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Molecular and morphological evolution of the amphipod radiation of Lake Baikal

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Abstract

Lake Baikal, in Siberia, Russia, contains the highest biodiversity of any extant lake, including an impressive radiation of gammaroidean amphipods that are often cited as a classic case of adaptive radiation. However, relationships among Baikal's amphipods remain poorly understood. The phylogenetic history of 32 Lake Baikal amphipod species, representing most major lineages of the endemic fauna, was examined using three genes (COI, 16S rRNA, and 18S rRNA), and 152 morphological characters. Results support monophyly of the largest and most diverse of the Baikalian families, the Acanthogammaridae. Analyses suggest that a second Baikalian family, the fossorial Micruropodidae, is paraphyletic and composed of two divergent clades, one of which includes *Macrohectopus branickii*, a morphologically specialized pelagic planktivore traditionally assigned its own family. The extreme morphological and ecological divergence of *Macrohectopus* from its close genetic relatives, and conversely, the large genetic distances among other morphologically similar micruropodids, suggest that morphological and molecular evolution have often been uncoupled during the radiation of Baikal's amphipods. This study suggests that the amphipod fauna of Lake Baikal is polyphyletic; originating from two independent invasions of the lake.

Keywords: COI; 16S rRNA; 18S rRNA; Morphology; Amphipoda; Lake Baikal; Gammaroidea

1. Introduction

The Siberian Lake Baikal is the oldest lake in the world and has the most diverse fauna and the highest level of endemism of any extant lake (Kozhov, 1963; Martin, 1994). Lake Baikal is also the world's deepest (1637 m maximum depth), and volumetrically largest lake, containing 20% of the planet surface's liquid fresh water (Martin, 1994). Baikal likely originated around 72 million years ago (ma) as a series of scattered, shallow, marsh-like lakes. A permanent lake probably originated around 27 ma, but it was not until the last 3 ma that the lake substantially deepened and became the cold, extremely deep lacustrine environment that exists today (Mats et al., 2000). Baikal's great age and geological isolation likely have been important in generating its impressive endemic fauna, which contains radiations from several disparate taxa, including the Cottoidei (sculpins), Ostracoda, Rhabdocoela and Tricladida (flatworms), Copepoda, Gastropoda, and Amphipoda (Bazikalova, 1945; Brooks, 1950; Kozhov, 1963; Martin, 1994; Sherbakov, 1999).

Because of the great number of species (⩾265; Kamaltynov, 1999b), as well as their morphological and ecological diversity, amphipods are often considered the most remarkable of Lake Baikal's radiations. The endemic Baikalian amphipods represent a substantial

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and distinctive portion of the superfamily Gammaroidea, a large, diverse, and cosmopolitan amphipod group that is ecologically important in fresh and coastal waters throughout the northern hemisphere (Barnard and Barnard, 1983; Bousfield, 1977, 1982). The Baikalian gammaroideans currently are divided into 51 genera and 265 species (Kamaltynov, 1999b), although some species appear to be cryptic species complexes (Väinölä and Kamaltynov, 1999). Baikal's amphipod fauna comprises over 40% of the world gammaroidean species, and its species are extremely diverse morphologically, ranging from generalized forms (Fig. 1B), similar to the cosmopolitan freshwater genus *Gammarus* (Fig. 1F), to highly armored, processiferous forms (Fig. 1C), to a uniquely specialized pelagic gammaroidean (Fig. 1A). They are also ecologically diverse, with benthic, fossorial, and nektonic forms, including the world's only pelagic gammaroidean (Freyer, 1991; Kozhov, 1963). In addition to the benthic detritivore habit common among gammaroideans and the pelagic planktivore, there are also predators and parasites (Bazikalova, 1945; Freyer, 1991; Kozhov, 1963).

Taxonomically, amphipods have a history of instability (Bousfield and Shih, 1994). In fact, the higher-level relationships within Amphipoda as a whole are so uncertain that several taxonomic treatments simply list families alphabetically (Barnard and Barnard, 1983; Barnard and Karaman, 1975; Martin and Davis, 2001). Especially problematic are the amphipods of the superfamily Gammaroidea, in which high morphological diversity, apparent morphological convergence of unrelated lineages in similar environments (e.g., fossorial amphipods), and evolutionary plasticity of many characters lead to great difficulties in identification of homology (Barnard and Barnard, 1983; Barnard and Karaman, 1975; Bousfield, 1977; Pinkster, 1983). The Baikal amphipods in particular are by far the most morphologically diverse gammaroideans (Bousfield, 1982; Brooks, 1950; Kozhov, 1963),

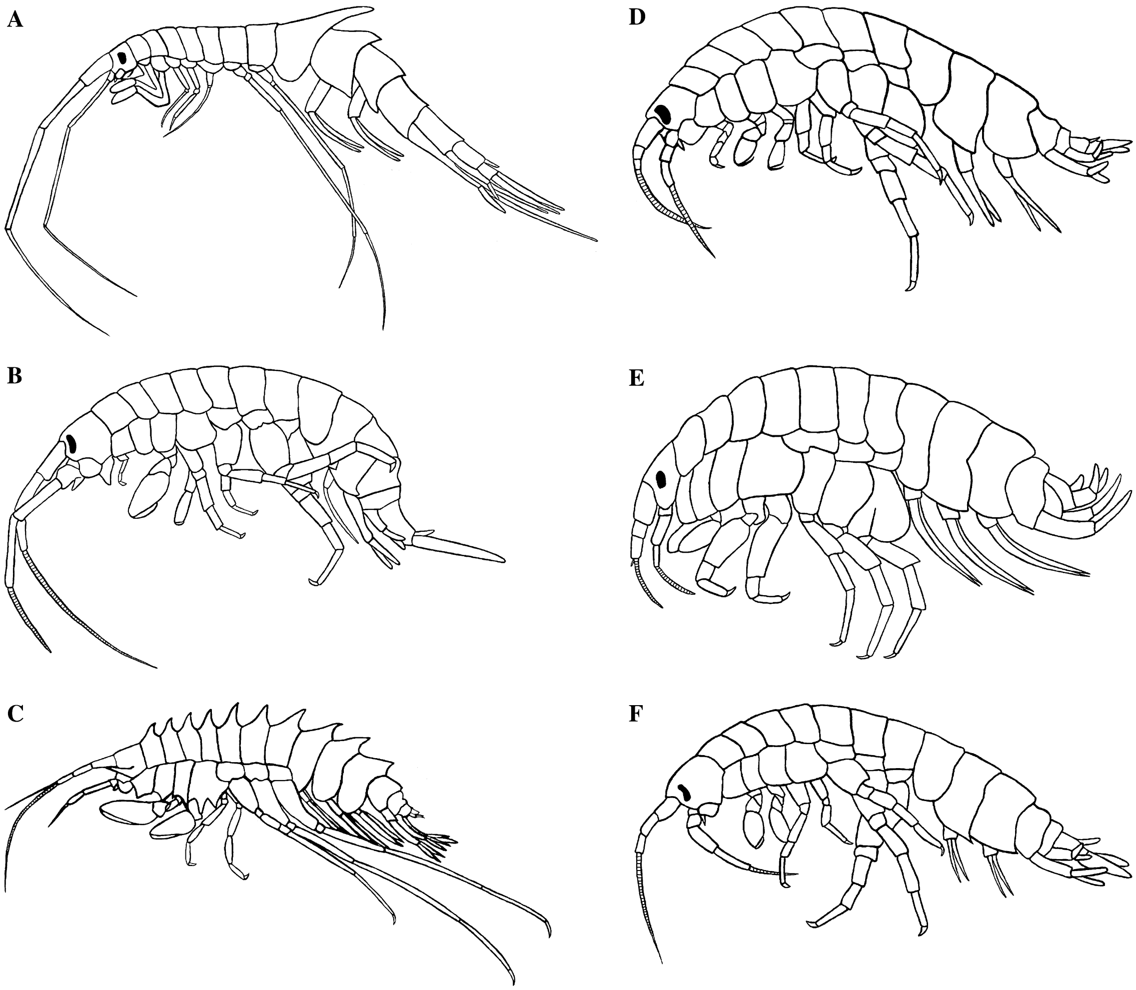

Fig. 1. Representatives from the three major lineages (Kamaltynov, 1999b) of Baikalian amphipods and a Eurasian *Gammarus* species. (A) *Macrohectopus branickii* (Macrohectopidae). (B) *Eulimnogammarus cruentus* (Acanthogammaridae). (C) *Acanthogammarus grewingkii* (Acanthogammaridae) (D). *Micruropus wahli* (Micruropodidae) (E). *Crypturopus pachytus* (Micruropodidae) (F). *Gammarus duebeni* (Gammaridae) (A, C, E modified from Barnard and Barnard, 1983).

consequently there has been much contention concerning their classification and phylogenetic relationships (Barnard and Barnard, 1983; Bazikalova, 1945; Bousfield, 1977; Kamaltynov, 1999a,b). Thus, the taxonomic history of the Baikal amphipods is long, unstable, and currently not well resolved.

The most recent classification of Baikal's amphipods is by Kamaltynov (1999b), who placed all Baikal amphipods into the superfamily Gammaroidea, and assigned them to four families. The largest family is the endemic Acanthogammaridae. Whereas this family historically was restricted to the characteristic armored amphipods (Bazikalova, 1945; Bousfield, 1977, 1982; Kamaltynov, 1992), Kamaltynov's diagnosis of Acanthogammaridae now includes smooth-bodied, generalized amphipods with elongate antennae and pereopods and a dominant first gnathopod (see Fig. 1B), as well as the armored, carinate and/or processiferous amphipods, considered uniquely Baikalian (Fig. 1C). Unfortunately, there seem to be no diagnostic characters for Acanthogammaridae in this conception. Kamaltynov (1999b, p. 935) simply stated that this family is 'Distinguished from all other gammaroideans by high development and morphological diversity of body processes and body appendages.' The second Baikalian family is Micruropodidae. These amphipods are smooth-bodied, small, compact with shortened antennae, and pereopods, apparently adapted to their fossorial (burrowing) lifestyle (Figs. 1D and E). The third family, Macrohectopidae, is monotypic, comprising the species *Macrohectopus branickii*. This bizarre species is highly modified for a strictly pelagic lifestyle, with a streamlined body and elongated appendages (Fig. 1A). The fourth family is Pachyschesidae, a small group containing 16 species that are commensals or parasites in the marsupia of larger amphipods.

Only in the last several years have molecular studies attempted to address the phylogeny of the major amphipod groups within Lake Baikal. Ogarkov et al. (1997) examined phylogenetic relationships among selected Baikal gammaroideans using the mitochondrial cytochrome *c* oxidase subunit III (COIII) gene. They focused on two genera, namely *Pallasea*, an acanthogammarid, and *Eulimnogammarus*, at the time considered a member of the widespread family Gammaridae, but they did not include any non-Baikalian outgroups. Phylogenetic resolution was poor but the study did suggest that the family Acanthogammaridae (at the time comprising only the armored amphipods) was not monophyletic. A subsequent study by Sherbakov et al. (1998) examined the phylogeny of 18 selected amphipod taxa from Lake Baikal by sequencing a segment of the 18S rRNA gene. Their resultant phylogenetic hypothesis contained only a single well supported clade, unexpectedly uniting the unique, pelagic *Macrohectopus branickii* with *Gammarus pulex*, a morphologically generalized freshwater amphipod found through much of Europe. Finally, the most recent study was an update by Sherbakov et al. (1999), reexamining their previous 18S rRNA sequences and adding preliminary cytochrome *c* oxidase subunit I (COI) data. Their results were little changed from the 1998 paper, except to suggest that several of the genera within Lake Baikal (*Acanthogammarus*, *Pallasea*) may not be monophyletic. Unfortunately, no molecular study to date has produced sufficient resolution or support to elucidate either relationships within and among the families of amphipods in Lake Baikal, or the origin of Baikal's fauna by including data on relevant outgroup taxa.

A question of more general interest to evolutionary biology is how many invasions from nearby waters gave rise to the radiation of the current Baikalian amphipod fauna. It is universally accepted that Baikal's amphipods resulted from multiple invasions, and past estimates range from four to more than 18 (Barnard and Barnard, 1983; Bousfield, 1977, 1982; Brooks, 1950; Kamaltynov, 1992, 1999a; Kozhov, 1963; Ogarkov et al., 1997; Sherbakov, 1999; Sherbakov et al., 1998). However, these estimates have never been tested through rigorous phylogenetic analysis. Additionally, no studies on Baikal's amphipods have been conducted that combine morphological characters with molecular sequences in a cladistic framework. In this study, we examine the phylogenetic relationships among most major lineages of Baikalian amphipods using molecular data from portions of three genes and 152 morphological characters.

2. Materials and methods

2.1. Sampling

We sequenced portions of the mitochondrial 16S rRNA and cytochrome *c* oxidase subunit I (COI) genes, and two portions of the nuclear 18S rRNA gene, one at the 5′ end and the other at the 3′ end of the gene, for 62 amphipod species from Lake Baikal, northern Europe, the Ponto-Caspian region, and North America (Table 1). Additionally, 152 morphological characters for these same species were scored. Species were chosen to sample as broadly as possible the great diversity of Baikal's amphipods, and included several representative genera from each of Kamaltynov's (1999b) hypothesized families, except for the small, parasitic family, Pachyschesidae, which we were unable to obtain. Additionally, non-Baikalian gammaroidean amphipods were sampled, including several *Gammarus* and *Chaetogammarus* species (Gammaridae) from Eurasia and North America, species from the Caspian and Black Seas (Pontogammaridae), and members of Anisogammaridae, a family found exclusively along the Pacific Rim, to assist in clarifying the origin of Baikal's fauna.

The uncertainty of higher-level amphipod systematics makes the selection of an appropriate outgroup difficult.

Table 1
List of all species sequenced, sorted by taxonomic family (Kamaltynov, 1999a, 1999b), with sampled location and Genbank Accession numbers for all genes

	Collecting locality	Accession Nos.			
		COI	16S	18S-1	18S-3
Gammaroidea					
Acanthogammaridae					
Abludogammarus flavus	Listvyanka, Lake Baikal	—	AY926692	AY926752	AY926814
Abyssogammarus gracilis	Listvyanka, Lake Baikal	—	AY926693	AY926753	AY926815
Acanthogammarus brevispinus	Off Selenga Delta, Lake Baikal	AY926651	AY926694	AY926754	AY926816
Acanthogammarus victorii	Ushkani, Lake Baikal	AY926652	AY926695	AY926755	AY926817
Brandtia lata	Bolshoi Kotoye, Lake Baikal	AY926654	AY926698	AY926758	AY926820
Eulimnogammarus cruentus	Bolshoi Kotoye, Lake Baikal	AY926661	AY926709	AY926769	AY926831
Eulimnogammarus inconspicuous	Bolshoi Kotoye, Lake Baikal	AY926662	AY926710	AY926770	AY926832
Eulimnogammarus maacki	Ushkani, Lake Baikal	AY926663	AY926711	AY926771	AY926833
Eulimnogammarus testaceus	Olkhon, Kurkutskaya, Lake Baikal	—	AY926712	AY926772	AY926834
Eulimnogammarus verrucosus	Olkhon, Khorgojskaya, Lake Baikal	—	AY926713	AY926773	AY926835
Eulimnogammarus viridis	Bolshoi Kotoye, Lake Baikal	AY926664	AY926714	AY926774	AY926836
Eulimnogammarus viridulus	Ushkani, Lake Baikal	AY926665	AY926715	AY926775	AY926837
Eulimnogammarus vittatus	Olkhon, Khorgojskaya, Lake Baikal	AY926666	AY926716	AY926776	AY926838
Hakonboekia strauchi	Olkhon Gates, Baikal	AY926676	AY926731	AY926792	AY926853
Odontogammarus calcaratus	Listvyanka, Lake Baikal	AY926685	AY926739	AY926801	AY926862
Ommatogammarus albinus	Ushkani, Lake Baikal	AY926686	AY926740	AY926802	AY926863
Pallasea cancelloides	Ushkani, Lake Baikal	—	AY926741	AY926803	AY926864
Pallasea cancellus	Olkhon, Kharin-Irgi, Lake Baikal	AY926687	AY926742	AY926804	AY926865
Pallasea grubei	Maloye More, Lake Baikal	AY926688	AY926743	AY926805	AY926866
Pallasea viridis	Olkhon, Kharin-Irgi, Lake Baikal	—	AY926744	AY926806	AY926867
Parapallasea borowskii	Ushkani, Lake Baikal	—	AY926745	AY926807	AY926868
Plesiogammarus brevis	Off Selenga Delta, Lake Baikal	AY926689	AY926746	AY926808	AY926869
Poekilogammarus pictoides	Olkhon Gates, Baikal	AY926690	AY926747	AY926809	AY926870
Micruropodidae					
Carinogammarus ”sp”	Off Selenga Delta, Lake Baikal	—	AY926699	AY926759	AY926821
Crypturopus pachytus	Olkhon Gates, Baikal	—	AY926704	AY926764	AY926826
Gmelinoides fasciatus	Olkhon, Khorgojskaya, Lake Baikal	AY926675	AY926730	AY926791	AY926852
Micruropus crassipes	Olkhon Gates, Baikal	AY926680	AY926734	AY926796	AY926857
Micruropus fixseni	Olkhon Gates, Baikal	AY926681	AY926735	AY926797	AY926858
Micruropus glaber	Olkhon Gates, Baikal	AY926682	AY926736	AY926798	AY926859
Micruropus wahli	Ushkani, Lake Baikal	AY926683	AY926737	AY926799	AY926860
Pseudomicruropus rotundatulus	Ushkani, Lake Baikal	—	AY926750	AY926812	AY926873
Macrohectopidae					
Macrohectopus branickii	Bolshoi Kotoye, Lake Baikal	AY926677	AY926732	AY926793	AY926854
Gammaridae					
Chaetogammarus marinus	Bergin, Norway	AY926655	AY926700	AY926760	AY926822
Chaetogammarus obtusatus	Novia Scotia, CA	AY926656	AY926701	AY926761	AY926823
Chaetogammarus stoerensis	Maine, USA	AY926657	AY926702	AY926762	AY926824
Gammarus aequicauda	Black Sea	AY926667	AY926718	AY926778	AY926840
Gammarus annulatus	Massachusetts, USA	AY926668	AY926719	AY926779	AY926841
Gammarus balcanicus	Alma-Ata, Kazahstan	—	AY926720	AY926780	AY926842
Gammarus duebeni	Maine, USA	AY926669	—	AY926781	AY926843
Gammarus fasciatus	Washington DC, USA	—	AY926721	AY926782	AY926844
Gammarus lacustris BK	Olkhon Island, Lake Baikal	AY926671	AY926723	AY926784	AY926846
Gammarus lacustris HV	Lake Hovsgol, Mongolia	AY926670	AY926722	AY926783	AY926845
Gammarus lacustris VI	Vancouver Island, CA	AY926672	AY926724	AY926785	AY926847
Gammarus lacustris WA	Washington, USA	AY926673	AY926725	AY926786	AY926848
Gammarus locusta	Roscoff, FR	—	AY926726	AY926787	AY926849
Gammarus mucronatus	Chesapeake Bay, USA	—	AY926727	AY926788	AY926850
Gammarus oceanicus	Massachusetts, USA	AY926674	AY926728	AY926789	AY926851
Gammarus pseudolimnaeus	Virginia, USA	—	AY926729	AY926790	—
Anisogammaridae					
Eogammarus confervicolus	Columbia River, Washington, USA	AY926659	AY926707	AY926767	AY926829
Eogammarus oclairi	Vancouver Island, CA	AY926660	AY926708	AY926768	AY926830
Ramellogammarus vancouverensis	Vancouver Island, CA	—	AY926751	AY926813	AY926874

Table 1 (*continued*)

	Collecting locality	Accession Nos.			
		COI	16S	18S-1	18S-3
Pontogammaridae					
Amathillina pusilla	?	—	AY926696	AY926756	AY926818
Dikkerogammarus caspius	Caspian Sea	—	AY926705	AY926765	AY926827
Dikkerogammarus villosus	Black Sea	—	AY926706	AY926766	AY926828
Pontogammarus abbreviatus	Caspian Sea	AY926691	AY926748	AY926810	AY926871
Pontogammarus crassus	Black Sea	—	AY926749	AY926811	AY926872
Non-gammaroidean					
Melitidae					
Megomaera subtener	Washington, USA	AY926678	—	AY926794	AY926855
Melita nitida	Chesapeake Bay, USA	AY926679	AY926733	AY926795	AY926856
Crangonyctidae					
Crangonyx serratus	VA, USA	AY926658	AY926703	AY926763	AY926825
Gammarellidae					
Gammarellus angulosus	Maine, USA	—	AY926717	AY926777	AY926839
Pontoporeidae					
Monoporeia affinis	?	AY926684	AY926738	AY926800	AY926861
Ampithoidae					
Ampithoe longimana	Chesapeake Bay, USA	AY926653	AY926697	AY926757	AY926819

Thus, a range of non-gammaroidean species were sampled as potential outgroups. Some of these were members of the family Gammaridae before Bousfield's (1977, 1982) revision. These include *Melita nitida* and *Megomaera subtener*, two members of the family Melitidae, and *Crangonyx serratus*, a member of the family Crangonyctidae, often considered one of the oldest freshwater amphipod groups. Other sampled taxa have historically been considered more distantly related. These include *Monoporeia affinis*, a freshwater pontoporeid from Europe, the marine amphipod *Gammarellus angulosus*, which Barnard and Barnard (1983) suggested may have an affinity to *Macrohectopus branickii*, although this hypothesis has not been mentioned elsewhere, and *Ampithoe longimana*, a marine ampithoid from the Atlantic. Voucher specimens of all sampled species will be deposited in the American Museum of Natural History in New York and in the National Museum of Natural History in Washington, DC.

The amphipods of Lake Baikal were collected using dipnets on sublittoral cobble shorelines; by hand and net from large rubble and associated aquatic sponges using SCUBA; by bottom dredging at depths of 30–90 m; with a spotlight and plankton net at night; and using meat-baited traps at depths of 25–100 m. Most non-Baikalian amphipods were collected by dipnetting in nearshore aquatic vegetation. Specimens were collected from Baikal in the summers of 1995 and 2002, Moscow in 1998, the Caspian and Black Seas in 1999, and from North America from 1997 through 2003, and were preserved and stored in 95% EtOH or in Vodka.

2.2. Molecular methods

DNA was isolated from all specimens using QIAamp or DNeasy (Qiagen, Valencia, California) tissue preparation kits. Primers for amplification are listed in Table 2.

Table 2
Primers used for amplification and sequencing in this study

Primer	Sequence (5′–3′)	Source
18S rRNA		
18SF	CCTAYCTGGTTGATCCTGCCAGT	Englisch et al. (2003)
18S700R	CGCGGCTGCTGGCACCAGAC	Englisch et al. (2003)
18S1500R	CATCTAGGGCATCACAGACC	Englisch et al. (2003)
18SR	TAATGATCCTTCCGCAGGTT	Englisch et al. (2003)
16S rRNA		
16STf	GGTAWHYTRACYGTGCTAAG	Developed by author (K.S.M.)
16Sbr	CCGGTTTGAACTCAGATCATGT	Palumbi et al. (1991)
COI		
HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. (1994)
LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer et al. (1994)

Typical 50 µl PCRs for the 16S rRNA and COI sequences contained 5 µl 10× PCR buffer, 2 mM (2 µl) $MgCl_2$, 0.2 mM (2 µl) dNTP mixture (Sigma), 10µM (0.5 µl) of each primer, 1 U (0.25 µl) Amplitaq DNA polymerase (Perkin-Elmer, Foster City, California), and 1 µl template DNA solution. Reactions were cycled on a MJResearch PTC200 thermocycler, and started with a 4 min 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 s. Reactions finished with a single 72 °C, 7 min elongation step. PCR products were cleaned using Wizard PCR Preps (Promega, Madison, WI). Gene segments were sequenced using Sequenase 2.0 kits (Epicenter), and were run through 5 1/2% Long Ranger acrylamide gels (FMC Bioproducts, Rockland, ME) on a LI-COR DNA4200L automated DNA sequencer.

18S rRNA gene fragments were amplified with Ready-To-Go PCR beads (Amersham–Pharmacia Biotech, Piscataway, NJ) with 0.5 µl of each 10 µM primer, 1 µl of template solution, and 23 µl H_2O. Reactions were cycled on a GeneAmp PCR System 9700 (PE Applied Biosystems), with an initial 5 min denaturing step at 95 °C, followed by 35 cycles of the following reaction: 95 °C for 20 s, 50 °C for 20 s, and 72 °C for 45 s. Reactions finished with a single 72 °C, 7 min elongation step. Amplification products were purified using QIAquick PCR Purification Kits (Qiagen). Each sequencing reaction mixture included 2 µl BigDye (Applied Biosystems, Perkin-Elmer), 2 µl of 1 µM primer, and 5 µl DNA template, and ran for 40 cycles of 96 °C (10 s), 50 °C (10 s), and 60 °C (4 min). Sequencing products were purified by 70% isoproponal/70% ethanol precipitation and were run on an ABI Prism 3700 sequencer (Applied Biosystems).

2.3. Morphological methods

We chose and scored 152 morphological characters for 62 taxa (see Appendix A for character list). Character states were scored by a single worker (KSM) by examination of a minimum of three male specimens per species, where possible (see Appendix B for data matrix). Nine characters were found to be autapomorphic. As these characters may become informative with the future addition of taxa, they were retained. All characters were treated as unordered, thus character state does not imply polarity.

2.4. Phylogenetic analysis

Sequences were edited using CodonCode Aligner (http://www.codoncode.com/aligner/). 16S rRNA and 18S rRNA sequences were aligned with BioEdit (Isis Pharmaceuticals), which aligns using the ClustalW program (Thompson et al., 1994) using the following gap opening/extensions costs: 10/5; 10/1; 5/5; 5/1; 2/2; 2/1. COI sequences were conserved in length and required no insertion/deletion events (indels). Phylogenetic hypotheses of relationships among taxa were constructed by analyzing the aligned nucleic acid sequences using parsimony and maximum likelihood in PAUP* (Swofford, 2002), and Bayesian inference in MrBayes v3.01 (Huelsenbeck, 2000; Ronquist and Huelsenbeck, 2004). Indels were considered missing data in all analyses. First, each dataset (three gene segments and the morphological data) was analyzed separately. Next, the three molecular datasets were analyzed together using both parsimony and likelihood. Finally, the molecular and morphological data were used in combined-data parsimony and Bayesian analyses.

All parsimony analyses assumed equal weights for all character changes, and used the heuristic search option with 100 random addition replicates and TBR branch swapping in PAUP*. The likelihood analysis used the $GTR + \Gamma + I$ model, which was determined to be the best-fit model using the Akaike information criterion (AIC) in Modeltest (Posada and Crandall, 1998). The likelihood analysis was conducted using the heuristic search option in PAUP* and a single stepwise addition starting tree. Because a single replicate random addition sequence took over one week of computation time using a 1.5 GHz PowerBook G4, we were not able to perform more replicates. Two Bayesian MCMCMC (Metropolis-coupled Markov chain Monte Carlo) searches were run with four simultaneous chains for 5,000,000 and 10,000,000 generations, respectively, sampling every 1000 chains (for a total of 5000 and 10,000 sampled trees, respectively). For the Bayesian analysis, the $GTR + \Gamma + I$ model was used for the molecular data, with parameter values determined independently for each gene, while the Mk model of Lewis (2001) was used for the morphological data. Stationarity in the Bayesian analyses was determined by plotting $\ln L$, α, m, and a range of substitution parameters for all partitions versus generation (as suggested by Nylander et al., 2004) and examining for leveling of values. These same parameters were also plotted separately for all eight chains to ensure chain convergence. Using this criterion, stationarity was reached by the 30,000th tree (the 30th saved tree), for the first run, and the 110,000th tree (110th saved tree) for the second. Convergence of all chains for both runs was also reached by the 30th and 110th saved trees, respectively. Giving a small margin for error, trees 50–5000 from the first search and trees 120–10,000 from the second search were used to construct separate 50% majority-rule consensus tree, and were combined in a single majority-rule consensus tree.

For parsimony and likelihood analyses, non-parametric bootstrap support values for clades were obtained using the heuristic bootstrap search command (with 1000 parsimony and 100 likelihood pseudoreplicates) in PAUP*. Bremer support values (Bremer, 1988, 1994) for the parsimony analyses were calculated using the program TreeRot (Sorenson, 1999).

Monophyly of the Baikal amphipods as a whole, the families Micruropodidae and Acanthogammaridae, and several of the well-sampled genera were examined using the molecular data and the SH (Shimodaira and Hasegawa, 1999) test as implemented in PAUP*.

3. Results

Parsimony analyses of the 16S rRNA and 18S rRNA gene segments indicate that both datasets were robust to changes in alignment parameters. Analyses of the six different aligned datasets produced almost identical trees. The few differences between these trees were in poorly supported nodes. As there was no objective justification for choosing any particular set of alignment parameters, we chose to use the 5/1 (gap opening/gap extension) alignment. After alignment, the COI dataset consisted of 709 bp (of which 354 were parsimony informative and 294 were constant), the 16S rRNA consisted of 621 bp (326 parsimony informative, 211 constant), the 5′ 18S rRNA gene portion consisted of 867 bp (145 parsimony informative, 646 constant), and the 3′ 18S rRNA gene portion consisted of 922 bp (192 parsimony informative, 627 constant).

The trees resulting from the analyses of individual genes (not shown) were generally in agreement, and did not disagree on any node with bootstrap support >50% or Bremer support >2. There were also no well-supported node differences between any single-gene tree and the combined molecular data tree. The parsimony analysis of combined molecular data resulted in seven most parsimonious trees (strict consensus presented in Fig. 2A) with a length of 6668 steps. The likelihood analysis of the same data resulted in a single most likely tree, with $-\ln L = 31790.87614$ (Fig. 3). The results of both analyses share many points of agreement, although an SH test comparing the seven parsimony derived trees and the likelihood tree indicated that the likelihood topology was significantly more likely to give rise to the data than any of the parsimony topologies (with p values ranging from 0.031 to 0.042), while the likelihood topology required 34 additional steps compared to the most parsimonious trees. Neither analysis supports monophyly of the Baikal amphipods, since some members of the genus *Gammarus* are nested within the Baikal clade. The most likely tree with a monophyletic Baikal clade is not significantly less likely than the unconstrained most-likely tree using the SH test ($p = 0.053$), although this comparison is only marginally non-significant, and only five additional steps are required for a parsimony tree containing a monophyletic Baikal fauna. Within the Baikal fauna, both parsimony and likelihood analyses support the monophyly of the morphologically diverse family Acanthogammaridae (as designated by Kamaltynov, 1999b), although this is not significant according to the SH test (bootstrap = 96%, Bremer support = 16 for parsimony analysis, likelihood bootstrap = 100%, SH test $p = 0.10$). In contrast to the consistent support for monophyly of Acanthogammaridae, both parsimony and ML analyses find the morphologically more homogeneous family Micruropodidae to be paraphyletic, showing strong support for inclusion in this clade of *Macrohectopus branickii*, the sole member of the family Macrohectopidae. Constraining a monophyletic Micruropodidae adds 30 steps to the parsimony analysis, and yields a significantly less likely tree according to a SH test ($p = 0.001$). One potential difference between the parsimony and ML analyses regards the relationship of the Baikal amphipods to the cosmopolitan freshwater genus *Gammarus*. The parsimony analysis unites two *Gammarus* species, the Holarctic *G. lacustris* and the North American *G. pseudolimnaeus*, with the micruropodid/*Macrohectopus* clade, albeit with little support, while uniting the remaining *Gammarus* species with the Baikalian acanthogammarids. The likelihood analysis, in contrast, unites all the sampled *Gammarus* species with the micruropodid/*Macrohectopus* clade, although *Gammarus* remains paraphyletic. Within Micruropodidae–Macrohectopidae, both analyses support two distinct groups, henceforth referred to as the *Gmelinoides*-group (parsimony bootstrap and Bremer support values of 95% and 10, respectively, likelihood bootstrap = 77%) and the *Crypturopus*-group (76% and 4 for parsimony, 73% for likelihood), the latter including *Macrohectopus*. These two groups are sister to each other, but are highly divergent when compared to other Baikalian groups, with uncorrected-parameter distances based on 16S rRNA data between these two micruropodid groups (0.16–0.27) similar to distances between each group and the Acanthogammaridae (0.17–0.32 between the Acanthogammaridae and *Gmelinoides*-group, 0.18–0.32 between the Acanthogammaridae and *Crypturopus*-group). The pelagic *Macrohectopus* is strongly supported as a member of the predominantly fossorial *Crypturopus*-group. A tree without a monophyletic *Crypturopus*-group + *Macrohectopus* requires an additional four steps, and is significantly less likely according to the SH test ($p = 0.033$). Additionally, neither the parsimony nor the likelihood analysis supports the monophyly of the armored Baikal amphipods (dashed lines in Figs. 2 and 3).

Finally, both parsimony and ML analyses of the molecular data support polyphyly of several genera within the Baikal fauna. The genus *Micruropus* is one of these, with members in both the *Gmelinoides*-group and the *Crypturopus*-group (31 additional steps required for monophyly and $p < 0.001$ in the SH test). The genus *Pallasea* is also polyphyletic, with *Pallasea cancellus* distantly related to the other members of the genus (16 steps required for monophyly, but results of the SH test are not significant, with $p = 0.482$). Overall, there is little support for most clades within the family

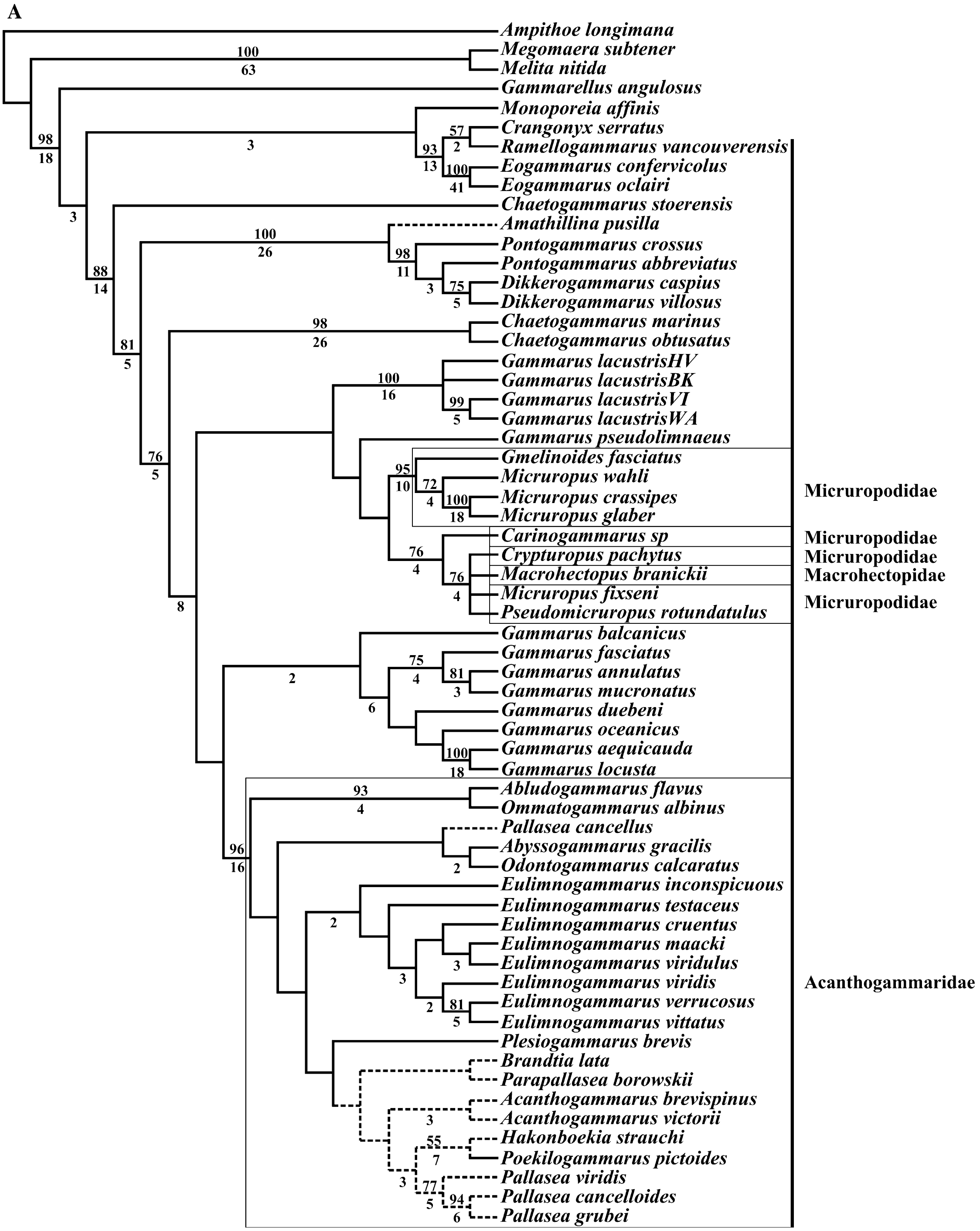

Fig. 2. A. Strict consensus of seven most parsimonious trees (tree length = 4022) from analysis of all molecular data (16S rRNA, 18S rRNA, and COI). B. Strict consensus of four most parsimonious trees from analysis of 152 morphological characters (tree length = 1209). Numbers above branches are bootstrap values (1000 pseudoreplicates), and those below branches are Bremer branch support values. Boxes enclose all sampled Baikal amphipods. Each box encloses a monophyletic group and is labeled with family designation, based on Kamaltynov (1999b). Black vertical line to right of species names unites Bousfield's (1978, 1928) Gammaroidea. Dashed branches denote armored/processiferous lineages.

Acanthogammaridae, with only five clades (out of 21) exhibiting bootstrap support values >50%.

Parsimony analysis of the morphological data (143 parsimony informative characters) resulted in four most parsimonious trees with a length of 1209 (Fig. 2B; see Appendix B for character state matrix). These trees greatly differ from those of both molecular data analyses. Notably, the morphological analysis does not support a monophyletic Acanthogammaridae. Nor does it support a monophyletic Baikal fauna, but instead

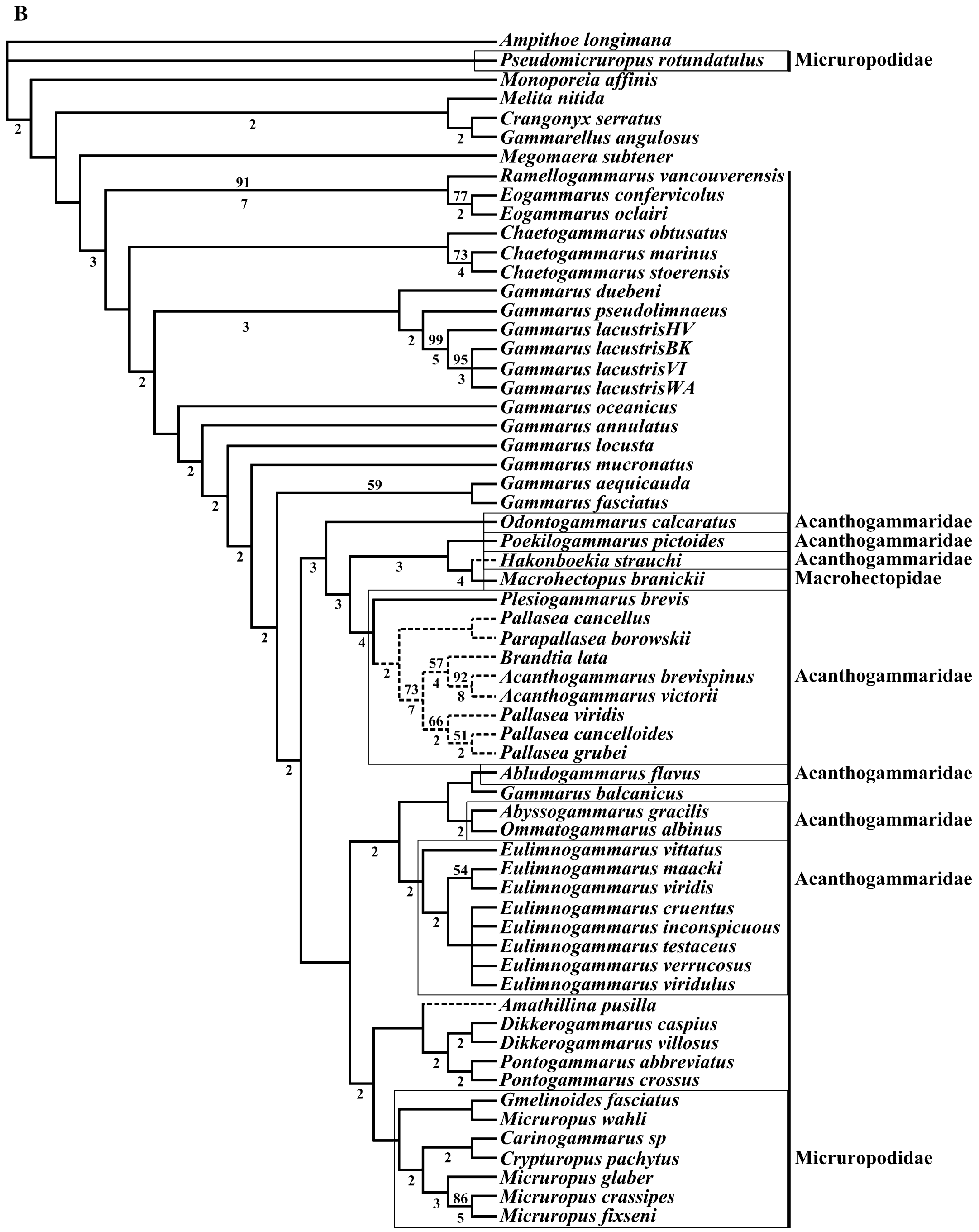

Fig. 2. (*continued*)

includes a clade comprising most of the Baikalian fauna (with the lone exception being *P. rotundatulus*) with the Ponto-Caspian family Pontogammaridae. The morphological analysis also does not support the monophyly of the processiferous Baikal gammaroideans, although only *Hakonboekia strauchi* is excluded. However, it does show a monophyletic Micruropodidae with the exception of *Pseudomicruropus rotundatulus*, which has a basal position in the morphological analysis. Overall, most clades differing between the morphological and molecular topologies are poorly supported, and the morphological analysis generally exhibits poor support, with only 10 nodes having bootstrap support values >50%, and only 5 nodes with bootstrap support values >75%.

Combining the morphological and molecular data in a parsimony analysis yields a single tree with a length of 8017 steps (Fig. 4). Majority-rule consensus trees resulting from the two Bayesian analyses of the entire dataset were identical, with almost identical posterior probabilities (three probabilities differed by a maximum of 0.02),

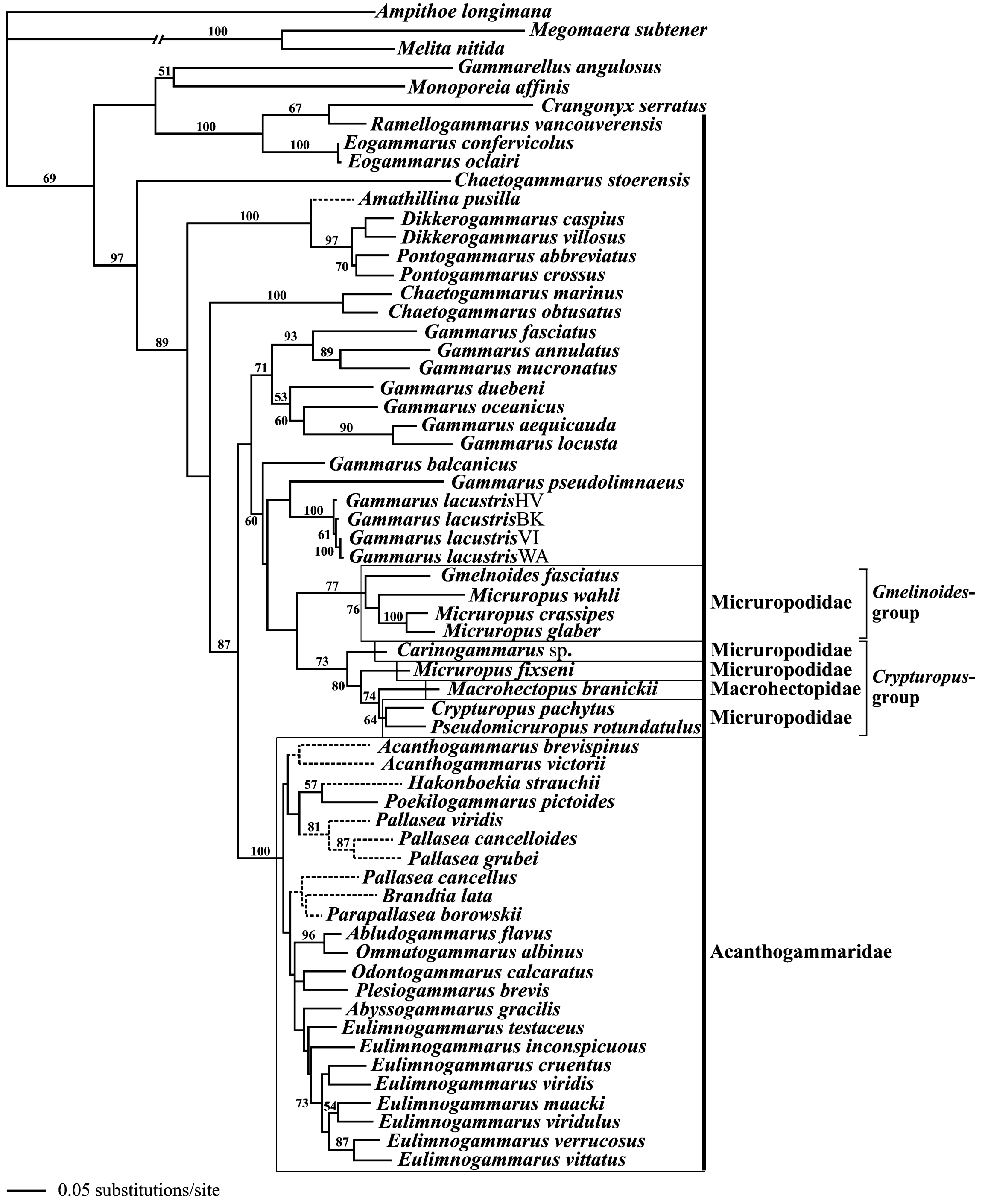

Fig. 3. Single best tree ($-\ln L = 31790.87614$) from likelihood analysis of all molecular data (16SrRNA, 18SrRNA, COI). Numbers above branches are bootstrap values (100 pseudoreplicates). Brackets enclose two distinct, reciprocally monophyletic micruropodid groups found in this study. Other symbols as in Fig. 2. The extraordinarily long branch uniting *Megomaera subtener* and *Melita nitida* was shortened by 50% (denoted by //) to allow better visualization of branch lengths for the rest of the tree.

thus the topology and posterior probabilities from the majority-rules consensus tree of all 14,850 trees were used (Fig. 5). Both parsimony and Bayesian analyses of the combined dataset strongly support a monophyletic Acanthogammaridae (100% bootstrap and Bremer support of 22 for the parsimony analysis, Bayesian posterior probability of 1.00), and a monophyletic micruropodid/*Macrohectopus* clade (82% bootstrap, 11 Bremer support, 1.00 posterior probability) composed of two monophyletic groups. Both analyses also show *Gammarus lacustris* and *G. pseudolimnaeus* as sister to the micruropodid/*Macrohectopus* clade. A difference between the parsimony and Bayesian combined data analyses is the placement of the remaining *Gammarus* species, which are sister to the

Fig. 4. Single most parsimonious tree (tree length = 8017) from combined analysis of all data (16S rRNA, 18S rRNA, COI, and morphology). Numbers above branches (or numbers before "/" for short branches) are bootstrap values (1000 pseudoreplicates), and those below branches (or after "/" for short branches) are Bremer branch support values. Other symbols as in Fig. 3.

G. lacustris/micruropodid clade in the Bayesian analysis, and are sister to the acanthogammarid/*G. lacustris* / micruropodid clade in the parsimony analysis. However, the placement of these taxa is not well supported in either analysis. There are also differences between the Bayesian and parsimony analyses in the relationships within the Baikalian families, but they are all on nodes that are poorly supported in the parsimony tree.

The addition of the morphological data increases support for many clades over values found in the molecular parsimony analysis. Of clades with bootstraps >60% and Bremer values ⩾3 in the molecular analysis, all were present and supported in the combined-data parsimony analysis, with 17 clades showing increased support and 8 clades showing decreased support. Additionally, 5 clades that were present but poorly supported (<50% bootstrap and <3 Bremer value) in the molecular analysis increased support in the combined data analysis.

4. Discussion

4.1. Phylogeny of Acanthogammaridae

Our analyses provide the first rigorous support for monophyly of the diverse, Baikalian endemic amphipod

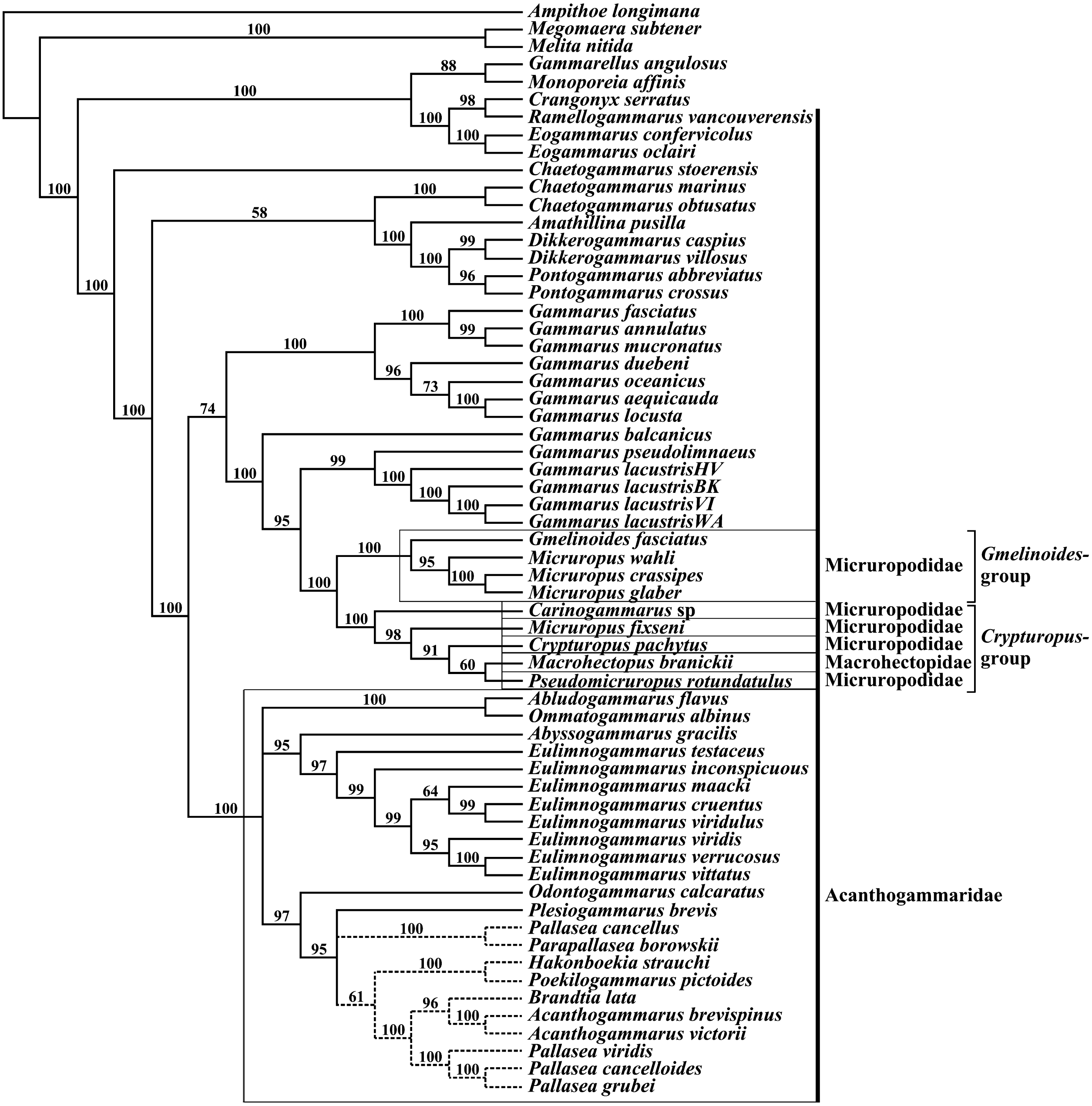

Fig. 5. Majority rules consensus tree of 14,850 trees resulting from Bayesian analysis of combined molecular and morphological data. Values above branches are Bayesian posterior probabilities (%). Other symbols as in Fig. 3.

family Acanthogammaridae as defined by Kamaltynov (1999b). Many of the taxa within this recently redefined family historically have been classified in different families due to their highly divergent morphologies (Kamaltynov, 1999a,b). Kamaltynov's inclusion of many smooth-bodied, morphologically generalized amphipods in a family that was once primarily diagnosed by armor or large processes seems mostly a response to preliminary molecular data that showed little genetic differentiation between armored and non-armored Baikalian species (Sherbakov et al., 1998, 1999). In fact, Kamaltynov (1999b) listed no diagnostic characters for Acanthogammaridae, which he defined as consisting of Baikal amphipods that are neither small and smooth-bodied fossorial forms (Micruropodidae), nor streamlined with elongated appendages built for a pelagic lifestyle (i.e., *Macrohectopus*). Our analysis also has not identified diagnostic morphological characters for Acanthogammaridae. Despite the lack of clear morphological synapomorphies, however, Acanthogammaridae as defined by Kamaltynov (1999b) was strongly supported as a natural taxonomic group in the parsimony and Bayesian analyses. This suggests that the amazing diversity in body form and ecology within this endemic family diversified from a single ancestral lineage. As all of the many species are restricted to Lake Baikal and its watershed, this spectacular radiation presumably occurred within the lake.

Unfortunately, despite application of three gene segments totaling more than 3100 bp and 143 informative morphological characters, little resolution or support was found among lineages within Acanthogammaridae. This is likely due to the many short internal branches uniting the species in this family, coupled with proportionally long terminal branches (see likelihood analysis, Fig. 3), a pattern similar to those found in several other diverse taxa such as African cichlids (Sturmbauer et al., 1994), saxifragalean plants (Fishbein et al., 2001), New World warblers (Lovette and Bermingham, 1999), and snapping shrimp (Morrison et al., 2004). A combination of short internal and long terminal branches has often been interpreted as indicative of an ancient rapid radiation (Donoghue and Sanderson, 1992), and departures from null model expectations in several of the foregoing examples support this conclusion. It is tempting to ascribe the similar pattern in Baikal's amphipods to a rapid ancient radiation as well, although our incomplete sampling of the lake's tremendous species diversity advises caution. If Acanthogammaridae indeed diverged anciently and rapidly, relationships within this family may never be fully resolved.

The most morphologically generalized of Baikal's amphipod lineages, the genus *Eulimnogammarus*, is monophyletic with low internal support in all parsimony analyses, and relationships within the genus were almost completely unresolved in our analysis of morphological data. Our Bayesian analysis does show high posterior probability values for relationships within this genus, but values such as these are generally acknowledged to be sometimes unreasonably large (Cummings et al., 2003; Douady et al., 2003; Erixon et al., 2003; Huelsenbeck et al., 2002; Suzuki et al., 2002). The processiferous genus *Pallasea* is a well-supported clade in all analyses, with the exception of *Pallasea cancellus* (the type species of the genus), which falls well outside the rest of the genus. Sequencing of multiple *P. cancellus* individuals verified this unexpected result. The outlier status of *P. cancellus* is supported by the morphological data as well (Fig. 2B), with 22 characters uniting the rest of the *Pallasea* species to the exclusion of *P. cancellus*. All analyses show a non-monophyletic armored Baikal fauna, albeit with generally low support. These results suggest that the exaggerated body processes characteristic of many endemic Baikalian amphipods may have evolved or been lost in parallel multiple times within the lake, consistent with opinions held by some previous researchers (Kamaltynov, 1999a), and recalling the parallel evolution characteristic of several other adaptive radiations such as those of the African cichlids (Kocher et al., 1993).

4.2. Phylogeny of Micruropodidae

The second major family of endemic Baikalian gammaroideans in Kamaltynov's (1999b) classification is the taxonomically diverse but morphologically rather uniform Micruropodidae. This new family, consisting mostly of burrowers in sediments, was proposed largely on the basis of preliminary molecular studies and older immunological work (Kamaltynov, 1999b), yet its members have long been considered to have a close affinity (Barnard and Barnard, 1983; Bazikalova, 1945). Despite their morphological uniformity and fossorial habit and body-form, our analysis indicates that Micruropodidae is paraphyletic, containing the morphologically divergent, pelagic species *Macrohectopus branickii*, and that even the type genus *Micruropus* is polyphyletic. Micruropodidae consists of two distinct, reciprocally monophyletic groups: the *Gmelinoides*-group (*Gmelinoides fasciatus*, *Micruropus crassipes*, *M. glaber*, *M. wahli*), and the *Crypturopus*-group (*Crypturopus pachytus*, *Carinogammarus* sp., *Macrohectopus branickii*, *Micruropus fixseni*, and *Pseudomicruropus rotundatulus*). These clades are highly divergent, with between-group distances comparable to distances between each group and the family Acanthogammaridae. Despite this level of divergence, all analyses strongly support monophyly of the micruropodid/*Macrohectopus* clade, as well as both the *Crypturopus*-group and *Gmelinoides*-group. Our results suggest a deep divergence within Micruropodidae, and that the common ancestor of this fossorial family also gave rise to the morphologically divergent pelagic *Macrohectopus branickii*.

While the molecular divergence of the two micruropodid clades may roughly equal their respective divergences from Acanthogammaridae, micruropodids have not diversified morphologically to nearly the same extent as the latter taxon. Members of the *Gmelinoides*-group and the *Crypturopus*-group are morphologically very similar, as reflected in the parsimony analysis of the morphological data (Fig. 2B), yet are genetically diverse, with genetic distances ranging from 16 to 27%. In contrast, distances between the genus *Eulimnogammarus* and the genus *Acanthogammarus* range from 13 to 17% yet these two genera are morphologically highly distinct (Figs. 1B and C; 2B). The apparent morphological stasis in the micruropodid species may result from their predominantly fossorial (burrowing) lifestyle, and its probable constraints on their morphology—short, compact bodies and appendages, and numerous setae. This explanation is supported by the main exception within the group, the pelagic planktivore *Macrohectopus*. Although *Macrohectopus* is strongly supported as a member of the *Crypturopus*-group, it is one of the few within the Micruropodidae to have completely given up the fossorial lifestyle. Probably not coincidentally, it is also drastically different morphologically from the rest of the micruropodids (Fig. 1), retaining only the setation characteristic of this group, which also may be beneficial for a pelagic lifestyle. Interestingly, even some of the burrowing species are sometimes found in the water column. *Micruropus wahli*, which suspension feeds from burrows in muddy substrate, was caught in large quantities in surface waters swarming around a light at night. Some (Barnard and Barnard, 1983; Kamaltynov, 1999b) have considered micruropodids to be the 'Baikalian analogue' in morphology to *Pontogammarus*, a predominantly burrowing genus in the Ponto-Caspian region. Yet these groups are undoubtedly not closely related (with 79 additional steps required for monophyly using the molecular data, and 74 required using all data), further supporting the idea that the relatively homogeneous morphology of micruropodids has been shaped by their fossorial lifestyle.

4.3. Phylogeny of the Lake Baikal fauna

None of our analyses supported monophyly of the Baikal amphipod fauna as a whole, yet nowhere was overwhelming evidence against Baikal monophyly. Nonmonophyly of the Baikal fauna would not be unexpected, for it has been generally believed that this fauna consists of multiple lineages (Bazikalova, 1945; Bousfield, 1977; Kamaltynov, 1999b; Kozhov, 1963). Additionally, a close relationship between a member of the genus *Gammarus* and the micruropodid/*Macrohectopus* clade recalls the similar results of Sherbakov et al. (1998), who found a close relationship between *Macrohectopus* and *Gammarus pulex* (they included no other *Crypturopus*-group amphipod, nor *G. lacustris*, in their analyses). Thus, this study suggests that the Baikal fauna is polyphyletic, consisting of two lineages, one consisting of Acanthogammaridae, and the other consisting of Micruropodidae (including *Macrohectopus*).

Relationships within both Micruropodidae and Acanthogammaridae support the conclusion that molecular and morphological evolution became uncoupled during the radiation of the Baikalian amphipods. The apparent parallel evolution of well-developed armor in multiple acanthogammarid lineages and the divergence of the pelagic *Macrohectopus* suggest evolutionary responses to ecological opportunity. Conversely, the relatively uniform morphology of the genetically divergent micruropodid clades suggest constrained morphological evolution within fossorial niches. Similar instances of decoupling between molecular and morphological evolution have been found in other well-studied examples of adaptive radiation, such as the cichlids of the African Rift Lakes (Kocher et al., 1993; Rüber et al., 1999; Sturmbauer and Meyer, 1992), and the songbirds of Hawaii (Lovette and Bermingham, 1999). Our results may contradict Greenwood's (2000) conclusions (discussed and supported by Sturmbauer, 1998) that radiations with morphologically similar species are younger, while morphologically diverse radiations indicate an older species flock. Baikal's two radiations seem to be relatively old, inferring from the large pairwise distances and branch lengths, yet the Baikal clades exhibit both diverse and conservative morphologies.

Admittedly, this study does not take into account members of the fourth of Kamaltynov (1999b) families, Pachyschesidae, which we were not able to obtain. However, Sherbakov et al.'s (1998) study did include a *Pachyschesis* species, which was found to belong to a group including *Parapallasea*, *Pallasea*, and *Eulimnogammarus*, so our conclusions likely would not change significantly with the addition of the missing taxa.

4.4. Origin of Lake Baikal fauna

Understanding the origins of the Lake Baikal fauna requires one to examine how they relate to non-Baikalian amphipods. Some researchers have suggested a close affinity between the Baikalian and Ponto-Caspian amphipod fauna, and their views have been reviewed by Bazikalova (1945) and Kozhov (1963). In addition to the similarities between the micruropodids and some pontogammarids, other members of the Ponto-Caspian amphipod fauna, such as *Amathillina pusilla*, exhibit well-developed processes similar to the armored Baikalian species. Our morphological results (Fig. 2B) suggest similarities between the Baikalian and Ponto-Caspian faunas, especially between the pontogammarids and micruropodids. However, our molecular and combined analyses show that the Baikal fauna is actually more closely related to the Holarctic genus *Gammarus*, especially *Gammarus lacustris*, the most widespread freshwater gammaroidean. *G. lacustris*, has not been found within the main body of Lake Baikal, but is common in ephemeral pools around its margins, as well as in a pond on Olkhon Island, surrounded entirely by Baikal. An individual sampled from the pond on Olkhon (designated *Gammarus lacustris* BK) is part of a well supported monophyletic *G. lacustris*, with individuals sampled from Lake Hovsgol, Mongolia, Washington, USA, and Vancouver Island, Canada. Other Eurasian gammaroideans have also been hypothesized to be closely related to members of the Baikalian fauna. *Chaetogammarus obtusatus* has often been referred to as *Eulimnogammarus obtusatus*, for it shares some distinctive morphological characteristics with the Baikalian genus *Eulimnogammarus* (Pinkster, 1973; Pinkster and Stock, 1970; Stock, 1969). However, our analyses show that this species is instead closely related to *Chaetogammarus marinus*, and is basal to both the Baikal/*Gammarus*, and pontogammarid clades. Finally, no analyses support the monophyly of Bousfield's (1977, 1982) superfamily Gammaroidea. *Eogammarus* and *Ramellogammarus*, both members of the North Pacific family Anisogammaridae, are apparently not closely related to the other gammaroideans, instead being more closely related to *Crangonyx serratus*, *Monoporeia affinis*, and possibly

Gammarellus angulosus, all non-gammaroideans. The family Anisogammaridae may not even be monophyletic, with the molecular likelihood and combined-data Bayesian analyses both showing a relationship between *Ramellogammarus* and *Crangonyx*.

While our results do not resolve completely the origins of Baikal's amphipods, they do reduce the number of invading taxa from historical estimates that range from 4 to 18 (Barnard and Barnard, 1983; Bousfield, 1977, 1982; Brooks, 1950; Kamaltynov, 1992, 1999a; Kozhov, 1963; Ogarkov et al., 1997; Sherbakov, 1999; Sherbakov et al., 1998). The most parsimonious reconstruction suggests that the Baikal amphipod fauna arose from two independent invading ancestor lineages, one giving rise to the Acanthogammaridae, the other to a Micruropodidae/*Macrohectopus* clade. The Micruropodidae/*Macrohectopus* clade probably arose from a freshwater *Gammarus lacustris*-like ancestor. Acanthogammaridae may have also arisen from a *Gammarus*-like ancestor, but the evidence for this is not as strong. Additional sampling of gammaroidean species from Eastern Europe and Asia is needed to develop a better understanding of the origins of the spectacular gammaroidean fauna of Baikal, especially the principal endemic family Acanthogammaridae, as well as the relationships between Baikalian and other non-Baikalian amphipods.

5. Conclusions

This study clarifies the evolutionary history of the highly diverse, endemic amphipods of Lake Baikal, and reveals some new and unexpected relationships. While there is strong molecular support for the endemic, morphologically and ecologically diverse Acanthogammaridae as a natural taxonomic family, albeit without a reliable morphological diagnosis, the family Micruropodidae is comprised of two highly divergent monophyletic groups. Most surprisingly, while the pelagic *Macrohectopus* has been considered morphologically distinct enough to merit creation of its own family, this distinctiveness is not reflected in the molecular data, which strongly supports the placement of the pelagic planktivore within the predominantly fossorial *Crypturopus*-group. These several results highlight the uncoupling of molecular and morphological evolution that appears to be common in adaptive radiations. Our results also emphasize that morphological data, long considered highly homoplastic in amphipods, adds support and resolution to molecular data analyses, even when the two data types are strongly incongruent. We hope that with additional taxonomic sampling, mainly of the non-Baikalian species found throughout Europe and Asia, we can better resolve the phylogeny and obtain a better detailed understanding of how these amphipods evolved into one of the most impressive invertebrate endemic faunas in existence.

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Appendix A. Morphological characters used in this study

1. Accessory flagellum: 0 = absent; 1 = 1 article; 2 = 2–6 articles; 3 = 7+ articles
2. Calceoli, antenna 1 flagellum: 0 = absent; 1 = present
3. Calceoli, antenna 2 flagellum: 0 = absent; 1 = present
4. Peduncle article 2, antenna 2: 0 = bare; 1 = setae only present; 2 = spine present
5. Anterior-most segment with dorsal spines: 0 = head; 1 = pareon 1; 2 = pareon 2; 3 = pareon 3; 4 = pareon 4; 5 = pareon 5; 6 = pareon 6; 7 = pareon 7; 8 = pleon 1; 9 = pleon 2; 10 = pleon 3; 11 = urosome 1; 12 = urosome 2; 13 = urosome 3; 14 = no dorsal spination
6. Anterior-most segment with spines on dorso-posterior margin: 0 = head; 1 = pareon 1; 2 = pareon 2; 3 = pareon 3; 4 = pareon 4; 5 = pareon 5; 6 = pareon 6; 7 = pareon 7; 8 = pleon 1; 9 = pleon 2; 10 = pleon 3; 11 = urosome 1; 12 = urosome 2; 13 = urosome 3; 14 = no dorsal spination
7. Anterior-most segment with dorsal setae: 0 = head; 1 = pareon 1; 2 = pareon 2; 3 = pareon 3; 4 = pareon 4; 5 = pareon 5; 6 = pareon 6; 7 = pareon 7; 8 = pleon 1; 9 = pleon 2; 10 = pleon 3; 11 = urosome 1; 12 = urosome 2; 13 = urosome 3; 14 = no dorsal spination
8. Anterior-most segment with setae on dorso-posterior margin: 0 = head; 1 = pareon 1; 2 = pareon 2; 3 = pareon 3; 4 = pareon 4; 5 = pareon 5; 6 = pareon 6; 7 = pareon 7; 8 = pleon 1; 9 = pleon 2; 10 = pleon 3; 11 = urosome 1; 12 = urosome 2; 13 = urosome 3; 14 = no dorsal spination
9. Setae on ventral margin of coxal plates 1–4: 0 = bare/few setae; 1 = highly setose
10. Spines on ventral margin of Epimeron 1: 0 = absent; 1 = present
11. Spines on ventral margin of Epimeron 2: 0 = absent; 1 = present
12. Setae on ventral margin of Epimeron 2: 0 = absent; 1 = singly inserted; plain; 2 = inserted in sets >2; plain; 3 = singly inserted; plumose; 4 = inserted in sets >2; plumose
13. Spines on ventral margin of Epimeron 3: 0 = absent; 1 = present

14. Setae on ventral margin of Epimeron 3: 0 = absent; 1 = singly inserted; plain; 2 = inserted in sets >2; plain; 3 = singly inserted; plumose; 4 = inserted in sets >2; plumose
15. Mid ventral lateral margin, uronite 1: 0 = bare; 1 = plain setae present; 2 = plumose setae present; 3 = spine present
16. Posterior ventral lateral margin, uronite 1: 0 = bare; 1 = plain setae present; 2 = plumose setae present; 3 = spine present
17. Medial armor of head segment: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
18. Lateral armor of head segment: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
19. Medial armor of 1st pareon: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
20. Lateral armor of 1st pareon: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
21. Marginal armor of 1st pareon: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
22. Medial armor of 2nd pareon: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
23. Lateral armor of 2nd pareon: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
24. Marginal armor of 2nd pareon: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
25. Medial armor of 3rd pareon: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
26. Lateral armor of 3rd pareon: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
27. Marginal armor of 3rd pareon: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
28. Medial armor of 4th pareon: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
29. Lateral armor of 4th pareon: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
30. Marginal armor of 4th pareon: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
31. Medial armor of 5th pareon: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
32. Lateral armor of 5th pareon: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
33. Marginal armor of 5th pareon: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
34. Medial armor of 6th pareon: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
35. Lateral armor of 6th pareon: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
36. Marginal armor of 6th pareon: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
37. Medial armor of 7th pareon: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
38. Lateral armor of 7th pareon: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
39. Marginal armor of 7th pareon: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
40. Medial armor of 1st pleon: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
41. Lateral armor of 1st pleon: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
42. Marginal armor of 1st pleon: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
43. Medial armor of 2nd pleon: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
44. Lateral armor of 2nd pleon: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
45. Marginal armor of 2nd pleon: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
46. Medial armor of 3rd pleon: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
47. Lateral armor of 3rd pleon: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
48. Marginal armor of 3rd pleon: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
49. Armor of 1st uronite: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
50. Armor of 2nd uronite: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
51. Armor of 3rd uronite: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
52. Armor of 1st coxal plate: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
53. Armor of 2nd coxal plate: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
54. Armor of 3rd coxal plate: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
55. Armor of 4th coxal plate: 0 = none; 1 = tubercles; 2 = mounds/swellings; 3 = pronounced (keels, teeth), 4 = mucronations (extension of posterior margin)
56. Proximal lateral face, peduncle of uropod 1: 0 = bare; 1 = setae only present; 2 = spine present
57. Number of sets of setae/spines on proximal lateral face, peduncle of uropod 1: 0 = none; 1 = 1; 2 = 2+
58. Proximal medial face, peduncle of uropod 1: 0 = bare; 1 = setae only present; 2 = spine present
59. Number of sets of setae/spines on proximal medial face, peduncle of uropod 1: 0 = none; 1 = 1; 2 = 2+
60. Dorso-lateral margins, peduncle of uropod 1: 0 = bare; 1 = setae only present; 2 = spines present

61. Lateral interramal margin, peduncle of uropod 1: 0 = bare; 1 = setae only present; 2 = 1 spine present; 3 = 2 spines present
62. Lateral margins, outer ramus of uropod 1: 0 = bare; 1 = setae only present; 2 = spines present
63. Lateral margins, inner ramus of uropod 1: 0 = bare; 1 = setae only present; 2 = spines present
64. Proximal lateral face, peduncle of uropod 2: 0 = bare; 1 = setae only present; 2 = spine present
65. Number of sets of setae/spines on proximal lateral face, peduncle of uropod 2: 0 = none; 1 = 1; 2 = 2+
66. Dorso-lateral margins, peduncle of uropod 2: 0 = bare; 1 = setae only present; 2 = spines present
67. Lateral interramal margin, peduncle of uropod 2: 0 = bare; 1 = setae only present; 2 = 1 spine present; 3 = 2 spines present
68. Lateral margins, outer ramus of uropod 2: 0 = bare; 1 = setae only present; 2 = spines present
69. Lateral margins, inner ramus of uropod 2: 0 = bare; 1 = setae only present; 2 = spines present
70. Apical spines, rami of uropods 1 & 2: 0 = 5 spines; 1 = 3 spines; 2 = 1 spine
71. Lateral face, peduncle of uropod 3: 0 = bare; 1 = setae only present; 2 = spines present
72. Number of sets of setae/spines on lateral face, peduncle of uropod 3: 0 = none; 1 = 1; 2 = 2+
73. Distal dorso-lateral margin, peduncle of uropod 3: 0 = bare; 1 = setae only present; 2 = spines present
74. Distal dorso-medial margin, peduncle of uropod 3: 0 = bare; 1 = setae only present; 2 = spines present
75. Distal ventral margin, peduncle of uropod 3: 0 = bare; 1 = setae only present; 2 = spines present
76. Number of articles, outer ramus of uropod 3: 0 = 0; 1 = 1; 2 = 2
77. Lateral margin spines, outer ramus of uropod 3: 0 = absent; 1 = present
78. Lateral margin setae, outer ramus of uropod 3: 0 = absent; 1 = plain setae only; 2 = plumose setae present
79. Medial margin spines, outer ramus of uropod 3: 0 = absent; 1 = present
80. Medial margin setae, outer ramus of uropod 3: 0 = absent; 1 = plain setae only; 2 = plumose setae present
81. Apical spines, outer ramus of uropod 3: 0 = absent; 1 = present
82. Apical setae, outer ramus of uropod 3: 0 = absent; 1 = plain setae only; 2 = plumose setae present
83. Inner ramus of uropod 3: 0 = absent; 1 = vestigial (scale-like); 2 = < 1/2 length of outer ramus; 3 = < length of outer (>1/2 length of outer); 4 = subequal length of outer; 5 = > length of outer
84. Lateral margin spines, inner ramus of uropod 3: 0 = absent; 1 = present
85. Lateral margin setae, inner ramus of uropod 3: 0 = absent; 1 = plain setae only; 2 = plumose setae present
86. Medial margin spines, inner ramus of uropod 3: 0 = absent; 1 = present
87. Medial margin setae, inner ramus of uropod 3: 0 = absent; 1 = plain setae only; 2 = plumose setae present
88. Apical spines, inner ramus of uropod 3: 0 = absent; 1 = present
89. Apical setae, inner ramus of uropod 3: 0 = absent; 1 = plain setae only; 2 = plumose setae present
90. Shape of telson: 0 = entire; 1 = notched; 2 = partially split (split < 3/4 of length), lobes together; 3 = partially split, lobes separated; 4 = entirely split (split > 3/4 of length), lobes together; 5 = entirely split, lobes separated
91. Dorso-lateral margin of telson: 0 = bare; 1 = setae only present; 2 = spines present
92. Number of sets of setae/spines on dorso-lateral margin of telson: 0 = none; 1 = 1; 2 = 2+
93. Dorso-medial margin of telson: 0 = bare; 1 = setae only present; 2 = spines present
94. Number of sets of setae/spines on dorso-medial margin of telson: 0 = none; 1 = 1; 2 = 2+
95. Apex of telson: 0 = bare; 1 = setae only present; 2 = spines present
96. Free-hanging distal lobe of posterior margin, basis of pereopod 7: 0 = absent; 1 = present
97. Anterior distal margin spines, basis of pereopod 7: 0 = absent; 1 = present
98. Anterior distal margin setae, basis of pereopod 7: 0 = absent; 1 = plain setae only; 2 = plumose setae present
99. Shape of 3rd article, mandible palp: 0 = normal; 1 = reduced (rounded)
100. Comb-setae (D spines/setae of Karaman, 1969), 3rd article, mandible palp: 0 = absent; 1 = plain setae only; 2 = plumose setae present
101. Apical setae, 3rd article, mandible palp: 0 = absent; 1 = plain only; 2 = plumose present
102. Medial setae, 2nd article, mandible palp: 0 = absent; 1 = plain only; 2 = plumose present
103. Lateral setae, 2nd article, mandible palp: 0 = absent; 1 = plain only; 2 = plumose present
104. Apical elongate setae, 3rd article, maxilliped palp: 0 = absent; 1 = plain only; 2 = plumose present
105. Short, fuzz-like setae, lateral distal tip 3rd article, maxilliped palp: 0 = absent; 1 = present
106. Distal modified setae (similar to the type 3aiii, setulate-serrate pore-bearing setae, of Oshel and Steele, 1988), 3rd article, maxilliped palp: 0 = absent; 1 = present
107. Medial setae, dactyl (4th article), mandible palp: 0 = absent; 1 = present
108. Lateral distal margin setae, 2nd article, maxilliped palp: 0 = absent; 1 = plain only; 2 = plumose present
109. Medial setae, 2nd article, maxilliped palp: 0 = absent, 1 = inserted singly; 2 = inserted in sets > 2
110. Medial setae, 2nd article, maxilliped palp: 0 = absent, 1 = plain only; 2 = plumose present
111. Lateral setae, 2nd article, maxilliped palp: 0 = absent, 1 = plain only; 2 = plumose present
112. Gnathopod propod (6th article), relative size: 0 = 1st gnathopod larger; 1 = subequal in size; 2 = 2nd gnathopod larger
113. Propod palmar margin, gnathopod 1: 0 = oblique (no evident angle); 1 = intermediate (angle between 0° and 90°); 2 = acute (angle ⩾ 90°)
114. Propod mid-palmar spine, gnathopod 1: 0 = absent; 1 = short/blunt (truncate); 2 = long/sharp
115. Propod palmar angle spine, lateral face, gnathopod 1: 0 = absent; 1 = short/blunt (truncate); 2 = long/sharp
116. Propod palmar angle spine, medial face, gnathopod 1: 0 = absent; 1 = short/blunt (truncate); 2 = long/sharp
117. Propod posterior margin spines (extending proximally from palm), gnathopod 1: 0 = absent; 1 = present
118. Propod posterior margin setae, gnathopod 1: 0 = absent; 1 = inserted singly; 2 = inserted in sets > 2
119. Propod posterior margin setae, gnathopod 1: 0 = absent; 1 = plain only; 2 = plumose present
120. Propod lateral face setae, gnathopod 1: 0 = absent; 1 = inserted singly; 2 = inserted in sets > 2
121. Propod lateral face setae, gnathopod 1: 0 = absent; 1 = plain only; 2 = plumose present
122. Propod medial face setae, gnathopod 1: 0 = absent; 1 = inserted singly; 2 = inserted in sets > 2
123. Propod medial face, anterior margin setae, gnathopod 1: 0 = absent; 1 = plain only; 2 = plumose present
124. Propod lateral face, anterior margin setae, gnathopod 1: 0 = absent; 1 = inserted singly; 2 = inserted in sets > 2
125. Propod lateral face, anterior margin setae, gnathopod 1: 0 = absent; 1 = plain only; 2 = plumose present

126. Propod medial face, anterior margin setae, gnathopod 1: 0 = absent; 1 = inserted singly; 2 = inserted in sets > 2
127. Propod medial face, anterior margin setae, gnathopod 1: 0 = absent; 1 = plain only; 2 = plumose present
128. Propad palm: 0 = normal; 1 = serrate
129. Elongated setae, base of palm (near conjunction with dactyl), propod, gnathopod 1: 0 = absent; 1 = plain; 2 = plumose
130. Carpus (5th article) posterior margin setae, gnathopod 1: 0 = absent; 1 = inserted singly; 2 = inserted in sets > 2
131. Carpus posterior margin setae, gnathopod 1: 0 = absent; 1 = plain only; 2 = plumose present
132. Carpus posterior margin modified setae (similar to the type 3ai, serrate pore-bearing setae, of Oshel and Steele, 1988), gnathopod 1: 0 = absent; 1 = present
133. Propod palmar margin, gnathopod 2: 0 = oblique (no evident angle); 1 = intermediate (angle between 0° and 90°); 2 = acute (angle ⩾ 90°)
134. Propod mid-palmar spine, gnathopod 2: 0 = absent; 1 = short/blunt (truncate); 2 = long/sharp
135. Propod palmar angle spine, lateral face, gnathopod 2: 0 = absent; 1 = short/blunt (truncate); 2 = long/sharp
136. Propod palmar angle spine, medial face, gnathopod 2: 0 = absent; 1 = short/blunt (truncate); 2 = long/sharp
137. Propod posterior margin spines (extending proximally from palm), gnathopod 2: 0 = absent; 1 = present
138. Propod posterior margin setae, gnathopod 2: 0 = absent; 1 = inserted singly; 2 = inserted in sets > 2
139. Propod posterior margin setae, gnathopod 2: 0 = absent; 1 = plain only; 2 = plumose present
140. Propod lateral face setae, gnathopod 2: 0 = absent; 1 = inserted singly; 2 = inserted in sets > 2
141. Propod lateral face setae, gnathopod 2: 0 = absent; 1 = plain only; 2 = plumose present
142. Propod medial face setae, gnathopod 2: 0 = absent; 1 = inserted singly; 2 = inserted in sets > 2
143. Propod medial face, anterior margin setae, gnathopod 2: 0 = absent; 1 = plain only; 2 = plumose present
144. Propod lateral face, anterior margin setae, gnathopod 2: 0 = absent; 1 = inserted singly; 2 = inserted in sets > 2
145. Propod lateral face, anterior margin setae, gnathopod 2: 0 = absent; 1 = plain only; 2 = plumose present
146. Propod medial face, anterior margin setae, gnathopod 2: 0 = absent; 1 = inserted singly; 2 = inserted in sets > 2
147. Propod medial face, anterior margin setae, gnathopod 2: 0 = absent; 1 = plain only; 2 = plumose present
148. Propad palm: 0 = normal; 1 = serrate
149. Elongated setae, base of palm (near conjunction with dactyl), propod, gnathopod 2: 0 = absent; 1 = plain; 2 = plumose
150. Carpus (5th article) posterior margin setae, gnathopod 2: 0 = absent; 1 = inserted singly; 2 = inserted in sets > 2
151. Carpus posterior margin setae, gnathopod 2: 0 = absent; 1 = plain only; 2 = plumose present
152. Carpus posterior margin modified setae (similar to the type 3ai, serrate pore-bearing setae, of Oshel and Steele, 1988), gnathopod 2: 0 = absent; 1 = present

Appendix B

Character states of all taxa used in this study. Question Marks denote unknown states. Dashes denote non-applicable character. ! = 10, # = 11, $ = 12, % = 13, & = 14

		10	20	30	40	50	60	70
Abludogammarus flavus	2001&%&800	1010010000	0000000000	0000000000	0000000000	0000021002	3220032330	2122221112
Abyssogammarus gracilis	3001671701	1010030000	0000000000	0000000000	0000000000	0000021002	3220032330	2222221212
Acanthogammarus brevispinus	3000008&10	0202110022	3023023303	3033023023	2032032030	0000012121	1001211001	1201210202
Acanthogammarus victorii	3001%%2%10	0202011030	3303303303	3133133133	1031031030	0000212001	1001211002	1202010102
Amathilina pusilla	2001#%#%00	1111200000	0000000000	0003003003	0030030020	0000021002	2220022220	0022221100
Ampithoe longimana	0001%%%%00	0000000000	0000000000	0000000000	0000000000	0000000002	2220022220	0002110000
Brandtia lata	10010%0%00	0101013030	3303303303	2033033033	1231231000	0000000002	3000033000	001?010100
Carinogammarus sp.	2001%&0910	0111030000	0000000000	2002002002	0020020022	2000022002	2110032110	2122221002
Chaetogammarus marinus	2001&%&%00	1010330000	0000000000	0000000000	0000000000	0000021002	2220022220	2122221111
Chaetogammarus obtusatus	2001&%&%00	1010220000	0000000000	0000000000	0000000000	0000021002	2220022220	2122221111
Chaetogammarus stoerensis	2001&%&%00	1010330000	0000000000	0000000000	0000000000	0000000002	2220022220	2122221010
Crangonyx serratus	2010%%##11	1010000000	0000000000	0000000000	0000000000	0000000002	2220022220	2102211010
Crypturupous pachytus	1001%%0%10	0100000000	0000000000	0000000000	0000000000	0000000002	0220030330	0020011000
Dikkerogammarus caspius	2000&%&%01	1111000000	0000000000	0000003003	0030030033	0000021002	2220032330	0020221212
Dikkerogammarus villosus	2001&%&%00	0210000000	0000000000	0000000000	0000000033	0000021002	2020032030	0022221202
Eogammarus confervicolus	2012&%&800	1110200000	0000000000	0000000000	0000000000	0000021112	3220022220	0022221212
Eogammarus oclairi	2012&%%800	1010300000	0000000000	0000000000	0000000000	0000021112	3220022220	0022221212
Eulimnogammarus cruentus	2001981800	1011030000	0000000000	0000000000	0000000022	0000021002	3220032330	2222221111
Eulimnogammarus inconspicuous	3011!9&$00	1010030000	0000000000	0000000000	0000000000	0000021002	3220032330	2122221111
Eulimnogammarus maacki	20018%8%00	1010030000	0000000000	0000000000	2002002002	2000021002	3220032330	2122211111
Eulimnogammarus testaceus	2001!8!800	1111020000	0000000000	0000000000	0000000022	0000021002	3220032330	2122221112
Eulimnogammarus verrucosus	2012785800	1111030000	0000000000	0000000000	0000000000	0000021002	3220032330	2122211111
Eulimnogammarus viridis	20018%8%00	1010030000	0000000000	0000000000	0000000022	0000021002	3220032330	2122221111
Eulimnogammarus viridulus	2001!%!%00	1111030000	0000000000	0000000000	0000000000	0000021002	3220032330	2122221111
Eulimnogammarus vittatus	2001989%00	1010030000	0000000000	0000000000	0000000000	0000021002	3220032330	2122221112
Gammarellus angulosus	2010%%%%01	1010020030	0200200200	2003003003	0030030030	0000000002	2220022220	0022011012
Gammarus aequicauda	2012&%&%00	1010300000	0000000000	0000000000	0000000022	0000022112	3220032330	2122221202
Gammarus annulatus	2002&%%%10	1111200000	0000000000	0000000000	0000000000	0000022002	3220022220	2122221212
Gammarus balcanicus	2001&%7900	1010330000	0000000000	0000000000	0000000000	0000021002	3220032330	2122221112
Gammarus duebeni	2011&%&%00	1111010000	0000000000	0000000000	0000000000	0000021002	3220022220	2122221112
Gammarus fasciatus	3002&%8800	1111300000	0000000000	0000000000	0000000022	0000022112	3220032330	2122220212

Appendix B (*continued*)

		10	20	30	40	50	60	70
*Gammarus lacustris*BK	2011&%&800	1111120000	0000000000	0000000000	0000000000	0000021002	3220022220	2122221202
Gammarus lacustris HV	2011&%&800	1111120000	0000000000	0000000000	0000000000	0000021002	3220022220	2122221202
Gammarus lacustris VI	2011&%&800	1111120000	0000000000	0000000000	0000000000	0000021002	3220022220	2122221202
Gammarus lacustris WA	2011&%&800	1111120000	0000000000	0000000000	0000000000	0000021002	3220022220	2122221202
Gammarus locusta	3012&%&%00	1010300000	0000000000	0000000000	0000000022	0000022002	3220022220	2122221202
Gammarus mucronatus	2002&%&801	1010300000	0000000000	0000000004	0040040000	0000022112	3220032330	2122221102
Gammarus oceanicus	2001&%&800	1010310000	0000000000	0000000000	0000000000	0000021112	3220022220	2122221212
Gammarus palustris	200?$%0800	1011300000	0000000000	0000000000	0000000000	0000021112	3220032330	2122221202
Gammarus pseudolimnaeus	2011&%%800	1212030000	0000000000	0000000000	0000000000	0000021002	3220022220	2122221202
Gmelinoides fasciatus	1001%%8%00	0101010000	0000000000	0000000002	0020020000	0000011112	3220032330	1121221102
Hakonboekia strauchi	200?%%3700	1010030302	3023023023	0234034004	0040040000	0222222000	2220032030	1210010102
Macrohectopus branickii	0000&8%%00	1010030000	0000000000	0000000004	0040040000	0000022000	2200022221	1221010202
Megomaera subtener	2001%$&%00	1010000000	0000000000	0000000004	0040040044	4000000002	3220023220	2122221111
Melita nitida	2000%#%800	1010000000	0000000000	0000000000	0000000000	0000021002	3220022220	2120211010
Micruropus crassipes	1000#50710	0211010000	0000000000	0000000000	0000000000	0000021112	2220022220	0020211000
Micruropus fixseni	1000#&0810	0211000000	0000000000	0000000000	0000000000	0000021111	2220012220	002?211000
Micruropus glaber	1000$70800	0110000000	0000000000	0000000000	0000000000	0000021002	2220022220	2120221110
Micruropus wahli	1110&%&%10	1212010000	0000000000	0000000000	0000000000	0000012122	2220002330	1122211102
Monoporeia affinis	??0?7%%%20	0313300000	0000000000	0000000000	0000000020	0000000???	2??0022???	001?21????
Odontogammarus calcaratus	3001&%3800	1010030000	0000000000	0000000000	0000000000	0000022002	3220032330	2122221112
Ommatogammarus albinus	3001#%&%00	1010030000	0000000000	0000000000	0000000000	0000021002	3220032330	2122221102
Pallasea cancelloides	2001%%0%00	0101012303	3133133133	1331331332	3023023020	0000312000	1000001002	1110010102
Pallasea cancellus	2001&%1%01	1010030323	0232232232	2322322322	3023023022	2002322002	3220002331	1222210202
Pallasea grubei	2001%%0%00	0101011320	3123123123	1331331331	3013013020	2000212000	1000001002	1211010102
Pallasea viridis	2000%%1&00	0101000303	3033033033	0330321321	3003003000	0000212000	1001201002	1210120102
Parapallasea borowskii	3001&%6%00	1111030000	2002002002	0320320320	3203203222	0000022002	3220032330	1222221212
Plesiogammarus brevis	2000&09700	0202030000	2002002002	0020020020	0200200200	0000000001	1220012330	2222010100
Poekilogammarus pictoides	2001&92601	1010030000	0000000000	0000000000	0000000000	0000022002	1220032330	1221010202
Pontogammarus abbreviatus	1000#%&%10	1411000000	0000000000	0000000000	0000000002	0000021002	2020032030	0022221202
Pontogammarus crossus	2000&%&%10	0401000000	0000000000	0000000000	0000000002	0000021002	2220032330	0022221202
Pseudomicruropus rotundatulus	100?%%0%00	0101000000	0000000000	0001001001	0000000000	0000021002	2220022330	00???11000
Ramellogammarus vancouverensis	20019%9810	1111400000	0000000000	0000000000	0000000000	0000021112	3220022220	0020221111

	80	90	100	110	120	130	140	150
Abludogammarus flavus	1120112125	0000211001	2201110121	1112221212	1002121012	2020220222	1002121012	20
Abyssogammarus gracilis	1130112125	2222211101	1101011121	1101220212	1002121012	1022220221	1002121012	20
Acanthogammarus brevispinus	0130202013	1212100102	2101111021	1000221212	1002121002	1100200220	0002121002	20
Acanthogammarus victorii	0231212022	1200101102	2201010021	0002221210	0002121002	2112220220	0002121002	20
Amathilina pusilla	1120000115	0000011002	2201010121	0112220211	1002121012	2012220221	1002121012	20
Ampithoe longimana	1050010110	12–100110	2101011021	0120000210	0212121012	1010000210	0212121012	10
Brandtia lata	0040001014	1100100102	2201110121	0110220211	1002121012	2010220222	1002121012	20
Carinogammarus sp.	1230202125	0000200102	1201111121	0110220222	0002121102	2010220222	1002121102	20
Chaetogammarus marinus	1121000105	2100201002	2201110121	0012221210	0210021012	2122220220	0212121012	21
Chaetogammarus obtusatus	1120011115	2100201102	2101111121	0010220210	0212121012	2010220220	0212121012	20
Chaetogammarus stoerensis	1020000105	2100211002	2201111121	0210220210	0210000012	2110220220	0210000012	21
Crangonyx serratus	1010000003	0022211001	1101101111	0216330220	0002121011	2106220220	0002121001	21
Crypturupous pachytus	1120010015	0000200102	2101111021	0110220212	1002121102	2020220212	1002121102	20
Dikkerogammarus caspius	1120100105	0000201101	1101011121	0212220212	1002121012	2012220222	1002121012	20
Dikkerogammarus villosus	1120001104	0000211002	2201111121	0202210212	1002121012	2011110212	1002121012	20
Eogammarus confervicolus	1220012125	2100201002	1201011121	0123110212	1212121012	1023110210	0212121012	21
Eogammarus oclairi	1120010125	2100201002	1201011121	0123110110	0210021002	1023110220	0210021012	21
Eulimnogammarus cruentus	1120111114	2100201102	2201111121	1001221212	1002121012	1022200222	1002121012	21
Eulimnogammarus inconspicuous	1120111115	2100211102	2202111121	1002221212	1002121012	2012220222	1002121012	21
Eulimnogammarus maacki	1120001114	2100201102	2202111121	1102220212	1002121012	2022220222	1002121012	20
Eulimnogammarus testaceus	1130112115	2100211102	2201110121	1002221212	1002121012	1112220222	1002121012	21
Eulimnogammarus verrucosus	1120011115	2100201102	22?1110121	0102221212	1002121012	2112220222	1002121012	21
Eulimnogammarus viridis	1120011115	2100200102	2201011121	1102220212	1112121012	2010220222	1002121012	21
Eulimnogammarus viridulus	1120100115	2100201102	2201111121	10012?0212	1002121012	20222?0222	1002121012	21
Eulimnogammarus vittatus	1121012115	2200211102	2201111121	1102220212	1002121012	2022210222	1002121012	21
Gammarellus angulosus	1031212101	0011011002	2201010111	0104221210	0222222002	2004221210	0222222002	20
Gammarus aequicauda	1131212015	2200201102	2201110121	0202220211	1002121112	2112220221	1002121112	20
Gammarus annulatus	1231212125	2221201102	2?01011121	0211120210	0212121112	2011220220	0210021112	20

(*continued on next page*)

Appendix B (*continued*)

		10	20	30	40	50	60	70
Gammarus balcanicus	1130112115	0000211002	2201110121	1102221212	1002121012	2012220222	1002121012	20
Gammarus duebeni	1131212115	2200211102	2201111121	0202221210	0212121112	2112220220	0212121112	21
Gammarus fasciatus	1131212115	2100201102	2201111121	0202220221	1002121112	2011220221	1002121112	20
*Gammarus lacustris*BK	1230212125	0000211002	1201111121	1102221210	0212100012	2012220220	0210000012	20
Gammarus lacustris HV	1230212125	0000211002	1201111121	0102221210	0212121012	2012220220	0212121012	20
Gammarus lacustris VI	1230212125	0000211002	1201111121	1102221210	0212121012	2012220220	0210021012	20
Gammarus lacustris WA	1230212125	0000211002	1201111121	1102221210	0212121012	2012220220	0210021012	20
Gammarus locusta	0241212025	2200201102	22011?1121	0201220210	0210021112	2111220220	0212121112	20
Gammarus mucronatus	1231212125	2221201002	22011?0111	0201221210	0102121112	2011210211	1002121112	20
Gammarus oceanicus	1231212125	2100201002	2201110021	0110220220	0212121112	2110220220	0212121112	21
Gammarus palustris	1230212125	2100201002	2201110111	0201220210	0002100112	2111220210	0002111112	20
Gammarus pseudolimnaeus	1131212115	2121201102	1201110121	0202221210	0212121012	2012220220	0212121012	20
Gmelinoides fasciatus	0120000015	0000200102	2201110011	0111220211	1002121012	2012220221	1002121012	20
Hakonboekia strauchi	0130202012	1212110001	?101110111	1012220210	0001111002	2122220220	0110121002	20
Macrohectopus branickii	0?402120?3	0021210110	2112000011	0102220220	0002121112	2002220220	0002121112	20
Megomaera subtener	1120000115	0021211110	1101111021	0200010210	0002121012	2000020210	0002121012	20
Melita nitida	1020010005	0021211110	1101111021	0220000210	0002121002	1014200210	0212121002	20
Micruropus crassipes	1120000012	0000200102	2201010021	0112221212	1002121012	2012220222	1002121012	20
Micruropus fixseni	0130000015	0000200102	2201110021	0102221212	1002121002	2012220222	1002121002	20
Micruropus glaber	1120000015	0000200102	1??1110011	0101210112	1002111012	2011220222	1002121012	20
Micruropus wahli	1220002024	0000200100	1101010121	0102220211	1002121112	2012220211	1002121112	20
Monoporeia affinis	??3??????4	0000?10210	110100-122	1?????????	??????????	??????????	??????????	??
Odontogammarus calcaratus	1131212104	2200211102	2201110011	0112220211	1002121012	2022220221	1002121012	20
Ommatogammarus albinus	1120212125	2100211101	2101000121	1110220212	1002121012	2020220222	1002121012	20
Pallasea cancelloides	0130202011	1200101102	2201110021	0000221211	1002121012	2010220221	1002121012	20
Pallasea cancellus	0130212011	0000210102	2201111121	0102221212	1002121012	2112220222	1002121012	20
Pallasea grubei	0120002011	1100100102	2201111021	0102220212	1002121012	2012220222	1002121012	20
Pallasea viridis	0130202011	1200111102	2201110021	0102221212	1002111012	2012220222	1002121012	20
Parapallasea borowskii	1131212114	1100201102	2201011021	0102220210	0002121012	2012220220	0002121012	20
Plesiogammarus brevis	1130001115	0000101102	2201010011	0100201221	1001111002	2110201221	1001111002	22
Poekilogammarus pictoides	0140212014	1212111002	2201110021	0102220211	1002111012	2122220221	1002111012	20
Pontogammarus abbreviatus	1220002125	0000211102	2101011121	0212220210	0002121012	2020221220	0002121012	20
Pontogammarus crossus	1120002115	0000211102	2201010121	0202220211	1002121012	2012220221	1002121012	20
Pseudomicruropus rotundatulus	1020000015	1200211110	1101000011	0112220210	0112121002	1022221100	0112121012	20
Ramellogammarus vancouverensis	1120001115	0000201102	1201011121	0113110212	1112121012	1013110210	0212121012	20

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