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Molecular Phylogeny of Lake Baikal Amphipods

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Molecular Phylogeny of Lake Baikal Amphipods

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Abstract

Lake Baikal, in Siberia, Russia, contains the highest biodiversity of any extant lake, including one of the most impressive species radiations known, the endemic gammaroidean amphipods. The amphipods of Lake Baikal are incredibly diverse, both morphologically and ecologically, and are often cited as a classic case of adaptive radiation. However, the taxonomy of these amphipods is poorly resolved, is based solely on morphology, and little is known about the history of their speciation, and how it relates to the history of the lake.

The phylogenetic history of the Lake Baikal amphipods was examined using nucleic acid sequences of a 659 bp segment of the mitochondrial cytochrome c oxidase subunit I (COI) gene. Phylogenetic hypotheses of relationships among selected Baikal amphipods were constructed based on 303 parsimony-informative characters from this segment. Monophyly was tested for two families of Baikal amphipods: the Acanthogammaridae and the Gammaridae. Divergence times of taxa were estimated using a molecular clock calibrated to *Alpheus* shrimp separated by the Isthmus of Panama.

The trees resulting from phylogenetic analyses of the sequence data were not greatly resolved, and few clades were well supported. A 6-parameter weighted parsimony analysis suggested the monophyly of the Lake Baikal amphipods, while maximum likelihood analysis weakly supported the non-monophyly of Baikal's amphipods, suggesting a sister-group relationship between the pelagic Baikalian *Macrohectopus* and the cosmopolitan *Gammarus lacustris*. All analyses supported the monophyly of Baikal's amphipods excluding *Macrohectopus*. Most analyses also found that the endemic and morphologically distinct family Acanthogammaridae is not a monophyletic group, suggesting that its distinctive characters (spines, keels, body armor) evolved more than once in the lake. Estimated times of divergence of lake species ranged from 7 to 16 ma, although these estimates may not be valid.

Additional data are needed to resolve the phylogeny and test for the monophyly of Lake Baikal's amphipods. COI sequences from additional taxa are needed, including species from both major Baikalian families, and gammarid species from waters surrounding Baikal. Additionally, a second, more slowly evolving gene is needed. This gene needs to resolve relationships at the generic and familial levels. A possibility is the 16S rRNA gene, which has been used successfully to complement the COI gene in resolving multi-level phylogenies.

Phylogeny of Lake Baikal Amphipods

Introduction

Ancient lakes have long fascinated researchers due to their complex geological history and often unique biota. Most of the world's lakes are less than 10,000 years old, forming after the last ice age, and will probably disappear in the next 100,000 years, filling with sediment and plant biomass (Gorthner, 1994). However, a few lakes (~10), most of which were created through tectonic subsidence, are vastly older (>1 ma) (Gorthner, 1994). Many of these ancient lakes contain species flocks: unusually large, geographically limited, assemblages of closely related endemic species (Greenweed, 1984; Ribbink, 1984). The existence of these species flocks raises several questions: (1) What is the origin of the flock? (2) How long has the flock been in existence? (3) How did the flock evolve? (Martens et al., 1994).

The first two questions are typically dependent upon the taxa and body of water involved. The origin of a species flock depends on the history of the body of water, and the taxonomic groups found in the immediate surroundings (Martens, et al., 1994; Martens, 1997). Some flocks are believed to have arisen from several invasions with subsequent radiations, such as the cichlids of Lake Tanganyika (Kocher et al, 1993), and the ostrocods (Mazepova, 1994) and turbellarians (Timoshkin, 1994) of Lake Baikal. Other species flocks seem to have arisen from a single invading species, such as the cichlids of Lakes Malawi and Victoria (Meyer et al., 1990, Kocher et al., 1993) and the cottoids (sculpins) of Lake Baikal (Sideleva, 1994; Hunt et

al., 1997). However, the origin of most flocks is not known.

The age of a species flock is also often dependent upon the taxa and body of water. Some are considered young, such as the cichlid flocks of Lakes Victoria (< 200 ky, and possibly ~ 12.4 ky; Meyer et al., 1990; Meyer et al., 1994; Johnson et al., 1996) and Malawi (200 ky - 2 ma; Meyer et al., 1994; Kocher et al., 1993), while others are considered old, such as the cichlid flocks of Lake Tanganyika (up to 5 ma; Nishida, 1991). The relative terms old and young are lake-dependent, as the sculpin flock of Lake Baikal is also considered young at 3-5 ma (Kiril'chik et al., 1995; Hunt et al., 1997). Species flocks within the same lake can also be of different ages, such as the younger cottoids and baicaliid gastropods and the much older *Choanomphallus* gastropods of Lake Baikal (Sherbakov, 1999). The fossil record in most of these lakes is "disturbingly" scant (Martens et al., 1994), so most of the age estimates are based solely on "molecular clocks". However, these estimates are often questionable because different genes often give different age estimates (< 3 ma vs. 4.9 ma for Baikal's sculpins; Kiril'chik et al., 1995; Hunt et al., 1997), and there is often disagreement between molecular clock estimates and those based upon geological history. In the case of Lake Malawi's cichlids, different genes give different estimates of maximum flock age (2 ma for Kocher et al., 1993; 0.2 ma for Meyer et al., 1994), and both disagree with geological history (a maximum age of 0.014 ma, Johnson et al., 1996).

The first two questions discussed above lead to the third question of how a species flock has evolved. This question has a much wider scientific audience, for it delves into tempo and modes of speciation. Much of the attention on tempo focuses on the degree of continuity

of the speciation events, specifically gradualism vs. punctuated equilibrium (Martens et al., 1994). The fauna of Tanganyika seems to show both modes: gradualism in the pelagic taxa, and stasis followed by rapid speciation in littoral taxa (Coulter, 1991). However, periods of rapid radiations, which are consistent with punctuated equilibrium and are often correlated to changes in water level, seem more common than slow and continuous speciation (Martens et al., 1994; Martens, 1997). A more controversial discussion arises concerning modes of speciation of species flocks, for this raises questions concerning the predominance of allopatric vs. sympatric speciation (McCune, 1987). While the origin of 400 species in a relatively short period of time and in a limited geographical area (i.e., Lake Malawi) seems like a prime opportunity to find strong evidence for the existence of sympatric speciation, definitive evidence is difficult to find, and sympatric speciation has not been convincingly demonstrated in the well studied ancient lakes. However, Schliewen et al. (1994) show strong evidence for sympatric speciation of cichlids in two crater lakes. While some researchers remain adamantly opposed to the possibility of sympatric speciation in these flocks (Mayr, 1963; Mayr, 1994), most acknowledge the possibility of sympatric speciation while stating that it is unnecessary. They argue that allopatric speciation can adequately account for most of these radiations, and can rarely be ruled out (Fryer, 1991; Martens et al., 1994). Much of the speciation creating species flocks has been attributed to isolation of populations during lake-level fluctuations and habitat/depth segregation (Coulter, 1991; Fryer, 1991, Martin et al., 1994; Mazepova, 1994), although no single factor seems likely to explain all intra-lacustrine speciation events (Martens et al., 1994).

The Gammarid Amphipods of Lake Baikal

Lake Baikal, an ancient lake in Siberia, Russia, has the most highly diverse and endemic fauna of any extant lake, and includes many species flocks (Kozhov, 1963; Martin, 1984). Although it is not the largest lake in the world in surface area, it is the deepest (up to 1637 m maximum depth), and consequently the largest lake volumetrically, containing 20% of the planet surface's liquid fresh water (Martin, 1994). Lake Baikal is also the oldest lake in the world, and while the specifics of the geological history of the lake are much disputed, most authors agree that it originated between 10 and 60 ma, and was created in a two-stage rifting process (Logatchev and Florensov, 1978; Logatchev and Zorin, 1987; Artyushkov et al., 1990; Hutchinson et al., 1992, Logatchev, 1993; Logatchev, 1994). An early slow-rift stage created a predominantly shallow, marsh-like lake, and lasted until ~0.4-4 ma. This was followed by a second stage of fast-rifting which substantially deepened the lake and created the cold, deep-water lacustrine environment that exists today (Logatchev and Florensov, 1978; Logatchev and Zorin, 1987; Artyushkov et al., 1990; Hutchinson et al., 1992, Logatchev, 1993; Logatchev, 1994).

Lake Baikal has possibly the most impressive endemic fauna in the world. It contains many species flocks from several disparate taxa, including the Cottoidei (sculpins), Ostracoda, Turbellaria (flatworms), Copepoda, Gastropoda (snails), and Amphipoda (Bazikalova, 1945; Brooks, 1950; Kozhov, 1963; Martin, 1994; Sherbakov, 1999). Because of the numbers of species and their morphological and ecological diversity, the amphipods are often considered the most remarkable of Baikal's species flocks.

The amphipods of Lake Baikal are part of the superfamily Gammaroidea, a large, diverse, cosmopolitan amphipod group (Bousfield, 1977, 1982; Barnard and Barnard, 1983; Kamaltynov, 1992). The Baikalian gammaroideans are divided into 49 genera and 259 species (Kamaltynov, 1992), all endemic to Baikal and its watershed. They comprise roughly 20% of the world gammaroidean genera and species (Kamaltynov, 1992), and are extremely diverse morphologically, ranging from relatively generalized forms, similar to the cosmopolitan genus *Gammarus*, to highly armored, spinous forms (Fig. 1). They are also ecologically diverse, including benthic, fossorial, and nektonic forms, and the world's only pelagic gammaroid (Kozhov, 1963; Fryer, 1991). In addition to the benthic detritivore habit typical of gammarids, there are also predators, parasites, and a pelagic planktivore (Bazikalova, 1945; Kozhov, 1963; Fryer, 1991) .

The taxonoma of the Baikal amphipods has a long, convoluted history, and is unresolved at present. The current classification was established by Bousfield (1977, 1982) and reviewed by Kamaltynov (1992). This classification places all Baikal amphipods into the superfamily Gammaroidea, and divides them into three families and one informal family group (see Table 1 for a species list and current classification). The morphologically unspecialized, hypothetically ancestral genera were placed into the cosmopolitan family Gammaridae. These include such genera as *Eulimnogammarus*, *Heterogammarus*, *Baikalogammarus*, *Abyssogammarus*, and *Micruropus*. The large, carinate (keeled) and/or spinous amphipods were placed in the endemic family Acanthogammaridae. This family contains most of the taxa considered uniquely Baikalian; the characters that identify the acanthogammarids (carinae,

teeth, spines) are considered Baikalian amphipod characters. Although some of these characters are present in certain deep-sea amphipods from the Antarctic and shallow-water taxa from the Caspian Sea, these species lack the immense variety or extreme development of the characters found in Baikal. Within the Acanthogammaridae were placed genera such as *Acanthogammarus*, *Eucarinogammarus*, *Gmelinoides*, *Spinacanthus*, *Pallasea*, *Crypturopus*, *Brandtia*, and *Poekilogammarus*. The third family, Macrohectopidae, is monotypic (containing only one species), comprising the species *Macrohectopus branickii*. This species is strictly planktonic and is highly modified for a pelagic lifestyle (Fig 1f). The fourth, informal amphipod group in Baikal is the *Iphigenella-Pachyschesis* family group. This aberrant, relatively unspecialized, and possibly polyphyletic group is endemic to the Caspian Sea and Lake Baikal (Bousfield, 1982). In Lake Baikal the group is represented by the genus *Pachyschesis*.

Understanding the origins of the amphipod diversity in Lake Baikal means focusing on two important questions. The first concerns the origin of the first amphipods in the lake. *How many invasions from nearby waters formed the basis of the current amphipod fauna*? Past estimates range from 4 to more than 18, and it is universally accepted that Baikal's amphipods resulted from multiple invasions (Brooks, 1950; Kozhov, 1963; Bousfield, 1977; 1982; Barnard and Barnard, 1983; Kamaltynov, 1992; Ogarkov et al., 1997; Sherbakov et al., 1998; Sherbakov, 1999). However, this idea has never been tested through any rigorous phylogenetic analysis.

Knowing how many ancestral amphipod species invaded Lake Baikal leads to the

second question. *How did these putatively few invaders evolve into the vastly diverse fauna that exists today*? This question potentially leads to more controversy than the previous one, for it delves into questions concerning the origin of species, including the contentious issue of sympatric speciation (Brooks, 1950; Mayr, 1963; McCune, 1987; Schliewen, et al., 1994). Unfortunately, the history of speciation in the lake is not well understood. Lake Baikal is extremely old, and has been through several bathymetric and configuration changes (Martin, 1994). It is uncertain, for example, if speciation has occurred at a relatively constant rate throughout the history of the lake, creating a gradual buildup of diversity (gradualism); or if the amphipods of Baikal have experienced times of intense speciation between times of relative stasis (punctuated equilibrium). Knowing the history of speciation in Lake Baikal amphipods may lead to a better understanding of the environmental context of the speciation events that create great diversity both in Lake Baikal and elsewhere.

Previous Molecular Studies of the Baikal Gammarids

To date, all taxonomic classifications of Baikal amphipods have been based solely on morphologal characters (Bousfield, 1978, 1982; Barnard and Barnard, 1983; Fryer, 1991; Kamaltynov, 1992). This approach may have problems identifying convergence of characters and recognizing common ancestry when individuals show extreme morphological specialization. This is especially a concern for gammaroidean amphipods, which have high diversity of morphological adaptations and exhibit general evolutionary plasticity of many characters (Pinkster, 1983; Barnard and Karaman, 1975; Bousfield, 1977; Barnard and Barnard, 1983).

In fact, the higher-level relationships of the amphipods are so uncertain that many taxonomic treatments simply list families alphabetically (Barnard and Karaman, 1975; Barnard and Barnard, 1983) and some even question the usefulness of the Linnean system in classifying amphipods (Barnard and Karaman, 1975).

Although several molecular studies of the Baikal gammarids have been done, until recently none has attempted to examine the deep phylogeny of the major groups within Baikal. Yampolsky et al. (1994) used allozymes to study population subdivision and genetic distances within and between two closely related genera, *Spinacanthus* and *Brandtia* but did not specifically address phylogenetic relationships. Ogarkov et al. (1997) examined phylogenetic relationships among Baikal gammarids using the mitochondrial cytochrome c oxidase subunit III (COIII) gene. They examined many Baikal amphipod species, but no non-Baikalian outgroup species. Their main focus was on phylogenetic relationships within and amoung two genera: *Pallasea* (an acanthogammarid) and *Eulimnogammarus* (a gammarid), and on estimating their divergence times using a molecular clock calibrated from 3rd position transitions in molluscs. Phylogenetic resolution was poor, but did suggest that the family Acanthogammaridae was not monophyletic. They estimated that minimal time of divergence for the two closest congeners was 2.6 ma. Other congeners may have diverged up to 8.5 ma, but 3rd position transitions became too saturated for estimating divergence times between genera.

Recently, Sherbakov et al. (1998) examined the phylogeny of selected amphipod taxa from Lake Baikal by sequencing a segment of the 18S rRNA gene. They report finding two major clades of amphipods, with Baikal endemics *Eulimnogammarus*, *Brandtia*,

Spinacanthus, *Pallasea*, *Micruropus*, *Macrohectopus*, and the cosmopolitan *Gammarus pulex* in one group, and the Baikal endemics *Abyssogammarus*, *Ommatogammarus* and *Acanthogammarus* in the other group, although neither clade was well supported. The only clade strongly supported in their analysis was a sister-group relationship between *Macrohectopus* and *Gammarus pulex,* evidence against the monophyly of Lake Baikal's amphipods. However, there were some significant problems with this study. Each of the two major lineages had a Bremer (1988) support value of 1 (i.e. the shortest tree without this clade was a single step longer), and bootstrap values under 35%, both low levels of support. The most parsimonious (MP) tree was 198 steps, but there are more than 41 000 trees within four steps of the MP tree (58 trees at 199 steps; 1066 trees at 200 steps; 11 988 trees at 201 steps; 28 461 trees at 202 steps). Another problem with the study was the taxa used. One species from each of 21 genera were sequenced, yet specific species were not identified in the publication. Some of the amphipod genera found in Lake Baikal are probably not monophyletic, so analyzing single unidentified species from each genus may misrepresent some intergeneric relationships. Additionally, as the purpose of the study was to examine the phylogeny of Lake Baikal's amphipods and relate it to their morphological and ecological characters, no amphipods from the Caspian Sea or elsewhere in Eurasia (except *Gammarus pulex,* collected from Belgium) was analyzed; their outgroup, *Tryphosella murrayi*, is a non-Gammaroidean Antarctic marine amphipod.

Objectives

This study addresses several objectives. First, I construct phylogenetic hypotheses for relationships among selected Baikal taxonomic groups. Then I test the monophyly of the two non-monotypic families within the lake, and of the Baikal amphipods as a group. In the process, the number of possible invasions into the lake was addressed. Finally, I examine the sister-group relationship found between the endemic *Macrohectopus brannickii* and the cosmopolitan *Gammarus* sp. by Sherbakov et al.(1998).

For this phylogenetic analysis, the sequence data were needed to be versatile, able to differentiate closely related species without becoming saturated when comparing families. One possible way to accomplish this is to use a protein-coding gene. These typically have differing substitution rates at different positions within a codon. The first and second positions usually change slowly (and thus are useful for more divergent taxa) while the third position shows more rapid change (useful for more closely related taxa) (Brown, 1985). Additionally, sequences with extremely high variability can be translated into more conservative amino acid sequences to resolve older divergences.

The mitochondrial genome has been a useful tool in studying phylogeny (Brown,1985; Moritz et al., 1987), including that of crustaceans (Cunningham et al., 1992; Knowlton et al., 1992; Tam et al., 1996; Duffy et al., in press). Different genes within the mitochondrial genome evolve at different rates, and facilitate examination at different taxonomic levels (Xiong and Kocher, 1991). The mitochondrially coded cytochrome c oxidase subunit I (COI) gene codes for one of the three large subunits of the cytochrome c oxidase protein complex, which plays an

important role in the electron transfer chain of the oxidative phosphorylation reaction (Stryer, 1988). Its fundamental role in this essential metobolic pathway constrains its form, therefore the amino acid sequence of COI is highly conserved (Brown, 1985); yet the gene tends to have many synonymous (silent) substitutions. Because of these varying levels of conservativeness, COI is a very versatile gene. It is useful not only for resolving relationships at the phylum and class level (Folmer et al., 1994; Cummings et al., 1995, Cunningham, 1997) but also at the family and generic levels (Carlini and Graves, 1999), and has been used often at this level in crustaceans (Palumbi and Benzie, 1991; Knowlton et al., 1992, Van Syoc, 1994; Tom et al., 1996)

This study reexamines the taxonomy and phylogeny of the Baikalian amphipods using molecular characters by sequencing a 657 bp segment of the COI gene from a sample of Baikal amphipod taxa intended to encompass the major lineages within the lake, as well as several gammaridean amphipods from northern Eurasia.

Materials and Methods

I sequenced a 659 bp portion of the COI gene for 18 amphipod species from Lake Baikal, northern Eurasia, and North America (Table 1). Selection of species was based predominantly on their hypothesized phylogenetic positions, but availability of specimens and difficulties with amplification and sequencing also influenced the selection. Sequences from multiple genera of each of the three Baikalian families hypothesized by Bousfield (1977, 1982), and several representative species of larger (or taxonomically ambiguous) genera were obtained. Additionally, I sequenced two unspecialized cosmopolitan amphipods common in Eurasian fresh waters, a gammaroidean and a closely related pontoporeioidean. Finally, *Cymadusa compta,* a North American non-gammaroidean amphipod, was sequenced as an outgroup.

All species were collected by J. E. Duffy and L. Yampolsky (Baikal, summer 1995), L. Yampolsky (Caspian, summer 1996), Nikolai Mugue (Moscow, Russia, 1998), and me (North American, summer 1997). Specimens were preserved and stored in 95% EtOH.

Molecular Methods

DNA was isolated from the specimens using QIAamp (QIAGEN) tissue preparation kits. Whole animals or abdominal muscle of larger individuals was placed in a microcentrifuge tube with 120 µl lysis buffer (AL buffer, QIAGEN), 25 µl of 20 mg/ml proteinase K, and

incubated at 55°C overnight to degrade tissue and lyse cells. The resultant liquid was placed into a QIAamp column. Columns were centrifuged twice at high speed to remove non-genomic material. 500 µl ATL Buffer was added to the column and centrifuged to clean the DNA. This step was then repeated once. The DNA was finally removed from the columns by eluting and centrifuging twice with 200 µl AE buffer heated to 60°C. The DNA solution was stored at 4°C.

A fragment of the COI gene was then amplified using the Polymerase Chain Reaction (PCR) and the universal metazoan primers of Folmer et al. (1994) with the addition of M13 Forward and Reverse primer tails:

(LCO 1490, 5'-GGTCAACAAATCATAAAGATATTGG-3' attached to

M13F, 5'-CACGACGTTGTAAAACGAC-3';

HCO 2198, 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' attached to

M13R, 5'-GGATAACAATTTCACACAGG-3').

Typical 50 µl PCR reactions were run using a PCR reagent system (BLR/GibCo) with 5 µl 10X PCR Buffer (GibcoBRL), 2 mM (2 µl) $MgCl_2$, 0.2 mM (2 µl) dNTP mixture (GibcoBRL), 10µM (1 µl) of each primer, 1 unit (0.25 µl) *Taq* polymerase (GibcoBRL), and 2 µl template DNA solution. Typical reactions were cycled on a MJResearch PTC200 thermocycler for an initial 4 minute denaturing step at 95°C, followed by 40 cycles of the following reaction: 95°C for 1 min, 45°C for 1 min, and 72°C for 2 min 30 sec, finishing with a single 72°C, 7 min elongation step. PCR products were cleaned using Wizard PCR Preps or 5'-3' PCR Preps. Attachment of M13 tails onto primers for the original amplification allowed for the direct use of the PCR product and fluorescently labeled M13 primers in the sequencing

reactions. Gene segments were sequenced by the dideoxy chain termination method of Sanger et al. (1977), using Sequenase 2.0 kits (United States Biochemical). Sequencing reaction products were run on 5 1/2% Long Ranger acrylamide gels (FMC Bioproducts, Rockland, ME) on a LI-COR DNA4000L automated DNA sequencer, and were read using the Base ImagIR version 4.0 software package.

Data Analysis

Sequence data were aligned using a clustal algorithm in GeneJockey II v2.11 (Taylor 1993). MacClade (Maddison and Maddison, 1992) was used to assign codon position, and translate nucleic acid sequences into amino acid sequences. Phylogenetic hypotheses of relationships among taxa were obtained by analyzing the aligned nucleic acid sequences using PAUP* (Swofford, 1999) with three methods: 1) equally weighted parsimony, 2) 6-parameter weighted parsimony with down-weighted 3rd positions, and 3) maximum likelihood. Amino acid sequences were analyzed with equally weighted parsimony. The heuristic search option was used in all cases.

For all analyses, nonparametric bootstrap support values for clades were obtained using the heuristic bootstrap search command (with 100 replicates) in PAUP*. Additionally, for all the parsimony analyses, consistency index (CI) and retention index (RI; Farris, 1989) values were calculated in PAUP*. CI values represent the amount of homoplasy present in the data. A value of 0 means that every character is homoplastic, while a value of 1 means no characters are homoplastic. However, this value tends to be inflated by autapomorphies, so RI

values were also calculated. The RI represents the proportion of non-homoplastic, non-autapomorphic characters. The RI is calculated as the maximum number of extra changes (number of changes on a tree with all characters homoplastic minus the number of changes if no characters were homoplastic), minus the observed number of extra changes (number of homoplastic changes on actual tree), divided by the maximum number of extra changes (Farris, 1989). This value also ranges from 0 to 1, with a value of 0 representing all characters are homoplastic, and 1 representing no homoplastic or autapomorphic characters. Bremer support values (Bremer, 1988; 1994) were calculated using the program TreeRot (Sorenson, 1996) for the equally weighted parsimony analyses. Bremer support values indicate the additional steps needed to collapse a clade. In the maximum likelihood analysis, support for clades was also estimated by calculating quartet puzzling support values (Strimmer and von Haeseler, 1996). Quartet puzzling finds the tree with the highest likelihood for every possible four-taxon combination, and the support value of a clade indicates the percentage of those trees containing the observed clade.

6-parameter weighted parsimony attempts to account for variation in frequency among different substitution types (Cunninham, 1997; Stranger-Hall and Cunningham, 1998). Nucleotide substitutions that are more common are also more likely to show homoplastic changes, and therefore will, on average, be less informative about the topology of a tree than substitutions that are rare and therefore less likely to be homoplastic. To account for this, I first obtained the equally weighted parsimony trees. Maximum likelihood values using the General Time Reversible (GTR) model were then found for this tree. Proportional substitution rate

values were given in the r-matrix output. The r-matrix value for each substitution type (A↔C, A↔G, A↔T, etc.) was divided by the sum of all r-matrix values to determine the proportion of each value. The negative natural log of this proportion was calculated, giving the weight for that substitution type. Additionally, because substitutions were found to be much more common at the 3rd position (see results), these were downweighted to 10% of the weight of the1st and 2nd positions. This weighting method has been found to be more effective in correctly resolving well corroborated phylogenies than equally weighted parsimony, is theoretically justified, and rarely violates the triangle-inequality rule (Felsenstein, 1981; Cunningham, 1997; Stanger-Hall and Cunningham, 1998).

The maximum likelihood method uses a nested hierarchical approach as described by Huelsenbeck and Crandall (1997). A hierarchy of likelihood ratio tests was used to compare likelihoods of successively more parameter-rich models to less constrained models, starting with the most constrained model, JC69 (Jukes and Cantor, 1969). The JC69 model assumes equal base frequencies, equal probabilities for all substitution types, and equal probabilities of substitutions across sites. The log likelihood of the most likely tree based on JC69 was then compared to the log likelihood of the best tree obtained under the F81 (Felsenstein, 1981) model, which allows for unequal base frequencies, using a likelihood ratio test. If the F81 model found a tree that was significantly more likely than the JC69 tree, the former was then compared to the F81 model with site heterogeneity (F81+). This model allows for different probabilities of substitution across sites. It determined two site parameters, one for 1st and 2nd positions, and the another for the 3rd position. The F81+ tree was then compared to the tree

obtained from the general time reversible model with site heterogeneity (GTR+). This model allows for different probabilities of the different substitution types. The likelihood tests are complete when a tree from a less constrained model is not significantly more likely than a more constrained model (in which case, the more constrained model is used). The GTR+ model was the least constrained (most parameter-rich) model tested.

Monophyly of clades was tested using the GTR parsimony tree and two methods. First, the T-PTP test (Faith, 1991) was performed. This test finds the difference in tree length between the most parsimonious tree with the group of interest constrained to be monophyletic and the tree with that group unconstrained. It then randomizes the data matrix, and reanalyzes the data, again finding a constrained monophyletic and non-monophyletic tree length difference. It performs the randomization and analysis a total of 100 times, and creates a distribution of tree length differences. With this distribution, a p-value can be obtained. The p-value is the probability that a tree length difference as large as the one found in the data could occur by chance. Monophyly was also analyzed using the non-parametric Templeton (1983) Test. This test finds the number of steps required by each character for both the monophyletic and non-monophyletic trees. It then uses a Wilcoxan ranked sums test to test whether the difference between two trees is more significant than can be expected due to random error.

Results

The nucleotide sequences obtained are shown in Figure 2. Of 659 total sites, 360 were variable, with a majority of those in the 3rd position (208 of 220 total characters). The 1st position had 94 (of 220 characters) and the 2nd postition had 49 (of 219) variable sites. There were 303 parsimony informative sites, of which 72 were in the first position, 23 were in the 2nd position, and 208 were in the third position. Uncorrected pairwise distances (p) ranged from a minimum of 0.138 between species within the genus *Eulimnogammarus* to a maximum of 0.294 between *Macrohectopus* and *Gmelinoides* (Table 2). A plot of uncorrected pairwise distances as a function of branch length is shown in Fig. 3. A 1st order regression of the 3rd positions has a r^2=0.18, while a 2nd order regression has an r^2=0.23, indicating a flattening of the curve, which suggests 3rd posititon substitutions may start becoming saturated at longer branch lengths.

The equally weighted parsimony analysis of the nucleotide sequence data yielded 3 most parsimonious trees (Fig. 4) with a length of 1483, a CI of 0.43 and an RI of 0.31. This analysis does not have the resolution to evaluate monophyly of the Lake Baikal amphipods, but it does suggest a non-monophyletic family Acanthogammaridae and genus *Acanthogammarus*. It also is consistent with the monophyly of the Baikal amphipods. However, support in this phylogeny is low, with strong bootstrap values and moderate Bremer support for only two clades, the genus *Pallasea*, and a clade containing *Micruropus* and *Gmelinoides*.

The 6-parameter weighted parsimony analysis yielded 2 most parsimonious trees (Fig. 5) with a length of 704.4, a CI of 0.51 and an RI of 0.42. This analysis has better bootstrap support than the equally weighted parsimony analysis. This tree suggests that neither the acanthogammarids, nor the genus *Acanthogammarus* are monophyletic, although there is little support for this result. The 6-parameter tree supports the monophyly of the Lake Baikal amphipods, although with <50% bootstrap support. It also supports (with moderate bootstrap values) the monophyly of the Baikal amphipods excluding *Macrohectopus* and supports (with high bootstrap values) the monophyly of the Baikal amphipods plus *Gammarus lacustris*. This analysis also gives strong bootstrap support for the genus *Pallasea*, and the (*Micruropus, Gmelinoides*) clade.

The GTR+ model showed the best fit to the data in the maximum likelihood analysis. The tree generated from this model is shown in Fig. 6. This tree does not support the monophyly of the Baikal amphipods, the family Acanthogammaridae, nor the genus *Acanthogammarus*. It does give weak bootstrap and quartet puzzling values supporting a monophyletic Baikal clade exclusive of *Macrohectopus*, and a monophyletic clade including the Baikal species plus *Gammarus lacustris*. It also reveals a sister-group relationship between *Macrohectopus* and *Gammarus lacustris*, although with little bootstrap or quartet puzzling support. This analysis gives strong bootstrap and quartet puzzling support for the genus *Pallasea* and the (*Micruropus, Gmelinoides*) clade, and good support for the genus *Eulimnogammarus*.

The amino acid sequences were deduced from nucleotide sequences. Of 219 total

characters, 96 were variable, and of those, 46 were parsimony-informative. The equally weighted parsimony analysis of the amino acid sequence data yielded 42 most parsimonious trees (Fig. 7) with a tree length of 269 steps, a CI of 0.76, and an RI of 0.43. This analysis shows very little resolution amoung the genera of the Lake Baikal amphipods. It does, however, show good bootstrap and Bremer support for the monophyly of the Baikal amphipods excluding *Macrohectopus*, and for the Baikal amphipods plus *Gammarus lacustris*. Like all of the other analyses, it also shows strong support for a monophyletic *Pallasea*, and for the (*Micruropus Gmelinoides*) clade.

The monophyly of 2 clades from the 6-parameter parsimony tree were tested: the family Acanthogammaridae, and the genus *Acanthogammarus*. Neither of these clades was significantly non-monophyletic using the Templeton test ($p = 0.35$ and 0.24 respectively). The acanthogammarids were significantly non-monophyletic using the t-PTP ($p = 0.01$) but not *Acanthogammarus* ($p = 0.74$). The Baikal members of the Gammaridae were significantly non-monophyletic with both tests (Templeton test, $p = 0.0002$; T-PTP test, $p = 0.04$).

Discussion

The 659 bp segment of the COI gene sequenced for this study does not have the resolution to provide definitive answers to the questions concerning the origin and phylogenetic history of Lake Baikal's amphipods posed in the Introduction. However, it does hint at some possibilities, and gives us insight into the future work that is needed to help us better understand this radiation. All the analyses except the equally weighted parsimony supported a monophyletic relationship of the Baikal amphipods excluding *Macrohectopus branickii*. *Macrohectopus* was always basal to or showed a polyphyletic relationship with the rest of Baikal's amphipods. This contradicts Bazikalova (1945) and other morphologists who felt that *Macrohectopus* was descended from an acanthogammarid, most likely *Poekilogammarus* sp. It also differs from the results of Sherbakov et al., (1998), who found that *Macrohectopus* was not descended from any Baikalian amphipod, but formed a clade with a common Eurasian gammarid, *Gammarus pulex*. This is not inconsistent with my findings. However, the (*Macrohectopus, Gammarus pulex*) clade fell between two clades encompassing the rest of Baikal's amphipods, suggesting at least two Baikal radiations and possibly a second invasion of the ancestral *Macrohectopus*. This study includes several species from both of Sherbakov's Baikal clades, but supports their monophyly, suggesting a single radiation of most Baikal amphipods, with a possible second invasion by an ancestor of *Macrohectopus*. Regardless of who the ancestor of *Macrohectopus* was, it is likely that it has evolved <4 ma, when the first

truly pelagic (deep water) environment appeared in Lake Baikal (Logatchev and Florensov, 1978; Logatchev and Zorin, 1987; Artyushkov et al., 1990; Hutchinson et al., 1992, Logatchev, 1993; Logatchev, 1994).

All analyses (except the equally weighted parsimony analysis of the amino acid sequences, which had little or no resolution among genera within Baikal) suggested that the family Acanthogammaridae is not monophyletic. Although this non-monophyly was not deemed significant using the Templeton test, it was significant using the T-PTP test. However, the validity of the T-PTP for testing monophyly has been strongly questioned by Swofford et al. (1996). If the acanthogammarids are not monophyletic, then the evolution of their distinctive body armature (spines, keels, etc.) represents an extreme example of convergence. Similarly armed amphipods are also found in other ancient lakes (Lake Titicaca and Lake Ohrid), in the Caspian Sea, in the deep sea, and in antarctic waters (Kozhov, 1963; Martens et al., 1994; Martens, 1997), indicating these body types have evolved several times in different locations. The multiple origins of such extreme morphological characters exemplifies the difficulties with creating a natural morphological classification in amphipods.

These results also support the hypothesis that amphipods of the family Gammaridae in Lake Baikal are not monophyletic. This was somewhat expected, for this family has long been considered an unnatural group (Bousfield, 1977; Bousfield, 1982; Barnard and Barnard, 1973). In fact, in Bousfield's (1977) discussion of the use of morphological characters to create a taxonomy based upon cladistic analysis, the character states he lists as "plesiomorphic" are all characters used to describe the family Gammaridae.

One important goal towards understanding the evolution of Baikal's amphipods is to determine the age of this radiation. Unfortunately, a molecular clock has yet to be found for amphipods. However, a rough correlation of pairwise distances and divergence times is available from other crustaceans. Knowlton et al. (1992) used the final closure of the Panama seaway to calibrate a molecular clock for *Alpheus* shrimp using a segment of the COI gene. They estimate 2.2 - 2.6% sequence divergence (using Kimura's corrected distances) per million years. Even using the conservative end of this estimate, the Baikal amphipods are extremely old. According to Knowlton's clock, the two most similar species, *Eulimnogammarus cruentus* and *E. maacki,* diverged over 7 ma. Other Baikal amphipods may have diverged as long as 16 ma (*E. maacki* and *Micruropus whali*) If actual divergence times are similar to these estimates, the radiation occurred long before Baikal started forming its current deep basin. Early in its history, Baikal was predominantly shallow and marsh-like. If the radiation occurred during this period, the speciation events could not be due to depth segregation as hypothesized by Fryer (1991), but could possibly be due to isolation of parts of the marsh with changing water levels. However, the validity of these divergence times is doubtful. Using these estimates, *Cymadusa compta*, an estuarine corophioid amphipod from the Eastern U.S., is more closely related to some Baikalian amphipods than many Baikalian amphipods are too each other. This seems highly unlikely. It is more likely that saturation of 3rd position substitutions (which are the major source of variation among species) have caused more distantly related taxa to appear less divergent due to reversals and convergent substitutions.

The equally weighted parsimony analysis of the nucleotide sequence data showed very little resolution among genera and families. This was most likely due to the extremely high level of substitutions in 3rd positions and the relative paucity of substitutions in 1st and 2nd positions. 95% of third positions were variable, so variation at this position is most likely saturated and many substitutions at this position are likely homoplastic. To look for 3rd position saturation, branch lengths were plotted vs. uncorrected pairwise distances (Fig. 3), and signs of saturation were found (as branch lengths increase to a certain level, distances stop increasing and the plot starts to plateau, supported by a better fitting 2nd order regression than 1st order regression). The effect of this high level of homoplasy on an analysis can often be minimized by character weighting. Proportionally downweighting the more common substitution types and the highly variable 3rd positions helped the resolution and support in this analysis, mostly for the deeper (and therefore older) nodes, which tend to be more prone to homoplasies. Maximum likelihood analysis is designed to take into account multiple substitutions, heterogeneity across sites, and differences in substitution type rates. Although the maximum likelihood analysis found a tree with better support than the equally weighted parsimony, there was little difference in support between the ML and 6-parameter parsimony trees. This, along with overall stronger bootstrap support than the equally weighted tree, indicates that 6-parameter parsimony may reduce some of the problems inherent in the maximum parsimony method, and provide similar results to the ML analysis with significantly less computer time.

Amino acid sequences are often used to resolve deeper nodes, especially in highly variable data. The amino acid analysis did this, giving much better support for the older clades

(the Baikal amphipods except *Macrohectopus*, and the Baikal amphipods with *Gammarus lacustris*), but at the price of losing practically all resolution among genera within Baikal.

An obvious conclusion from this study is that much more data is needed to resolve better the phylogeny of the Baikal amphipods. Two forms of additional data are needed. First, more taxa need to be added to this phylogenetic analysis of the COI gene. One potential problem apparent in all the analyses is long terminal branches. Long branches are especially problematic in parsimony analyses (Felsenstein, 1978), because long branches by definition have more substitutions, and consequently are likely to have more homoplastic characters. The addition of internal taxa may significantly shorten branches, and has been shown to increase resolution (Graybeal, 1998; Hillis, 1998). Also, adding some taxa may help answer some specific questions. First, *Gammarus pulex* needs to be sequenced and added to this analysis to determine if it forms a clade with *Macrohectopus*, as found by Sherbakov et al. (1998) using 18S rRNA sequences. This could significantly increase the support for the non-monophyly of Baikal's amphipods. Additional species from the genus *Acanthogammarus* also need to be added. The findings suggest that *Acanthogammarus* is not a natural taxonomic group. Interestingly, the distinctive long lateral spines characteristic of many *Acanthogammarus* species are similar to those found in the ancient Lake Titicaca (Dejoux, 1992), which suggests that a polyphyletic *Acanthogammarus* is not unreasonable. However, while all analyses separate the two species of *Acanthogammarus*, their actual placement depends upon the analysis, and the separation of the two clades is not strongly supported in any of the analyses. Adding closely related taxa may stabilize their placement within the phylogeny,

and give stronger support for their non-monophyly, or it could possibly bring them together into monophyly. Additionally, the number of acanthogammarid taxa in the analysis needs to be increased to examine better their monophyly. Finally, to test the monophyly of Baikal's amphipods rigorously, a more exhaustive sampling of other Eurasian amphipods is needed to better guarantee the inclusion of any possible ancestral taxa.

Additional characters are also needed in this analysis. Specifically, another gene is needed that can resolve relationships among the genera of Lake Baikal, a gene that has a level of variation somewhere between that of the $1^{st}/2^{nd}$ positions and the 3^{rd} position of the COI gene. Sherbakov et al. (1998) used the nuclear 18S rRNA gene, but that gene contained too little variation. A possibility is the mitochondrial 16S rRNA gene. The 16S gene has successfully resolved phylogenies among and within hermit crab genera (Cunningham et al., 1992), and within mole crab genera that had COI divergences similar to mine (Tam et al., 1996). Adding 16S sequences also greatly increased the resolution of a phylogeny of *Synalpheus* shrimp over the COI phylogeny alone (Duffy, et al., in press). The addition of another gene such as 16S may result in a more resolved and well-supported phylogeny, leading to greater insight into the history of amphipods in Lake Baikal and possibly a better understanding of how species flocks such as the Baikal gammarids originate and flourish.

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Table 1.

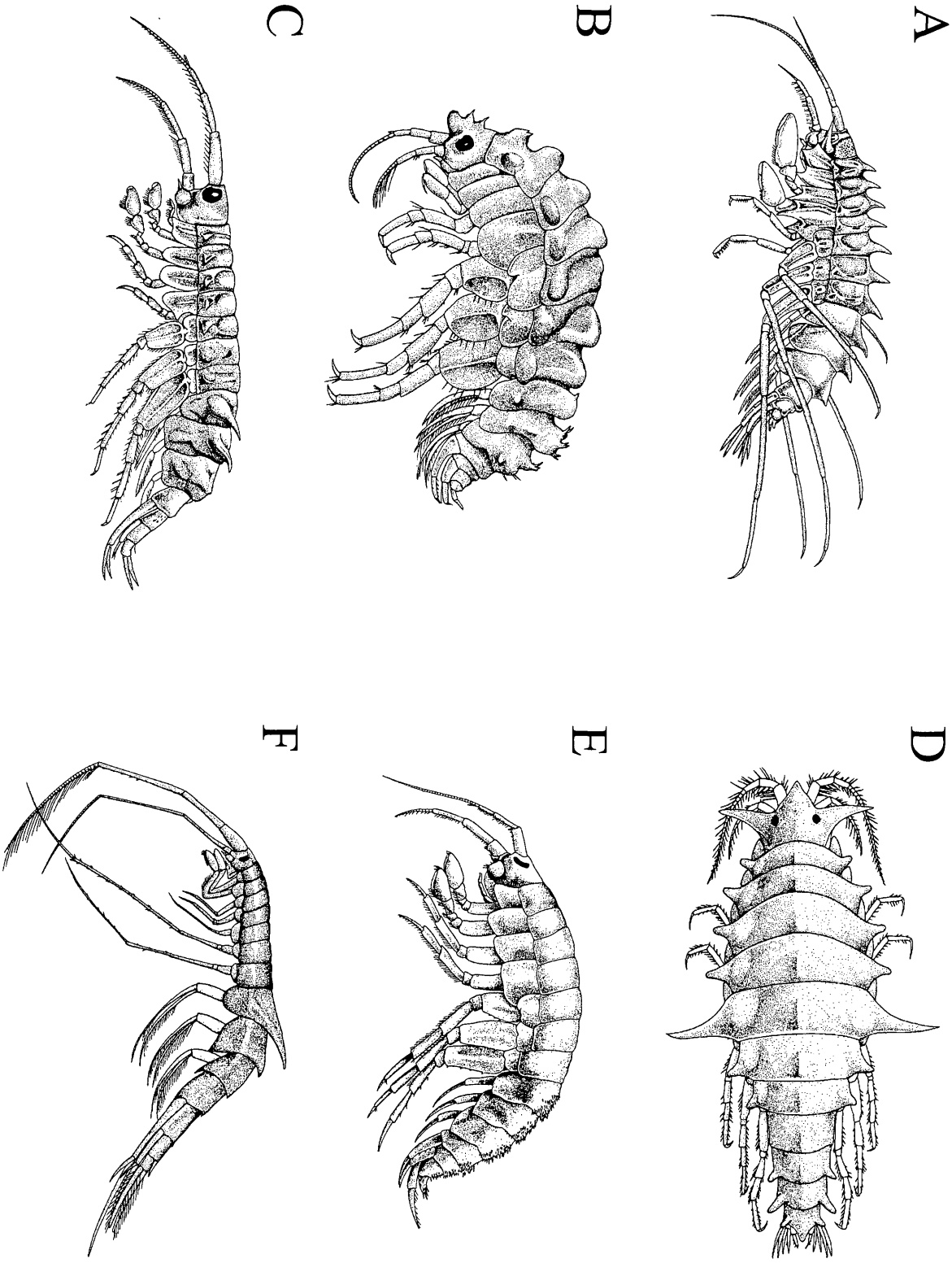
	Gammaroidea			Pontoporeioidea	Corophioidea
Source	Acanthogammaridae	Gammaridae	Macrohectopidae	Pontoporeidae	Ampithoidae
Lake Baikal	*Spinacanthus parasiticus* *Brandtia latissima* *Acanthogammarus maximus* *Acanthogammarus victorii* *Pallasea baikali* *Pallasea kessleri* *Gmelinoides fasciatus* *Poekilogammarus pictus* *Eucarinogammarus wagi*	*Ommatogammarus albinus* *Eulimnogammaurs maacki* *Eulimnogammarus vittatus* *Eulimnogammarus verrucosus* *Odontogammarus calceratus* *Micruropus whali*	*Macrohectopus branickii*		
Other		*Gammarus lacustris* (Palearctic: Siberia)		*Pontoporeia sp.* (Palearctic: Eastern Europe)	*Cymadusa compta* (N. America: Chesapeake Bay)

Representative Baikalian and non-Baikalian amphipods used in this study, ordered by superfamily and family (from Bousfield, 1977, 1982; Kamaltynov, 1991). Species shaded in grey are endemic to Lake Baikal. Parenthesis enclose species range and origin of specimens.

Table 2.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	*Eulimnogammarus cruentus*	-	0.318	0.156	0.256	0.309	0.246	0.258	0.176	0.193	0.193	0.290	0.171	0.178	0.320	0.271	0.176	0.212	0.272	0.240
2	*Pontoporeia sp.*	0.257	-	0.340	0.319	0.349	0.351	0.353	0.326	0.368	0.285	0.327	0.347	0.356	0.315	0.301	0.295	0.313	0.368	0.331
3	*Eulimnogammarus maacki4*	0.138	0.269	-	0.290	0.372	0.350	0.360	0.220	0.226	0.234	0.349	0.193	0.234	0.348	0.314	0.235	0.312	0.286	0.263
4	*Poekilogammarus pictus*	0.212	0.257	0.237	-	0.306	0.286	0.286	0.255	0.268	0.230	0.343	0.275	0.279	0.313	0.341	0.268	0.287	0.299	0.307
5	*Micruropus wahli*	0.248	0.277	0.288	0.248	-	0.305	0.327	0.280	0.285	0.273	0.265	0.337	0.335	0.355	0.331	0.273	0.311	0.314	0.322
6	*Pallasea kessleri*	0.207	0.278	0.275	0.233	0.249	-	0.213	0.225	0.240	0.237	0.342	0.294	0.289	0.338	0.329	0.267	0.313	0.288	0.290
7	*Pallasea baikali*	0.213	0.278	0.279	0.232	0.263	0.184	-	0.237	0.225	0.238	0.334	0.285	0.299	0.360	0.357	0.274	0.287	0.243	0.292
8	*Odontogammarus calceratus*	0.155	0.261	0.189	0.212	0.231	0.192	0.200	-	0.175	0.184	0.301	0.232	0.195	0.278	0.283	0.179	0.241	0.224	0.218
9	*Ommatogamarus albinus*	0.168	0.287	0.192	0.222	0.234	0.203	0.191	0.154	-	0.183	0.312	0.226	0.241	0.322	0.269	0.184	0.252	0.254	0.243
10	*Acanthogammarus maximus*	0.167	0.234	0.198	0.195	0.226	0.202	0.201	0.161	0.160	-	0.295	0.246	0.219	0.318	0.293	0.171	0.268	0.226	0.245
11	*Gmelinoides fasciatus*	0.237	0.262	0.276	0.270	0.218	0.272	0.265	0.245	0.252	0.240	-	0.333	0.342	0.384	0.342	0.251	0.320	0.315	0.309
12	*Eulimnogammarus maacki2*	0.149	0.274	0.166	0.227	0.268	0.240	0.234	0.196	0.193	0.207	0.266	-	0.178	0.341	0.303	0.247	0.276	0.307	0.275
13	*Eulimnogammarus vittatus*	0.155	0.280	0.196	0.230	0.267	0.237	0.242	0.170	0.201	0.188	0.272	0.156	-	0.315	0.310	0.209	0.285	0.297	0.276
14	*Macrohectopus branickii*	0.257	0.255	0.276	0.253	0.279	0.270	0.282	0.229	0.258	0.256	0.294	0.270	0.254	-	0.360	0.301	0.309	0.366	0.345
15	*Cymadusa compta*	0.226	0.244	0.254	0.271	0.264	0.266	0.281	0.233	0.225	0.240	0.272	0.248	0.252	0.279	-	0.269	0.302	0.314	0.331
16	*Eucarinogammarus wagi*	0.155	0.242	0.198	0.221	0.227	0.222	0.225	0.157	0.160	0.151	0.212	0.208	0.180	0.245	0.225	-	0.258	0.245	0.222
17	*Gammarus lacustris*	0.182	0.252	0.251	0.234	0.252	0.253	0.236	0.202	0.211	0.222	0.257	0.228	0.234	0.248	0.246	0.214	-	0.306	0.289
18	*Acanthogammarus victorii*	0.223	0.285	0.234	0.240	0.253	0.235	0.204	0.192	0.212	0.191	0.253	0.248	0.241	0.283	0.254	0.206	0.246	-	0.257
19	*Brandtia latissima*	0.201	0.265	0.218	0.245	0.258	0.238	0.237	0.187	0.205	0.205	0.251	0.227	0.226	0.272	0.264	0.189	0.236	0.214	-

Pairwise differences between taxa. Numbers above diagonal are Kimura corrected pairwise distances, numbers below are uncorrected pairwise distances (p).


A
B
C
D
E
F

[	10 20 30 40 50 60 70]	
Eulimnogammarus vittatus	CACTTTATACTCCATTTTAGGTGCTTGAGCTAGAATAGTCGGTACCTCTATAAGGGTAATTATCCGATCT	[70]
Eulimnogammarus maacki2	NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN	[70]
Eulimnogammarus cruentus	NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN......T..G...	[70]
Eulimnogammarus maacki4	...A.....T.TT.....TR.G........CG.......G.CACTTCTAT....A........T..G...	[70]
Poekilogammarus pictus	NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN....T..A	[70]
Micruropus wahli	G..CC.T....TT..CC.......A........CG.T..A..A..AG..T....A..T........C..A	[70]
Pallasea kessleri	T...C....T.TT..GC.C...TTA..SR.......GT.A...T.T..A.....AC.T..CC.A.....A	[70]
Pallasea baikali	T..GC.T..T.TT..GC....CTTA..GT....C........G..T........AC.G..CC.G.....A	[70]
Odontogammarus calceratus	NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN	[70]
Ommatogammarus albinus	NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN	[70]
Acanthogammarus victorii	NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN	[70]
Acanthogammarus maximus	...GC....T.TT..A.....A.................A..............A...........C...	[70]
Gmelinoides fasciatus	NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN	[70]
Macrohectopus branickii	A...A..NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN	[70]
Eucarinogammarus wagi	...C.....T.TT........G..........................C.....A........N..C..C	[70]
Brantia latissima	...C.....T.TT........G..C..............A..C..T........C...........G...	[70]
Gammarus lacustris	G..A.....T.TTG.......G..A.........G.T..T.....T...C.T..C..G........G..A	[70]
Pontoporeia sp.	NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN	[70]
Cymadusa compta	A..A.....T.TT........A.GG.....AG.......A..AG.T...T.G..AA.......T...A..	[70]

[	80 90 100 110 120 130 140]	
Eulimnogammarus vittatus	GAGTTAAGAACACCTGGTAATTTAATCGGAGACGATCAACTATATAATGTTATAGTGACAGCTCACGCTT	[140]
Eulimnogammarus maacki2	NNNNNNNNNNNNNNNNNNNNNNA...T..C.....C...T....C.....C........T..A..T..C.	[140]
Eulimnogammarus cruentus	R.A.....GG.C..A.....C.....T..T..T..C........C..C..C..G..A..C..G.......	[140]
Eulimnogammarus maacki4	..A.....GG..........C.....T.....T..C..G..G........C..G.....T..C.......	[140]
Poekilogammarus pictus	...C.....G.T..C....GC..............C........C..C..G.....A........T....	[140]
Micruropus wahli	...C...T.G.T..A.....C.....A........C........C........G.....G..........	[140]
Pallasea kessleri	..AC....TT.........GAC..........T..C..R........C..A........C.....TSYB.	[140]
Pallasea baikali	..AC.T...G.C.....C.GC..........T...C...T..........A.....A.....C..T....	[140]
Odontogammarus calceratus	NNNNNNNNNNNN..A.....CC.G..............G.......................C.......	[140]
Ommatogammarus albinus	NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN...A.....C..T..C.	[140]
Acanthogammarus victorii	...C.....G.C..G..G..CC....T..G..T..C..GT.G........C.....C..T..C..T....	[140]
Acanthogammarus maximus	..AC.....G.C..A.....C.....T.....T..C...........C........A........T....	[140]
Gmelinoides fasciatus	NNNNNNNNNNNNNNA....T.C....G..G..T..C..G..C..............C.....A.......	[140]
Macrohectopus branickii	NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN.C..G..N..C.....C.....C.	[140]
Eucarinogammarus wagi	.........G.C..C..C........T..T..T..C...T.............G........C.......	[140]
Brantia latissima	.........G.G....A......G.....C.....C..G..G........A........T..C..T....	[140]
Gammarus lacustris	...C.G..CG.C........C...........T..C.....T..C..C..A.....T..T........C.	[140]
Pontoporeia sp.	N.......CG.C..A.....C..............C...G....C..C..C........G..C..T....	[140]
Cymadusa compta	..A...........A..A..C.....T..T..T..C...A.T......AC...T..A.....C..T....	[140]

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[                                150       160       170       180       190       200       210]

Eulimnogammarus vittatus     TTGTTATAATTTTTTTCATAGTAATACCTATTATAATCGGGGGTTTTGGTAACTGGTTAGTTCCTTTGAT   [210]
Eulimnogammarus maacki2      ....A..........T.................G........G........T..A.....C.....A..   [210]
Eulimnogammarus cruentus     ................T.....G..G.................A..C.........AC.G........A..   [210]
Eulimnogammarus maacki4      ................T.......................C..A..C.....T...C..........A..   [210]
Poekilogammarus pictus       ................TG..........A.....G..T.....G........T..AC..........A..   [210]
Micruropus wahli             .C........C.....T..G.....G...........T.....A.....A..T..A..GACC.....A..   [210]
Pallasea kessleri            B..............T.....T..G...........T..A..G............C....A.....A..   [210]
Pallasea baikali             ..A....G........T.....................T..A..G..C.........C..A.C..C..A..   [210]
Odontogammarus calceratus    ....C...........T.....T.............T.....A..C..A..T.............A..   [210]
Ommatogammarus albinus       ....A...........T.....................T..A.....C.....T..AC....A..G..A..   [210]
Acanthogammarus victorii     ..A.C...........T..G..T..G.....C..G..T..C..G.....C.............A..A..   [210]
Acanthogammarus maximus      ................T.......................A..G............C....A.....A..   [210]
Gmelinoides fasciatus        ..A....G.....C........G.....A..C.....T..............T..A...ACC.....A..   [210]
Macrohectopus branickii      .CNCA...........T.....TN.G..NN.......K.....C............AC....C...C.T..   [210]
Eucarinogammarus wagi        ..A.............T.....T.....A........T..A..A........T..A..G..A.....A..   [210]
Brantia latissima            ................T.....T.....G..C........A..A............AC....C.....A..   [210]
Gammarus lacustris           ................T.....C..G.....C.....T..A..G..C..C.....AC....G.....A..   [210]
Pontoporeia sp.              ....A........C..T.....T.....A........T..............T........A.....A..   [210]
Cymadusa compta              ....A...........T.....................T..A..A........T..A............A..   [210]
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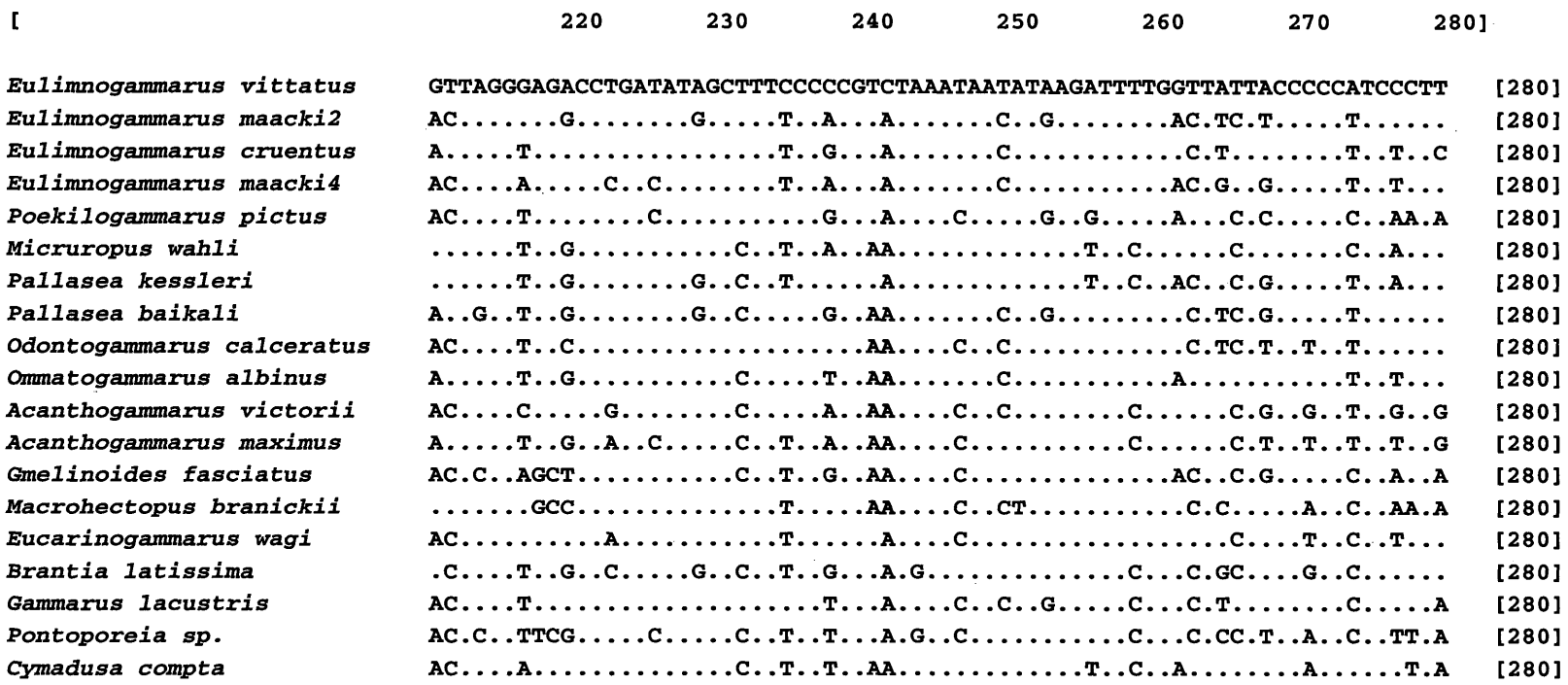
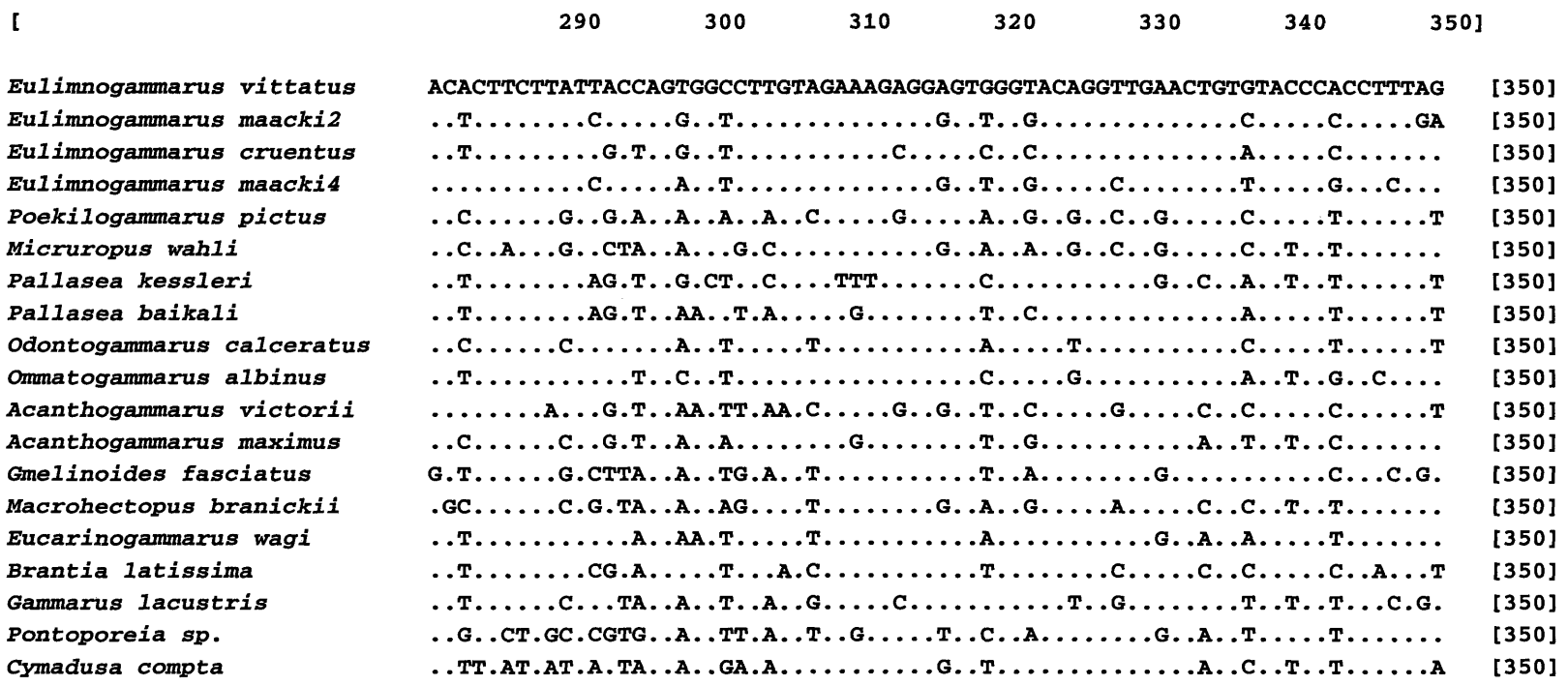

[220 230 240 250 260 270 280]
Eulimnogammarus vittatus GTTAGGGAGACCTGATATAGCTTTCCCCCGTCTAAATAATATAAGATTTTGGTTATTACCCCCATCCCTT [280]
Eulimnogammarus maacki2 AC.....G.....G.....T..A...A.....C..G.....AC.TC.T.....T..... [280]
Eulimnogammarus cruentus A.....T.....T..G...A.....C.....C.T.....T..T..C [280]
Eulimnogammarus maacki4 AC....A.....C..C.....T..A...A.....C.....AC.G..G.....T..T... [280]
Poekilogammarus pictus AC....T.....C.....G...A....C.....G..G.....A...C.C.....C..AA.A [280]
Micruropus wahli .....T..G.....C..T..A..AA.....T..C.....C.....C..A... [280]
Pallasea kessleri .....T..G.....G..C..T.....A.....T..C..AC..C.G.....T..A... [280]
Pallasea baikali A..G..T..G.....G..C.....G..AA.....C..G.....C.TC.G.....T..... [280]
Odontogammarus calceratus AC....T..C.....AA....C..C.....C.TC.T..T..T..... [280]
Ommatogammarus albinus A.....T..G.....C.....T..AA.....C.....A.....T..T... [280]
Acanthogammarus victorii AC....C.....G.....C.....A..AA....C..C.....C.....C.G..G..T..G..G [280]
Acanthogammarus maximus A.....T..G..A..C.....C..T..A..AA....C.....C.....C.T..T..T..T..G [280]
Gmelinoides fasciatus AC.C..AGCT.....C..T..G..AA....C.....AC..C.G.....C..A..A [280]
Macrohectopus branickii .....GCC.....T.....AA....C..CT.....C.C.....A..C..AA.A [280]
Eucarinogammarus wagi AC.....A.....T.....A....C.....C....T..C..T... [280]
Brantia latissima .C....T..G..C.....G..C..T..G...A.G.....C...C.GC....G..C..... [280]
Gammarus lacustris AC....T.....T...A....C..C..G.....C...C.T.....C.....A [280]
Pontoporeia sp. AC.C..TTCG.....C.....C..T..T...A.G..C.....C...C.CC.T..A..C..TT.A [280]
Cymadusa compta AC....A.....C..T..T..AA.....T..C..A.....A.....T.A [280]


[290 300 310 320 330 340 350]
Eulimnogammarus vittatus ACACTTCTTATTACCAGTGGCCTTGTAGAAAGAGGAGTGGGTACAGGTTGAACTGTGTACCCACCTTTAG [350]
Eulimnogammarus maacki2 ..T.....C.....G..T.....G..T..G.....C.....C.....GA [350]
Eulimnogammarus cruentus ..T.....G.T..G..T.....C.....C..C.....A.....C..... [350]
Eulimnogammarus maacki4 .....C.....A..T.....G..T..G.....C.....T.....G...C... [350]
Poekilogammarus pictus ..C.....G..G.A..A..A..C.....G.....A..G..G..C..G.....C.....T.....T [350]
Micruropus wahli ..C..A...G..CTA..A...G.C.....G..A..A..G..C..G.....C..T..T..... [350]
Pallasea kessleri ..T.....AG.T..G.CT..C....TTT.....C.....G..C..A..T..T.....T [350]
Pallasea baikali ..T.....AG.T..AA..T.A.....G.....T..C.....A.....T.....T [350]
Odontogammarus calceratus ..C.....C.....A..T.....T.....A.....T.....C.....T.....T [350]
Ommatogammarus albinus ..T.....T..C..T.....C.....G.....A..T..G..C.... [350]
Acanthogammarus victorii .....A...G.T..AA.TT.AA.C.....G..G..T..C.....G.....C..C.....C.....T [350]
Acanthogammarus maximus ..C.....C..G.T..A..A.....G.....T..G.....A..T..T..C..... [350]
Gmelinoides fasciatus G.T.....G.CTTA..A..TG.A..T.....T..A.....G.....C...C.G. [350]
Macrohectopus branickii .GC.....C.G.TA..A..AG....T.....G..A..G.....A.....C..C..T..T..... [350]
Eucarinogammarus wagi ..T.....A..AA.T.....T.....A.....G..A..A.....T..... [350]
Brantia latissima ..T.....CG.A.....T...A.C.....T.....C.....C..C.....C..A...T [350]
Gammarus lacustris ..T.....C...TA..A..T..A..G.....C.....T..G.....T..T..T...C.G. [350]
Pontoporeia sp. ..G..CT.GC.CGTG..A..TT.A..T..G.....T..C..A.....G..A..T.....T..... [350]
Cymadusa compta ..TT.AT.AT.A.TA..A..GA.A.....G..T.....A..C..T..T.....A [350]

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[                                  360        370        380        390        400        410        420]

Eulimnogammarus vittatus      CAGGAGCCATAGCCCATAGAGGAGGGAGTGTCGATCTAGCTATTTTTTCTCTTCATTTAGCGGGGGCCTC   [420]
Eulimnogammarus maacki2       GG...A..G.G........C......GC...T..C..G..............A........A........   [420]
Eulimnogammarus cruentus      .G...A.T.....T...........CGC...T...T..................C..G..T........   [420]
Eulimnogammarus maacki4       .G..T.TAG....T..C.....T..AGC...T..C..G..............A...C.G..A........   [420]
Poekilogammarus pictus        ....GT..........C..T....CTGC...A..CT.............A........G..C..C.....   [420]
Micruropus wahli              .TA.CAAT.....T.....C..G.CAGCA..T..C..............CT.A...C.T..C..A..T..   [420]
Pallasea kessleri             ..A..T.T..T..T..C..C...A.CGCC..A........A.....C..C.....CC.C..C..T..T..   [420]
Pallasea baikali              .TA.G....C...T........TA.AGC...T..C...........C..A.....C.....A..T..T..   [420]
Odontogammarus calceratus     ......TA.CT..T.....T..TA.AGCG..A...T..................CC.G..C.....A..   [420]
Ommatogammarus albinus        .C..T.TA.....T.....T..TA.AGCC..G..C..G.....................A.....T..   [420]
Acanthogammarus victorii      ..A..CTA..........G..T..AGC...G.............C..A..A..CC....C.....T..   [420]
Acanthogammarus maximus       .T..T.TA.CG..........TA.AGCC..A..CT.............A..A........C..A..T..   [420]
Gmelinoides fasciatus         .TA..AATG....T.....G..GCCAGCA..T...T.............GT.G...C....T..A.....   [420]
Macrohectopus branickii       .G....TAGCT.....C..G..T..AGCC..A..C..G...........C.....C..G..C..A..T..   [420]
Eucarinogammarus wagi         .T..C.TA.....T..C.....TA.AGC...A...T.............C......A....T..A..T..   [420]
Brantia latissima             ....T.TT.CT..T.....G..TA.AGC...T...A.G...........G..C...C....A..A.....   [420]
Gammarus lacustris            .T.....A.CC........G.....TGC...A..CT..........C........C..G..T.....T..   [420]
Pontoporeia sp.               .G.CTA.AGCC..T..CG.G..C..CTC...T...T.......C.....A..A..C.....C..C..TAG   [420]
Cymadusa compta               G......A..T..T...........TGCA..T...T..........C..............A.....AAG   [420]
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[                                  430        440        450        460        470        480        490]

Eulimnogammarus vittatus     TTCTATTTTAGGGGCTGTGAATTTTATCTCTACAATCATTAATATGCGTAGACCTGGTATGACAATAGAC   [490]
Eulimnogammarus maacki2      ..................A........T........T........A........A..C....G.......   [490]
Eulimnogammarus cruentus     C.................A..........C.....T........A.....G..C.....A.G.......   [490]
Eulimnogammarus maacki4      ..................A..........C..........C.....C..G..AT....A.G.......   [490]
Poekilogammarus pictus       .......C.G.....C..A.....................C..A..AGCG..C.......GG......   [490]
Micruropus wahli             ......CC....T..AA.C..C.....T..A..T..T..C..........C...CA...AT.TT....G   [490]
Pallasea kessleri            A.....C........C..A..........A..TGCT..................AA..A.A.......   [490]
Pallasea baikali             C.....C..........A.............CTC.G..........GC......G..A.A.G.....   [490]
Odontogammarus calceratus    C.................A..........C.....T........A.............A.G.......   [490]
Ommatogammarus albinus       C.....C.....C..A..A........T........T..C.....A.....G..C.....RGG.......   [490]
Acanthogammarus victorii     G......C..........A..C..........T..........A..A..C...A....A.G.T.....   [490]
Acanthogammarus maximus      A......C..........A..........C..T..........A.....C.....C..A.G.......   [490]
Gmelinoides fasciatus        .........G..T..AA.T..........C..T...T.......A.AA..C..C.....A.AT.....A   [490]
Macrohectopus branickii      C..C...C.......C..T..N..........G.AC....C...AACCT..AA...G..T.G......   [490]
Eucarinogammarus wagi        C......C....T.....A........T........T.............G......G.A.G.......   [490]
Brantia latissima            C.................A..C......A.A..T..TT.A..C..A..GG.G..C...T.A...T.....   [490]
Gammarus lacustris           ......C.....T...A.C..C........C..CG.GC.A..........T..G......C........   [490]
Pontoporeia sp.              A..A..CC........A.T.............TG.G.....C.....GGCC..G..A..AT.T......   [490]
Cymadusa compta              A..A..........CA.T........T......G.T........A..AGCTGAAAA...ATTT......   [490]
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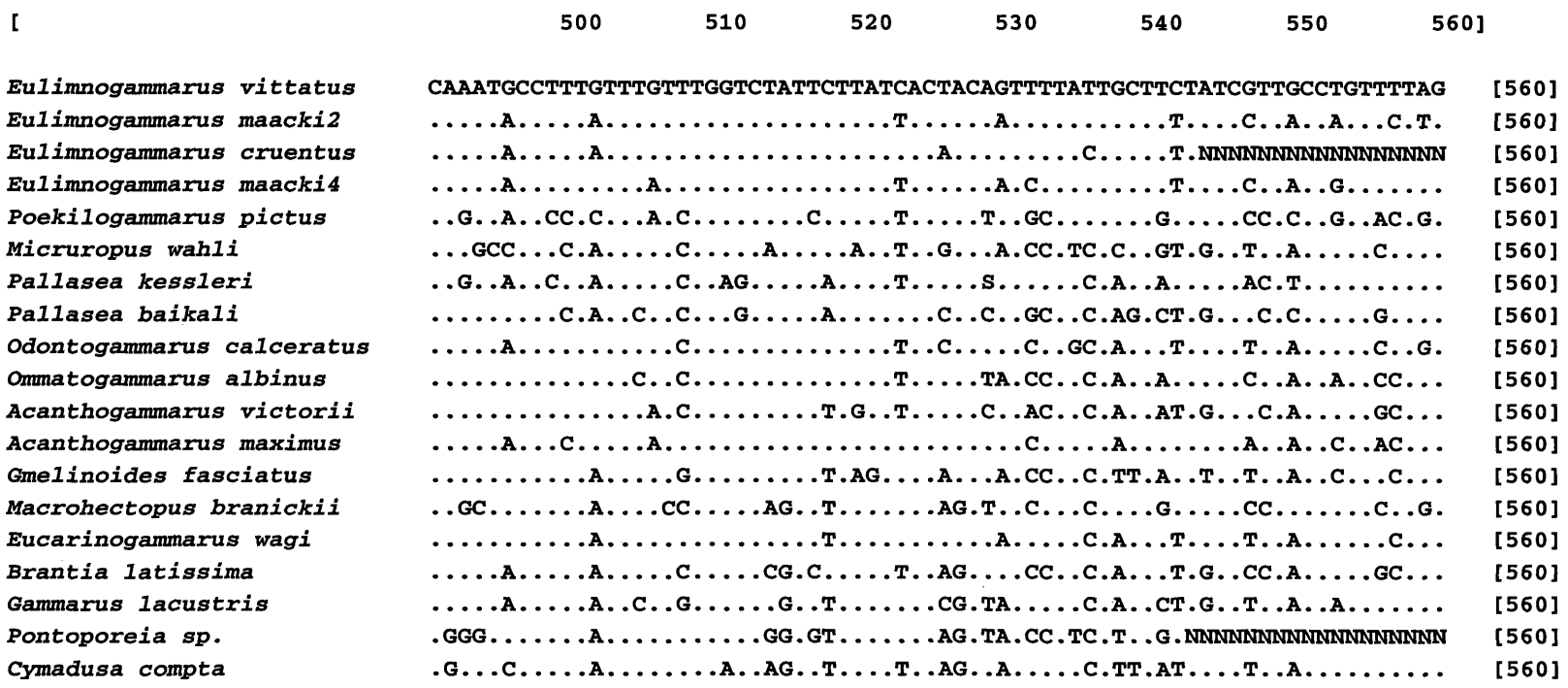
[500 510 520 530 540 550 560]
Eulimnogammarus vittatus CAAATGCCTTTGTTTGTTTGGTCTATTCTTATCACTACAGTTTTATTGCTTCTATCGTTGCCTGTTTTAG [560]
Eulimnogammarus maacki2 .....A.....A.....T.....A.....T....C..A..A...C.T. [560]
Eulimnogammarus cruentus .....A.....A.....A.....C.....T.NNNNNNNNNNNNNNNNN [560]
Eulimnogammarus maacki4 .....A.....A.....T.....A.C.....T....C..A..G..... [560]
Poekilogammarus pictus ..G..A..CC.C...A.C.....C.....T.....T..GC.....G.....CC.C..G..AC.G. [560]
Micruropus wahli ...GCC...C.A.....C.....A.....A..T..G...A.CC.TC.C..GT.G..T..A.....C.... [560]
Pallasea kessleri ..G..A..C..A.....C..AG.....A....T.....S.....C.A..A.....AC.T..... [560]
Pallasea baikali .....C.A..C..C...G.....A.....C..C..GC..C.AG.CT.G...C.C.....G.... [560]
Odontogammarus calceratus .....A.....C.....T..C.....C..GC.A...T....T..A.....C..G. [560]
Ommatogammarus albinus .....C..C.....T.....TA.CC..C.A..A.....C..A..A..CC... [560]
Acanthogammarus victorii .....A.C.....T.G..T.....C..AC..C.A..AT.G...C.A.....GC... [560]
Acanthogammarus maximus .....A...C.....A.....C.....A.....A..A..C..AC... [560]
Gmelinoides fasciatus .....A.....G.....T.AG....A...A.CC..C.TT.A..T..T..A..C...C... [560]
Macrohectopus branickii ..GC.....A....CC.....AG..T.....AG.T..C...C....G.....CC.....C..G. [560]
Eucarinogammarus wagi .....A.....T.....A.....C.A...T....T..A.....C... [560]
Brantia latissima .....A.....A.....C.....CG.C.....T..AG....CC..C.A...T.G..CC.A.....GC... [560]
Gammarus lacustris .....A.....A..C..G.....G..T.....CG.TA.....C.A..CT.G..T..A..A..... [560]
Pontoporeia sp. .GGG.....A.....GG.GT.....AG.TA.CC.TC.T..G.NNNNNNNNNNNNNNNNNN [560]
Cymadusa compta .G...C.....A.....A..AG..T....T..AG..A.....C.TT.AT....T..A..... [560]

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[                                                     570       580       590       600       610       620       630]

Eulimnogammarus vittatus     CTGGAGCTATTACTATACTACTTACTGACCGTAACCTAAATACTTCTTTTTTTGACCCTAGGGGAGGGGG  [630]
Eulimnogammarus maacki2      ..........C.....GT................T.....C..C....................G..A..  [630]
Eulimnogammarus cruentus     NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN  [630]
Eulimnogammarus maacki4      .C..C.....C......T..........................CT......GAC...AGT..CG.R..C  [630]
Poekilogammarus pictus       .......C..C......T.G.....A........TT....C..G...............GC...C.....  [630]
Micruropus wahli             .A.....CC.A........C.....A.....A..TA.C..C..C.....C........A..A.....A..  [630]
Pallasea kessleri            .C........C..............A..S.....TA.G.....A...........SM...RA........  [630]
Pallasea baikali             .C........C..C...........A........TA....C..G...........T..Y..T.....A..  [630]
Odontogammarus calceratus    ....G.....C...........A..A.................C..........................  [630]
Ommatogammarus albinus       .C..G.....C......T.......A..T...........C..C.................T........  [630]
Acanthogammarus victorii     .A..G........A.....G..C..A..T..............C..C..............T.....A..  [630]
Acanthogammarus maximus      ..........C..C...T.......A...........T..C..A...........T.....A........  [630]
Gmelinoides fasciatus        .A.....CT.A..C..G..T..C..A.....A...ACC..C..A..A..............C..G..A..  [630]
Macrohectopus branickii      .A........C..A.....C..G..A........TG.T..C........C..C.....CG.A..G.....  [630]
Eucarinogammarus wagi        .G...............T.......G..............C..A..A...........G..T..G.....  [630]
Brantia latissima            .C..G...........G..T.....G.....C...........G....................G..C..  [630]
Gammarus lacustris           .C........C.....GT..T.A........A..TT....C..A.....C..............T..A..  [630]
Pontoporeia sp.              NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN  [630]
Cymadusa compta              .A..G..A.........T..T.A..A..T.....TT...................T...TTA.....A..  [630]
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[                                   640        650        ]

Eulimnogammarus vittatus      GGACCNTATTCTATACCAGCACTTATTTT   [659]
Eulimnogammarus maacki2       T....NA...........A..T.....C.   [659]
Eulimnogammarus cruentus      NNNNNNNNNNNNNNNNNNNNNNNNNNNNN  [659]
Eulimnogammarus maacki4       .AC.MA.TC....NNNNNNNNNNNNNNNN  [659]
Poekilogammarus pictus        T..T.CC...........A...C.C...N   [659]
Micruropus wahli              T....C............A..T......N   [659]
Pallasea kessleri             A..T.C...S........A..T......N   [659]
Pallasea baikali              A....CA..C........A.....G...N   [659]
Odontogammarus calceratus     C..T.CA..C..T.....A..T.......   [659]
Ommatogammarus albinus        C....CG.....C.....A..T.....C.   [659]
Acanthogammarus victorii      .....CA...........A..TC.G...N   [659]
Acanthogammarus maximus       .....C............A..T.......   [659]
Gmelinoides fasciatus         A....C......T.....A..T.....C.   [659]
Macrohectopus branickii       .....CA..C..GNNNNNNNNNNNNNNNN  [659]
Eucarinogammarus wagi         A..T.C............A..T.....C.   [659]
Brantia latissima             C..T.C......G.............N   [659]
Gammarus lacustris            .....CA...T.......A...C....C.   [659]
Pontoporeia sp.               NNNNNNNNNNNNNNNNNNNNNNNNNNNNN  [659]
Cymadusa compta               T....C....T.......A..TC..A..N   [659]
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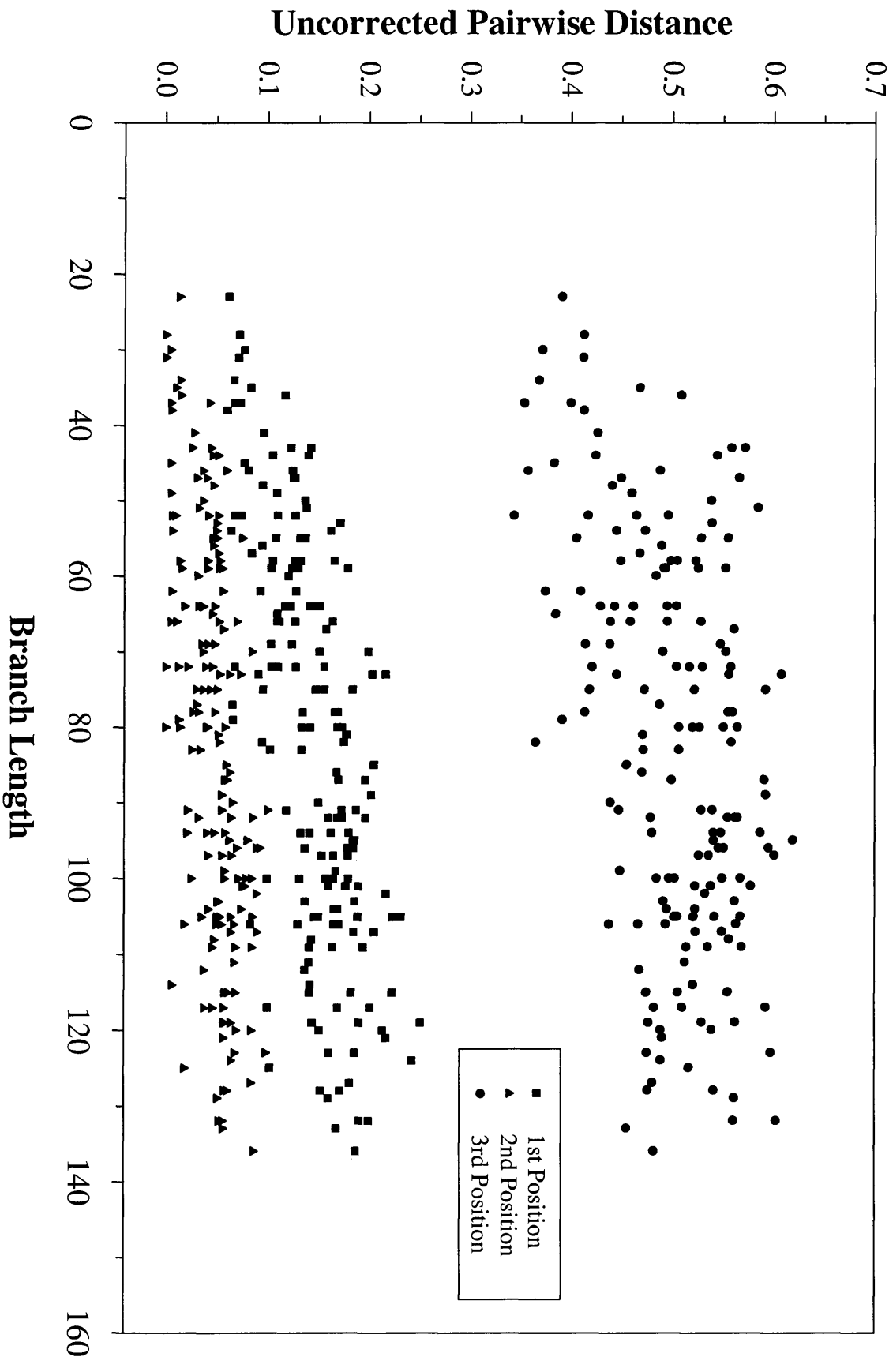
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0.2
0.3
0.4
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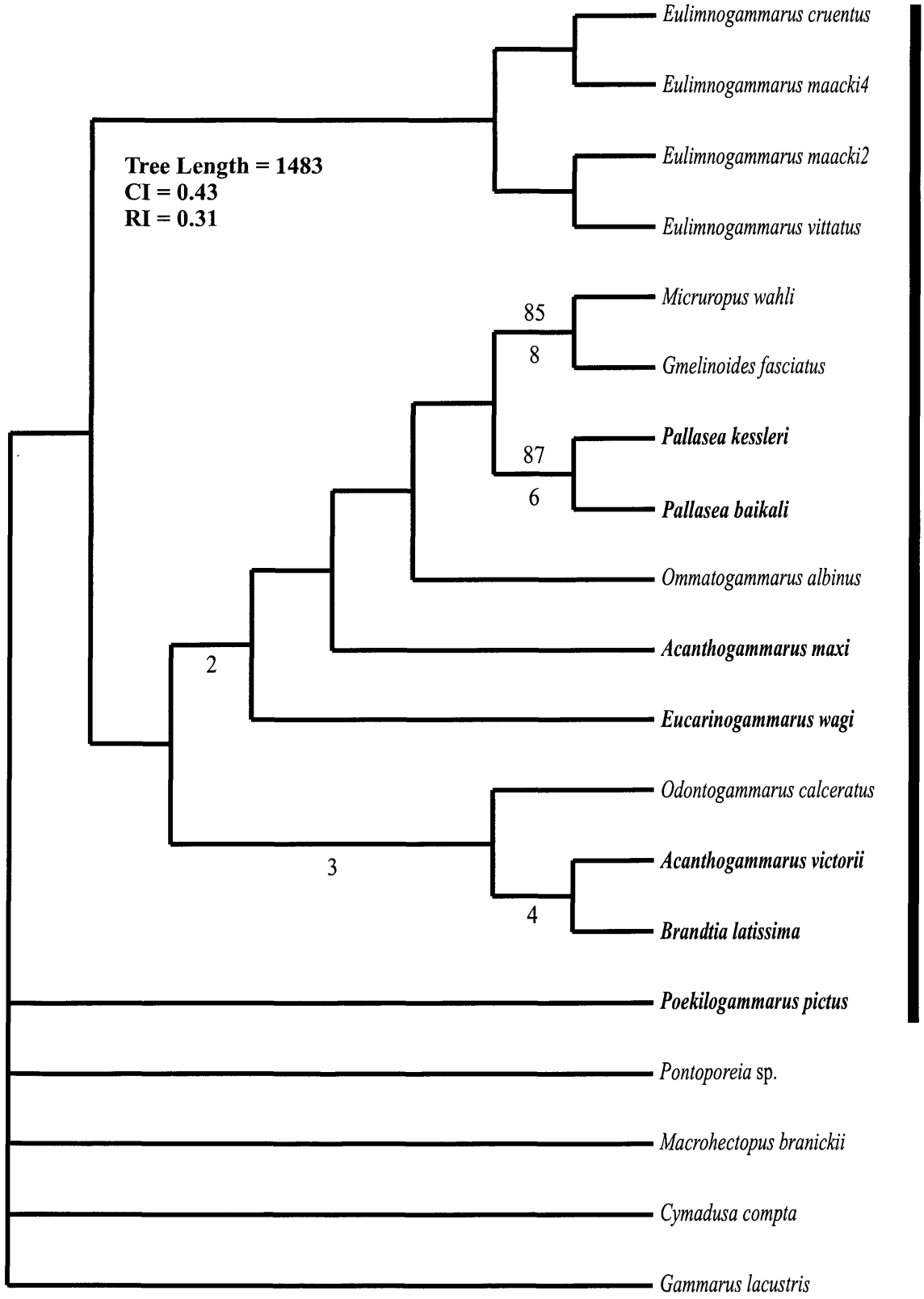
100
120
140
160
Branch Length
1st Position
2nd Position
3rd Position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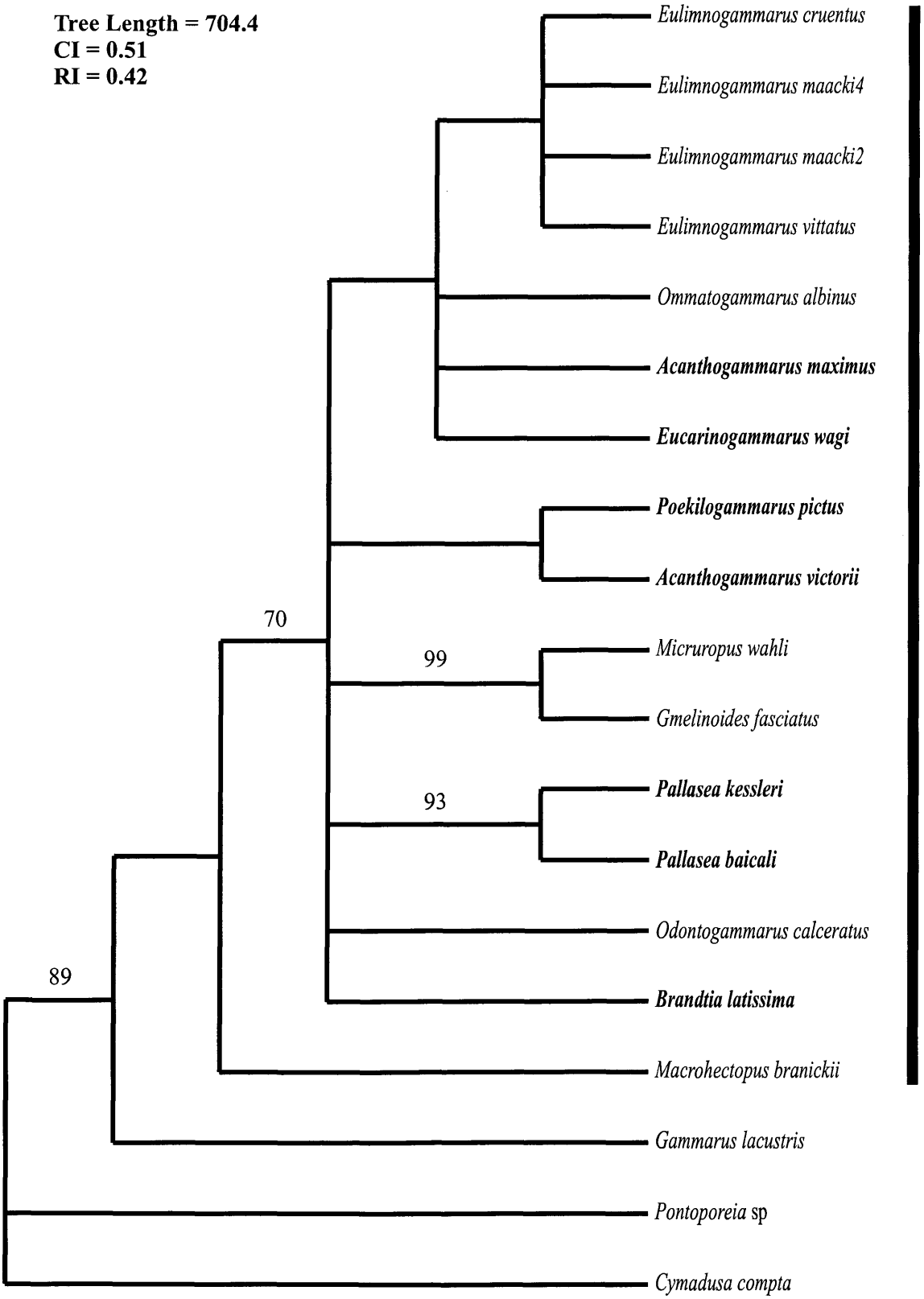
Eulimnogammarus maacki2
Eulimnogammarus vittatus
Micruropus wahli
Gmelinoides fasciatus
Pallasea kessleri
Pallasea baikali
Ommatogammarus albinus
Acanthogammarus maxi
Eucarinogammarus wagi
Odontogammarus calceratus
Acanthogammarus victorii
Brandtia latissima
Poekilogammarus pictus
Pontoporeia sp.
Macrohectopus branickii
Cymadusa compta
Gammarus lacustris
85
8
87
6
2
3
4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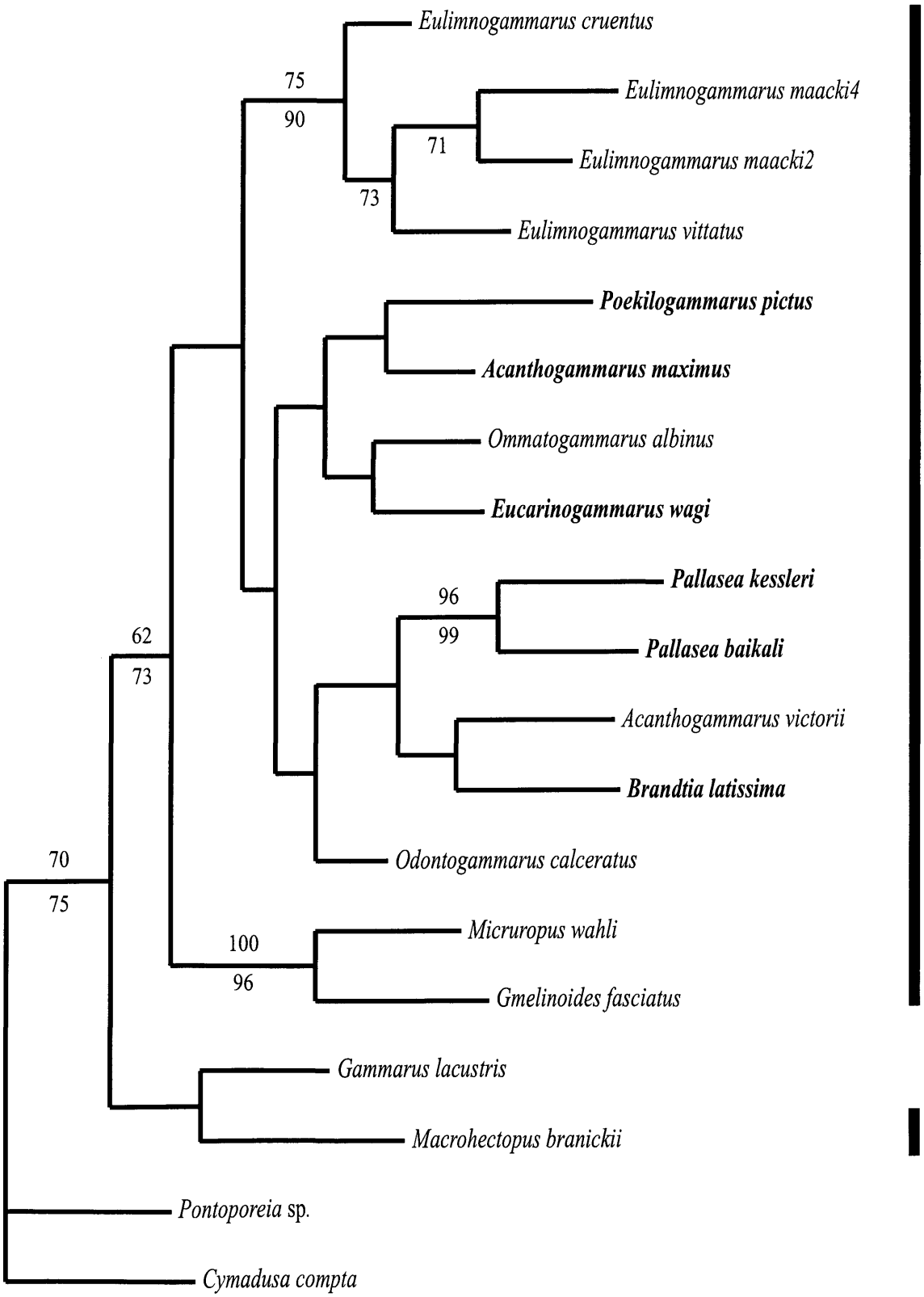
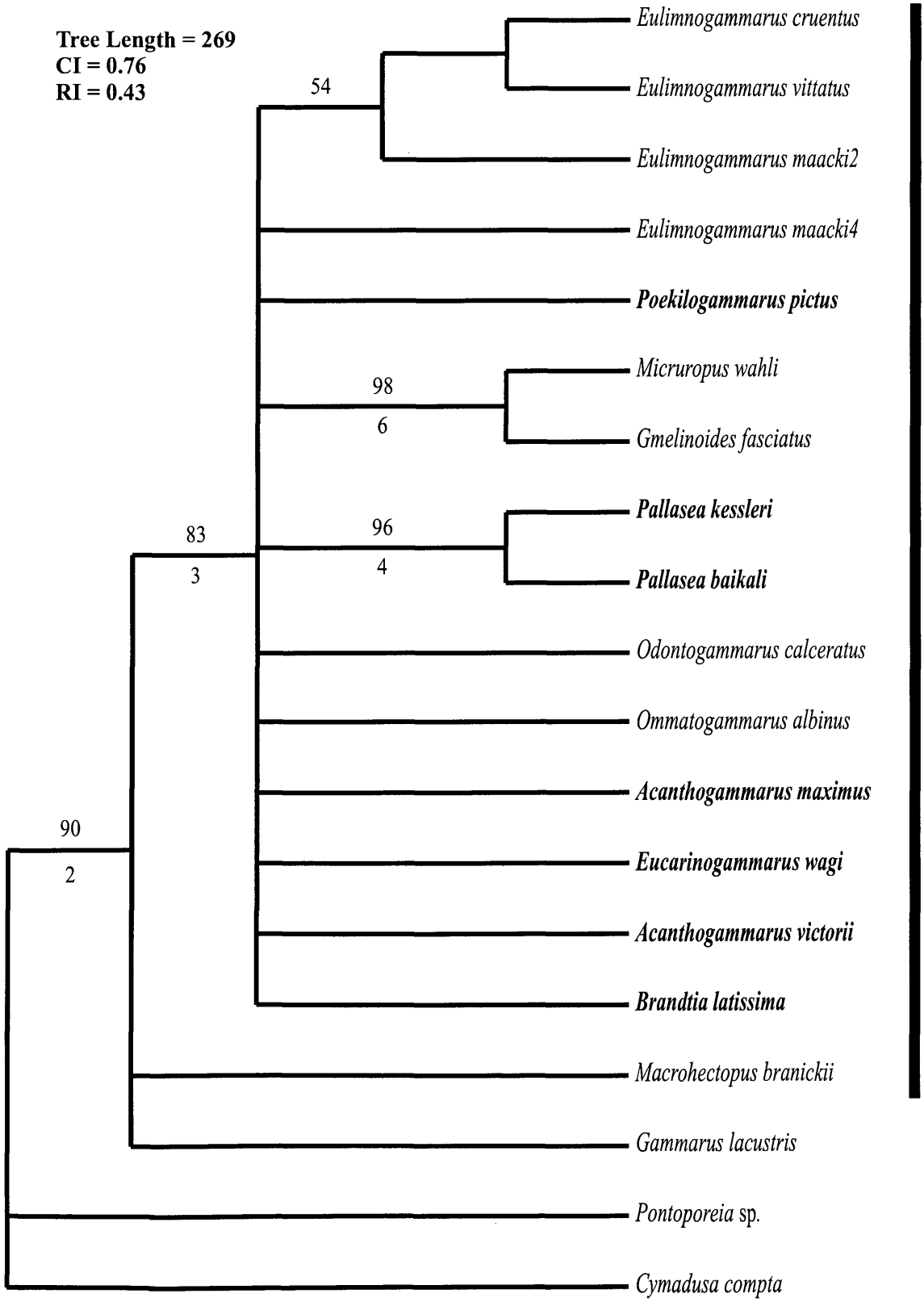
Eulimnogammarus maacki2
Eulimnogammarus vittatus
Ommatogammarus albinus
Acanthogammarus maximus
Eucarinogammarus wagi
Poekilogammarus pictus
Acanthogammarus victorii
Micruropus wahli
Gmelinoides fasciatus
Pallasea kessleri
Pallasea baicali
Odontogammarus calceratus
Brandtia latissima
Macrohectopus branickii
Gammarus lacustris
Pontoporeia sp
Cymadusa compta
70
99
93
89


Eulimnogammarus cruentus
75
90
Eulimnogammarus maacki4
71
Eulimnogammarus maacki2
73
Eulimnogammarus vittatus
Poekilogammarus pictus
Acanthogammarus maximus
Ommatogammarus albinus
Eucarinogammarus wagi
Pallasea kessleri
96
99
Pallasea baikali
62
73
Acanthogammarus victorii
Brandtia latissima
Odontogammarus calceratus
70
75
Micruropus wahli
100
96
Gmelinoides fasciatus
Gammarus lacustris
Macrohectopus branickii
Pontoporeia sp.
Cymadusa compta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54
98
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83
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96
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90
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Eulimnogammarus cruentus
Eulimnogammarus vittatus
Eulimnogammarus maacki2
Eulimnogammarus maacki4
Poekilogammarus pictus
Micruropus wahli
Gmelinoides fasciatus
Pallasea kessleri
Pallasea baikali
Odontogammarus calceratus
Ommatogammarus albinus
Acanthogammarus maximus
Eucarinogammarus wagi
Acanthogammarus victorii
Brandtia latissima
Macrohectopus branickii
Gammarus lacustris
Pontoporeia sp.
Cymadusa compta

VITA

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Born in Washington, D.C., 5 September, 1971. Graduated from Walkersville High School, Walkersville, MD in 1989. Earned B.S. in Biological Sciences from the University of Alaska Fairbainks in 1995. Entered master's program at the College of William and Mary, School of Marine Science in 1995. Entered Ph.D. program at the College of William and Mary, School of Marine Science in 1999.