PHYLOGENY OF FRESHWATER CRAYFISH

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By

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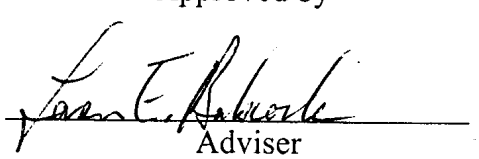

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ABSTRACT

The phylogeny of freshwater crayfish, including both fossil and extant taxa, is assessed using morphologic analysis and nucleotide sequencing. Based on external morphologic characters, primarily characters of the carapace and appendages, the crayfish are reconfirmed as a monophyletic group. The nearest sister taxon to the crayfish is the Chilenophoberidae. Within the crayfish, the longstanding distribution of species among three families is supported. The superfamily Astacoidea is redefined to include three families, Astacidae, Cambaridae, and Parastacidae. By including the Parastacidae in the Astacoidea, the superfamily Parastacoidea becomes superfluous and is now suppressed. The Astacoidea is characterized by several synapomorphies: a diaresis of the telson, mobility of the last thoracic segment, and carapace groove pattern. Species in the Parastacidae are characterized by change in calcification of the telson. Species in the Cambaridae are characterized by the apomorphous annulus ventralis in the female and hooks on the ichiopodites of one or more pereiopods in the male. Species in the Astacidae are characterized by an apomorphous medial rostral ridge. Nucleotide sequencing (18s and 16s ribosomal mtDNA) of extant crayfish species supports the phylogenetic pattern inferred from character analysis.

DEDICATION

This work is dedicated to my family and Phil in appreciation for their love and support.

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INTRODUCTION

The freshwater crayfish are decapod crustaceans belonging to the infraorder Astacidea (Fig. 1; Hobbs, 1988). Crayfish are generally freshwater aquatic to terrestrial in habit. Crayfish are historically classified among three families and two superfamilies (Table 1). The superfamilies are Astacoidea and Parastacoidea (Hobbs, 1988). The Astacoidea historically comprises the families Astacidae and Cambaridae. The Parastacoidea contains the family Parastacidae. Members of the Parastacidae are confined to the Southern Hemisphere, and members of the other two families are confined to the Northern Hemisphere. Modern crayfish are a relatively small group of organisms. The extant crayfish, 29 genera, embracing approximately 478 species, are recognized (Holdrich and Lowery, 1988). Fossil crayfish have been assigned to eight species divided among seven genera. Only one known genus of crayfish is extinct. The number and diversity of species vary among the three families and show a correlation to the diversity of habitats occupied.

Crayfish are an economically important biologic crop throughout much of the world. Various species are cultured and harvested for human consumption. They also provide food for other animals. Most research about modern crayfish concerns their ecology, fecundity, and disease, all which are of interest for the purposes of aquaculture management.

Modern crayfish inhabit every continent except Africa and Antarctica; they also inhabit a number of islands, such as Madagascar and Cuba (Fig. 2; Hobbs, 1988). The family Astacidae has a geographical distribution ranging across Europe, eastern and western Asia, and western North America. The geographic range of the Cambaridae includes North America east of the Rocky Mountains, Central America, and the Caribbean. The Parastacidae range through South

America, Madagascar, Australia, New Zealand, and Indonesia.

Crayfish live in a variety of habitats. The number and diversity of different habitats occupied vary among the families. The Astacidae reside in streams and lakes. Their burrows extend only into the nearby stream beds and banks (tertiary burrowing) (Hobbs, 1988). The Cambaridae exhibit the greatest ecological diversity. In addition to the environments inhabited by the Astacidae, the Cambaridae may reside in caves, remain completely in burrows throughout their life cycle (primary burrowing), or exhibit burrowing behavior intermediate to the primary and tertiary burrowers (secondary burrowing) (Hobbs, 1988). The Parastacidaes include species that live in stream and lake environments as well as in burrows (at all three levels). In fact, some parastacids may even be considered terrestrial in that they burrow in only dry or moist soil (Hogger, 1988).

Little has been published concerning fossil crayfish and the zoological affinities of the group. This is largely because of the scarcity of pre-Cenozoic fossils from freshwater habitats (Gray, 1988). Crayfish have a poor fossil record beyond 30 million years, although the clade that includes the crayfish putatively dates to the late Paleozoic or early Mesozoic (Schram, 1977; Hobbs, 1988; Miller and Ash, 1988; Babcock et al., 1998).

The purpose of this thesis is to reconstruct the phylogeny of freshwater astacideans, including both extinct and extant species. Relationships between the freshwater crayfish and several sister groups of marine lobsters are evaluated. Relationships are assessed cladistically using morphological criteria and through nucleotide sequencing. New information provides clues to the ancestry of crayfish and clarifies relationships within the group. Freshwater astacideans are interpreted here as a monophyletic group. In addition to providing information about the evolutionary history of freshwater crayfish, this work provides further insight into the

relationships among all astacidean groups, including the marine lobsters (chilenophoberids and nephropids) and the marine to freshwater erymids. This work adds support to the hypothesis that various decapod crustacean groups (crayfish, erymids, and brachyurans) invaded freshwater habitats separately and at different times during geologic history.

APPROACHES TO THE CLASSIFICATION AND PHYLOGENY OF FRESHWATER CRAYFISH

With the development and implementation of new procedures and technologies in the biological sciences, the approaches to the classification of crayfish and many other organisms, and subsequently the interpretations of phylogeny, have changed. For crayfish, traditional methods of investigation were centered around comparative morphology (Huxley, 1880; Faxon, 1885; Riek, 1972; Hobbs, 1988), whereas more recent methods place emphasis on nucleotides and other molecular indicators of evolutionary history (see summary in Crandall, 1998). In the work reported here, both approaches have been followed. This methodology allows comparative information from recent and ancient species to be integrated with current molecular information obtained from recent species.

Using comparative morphology, Huxley (1880) produced one of the earliest interpretations of the evolutionary history of crayfish (Fig. 3). Huxley's classification of genera into suprageneric categories relied primarily on gill structure and ornamentation of the carapace. Following the work of Huxley, crayfish phylogeny received little attention until the work of Hobbs (1942, 1974, 1988) and Riek (1969, 1971, 1972). These investigators also based inferences about taxonomic relations (Fig. 4) on morphology. Hobbs dealt primarily with the taxonomy of the Cambaridae and Astacidae, whereas Riek worked with the Parastacoidea. In the classification of Hobbs (1988), the primary characters used to classify modern crayfish (Fig. 5) are accessory appendages and branchial arrangement. External characters, such as rostral appearance and cheliped morphology, were also used but carried less taxonomic weight. In the classification of Riek (1969, 1972), the primary characters used to classify modern crayfish (Fig.

6) were the extent of separation between grooves, the resting position of the chelae, telson structure, and carapace ornamentation. In a cladistic study of morphologic characters in decapods, Scholtz and Richter (1995; Fig. 7) provided support for the separation of the Nephropoidea (or Homarida) and freshwater crayfish, and reassigned the crayfish to either: 1, a clade containing the Brachyura, Anomala, and Thalassinida; or 2, a branch that emerged from the Thalassinida.

As studies of molecular markers became more common in the 1980s and 1990s, a number of studies of crayfish phylogeny based on several different markers were published (Patak et al, 1989; Austin, 1995; Crandall and Fitzpatrick, 1996; Crandall and Cronin, 1997; Lawler and Crandall, 1998; Crandall et al., in press). Chromosome variation, allozyme variation, and nucleotide variation were some of the factors studied. In general, more research about the phylogeny of parastacid crayfish was generated than was research about the astacid and cambarid crayfish. Researchers working on the Astacidae and Cambaridae have concentrated on genetic variation within populations, and less on the phylogeny of the group (Crandall, 1998).

Within the Parastacidae (the Southern Hemisphere crayfish species) a number of phylogenies based on evidence from molecular methods have been published in recent years. Patak and Baldwin (1984) studied electrophoretic and immunochemical markers among genera. Patak et al. (1989) published another immunochemical analysis of parastacid genera (Fig. 8). Austin (1995) used allozymes to reconstruct the phylogeny of several parastacids (Fig. 9). At least three published studies (Lawler and Crandall, 1998; Ponniah and Hughes, 1998; Crandall et al., in press) used 16s mitochondrial DNA to reconstruct the phylogeny of parastacid crayfish (Fig. 10 and 11).

Within the Astacidae and Cambaridae (the Northern Hemisphere crayfish species, almost

all phylogenetic research based on molecular information has concerned the Cambaridae. Cambarid phylogeny has been assessed using 16s mitochondrial DNA (Crandall and Fitzpatrick, 1996; Crandall, 1998). Crandall and Cronin (1997) examined the molecular evolution of rhodopsin, a visual pigment, in four cambarid genera and produced a phylogenetic hypothesis (Fig. 12).

Relevant supporting studies of crayfish phylogeny includes studies that involved teloblasts, sperm ultrastructure, and 18s ribosomal RNA (rRNA) to address issues related to the evolutionary origin of the group. In studies of teloblasts (cells in the posterior growth zones of embryos) and sperm ultrastructure, Scholtz (1993) and Jamieson (1991) discovered evidence supporting a monophyletic origin for the crayfish. In a study of 18s rRNA among decapods, Kim and Abele (1990) showed that *Procambarus* has a close common ancestry with *Callinectes* (infraorder Brachyura).

TAXONOMY OF THE INFRAORDER ASTACIDEA

Tables 1 and 2 summarize the current classification of crayfish species. Table 1 summarizes the major crustacean groups relevant to understanding crayfish relationships (together with salient morphological features of each group), and Table 2 lists species of crayfish and closely related decapod crustaceans. Despite recent technological innovations that improved our ability to resolve relationships among biological organisms, the current classification (and inferred phylogeny) of decapod groups remains remarkably similar to classifications published decades ago (e.g., Glaessner, 1969)

Relationships within the crayfish

The evolutionary origin of the freshwater crayfish is a longstanding unresolved issue. The central question, that of a monophyletic versus polyphyletic origin, has been debated for more than a century. Early investigators, such as Huxley (1880), viewed crayfish as polyphyletic, an interpretation supported by the modern geographical separation of the superfamilies. On the other hand, recent investigations into embryology and postembryonic development yielded information suggesting a monophyletic origin of the freshwater crayfish (Scholtz, 1998).

Studies, such as those of Huxley (1880), predate the acceptance of plate tectonic theory. Because the continents were viewed as immobile, the simplest and most understandable reason for the two groups of freshwater crayfish is separate invasions of the freshwater habitat. Indeed, this view held even after the acceptance of plate tectonics due to the strong separation of the crayfish families between Gondwanan and Laurasian habitats (Hobbs, 1974; Albrecht, 1983).

Traditional interpretations of the taxonomy of the freshwater crayfish are based solely on

modern genera. Primary characters used in the classification of modern crayfish include accessory appendages and branchial arrangement. Secondary characters used in classification include rostral appearance and cheliped morphology. These external characters can also be used to classify fossil material; however, the great emphasis of neo-astacologists on characters that do not readily fossilize makes the connection between modern and some ancient crayfish uncertain.

A monophyletic origin of crayfish was proposed during the early Twentieth Century (Ortmann, 1902), but during the following decades, this hypothesis fell out of favor. However, during the past decade, biological studies based on development and nucleotide sequences tended to support a monophyletic origin of the freshwater crayfish. Several studies of developmental and reproductive phases of the life cycle provided support for a common ancestor for both Northern Hemisphere and Southern Hemisphere groups (Jamieson, 1991; Scholtz, 1993). For example, the posterior growth zone of decapod embryos is composed of teloblasts, large cells that produce smaller cells through unequal division in the anterior direction. For malacostrocan decapods, the original number of teloblasts was 19 ectoteloblasts and eight mesoteloblasts. However, in crayfish a derived character of approximately 40 ectoteloblasts are present, while the original eight mesoteloblasts are maintained (Scholtz, 1993). This character is interpreted as a synapomorphy that defines the freshwater crayfish.

Studies based on nucleotide sequence data have led to new progress in understanding the phylogeny of freshwater crayfish. However, most studies have been limited in scope addressing only the relationships between several genera (Crandall and Fitzpatrick, 1996; Crandall, 1998; Lawler and Crandall, 1998; Ponniah and Hughes, 1998). Some recent studies examined nucleotide sequences in a larger context (Lawler and Crandall, 1998; Crandall, in press). These studies tended to support the higher taxonomic groupings of the freshwater crayfish but also

showed several distinct differences from traditional taxonomy at the generic level (e.g., Lawler and Crandall, 1998).

Relationships with other decapods

Although evolutionary relationships are not well known within Astacidea, the superfamilies Astacoidea and Parastacoidea historically have been interpreted as sister groups to the Nephropoidea, the marine clawed lobsters (Glaessner, 1969; Tshudy and Babcock, 1997). In this interpretation, the Erymidae are considered the ancestral group to the Astacidae. However, a number of recent authors considered the Northern and Southern Hemisphere groups to have evolved independently from an erymid stock (Albrecht, 1983; Scholtz, 1998).

The relationships between freshwater crayfish and other clades of decapod crustaceans have also been included in recent studies involving nucleotide sequencing and phylogenetic methods (Kim and Abele, 1990; Scholtz and Richter, 1995). Although monophyly of the freshwater crayfish was supported, their phylogenetic position within the repantian decapods remains unclear (Scholtz and Richter, 1995). Interpretations such as that of Scholtz and Richter (1995) disputed the traditional assignment of the Nephropidae (clawed lobsters) as a sister group to the modern freshwater crayfish.

Along with the study of modern crayfish genera, some interest has been taken in the fossil record of crayfishes. Albrecht (1983) proposed a new family, Protastacidae, as direct ancestors to modern crayfish on the basis of suture patterns. The Protastacidae were thought to have been derived from an erymid ancestor; protastacids include the genera *Pseudastacus* and *Protastacus* (Albrecht, 1983). In a revision of the Nephropidae, Tshudy and Babcock (1997) created a new family, Chilenophoberidae, containing the genera *Chilenophoberus*, *Paleophoberus*, *Tillocheles*,

and *Pseudastacus*. The Chilenophoberidae were considered an earlier derivative of the erymids than the nephropid lobsters (Tshudy and Babcock, 1997). The Chilenophoberidae contains one genus formerly included in the Protastacidae by Albrecht (1983). Potentially, either the Protastacidae or Chilenophoberidae could have given rise to the freshwater crayfish.

FOSSIL ASTACIDS

Freshwater astacids have a poor fossil record. Only nine described species in eight genera are currently recognized. One of the fossil astacids is an erymid. Some of the fossil species were described based on a single, incomplete specimen (i.e., Miller and Ash, 1988; Feldmann and Pole, 1994). However, a few of the described species are represented by a large number of specimens (Aguirre-Urreta, 1992; Garassino, 1997).

Marine members of the Astacidea have a more substantial fossil record than the freshwater members of the infraorder. These groups of animals resided on the continental shelf or, less commonly, in brackish water. Because these types of environments are more likely to preserve body fossils than terrestrial environments, the diversity and record of these groups are more extensive than those of the crayfish.

Of the fossil crayfish species, most are assigned to Northern Hemisphere families. Species described from North America are *Pacifastacus chenoderma* (Cope, 1870), an astacid, and *Procambarus primeavus* (Packard, 1880), a cambarid. An additional unidentified cambarid of Teritiary age was collected in Oklahoma (Feldmann and May, 1991). Two species, described as *Astacus licenti* van Straelen, 1928 and *Astacus spinirostris* Imaizumi, 1938, have been described from Mongolia and Jehol, respectively. Although originally assigned to *Astacus, A. licenti* and *A. spinirostris* require of generic reevaluation. European fossil crayfish belong to the family Astacidae. They are assigned to *Austropotamobius* and *Astacus*. However, several of the proposed *Astacus* species were reassigned recently to potentially ancestral decapod groups, such as the chilenophoberids and protastacids. Three parastacid fossils have been described so far:

Lammuastacus longirostris Aguirre-Urreta, 1992, *Paranephrops fordycei* Feldmann and Pole, 1994, and a claw resembling a member of the modern genus *Euastacus* (Sokal, 1987).

Because the generic scheme of modern crayfish has changed greatly in the past 60 years, many of the original generic names for the fossil crayfish are outdated. During the late Nineteenth and early Twentieth centuries, all astacid and cambarid crayfishes were assigned to the genus *Astacus* Linnaeus. However, although the recent taxa were reclassified, most of the fossil species were not reassigned. Two species that have been reassigned are *Astacus chenoderma*, reassigned to *Pacifastacus* (Hobbs, 1974), and *Cambarus primaevus*, reassigned to *Procambarus* (Feldmann et al., 1981). Recent descriptions of new crayfish species have primarily placed these animals into extant genera, with the exception of *Lammuastacus longirostris* (Aguirre-Urreta, 1992).

Several other freshwater or brackish-water decapods have been described. The most recent of these is *Enoploclytia porteri* Miller and Ash, 1988, an erymid, from the Triassic (late Carnian) of the Colorado Plateau. Several other crayfish-like creatures have also been collected from brackish-water deposits in Europe. These include the genera *Protastacus*, *Pseudastacus*, and *Palaeastacus* (Albrecht, 1983).

HISTORICAL INTERPRETATIONS OF THE TIMING AND PATTERN OF CRAYFISH EVOLUTION

Historically, crayfish are thought to have evolved from nephropid lobsters at low paleolatitudes during the early Mesozoic (Hobbs, 1988; Schram, 1977), although, the first definitive appearance of crayfish is in the Late Jurassic or Early Cretaceous (Imaizumi, 1938). Body fossils (Miller and Ash, 1988) and trace fossils (Hasiotis and Mitchell, 1993) collected from the Upper Triassic of the Colorado Plateau were considered support for this interpretation (Hasiotis and Mitchell, 1993). These strata were located in low paleolatitudes during of deposition (Babcock et al., 1998). The interpretation of crayfish evolution and distribution based on these fossils suggests that astacids invaded freshwater during the early Mesozoic Era (~230 mya). Body fossils from the Colorado Plateau sites are the erymid, *Enoploclytia porteri* Miller and Ash (1988). Although erymids are commonly interpreted as crayfish (e.g., Gall and Fischer, 1965; Miller and Ash, 1988, Hasiotis and Mitchell, 1993), this view has not received universal support (e.g., Glaessner, 1969; Feldmann, 1979). Based on phylogenetic studies reported here, the erymids are considered to be a sister group of crayfish. Therefore, the Colorado Plateau specimens are not useful for interpretations of crayfish evolution. Crustacean remains from the Permian of Antarctica (Babcock et al., 1998) are too fragmentary to render definitive judgment. However, work based on recently collected material indicates that they represent another sister group to the crayfish (L.E. Babcock, personal communication, 1999).

The first comprehensive approach to understanding the evolutionary distribution and paleobiogeographic movements of the crayfish was by Ortmann (1902). In fact, this view was so widely accepted that it was not challenged for more than 50 years (Hobbs, 1988). Ortmann

(1902) advocated a monophyletic origin for freshwater crayfish. He hypothesized, without benefit of physical evidence, that the ancestral crayfish lived in Sino-Australia and Antarctica during the Early Cretaceous. During the Middle Cretaceous, the hypothetical ancestral crayfish migrated to Madagascar, giving rise to *Astacoides*. During the Late Cretaceous, the Astacoidea (genus *Potamobius)* and Parastacoidea diverged. The Astacidae extended their range into North America. During the Early Tertiary, *Potamobius* gave rise hypothetically to the genus *Cambarus* and the Parastacidae became into a South American group and an Australian group. During the Late Tertiary, the Astacidae from eastern Asia reached Europe, and the Parastacidae became restricted to their present ranges (Ortmann, 1902).

Although the interpretation by Ortmann (1902) has several inconsistencies with modern data, a further attempt was not made to synthesize an evolutionary hypothesis for crayfish until the work of Hobbs (1988). In this more recent attempt to explain crayfish evolution, Hobbs (1988) followed the example of Huxley (1880) in postulating separate ancestries for the two superfamilies. Hobbs (1988) hypothesized that crayfish (Astacidae) invaded freshwater in the Ponto-Caspian basin during the Cretaceous. The origin of the Cambaridae was unclear to Hobbs (1988), but the genus *Procambarus* was regarded as the ancestral stock for the family. The parastacids were considered an entirely independent group of animals, but the evolutionary pathway that gave rise to them was unclear to Hobbs (1988).

A recent attempt to explain the distribution of crayfish families based on a monophyletic ancestry was proposed by Scholz (1998). Scholz (1998) advocated a single invasion into freshwater occurring possibly as late as the Triassic, before the breakup of Pangea. With the separation of Pangea during the Jurassic, the superfamilies separated with the Astacidae and Cambaridae becoming restricted to the Northern Hemisphere continents and the Parastacoidea

becoming restricted to the Southern Hemisphere continents.

METHODS

Crayfish phylogeny was investigated using a phylogenetic approach incorporating both morphological characters and nucleotide sequences. Relationships within modern taxa of crayfish are commonly inferred from the external morphology of appendages (especially reproductive appendages), development of sutures, and other external characteristics (Hobbs, 1981). These external characters are the same as those used in cladistic analysis of fossil material. Therefore, analyses that include both the modern and ancient taxa are appropriate. Phylogenetic relationships of extant species can also be identified through the comparison of nucleotide signatures and other biochemical methods. Comparison of the similarities between large ribosomal nucleotide sequences at specific locations provides data that are useful for determining the degree of relatedness between groups (Field et al., 1988). Because these molecules tend to be conservative in organisms over geologic time, they are suitable for phylogenetic reconstruction (Raff, 1988). However, these molecules are not preserved in fossil material, so this analysis can only be applied to extant taxa. The combination of morphological phylogenetic analysis and molecular methods provides a more complete data set on which to base phylogenetic interpretations (Budd, 1996).

Morphological phylogenetic analysis

Morphologic analysis was performed on available fossil and representative modern species as a prelude to phylogenetic analysis. Representative species of both modern and fossil taxa were obtained from museums and collections. Character states used in the morphological analysis include external characters based primarily on features of the carapace and appendages

(Table 3). One species per genus was chosen as a representative for each modern crayfish genus. These species were most commonly the type species, unless only an alternate species was available from museum or personal collections. Genera in which preserved specimens could not be directly observed were scored based on published descriptions and illustrations. All described fossil crayfish species were included based on data extracted from publications. Additional taxa were analyzed as potential ancestral groups. These additional taxa include members of the families Chilenophoberidae, Nephropidae, Erymidae, and Protastacidae, and Palaeopalaemonidae (Table 1).

Phylogenetic analysis or cladistics, is the method of reconstructing phylogenies using synapomorphies, or shared derived characters (Wiley, 1981). Taxa are grouped into monophyletic groups, or clades, by the possession of these shared adaptations, not by overall similarities and differences. The strength of this type of analysis is that relationships are interpreted based only on characters that reflect phylogeny, not merely on the overall similarities of organisms (Wiley, 1981). The weaknesses of this type of analysis is that homology of all characters, particularly in ancient organisms, is not always easy to determine. Homoplasy, or character convergence, or parallelism can be difficult to distinguish, especially among fossils. Character reversal poses another problem in phylogenetic interpretation. Data sets that include a large number of characters can be used to overcome the problems of homoplasy and reversal. Characters that are determined to be homoplastic after initial analysis can be easily removed from the data set (Swofford, 1993). Maximum parsimony methods may be used to produce a phylogenetic interpretation of the taxa. This method allows for phylogenetic construction using the least number of evolutionary changes (Maddison and Maddison, 1992).

Cladistic (phylogenetic) interpretation of relationships was performed using PAUP

(Phylogenetic Analysis Using Parsimony) 3.1.1 (Swofford, 1993). A heuristic search under standard settings was performed on the data set (Table 4). Characters were unordered and unweighted with multiple character states interpreted as polymorphism. Character states were polarized using the outgroup method. *Palaeopalaemon newberryi*, the oldest known decapod (Schram et al., 1978), was used as the outgroup taxon for the initial analyses. In more refined analyses less distantly related sister groups were used as outgroups for polarizing data.

Nucleotide analysis

Nucleotide analysis is performed in a very similar manner to that of cladistic analysis of morphology. Following the acquisition of sequences of nucleotide bases, the sequences were aligned. Alignment compensates for sequences of different lengths of bases (Thompson et al., 1994). The length differences may be due to different preparations of the nucleotides for sequencing (such as incomplete isolation of the nucleotide fragment, loss of material during preparation, or poor condition of original material from which to extract the nucleotides) (Crandall and Fitzpatrick, 1996). Alignment produces a set of nucleotide sequences that are most closely related to each other by shifting bases through inserting gaps (Thompson et al., 1994). This alignment is controlled by parameters of the alignment program, but errors in sequencing may lead to inaccurate results (Thompson, et al., 1994). Once the sequences are aligned, they can be analyzed in the same manner as morphological data using maximum parsimony methods to produce a phylogenetic interpretation (Swofford, 1993).

The use of nucleotide sequences has proved to be a useful tool for clarifying phylogenetic relationships; however, the phylogeny produced is not always consistent with morphological data (Budd, 1996). Nucleotide sequences undergo molecular evolution randomly. The rate of change

is commonly considered to be relatively constant, although this is not always the case (Raff, 1988). The rate of change varies both among organisms and among molecules (Wilson et al., 1988). Therefore, either sudden rapid changes in nucleotide structure or slower changes may alter a phylogenetic interpretation. Problems with aligning sequences produced by various methods, such as different oligonucleotide primers, may also hinder phylogenetic interpretation (Thompson et al, 1994). Another weakness of nucleotide analysis is that analysis is limited to modern or very recent organisms, because genetic material normally breaks down quickly (using evolutionary time scales for reference) (Raff, 1988).

Nucleotide analysis was performed based on published data sets of both the 16s and 18s regions of mitochondrial nucleotides (Table 5) (GenBank, 1999; Crandall, 1996; Crandall, in press). Neither nuclear region has published sequences for all three crayfish families. The parastacid genera have been widely sequenced in the 16s mitochondrial DNA (mtDNA) region, but no published data exist for the 18s nuclear region. A few genera of the Cambaridae have been sequenced for both the 16s and 18s regions. In the Astacidae, only *Astacus astacus* has been sequenced for the 18s region.

Nucleotide sequences were aligned using Clustal W 1.4 (Thompson et al., 1994). Aligned nucleotide sequences are presented in Tables 6 and 7. Aligned sequences were analyzed using PAUP 3.1.1 (Swofford, 1993). All changes were assigned equal weight. The 18s data set was analyzed using an exhaustive search. The 16s data set, because of its larger size, was analyzed using a heuristic search under standard settings. The nephropid lobster, *Nephrops norvegicus* was used as the outgroup. Additional possible outgroups were considered, but provided neither further resolution nor changes in any positions within the most-parsimonious trees.

RESULTS

Results of the morphological and phylogenetic analysis (Fig. 13) support longstanding ideas of higher order classification within the freshwater crayfish; they also provide new insight into crayfish ancestry. The majority-rule consensus tree (Fig. 13) of the morphological data set supports three clades of freshwater crayfish that roughly correspond to the established family-level taxonomic groupings. The nearest sister group to the crayfish is *Chilenophoberus*, suggesting an evolutionary origin different from the historically interpreted sister group, the Nephropidae. Additional most-parsimonious arrangements of data sets with certain sister group taxa eliminated result in minor rearrangements of some sister taxa and some inferred relationships of parastacid genera. However, the general topology of all discovered trees is consistent with the consensus tree presented in Figure 13. The majority-rule consensus tree (Fig. 13) groups most cambarid and all parastacid crayfish into well-defined clades. Similarly, astacids appear as a well-defined clade.

Each clade in the morphological phylogenetic interpretation is distinguished by one or more synapomorphy. The Erymidae are characterized by a synapomorphous pattern of carapace grooves that includes three parallel, essentially linear, grooves along the lateral side of the carapace, the cervical, post-cervical, and branchiocardiac grooves. The Nephropidae possess synapomorphies of marginal telson spines, a shortened cervical groove, and converging post-cervical and branchiocardiac grooves. The Chilenophoberidae are characterized by a synapomorphous groove pattern in which the cervical groove extends across the dorsal surface, and both the post-cervical and branchiocardiac grooves are reduced. The Astacoidea possess a synapomorphous diaresis of the telson, movable fifth thoracic segment, and groove pattern. The

family Astacidae is characterized by a synapomorphous a medial rostal ridge. Species in the family Cambaridae are characterized by an apomorphous annulus ventralis in the female and hooks on the ichiopodites of one or more pereiopods in the male. The family Parastacidae is characterized by a reduction resulting in the lack of first pair of abdominal appendages and a synapomorphous change in calcification in the distal portion of the telson..

The most-parsimonious arrangement of the 16s nuclear region is given in Figure 14. In general, there is reasonably good agreement between the morphology-based consensus tree (Fig. 13) and the nucleotide-based tree (Fig. 14). The clustering of *Engaewa* into the cambarid clade, which differs from its placement within in the morphological consensus tree, may be due to either sequencing problems or convergent evolution. The inferred clustering of parastacids based on nucleotide sequences differs from the clustering based on morphology. Most notable are the positions of *Gramastacus, Tenuibranchiurus,* and *Parastacoides*. Several factors may contribute to the difference in positions of these genera between Figures 13 and 14, including missing regions of DNA or homoplasy in the morphological data set.

The 18s DNA data set provided essentially no information about the phylogenetic affinities of the crayfish (Fig. 15). Each of the three possible tree arrangements within the crayfish was equally parsimonious, and the consensus tree has a polytomy in the crayfish clade. In part, the lack of clear resolution of relationships using 18s DNA is due to the large gaps in the sequences of some of the taxa evaluated in the study (Table 7).

INTERPRETATION

The three data sets used in this study provide various levels of insight into crayfish relationships. Morphological information is especially useful for interpretation of the ancestry of crayfish and for crayfish relationships at the family level. Within the Astacoidea, information about generic relationships is also provided by morphological information. The 16s nucleotide data provide high resolution of generic relationships within the Parastacidae, but the data provide relatively little information about the Astacoidea in general. The 18s nucleotide data provide little specific information about the phylogenetic relations of astacoidean genera.

Crayfish ancestry and monophyly

The morphological data (Fig. 13) support the interpretation of the crayfish as a monophyletic group. The Chilenophoberidae represents the most likely sister taxon to the Astacoidea. The Chilenophoberidae share a close common ancestry with the Nephropidae, and the Nephropidae share a close common ancestry with the Erymidae.

Nucleotide data provide further support for the monophyly of the freshwater crayfish. All crayfish families analyzed in either study were shown to be more closely related to each other than to the Nephropidae, the closest living relatives. Due to the limitations of molecular data, extinct groups such as the chilenophoberids and erymids could not be analyzed

Contrary to previous interpretations (e.g., Glaessner, 1969; Albrecht, 1983), the crayfish are interpreted as having an ancestor among the chilenophoberid lobsters. Previously, erymid lobsters were usually considered to have given rise to the crayfish (Glaessner, 1969; Albrecht, 1983). However, shared modifications in carapace groove patterns: extension of the cervical

groove, reduction of the post-cervical groove, and horizontal trend of the branchiocardiac groove, suggest a chilenophoberid-to-crayfish evolutionary pathway. By constast, erymid lobsters lack this character state. The evident monophyletic origin of crayfish lends support to the hypothesis (Ortmann, 1902; Scholz, 1993) that crayfish or their immediate ancestors invaded freshwater habitats only once.

Due to the well-supported monophyly of the freshwater crayfish, reassignment of all families into a single superfamily is warranted. Because the previous classification placed the Southern Hemisphere group and Northern Hemisphere groups in separate superfamilies within the Astacidea, each were give equal rank with the nephropid lobsters and interpreted as equally closly related to the nephropids as to each other. Placement of the crayfish into a single superfamily illustrates the monophyletic origin of the group. Assignment of all crayfish families to a single superfamily will also place the crayfish at equal taxonomic ranking with the nephropid and erymid superfamilies. The superfamily Parastacoidea is suppressed due to its monofamilial status and all three families are placed into the redefined superfamily Astacoidea.

Several other reassignments may be warranted among the outgroup taxa. The family Protastacidae is not supported, because its contained species are grouped within the crayfish clade rather than elsewhere. *Protastacus,* which was previously defined as the type genus for the family Protastacidae (Albrecht, 1983), is included in the crayfish clade. Here, *Protastacus* is interpreted as an astacid crayfish. Among sister groups of the crayfish, some decapods generally considered to be erymids cluster in separate regions of the majority-rule consensus tree (Fig. 13). Pending further investigation, two genera, *Paleastacus* and *Pseudastacus*, are tentatively reassigned to the Chilenophoberidae (Fig. 13).

The interpretation of crayfish ancestry presented here is supported by the inferred

evolution of carapace suture patterns (Fig. 16). The pattern of sutures probably represents sites of muscle attachment and potential somite boundaries (Glaessner, 1969; Chong and Foster, 1976; Albrecht, 1983; Tshudy and Babcock, 1997). In the Erymidae, three grooves extend across the dorsal surface in a nearly parallel manner (Fig. 16b). The suture patterns of the nephropids and chilenophoberids are both derivations of this basic three-suture pattern. Evolution of suture patterns in the Nephropoidea involved reduction of the cervical, inferior, and gastro-orbital grooves (Fig. 16c). The postcervical groove extended across the dorsal surface, and the branchiocardiac groove became more horizontal near the dorsal surface. In the Chilenophoberidae, the cervical groove extended across the dorsal surface, whereas the postcervical groove became reduced and joined with the cervical groove laterally (Fig. 16d). The branchiocardiac groove became less strongly impressed laterally and more horizontally trending near the dorsal surface. In both the Astacoidea and Parastacoidea, the suture pattern appears to be a further modification of the chilenophoberid condition (Fig. 16f). The hepatic, inferior, and gastro-orbital grooves became obsolete. The postcervical groove is further reduced and is present only near the dorsal surface where it branches from the cervical groove. The cervical groove became more sinuous in shape along the lateral side. The branchiocardiac groove is only present as a horizontal groove near the dorsal surface. Minor modifications of the basic crayfish suture pattern occur within the clade (Fig. 17). For example, in the Cambaridae, the postcervical groove is weekly impressed. The extent of separation between the postcervical and branchiocardiac grooves also varies, especially within the Parastacidae. Reconstructions of the suture patterns in the Protastacidae are not different from the crayfish condition (Fig 16e).

The monophyly and ancestry of the crayfish are also expressed in the development of the telson (Fig. 16). Over evolutionary time, the shape of the telson changed from triangular (Fig.

16a) to more quadrate in shape (Fig. 16f). This change was accompanied by the development of marginal spines in the Nephropidae. In the freshwater crayfish, a diaresis was developed; the diaresis allows for bending of the telson. In the Parastacidae, bending the telson is accomplished by a change in calcification, such as in distal membranous sections of the exoskeleton. The Astacoidea have an actual break between the proximal and distal portions of the telson. The Protastacidae also possess a diaresis of the telson. None of the marine lobsters possesses a diaresis.

Several additional characters unite the crayfish as a monophyletic taxon. One of these is the mobility of the last thoracic sternite. In the Nephropoidea and the Erymidae, the last thoracic sternite is fused rather than mobile. The lack of a longitudinal medial suture along the carapace (Fig. 17) also separates the crayfish from all additional groups in the analysis. Developmental aspects, such as lack of a larval stage and number of ectoteloblasts in the embryonic growth zone, further support a monophyletic grouping of freshwater crayfish (Table 2; Albrecht, 1983; Scholtz, 1995).

Relationships among the freshwater crayfish families

Data presented in the morphological and 16s nucleotide study support the division of crayfish in to two super-familial groups. The separation of the crayfish into two clades is based on several apomorphic characters. Synapomorphies uniting the crayfish of the Southern Hemisphere include the diaresis of the telson marked by a change in calcification. Characters such as modification of the first pair of pleopods in the male for sperm transport and presence of a true diaresis of the telson unite the crayfish of the Northern Hemisphere. Within the Northern Hemisphere group, two clades are present. The clade including *Cambarus* is united by a number

of synapomorphies including the presence of an annulus ventralis in the female and hooks present on the ischia of one or more pereiopods of the male. The clade containing *Astacus* is united by the presence of an apical median ridge on the rostrum and the absence of the annulus ventralis and hooks on the ischia of the male pereiopods.

Grouping freshwater crayfish genera into these three clades reflects, to a large extent, the historical interpretation of crayfish relationships. The three clades have been interpreted as separate families, the Parastacidae, Cambaridae, and Astacidae (Hobbs, 1988). The monophyly of each of these groups is strongly supported by the morphological data. Monophyly of the Astacidae has been questioned based on the lack of good apomorphic characters and the question of evolutionary distribution (Scholtz, 1998). However, the morphological data presented here strongly support monophyly of the Astacidae (Fig. 13). The 18s nucleotide data set, however, does raise some questions (Fig. 15). The genetic difference is not greatly different between members of the same cambarid genus (*P. clarkii* and *P. leonensis*) and *Astacus*, a member of the Astacidae, because all arrangements between the three taxa were equally parsimonious. However, due to the limited data set of 18s nucleotide sequences, the morphological data may provide better resolution for phylogenetic relationships.

Relationships among genera of the Parastacidae

The Parastacidae are separated into two clades in the morphological interpretation of phylogeny and several small clades in the 16s nucleotide interpretation. The placement of *Gramastacus* as the sister group to the other freshwater crayfish in the morphological analysis is not supported by the nucleotide interpretation. Much of the groupings and tree topology of the two trees are similar; however, key differences are in the placement of *Tenuibranchiurus,*

Parastacoides, and *Engaewa.* Part of the reason for the change in relative positions of the taxa may be due to the incorporation of more species within the morphological data set, including the South American genera *Virilastacus, Samastacus,* and *Parastacus.* Support for some of the placements within the Parastacoidea is not strong, as is shown by a low percentage of trees supporting a particular grouping (Fig. 15).

Both phylogenetic interpretations differ from historical interpretations. Although the number of clades in the interpretation by Riek (1972; Fig. 6) is not consistent with the interpretations from these data sets, several relationships have been preserved to some degree, especially within the morphological interpretation. Some of the Riek (1972) groupings, for example, the *Tenuibranchiurus*, *Engaewa*, *Engaeus*, and *Parastacus* group, are each included in the same clade within the morphological interpretation. The 16s nucleotide data set matches well with the Crandall et al. (in press) interpretation. Crandall separates the Parastacidae into three clades: the first containing *Cherax*, *Geocherax*, *Gramastacus*, *Tenuibranchiurus*, and *Engaeus*; the second containing *Paranephrops*, *Parastacoides*, *Euastacus*, and *Astacopsis*; and the third clade containing *Engaewa.* This division is closely approximated in the 16s investigation, although the placements of *Cherax* and *Engaewa* are different. Placement of *Engaewa* within the cambarid crayfish clade is due to an arbitrary effect based on the amount of separation from the remainder of the parastacids.

Relationships among genera of the Cambaridae

Historical interpretations of the Cambaridae recognized three subfamilies, Cambarellinae, Cambaroidinae, and Cambarinae. Cambarinae encompasses the majority of the genera, with both of the other two subfamilies being monogeneric (Fig. 5) (Hobbs, 1974). The Cambarellinae

included the genus *Cambarellus*, and Cambaroidinae included *Cambaroides*. The morphological study did not support the concept of three distinct subfamilies. In fact, *Cambaroides* was placed within the Astacidae but in a sister group position to the remainder of the astacid genera. The Cambaroidinae as previously viewed were a transitional group between the two families of the Astacoidea (Hobbs, 1988) due to the presence of only a partial suite of cambarid characters. The phylogenetic position of *Cambaroides* in the consensus tree reinforces that interpretation. The placement of *Cambarellus* within the remainder of the Cambaridae reflects the lack of significant characters for separation of the subfamily. Initial separation was based on a slightly different branchial formula, mobility of the annulus ventralis, and placement of ischial hooks on the pereiopods (Hobbs, 1974). These features are relatively plastic as is shown by the variation of these characters within the subfamily Cambarinae (Hobbs, 1974).

Relationships among genera of the Astacidae

Within this study, the Astacidae have been reconstructed to include the modern astacids, *Cambaroides*, and all crayfish fossils known from the Northern Hemisphere. The relationships between the three extant genera, *Astacus*, *Austropotamobius*, and *Pacifastacus* are identical to those proposed by Hobbs (1988). The incorporation of *Cambaroides* was discussed earlier in this thesis. Placing all of the Northern Hemisphere fossil species into one clade has interesting implications in terms of crayfish evolution. Only one of the fossil species was originally placed in another family. *Procambarus primeavus* was originally interpreted as a cambarid. However, this species does possess the synapomorphic medial rostral ridge of the Astacidae (Feldmann et al., 1981). In the case of *P. primeavus*, it is uncertain whether the species should be reassigned taxonomically because preserved fossils lack some key morphological characters. Recent

interpretation of *Protastacus politus* by Albrecht (1983) placed this brackish-water organism into *Protastacus*; however, the original generic designation was *Astacus*. The fact that the this species was collected from brackish-water deposits does not negate its affinities with true crayfish, for several modern species along the Pacific coast migrate into brackish water during part of their yearly life cycle (Hobbs, 1988). As a result of including *Protastacus* in the Astacidae, the family Protastacidae is abandoned.

Evolutionary considerations

It is likely that crayfish only invaded the freshwater environment once. Although a monophyletic origin does not necessarily imply a single invasion, it certainly provides support for this interpretation. The fact that crayfish have quite similar habitats suggests that the ancestor lived in the same way. The loss of a free-living larval state is a condition common to freshwater arthropods (Grey, 1988). However, the crayfish, are united in several characteristics of the young hatchlings. Hatchlings possess all appendages except the first pleopods and the uropods, the telson has the adult shape, and the pereiopods are without setose exopods (Schram, 1993).

Although the fossil record of freshwater crayfish is poor, some interpretations can be made regarding the timing of evolution within the group. The Chilenophoberidae, from which the crayfish diverged, probably arose from an erymid ancestry by the end of the Permian (Tshudy and Babcock, 1997). The oldest definitive crayfish fossils date from the Late Jurassic to Early Cretaceous (Table 8) (Imaizumi, 1938). These fossils, *Astacus licenti* and *A. spinirostris,* represent crayfish that possess good astacid characters, indicating that the radiation of crayfish into separate families had already occurred. Scholz (1998) proposed a Triassic invasion of freshwater by astacoideans. This invasion was followed by establishment of separate families as

the supercontinent Pangea separated during the Jurassic (Scholz, 1998). Further dispersal and diversification of the two crayfish stocks into families and genera remains unclear due to insufficient fossil evidence. These features, along with the inferred monophyletic origin of crayfish, suggest a single invasion into freshwater.

SUMMARY

Cladistic and nucleotide sequence analysis of all fossil and modern crayfish provides information about crayfish affinities and relationships. The closest relatives of the freshwater crayfish are the Chilenophoberidae. The Chilenophoberidae and the Nephropoidea are interpreted to have evolved from an erymid ancestor. The family Protastacidae groups within the crayfish clade, which leads to the suppression of the name Protastacidae. Primary support for crayfish ancestry is based the evolution of carapace groove patterns.

The freshwater crayfish are a monophyletic group consisting of three families. Monophyly of the crayfish is established based on diaresis of the telson, carapace groove pattern, mobility of last thoracic sternite, and developmental characters. The three monophyletic families within the freshwater crayfish are each recognized by a set of synapomorphies. These families are consistent in a general way with the traditional interpretation of the Parastacidae, Astacidae, and Cambaridae. The subfamilies within the Cambaridae were not supported by this study. A single freshwater invasion by crayfish is supported by the similarity of habitats and developmental adaptations. On the best available evidence, the timing of the astacid invasion of freshwater is inferred to have occurred during the Triassic.

REFERENCES

Aguirre-Urreta, M.B. 1992. Tertiary freshwater decapod (Crustacea: Parastacidae) from the Ñirihuau Basin, Patagonia, Argentina. Journal of Paleontology 66: 817-825.

Albrecht, H. 1983. Die Protastacidae n. fam., fossile Vorfahren der Flußkrebse? Neues Jahrbuch für Geologie und Paläontologie, Monatshefte 1: 5-15.

Austin, C.M. 1995. Evolution in the genus *Cherax* (Decapoda: Parastacidae) in Australia: A numerical cladistic analysis of allozyme and morphological data. Freshwater Crayfish 8.

Babcock, L.E., Miller, M.F., Isbell, J.I., Collinson, J.W., and Hasiotis, S.T. 1998. Paleozoic-Mesozoic crayfish from Antarctica: earliest evidence of freshwater decapod crustaceans. Geology 26: 539-542.

Budd, G.E. 1996. Progress and problems in arthropod phylogeny. Trends in Ecology and Evolution 11: 356-358.

Chong, G. and Förster, R. 1976. *Chilenophoberus atacamensis*, a new decapod crustacean from the Middle Oxfordian of the Cordillera de Domoyko, northern Chile. Neues Jahrbuch für Geologie und Paläontologie, Monatshefte 3: 145-156.

Cope, E.D. 1870. On three extinct Astaci from the fresh-water Tertiary of Idaho. Proceedings of the American Philosophical Society 11: 605-607.

Copeland, M.J. 1960. *Erymastacus bordensis* a new Mesozoic decapod from the Canadian Arctic. Geological Survey of Canada, Bulletin 60: 55-57.

Crandall, K.A.. 1998. Genetic variation within and among crayfish species. Freshwater Crayfish 11: 135-145.

Crandall, K.A. and Cronin, T.W. 1997. The molecular evolution of wavelength regulation in

freshwater crayfishes (Decapoda: Cambaridae). Journal of Molecular Evolution 45: 524-534.

Crandall, K.A., Fetzner, J.W. Jr., Lawler, S.H., Kinnersley, M., and Austin, C.M. in press. Phylogenetic relationships among the Australian and New Zealand genera of freshwater crayfish. Australian Journal of Zoology. In press.

Crandall, K.A., and Fitzpatrick, J.F., Jr. 1996. Crayfish molecular systematics: using a combination of procedure to estimate phylogeny. Systematic Biology 45: 1-26.

Crandall, K.A., Lawler, S.H., and Austin, C.M. 1995. A preliminary examination of the molecular phylogenetic relationships of some crayfish genera from Australia (Decapoda: Parastacidae). Freshwater Crayfish 10: 18-30.

Faxon, W. 1885. A revision of the Astacidae, Part 1. Memoirs of the Museum of Comparative Zoology at Harvard College 10, no. 4., 156 p.

Feldmann, R.M. 1979. *Eryma foersteri*, a new species of lobster (Decapoda) from the Jurassic (Callovian) of North America. American Museum Novitates 2668: 1-6.

Feldmann, R.M., Grande, L., Birkheimer, C.P., Hannibal, J.T., and McCoy, D.L. 1981. Decapod fauna of the Green River Formation (Eocene) of Wyoming. Journal of Paleontology 55: 788-799.

Feldmann, R.M., and May, W. 1991. Remarkable crayfish remains (Decapoda: Cambaridae) from Oklahoma--evidence of predation. Journal of Paleontology 65: 884-886.

Feldmann, R.M., and Pole, M. 1994. A new species of *Paranephrops* White, 1842: A fossil crayfish (Decapoda: Parastacidae) from the Manuherikia Group (Miocene), central Otago, New Zealand. New Zealand Journal of Geology and Geophysics 37: 163-167.

Feldmann, R.M., Tshudy, D.M., and Thomson, M.R. 1993. Late Cretaceous and Paleocene

Decapod Crustaceans from the James Ross Basin, Antarctic Peninsula. Paleontological Society Memoir 28, 41 p.

Field, K.G., Olsen, G.J., Lane, D.J., Giovannoni, S.J., Ghiselin, M.T., Raff, E.C., Pace, N.R., Raff, R.A. 1988. Molecular phylogeny of the animal kingdom. Science 239: 748-753.

Förster, R., and Rieber, H. 1982. Der älteste Vertreter der Gattung *Palaeastacus* (Crustacea: Decapoda), *Palaeastacus argoviensis* n.sp., aus dem Unteren Dogger der Nordschweiz. Eclogae Geological Helvetiae 75: 773-778.

Gall, J. and Fischer, Y. 1965. Révision du genre *Clytiopsis* Bill, décapode du Buntdandstein supérieur. Bulletin Serv. Carte géol. Als. Lorr. 18: 43-48.

Garassino, A. 1997. The macuran decapod crustaceans of the Lower Cretaceous (Lower Barremian) of Las Hoyas (Cuence, Spain). Atti de la Società Italiana di Scienze Naturali e Museo Civicio di Storia Naturale di Milano 137: 101-126.

GenBank sequence database. 1999. Los Alamos National Laboratory, Los Alamos, New Mexico. http://www.ncbi.nlm.nih.gov/.

Glaessner, M.F. 1969. Decapoda. *In* Moore, R.C., ed., Treatise on Invertebrate Paleontology, Part R, Arthropoda 4: Geological Society of America and University of Kansas Press, Lawrence, Kansas, R339-R533.

Gray, J. 1988. Evolution of the freshwater ecosystem: the fossil record. Palaeogeography, Palaeoclimatology, Palaeoecology 62: 1-214.

Hall, J., and Clarke, J.M. 1888. Descriptions of the trilobites and other Crustacea of the Oriskany, Upper Helderberg, Hamilton, Portage, Chemung, and Catskill Groups. New York State Geological Survey, Paleontology of New York 7, 236 p.

Hannibal, J.T. 1996. Phylum Arthropoda: phyllocarids, millipedes, insects, and other less

common forms. *In* Feldmann, R.M., ed., Fossils of Ohio. Ohio Division of Geological Survey, Bulletin 70: 124-131.

Hasiotis, S.T., and Mitchell, C.E. 1993. A comparison of crayfish burrow morphologies: Triassic and Holocene fossil, paleo- and neo-ichnological evidence, and the indentification of their burrowing signatures. Ichnos 2: 291-314.

Hobbs, H.H., Jr. 1942. The crayfishes of Florida. University of Florida Publications, Biological Sciences Series 3, no. 2, 179 p., 28 plates.

Hobbs, H.H., Jr. 1974. Synopsis of the families and genera of crayfishes (Crustacea: Decapoda). Smithsonian Contributions to Zoology 164, 32 p.

Hobbs, H.H., Jr. 1988. Crayfish distribution, adaptive radiation, and evolution. *In* Holdrich D.M., and Lowery, R.S., eds., Freshwater crayfish: biology, management and exploitation: London, Croom Helm, p. 52-82.

Hobbs, H.H., Jr. 1989. An illustrated checklist of the American crayfishes (Decapoda: Astacidae, Cambaridae, Parastacidae). Smithsonian Contributions to Zoology 480, 236 p.

Hobbs, H.H., Jr. 1991. A new generic assignment for a South American crayfish (Decapoda: Parastacidae) with revised diagnoses of the South American genera and comments on the parastacid mandible. Proceedings of the Biological Society of Washington 104: 800-811.

Holdrich, D.M., and Lowery, R.S., eds. 1988. Freshwater crayfish: biology, management and exploitation. London, Croom Helm, 498 p.

Holdrich, D.M., and Reeve, I.D. 1988. Functional morphology and anatomy. *In* Holdrich D.M., and Lowery, R.S., eds., Freshwater crayfish: biology, management and exploitation: London, Croom Helm: 11-51.

Huxley, T.H. 1880. The Crayfish: An Introduction to the Study of Zoology. New York, D.

Appleton and Company, 371 p.

Imaizumi, R. 1938. Fossil crayfishes from Jehol. Science Reports of the Tokyo Imperial University, Sendal, Japan, second series 19: 173-179.

Jamieson, B.G.M. 1991. Ultrastructure and phylogeny of crustacean spermatozoa. Memoirs of the Queensland Museum 31: 109-142.

Kim. W., and Abele, L.G. 1990. Molecular phylogeny of selected decapod crustaceans based on 18s rRNA nucleotide sequences. Journal of Crustacean Biology 10: 1-3.

Lawler, S.H, and Crandall, K.A. 1998. The relationship of the Australian freshwater crayfish genera *Euastacus* and *Astacopis*. Proceedings of the Linnean Society of New South Wales 119: 1-8.

Maddison, W.P., and D.R. Maddison. 1992. MacClade: Analysis of phylogeny and character evolution. Version 3.0. Sinauer Associates, Sunderland, Massachussetts.

Miller, G.L., and Ash, S.R. 1988. The oldest freshwater decapod crustacean from the Triassic of Arizona. Paleontology 31: 273-279.

Ortmann, A.E. 1902. The geographical distribution of freshwater decapods and its bearing upon ancient geography. Proceedings of the American Philisophical Society 41: 267-400.

Packard, A.S., Jr. 1880. Fossil crawfish from the Tertiaries of Wyoming. American Naturalist 14: 222-223.

Packard, A.S., Jr. 1881. A fossil Tertiary crayfish. American Naturalist 15: 832-834.

Patak, A., and Baldwin, J. 1984. Electrophoretic and immunochemical comparisons of haemocyanins from Australian freshwater crayfish (family Parastacidae): Phylogenetic implications. Journal of Crustacean Biology 4: 528-535.

Patak, A., Baldwin, J., and Lake, P.S. 1989. Immunochemical comparisons of haemocyanins of

freshwater Australian crayfish: Phylogenetic implications. Biological Systematics and Ecology 17: 249-252.

Ponniah, M., and Hughes, J.M. 1998. Evolution of the Queensland spiny mountain crayfish of the genus *Euastacus* Clark (Decapoda: Parastacidae): preliminary 16s mtDNA phylogeny. Proceedings of the Linnean Society of New South Wales 119: 9-19.

Raff, R.A. 1988. Ribosomal RNA sequences and the early history of the Metazoa. *In* Broadhead, T.W., ed., Molecular Evolution and the Fossil Record. Paleontological Society Short Courses in Paleontology 1: 63-74.

Rathburn, M.J. 1926. The fossil stalk-eyed crustacea of the Pacific slope of North America. United States National Museum, Bulletin 138, 155 p.

Riek, E.F. 1969. The Australian freshwater crayfish (Crustacea: Decapoda: Parastacidae), with descriptions of new species. Australian Journal of Zoology 17: 855-918.

Riek, E.F. 1971. The freshwater crayfishes of South America. Proceedings of the Biological Society of Washington 84: 129-136.

Riek, E.F. 1972. The phylogeny of the Parastacidae (Crustacea: Decapoda: Parastacidae), with descriptions of new species. Australian Journal of Zoology 17: 855-918.

Scholtz, G. 1993. Teloblasts in decapod embryos: an embryonic character reveals the monophyletic origin of the freshwater crayfishes (Crustacea, Decapoda). Zoologishe Anzeiger 230: 45-54.

Scholtz, G. 1998. Von Zellen und Kontinenten--die Evolution der Flußkrebse (Decapoda, Astacida). Stapfia 58, zugleich Kataloge des OÖ, Landesmuseums, Neue Folge 137: 205-212.

Scholtz, G. and Richter, S. 1995. Phylogenetic systematics of the repantian Decapoda

(Crustacea, Malacostraca). Zoological Journal of the Linnean Society 113: 289-328.

Schram, F.R. 1977. Paleozoogeography of late Paleozoic and Triassic Malacostraca. Systematic Zoology 26: 367-379.

Schram, F.R., Feldmann, R.M., and Copeland, M.J. 1978. The Late Devonian Palaeopalaemonidae and the earliest decapod crustaceans. Journal of Paleontology 52: 1375-1387.

Sokol, A. 1987. A note on the existence of pre-Pleistocene fossils of parastacid crayfish. Victorian Naturalist 104: 81-82.

Swofford, D.L. 1993. PAUP, Phylogenetic analysis using parsimony, version 3.1.1. Computer program distributed by the Illinois Natural Historical Survey, Champaign, Illinois.

Thompson, J.D., Higgins, E.G, and Gibson, T.J. 1994. Clustal W: improving the sensitivity of multiple sequence alignment through sequence weighting, position specific gap penalty, and weight matrix choice. Nucleic Acids Research 22: 4673-4680.

Tshudy, D., and Babcock, L.E. 1997. Morphology-based phylogenetic analysis of the clawed lobsters (family Nephropidae and the new family Chilenophoberidae). Journal of Crustacean Biology 17: 253-263.

van Straelen, V. 1928. On a fossil freshwater crayfish from eastern Mongolia. Bulletin of the Geological Society of China 7: 173-178.

Wiley, E.O. 1981. Phylogenetics: The Theory and Practice of Phylogenetic Systematics. New York: Wiley-Liss, 439 p.

Wilson, A.C., Ochman, H., Prager, E.M. 1988. Molecular time scale for evolution. *In* Broadhead, T.W., ed. Molecular evolution and the fossil record. Short Courses in Paleontology 1: 49-62.

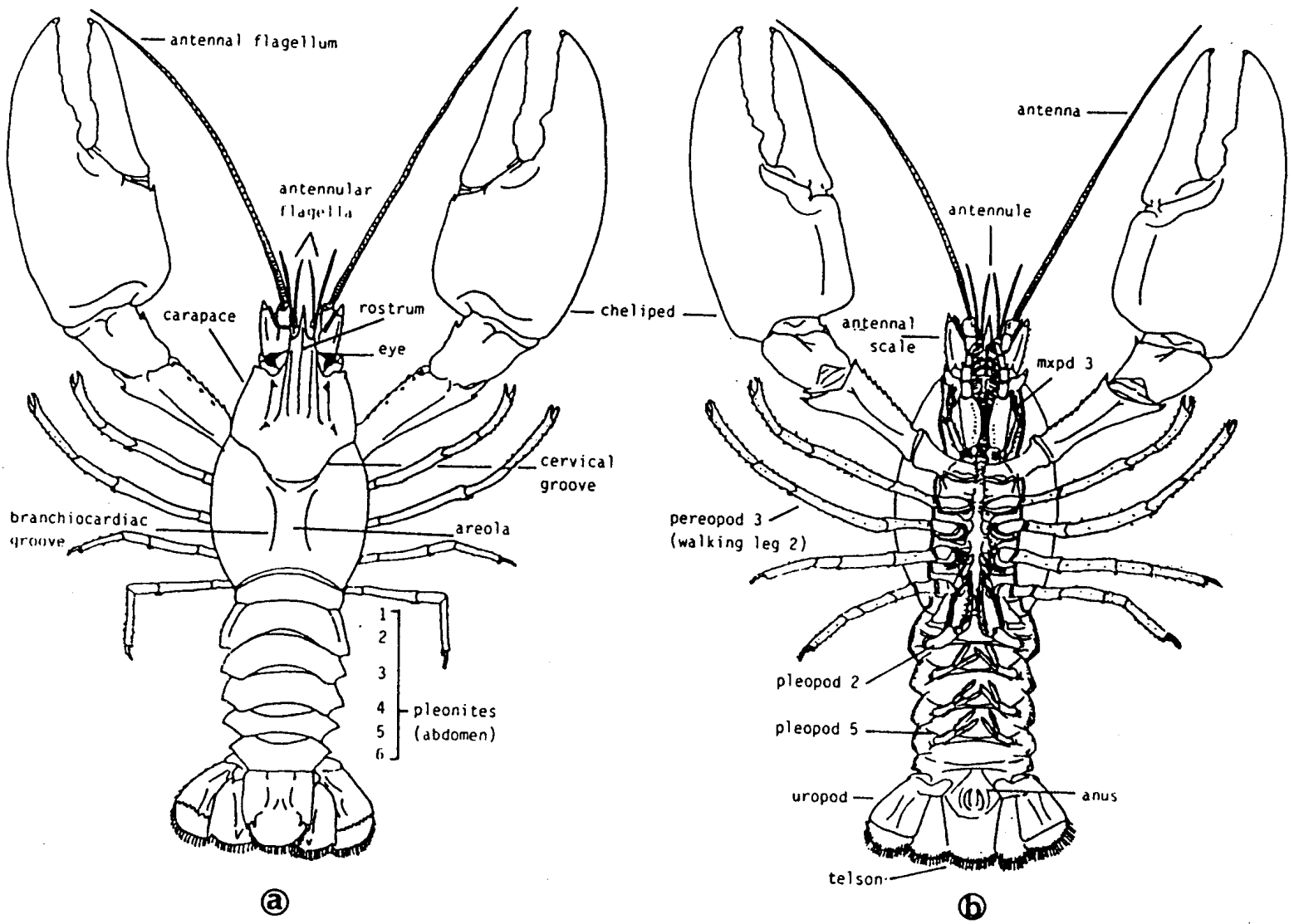

Figure 1. Dorsal (a) and ventral (b) views of male *Pacifastacus leniusculus* (Holdrich and Reeve, 1988).

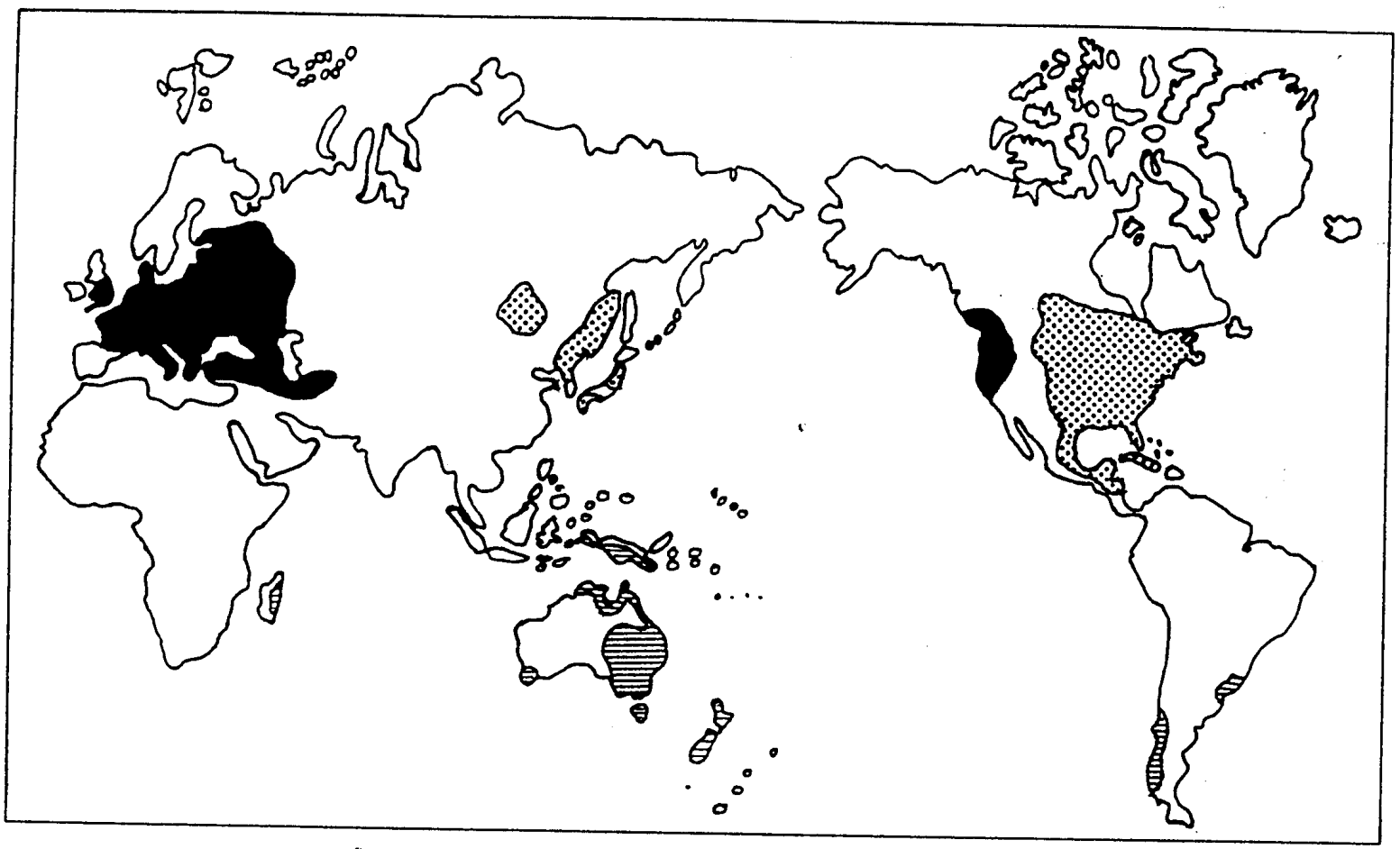

Figure 2. Global distribution of freshwater crayfish (from Scholz, 1998). Symbols: Astacidae, black; Cambaridae, stippled; Parastacidae, striped. Based on information presented here, Cambaridae from eastern Asia are reassigned to the Astacidae.

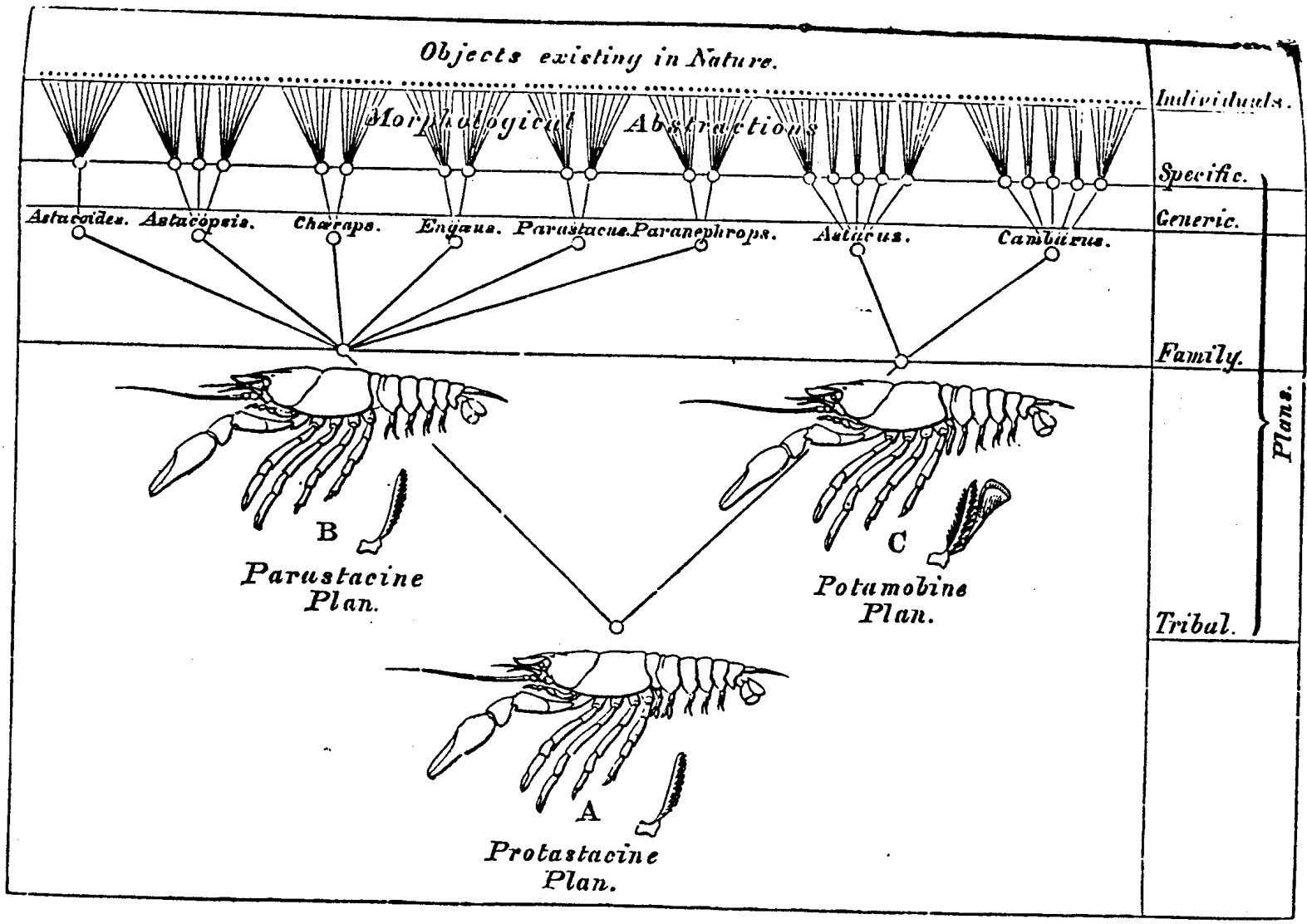

Figure 3. Classification of crayfish proposed by Huxley (1880), based on comparative morphologic criteria.

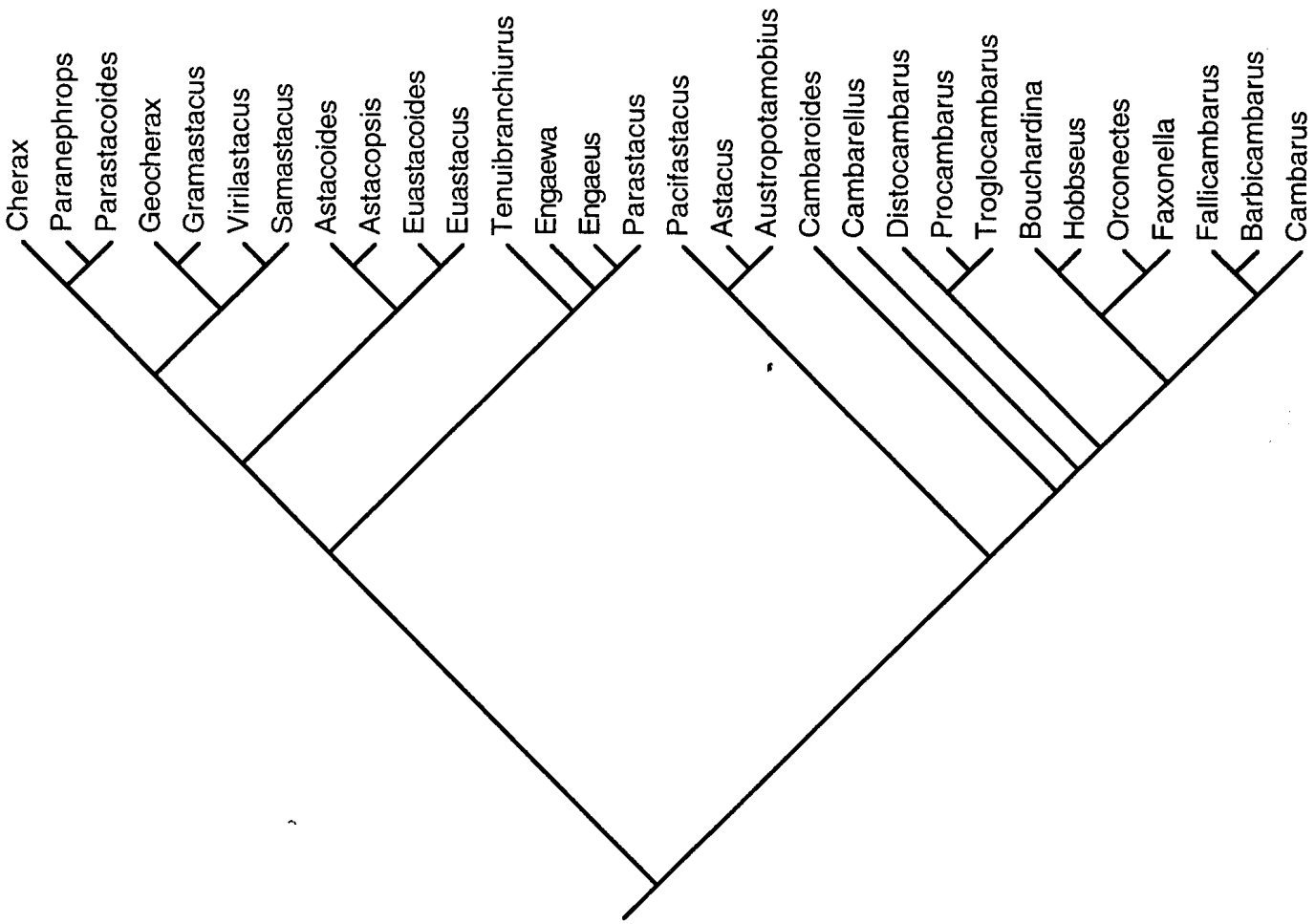

Figure 4. Cladogram showing relationships among crayfish genera, based on the works of Hobbs (1988) and Riek (1972). Relationships were assessed using morphologic criteria.

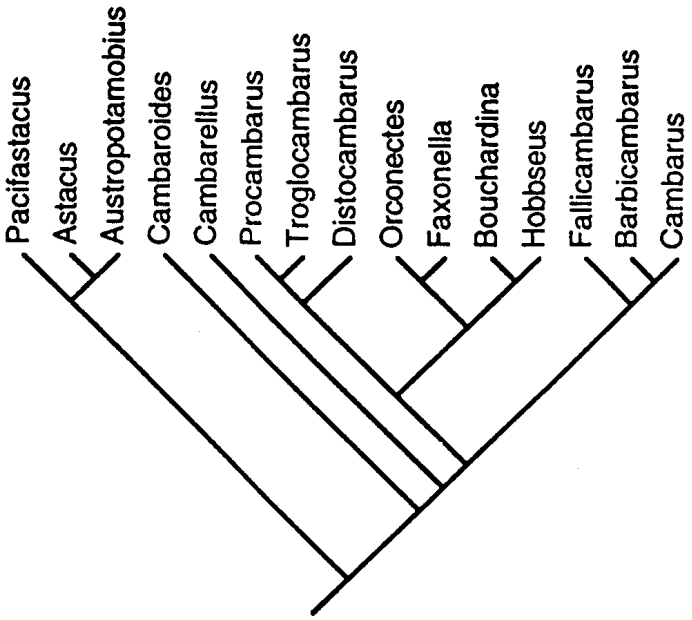

Figure 5. Cladogram showing astacid and cambarid relationships based on the work of Hobbs (1988). Relationships were assessed using morphologic criteria.

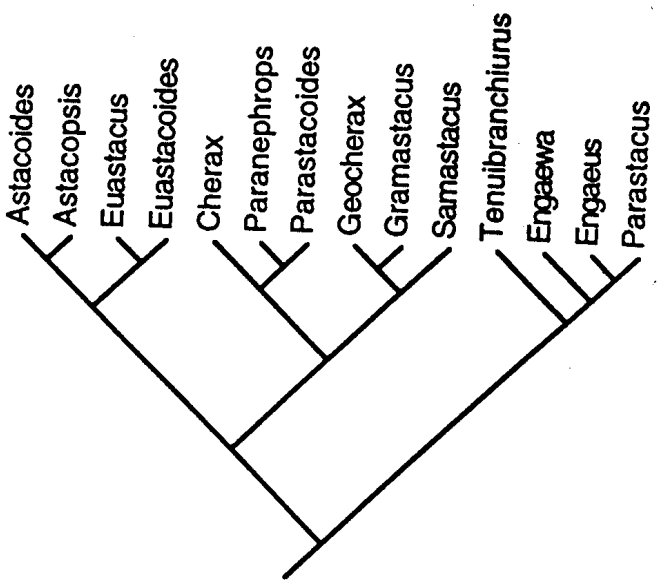

Figure 6. Cladogram showing parastacid relationships based on the work of Riek (1972). Relationships were assessed using morphologic criteria.

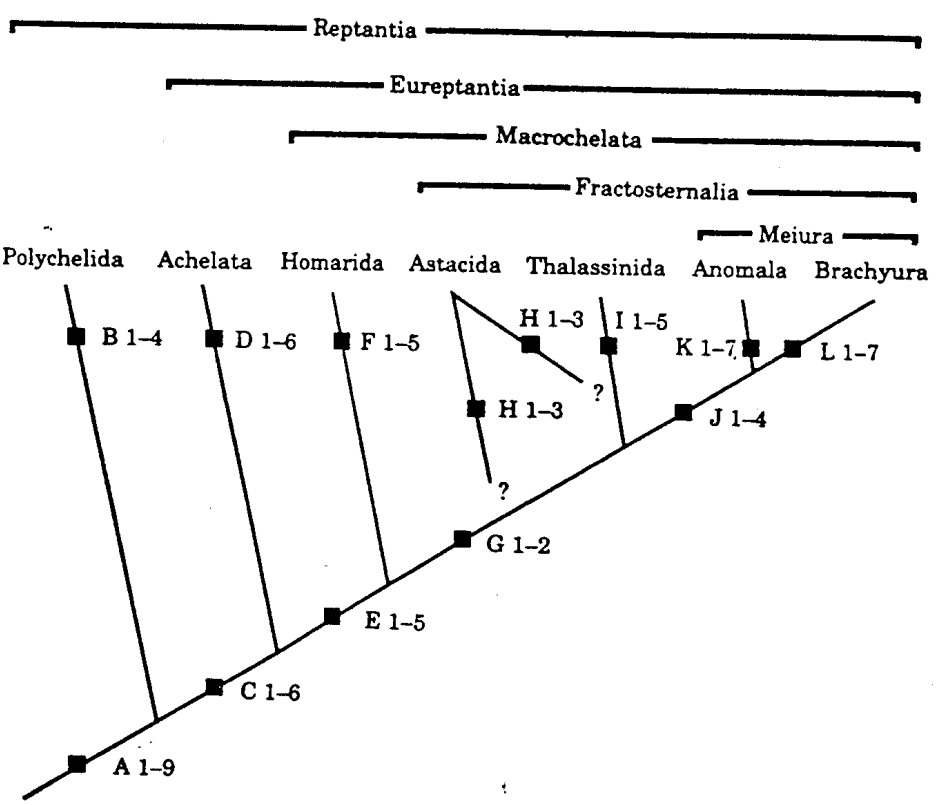

Figure 7. Cladogram showing relationships among major groups of repantian decapods. Numbers refer to apomorphies listed in the original paper (Scholtz and Richter, 1995).

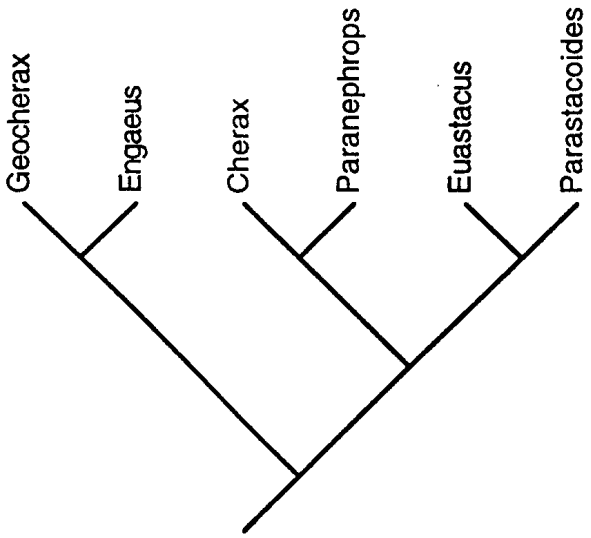

Figure 8. Cladogram showing relationships among several parastacid genera based on immunochemical studies (Patak et al., 1989).

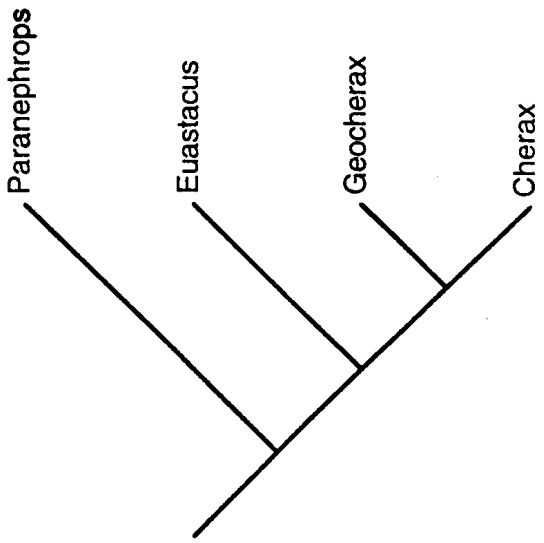

Figure 9. Cladogram showing relationships among several parastacid genera based on allozymes (Austin, 1995).

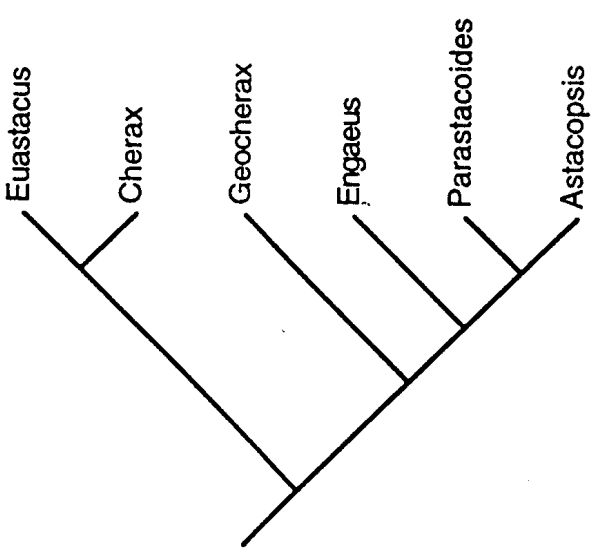

Figure 10. Cladogram showing relationships among several parastacid genera based on 16s mtDNA data (Lawler and Crandall, 1998).

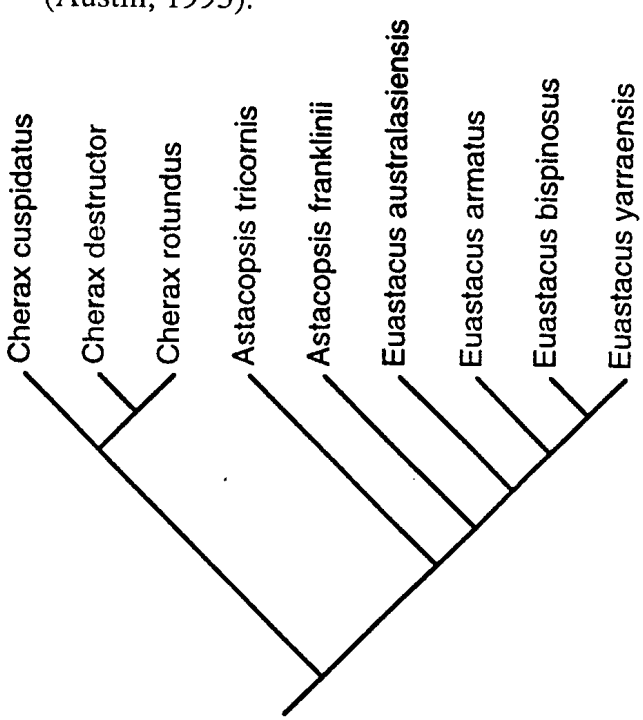

Figure 11. Cladogram showing relationships among several parastacid genera based on 16s mtDNA data (Crandall et al., in press).

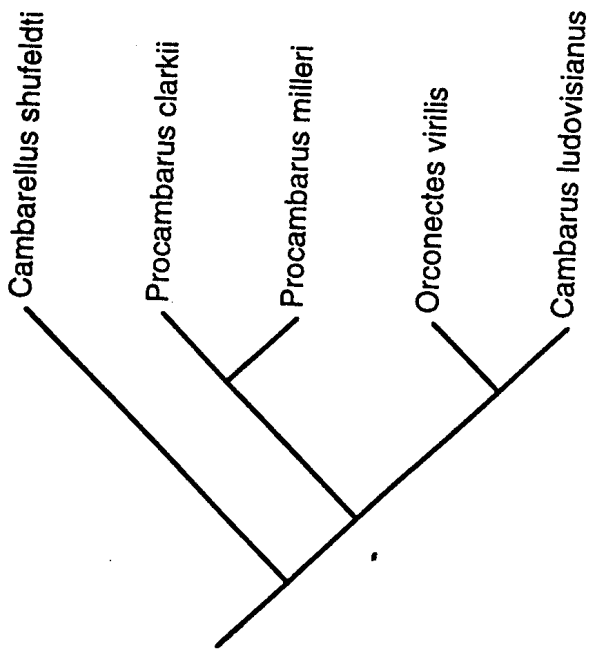

Figure 12. Cladogram showing relationships among several cambarid genera based on rhodopsin (Crandall and Cronin, 1997).

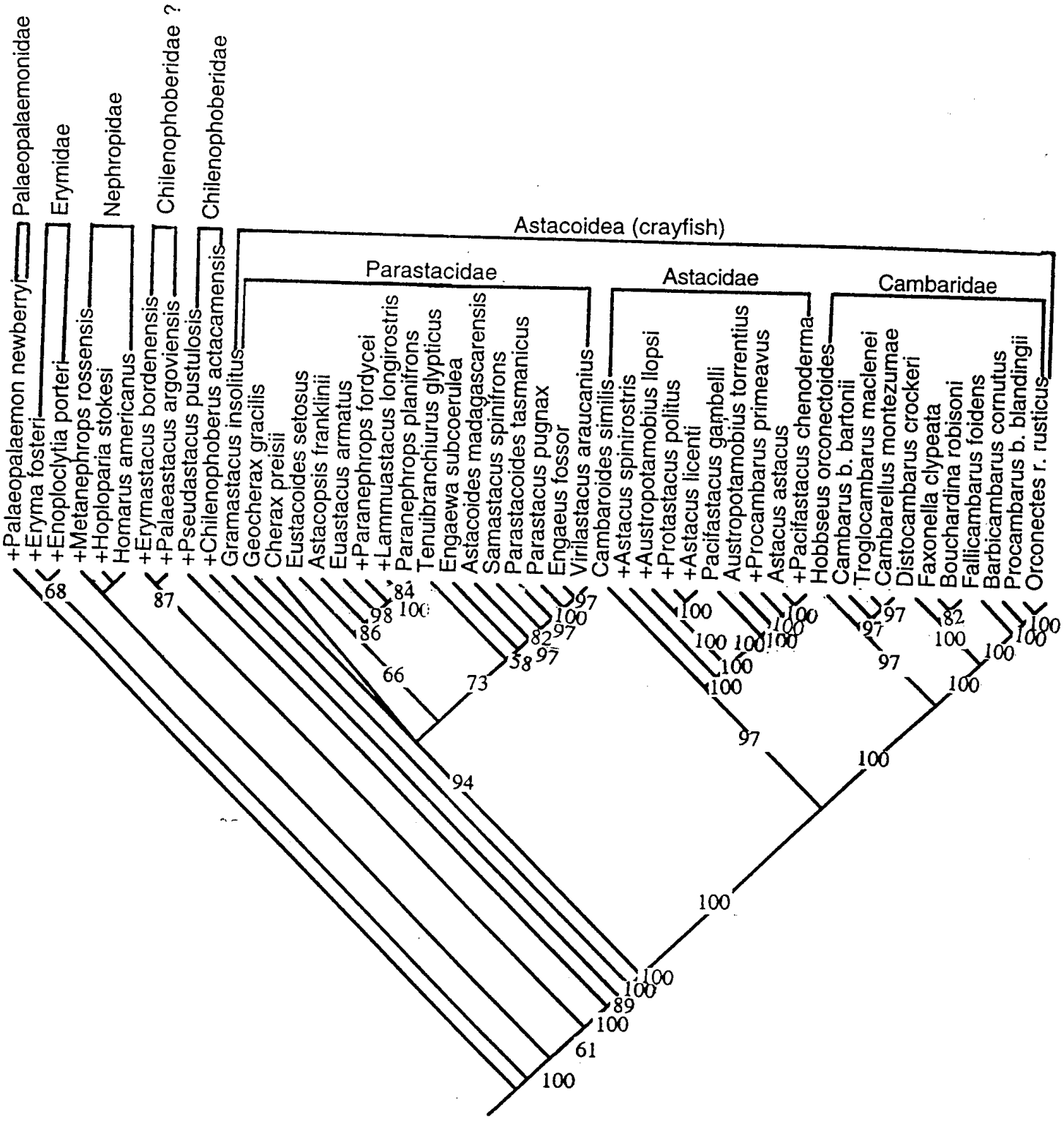

Figure 13. Majority-rule consensus tree based on 1500 most-parsimonious trees using the morphological data set (see Table 4) determined using a heuristic search. Tree length equals 193.

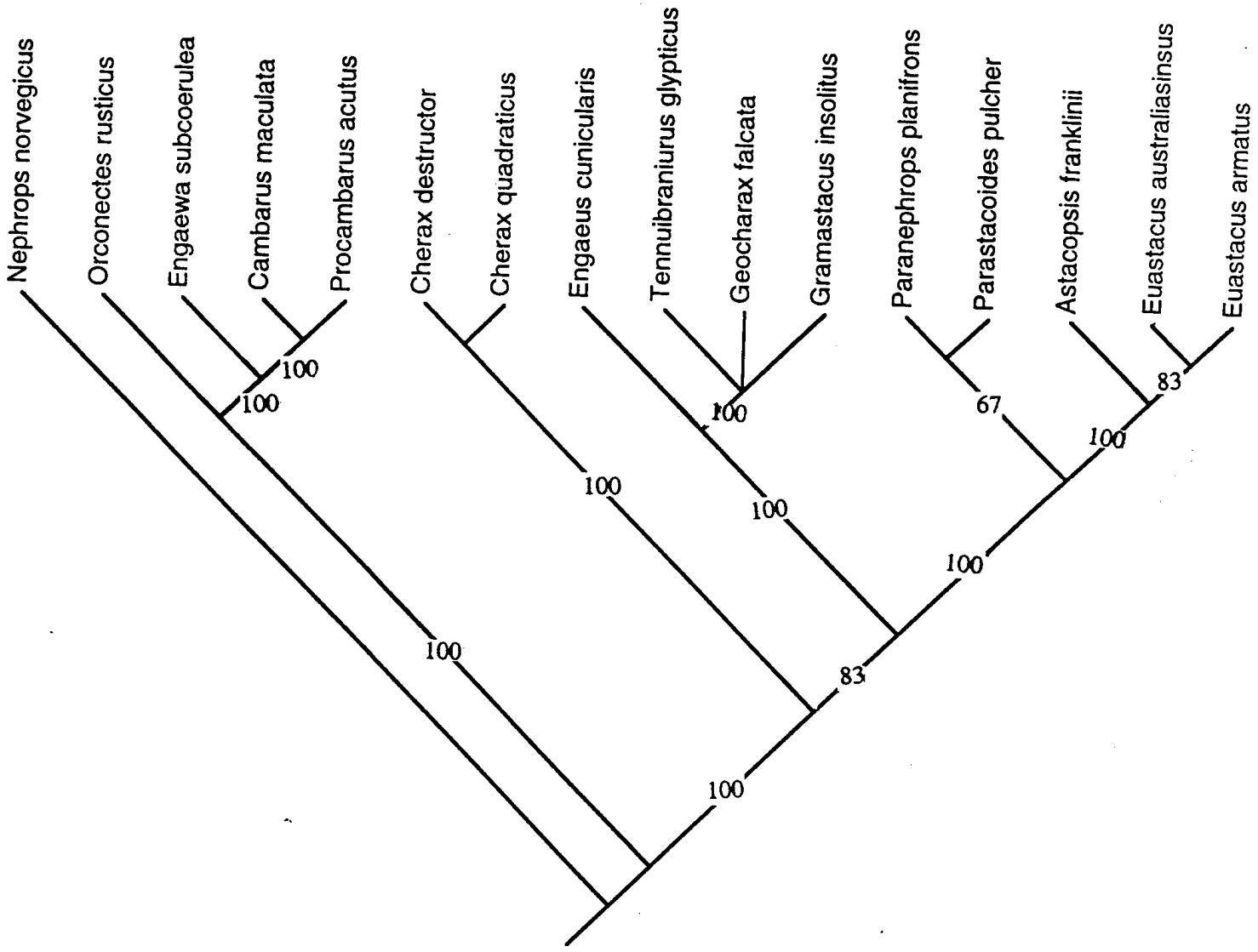

Figure 14. Majority-rule consensus tree based on 6 most-parsimonious trees of the 16s mtDNA data set (see Table 6) determined using a heuristic search. Tree length equals 669. Sequences from Genbank (1999), Crandall and Fitzpatrick (1996), and Crandall et al. (in press).

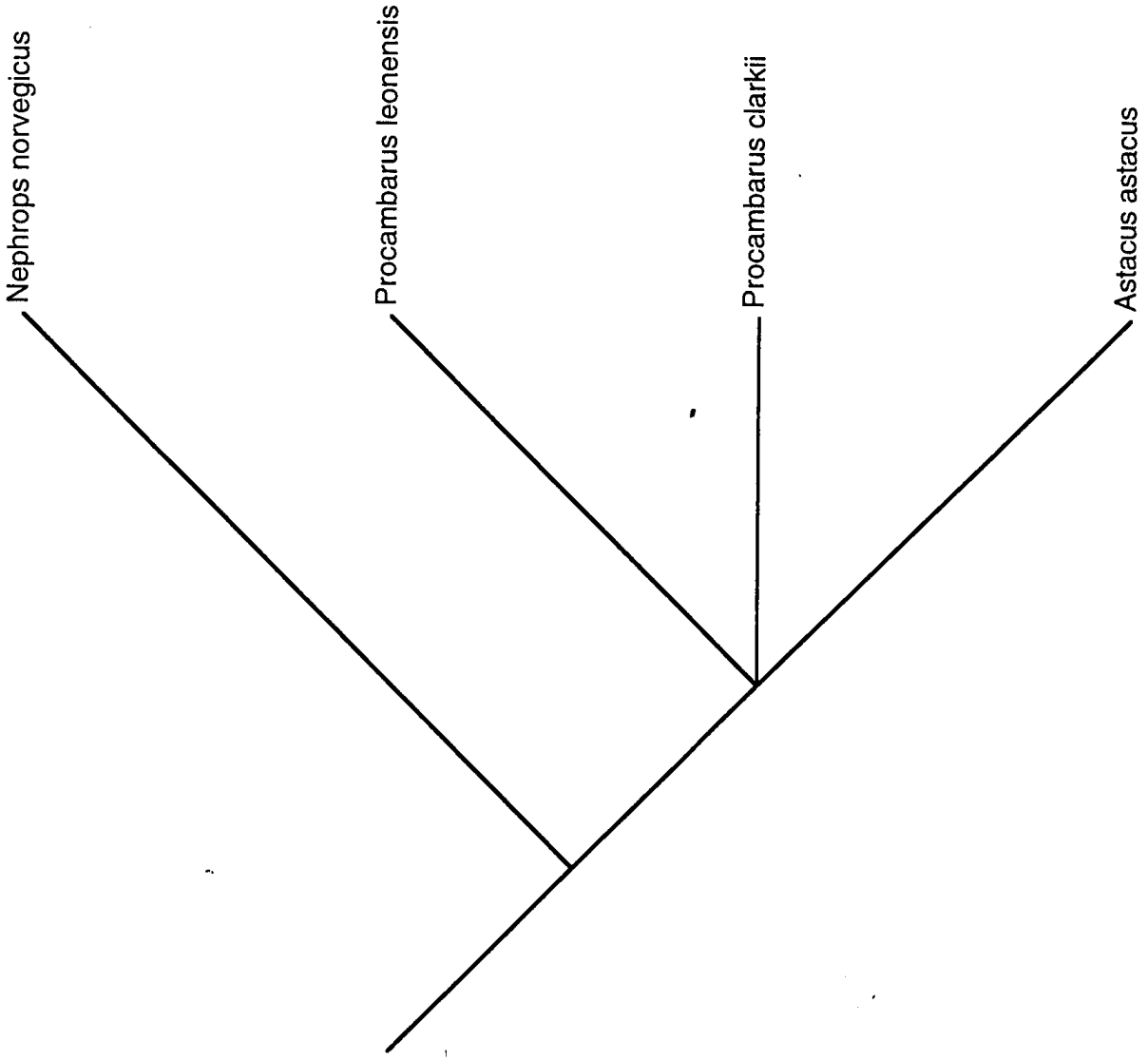

Figure 15. Majority-rule consensus tree based on 3 most-parsimonious trees of the 18s mtDNA data set (see Table 7) determined using a heuristic search. Tree length equals 113. Sequences from Genbank (1999), Crandall and Fitzpatrick (1996), and Crandall et al. (in press).

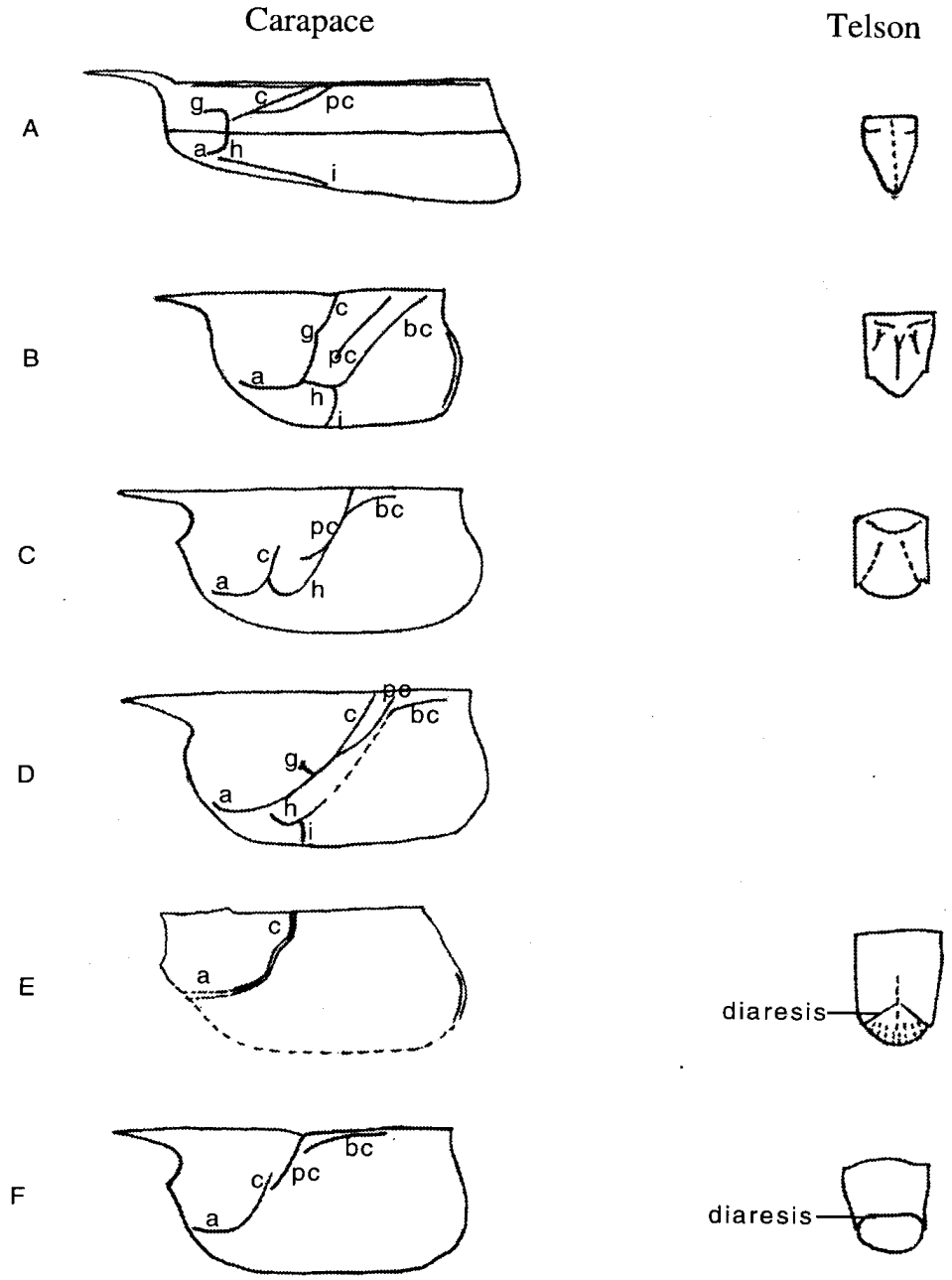

Figure 16. Generalized carapace suture patterns and telson structure of taxa included in the morphological study: A) Palaeopalaemonidae (after Schram et al., 1978); B) Erymidae (after Glaessner, 1969); C) Nephropidae (after Tshudy and Babcock, 1997); D) Chilenophoberidae (after Chong and Forster, 1976); E) Protastacidae (after Albrecht, 1983); F) Astacoidea and Parastacoidea. Grooves are identified as follows: a, antennular; bc, branchiocardiac; c, cervical; g, gastro-orbital; h, hepatic; i, inferior; pc, postcervical.

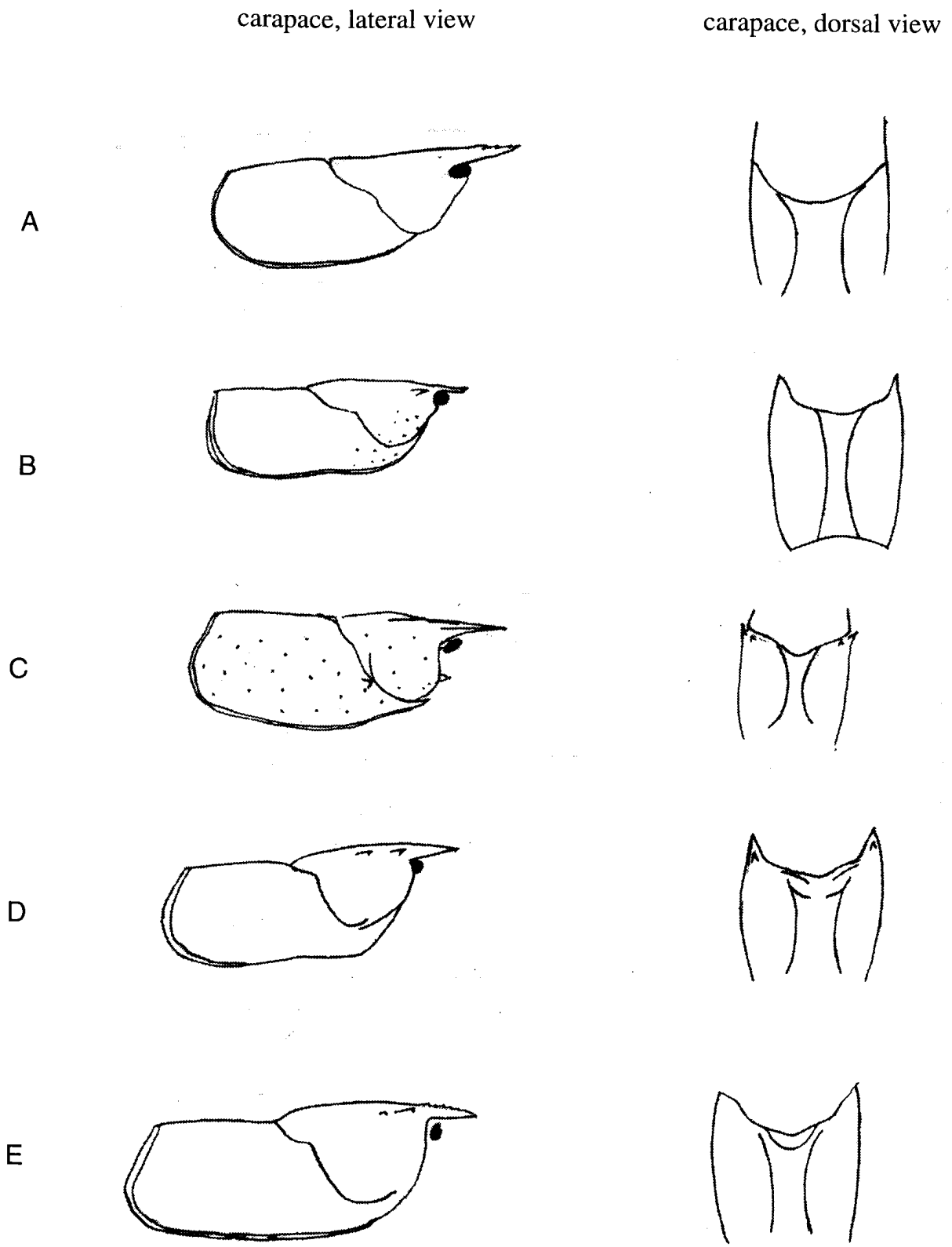

Figure 17. Lateral and dorsal views of freshwater crayifsh suture patterns: A) *Chreax preisii*; B) *Cambarus bartonii bartonii*; C) *Procambarus blandigii blandigii*; D) *Astacus astacus*; E) *Pacifastacus gambellii.*

Table 1. Selected crustacean groups and their key morphological characters

This is a list of the families of freshwater crayfish, selected sister groups, and nomenclatural categories of both crayfish and lobsters (as currently recognized) above the family level. Key morphological characters of each taxonomic group are included.

Subphylum Crustacea (Glaessner, 1969; Hobbs, 1988)
–arthropods that in at least one stage in their life history possess two pairs of antennae and three pairs of postoral appendages
–typically gill-bearing and aquatic

Class Malacostraca (Glaessner, 1969; Hobbs, 1988)
–body composed of eight thoracic and six abdominal segments
–female genital aperture on the sixth thoracic segment, male genital aperture located on the eighth thoracic segment
–carapace enveloping thoracic region
–movable paired stalked eyes
–biramous antennules
–flattened scale-like exopod on the antennae
–generally elongate, ventrally flexed abdomen
–commonly developed tail fan composed of uropods and the telson

Order Decapoda (Glaessner, 1969; Hobbs, 1988)
–carapace is fused dorsally with all of the thoracic segments
–gills are typically arranged into three series: podobranchiae, arthrobranchiae, pleurobranchie
–first 3 pairs of thoracic appendages modified as maxillopods
–no more than 5 pairs are locamotory pereiopods
–one or more pairs of pereiopods are chelate, with the first pair commonly strongly chelate
–exopods of pereiopods are typically lost in adults

Infraorder Astacidea (Glaessner, 1969; Hobbs, 1988)
–cephalothorax subcylindrical, rarely strongly compressed
–rostrum well developed
–antennae with five-segmented stalk and scale
–carapace not fused with epistome
–abdomen extended and bears well developed pleura and uropods
–abdominal pleura well deveoloped
–uropod lateral ramus divided by diaresis (transverse suture)
–first three pairs of pereiopods chelate
–all pleopods lack an appendix interna
–genital openings coxal

Family Erymidae (Glaessner, 1969)
–carapace with well developed, roughly parallel cervical, postcervial, and branchiocardiac grooves
–typically with median suture and fusiform intercalated plate

Family Chilenophoberidae (Tsudy and Babcock, 1997)
–carapace with well developed cervical and branciocardiac grooves and weakly developed post-cervical and inferior grooves
–lack of fusiform, intercalated plate
–medial carina present on the cepahalic region

Superfamily Nephropoidea (Glaessner, 1969; Hobbs, 1988)
–carapace median longitudinal suture or spiniform ridge
–first abdominal segment of male with pleopods that serve together to transport spermatophore to female
–young hatch as larvae
–carapace with well developed postcervical and branchiocardiac grooves
–sternal plate between fifth pereiopods is fused to the anterior complex sternal element
–embryo with 19 ectoteloblasts in posterior growth zone (Scholtz, 1995)

Superfamily Astacoidea (Glaessner, 1969; Hobbs, 1988)
–lack of medial longitudinal suture
–carapace with well developed cervical and branchiocardiac grooves
–some podobranchiae provided with bilobed plaited laminae
–first rami of antennules subequal in size
–telson and exopods of uropods with diaresis,
–podobranchiae of second and third maxillipeds and first three pereiopods with broad plaited laminae
–embryo with around 40 ectoteloblasts in posterior growth zone (Scholtz, 1995)
–pleopods in male modified for individual sperm transfer

Family Astacidae (Hobbs, 1988)
–distal part of first pereiopod is subtubular and devoid of ornamentation other than apical spoon-like lobes
–young hatch as miniatures of the adult and are attached to the pleopods of the mother by a telson thread
–first pair of male pleopods modified for individual sperm transfer
–pleopods of male second abdominal segment posses spiral appendix to the endopod

Family Cambaridae (Hobbs, 1988)

–cyclic dimorphism present in males in which distal part of pleopod bears a shallow (open) or deeply embedded sperm groove and ornamentation often consisting of spines, plates, knobs, or setal tuffs
–hooks present of ischia of one or more pereiopods
–females (except for Cambaroidinae) with annulus ventralis in median between fourth and fifth pereiopods
–absence of postcoxal lappets on articular membrane just posteriodorsal to base of fourth pereiopod
–young hatch as miniatures of the adult and are attached to the pleopods of the mother by a telson thread
–first pair of male pleopods modified for individual sperm transfer
–pleopods of male second abdominal segment posses spiral appendix to the endopod

Family Parastacidae (Hobbs, 1988)
–pleopods absent from first abdominal segment
–pleopods on second segment of male lack spiral appendix,
–podobranchiae provided with rudimentary laminae
–young hatch as miniatures of adult, but cling to the pleopods of the mother by their pereiopods
–telson usually without transverse suture, but diaresis is marked by change in calcification

Table 2. Classification of species included the morphological study

Phylum Arthropoda Siebold and Stannius, 1845
Subphylum Crustacea Pennant, 1777
Class Malacostraca Latreille, 1806
Order Decapoda Latreille, 1803
Infraorder Caridea Dana, 1852
†Family Palaeomonidae Rafinesque, 1815
†*Palaeopalaemon newberyii* Whitfield, 1880
Infraorder Palinura Latreille, 1803
†Family Eryonidae de Haan, 1841
†*Eryon arctiformis* (von Schlotheim, 1820)
Infraorder Astacidea Latreille, 1802-1803
†Family Erymidae Van Straelen, 1924
†*Eryma fosteri* Feldmann, 1979
†*Enoploclytia porteri* Miller and Ash, 1988
Family Nephropidae Dana, 1852
Homarus americanus Milne Edwards, 1837
†*Hoploparia stokesi* (Weller, 1903)
†*Metanephrops rossensis* Feldmann, Tshudy, and Thomson, 1993
†Family Chilenophoberidae Tshudy and Babcock, 1997
†*Chilenophoberus actacamensis* Chong and Förster, 1976
†*Pseudastacus pustulosis* (Münster, 1839)
†Family Chilenophoberidae ? Tshudy and Babcock, 1997
†*Palaeastacus argoviensis* Förster and Rieber, 1982 [tentatively reassigned from family Eryimidae Van Straelen, 1924]
†*Erymastacus bordenensis* Copeland, 1960 [tentatively reassigned from family Eryimidae Van Straelen, 1924]
Superfamily Astacoidea De Haan, 1841
Family Astacidae Latreille, 1802
Astacus astacus (Linnaeus, 1758)
†*Astacus licenti* (van Straelen, 1928)
†*Astacus spinirostris* (Imaizumi, 1838)
†*Austropotamobius llopsi* (Via, 1971)
Austropotamobius torrentius (Schrank, 1803)
Cambaroides similis (Koebel, 1892) [reassigned from family Cambaridae, subfamily Cambaroidinae Villalobos, 1955]
Pacifastacus gambelli (Girard, 1852)
†*Pacifastacus chenoderma* (Cope,1870)
†*Protastacus politus* (Schlüter, 1868) [reassigned from †Family Protastacidae Albrecht, 1983]
Family Cambaridae Hobbs, 1942 [formerly subfamily Cambarinae Hobbs, 1942]
Barbicambarus cornutus (Faxon, 1884)
Bouchardina robisoni Hobbs, 1977

Cambarellus montezumae (Saussure, 1857) [reassigned from subfamily Cambarellinae Laguarda, 1961]
Cambarus bartonii (Fabricius, 1798)
Distocambarus crockeri Hobbs and Carlson, 1983
Fallicambarus foidens (Cottle, 1863)
Faxonella clypeata (Hay, 1899)
Hobbseus orconectoides Fitzpatrick and Payne, 1968
Orconectes rusticus (Girard, 1852)
Procambarus blandingii (Harlan, 1830)
†*Procambarus primeavus* (Packard, 1880) [assignment uncertain]
Troglocambarus maclenei Hobbs, 1942

Family Parastacidae Huxley, 1879
Astacoides madagascarensis Petit, 1923
Astacopsis franklinii Huxley, 1878
Cherax preisii (Erichson, 1846)
Engaeus fossor (Erichson, 1846)
Engaewa subcoerulea Riek, 1967
Euastacoides setosus Riek, 1956
Euastacus armatus (von Martens, 1866)
Geocherax gracilis Clark, 1936
Gramastacus insolitus Riek, 1972
†*Lammuastacus longirostris* Aguirre-Urreta, 1992
†*Paranephrops fordycei* Feldmann and Pole, 1994
Paranephrops planifrons White, 1842
Parastacoides tasmanicus (Erichson, 1846)
Parastacus pugnax (Poeppig, 1865)
Samastacus spinifrons (Philippi, 1882)
Tenuibranchiurus glypticus Riek, 1951
Virilastacus araucanius (Faxon, 1914)

† denotes an extinct taxon

Table 3. Character states used in morphological study

1) rostral ornamentation
- 0—smooth, no indication of break between acumen and rest of rostrum
- 1—margins interrupted without bearing lateral spines
- 2—with lateral spines
- 3—serrate, spiny
- 4—rostrum absent

2) apical median ridge of rostrum
- 0—absent
- 1—present

3) rostrum length
- 0—long
- 1—reduced

4) lateral ridges on rostrum
- 0—absent
- 1—present

5) pereiopods with chelae
- 0—one through four
- 1—one through three

6) chelae of the first pereiopod (length/width)
- 0—elongate (>2.5)
- 1—slender (1.5-2.5)
- 2—ovate, broad (<1.5)

7) ventro-lateral margin of the chelae
- 0—without tubercles
- 1—with few tubercles
- 2—with many tubercles
- 3—tubercles arranged in discrete rows

8) medial portion of dactyl
- 0—without tubercles
- 1—with randomly arranged tubercles
- 2—one row of tubercles
- 3—two rows of tubercles

9) interior spines on the carpus
- 0—absent
- 1—present
- 2—reduced

10) transverse suture across the telson
- 0—absent
- 1—partial
- 2—complete

11) transverse suture on exopods of uropods
- 0—absent
- 1—present

12) distomedial spine of mesial uropod ramus
- 0—absent
- 1—present

13) hooks on ishiopodites of pereiopods
- 0—absent
- 1—only on third
- 2—on second and third
- 3—on third and fourth

14) annulus ventralis
- 0—absent
- 1—present

15) first pair of pleopods in male
- 0—present
- 1—absent
- 2—modified for sperm transport

16) carapace medial ridge
- 0—present

1—absent

17) fifth throracic segment
0—fused to fourth segment
1—moveable

19) lateral view of main groove
0—straight
1—sinuous

20) post-cervical groove
0—u-shaped
1—too close to cervical groove to differentiate
2—v-shaped

21) antennular groove
0—present
1—absent

22) areola
0—absent
1—broad, but curving inward
2—joined together

23) pairs of post-orbital ridges
0—none
1—one, prominent
2—two, prominent
3—one, greatly reduced
4—three

24) post-orbital spine
0—absent
1—present

25) cervical spine
0—absent
1—present

26) branchiostegal spine
0—absent
1—present

18) dorsal aspect of cervical groove
0—u-shaped
1—v-shaped
2—absent (lateral grooves do not join)

27) ventral keel
0—absent
1—present

28) hepatic groove
0—present
1—absent

29) gastro-orbital groove
0—present
1—reduced
2—absent

30) antennal carina
0—carina present with or without spines
1—spines only
2—spines and carina absent

31) trend of branchiocardiac groove
0—vertical
1—horizontal

Table 4. Data matrix for morphological study

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	+Palaeopalaemonidae newberri	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0
2	+Eryma fosteri	0	0	0	0	1	2	1	1	0	0	1	?	0	0	0
3	+Erymastacus bordenensis	2	0	0	0	1	1	1	1	?	?	?	?	0	0	0
4	+Enoploclytia porteri	3	1	0	?	1	0	1	0	0	0	0	0	0	0	0
5	+Palaeastacus argoviensis	2	0	0	0	1	1	2	1	0	0	0	1	0	0	0
6	+Chilenophoberus actacamensis	?	?	?	?	1	0	0	0	0	0	1	?	0	0	0
7	+Pseudastacus pustulosis	2	0	0	0	1	1	2	0	0	0	1	?	0	0	0
8	+Protastacus politus	?	?	?	?	1	?	?	?	?	1	1	0	0	0	0
9	+Metanephrops rossensis	2	0	0	0	1	1	0	0	0	0	0	0	0	0	0
10	+Hoploparia stokesi	3	0	0	1	1	0	2	1	0	0	1	0	0	0	0
11	Homarus americanus	2	0	0	0	1	2	2	0	0	0	1	0	0	0	0
12	+Astacus licenti	0	1	0	0	1	1	1	1	1	1	1	1	?	?	2
13	+Astacus spinirostris	2	0	0	0	1	1	3	1	2	1	1	1	?	?	2
14	Astacus astacus	2	1	0	1	1	2	1	1	2	2	1	1	0	0	2
15	+Austropotamobius llopsi	2	1	0	0	1	1	2	1	0	2	1	1	0	0	2
16	Austropotamobius torrentius	2	1	0	1	1	2	2	1	2	2	1	1	0	0	2
17	+Pacifastacus chenoderma	0	0	0	1	1	2	2	1	0	2	1	1	0	0	2
18	Pacifastacus gambelli	2	1	0	1	1	2	2	1	0	1	1	1	0	0	2
19	+Procambarus primeavus	2	1	0	1	1	2	2	1	0	2	1	1	?	1	2
20	Procambarus b. blandingii	2	0	0	1	1	0	1	1	1	2	1	1	3	1	2
21	Cambarus b. bartonii	0	0	0	1	1	1	0	0	1	2	1	1	3	1	2
22	Barbicambarus cornutus	2	0	0	1	1	2	3	0	1	2	1	1	1	0	2
23	Distocambarus crockeri	0	0	0	1	1	1	3	2	2	2	1	1	1	1	2
24	Fallicambarus foidens	0	0	0	1	1	1	0	2	1	2	1	1	1	1	2
25	Faxonella clypeata	0	0	0	1	1	1	0	0	2	2	1	1	1	1	2
26	Hobbseus orconectoides	0	0	1	0	1	1	2	0	1	2	1	1	1	1	2
27	Orconectes r. rusticus	2	0	0	1	1	1	0	1	1	2	1	1	1	1	2
28	Troglocambarus maclenei	0	0	0	0	1	0	0	0	0	2	1	0	3	1	2
29	Bouchardina robisoni	1	0	0	1	1	0/1	0	0	1	2	1	?	1	1	2
30	Cambarellus montezumae	2	0	0	0	1	0	0	0	0	2	1	?	2	1	2
31	Cambaroides similis	0	0	0	0	1	2	0	1	1	0	1	1	0/2	0/1	2
32	+Lammuastacus longirostris	3	0	0	1	1	0/1	3	1	2	?	1	1	0	0	1
33	+Paranephrops fordycei	3	0	0	1	1	0	3	2	1	0	1	?	0	0	1
34	Engaeus fossor	0	0	1	1	1	0/1	0	2	0	0	1	1	0	0	1
35	Engaewa subcoerulea	2	1	1	0	1	1	0	0	0	0	1	1	0	0	1
36	Gramastacus insolitus	2	0	0	1	1	1	0	0	0	0	1	?	0	0	1
37	Parastacus pugnax	0	0	1	1	1	1	0/1	0	2	0	1	?	0	0	1
38	Tenuibranchiurus glypticus	0	0	1	0	1	0/1	0	1	0	0	1	?	0	0	1
39	Geocherax gracilis	0	0	0	0	1	1	2	0	1	0	1	1	0	0	1
40	Astacoides madagascarensis	2	0	1	0	1	0	1	0	1	0/1	1	?	0	0	1
41	Astacopsis franklinii	2	1	0	1	1	1	3	2	1	0	1	1	0	0	1
42	Cherax preisii	0	0	0	0	1	0/1	3	0	1	0	1	1	0	0	1
43	Eustacoides setosus	0	0	0	0	1	1	3	2	1	1	1	0	0	0	1
44	Euastacus armatus	3	0	0	1	1	1	3	0	1	1	1	0	0	0	1
45	Paranephrops planifrons	3	0	0	0	1	0	3	1	1	0	1	?	0	0	1
46	Parastacoides tasmanicus	0	0	1	0	1	1	0	1	0	0	1	?	0	0	1
47	Samastacus spinifrons	2	0	0	0	1	2	0	1	2	0	1	?	0	0	1
48	Virilastacus araucanius	0	0	1	1	1	2	2	1	2	0	1	1	0	0	1

		16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	+Palaeopalaemonidae newberri	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0
2	+Eryma fosteri	0	0	1	0	2	0	0	0	0	0	0	0	0	2	0
3	+Erymastacus bordenensis	0	?	0	0	2	0	0	0	0	0	0	0	0	0	1
4	+Enoploclytia porteri	1	?	1	0	2	0	0	0	0	0	0	0	1	2	0
5	+Palaeastacus argoviensis	0	0	0	0	2	0	0	0	0	0	0	0	1	1	1
6	+Chilenophoberus actacamensis	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0
7	+Pseudastacus pustulosis	0	?	0	1	0	0	1	0	0	1	0	0	0	1	1
8	+Protastacus politus	0	?	0	1	1	0	?	1	0	0	1	0	1	2	1
9	+Metanephrops rossensis	0	0	0	0	0	0	0	4	1	0	0	0	0	0	0
10	+Hoploparia stokesi	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0
11	Homarus americanus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	+Astacus licenti	1	1	1	1	1	0	1	1	0	0	1	0	1	2	1/2
13	+Astacus spinirostris	1	1	0	1	1	0	1	0	0	0	0	0	1	2	1/2
14	Astacus astacus	1	1	0	1	0	0	1	2	1	1	0	0	1	2	2
15	+Austropotamobius llopsi	0	1	0	1	1	0	1	1	0	0	0	0	1	0	1
16	Austropotamobius torrentius	1	1	0	1	0	0	1	1	0	0	0	0	1	2	2
17	+Pacifastacus chenoderma	1	1	0	1	0	0	1	2	1	0	0	0	1	2	1/2
18	Pacifastacus gambelli	1	1	0	1	2	0	1	2	0	0	0	0	1	2	2
19	+Procambarus primeavus	1	1	0	1	0	0	2	1	1	0	0	0	1	2	1/2
20	Procambarus b. blandingii	1	1	0	1	1	0	1	0	1	1	1	0	1	2	2
21	Cambarus b. bartonii	1	1	0	1	1	0	3	1	0	0	0	0	1	2	2
22	Barbicambarus cornutus	1	1	0	1	1	0	1	0	1	1	0	0	1	2	2
23	Distocambarus crockeri	1	1	0	1	2	0	2	1	0	0	1	0	1	2	2
24	Fallicambarus foidens	1	1	0	1	1	0	3	0	0	1	0	0	1	2	2
25	Faxonella clypeata	1	1	0	1	1	0	1	1	0/1	0	1	0	1	2	2
26	Hobbseus orconectoides	1	1	0	1	1	0	1	1	0	0	0	0	1	2	2
27	Orconectes r. rusticus	1	1	0	1	1	0	1	0	1	1	0	0	1	2	2
28	Troglocambarus maclenei	1	1	0	1	0	0	1	1	0	0	0	0	1	2	2
29	Bouchardina robisoni	1	1	0	1	1	0	1	1	1	0	1	0	1	2	2
30	Cambarellus montezumae	1	1	0	1	1	0	1	1	0/1	0	0	0	1	2	2
31	Cambaroides similis	1	1	0	1	1	0	1	1	0	0	0	0	1	2	2
32	+Lammuastacus longirostris	1	1	0	1	0	0	1	1	1	0	0	0	1	2	0
33	+Paranephrops fordycei	1	1	0	1	0	0	1	1	1	1	1	0	1	2	0
34	Engaeus fossor	1	1	1	1	2	0	1/2	3	0	0	0	1	1	2	2
35	Engaewa subcoerulea	1	1	0	1	1	0	1	0	1	0	0	1	1	2	2
36	Gramastacus insolitus	1	1	0	1	0	0	1	1	0	0	0	0	1	2	2
37	Parastacus pugnax	1	1	1	1	0	0	1	1	0	0	0	0	1	2	2
38	Tenuibranchiurus glypticus	1	1	0	1	1	0	2	3	1	0	0	1	1	2	2
39	Geocherax gracilis	1	1	0	1	0	0	1	1	1	0	0	1	1	2	2
40	Astacoides madagascarensis	1	1	0	1	1	0	1	1	1	1	1	0	1	2	2
41	Astacopsis franklinii	1	1	0	1	0	0	2	1	1	0	0	1	1	2	2
42	Cherax preisii	1	1	0	1	1	0	1	1	0	0	0	1	1	2	2
43	Eustacoides setosus	1	1	0	1	1	0	2	1	1	0	0	1	1	2	2
44	Euastacus armatus	1	1	0	1	0	0	2	1	1	1	1	1	1	2	2
45	Paranephrops planifrons	1	1	0	1	0	0	1	1	1	1	1	0	1	2	2
46	Parastacoides tasmanicus	1	1	0	1	0	0	1	1	1	0	0	0	1	2	2
47	Samastacus spinifrons	1	1	0	1	1	0	1	1	1	0	0	0	1	2	2
48	Virilastacus araucanius	1	1	1	1	2	0	1	0	0	0	0	0	1	2	2

		31
1	+Palaeopalaemonidae newberri	0
2	+Eryma fosteri	0
3	+Erymastacus bordenensis	0
4	+Enoploclytia porteri	0
5	+Palaeastacus argoviensis	0
6	+Chilenophoberus actacamensis	1
7	+Pseudastacus pustulosis	1
8	+Protastacus politus	1
9	+Metanephrops rossensis	0
10	+Hoploparia stokesi	0
11	Homarus americanus	0
12	+Astacus licenti	1
13	+Astacus spinirostris	1
14	Astacus astacus	1
15	+Austropotamobius llopsi	1
16	Austropotamobius torrentius	1
17	+Pacifastacus chenoderma	1
18	Pacifastacus gambelli	1
19	+Procambarus primeavus	1
20	Procambarus b. blandingii	1
21	Cambarus b. bartonii	1
22	Barbicambarus cornutus	1
23	Distocambarus crockeri	1
24	Fallicambarus foidens	1
25	Faxonella clypeata	1
26	Hobbseus orconectoides	1
27	Orconectes r. rusticus	1
28	Troglocambarus maclenei	1
29	Bouchardina robisoni	1
30	Cambarellus montezumae	1
31	Cambaroides similis	1
32	+Lammuastacus longirostris	1
33	+Paranephrops fordycei	1
34	Engaeus fossor	1
35	Engaewa subcoerulea	1
36	Gramastacus insolitus	1
37	Parastacus pugnax	1
38	Tenuibranchiurus glypticus	1
39	Geocherax gracilis	1
40	Astacoides madagascarensis	1
41	Astacopsis franklinii	1
42	Cherax preisii	1
43	Eustacoides setosus	1
44	Euastacus armatus	1
45	Paranephrops planifrons	1
46	Parastacoides tasmanicus	1
47	Samastacus spinifrons	1
48	Virilastacus araucanius	1

Table 5. Species analyzed in nucleotide study

Species analyzed in 16S mtDNA phylogeny

Infraorder Astacidea	
Superfamily Nephropoidea	
Nephrops novegicus	U96083 (GenBank, 1999)
Superfamily Parastacoidea	
Astacopsis franklinii	(Crandall et al, 1999)
Cherax destructor	(Crandall et al, 1999)
Cherax quadraticus	(Crandall et al, 1999)
Engaeus cunicularis	(Crandall et al, 1999)
Engaewa subcoerulea	(Crandall et al, 1999)
Euastacus armatus	(Crandall et al, 1999)
Euastacus australasinsis	(Crandall et al, 1999)
Geocherax falcata	(Crandall et al, 1999)
Gramastacus insolitus	(Crandall et al, 1999)
Paranephrops planifrons	(Crandall et al, 1999)
Parastacoides pulcher	(Crandall et al, 1999)
Tennuibranchiurus glypticus	(Crandall et al, 1999)
Superfamily Astacoidea	
Family Cambaridae	
Orconectes rusticus	(Crandall and Fitzpatrick, 1996)
Cambarus macualata	(Crandall and Fitzpatrick, 1996)
Procambarus acutus	(Crandall and Fitzpatrick, 1996)

Species analyzed in 18S mtDNA phylogeny

Astacidea	
Nephropoidea	
Nephrops norvegicus	Y14812 (GenBank, 1999)
Astacoidea	
Family Astacidae	
Astacus astacus	U33181 (GenBank, 1999)
Family Cambaridae	
Procambarus clarkii	X90672 (GenBank, 1999)
Procambarus leonensis	M34363 (GenBank, 1999)

*letter-number combinations refer to GenBank accession numbers

Table 6. CLUSTAL W(1.4) multiple sequence alignment of 16s mitochondrial nucleotide sequences. Sequence length is 559 bases.

```
Nephrops_n      ----------------------------------------GGCCGCGGTATTTTAAC
Astacopsis      ----------------------------------------CGCGGTATATTGAC
Cherax_des      ----------------------------------------CGCGGTATTATGAC
Cherax_qua      ----------------------------------------CGCGGTATTTTGAC
Engaeus_cu      ----------------------------------------CGCGGTATTTTGAC
Engaewa_su      ----------------------------------------CGCGGTATTATGAC
Euast_arma      ----------------------------------------CGCGGTATAGTGAC
Euast_aust      ----------------------------------------CGCGGTAT-GTGAC
Geocharax_      ----------------------------------------CGCGGTATTTTGAC
Gramastacu      ----------------------------------------CGCGGTATTTTGAC
Paranephro      ----------------------------------------CGCGGTATTATGAC
Parastacoi      ----------------------------------------CGCGGTATTGTGAC
Tennuibran      ----------------------------------------CGCGGTATTTTGAC
Orconectes      TGAGAGATATATAAAGTCTGACCTGCCCATTGGAAAACTAAAAGGCCGCGGTATTATGAC
Cambarus_m      TGAGAGATTTATAAGGTCTGACCTGCCCATTGGAGAACTAAAAGGCCGCGGTATTATGAC
Procambaru      TGAGAGGNNTATAAAGTCTAACCAGCCCATTGG-GAACTAAAAGGCCGCGGTATTATGAC
                                                 ********  * **

Nephrops_n      CGTGCGAAGGTAGCATAGTCACTAGTCTCTTAATTGGAGGCTTGTATGAATGGTTGGACA
Astacopsis      CGTGCGAAGGTAGCATAATCATTAGTCTTTTAATTGAGGGCTTGCATGAATGGTTAGACG
Cherax_des      CGTGCGAAGGTAGCATAATCATTAGTCTTTTAATTGAGGGCTGGTATGAAGGGTCGGACA
Cherax_qua      CGTGCGAAGGTAGCATAATCATTAGTCTTTTAATTGGAGGCTGGAATGAATGGTTGGACG
Engaeus_cu      CGTGCGAAGGTAGCATAATCATTAGTCCTTTAATTGGGGGCTTGTATGAAGGGTTGGACG
Engaewa_su      CGTGCAAAGGTAGCATAATCATTAGTTTTTTAATTGAAGGCTAGAATGAATGGTTGGACA
Euast_arma      CGTGCGAAGGTAGCATAATCATTAGTCTTTTAATTGAAGGCTTGTATGAATGGTTGGACG
Euast_aust      CGTGCGAAGGTAGCATAATCATTAGTCTTTTAATTGAAGGCTTGTATGAATGGTTGGACG
Geocharax_      CGTGCGAAGGTAGCATAATCATTAGTCTTTTAATTGAAGGCTTGTATGAAGGGTTGGACG
Gramastacu      CGTGCGAAGGTAGCATAATCATTAGTCTTTTAATTGAAGGCTTGTATGAAGGGTTAGACG
Paranephro      CGTGCAAAGGTAGCATAATCATTAGTCTTTTAATTGGGGGCTTGTATGAATGGTTGGACG
Parastacoi      CGTGCGAAGGTAGCATAATCATTAGTCTTTTAATTAAAGGCTTGTATGAATGGTTGAACG
Tennuibran      CGTGCGAAGGTAGCATAATCATTAGTCTTTTAATTGAAGGCTTGTATGAAGGGTTGGACG
Orconectes      CGTGCGAAGGTAGCATAATCATTAGTTTTTTAATTGAAGGCTAGAATGAATGGTTGGACA
Cambarus_m      CGTGCAAAGGTAGCATAATCATTAGTTTTTTAATTGAGGGCTAGAATGAATGGTTGGACA
Procambaru      CGTGCAAAGGTAGCATAATCATTAGTTTTTTAATTGAAGGCTAGAATGAATGGTTGGACA
                ***** *********** *** ****   ******   **** * ***** ***   **

Nephrops_n      AGAAGTAAGTTGTCTCAA-GTACAAAAATTGAATTTGACTTTTAAGTGAAAAGGCTTAAA
Astacopsis      AGAAGCGGACTGTCTTTA-TTGGGACAATTGAATTTAACTTTTTAGTGAAAAGGCTTAAA
Cherax_des      AAAAATGAGCTGTCTTAAATTTTGAAAATTGAATTTAACTCTTAAGTGAGAAGGCTTAAA
Cherax_qua      AGAAGGAAGCTGTCTCTA-TCTCGGAGATTGAATTTAACTTTTAAGTGAAAAGGCTTAAA
Engaeus_cu      AGAAATTAGCTGTCTTTA-TAAAAAAAGTAGAATTTAACTTTTAAGTGAGAAGGCTTAAA
Engaewa_su      AGAAATAATCTGTCTTAA-TTAAAGATATTGAATTTAACTTTTTAGTGAAAAGGCTTAAA
Euast_arma      AGAAGTAATCTGTCTCTG-TTGAAAAAATTGAATTTAACTTTTAAGTGAGAAGGCTTAAA
Euast_aust      AGAAGTAATCTGTCTCTA-TTAAAAAAATTGAATTTAACTTTTGAGTGAGAAGGCTCAAA
Geocharax_      AGAAATTAGCTGTCTTTA-TAGGAGAAATAAAATTTAACTTTTAAGTGAGAGGGCTTAAA
Gramastacu      AGAAGTCAGCTGTCTTTA-TTGAAAAGATGGAATTTAACTTTTAAGTGAGAGGGCTTAAA
Paranephro      AGAAGTTAGCTGTCTCTA-ATTAATGAATTGAATTTAACTTTTAAGTGAAAAGGCTTAAA
Parastacoi      AGGAATAAGCTGTCTCTA-TTAGGCTAGTTGAACTTAACTTTTAAGTGAAAAGGCTTAAA
Tennuibran      AGAAATTAGCTGTCTTTG-TATGAAAGATGGAACTTCACTTTTAAGTGAAAAGGCTTAAA
Orconectes      AGAAATAATCTGTCTTAA-ATTAAGATATTGAATTTAACTTTAAAGTGAAAAGGCTTTAA
Cambarus_m      AGAGATAGGCTGTCTTAG-ATTAAGATATTGAATTTAACTTTTGAGTGAAAAGGCTTAAA
Procambaru      AGAAATAATCTGTCTTAA-ATTAATATATTGAATTTAACTTTTAAGTGAAAAGGCTTAAA
                *         *****             *  ** ** *** *  ***** * ****  **
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Nephrops_n      TATTTTAAAGGGACGATAAGACCCTATAAAGCTTAATAATTTAATATATAACCAGATAAA
Astacopsis      TGATCCAGGGGGACGATAAGACCCTATAAAGTTTAACATAATAAGGA-TAAA-AAATTAA
Cherax_des      TAGGCTAGGGGGACGATAAGACCCTATAAAGTTTG-ACACTAAATTAATTAA-GGGTGAT
Cherax_qua      TAAGTTAGAGGGACGATAAGACCCTATAAAGTTTATACATGAAGTTGGTTAA-GAGTGAT
Engaeus_cu      TAATCTAAGGGGACGATAAGACCCTATAAAGTTTAACATATTTGTTATTAAA-AAAAAAA
Engaewa_su      TAATCTGAAGGGACGATTA-ACCCTATAAAACTTTATATTTG-AAAG-TAGA-AATTAGT
Euast_arma      TAATCTAGAGGGACGATAAGACCCTATAAAGTTTAATATTATAACAA-TAGA-GAATTAA
Euast_aust      TGACCTAGAGGGACGATAAGACCCTATAAAGTTTAACATTATAACAA-CAAC-AGGTTAA
Geocharax_      TACTCTAAAGGGACGATAAGACCCTATAAAGTTTGACACTTTATCTTTTGTT-GAGTCAG
Gramastacu      TATTCTAAGGGGACGATAAGACCCTATAAAGTTTGATATTTAATTTTTTAAA-AAATAAG
Paranephro      TGATCTAGAGGGACGATAAGACCCTATAAAGCTTTACATTGAATTTACTAAA-AAGTAAA
Parastacoi      TGATCTAGAGGGACGATAAGACCCTATAAAGTTTTACATCACATCTATTATA-AAATGGG
Tennuibran      TGTTCTAAAGGGACGATAAGACCCTATAAAGTTTGACGGTCTGGGTCTTAGA-AGACAAG
Orconectes      TGATCTAATGGGACGATAAGACCCTATAAAACTTTATATTTT-AATG-TAGA-AGTTAAT
Cambarus_m      TAATCTGAAGGGTCGATAAGACCCTATAAAACTTTATATTGA-GAGG-TGAG-AGGTAAT
Procambaru      TAATCTGAAGGGACGATAAGACCCTATAAAACTTTATATTTATAAGA-TAGT-AGCTAGT
                *        *** **** * **********  **

Nephrops_n      TTAAAAGTTTAATATTCTTTATATATTAAATTATTTCGTTGGGGCGACGATGATATAATT
Astacopsis      TTAGGTTTATAAAGTTTATTATTACAAATAATGTTTTGTTGGGGCGACAAGAATAAAAGT
Cherax_des      TTAGGTAATAAAGTCTTATTATTA-TATAAGTGTTTAGTTGGGGCGACTAGGATATAAGT
Cherax_qua      TTAAGGTGTTAAAGTTTATTATCA-GCAGGGTGTTTAGTTGGGGCGACTAGGATATAAAT
Engaeus_cu      ACAAAAAGGTAAATTTTATTAGTA-GGAGATTGTTTGGTTGGGGCGACTAGGATATAAAT
Engaewa_su      TT---CATTTAAAGGTTACTATTT--AAAAATGTTTGGTTGGGACNACAAGGATAGAAGG
Euast_arma      TTAGGTT-ATAAGATTTATTATTGTAGGTAGTATTTTGTTGGGGCGACAAGAACATAAAT
Euast_aust      TTGGGTT-ATAGAATTTATTATTGTGAATAGTGTTTTGTTGGGGCGACAAGAATATAAAT
Geocharax_      A---ATAATTAAAGTTTATCAGGA-GCAAG-TGTTTTGTTGGGGCGACAAGAATATAAAT
Gramastacu      ATAAATAGGTAAAGTTAGTTAGAA-AAAAGATGTTTTGTTGGGGCGACAAGAATATAAAT
Paranephro      TTAAATAATAAAAGTTTATTAGTA-AAGAGATGTTTTGTTGGGGTGACAAGAATATAATA
Parastacoi      TTGGATAAAAAAGATTTGCTAGTA-GTTCGATGTTTTGTTGGGGTGACAAGAATATAAGG
Tennuibran      TTAGGTAATTAGAGTTTGTTAGGA-GCGGGGTGTTTTGTTGGGGCGATAAGAATATAAAT
Orconectes      TT---TATTTTAAGTTCACTATTT--TAAAATTTTTTGTTGGGGCGACAAGGATATAAAA
Cambarus_m      TT---TGTTTAAAAGTTATTGCTT--TAAAGTATTTGGTTGGGGCGACAAGGATACAAGG
Procambaru      TT---TATTTAAAAGT-ATTATTT--TGGAGTATTTGGTTGGGGTGACAAGGATAAAATA
                          *                * *** ******   *  *  * * **

Nephrops_n      TGT----AACTGTTTAAATTT-TAAATACAGAGATATTTGTGTGTAATGATCCTTTTTAT
Astacopsis      AATTTNNAACTGTTCNTTTTTNNTTAATCAAAAATATTTGGGT-GGGTGATCTTTTCTAA
Cherax_des      TATTT--AACTGTTTCTTCAC-TCGAATCAAAAATTTTTGATT-TTATGATCCTTTTTTA
Cherax_qua      TATAT--AACTGTTT-TTTGT-TTAAATCAGAGATATTTGTTC-ATATGGTCCCTTTTTA
Engaeus_cu      AATTT--AACTGTTC-TATA-TTATAAACAGGGATATCTGTGT-TCTTGAACCTTATTAG
Engaewa_su      TTAGGNTAACTNTCTNTTTTTTTTNNNTACAGTAATATTTGGTT-TAATGATCCTAA-AAG
Euast_arma      AATTT--AACTGTTCTTTTTTGT---ATCAAAAATATTTGAGT-TGATGATCTTTT-TAA
Euast_aust      AGTTT--AACTGTTCTTTTTTGT---ATCAAAGATATTTGAAT-TAATGACTCTTT-TAA
Geocharax_      AATTT--AACTGTCTTTATG--TAGCTATAAAGATAATTGAAT-TTATGGTCCTTATTAA
Gramastacu      TATAT--AACTGTTTTTATA--TTCATACAACGATAATTGAAT-TTATGAACCTTTGTAA
Paranephro      AATAT--AACTGTTCTTTTTTATTT---CAAAAATATTTGAAT-AGGAGATCCTTAATAA
Parastacoi      GATGT--AACTGTTCTTTAAAAAC----CAAATATATTTGTTT-AAGTGATCTTGAATTT
Tennuibran      GATTTNNAACTGTTTNTATGGTTAGAGACAAAGGTAGTTGGGT-TTATGATCCTTGTTAA
Orconectes      A-ATGATAACTATCTTTTATTTT---TACAATAATATTTGATT-TATTGATCCTAA-AAG
Cambarus_m      T-AAAGTAACTGTTTTTTTTTTC---TACAATAATGTTTGAGT-GAATGATCCTAA-GAT
Procambaru      T-AAAATAACTGTCTTTTTTTTT---TACAGTAATGTTTGGTT-TAATGATCCTAA-AAG
                       **** *                 *    *   **        *

Nephrops_n      TGATTAAAAATTTAAGTTACTTTAGGGATAACAGCGTTATTTATTTTGAGAGTTCATATC
Astacopsis      -AAGTATTAGAGTAAATTACTTTAGGGATAACAGCGTAATTTTTTTTGAGAGTTCTTATC
Cherax_des      -GGATATTAGAGTAAATTACTTTAGGGATAACAGCGTAATTTTTTTTGAGAGTTCTTATC
Cherax_qua      -GGATTAGAGAATAAATTACTTTAGGGATAACAGCGTAATTTTTTTTAAGAGTTCTTATC
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Engaeus_cu      AGATAAT-AGAATAAATTACTTTAGGGATAACAGCGTAATTTTTTTTTGAGAGTTCTTATC
Engaewa_su      GGATTAAA-GATTAGGTTACTTTAGGGATAACAGCGTAATTTTCTTTGAGAGTTCTTATC
Euast_arma      -GAGTATTAGAGTAAATTACTTTAGGGATAACAGCGTAATTTTTTTTTGAGAGTTCTTATC
Euast_aust      -GAGTATTAGAGTAAATTACTTTAGGGATAACAGCGTAATTTTTTTTTGAGAGTTCTTATC
Geocharax_      AGAGTGA-AGAGTAAATTACTTTAGGGATAACAGCGTAATTTTTTTTTAAGAGTTCATATC
Gramastacu      AGGTAAT-AGAGTAAATTACTTTAGGGATAACAGCGTAATTTTTTTTTGAGAGTTCTTATC
Paranephro      -AGATGTTAGAGTAAGTTACTTTAGGGATAACAGCGTAATTTTTTTTTGAGAGTTCTTATC
Parastacoi      TAAGTGCTAGAATAAATTACTTTAGGGATAACAGCGTTATTTTTTTTTAAGAGTTCTTATC
Tennuibran      AGATTATTAGAATAAATTACTTTAGGGATAACAGCGTAATTTTTTTTTGAGA-TTCTTATC
Orconectes      GGATTAAA-GATTAAGTTACTTTAGGGATAACAGCGTAATTTTCTTTGAGAGTTCTTATC
Cambarus_m      GGATTAAAAGATTAAGTTACTTTAGGGATAACAGCGTAATTTTCTTTAAGAGTTCTTATC
Procambaru      GGATTAAA-GATTAAGTTACTTTAGGGATAACAGCGTAATTTTCTTTAAGAGTTCTTATC
                        **  ********************** ****  *** *** *** ****

Nephrops_n      GACAAAAAAGTTTGCGACCTCGATGTTGAATTAAAAA-TTNNCCATGGCG----------
Astacopsis      GACAAAATAGTTTGCGACCTCGATGTTGAATTAAAGG-GTCTTTATAATGTAGGAGTTAT
Cherax_des      GATAAAAGAGTTTGCGACCTCGATGTTGAATTAAAA-TTTCTTTGTAATGCAGCAGTTAC
Cherax_qua      GACAAGAGAGTTTGCGACCTCGATGTTGAATTAAAAATTTCTCTGTGGTGTAGCGGTTAC
Engaeus_cu      GACAGAAAAGTTTGCGACCTCGATGTTGAATTAAAGAATTCTTTGTAGCGAAGAGGTTAC
Engaewa_su      GACAGGAAAGTTTGCAACCTCGATGTTGAATTAAAGG-TTCTTTATAGAGT-GAGACTAT
Euast_arma      GACAAAAGAGTTTGCGACCTCGATGTTGAATTAAAG--TTCTTTATAGCGCAGAAGTTAT
Euast_aust      GACAAAAGAGTTTGCGACCTCGATGTTGAATTAAAGA-TTCTTTATAGTGTAGCAGTTAT
Geocharax_      GATAAAAAAGTTTGCGACCTCGATGTTGAATTAAAGG-TTCTTTGTAATGTAGAAGTTAC
Gramastacu      GACAAAAAAGTTTGCGACCTCGATGTTGAATTAAAG--TTCTTTGTAATGTAGCAGTTAC
Paranephro      GACAGAAAAGTTTGCGACCTCGATGTTGAATTAAAGA-TTCTTTATAATGTAGAAGTTAT
Parastacoi      AACAAAAAAGTTTGCGACCTCGATGTTGAATTAAAGG-CTCTTTGAGATGCAGAGGTCTC
Tennuibran      GACAAAGAAGTTTGCGACCTCGATGTTGAATTAAAAG-TTCTTTGTGGCGTAGCAGTTAG
Orconectes      GACAAGAAAGTTTGCGACCTCGATGTTGAATTAAAGG-TTCTTTATAGAGTAGAGACTAT
Cambarus_m      GACAAGAAAGTTTGCGACCTCGATGTTGAATTAAAAG-TTCTTTATGGAGTAGAGACTAT
Procambaru      GACAAGAAAGTTTGCGACCTCGATGTTGAATTAAAAG-TTCTTTATAGAGTAGAGACTAT
                 * *    ******* ********************    *         *

Nephrops_n      ------------------------------------------------------------
Astacopsis      A--AGAAGGTCTGTTCGACC-TTTAAATCTTTACATGATTTGA-----------------
Cherax_des      AAGAGAGGGTCTGTTCGACCCTTTAAATTTTTACATGATTTGA-----------------
Cherax_qua      AGGAGAAGGTCTGTTCGACCCTTTAAATTTTTACATGATTTGA-----------------
Engaeus_cu      AGAAGAAGGTCTGTTCGACC-TTTAAATCTTTACATGATTTGA-----------------
Engaewa_su      AATAGAAGGTCTGTTCGACC-TTTAACATTTTACATGATTTGA-----------------
Euast_arma      ATAAGAAGGTCTGTTCGCCC-TTTAAATCTTTACATGATTTGA-----------------
Euast_aust      ATAAGAAGGTCTGTTCGACC-TTTAA-TCTTTACATGATTTGA-----------------
Geocharax_      AGAAGAAGGTCTGTTCGACC-TTTAAATCTTTACATGATTTGA-----------------
Gramastacu      AGGAGAAGGTCTGTTCGACC-TTTAA-TCTTTACATGATTTGA-----------------
Paranephro      ATGAGAGGGTCTGTTCGACC-TTTAAATCTTTACATGATTTGA-----------------
Parastacoi      AAAAGAGGGTCTGTTCGACC-TTTAAATCTTTACATGATTTGAGTTCAAACCGGTGTGAG
Tennuibran      GAGAGAAGGTCTGTTCGACC-TTTAAATTTTTACATGATTTGA-----------------
Orconectes      AAAAGAAGGTCTGTTCGACC-TTTAAAGTTTTACATGATTTGAGTTCAGACCGG------
Cambarus_m      AAGAGAAGGTCTGTTCGACC-TTTAAAATTTTACATGATTTGAGTTCAGACCGG------
Procambaru      AACAGAAGGTCTGTTCGACC-TTTAAAATTTTACATGATTTGAGTTCAGACCGG------

Nephrops_n      ------------------
Astacopsis      ------------------
Cherax_des      ------------------
Cherax_qua      ------------------
Engaeus_cu      ------------------
Engaewa_su      ------------------
Euast_arma      ------------------
Euast_aust      ------------------
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Geocharax_      ------------------
Gramastacu      ------------------
Paranephro      ------------------
Parastacoi      CCAGGTTGGTTTCTATCTA
Tennuibran      ------------------
Orconectes      ------------------
Cambarus_m      ------------------
Procambaru      ------------------
```

--represents a gap in the sequence
* represents uniformity of a base at a specific locus

Table 7. CLUSTAL W(1.4) multiple sequence alignment of 18s mitochondrial nucleotide sequences. Sequence length is 1878 bases.

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Nephrops_n      ---CTGGTTGATTCTGCCAGTAGTCATATGCTTGTCTCAAAGATTAAGCCATGCATGTGT
Astacus_as      AACCTGGTTGATCCTGCCAGTAGTCATATGCTTGTCTCAAAGATTAAGCCATGCATGTGT
P._clarkii      ------------------------------------------------------------
P._leonens      ------------------------------------------------------------

Nephrops_n      AAGTACAAGCCGATTTAAGGCGAAACCGCGAATGGCTCATTAAATCAGCTATGTTTCATT
Astacus_as      AAGTACAAGCCGAGTTAAGGCGAAACCGCGAATGGCTCATTAAATCAGCTATGTTTCATT
P._clarkii      ----------------------AAACCGCGAATGGCTCATTAAATCAGCTATGTTTCATT
P._leonens      ------------------------------------------------------------

Nephrops_n      GGATCTGTAAACCCACTTACTTGGATAACTGTGGCAATTCTAGAGCTAATACATGCATTT
Astacus_as      GGATCTGTAAACCCACTTACTTGGATAACTGTGGTAATTCCAGAGCTAATACATGCATCA
P._clarkii      GGATCTGTAAACCCACTTACTTGGATAACTGTGGTAATTCTAGAGCTAATACATGCATCA
P._leonens      ------------------------------------------------------------

Nephrops_n      AGTCTCTGACCGCAAGG-AAGAGCGCTTTTATTAGTTCAAAACTGGTCGGGCCTCGGTCC
Astacus_as      CGTCTCTGACCGCAAGGGAAGAGCGCTTTTATTAGTTCAAAACTGGTCGGGCCTCGGTCC
P._clarkii      CGTCTCTGACCGCAAGGGAAGAGCACTTTTATTAGTTCAAAACTGGTCGGGCCTCGGTCC
P._leonens      ------------------------------------------------------------

Nephrops_n      GT-AACCCACCTGTGGTGAATCTGAATAACTTCCGGCTGAGCGCACGGTCTCCGCACCGG
Astacus_as      GTTAACCCACCCGTGGTGAATCTGAATAACTTTTTGCTGAGCGCACGGTCTCCGCACCGG
P._clarkii      GTTAACCCTCCCGTGGTGAATCTGAATAACTTTTTGCTGAGCGCACGGTCTCCGCACCGG
P._leonens      ------------------------------------------------------------

Nephrops_n      CGCCGCTTCTTTCAAGTGTCTGCCTTATCAGCTTTCGATTGTAGGTTATGCGCCTACAAT
Astacus_as      CGCCGCATCCTTCAAGTGTCTGCCTTATCAGCTTTCGATTGTAGGTTATGCGCCCACAAT
P._clarkii      CGCCGCATCCTTCAAGTGTCTGCCTTATCAGCTTTCGATTGTAGGTTATGCGCCTACAAT
P._leonens      ------------------------------------------------------------

Nephrops_n      GGCTATAACGGGTAACGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGC
Astacus_as      GGCTATAACGGGTAACGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGC
P._clarkii      GGCTATAACGGGTAACGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGC
P._leonens      ------------------------------------------------------------

Nephrops_n      TACCACATCTAAGGAAGGCAGCAGGCACGCAAATTACCCACTCCCGGCACGGGGAGGTAG
Astacus_as      TACCACATCTAAGGAAGGCAGCAGGCACGCAAATTACCCACTCCCGGCACGGGGAGGTAG
P._clarkii      TACCACATCTAAGGAAGGCAGCAGGCACGCAAATTACCCACTCCCGGCACGGGGAGGTAG
P._leonens      ------------------------------------------------------------

Nephrops_n      TGACGAAAAATAACGATGTGAGTCTCATCNGAGGCCTCGCAATCGGAATGAGTACACTTT
Astacus_as      TGACGAAAAATAACGATGCGAGACTCATCCGAGGCCTCGCAATCGGAATGAGTACACTTT
P._clarkii      TGACGAAAAATAACGATGCGAGACTCATCCGAGGCCTCGCAATCGGAATGAGTACACTTT
P._leonens      ----------------TGCGAGACTCATCCGAGGCCTCGCAATCGGAATGAGTACACTTT
                              ** *** ****** ****************************

Nephrops_n      AAATCCTTTAACGAGTATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCC
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Astacus_as      AAATCCTTTAACGAGGATCTATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCC
P._clarkii      AAATCCTTTAACGAGGATCTATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCC
P._leonens      AAANCCTTTAACGAGGATCTATTGGAGGGCNAGTCTGGTGCCAGCAGCCGCGGTAATTCC
                *** *********** *** ********** ******************************

Nephrops_n      AGCTCCAATAGCGTATATTAAAGTTGTTGCGGTTAAAAAGCTCGTAGTTGGATCTCAGTT
Astacus_as      AGCTCCAATAGCGTATATTAAAGTTGTTGCGATTAAAAAGCTCGTAGTTGGATCTCAGTT
P._clarkii      AGCTCCAATAGCGTATATTAAAGTTGTTGCGGTTAAAAAGCTCGTAGTTGGATCTCAGTT
P._leonens      AGCTCCAATANNGTATATTAAAGTTGTTGCGGTTNNAAAGCTCGTAGTTGGATCTCAGTT
                ********** ****************** **  ************************

Nephrops_n      CCGGACTGACGGTGCACCGCCCGGTGTTTACTGTCACGCTCCGAACAGCC-----GCCCC
Astacus_as      CCGGACTGACGGTACACCGCCTGGTGCTTACTGTCACGTTCCGAACAGCTAACTAGCCCC
P._clarkii      CCGGACTGACGGTACACCGCCTGGTGCTTACTGTCACGCTCCGAACAGCTAACTAGCCCC
P._leonens      CCGGACTGACGGTACAC-GCNNGGTGCTTACTGTCACGCTCCGAACAGCTAACTAGCCCC
                ************* *** **  **** *********** **********      *****

Nephrops_n      GCCGGCTCGCACGGGATGCTCTTTGTCGAGTGTCCCGAGTGGCCGG-AGGTTTACTTTGA
Astacus_as      GCCGGCTCGCACGGGGTGCTCTTCATCGAGTGTCCCGAGTGGCCGGCACGTTTACTTTGA
P._clarkii      GCCGGCTCGCACGGGGTGCTCTTCATCGAGTGTCCCGAGTGGCCGGCACGTTTACTTTAA
P._leonens      GCCGGCCAG--TGGGGTGCTCTTCATCGAGTGTCCCGAGTGGCCGGNNCGTTTACTTTGN
                ******  *   *** *******  *********************   *********

Nephrops_n      AAAAATTAGAGTGCTCAGAGCAGGCTATTTGAATGGCCCGAATGGTGATGCA-TGGAATA
Astacus_as      AAAAATTAGAGTGCTCAGAGCAGGCTACTTTAATGGCCTGAATGTCTATGCA-TGGAATA
P._clarkii      AAAAATTAGAGTGCTCAGAGCAGGCTACTTTAATGGCCTGAATGTCTATGCA-TGGAATA
P._leonens      NNNNATTAGAGTGCTCAGAGCNGGCNNCNNNNATGGCCTGAATGTCTATGCACTGGAATA
                    ***************** ***       ****** *****   ***** *******

Nephrops_n      ATGGAATAGGACCTCGGTTCTATTTTGTTGGTTTTCGGAACCAGAGGTAATGACTAATCG
Astacus_as      ATGGAATAGGACCTCGGTTCTATTTTGTTGGTTTTCGGAACCTGAGGTAATGACTAATAG
P._clarkii      ATGGAATAGGACCTCGGTTCTATTTTGTTGGTTTTCGGAACCTGAGGTAATGACTAATAG
P._leonens      ATGGAATAGGACCTCGGTTCTATTTTGTTGGTTTTCGGAACCTGAGGTAATGACTAATAG
                ****************************************** *************** *

Nephrops_n      GAACAGGCGGGGGCATTCGTATTGCGACGCTAGAGGTGAAATTCTTGGACCGTCGCAAGA
Astacus_as      GAACAGGCGGGGGCATTCGTATTGCGACGCTAGAGGTGAAATTCTTGGACCGTCGCAAGA
P._clarkii      GAACAGGCGGGGGCATTCGTACTGCGACGCTAGAGGTGAAATTCTTGGACCGTCGCAAGA
P._leonens      GAACAGGCGGGGGCATTCGTATTGCGACGCTAGAGGTGAAATTCTTGGACCGTCGCNAGA
                ********************* ********************************** ***

Nephrops_n      CGAACTACTGCGAAAGCATTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTAGAG
Astacus_as      CGAACTACTGCGAAAGCATTTGCCAAGGATGTTTTCATTAATCAGGAACGAAAGTTAAAG
P._clarkii      CGAACTACTGCGAAAGCATTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTAGAG
P._leonens      CGAACTACTGCGAAAGCATTTGCCAAGGATGTTTTCATTAATCAAGAANGAAAGTTAGAG
                ****************************************** *** ******** **

Nephrops_n      GTTCGAAGGCGATCAGATACCGCCCTAGTTCTAACCATAAACGATGCCAACTAGCGATCC
Astacus_as      GTTCGAAGGCGATCAGATACCGCCCTAGTTCTAACCATAAACGATGCCAACTAGCGATCC
P._clarkii      GTTCGAAGGCGATCAGATACCGCCCTAGTTCTAACCATAAACGATGCCAACTAGCGATCC
P._leonens      GTTCGAAGGCGATCAGATACCGCNCNNGTTNNAACCATAAACGATGCCAACTAGCGATCC
                ********************** *  ***  ****************************

Nephrops_n      GCCGGCGTTATTCCCATGACCCGGCGGGCAGCTTCCGGGAAACCAAAGTCTTTGGGTTCC
Astacus_as      GCCGGCGTTATTCCCATGACCCGGCGGGCAGCTTCCGGGAAACCAAGGTCTTTGGGTTCC
P._clarkii      GCCGGCGTTATTCCCATGACCCGGCGGGCAGCTTCCGGGAAACCAAAGTCTTTGGGTTCC
P._leonens      GCCGGCGTTATTCCCATGACCCGGCNGNCAGCTTCCGGGAAACCAAAGTCTTTGGGTTCC
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Nephrops_n      GGGGGAAGTATGGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGA
Astacus_as      GGGGGAAGTATGGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGA
P._clarkii      GGGGGAA-------------------------------------------------------
P._leonens      GGGGGAAGTATGGTTGCAAAGCTGAAACTCAAAGGAATTGACGGNNNNNNNNNNNNNNNN
                *******

Nephrops_n      GTGGAGCCTGCGGCTTAATTTGACTCAACACGGGA-AACCTCACCAGGCCCAGACACCGG
Astacus_as      GTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGGAACCTCACCAGGCCCAGACACCGG
P._clarkii      ------------------------------------------------------------
P._leonens      NNNNNNNNNNNNNNNNNNNNNNNNNAACACGGGG-AACCTCACCAGGCCCAGACACCGG

Nephrops_n      AAGGATTGACAGATTGAGAGCTCTTTCTCGATTCGGTGGGTGGTGGTGCATGGCCGTTCT
Astacus_as      AAGGATTGACAGATTGAGAGCTCTTTCTCGATTCGGTGGGTGGTGGTGCATGGCCGTTCT
P._clarkii      ------------------------------------------------------------
P._leonens      AAGGATNGACAGATTGAGAGCTCTTTCTCGATTCGGTGGGTGGTNGTGCATGGCCGTTCT

Nephrops_n      TAGTTGGTGGAGCGATTTGTCTGGTTAATTCCGATAACGAACGAGACTCTGGCCTACTAA
Astacus_as      TAGTTGGTGGAGCGATTTGTCTGGTTAATTCCGATAACGAACGAGACTCTGGCCTATTAA
P._clarkii      ------------------------------------------------------------
P._leonens      TAGTTGGTGGAGCGATTTGTCTGGTTAATTCCGATNNNNNNNGAGACTCTGGCCTATTAA

Nephrops_n      GTAGTCGACGGATCTCCAGAAAATGGTGTCCAGTTCGCAACTTCTTCTTAGAGGGATAAG
Astacus_as      CTAGTCGACGGATCTCCAGCAATTGGTGTCCAGTTCGCAACTTCTTCTTAGAGGGATTAG
P._clarkii      ------------------------------------------------------------
P._leonens      CTAGTCGACGGATCTCCAGCNNTTGGTGTCCAGTTCGCAACTTCTTCTTAGAGGGATTA-

Nephrops_n      CGGCAATTCTAGCCGCACGAGATTGAGCAATAACAGGTCTGTGATGCCCTTAGATGTTCT
Astacus_as      CGGCAATTCTAGCCGCACGAGATTGAGCAATAACAGGTCTGTGATGCCCTTAGATGTTCT
P._clarkii      ------------------------------------------------------------
P._leonens      CGGCAATTCTAGCCGCACGAGATTGAGCAATAACAGGTCTGTGATGCCCTTAGATGTTCT

Nephrops_n      GGGCCGCACGCGCGCTACACTGAAGGGATCAACGAGTTTTCCCCCTCCGAGAGGAGCGGG
Astacus_as      GGGCCGCACGCGCGCTACACTGAAGGGATCAACGTGTTCTCCCCCTCCGAGAGGAGCGGG
P._clarkii      ------------------------------------------------------------
P._leonens      GGGC-GCACGCGCGCTACACTGAAGAGATCAACGTGTTCTCCCCCTCCGAGAGGAGCGGG

Nephrops_n      TAACCCGTTCAAAGCCTTTCTTGATAGGGATTGGGGCTTGCAATTGTTTCCCATGAACGA
Astacus_as      TAACCCGTTCAAACCCCTTCATGATAGGGATTGGGGCTTGCAATTGTTTCCCATGAACGA
P._clarkii      ------------------------------------------------------------
P._leonens      NAACCCGTTCAATCCCCTTCATGATAGGGATTGGGGCTTGCAATTGTTTCCCATGAACGA

Nephrops_n      GGAATTCCCAGTAAGTGCAAGTCATCAGCTTGCGCTGACTACGTCCCTGCCCTTTGTACA
Astacus_as      GGAATTCCCAGTAAGTGCAAGTCATCAGCTTGCGCTGATTACGTCCCTGCCCTTTGTACA
P._clarkii      ------------------------------------------------------------
P._leonens      GGAATTCCCAGTAAGTGCAAGTCATCACGTTGCGCTGATTNNGTCCCTGCCCNTTGTACA

Nephrops_n      CACCGCCCGTCGCTACTACCGATTGAATGATTTAGTGAGGCCTTCGGACTGGCGCTCTTG
Astacus_as      CACCGCCCGTCGCTACTACCGATTGAATGATTTAGTGAGGCCTTCGGACTGGCGCTCTTG
P._clarkii      ------------------------------------------------------------
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P._leonens      CACNNNNNNTCGCTACTACCGATTGAATGATTTAGTGAGGCTTCGGACTGGCGCTCTTGG

Nephrops_n      GATTGTCTGCCCCGTAGCCCGCAAGGGTTTTCTGGGGTCGTCGCCTCGAGCTGACGGAAA
Astacus_as      GATGTTCTACCCCTCGCGTCTCGGCG----CAGGGGGTTCTCGCCTCGAGCTGACGGAAA
P._clarkii      ------------------------------------------------------------
P._leonens      ATGTTCTACCCCTCGCGTCTCGGCGC-----AAGGNNNTCTCGCCTCGAGCTGACGGAAA

Nephrops_n      GATGTCCAAACTTGATCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAA
Astacus_as      GATGTCCAAACTTGATCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAA
P._clarkii      ------------------------------------------------------------
P._leonens      GATGTCCAAACTTGATNNNNNNNNNNNNNNNNAAGTCGTAACAAGGTNNNNNNNNNNNN

Nephrops_n      CCTGCAGAAG--------
Astacus_as      CCTGCAGAAGGATCA---
P._clarkii      ------------------
P._leonens      NNNNNNNNNNNNNNNNN
```

--represents a gap in the sequence
* represents uniformity of a base at a specific locus

Table 8. Geological ages of fossil species

Palaeopalaemonidae	
Palaeopalaemon newberryi	Late Devonian
Erymidae	
Eryma fosteri	Late Jurassic (Callovian)
Erymastacus bordenensis	Early Jurassic (Sinemurian)
Enocloplytia porteri	Late Triassic (Carnian)
Nephropoidea	
Hoploparia stokesi	Late Cretaceous (Campanian) to Paleocene
Metenephrops rossensis	Late Cretaceous (Campanian)
Chilenophoberidae	
Chileophoberus atacamensis	Late Jurassic (Oxfordian)
Pseudastacus pustulosis	Early Jurassic (Tithonian)
Astacoidea	
Astacus licenti	Late Jurassic to Early Cretaceous
Astacus spinirostris	Late Jurassic to Early Cretaceous
Austropotamobius llopsi	Early Cretaceous (Barremian)
Protastacus politus	Late Cretaceous
Pacifastacus chenoderma	Miocene to Pliocene
Procambarus primeavus	Eocene
Lammuastacus longirostris	Oligocene
Paranephrops fordycei	Miocene
Euastacus? sp.	Paleocene