Phylogenetic Analysis of Caprellid and Corophioid Amphipods (Crustacea) Based on the 18S rRNA Gene, With Special Emphasis on the Phylogenetic Position of Phtisicidae

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Abstract. Members of the amphipod suborder Caprellidea exhibit degenerated abdomens and pereopods 3 and 4. Some genera of Podoceridae (Gammaridea, Corophioidea) such as *Dulichia* also show reduced abdomens and pereopods and thus are generally regarded as a sister group of the Caprellidea. In addition, one of the caprellid families, the Caprogammaridae, exhibits abdominal segments that are similar to those of the podocerids, as well as rudimentary pereopods 3 and 4, which are more consistent with those of other caprellids. Therefore, an evolutionary scheme has been suggested on the basis of the gradual degeneration of the pereopods and abdomen: [*Dulichia*, (caprogammarids, caprellids)]. However, the Phtisicidae (Caprellidea) contradict this hypothesis because they exhibit well-developed pereopods 3 and 4, along with degenerated abdomens. Therefore, previous studies have suggested that the Phtisicidae and other caprellids may be polyphyletic. We examined the phylogenetic position of the Phtisicidae and other caprellid amphipods, using 18S rRNA gene sequence data. The results strongly indicate that the Phtisicidae and other caprellid families form a monophyletic clade. However, a close phylogenetic relationship among *Dulichia* (Corophioidea) and taxa belonging to the Caprellidea was not definitively supported. This study is the first to use molecular data to investigate the phylogenetic relationships among the Caprellidea.

Introduction

Caprellidea, a suborder of the order Amphipoda (Crustacea, Malacostraca, Peracarida), comprises more than 300 described species and is classified into eight families (Caprellidae, Caprellinoididae, Caprogammaridae, Cyamidae, Paracercopidae, Pariambidae, Phtisicidae, and Protellidae; Laubitz, 1993; Martin and Davis, 2001). All Caprellidea species are marine and benthic and are typically found on substrata such as seaweed, hydroids, sponges, and sediment.

The general characteristics of the Caprellidea include a slender and cylindrical body, fusion of the head and the pereonite 1, rudimentary coxae, two pairs of gills, brood plates on pereonites 3 and 4, reduced or absent pereopods 3 and 4, and a degenerated abdomen and abdominal appendages. These characteristics are highly divergent from the body plan of other malacostracan crustaceans; therefore, Caprellidea are of great interest for understanding the evolution of morphological novelty. However, many questions remain regarding their evolution. For example, there is ongoing debate as to whether the Caprellidea should be considered a monophyletic group. Some morphology-based phylogenetic studies have suggested a phylogenetic affinity between the Caprellidea and the superfamily Corophioidea, which belongs to the suborder Gammaridea (Amphipoda; *e.g.,* Barnard, 1974; Kim and Kim, 1993). In particular, the family Podoceridae (Gammaridea, Corophioidea) is considered the closest taxon to the Caprellidea because some genera such as *Dulichia* exhibit characteristics similar to those of the Caprellidea (*e.g.,* McCain, 1968; Laubitz, 1979; Takeuchi, 1993; Bousfield and Shih, 1994). Members of *Dulichia* and its allied genera such as *Neoxenodice* are

characterized by almost cylindrical bodies and relatively small pereopods 3 and 4 and abdominal parts. The number of gills, brood plates, and uropods is also reduced (Laubitz, 1979). Conversely, the Caprogammaridae (Caprellidea) exhibit some features that are Caprellidea-like and others that are more characteristic of the Podoceridae genera. For example, the Caprogammaridae possess segmented pleonites and urosomites with appendages, as well as a caprellid-like cylindrical body, two pairs of gills and brood pouches, reduced coxae, and rudimentary and unsegmented pereopods 3 and 4 (Laubitz, 1976; Takeuchi and Ishimaru, 1991). These morphological data suggest that the Caprogammaridae are an intermediate taxon between podocerids (*Dulichia* and its allied genera) and other caprellids (Fig. 1). The Paracercopidae (Caprellidea) are also considered a primitive family of the Caprellidea, because members of the group retain the five-segmented abdomen with appendages (McCain, 1968; Laubitz, 1976; Takeuchi, 1993), although the abdominal appendages are more rudimentary than those of the Caprogammaridae (Laubitz, 1970, 1972, 1993).

Another Caprellidea family, the Phtisicidae, complicates this simplistic evolutionary scheme of the gradual degeneration of pereopods. Pereopods 3 and 4 of some genera of the Phtisicidae family are segmented into six parts, whereas all other caprellids, including the Caprogammaridae, either lack or exhibit very rudimentary pereopods 3 and 4. In addition, whereas other caprellids generally have two pairs of gills on pereonites 3 and 4, most phtisicids have an additional pair of gills on pereonite 2. Given these morphological distinctions, Takeuchi (1993) suggested that the Phtisicidae may have evolved *via* a distinct lineage from the Caprogammaridae and other caprellids, which evolved from podocerid-like ancestors, and thus that the Caprellidea are polyphyletic (Fig. 1). Laubitz (1993) also suggested the possibility that the Caprellidea are a polyphyletic group, basing the suggestion on several characters of the mouthparts, including the presence or absence of the mandible molar. According to Laubitz (1993), one lineage (which has retained the mandible molar)—including the Caprogammaridae, Pariambidae, Protellidae, and Caprellidae—derived from the Corophioidea, whereas another lineage (which has lost the mandible molar)—including the Phtisicidae, Caprellinoididae, Cyamidae, and Paracercopidae—derived from a different gammaridean amphipod group, the Leucothoidea. Recently, however, a morphology-based cladistic analysis of the Corophioidea (Gammaridea) and the Caprellidea supplied evidence that Caprellidea are indeed a monophyletic lineage (Myers and Lowry, 2003).

For the Amphipoda, which are a problematic group because of poorly defined morphological features that make it difficult to identify homology, the analysis of mitochondrial and nuclear gene sequences is important for understanding the evolutionary relationships at different taxonomic levels (*e.g.,* Meyran *et al.,* 1997; Englisch and Koenemann, 2001;

Englisch *et al.,* 2003; Lörz and Held, 2004; Davolos and Maclean, 2005; Macdonald *et al.,* 2005; Tomikawa *et al.,* 2007). In particular, molecular data have already indicated the monophyly of several families (Englisch *et al.,* 2003); however, the molecular phylogenetic relationships among the Caprellidea and their allies have not been analyzed. We examined the phylogenetic relationships among members of the Phtisicidae and other caprellid families, using 18S ribosomal RNA (18S rRNA) gene sequences to resolve the controversial issues of the caprellid phylogeny. Specifically, we focused on whether the Phtisicidae form a monophyletic clade with other caprellid families. In addition, we tested the phylogenetic relationship among Caprellidea and some corophioidean gammarids to determine whether the phylogenetic affinity between Caprellidea and Podoceridae, in particular the genus *Dulichia,* is supported by 18S rRNA gene sequence data.

Materials and Methods

Sampling

Sampling was conducted from the spring of 2005 to the summer of 2006. Seven species from five caprellid families (Caprellidae, Caprellinoididae, Pariambidae, Phtisicidae, and Protellidae) and nine species from four corophioidean families (Ampithoidae, Corophiidae, Ischyroceridae, and Podoceridae) were collected at various sites along the coast of Japan (Table 1). We followed the familial classification suggested by Laubitz (1993) for the Caprellidea and that of Barnard and Karaman (1991) for the Corophioidea. The sampling sites consisted of rocky shores, tidal flats, and subtidal sediment at depths ranging from 5 to 100 m. Scuba diving was used to collect samples from shallow waters in subtidal areas, whereas bottom dredging was used in deeper waters. The specimens collected were transported on ice and preserved in 100% ethanol or stored in a deep freezer until use. In addition, the 18S rRNA gene sequences of *Caprella geometrica* (Caprellidae) and two outgroup species, *Synurella dentata* (Gammaridea, Crangonyctidae) and *Niphargus fontanus* (Gammaridea, Niphargidae), were obtained from GenBank (National Center for Biotechnology Information, NCBI; Table 1). *Synurella dentata* and *N. fontanus* were used as outgroups because preliminary analyses that included all available 18S rRNA gene sequences of the gammarid families indicated that they were basal to the corophioidean families.

Molecular methods

Genomic DNA was extracted from the appendages of the specimens using the DNeasy Tissue kit from Qiagen. Polymerase chain reaction (PCR) was used to amplify the 18S rRNA gene from the extracted DNA using specific primers (Table 2; Englisch *et al*., 2003). PCR was performed with
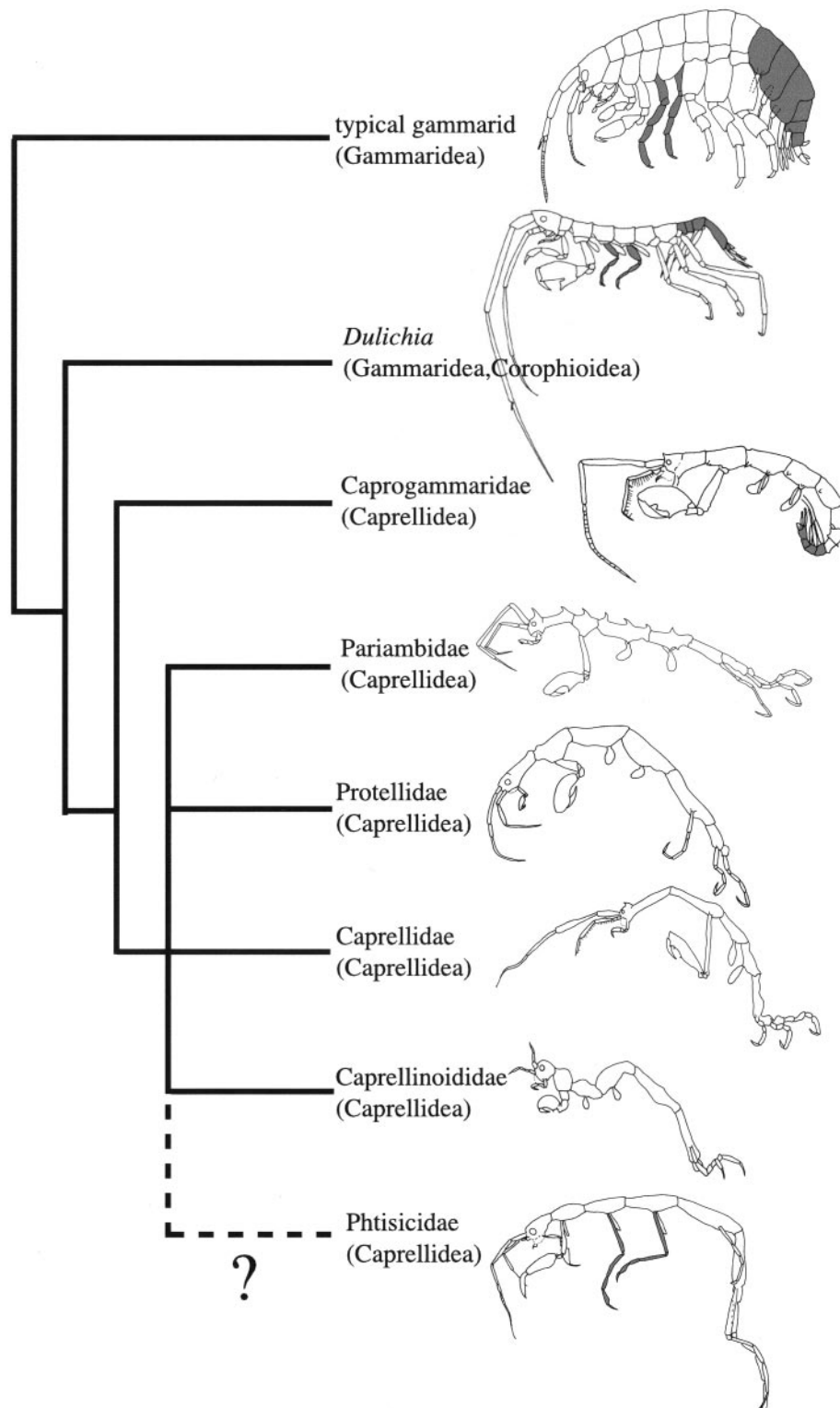
Figure 1. A simple evolutionary scheme of [*Dulichia* (caprogammarids, caprellids)] based on the gradual degeneration of the abdomen and pereopods 3–4; a dashed line shows the controversial position of the Phtisicidae. At hatching, pereopods 3 and 4 have six articles and the abdomen has more than five segments (pleosome and urosome). Two other caprellid families that were not included in the present analyses (Paracercopidae and Cyamidae) are not shown here. Pereopods 5–7 were omitted from the plate of the Caprogammaridae to show their abdominal segments. Figures are redrawn from fig. 1C of Laubitz (1983; *Dulichia*) and fig. 1 of Barnard and Karaman (1991; typical gammarid), with permission of the Australian Museum; and from figs. 21-173A, 21-175A, and 21-180A of Takeuchi (1995; Caprogammaridae, Caprellidae, and Phtisicidae), with permission of Hoikusha Publishing Co., Ltd.

Table 1

Summary of information for species included in the analyses

Species	Family	Collection site	Sequence length (bp)	GenBank accession number
Caprellidea				
Caprella danilevskii	Caprellidae	Tomioka Bay, Kumamoto	2194	AB295398
Caprella geometrica*	Caprellidae	—	2177	AY781423
Perotripus sp.	Caprellinoididae	Sotoura Bay, Shizuoka	2256	AB295401
Pseudoprotella sp.	Pariambidae	Tateyama Bay, Chiba	2310	AB295397
Monoliropus tener	Protellidae	Oura Bay, Shizuoka	2278	AB295395
Protella gracilis	Protellidae	Tomioka Bay, Kumamoto	2523	AB295396
Protogeton sp.	Phtisicidae	Oura Bay, Shizuoka	2286	AB295400
Protomima imitatrix	Phtisicidae	Oura Bay, Shizuoka	2339	AB295399
Corophioidea				
Ampithoe lacertosa	Ampithoidae	Sotoura Bay, Shizuoka	2279	AB295402
Corophium sp.	Corophiidae	Oura Bay, Shizuoka	2226	AB295404
Gammaropsis utinomii	Corophiidae	Tomioka Bay, Kumamoto	2273	AB295406
Grandidierella japonica	Corophiidae	Sanban-ze, Chiba	2326	AB295403
Bubocorophium sp.	Ischyroceridae	Oura Bay, Shizuoka	2398	AB295405
Ericthonius pugnax	Ischyroceridae	Suruga Bay, Shizuoka	2411	AB295407
Jassa slatteryi	Ischyroceridae	Tosa Bay, Kochi	2161	AB295408
Dulichia sp.	Podoceridae	Oura Bay, Shizuoka	2357	AB295394
Podocerus inconspicuus	Podoceridae	Oura Bay, Shizuoka	2325	AB295409
Outgroup				
Synurella dentata*	Crangonyctidae	—	2313	AF419233
Niphargus fontanus*	Niphargidae	—	2237	AF202981

* Sequence data were obtained from GenBank.

Ex Taq polymerase (Takara) using 1–20 ng of DNA as template. The conditions for PCR cycling using an iCycler (BIO-RAD) were as follows: 1×5 min at 94 °C, 35×1 min at 94 °C, 1 min at 60 °C, 2 min at 72 °C, and 1×5 min at 72 °C. The PCR products were separated by size using electrophoresis in 1% low-melting agarose gel and purified using the Wizard(R) SV Gel and PCR Clean-Up system (Promega). The purified PCR products were sequenced directly on a Beckman CEQ 2000 DNA sequencer using the GenomeLab DTCS Quick Start kit (Beckman Coulter, Fullerton, CA) or using the DNA sequence analysis service provided by Bio Matrix Research, Inc. (Chiba, Japan). The cycle sequencing reaction was conducted following the manufacturer's instructions.

Table 2

Primers used for PCR and cycle sequencing (Englisch et al., *2003)*

Primer	Sequence (5′–3′)
PCR	
Small SubunitF	CCTACCTGGTTGATCCTGCCAGT
Small SubunitR	TAATGATCCTTCCGCAGGTT
Cycle sequencing	
400F	ACGGGTAACGGGGAATCAGGG
400R	CCCTGATTCCCCGTTACCCGT
700F	GTCTGGTGCCAGCAGCCGCG
700R	CGCGGCTGCTGGCACCAGAC
1000F	CGATCAGATACCGCCCTAGTTC
1000R	GAACTAGGGCGGTATCTGATCG
1155F	CTGAAACTTAAAGGAATTGACGG
1155R	CCGTCAATTCCTTTAAGTTTCAG
1250F	CCGTTCTTAGTTGGTGGAGCG
1250R	CGCTCCACCAACTAAGAACGGCC
1500R	CATCTAGGGCATCACAGACC
1600F	CGTCCCTGCCCTTTGTACACACC

Phylogenetic analysis

Sequence data sets were initially aligned using Clustal X ver. 1.81 (Thompson *et al.,* 1997) with 10/1 pairwise gap opening/extension penalty and 10/2 multiple gap opening/extension penalty, then realigned by eye. Some nucleotide sites were difficult to align confidently because of substantial length heterogeneity and the lack of a conserved stretch of nucleotide sequences. We therefore excluded these sites from the analyses. We took a conservative approach to reduce the risk of comparing non-homologous sites; thus, the edges of the retained sites were highly conserved.

Phylogenetic trees were constructed using neighbor-joining (NJ; Saitou and Nei, 1987), maximum parsimony (MP), and maximum likelihood (ML; Felsenstein, 1981) methods in PAUP*4.0b (Swofford, 2002). The GTR + I + G model selected by the Akaike information criterion (AIC) in

Modeltest (Posada and Crandall, 1998) was used for the NJ analysis. The ML analysis was performed using the heuristic search with a stepwise addition algorithm, the axis option, and TBR branch swapping in PAUP*. The GTR + I + G model was used for the ML analysis as the substitution model. The MP analysis assumed equal weights for transitions and transversions and was conducted using the branch-and-bound algorithm. We performed 1000 bootstrap pseudoreplicates for the NJ and MP analyses and 100 bootstrap pseudoreplicates for the ML analysis to evaluate the confidence for each node. In addition, the SH test (Shimodaira and Hasegawa, 1999) using 1000 replicates estimated using the resampling estimated log-likelihood (RELL) method was performed to examine the validity of the ML tree.

Results

The 18S rRNA gene isolated from the 19 species examined ranged from 2161 to 2523 bp in length (Table 1), and the data set consisted of 1596 bp after alignment. The alignment data were registered in the EMBL Nucleotide Sequence Database (accession No. ALIGN_001210). We confirmed that the GC ratio of the sequence data fell in the range of 52% to 54%. Therefore, a biased G-C content was not likely to be problematic (Hasegawa and Hashimoto, 1993).

The monophyly of all caprellid families in this study (including Phtisicidae) was strongly supported by the three analyses (Fig. 2). All trees showed almost the same topology, and similar nodes were supported with high confidence. In addition, we performed the SH test to confirm the validity of the ML tree. Specifically, we constructed phylogenetic trees that did not contain the Phtisicidae and the Caprellinoididae within the clade of the Caprellidea and examined whether these alternative trees were rejected statistically. *Perotripus* sp. (Caprellinoididae) was also eliminated from the Caprellidea clade in alternative trees because some studies have considered it to belong to the Phtisicidae (*e.g.*, McCain, 1970). All alternative trees were significantly rejected by the SH test.

The 18s rRNA gene trees supported the sister grouping of *Podocerus inconspicuus* (Podoceridae) and *Jassa slatteryi* (Ischyroceridae) with the taxa of Caprellidea examined in the present study. Confidence values for this clade were high, particularly in the ML tree (Fig. 2); however, the SH test did not strongly support this result. Most alternative trees, including the sister grouping of the Caprellidea and *Dulichia*, could not be significantly rejected.

Discussion

Phylogenetic affinity of the Phtisicidae with other caprellid families

The Caprellidea have evolved fascinating morphological characters distinct from those of other malacostracan crustaceans. Their strongly degenerated pereopods and abdomen are particularly remarkable morphological novelties, probably associated with their clinging behavior. However, it remains a mystery how and why the Caprellidea developed such unique morphologies. Moreover, Caprogammaridae and Phtisicidae add further mystery, because the Caprogammaridae have a five-segmented abdomen with appendages, while the Phtisicidae have six-segmented pereopods 3 and 4 (Fig. 1). On the basis of these morphological distinctions, Takeuchi (1993) suggested that the Caprellidea may be a polyphyletic group. Laubitz (1993) also proposed two distinct evolutionary lines based on mouthpart structure and regarded the Phtisicidae as being derived from a different evolutionary process than that of the corophioid–caprogammarid–caprellid lineage. However, our analyses based on the 18S rRNA gene strongly support the phylogenetic affinity of five caprellid families, including the Phtisicidae (Fig. 2).

Assuming that the five-segmented abdomen and pereopods 3–4 with six articles are plesiomorphic characters (Takeuchi, 1993), the caprellid families Caprogammaridae and Phtisicidae show intermediate specialized morphologies (Fig. 3). Therefore, according to the phylogenetic trees in Figure 2, we assume that the degeneration of the abdomen and pereopods 3–4 occurred either simultaneously (*e.g.,* Caprellidae) or separately (*e.g.,* Phtisicidae) in the different evolutionary lines of the Caprellidea. The evolutionary plasticity of these morphological characters is probably a consequence of adaptive radiation of the caprellid amphipods. The availability of DNA sequences from caprogammarid species and other caprellid taxa will provide essential information to elucidate the phylogenetic relationships within the Caprellidea (Figs. 2, 3), and consequently to define in detail our hypothesis on the evolutionary patterns of the morphological diversity among the Caprellidea.

Figure 2. Phylogenetic trees of the examined caprellid and corophioidean taxa and two outgroup species (*Niphargus fontanus* and *Synurella dentata*) based on 18S rRNA gene sequence data. (A) Neighbor-joining tree constructed using the GTR + I + G model. (B) Strict consensus tree of three maximum parsimony trees (length = 587). (C) Maximum likelihood tree constructed using the GTR + I + G model (−lnL = 5428.54501). Numbers below branches are bootstrap values; values less than 50% are not shown. Thin gray bars indicate outgroups, thicker gray bars indicate corophioidean families, and thick black bars indicate caprellid families.
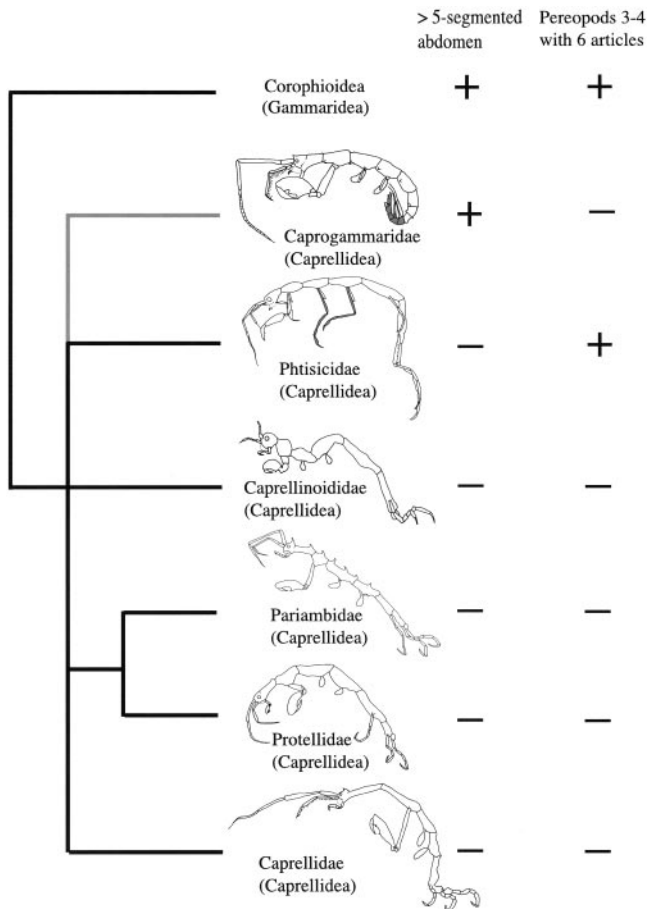
A

B

C
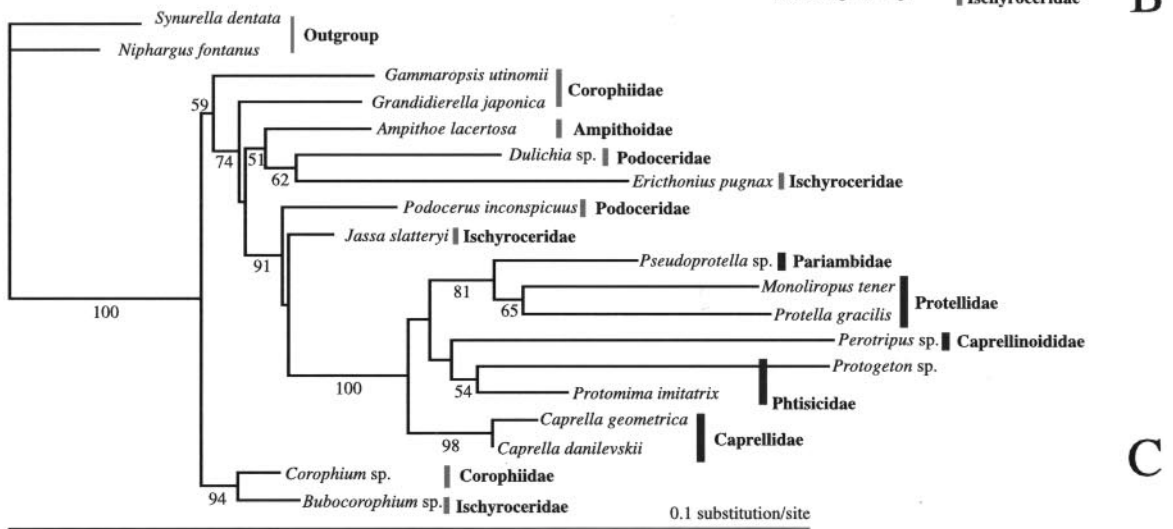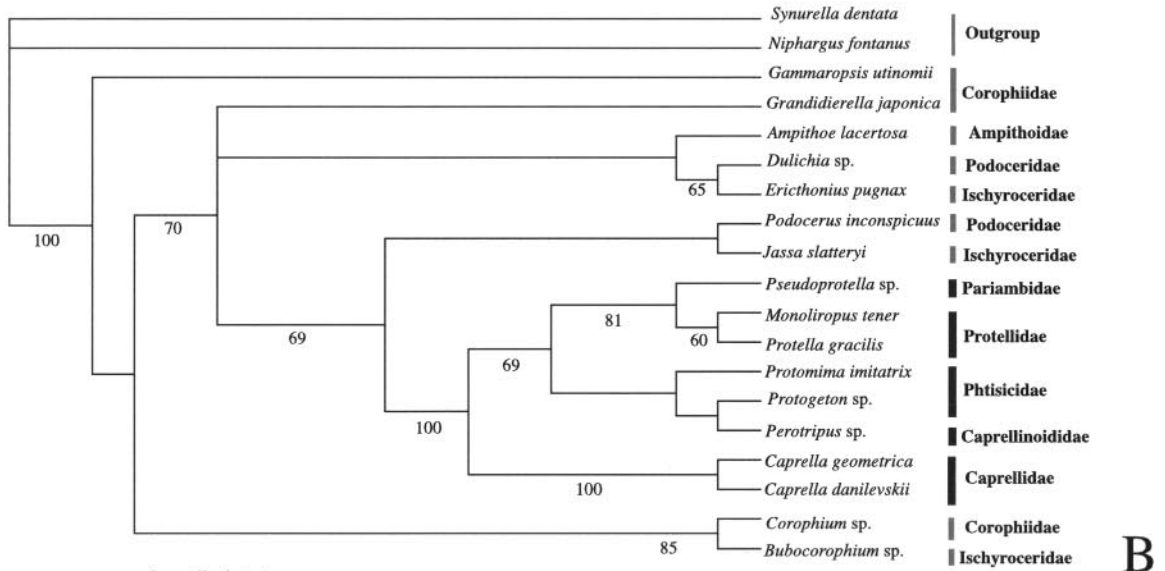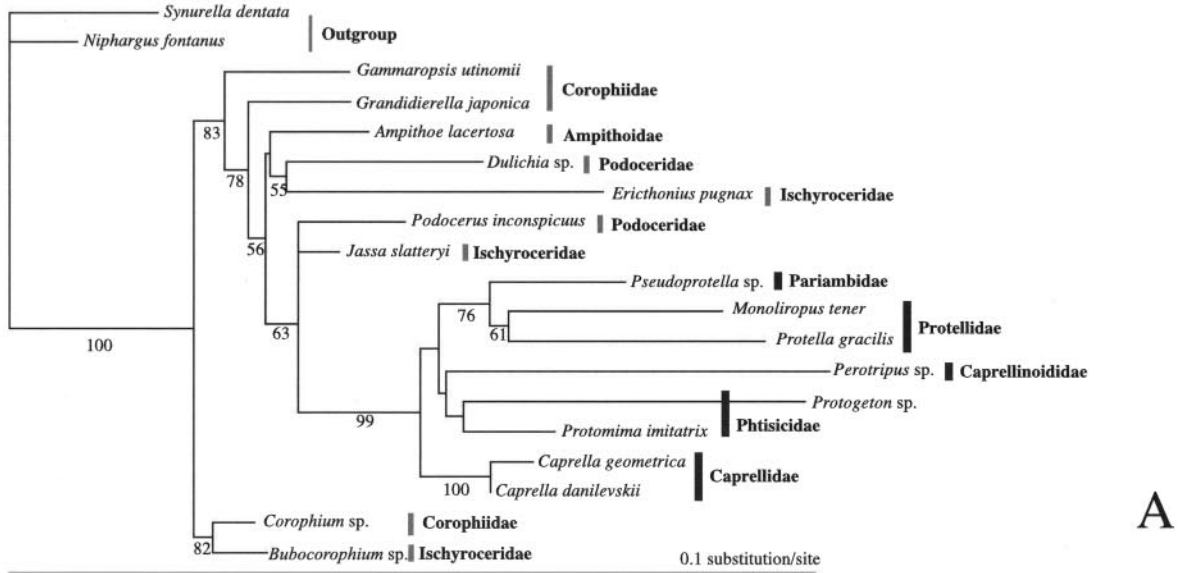
Figure 3. Schematic cladogram representing the phylogenetic relationships of caprellid families based on the present study. A branch leading to the Caprogammaridae is shown in gray because it was not included in the present analyses. Plus and minus signs indicate the presence or absence of pereopods 3 and 4 with six articles and an abdomen with more than five segments.

Phylogenetic relationship between the Caprellidea and Dulichia

On the basis of morphological characters, *Dulichia* (Podoceridae) and its allied genera have been considered to form a sister group of the Caprellidea (Fig.1; *e.g.,* McCain, 1968; Laubitz, 1979; Takeuchi, 1993). In addition, these podocerids and the caprellids show similar clinging behavior (Takeuchi, 1993). However, these observations are not in agreement with our results indicating that the Caprellidea and *Dulichia* are not closely related (Fig. 2). Homoplasious convergence of unrelated lineages could therefore be a plausible explanation for this apparent incongruence. However, there is molecular evidence in favor of phylogenetic affinities between caprellids and corophioids (Fig. 2). Caprellid amphipods may have arisen from a corophioid-like ancestor (Fig. 2), although more evidence from more taxa is necessary to support this hypothesis.

Our phylogenetic analyses (Fig. 2) suggest that the reduction of the pereopods and abdomen probably occurred independently in the Caprellidea and the corophioidean genera. However, our knowledge on the patterns of molecular evolution within these Amphipoda remains limited. Additional sampling and future studies based on a large set of molecular markers will help define the evolutionary relationships of these peculiar crustacean taxa and have a profound influence on our understanding of their stunning morphological diversity.

Acknowledgments

Literature Cited

Barnard, J. L. 1974. Evolutionary patterns in gammaridean Amphipoda. *Crustaceana* **27:** 137–146.

Barnard, J. L., and G. S. Karaman. 1991. *The Families and Genera of Marine Gammaridean Amphipoda (Except Marine Gammaroids)*, 2 vols. *Rec. Aust. Mus. Suppl.* **13 (Part 1 and 2),** Australian Museum, Sydney. 866 pp.

Bousfield, E. L., and C.-T. Shih. 1994. The phyletic classification of amphipod crustaceans: problems in resolution. *Amphipacifica* **1:** 76–134.

Davolos, D., and N. Maclean. 2005. Mitochondrial COI-NC-COII sequences in talitrid amphipods (Crustacea). *Heredity* **94:** 81–86.

Englisch, U., and S. Koenemann. 2001. Preliminary phylogenetic analysis of selected subterranean amphipod crustaceans, using small subunit rDNA gene sequences. *Org. Divers. Evol.* **1:** 139–145.

Englisch, U., C. O. Coleman, and J. W. Wägele. 2003. First observations on the phylogeny of the families Gammaridae, Crangonyctidae, Melitidae, Niphargidae, Megaluropidae and Oedicerotidae (Amphipoda, Crustacea), using small subunit rDNA gene sequences. *J. Nat. Hist.* **37:** 2461–2486.

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J. Mol. Evol.* **17:** 368–376.

Hasegawa, M., and T. Hashimoto. 1993. Ribosomal RNA trees misleading? *Nature* **361:** 23.

Kim, C. B., and W. Kim. 1993. Phylogenetic relationships among gammaridean families and amphipod suborders. *J. Nat. Hist.* **27:** 933–946.

Laubitz, D. R. 1970. Studies on the Caprellidae (Crustacea, Amphipoda) of the American North Pacific. *Natl. Mus. Can. Publ. Biol. Oceanogr.* **1:** 1–89.

Laubitz, D. R. 1972. The Caprellidae (Crustacea, Amphipoda) of Atlantic and Arctic Canada. *Natl. Mus. Can. Publ. Biol. Oceanogr.* **4:** 1–82.

Laubitz, D. R. 1976. On the taxonomic status of the family Caprogammaridae Kudrjaschov & Vassilenko (Amphipoda). *Crustaceana* **31:** 145–150.

Laubitz, D. R. 1979. Phylogenetic relationships of the Podoceridae (Amphipoda, Gammaridea). *Bull. Biol. Soc. Wash.* **3:** 144–152.

Laubitz, D. R. 1983. A revision of the family Podoceridae (Amphipoda, Gammaridea). Pp. 77–86 in *Papers from the Conference on the Biology and Evolution of Crustacea*, J. K. Lowry, ed. Australian Museum Memoir 18. Trustees of the Australian Museum, Sydney. 218 pp.

Laubitz, D. R. 1993. Caprellidea (Crustacea: Amphipoda): towards a new synthesis. *J. Nat. Hist.* **27:** 965–976.

Lörz, A. N., and C. Held. 2004. A preliminary molecular and morphological phylogeny of the Antarctic Epimeriidae and Iphimediidae (Crustacea, Amphipoda). *Mol. Phylogenet. Evol.* **31:** 4–15.

Macdonald, K. S. III, L. Yampolsky, and J. E. Duffy. 2005. Molecular and morphological evolution of the amphipod radiation of Lake Baikal. *Mol. Phylogenet. Evol.* **35:** 323–343.

Martin, J. W., and G. E. Davis. 2001. An updated classification of the recent Crustacea. *Nat. Hist. Mus. Los Angel. Cty. Sci. Ser.* **39:** 1–124.

McCain, J. C. 1968. The Caprellidae (Crustacea: Amphipoda) of the western North Atlantic. *U.S. Nat. Mus. Bull.* **278:** 1–147.

McCain, J. C. 1970. Familial taxa within the Caprellidea (Crustacea: Amphipoda). *Proc. Biol. Soc. Wash.* **82:** 837–842.

Meyran, J. C., M. Monnerot, and P. Taberlet. 1997. Taxonomic status and phylogenetic relationships of some species of the genus *Gammarus* (Crustacea, Amphipoda) deduced from mitochondrial DNA sequences. *Mol. Phylogenet. Evol.* **8:** 1–10.

Myers, A. A., and J. K. Lowry. 2003. A phylogeny and a new classification of the Corophiidea Leach, 1814 (Amphipoda). *J. Crustac. Biol.* **23:** 443–485.

Posada, D., and K. A. Crandall. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* **14:** 817–818.

Saitou, N., and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4:** 406–425.

Shimodaira, H., and M. Hasegawa. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Mol. Biol. Evol.* **16:** 1114–1116.

Swofford, D. L. 2002. *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods).* Ver. 4. Sinauer Associates, Sunderland, MA.

Takeuchi, I. 1993. Is the Caprellidea a monophyletic group? *J. Nat. Hist.* **27:** 947–964.

Takeuchi, I. 1995. Caprellidea. Pp. 193–205 in *Guide to Seashore Animals of Japan with Color Pictures and Keys,* Vol. 2, S. Nishimura, ed. Hoikusha, Osaka. (In Japanese).

Takeuchi, I., and S. Ishimaru. 1991. Redescription of *Caprogammarus gurjanovae* Kudrjaschov & Vassilenko, 1966 (Crustacea: Amphipoda) from Hokkaido, Japan, with notes on the taxonomic status of *Caprogammarus*. *Hydrobiologia* **223:** 283–291.

Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins. 1997. The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **25:** 4876–4882.

Tomikawa, K., N. Kobayashi, H. Morino, Z.-E. Hou, and S. F. Mawatari. 2007. Phylogenetic relationships within the genus *Jesogammarus* (Crustacea, Amphipoda, Anisogammaridae) deduced from mitochondrial COI and 12S sequences. *Zool. Sci.* **24:** 173–180.