCT TCTTT---CA CAAATTTCCG ACCTCAGATC GGACGAGATT ??
Austropeplea tomentosa NZN  ---------- ---TTGTCTT -------CTT CTTTC---CA CAAATTTCCG ACCTCAAATC GGACGAGATT ??
Austropeplea tomentosa NZS  ---------- ---TTGTCTT -------CTT CTTTC----A CAAATTTCCG ACGTCAGATC GGACGAGATT ??
Austropeplea tomentosa SA   ---------- -TTCTG---- ---TC-TTCT TCTTT---CA CAAATTTCCG ACCTCAGATC GGACGAGATT ??
Austropeplea tomentosa TAS  ---------- -TTCTTTTTT T--TCTTTCT TCTTT---CA CAAATTTCCG ACCTCAGATC GGACGAGATT ??
Austropeplea viridis       ---------- ---------- ------TTTT TTTTT---TA CCAATTTCCG ACCTCAGATC GGACGAGATT ??
Bullastra cumingiana       AGGGAGATTC -TTCTGTCTC TCTCTCTCGC TTTTC---GT TCGTTCCCCT TCTTTC???? ?????????? ??
Kutikina hispida           GAAGACTATG ---CTTTCTT AGACTCTTCT TCTTT---CA CAAATTTCCG ACCTCAGATC GGACGAGATT ??
Lymnaea stagnalis 1        --CTTGATGC TCGGCGCCCG GATCAAT--- -TGTT---TT TTTTCACTCG AAAAAAAAAT TAAC--ATT- CC
Pseudosuccinea columella 1 --CTTGAT-- -CGGCGCCTG ---------- TCTCC----A CATTTTTTTA AAAC????? ?????????? ??
Radix auricularia          ---TTCTTTT ---TTTTTTT TCATTTTCAT ATCTC????? ?????????? ?????????? ?????????? ??
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Radix natalensis      ---------- ---------- ------TTTT ACTTC---AA CATATCTCCG ACCTCAGATC GGACGAGATT ??
Radix ovata           ---TAACTGT ---GCGTTAT TTTTT-TTAT TTTTT---TT AATCTC???? ?????????? ?????????? ??
Radix peregra         ---TAACCGT ---GCGTTGC GCGTTATTTT TTTTT---TT CCTTTCTTTC ?????????? ?????????? ??
Radix quadrasi        ---CAGTTTT ---TTGTTTG TGTTTTTTCC CCCAA---TT CATATCCCCA ACCTC---TA ACCGCAGATT ??
Radix rubiginosa      ---CTGTTTT ---TTCTCTC ?????????? ?????????? ?????????? ?????????? ?????????? ??
Stagnicola corvus     --CTTGATGC ACGGCGCCCG GATTATTATA -TTTT---TT TTTCCACTCG AAGAAAAAAA AAATTAAAAT TC
Stagnicola palustris  --CTTGATGC TCGGCGCCCG GATCAGTTGT -TTTT---TT TTTTCACTCG AAAAAAAAAT AAAC----AT TC
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Appendix 4.5. Statistics for the anatomical analysis of the Lymnaeidae.

Character	Range	Min steps	Tree steps	Max steps	CI	RI	RC	HI	G-fit
1 shell umbilicus	2	2	4	7	0.500	0.600	0.300	0.500	0.600
3 number of whorls	7	7	18	25	0.389	0.389	0.151	0.611	0.214
4 colmella fold	2	2	11	16	0.182	0.357	0.065	0.818	0.250
5 shell sculpture	1	1	4	5	0.250	0.250	0.063	0.750	0.500
6 periostracum ornamentation	1	1	1	1	1.000	0/0	0/0	0.000	1.000
8 foot shape at posterior end	1	1	4	10	0.250	0.667	0.167	0.750	0.500
10 eye lobe	2	2	2	2	1.000	0/0	0/0	0.000	1.000
11 tentacle shape	3	3	5	7	0.600	0.500	0.300	0.400	0.600
12 lateral sides of snout	1	1	1	1	1.000	0/0	0/0	0.000	1.000
15 mantle expansion	3	3	6	8	0.500	0.400	0.200	0.500	0.500
16 exapanded mantle pigmentation	1	1	2	2	0.500	0.000	0.000	0.500	0.750
17 number of pnuemostomal ridges	1	1	1	1	1.000	0/0	0/0	0.000	1.000
18 anal flap	1	1	2	7	0.500	0.833	0.417	0.500	0.750
19 upper plate of pnuemostome	1	1	3	12	0.333	0.818	0.273	0.667	0.600
20 broadest part of kidney	3	3	7	12	0.429	0.556	0.238	0.571	0.429
21 kidney width to length ratio	2	2	4	16	0.500	0.857	0.429	0.500	0.600
22 right lobe of kidney	1	1	1	11	1.000	1.000	1.000	0.000	1.000
23 position of pulmonary vein	1	1	1	11	1.000	1.000	1.000	0.000	1.000
24 pulmonary vein length	2	2	2	5	1.000	1.000	1.000	0.000	1.000
25 ureter	1	1	5	9	0.200	0.500	0.100	0.800	0.429
26 opening of kidney	1	1	1	1	1.000	0/0	0/0	0.000	1.000
34 uterus/ vagina length	2	2	11	20	0.182	0.500	0.091	0.818	0.250
35 spermathecal duct length	2	2	11	17	0.182	0.400	0.073	0.818	0.250
36 spermathecal duct width	1	1	1	1	1.000	0/0	0/0	0.000	1.000
37 uterus shape	1	1	9	14	0.111	0.385	0.043	0.889	0.273
38 oviducal caecum size	4	4	12	17	0.333	0.385	0.128	0.667	0.273
39 oothecal gland shape	3	3	12	22	0.250	0.526	0.132	0.750	0.250
41 position of uterus vagina	2	2	9	17	0.222	0.533	0.119	0.778	0.300
42 velum shape	2	2	2	2	1.000	0/0	0/0	0.000	1.000
43 penis sheath length	4	4	14	23	0.286	0.474	0.135	0.714	0.231
50 upper prostate	1	1	4	7	0.250	0.500	0.125	0.750	0.500
51 length of prostate	3	3	8	14	0.375	0.545	0.205	0.625	0.375
52 shape of lower prostate	1	1	2	3	0.500	0.500	0.250	0.500	0.750
53 central tooth	3	3	7	13	0.429	0.600	0.257	0.571	0.429
54 position of smaller cusp on central	1	1	3	4	0.333	0.333	0.111	0.667	0.600
55 radula teeth shape	1	1	4	5	0.250	0.250	0.063	0.750	0.500
56 lateral teeth	2	2	5	8	0.400	0.500	0.200	0.600	0.500
57 marginal teeth	4	4	11	21	0.364	0.588	0.214	0.636	0.300
58 ureter length	2	2	5	9	0.400	0.571	0.229	0.600	0.500
60 insemination pocket	1	1	2	2	0.500	0.000	0.000	0.500	0.750
61 vaginal bulb	1	1	2	2	0.500	0.000	0.000	0.500	0.750
62 penal knot	1	1	2	3	0.500	0.500	0.250	0.500	0.750
63 prostate pouch	1	1	3	3	0.333	0.000	0.000	0.667	0.600
64 shell orientation	1	1	1	2	1.000	1.000	1.000	0.000	1.000
65 shell spire	3	3	6	15	0.500	0.750	0.375	0.500	0.500
66 tentacle form	1	1	1	2	1.000	1.000	1.000	0.000	1.000
67 columella digitations of mantle	1	1	1	2	1.000	1.000	1.000	0.000	1.000
68 number of flexures in ureter	1	1	2	2	0.500	0.000	0.000	0.500	0.750
69 ureter shape	1	1	1	1	1.000	0/0	0/0	0.000	1.000
70 spermatheca shape	1	1	5	14	0.200	0.692	0.138	0.800	0.429
71 penis shape	1	1	2	10	0.500	0.889	0.444	0.500	0.750
72 penis sheath head	1	1	3	8	0.333	0.714	0.238	0.667	0.600
73 width of penis sheath	2	2	6	9	0.333	0.429	0.143	0.667	0.429
74 praeputium retractor insertion	1	1	2	2	0.500	0.000	0.000	0.500	0.750
75 number of internal praeputium folds	1	1	1	1	1.000	0/0	0/0	0.000	1.000
76 preputial gland	1	1	1	2	1.000	1.000	1.000	0.000	1.000
77 prostate	1	1	1	2	1.000	1.000	1.000	0.000	1.000
78 prostate shape	3	3	6	9	0.500	0.500	0.250	0.500	0.500
79 internal prostate fold	2	2	4	5	0.500	0.333	0.167	0.500	0.600
80 Number of chromosome pairs	2	2	5	16	0.400	0.786	0.314	0.600	0.500

1 constant character not shown