Relationships within the Munnopsidae (Crustacea, Isopoda, Asellota) based on three genes

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The Munnopsidae are a diverse group of asellote isopods that are an important component of deep-sea fauna. Morphologically-based phylogenetic inference attempts have proven to be of limited use due to the ecological and morphological diversity within the clade. Monophyly of the family is well-established but relationships within the group remain unresolved. This project is the first molecularly-based effort focused specifically on resolving phylogenetic relationships within the Munnopsidae. Partial *28S* and *COI* and complete *18S* genes were sequenced for 28 asellotes, 15 additional taxa were included from which only one or two of the three target sequences could be obtained, and *18S* sequences for five additional taxa were available from GenBank. Sequences were analysed both as individual genes and in combination using Bayesian and maximum parsimony approaches. Each gene provided a phylogenetic signal that could be identified in the combined analyses, with *18S* analyses providing the most resolution of phylogenetic relationships. The available representatives of subfamilies Munnopsinae and Ilyarachninae were monophyletic, as was the genus *Munneurycope*. Relationships within the subfamily Munnopsinae were well-resolved by thorough taxon sampling, several new species were placed, and the need for taxonomic revision of *Munnopsis/Munnopsoides* was supported. These analyses supported putative *Eurycope* paraphyly and emphasized the need for careful revision of this highly variable genus. *Tytthocope* was sister to *Munnopsurus*. *Syneurycope* was suggested as the sister group to the ilyarachnines. Combined analyses provided increased support for clades suggested in at least two individual gene analyses and for clades not strongly contradicted by individual analyses. Further work is required to fully resolve the munnopsid phylogeny and should consist of increased taxon sampling for the complete *18S* sequence and possibly identification of at least one slowly evolving, nuclear protein-coding gene to resolve the basal polytomy and enable placement of the root.

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Introduction

Containing some 40 genera, the Munnopsidae (Crustacea, Malacostraca, Peracarida) is a diverse group of asellote isopods that is an important component of deep-sea fauna (Sanders & Hessler 1969; Wilson & Hessler 1987). Asellotes are divergent from other isopods in their morphology, ecology, and history, having evolved in the deep-sea since the late Palaeozoic or early Mesozoic (Hessler & Thistle 1975; Wilson 1999). This long history in the deep sea makes them relatively unique among deep-sea fauna and provides an exceptional opportunity to study diversification there. Munnopsids live below the seafloor surface (fossorial, *e.g. Ilyarachna*), on the seafloor (epibenthic, *e.g. Munnopsurus* and *Vanhoeffenura*), in the water column (holopelagic, *e.g.* several *Paramunnopsis*, *Acanthamunnopsis*, and some *Munneurycope*), or both on the seafloor and in the water column (benthopelagic, *e.g. Munnopsoides* and *Paropsurus*). This ecological diversity is reflected in similarly high levels of morphological variation within the group. If a reliable phylogenetic hypothesis for the Munnopsidae can be obtained, they will be an ideal group to examine the process of evolution from benthic to pelagic habit — the transition through which most planktonic groups are hypothesized to have evolved (Rigby & Milsom 1996; Bradford-Grieve 2002). Morphological data suggest that munnopsids have invaded the water column multiple times and possibly even returned to the seafloor from the pelagic realm (see below).

An accurate phylogenetic hypothesis using purely morphological features has proven problematic (Wägele 1989; Wilson 1989) due to the extensive divergence and ecological

convergence present within the group. Convergent evolution, and associated character homoplasy, is arguably one of the most difficult problems facing phylogenetic reconstructions. Homoplasy can easily confuse relationships between lineages, especially when related lineages move from an ancestral habitat to a vastly different one. In many cases, selection may shape these various ancestors similarly because the organisms have a limited range of variation and capabilities with which to meet the new set of challenges. Although certainly not devoid of homoplasy, molecularly based phylogenetic inference allows independent re-evaluation of morphological evolution hypotheses, as well as identification of homoplastic characters.

The phylogenetic relationships within the Munnopsidae and the identity of their sister group remain a series of unsupported hypotheses (Wilson 1987, 1989; Wägele 1989; Raupach *et al*. 2004). Thus, prior to Raupach *et al*. (2009), we had no phylogenetic hypothesis for a highly successful, monophyletic group (see below) that has successfully diversified in the largest habitat on earth, the deep, open ocean. The major evolutionary questions that rely on a phylogenetic hypothesis are thus unaddressed for this group and include: how munnopsid diversity arose in the deep-sea, how morphological, ecological, and behavioural features are linked to invasion of new habitats and ultimately to radiations, and more specifically, what features of their morphology enabled successful colonization of pelagic niches. A resolved and well-supported phylogenetic hypothesis for the Munnopsidae will provide the historical framework for future examination of transitions between benthic and pelagic habitat.

Figure 1 summarizes munnopsid systematics and the phylogenetic relationships among the Munnopsidae suggested before the current molecular work (Wolff 1962; Thistle & Hessler 1976; Wilson & Hessler 1980; Wägele 1989; Wilson 1989; Kussakin 2003; Malyutina 2003; Malyutina & Brandt 2006). Many of these relationships were mere suggestions based on similarity or gestalt; few deep relationships are supported by phylogenetic analyses of any kind.

The Munnopsidae contains at least six groups of uncertain affinity (*incertae sedis*), as well as what was long considered four separate families, yet monophyly of the family has not been seriously challenged since Wilson's (1989) revision. The enlarged, muscular, broadly joined fifth through seventh pereonites (body segments) containing a single mass of fused ventral nerve cord ganglia (Hult 1941) together with modifications (many long, plumose setae and carpi and propodi broadened and paddle like) of the legs of those segments (pereopods V–VII) unite the munnopsids as a natural, monophyletic clade which is not in question here. They are further united by: the presence of dactylar claws that enclose the distal sensillae in a hollow between the anterior and posterior claw, many distal plumose setae on the rami of pleopod III, and the broadened first articles of the antennulae (Wilson 1989). Thus, monophyly of the Munnopsidae is not tested here nor is the question of sister group explored; instead, resolving relationships within the family in order to examine questions of pelagic evolution is my goal.

The subfamily Munnopsinae, comprised of *Munnopsis*, *Acanthamunnopsis*, *Paramunnopsis*, *Munnopsoides*, and *Pseudomunnopsis*, contains the majority of the pelagic and benthopelagic munnopsids and thus are presumably well-adapted to life in the water column. They are united by the loss of the dactyli of pereopods V–VII. Their cuticle is generally not heavily calcified and thin, while the walking legs (pereopods III–IV) and second antennae are extremely elongate, in some species up to eight times the body length. The natapods (pereopods V–VII) of most munnopsids are modified such that two articles are broad and flat with plumose setae on the margins. These modified legs are used for digging and/or swimming. The natapods of many Munnopsinae are extreme in the amount of surface area achieved by these modifications and allow for effective swimming. The *Munneurycope* and Bathyopsurinae also contain holopelagic and benthopelagic members that show similar swimming adaptations. However, careful attention to morphology suggests that the Munnopsinae are neither closely related to *Munneurycope* nor to the bathyopsurines. If this is true, pelagic habit has evolved at least twice within the Munnopsidae and thus this single family may supply multiple lineages for study of pelagic evolution. Here, I test the monophyly of the subfamily Munnopsinae, of the genera *Acanthamunnopsis*, *Paramunnopsis*, and *Munneurycope*, and whether *Munneurycope* could be the sister group to the Munnopsinae. In addition, limited testing of relationships of non-pelagic groups was possible and included the following: *Munnopsurus* sister to *Tytthocope*, Storthyngurinae sister to *Acanthocope*, monophyly of Ilyarachninae, and *Syneurycope* sister to Ilyarachninae.

The purpose of this study was to obtain nucleotide sequence data for complete nuclear small-subunit ribosomal RNA (hereafter referred to as *18S*), the D1–3 region of the nuclear large-subunit ribosomal RNA (hereafter referred to as *28S*), and partial mitochondrial cytochrome oxidase I (hereafter referred to as *COI*) for representatives of all major munnopsid lineages in order to reconstruct the history of the group. The range of slowly to quickly evolving genes was chosen in hope of allowing inference of the relationships at various levels within the evolution of the Munnopsidae, from the origin/s of the pelagic clade/s to relationships within the primarily pelagic subfamily Munnopsinae. Additionally, I wanted to independently test the validity of morphologically based relationships within the Munnopsidae to provide direction for future morphological and molecular phylogenetic studies. The inference of relationships within non-pelagic clades was not the major focus of this study although some preliminary findings are presented. I also

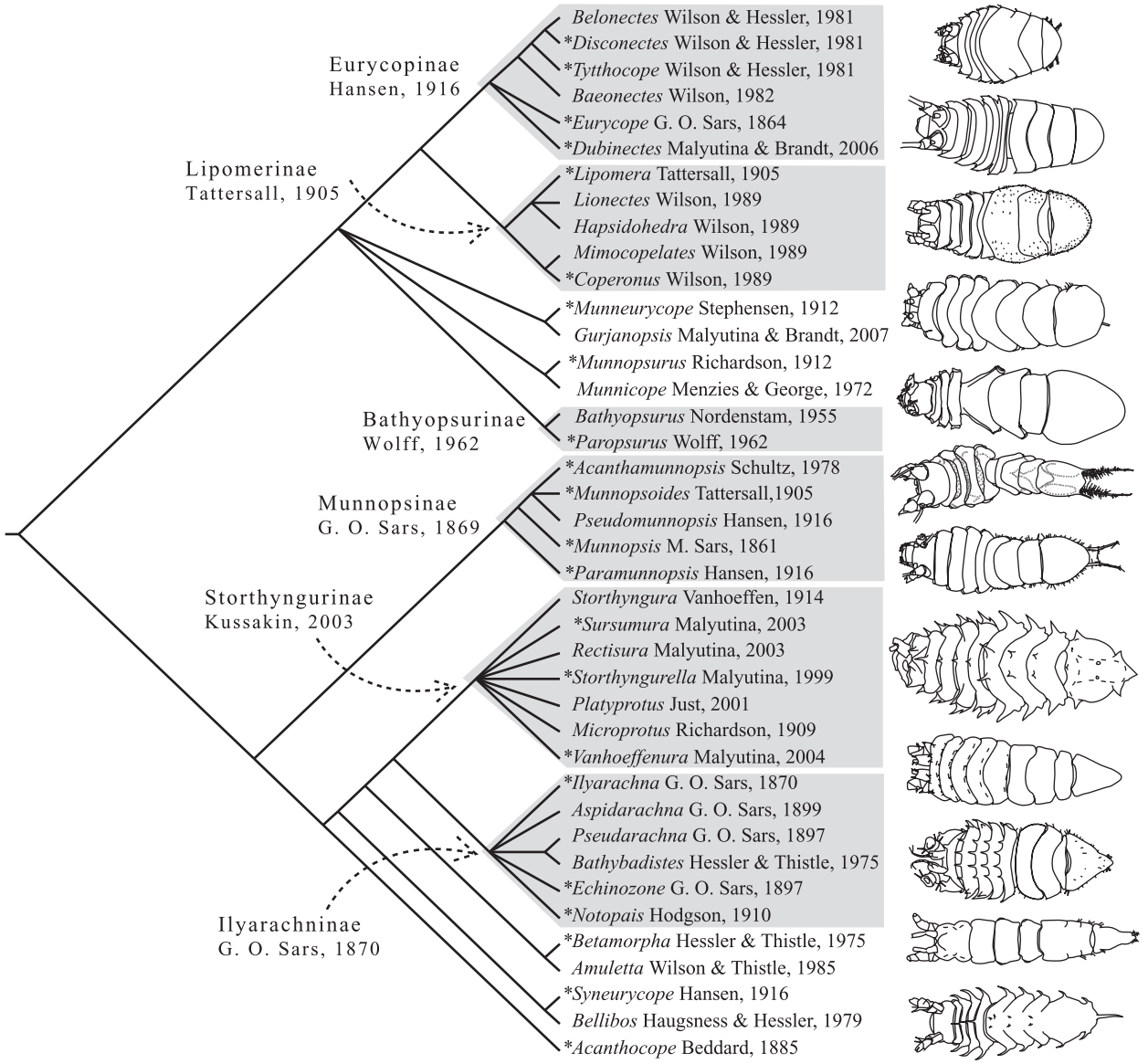

Fig. 1 Hypothetical tree of the Munnopsidae compiled from previous phylogenetic analyses and suggested relationships based on morphological similarity (Wolff 1962; Thistle & Hessler 1976; Wilson & Hessler 1980; Wägele 1989; Wilson 1989; Kussakin 2003; Malyutina 2003; Malyutina & Brandt 2006). Asterisks indicate taxa represented in this project. Gray boxes indicate major subfamilies. Representative munnopsids from top to bottom are *Disconectes phalangium* (redrawn from Wilson & Hessler 1981), *Eurycope* sp. MB, *Coperonus comptus* (redrawn from Wilson 1989), *Munneurycope* sp. 2, *Paropsurus giganteus*, *Munnopsis abyssalis*, *Paramunnopsis* sp. 1, *Vanhoeffenura* sp. MB, *Ilyarachna antarctica* (redrawn from Thistle 1980), *Echinozone magnifica* (redrawn from Vanhoeffen 1914), *Syneurycope parallela* (redrawn from Kussakin 2003), and *Acanthocope* sp. MB.

compare phylogenetic estimates based on individual genes and combined data sets, exploring the value of each gene for use in reconstructing the munnopsid tree.

Methods

Taxa

Asellote isopods were collected using a variety of techniques. *Ianiropsis epilittoralis* was collected from outdoor seawater tanks at Stanford University's Hopkins Marine Station. All other specimens from off California and Mexico (Table 1) were collected via the remotely operated vehicles *Ventana* and *Tiburon* with their high-flow suction samplers or detritus samplers (Robison 1993). *Munneurycope* sp. ABE was collected by the ROV *Jason II* with the suction sampler and *Munnopsis* sp. Aust. by trawl aboard the RV *Southern Surveyor* off Western Australia. All Antarctic specimens were collected

Table 1 Munnopsid and asellote taxa from which sequences were obtained for each gene with their GenBank and museum accession numbers and collection location.

Taxon	Voucher	*18S*	*28S*	*COI*	Locality
Subfamily Munnopsinae					
Acanthamunnopsis milleri Wilson, 1982	LACM-CR 2002-049.1	EF682219	EF682308	EF682261	Monterey Bay, California
Acanthamunnopsis longicornis (Hansen, 1895)	LACM-CR 2006-016.1	EF682220	EF682310	EF682265	Monterey Bay, California
Acanthamunnopsis sp. 2	LACM- CR 2004-031.1	EF682221	EF682309	EF682262	Monterey Bay, California
Acanthamunnopsis sp. 3	LACM-CR 2001-063.1	—	—	EF682264	Astoria Canyon, Oregon
Acanthamunnopsis sp. 4	LACM-CR 2004-033.1	EF682218	EF682307	EF682263	Monterey Bay, California
Acanthamunnopsis unknown	—	EF682226	EF682311	—	Monterey Bay, California
Munnopsis typica M. Sars, 1861	GenBank	AF496661	n/a	n/a	n/a
Munnopsis abyssalis Menzies & George, 1972	LACM-CR 2002-048.1	EF682222	EF682314	EF682273	Monterey Bay, California
Munnopsoides sp. MB	LACM-CR 2006-017.1	EF682224	EF682312	EF682271	Monterey Bay, California
Munnopsis sp. Aust.	AMS-P72075	EF682223	—	—	Perth Canyon, Western Australia
Munnopsis sp. 3	LACM-CR 2006-018.1	EF682225	EF682313	—	Monterey Bay, California
Paramunnopsis sp. 1	LACM-CR 2004-032.1	EF682227	EF682318	EF682267	Monterey Bay, California
Paramunnopsis sp. 2	LACM-CR 2003-051.1	EF682229	EF682316	EF682270	Gulf of California, Mexico
Paramunnopsis sp. 3	LACM-CR 2006-019.1	EF682231	—	—	Astoria Canyon, Oregon
Paramunnopsis sp. 4	LACM-CR 2000-072.1	EF682228	EF682317	EF682269	Monterey Bay, California
Paramunnopsis sp. 5	LACM-CR 2003-050.1	EF682230	EF682315	EF682266	Monterey Bay, California
Subfamily Eurycopinae					
Eurycope glabra Kensley, 1978	ZMH-42117	EF682255	EF682329	EF682280	Weddell Sea, Antarctica
Eurycope complanata Bonnier, 1896 complex	ZMH-42099	EF682256	EF682306	EF682281	Weddell Sea, Antarctica
Eurycope sp.	n/a	EF682257	EF682324	—	Weddell Sea, Antarctica
Eurycope sp. MB	LACM-CR 2006-015.1	EF682254	EF682323	—	Monterey Bay, California
Dubinectes acutitelson (Menzies, 1962)	ZMH-42073	EF682251	EF682330	EF682294	Weddell Sea, Antarctica
Disconectes 'antarcticus' (Vanhöffen, 1914)	n/a	EF682250	EF682325	EF682293	Weddell Sea, Antarctica
Tytthocope sp. 3 (or 5)	ZMH-42097	EF682252	EF682342	EF682290	Weddell Sea, Antarctica
Incertae sedis genera					
Munnopsurus sp. 1	ZMH-42100	EF682237	EF682340	EF682289	Weddell Sea, Antarctica
Munnopsurus sp. MB	LACM-CR 2006-020.1	EF682238	EF682341	EF682288	Monterey Bay, California
Munneurycope murrayi (Walker, 1903) *sensu* Wolff, 1962	LACM-CR 2003-049.2	EF682232	EF682319	EF682275	Monterey Bay, California
Munneurycope sp. 2	LACM-CR 2003-052.1	EF682233	EF682320	EF682276	Gulf of California, Mexico
Munneurycope sp. 3	LACM-CR 2005-041.1	EF682235	EF682321	EF682277	Monterey Bay, California
Munneurycope sp. ABE	LACM-CR 2005-041.1	EF682234	—	EF682283	New Hebrides Trench, S. Pacific
Subfamily Bathyopsurinae					
Paropsurus giganteus Wolff, 1962	LACM-CR 2002-047.1	EF682253	EF682339	EF682287	Monterey Bay, California
Subfamily Betamorphinae					
Betamorpha fusiformis (K.H. Barnard, 1920)	ZMH-42139	EF682247	EF682332	EF682291	Weddell Sea, Antarctica
Betamorpha africana (Menzies, 1962)	ZMH-42130	EF682248	EF682331	EF682292	Weddell Sea, Antarctica
Subfamily Lipomerinae					
Lipomerinae	ZMH-42088	—	EF682328	EF682297	Weddell Sea, Antarctica
Coperonus sp. 5	n/a	EF682258	EF682327	—	Weddell Sea, Antarctica
Coperonus sp. 1	n/a	EF682259	EF682326	—	Weddell Sea, Antarctica
Subfamily Syneurycopinae					
Syneurycope heezeni Menzies, 1962	ZMH-42079	EF682242	EF682334	EF682295	Weddell Sea, Antarctica
Syneurycope sp.	ZMH-42111	EF682243	EF682335	EF682296	Weddell Sea, Antarctica
Subfamily Ilyarachninae					
Ilyarachna triangulata Menzies, 1962	ZMH-42112	EF682244	EF682333	—	Weddell Sea, Antarctica
Ilyarachna antarctica Vanhöffen, 1914	n/a	EF682245	—	EF682299	Weddell Sea, Antarctica
Notopais magnifica (Vanhöffen, 1914)	FMK pending	EF682249	—	—	Weddell Sea, Antarctica
Echinozone sp. (JW2004)	GenBank	AY461480	n/a	n/a	n/a
Echinozone spinosa Hodgson, 1902	GenBank	AF496658	n/a	n/a	n/a
Subfamily Storthyngurinae					
Sursumura falcata (George & Menzies, 1968)	GenBank	AF498908	n/a	n/a	n/a
Storthyngurella triplospinosa (Menzies, 1962)	GenBank	AY461482	n/a	n/a	n/a
Vanhoeffenura sp. MB	LACM-CR 2002-047.2	EF682239	EF682338	EF682284	Monterey Bay, California
Subfamily Acanthocopinae					
Acanthocope sp. MB	LACM-CR 2005-040.1	EF682240	EF682336	EF682286	Monterey Bay, California
Acanthocope galatheae Wolff, 1962	ZMH-42084	EF682241	EF682337	—	Weddell Sea, Antarctica
Janiroidea outgroups					
Ianiropsis epilittoralis Menzies, 1952	LACM-CR 2002-050.1	EF682260	EF682305	EF682303	Monterey Bay, California
Ishnomesus sp. (MB in Suppl. Fig. 3)	LACM-CR 2003-049.1	EF682246	—	—	Monterey Bay, California

on ANDEEP II and III expeditions aboard the RV *Polarstern* by epibenthic sledge (Brandt *et al.* 2007).

Most specimens were either preserved in chilled 95% ethanol or RNALater (Ambion, Austin, TX) and stored at ≤ 4 °C for later DNA extraction. A few specimens collected early on in the project or by other researchers were frozen in liquid nitrogen and stored at –80 °C before extraction but this method was less reliable for obtaining high quality genomic DNA. Vouchers for all sequences from specimens collected in the Pacific Ocean were placed in the Natural History Museum of Los Angeles County (see Table 1 for accession numbers). Specimens from the South Atlantic and Weddell Sea are deposited in the Zoologisches Institut und Museum, Hamburg, Germany; M. Malyutina provided identification of these specimens and the names used here. The specimen identified as *Eurycope complanata* complex and Lipomerinae are both undescribed species, thus the names used are those currently available and allow matching to the voucher specimens. The specimen from Australia was deposited in the Australian Natural History Museum, Sydney.

Extraction to sequencing

Whenever possible, only 1–3 natapods were used for extraction so that the remainder of the specimen could serve as the voucher. Genomic DNA was extracted from specimens using DNAzol® Genomic DNA Isolation Reagent (Molecular Research Center, Inc., Cincinnati, OH) with the following modifications to the manufacturer's instructions. One to three legs, pleotelson tissue with the gut removed, or an embryo were homogenized in 250–500 μL DNAzol reagent and placed in a rotating incubator at room temperature for 24–72 h. Ten microliters of 10 mg/mL proteinase kinase were added each 24-h period. Two microliters of polyacryl carrier were added to each extraction to help visualize the DNA. DNA was extracted from *Eurycope glabra*, *Dubinectes acutitelson*, *Eurycope complanata* complex, *Syneurycope heezeni*, *Syneurycope* sp., Lipomerinae, *Ilyarachna triangulata*, *Ilyarachna antarctica*, *Notopais magnifica*, *Coperonus* sp. 1 and 5, *Acanthocope galatheae*, *Munnopsurus* sp. 1, *Betamorpha fusiformis*, *Betamorpha africana*, *Disconectes 'antarcticus'*, *Tytthocope* sp. 3, and *Eurycope* sp. using the Qiagen DNeasy Tissue Kit (Valencia, CA) according to the manufacturer's instructions by M. J. Raupach.

Approximately 1800 base pairs of *18S* were amplified using universal primers mitchA (5′-CAA CCT GGT TGA TCC TGC CAG T-3′) and mitchB (5′-TGA TCC TTC CGC AGG TTC ACC TAC-3′) modified from Medlin *et al.* (1988). The amplification profile was optimized for each extraction; 35 ramping cycles of 94 °C for 60 s, 58–64 °C for 60 s, 72 °C for 90–120 s, with an initial single denaturation step at 94 °C for 3 min and a final single extension step at 72 °C for 4–7 min.

Approximately 1100 base pairs surrounding the D1–3 region of *28S* were amplified using modified universal primers (Lenaers *et al.* 1989) LSUD1F (5′-ACC CGC TGA ATT TAA GCA TA-3′) and D3AR (5′-ACG AAC GAT TTG CAC GTC AG-3′). The amplification profile was optimized for each extraction, 35 cycles of 94 °C for 40–60 s, 60 °C for 30–60 s, 72 °C for 70–120 s, with an initial single denaturation step at 94 °C for 5 min, and a final single extension step at 72 °C for 5–7 min

Approximately 650 base pairs of the mitochondrial *COI* gene were amplified using primers LCO1490 (5′-TCA ACA AAT CAT AAA GAT ATT GG-3′) and HCO2198 (5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′; Folmer *et al.* 1994). The amplification profile was optimized for each extraction, with 5 cycles of 94 °C for 60 s, 45 °C for 90 s, 72 °C for 60 s, and then 35 cycles of 94 °C for 30–40 s, 51 °C for 30–90 s, 72 °C for 60 s, with an initial single denaturation step at 94 °C for 60–120 s, and a final single extension step at 72 °C for 5–7 min. Taq PCR Master Mix (Qiagen, Inc.) was used for all amplifications.

PCR products were either sequenced directly after spin column purification (Ultrafree-DA columns, Millipore, Billerica, MA) following the manufacturer's protocol or, in some cases, cloned according to the manufacturer's protocol with the Invitrogen TOPO cloning kit (Carlsbad, CA). In the latter case, three to six colonies were chosen for plasmid DNA purification using a QIAprep Spin Miniprep Kit (Qiagen). Plasmid DNA was digested with EcoRI to check for correct-size inserts. Cloned DNA was sequenced in both directions using M13 primers (forward 5′-GTA AAA CGA CGG CCA G-3′, reverse 5′-CAG GAA ACA GCT ATG AC-3′). All direct sequencing was carried out using the same primers that were used for amplification, with the addition of four internal primers for *18S (*514F 5′-TCT GGT GCC AGC AGC CGC GG-3′; 536R 5′-TGG AAT TAC CGC GGC TGC TG-3′; 1055F 5′-GGT GGT GCA TGG CCG-3′; 1055R 5′-CGG CCA TGC ACC ACC-3′). All sequencing was carried out with the BigDye Terminator v. 3.1 sequencing kit and analysed on an ABI 3100 capillary sequencer (Applied Biosystems, Foster City, CA). Sequences and alignments were deposited in GenBank (see Table 1 for accession numbers).

Analyses

Sequences were aligned with MUSCLE (v. 3.6, Edgar 2004) using default settings and proofread by eye in MacClade v. 4.04 OS X (Maddison & Maddison 2000). Several preliminary Bayesian analyses were run to determine the impact on tree topology and clade support of factors such as the inclusion of ambiguously aligned regions, inclusion of near saturated third positions, and inclusion of taxa that lacked sequence data for one or two of the three target genes in the combined analyses. Preliminary analyses were run with all nucleotide bases included, with positions for which the alignment was ambiguous excluded (defined as highly variable regions where

Table 2 Templeton's test results comparing null hypotheses.

Null hypothesis	Difference, tree length	Number alternative trees	*P*-value	Outcome
Munnopsinae monophyletic	0	1	n/a	Accept null
Acanthamunnopsis monophyletic	0	1	n/a	Accept null
Paramunnopsis monophyletic	12	3	0.521–0.563	Accept null
Paramunnopsis sister to Munnopsinae	25	1	0.193	Accept null
Munneurycope sister to Munnopsinae	15	2	0.327–0.360	Accept null
Munneurycope monophyletic	0	1	n/a	Accept null
Munnopsurus sister to *Tytthocope*	0	1	n/a	Accept null
Storthyngura sister to *Acanthocope*	0	1	n/a	Accept null
Ilyarachna monophyletic	0	1	n/a	Accept null
Ilyarachna sister to *Syneurycope*	8	1	0.576	Accept null

gaps were repeatedly required to align positions in more than 70% of the sequences), with known variable regions excluded, and in *COI* with third positions excluded. Neither ambiguously aligned bases nor third codon positions were removed from the final *18S* (2627 bp alignment), *28S* (1435 bp alignment), *COI* (684 bp alignment), or combined analyses, respectively, because their removal did not markedly impact the outcome. Additionally, combined analyses were performed on data sets that included all taxa for which at least one (shown here as the combined analysis), for which at least two (shown in Fig. S1 in Supporting Information), and for which all three gene sequences were obtained (shown in Fig. S2 in Supporting Information).

All analyses were run at least four times for at least 5 million generations. If stationarity of posterior probabilities was not reached, further generations were completed. The complete alignments were deposited in GenBank and are available from the author upon request. All trees shown are those from these final analyses that included all available sequence data.

At least two of the three gene sequences were obtained from most samples (Table 1). The topology of the trees did not change notably whether or not taxa missing sequences were included, as would be expected based on Wiens's (2006) research, but this did change the time to run analyses, increasing runtime in data sets with higher numbers of incomplete taxa. Sequences were concatenated for the combined analyses only when sequenced from the same individual. None of the munnopsid sequences available from GenBank met this criterion.

Parsimony analyses were conducted with the PAUP 4.0b10 software package (Swofford 2002). Parsimony trees were reconstructed from an equally weighted character matrix and the heuristic search option, using the tree-bisection-reconnection branch-swapping algorithm and 1000 random addition replicates. Gaps were treated as missing data because of the taxa with missing sequences. Bootstrap values were obtained with the same settings as the parsimony analysis except here only 100 random addition replicates were performed. Addition parsimony analyses (with the same parameters as above) with constraints as described in Table 2 were performed to test if the null or alternative hypotheses could be rejected.

Bayesian analyses of the data sets were conducted using MRBAYES 3.1.2 (Huelsenbeck & Ronquist 2001). Standard procedures based on MODELTEST 3.5 (Posada & Crandall 1998) were implemented in PAUP to select the most appropriate models for the analyses. The relative fit of models was assessed by the Akaike information criterion. Smaller values of *AIC* are preferred (Akaike 1974; Posada & Crandall 2001) and the General Time Reversible + Proportion Invariant + Gamma (GTR + I + Γ) represents the optimal model with respect to all three genes. Genes were unlinked in the concatenated analyses. Each Markov chain, three heated and one cold, was started from a random tree and all four chains were run simultaneously for 5–50 million generations, with trees being sampled so that the resulting data set from each run contained at least 10 000 data points after at least 25% had been discarded as burnin. The program AWTY (Wilgenbusch *et al*. 2007) was used to determine if a sufficient number of generations had been completed for posterior probabilities to stabilize, as well as to determine amount of required burnin before inference from the MCMC data set was made. Repetitions of each gene analysis and of the combined analyses converged on similar parameter estimates.

Results

Individual genes

Parsimony analyses resulted in the following: *18S*, a single best tree based on 351 parsimony informative characters (2276 constant or parsimony uninformative), *28S*, two most parsimonious trees based on 328 parsimony informative characters (797 constant or parsimony uninformative), and *COI*, four most parsimonious trees based on 428 parsimony informative characters (256 constant or parsimony uninformative characters).

Individual gene analyses resulted in no support for most deep relationships. The most resolution was found in the *18S* analyses (Fig. 2) followed by the *28S* analyses (Fig. 3) and the

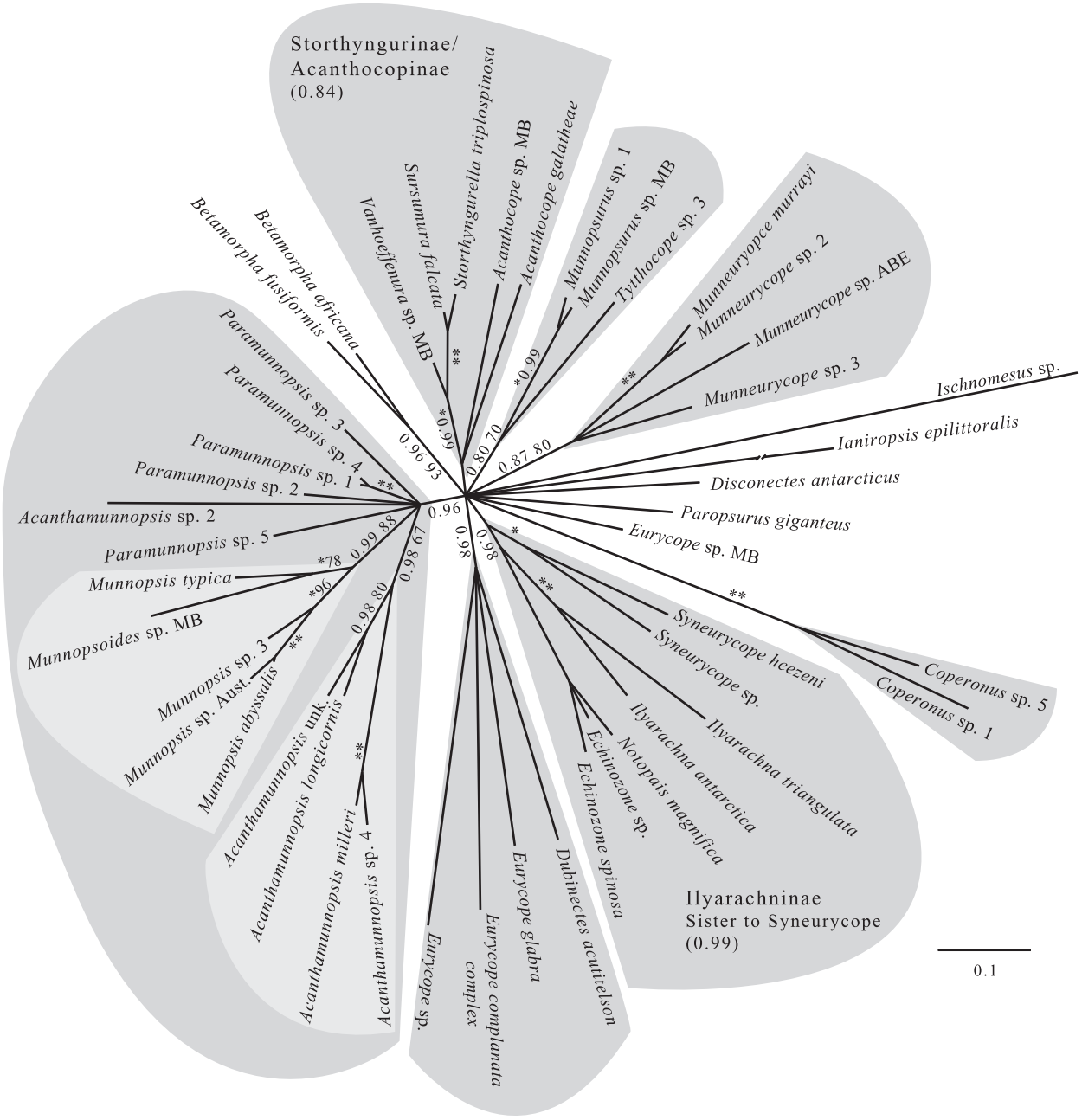

Fig. 2 The 80% majority rule *18S* gene tree from the Bayesian analysis of the Munnopsidae. Support values given are posterior probabilities then bootstrap values from the maximum parsimony analysis (distinguishable by order and the fact that posterior probabilities are in decimals and bootstraps given as the percentage). Asterisks indicate 1.0 posterior probability and 100% bootstrap values. All unsupported branches are collapsed and support values below 0.80 posterior probability and 60% bootstrap are not shown. Low support values shown are included because they are either supported by one of the analyses (Bayesian or maximum parsimony) or because they should be investigated further with increased taxon sampling and additional gene sequences. Gray shaded areas indicate clades of interest and support for the basal node of the indicated clade is given below the title of each when there is not space on the branch itself.

least resolution in the *COI* analyses (Fig. 4). The Munnopsidae cannot be rooted because of this lack of resolution and thus all trees are shown as unrooted in order to best represent our understanding of relationships within the family.

The monophyletic subfamily Munnopsinae was recovered with low support by the Bayesian analyses from the *18S* analyses (0.96 posterior probability, Fig. 2) and the Bayesian and parsimony analyses of the *28S* analyses (0.85 pp, 86

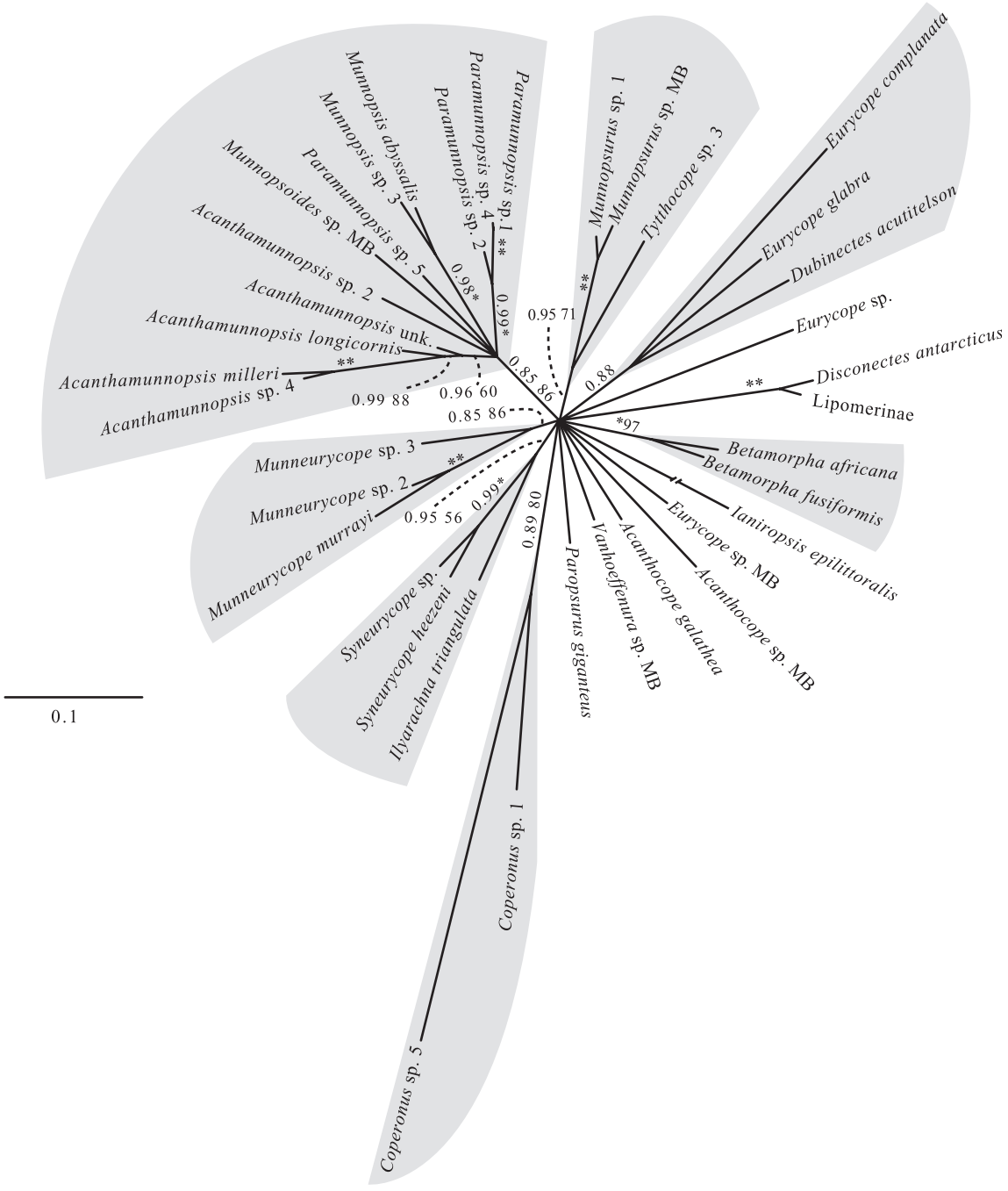

Fig. 3 The 80% majority rule *28S* gene tree from the Bayesian analysis of the Munnopsidae. Support values given are posterior probabilities then bootstrap values from the maximum parsimony analysis. Asterisks indicate 1.0 posterior probability and 100% bootstrap values. All unsupported branches are collapsed and support values below 0.80 posterior probability and 60% bootstrap are not shown. Gray shaded areas indicate clades of interest.

bootstrap Fig. 3) but was not recovered in the *COI* analyses (Fig. 4). Both the *18S* and *28S* analyses provided low support for *Acanthamunnopsis* monophyly when sp. 2 was excluded from the clade (0.98 pp, 67 bs, Fig. 2; 0.96 pp, 60 bs, Fig. 3). *Acanthamunnopsis* monophyly was also recovered with good support by the Bayesian *COI* analyses including sp. 2 (1.0 pp, Fig. 4). The genus *Munnopsis* was monophyletic according to both the Bayesian and parsimony *18S* analyses only when

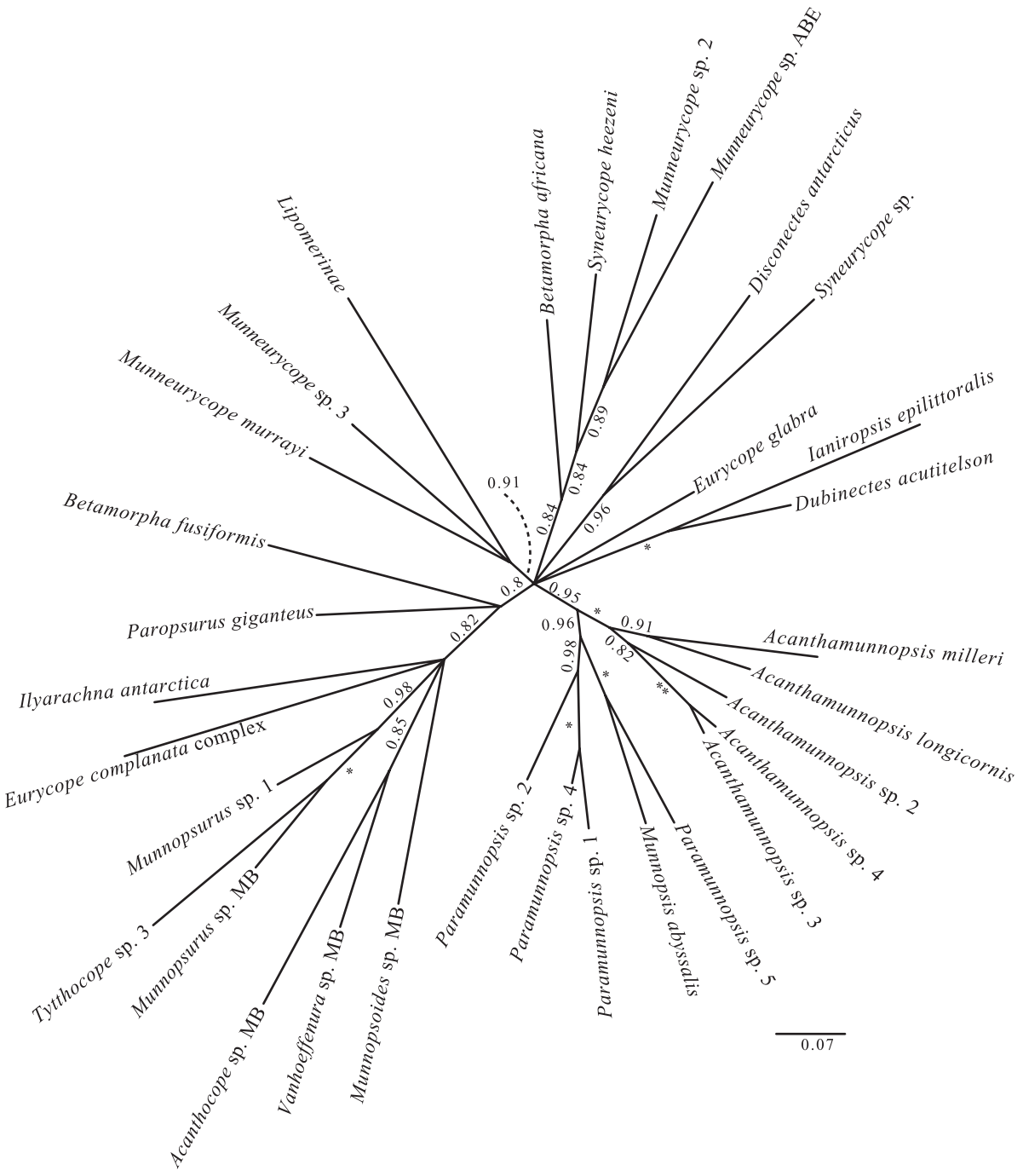

Fig. 4 The 80% majority rule *COI* gene tree from the Bayesian analysis of the Munnopsidae. Support values given are posterior probabilities then bootstrap values from the maximum parsimony analysis. Asterisks indicate 1.0 posterior probability and 100% bootstrap values. All unsupported branches are collapsed and support values below 0.80 posterior probability and 60% bootstrap are not shown. Gray shaded areas indicate clades of interest.

Munnopsis typica was excluded or *Munnopsoides* sp. MB was included in *Munnopsis* (0.99 pp, 88 bs, Fig. 2). Both Bayesian and parsimony *28S* analyses support the monophyly of *Munnopsis* based on the two species available (0.98 pp, 100 bs, Fig. 3). The species of *Paramunnopsis* formed an unresolved basal polytomy among the other monophyletic Munnopsinae genera according to the *18S* analyses (Fig. 2). In contrast, both the Bayesian and parsimony *28S* analyses support the

monophyly of the *Paramunnopsis* species included (0.99 pp, 100 bs, Fig. 3), with the exception of the morphologically unusual sp. 5 whose *28S*-based affinity seemed to lie closer to *Munnopsis abyssalis*. Similarly, the *COI* analyses provided moderate support for monophyly of all but *Paramunnopsis* sp. 5 (0.97 pp, Fig. 4). Relationships within individual *Acanthamunnopsis* and *Munnopsis/Munnopsoides* clades were well-supported in all analyses (Figs 2–4).

18S and *28S* analyses suggested, but only marginally supported, *Munneurycope* monophyly (0.87 pp, 80 bs, Fig. 2; 0.85 pp, 86 bs, Fig. 3) while *COI* did not support monophyly of the group. All individual gene analyses provided strong support for relationships within the *Munneurycope* (Figs 2–4).

Although never well-supported, a sister relationship was suggested by all individual gene analyses between *Munnopsurus* and *Tytthocope* (0.80 pp, 70 bs, Fig. 2; 0.95 pp, 71 bs, Fig. 3; 0.98 pp, Fig. 4).

The following clades contained two or more taxa and were monophyletic according to the *18S* and *28S* analyses: *Coperonus* (1.0 pp, 100 bs, Fig. 2; 0.89 pp, 80 bs, Fig. 3), *Syneurycope* (1.0 pp, Fig. 2; 0.89 pp, 80 bs, Fig. 3), *Betamorpha* (0.96 pp, 93 bs, Fig. 2; 1.0 pp, 97 bs, Fig. 3), and *Munnopsurus* (0.99 pp, 100 bs, Fig. 2; 1.0 pp, 100 bs, Fig. 3). Additionally, *Ilyarachna*, which contained two taxa in the *18S* analyses, was found to be monophyletic (1.0 pp, 100 bs, Fig. 2). *Notopais* and *Echinozone* formed a clade (1.0 pp, 100 bs, Fig. 2), as did *Dubinectes* and other *Eurycope* (0.98 pp, Fig. 2; 0.88 pp, Fig. 3). *18S* analyses suggested *Ilyarachna* was the sister group to *Notopais/Echinozone* (0.98 pp, Fig. 2), and *Syneurycope* was the sister group to the *Ilyarachna/Notopais/Echinozone* clade (0.99 pp, Fig. 2). *28S* analyses showed poor support for the relationship between *Syneurycope* and *Ilyarachna* (0.95 pp, 100 bs, Fig. 3), while *COI* data lent no information to this relationship. *18S* analyses suggested a relationship between *Acanthocope* and Storthyngurinae (0.84 pp, Fig. 2), which was not supported by *28S* or *COI* analyses.

Combined

The parsimony analysis resulted in a single most parsimonious tree of length 10 823 based on 1403 parsimony informative characters with a rescaled consistency index of 0.115. The monophyly of the subfamily Munnopsinae was well-supported by the Bayesian and parsimony combined analyses (1.0 pp, 95 bs, Fig. 5 and Figs. S1 and S2 in Supporting Information). The alternative hypothesis that it was not monophyletic could not be accepted according to the results of Templeton's test (Table 2, Larson 1994). Monophyly of the genus *Acanthamunnopsis*, excluding sp. 2, was well-supported in combined Bayesian analyses (1.0 pp, 67 bs) and could not be rejected based on the results of Templeton's test (Table 2). *Munnopsis/Munnopsoides* was monophyletic in combined analyses (1.0 pp, 93 bs). *Paramunnopsis* was not monophyletic when the unusual sp. 5 was included in combined analyses, yet monophyly of the group could not be rejected according to Templeton's test and parsimony analyses. Of the included *Paramunnopsis* species, sp. 1 and 4 were the most closely related and formed a clade with sp. 2 (0.96 pp, 70 bs).

The *Munneurycope* were monophyletic (0.98 pp, 66 bs, Fig. 5), with *M. murrayi* more closely related to *Munneurycope* sp. 2 than to others (1.0 pp, 95 bs, Templeton's test). *Munnopsurus* formed a clade that was sister to *Tytthocope* according to both Bayesian and parsimony analyses (1.0 pp, 98 bs, Fig. 5 and Table 2). The subfamily Storthyngurinae was a monophyletic group (1.0 pp, 98 bs) that was related to *Acanthocope* (0.98 pp) only in the Bayesian analyses and could not be rejected based on the results of Templeton's test. *Eurycope glabra*, *E. complanata* complex, *Eurycope* sp., and *Dubinectes acutitelson* were more closely related to each other than to other *Eurycope* available for these analyses (0.98 pp, 75 bs). *Coperonus* and *Betamorpha* each formed monophyletic clades (1.0 pp, 100 bs each). Ilyarachininae consisted of two monophyletic groups, *Ilyarachna* and *Notopais/Echinozone* (1.0 pp, 68 bs and 1.0 pp, 94 bs, respectively; Templeton's test, Table 2), which was sister to *Syneurycope* (1.0 pp; Templeton's test, Table 2) according to the Bayesian analyses only. Bayesian analyses further suggested the Ilyarachninae/*Syneurycope* clade was sister to *Betamorpha* (0.91 pp).

Individual vs. combined analyses

As expected, individual gene and combined analyses (Figs 2–5) showed differences in topology and clade support. No conflicting relationships were strongly supported in individual analyses and thus the combined analyses contained no surprising relationships. Relationships suggested in two or more individual gene analyses comprised those seen in the combined analyses (e.g. Munnopsinae monophyly, *Munnopsurus* monophyletic and sister to *Tytthocope*, the selective *Eurycope* and *Dubinectes* clade, *Ilyarachna* sister to *Syneurycope*, individual monophyly of *Munneurycope*, *Syneurycope*, *Coperonus*, and *Betamorpha*). Relationships that were supported by all three individual gene analyses were of course recovered in the combined analyses (e.g. *Acanthamunnopsis* sp. 2 outside a monophyletic *Acanthamunnopsis* and *Paramunnopsis* sp. 1 and 4 most closely related). Relationships that were supported by a single gene but that were not contradicted by the other two genes were also recovered in the combined analyses (e.g. *Munnopsoides* most closely related to *Munnopsis typica*, *Ilyarachna* monophyly, Lipomerinae sister to *Disconectes antarcticus*, *Notopais magnifica* with *Echinozone*).

Discussion

Undescribed species

Many species sequenced for this project are not yet described as is often the case when working with samples from poorly

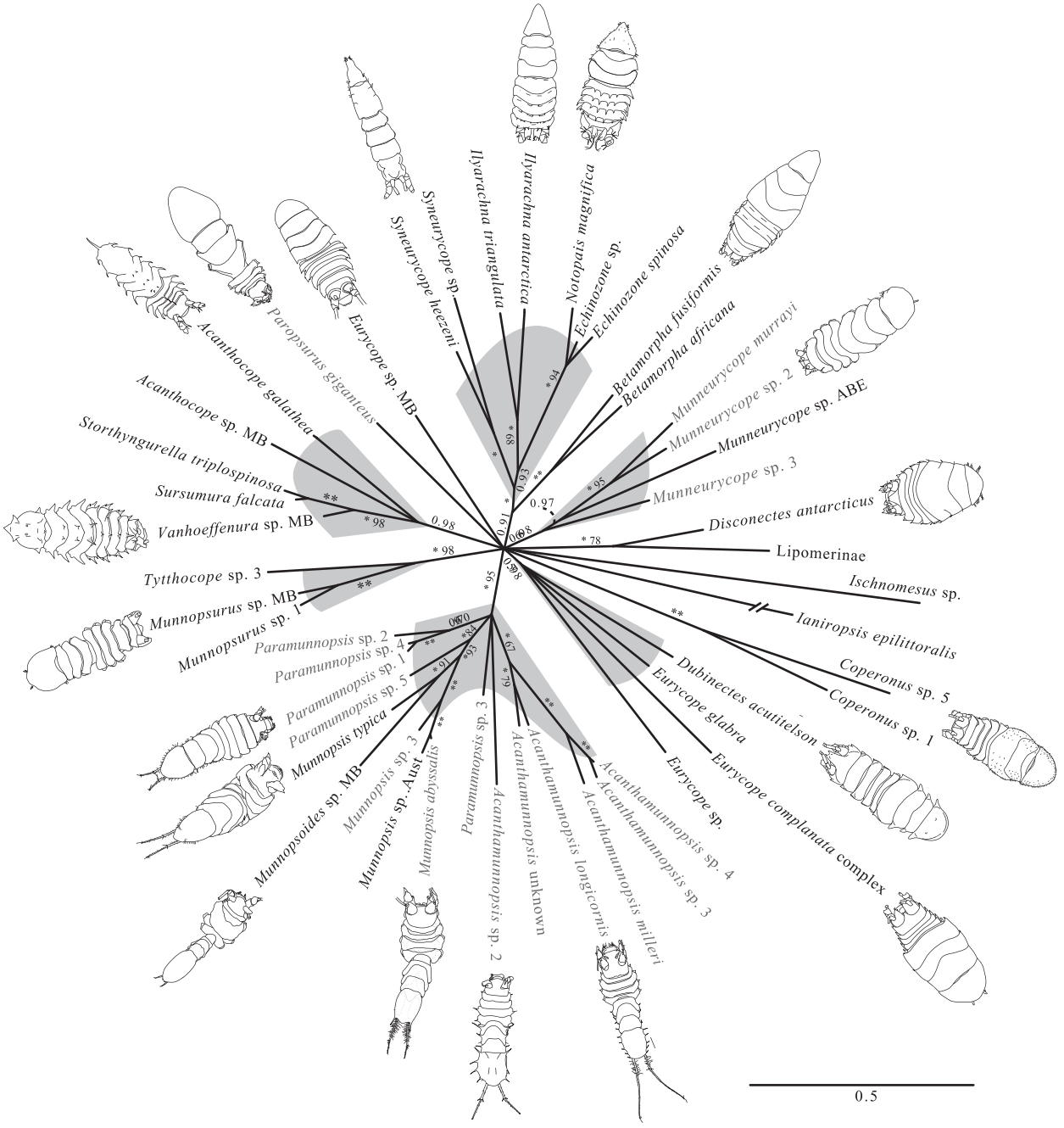

Fig. 5 The 90% majority rule tree from the combined data set of all three genes (*18S*, *28S* and *COI*) from the Bayesian analysis of the Munnopsidae. Support values given are posterior probabilities then bootstrap values from the maximum parsimony analysis. Asterisks indicate 1.0 posterior probability and 100% bootstrap values. All unsupported branches were collapsed and support values below 0.90 posterior probability and 60% bootstrap are not shown. Gray shaded areas indicate clades of interest. Gray species names indicate species collected from the water column. Dorsal views of animals are those shown in Fig. 1 with the following additions clockwise from top: *Betamorpha fusiformis* (redrawn from Kussakin 2003), *Dubinectes acutitelson* (redrawn from Malyutina & Brandt 2006), *Eurycope complanata* (redrawn from Wilson 1982), *Acanthamunnopsis longicornis*, *Acanthamunnopsis* sp. 2., *Munnopsoides* sp. MB, *Paramunnopsis* sp. 5, and *Munnopsurus* sp. MB.

sampled habitats such as the deep-sea and open-ocean (e.g. Brandt *et al.* 2007). Special care was taken to distinguish undescribed species from known species, to document the differences with drawings, and to provide voucher specimens for each sequence (Table 1). These species will be described in the near future but in many cases, this is no easy task because generic revisions are required due to the discovery of several new species with morphology distinct from or intermediate between current genera. This project provides the framework for revision and description of new species by clarifying relationships within and between several munnopsid groups.

Rooting the Munnopsidae and basal relationships

The munnopsid tree cannot be rooted based on the outgroup taxa included in these analyses due to the lack of resolution at the base of the tree. However, monophyly of the Munnopsidae is not in question due to numerous synapomorphies of the group that were defined by Wilson (1989) when he revised the family (see Introduction). Raupach *et al.* (2004) analysed a variety of asellote *18S* sequences and found that the 10 included munnopsids formed a monophyletic group according to both their Bayesian and parsimony analyses. They also found that the desmosomatids were the most likely sister group of those asellotes available for their analyses and, with no resolution between them, the ischnomesids, macrostylids, mesosignids, or janirellid were the next most likely possibilities. With additional taxa, Brandt *et al.* (2007) suggested nannoniscids as an additional possible sister group to the Munnopsidae. Raupach *et al.* (2009) provided more thorough sampling of possible sister groups but still were not able to find stability or support for any of these possible munnopsid sister groups.

The deep polytomy recovered both by the present analyses and that of Raupach *et al.* (2004, 2009) is not unexpected. The munnopsids are considered to be an ancient group because they are diverse, highly specialized, and widespread throughout all ocean basins (Hessler & Thistle 1975). Although no fossil record exists, they are considered to have persisted since at least the Mesozoic, possibly even the Palaeozoic (Wilson 1999). Thus more slowly evolving genes may be required to provide resolution in the distant past. Possibly the Munnopsidae diversified rapidly. If so, a rapid radiation would explain the difficulty resolving deep relationships because a relatively short time would have been available for genetic differences to accumulate during the rapid radiation and because of erosion of phylogenetic signal over the relatively long time since the radiation (Whitfield & Lockhart 2007).

Although research suggests that some ancient patterns of divergence are improbable to resolve using molecular sequence data (Whitfield & Lockhart 2007 and references therein), in this case, exploration of the problem has just begun. Further taxon sampling would serve to break up long branches and provide resolution of internal relationships (Graybeal 1998; Hillis *et al.* 2003 and references therein). Recovering monophyly of the well-sampled subfamily Munnopsinae while not recovering it for poorly sampled subfamilies, such as the Eurycopinae, suggests increased taxon sampling will indeed add resolution to internal relationships of the munnopsid tree as seen in Raupach *et al.* (2009 and Fig. S3 in Supporting Information). Alternatively, the Eurycopinae may be more distantly diverged than the Munnopsinae but this cannot be assessed simply based on the length of the branches leading to them (Phillippe & Laurent 1998; Brinkmann *et al.* 2005). Breaking up long branches by increased taxon sampling would also clarify the rooting of the tree because most often outgroup taxa are long branches and thus more often placed with internal long branches (Whitfield & Lockhart 2007).

Contribution of individual genes in resolving the munnopsid tree

18S sequences were by far the most useful in reconstructing the munnopsid phylogeny. *18S* provided resolution from well-established species to shallow subfamily level within the tree, providing by far the deepest resolution of this seemingly old group. Alignment of *18S* munnopsid sequences was straightforward because there were many highly conserved regions and few highly variable regions. No. *18S* pseudogenes were indicated in this munnopsid data.

Resolution deeper in the tree than genus level was not well-supported by *28S* analyses, suggesting that while *28S* sequences were somewhat useful in the combined analyses because they provided further support for groups suggested by the *18S* sequences, alone this segment of the *28S* gene is of limited usefulness in resolving the munnopsid tree. If further sequencing of this *28S* region were desired, the universal primers used here should be redesigned to be more isopod specific, because this was the most difficult gene to amplify with the primers used.

In this project, several *COI* sequences recovered were ultimately not reliable due to the use of universal primers and the ease of amplifying contamination or pseudogenes instead of target DNA (Williams & Knowlton 2001; Song *et al.* 2008; Buhay 2009). Considerable variability was found between clones and target organisms were sometimes difficult to sequence. *COI* sequences provided resolution only at the tips of the munnopsid tree but this did prove useful in the case of *Acanthamunnopsis* sp. 3 for which only the *COI* sequence was obtained and multiple closely related species were available. Despite this one exceptional case, *COI*'s usefulness for phylogenetic inferences within the Munnopsidae or even identification of the relative placement of an unknown species (e.g. barcoding) seems highly suspect unless multiple, closely related species are included in the analysis.

Individual gene trees vs. combined analyses

The increased phylogenetic resolution and support provided by analyses of combined gene sequences is well-established (e.g. Chippindale & Weins 1994; Wetzer 2002; Cho *et al*. 2004; Gontcharov *et al*. 2004; Frøslev *et al*. 2005), the value of the partition homogeneity test for partition congruency has been questioned (Hipp *et al*. 2004 and references therein), and Bayesian analysis tools are capable of applying different molecular evolution models to each data partition. It is now reasonable based on the above evidence to combine sequence data from multiple genes into a single analysis, yet some caution should remain (Cunningham 1997; Leebens-Mack *et al*. 2005) while new methods for examining incongruence in phylogenetic signals are developed (Bonnard *et al*. 2006; Whitfield & Lockhart 2007 and references therein). Individual analysis of each gene allowed understanding of the phylogenetic signal provided by each and the identification of the value of each gene for phylogenetic inference of the munnopsid tree, as discussed above. Combined analyses provided strong support for relationships that were suggested, but not strongly supported, in more than one individual analysis (e.g. *Munneurycope* monophyly and the *Munnopsis/Tytthocope* sister relationship). Combined analyses also provided strong support for relationships that were strongly supported by an individual gene analysis and not contradicted by other individual gene analyses (e.g. *Notopais* and *Echinozone*).

Missing sequences do not seem to pose a problem in large, multigene data sets because of the presence of sufficient informative characters (Fulton & Strobeck 2006). In fact, Fulton & Strobeck (2006) found that inclusion of more taxa, even if missing data, can have positive effects on phylogenetic inference by breaking up long branches. Twenty-one of the 49 included taxa were missing one or two of the three target gene sequences so the possibility remains that missing data could still impact phylogenetic inference in these moderately sized, combined analyses. Impact of missing data here was influenced by which gene sequence was missing and how well-sampled close relatives were. *18S* sequence was missing for two taxa; in the case of Lipomerinae, placement was questionable but in the case of *Acanthamunnopsis* sp. 3, where multiple, closely related species were available, placement was well-resolved by *COI* data. Taxa missing *28S* sequence data were variably resolved by other sequence data. For example, *Munneurycope* sp. ABE was unresolved within the *Munneurycope* by *18S* data and resolved by *COI* data. Placement of the taxa included from GenBank (*Munnopsis typica*, *Echinozone* sp., *Echinozone spinosa*, *Sursumura falcata*, and *Storthyngurella triplospinosa*, also Fig. S3 in Supporting Information) was well-supported and reasonable based only on their *18S* data. These relationships were maintained in the combined analyses, possibly because no data contradicted them. Missing only *COI* sequence had no impact on placement of taxa in combined analyses.

Munnopsinae

The monophyly of subfamily Munnopsinae is not in question due to several distinctive apomorphies (Wolff 1962; Wilson 1982). The limited support for the monophyly of the Munnopsinae (*Acanthamunnopsis*, *Munnopsis*, *Munnopsoides* and *Paramunnopsis*) in individual gene analyses is likely due to the lack of resolution deep in the tree, not uncertainty in the history of the group. The monophyly of this subfamily was well-supported in the combined analyses (Fig. 5).

Paramunnopsis is not a derived group and as a result contains a varied assortment of unmodified munnopsines (Hansen 1916; Wolff 1962). Wilson (1982) noted that *Paramunnopsis* is more similar to eurycopids than other Munnopsinae, thus it follows that *Paramunnopsis* is probably basal within the subfamily. The present analyses strengthen Wilson (1982) hypothesis although strong support is lacking because of limited resolution deep in the tree and the need to revise *Paramunnopsis* in the process of describing the multiple new species that form various clades stemming from the basal Munnopsinae polytomy.

Paramunnopsis sp. 5 is an unusual species due to an excavated notch distally located on pereopods III–IV propodi and enclosed by curved, comb-like setae (Fig. 6A–C). This character has understandably never been reported for any munnopsid because the elongate, delicate pereopods are seldom recovered on trawled animals. The present molecular analyses further support the uniqueness of this species, which was shown to be more closely allied to the *Munnopsis/Munnopsoides* clade (Fig. 5) than to other *Paramunnopsis*, which it grossly resembles. Exclusion of this species from *Paramunnopsis* results in monophyly of the remaining *Paramunnopsis* suggesting it should not be included in *Paramunnopsis* when described.

Excluding sp. 2, *Acanthamunnopsis* was monophyletic based on the individual and combined data. *Acanthamunnopsis* sp. 2 (Fig. 6D–F) is found in the water column yet often covered in sediment (Osborn, unpublished data) suggesting a benthopelagic lifestyle. Although clearly a member of the Munnopsinae, this species has body spines that are stouter and more heavily calcified than other known *Acanthamunnopsis* and possesses a variety of apomorphies that, with further analyses, may be sufficient to separate it from *Acanthamunnopsis* as is indicated by the present molecular data. In *18S* and *28S* analyses, *Acanthamunnopsis* sp. 2 appears in the basal polytomy of the Munnopsinae while Bayesian *COI* analyses place it just inside the *Acanthamunnopsis*. Morphological work and further sequencing may reveal a sister relationship between sp. 2 and the rest of the *Acanthamunnopsis* or may place it as an intermediate between *Munnopsoides*, with which it shares some characters, and *Acanthamunnopsis*.

An unknown specimen was sequenced early on in this project for which there is no voucher material (*Acanthamunnopsis* unknown). I assumed that I would eventually match

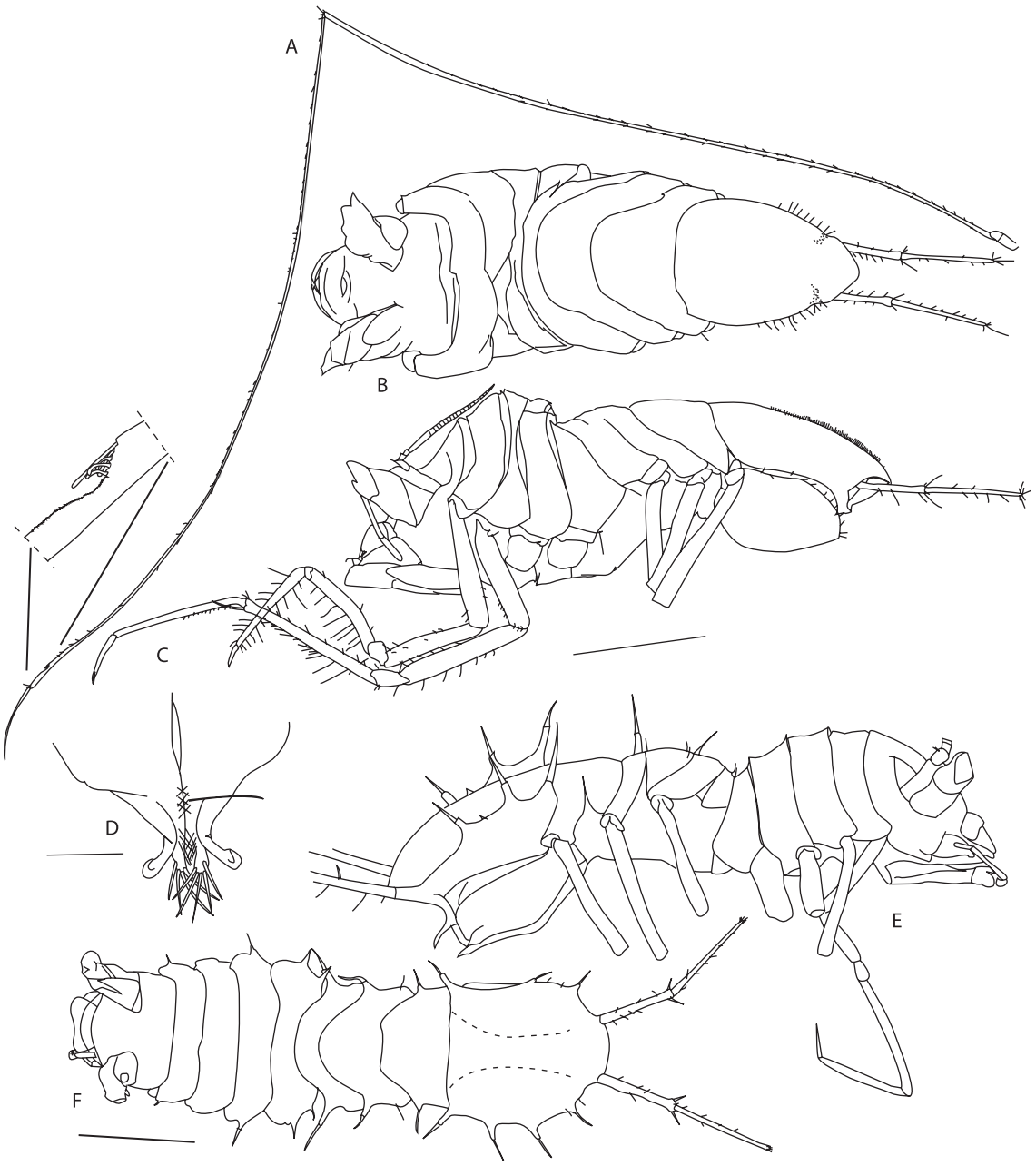

Fig. 6 A–F. *Paramunnopsis* sp. 5 —A. Pereopod 3 and detail of unusual structure found on distal portion of propodus. —B. Dorsal view of habitus (pereonites 2–4 crumpled). —C. Lateral view of habitus. —D–F. *Acanthamunnopsis* sp. 2 —D. Distal tip of male pleopod 1. —E. Lateral view of habitus. —F. Dorsal view of habitus. Scale bars: C & F = 1 mm, D = 0.1 mm.

sequences from another specimen to the sequences obtained from this specimen, but never came across matching sequences in my samples. This specimen was collected by another researcher, not identified beyond Munnopsidae, and frozen in liquid nitrogen precluding morphological examination. The sequence, although not vouchered, is included in the analyses in hope of breaking up a long branch. Based on *18S* and *28S* analyses, this specimen was clearly a member of *Acanthamunnopsis* and while closely related to *A. longicornis* was significantly different from it. These sequences show that the total diversity of pelagic munnopsids off the coast of California has not yet been fully sampled.

Munnopsis and *Munnopsoides* have been allied, synonymized, and confused (Tattersall 1905; Hansen 1916; Wolff 1962; Menzies & George 1972; Shimomura & Ohtsuka 2005; Brandt *et al*. 2007) for the past century although adequate diagnoses have been published (Hansen 1916; Menzies & George 1972). The present analyses support the idea that these genera are closely allied and form a monophyletic clade within the Munnopsinae. The present findings also suggest that a comprehensive revision of *Munnopsis*, *Munnopsoides*, and the closely allied *Pseudomunnopsis* is required because *Munnopsis typica*, the type species for *Munnopsis*, is more closely allied to the available *Munnopsoides* species than to other available *Munnopsis*. The current number of undescribed *Munnopsoides* (on loan from the Australian Museum, Melbourne) and *Munnopsis* species further support this suggestion.

Eurycopinae and Munneurycope

Munneurycope has long been a problem for munnopsid taxonomists. Wolff (1962) separated it from *Eurycope*, Wilson (1989) retained that separation and placed it as *incertae sedis*, and Aydogan *et al*. (2000) noted the continued confusion between the two genera when describing *Munneurycope hadalis* (which incidentally, they placed in the wrong genus). The confusion arises because both *Eurycope* and *Munneurycope* include at least two subgroups, as well as intermediate forms between the genera. Although Wilson & Hessler (1980, 1981), Wilson (1982, 1983, 1989) and Malyutina & Brandt (2006) have made steady progress toward sorting out the eurycopines, diagnostic features uniting remaining members of *Eurycope* and *Munneurycope* have not been identified; there continue to be exceptions and not yet revised taxa that cause confusion. Thus, *Eurycope* and *Munneurycope* both require extensive revision, a project that would be enhanced by inclusion of molecular data.

One would expect *Eurycope* and *Munneurycope* to be closely related because of what appears to be a continuum from the extreme of one form to the other, but according to these analyses either they are not or, more likely, the relationship is older than those resolved in the present tree. Within the *Munneurycope*, lifestyle seems to plays an important role in shaping the morphology (Osborn, unpublished data) because morphology of pelagic and benthic species differs dramatically. With the exception of *Munneurycope* sp. ABE, which was collected from the seafloor, this project was unable to include benthic *Munneurycope*. Thus, one would parsimoniously expect all swimming *Munneurycope* to be monophyletic. Although suggested in both the *18S* and *28S* analyses, the monophyly of the *Munneurycope* was only significant in the combined analyses. Not resolving a sister group for the *Munneurycope* and finding only a weak relationship between those members included here, suggests that the *Munneurycope* split from their sister group prior to cladogenic events resolved by these data. Raupach *et al*. (2009) included a benthic *Munneurycope* and found it most closely related to *Eurycope sarsi* in their analysis. Inclusion of this sequence in a postsubmission-analysis showed it to be most closely related to my *Eurycope* sp. MB (Fig. S3 in Supporting Information).

The heterogeneity within the current *Eurycope* (Wolff 1962; Wilson & Hessler 1981; Wilson 1989; Kussakin 2003; Malyutina & Brandt 2006) suggests that the genus is paraphyletic. Raupach *et al*. (2009) supported this idea based on the location of *E. sarsi* within their tree. However, *E. sarsi* was identified by Wilson & Hessler (1981) as not belonging to *Eurycope* although taxonomic confirmation of this remains to be completed. Paraphyly of *Eurycope* is supported by the present analyses that show *Eurycope* and eurycopines throughout the tree, grouped with various taxa and in small groups of their own (Figs 2–5). A particularly interesting relationship is suggested by the *18S*, *COI*, and the combined analyses, that *Dubinectes* was sister to *Eurycope glabra*. Currently, *E. glabra* is an *incertae sedis* clade (Wilson & Hessler 1981; Malyutina & Brandt 2006) and *Dubinectes* was recently created to contain several previously *incertae sedis* taxa of the *Eurycope*. *Dubinectes* and *E. glabra* share the presence of tubercles on the anterior half of the dorsal surface of the pleotelson (Malyutina & Brandt 2006). The present analyses also suggest the member of the *Eurycope complanata* complex included here, may be more closely related to *E. glabra* than to any other taxa available for these analyses.

None of the present analyses returned monophyly of the Eurycopinae as currently defined, which was represented in the present analyses by *Eurycope*, *Disconectes*, *Dubinectes*, and *Tytthocope*. Neither *Disconectes* nor *Tytthocope* were ever allied to *Eurycope* representatives in the present analyses. This may be explained by limited taxon sampling for each of the represented genera or by the great need for revision of the *Eurycope*. The lack of a relationship shown between the members of the Eurycopinae suggests revision is required within the subfamily in addition to that required within the type genus.

Munnopsurus and Tytthocope

The idea of *Tytthocope* as sister to the *Munnopsurus* is not without merit. They share the following: reduction in pereonite 7 of some species (M. Malyutina, personal communication), the inflated pleotelson, simplification of the molar process, the T-shaped cross section of the mandibular condyle, and the narrow distal tip of male pleopod II. All genes minimally suggested that *Tytthocope* was sister to *Munnopsurus* and thus the combined analyses strongly supported the idea (Fig. 5), as did the findings of Raupach *et al*. (2009). Morphological work currently underway (Malyutina, personal communication) may further strengthen the support for this relationship, in turn linking the *Munnopsurus* to the *Eurycope* from which *Tytthocope* was originally separated (Wilson & Hessler 1981).

Ilyarachninae and Syneurycope

The Ilyarachninae, represented in this project by *Ilyarachna*, *Echinozone*, and *Notopais*, were monophyletic according to the *18S* and *28S* Bayesian analyses (Figs 2 and 3) and thus this relationship was also seen in the combined analyses. Merrin (2004) took *Notopais* out of synonymy with *Echinozone* and *Pseudarachna* after a long history of changing taxonomy (Hodgson 1910; Vanhöffen 1914; Hult 1941; Wolff 1962; Hessler & Thistle 1975; Schultz 1976; Brandt 1990). Inclusion of two *Echinozone* and one *Notopais* in the current *18S* analyses did not clarify the relationship between these taxa. Increased taxon sampling within the Ilyarachininae is needed to resolve the relationship.

The Bayesian *18S* analyses suggest that *Ilyarachna* is sister to the *Echinozone/Notopais* clade and in turn; Raupach *et al*. (2009) also found a sister relationship between their included *Ilyarachna* and *Echinozone/Notopais*. *Syneurycope* is sister to the ilyarachnines (Fig. 2) according to *18S*, *28S*, and combined analyses (Figs 3 and 5; Raupach *et al*. 2009). The relationship between *Echinozone* and *Ilyarachna* has long been supported by morphological work (Hansen 1916; Hult 1941; Wolff 1962; Hessler & Thistle 1975). *Syneurycope* was once included in the Eurycopinae (Menzies 1956) but few suggestions of its affinities have been made otherwise (Haugness & Hessler 1979).

Storthyngurinae and Acanthocope

The members of Storthyngurinae and *Acanthocope* superficially resemble each other because of their prominent body spines, narrow natapods, and the form of their uropods. Wägele (1989) even combined them under the Acanthocopinae but Malyutina (1999, 2003) showed this to be unlikely. Bayesian *18S* analyses suggest that further investigation into the relationship between *Acanthocope* and storthyngurines may reveal a deep relationship between at least some members of the groups. The present limited sampling of the Storthyngurinae together with *18S* sequences available from GenBank supports Malyutina's (1999; 2003) idea of relationships within the group. As seen in the *18S* analyses (Fig. 2), inclusion of additional taxa begins clarifying the nature of the relationships even between more distantly related groups such as the Storthyngurinae and *Acanthocope* although this was not recovered in the Raupach *et al*. (2009) Bayesian analysis.

Conclusions

This project introduced 17 undescribed munnopsid species, collected from the Pacific Ocean, and used them as a basis for a molecularly based phylogenetic hypothesis for the family Munnopsidae. All trees recovered were unrooted because the base of the munnopsid tree was unresolved. The subfamily Munnopsinae, which contains the majority of the pelagic species, was monophyletic and relationships within the clade were better resolved than other infrasubfamilial relationships due to the most complete taxon sampling of any included subfamily. The *Munneurycope* included in this study were monophyletic but no sister group was identified. Based on the present data, a sister relationship between the Munnopsinae and *Munneurycope* could not be rejected (Table 2). Additionally, the sister relationship of *Paropsurus giganteus*, a good swimmer, to other munnopsids was unresolved, leaving the question of whether use of pelagic habitat has arisen more than once within the Munnopsidae unanswered. Although sampling was limited within the Eurycopinae, these analyses supported the putative paraphyly of the group and reinforced the need for careful revision of the remaining *Eurycope*, as well as a reexamination of the inclusion of *Disconectes* and *Tytthocope* in this subfamily. The ilyarachnines included here, *Echinozone*, *Notopais*, and *Ilyarachna*, were monophyletic according to the *18S* analyses and *Syneurycope* was unexpectedly suggested as their sister group. *Munnopsurus* and *Tytthocope* were sister groups but the relationship of this group to the remainder of the included taxa was not resolved. Analyses of combined data provided increased support for clades suggested in at least two individual gene analyses and for clades that were not contradicted by other individual analyses while being supported by analyses of a single gene.

Further work is required to resolve the history of the munnopsids well enough to begin examining the evolution of pelagic life. Further taxon sampling to break up long branches and to better represent the diversity of the group should be the first objective of further work. Based on the resolution achieved, *18S* is the most promising of the three target genes to pursue for further taxon sampling. Additional sequencing of *COI* is not recommended. Likewise, further sequencing of *28S* is not recommended (although sequencing of complete *28S* would likely add to support for clades already suggested by *18S* data) because a gene unlinked to the ribosomal operon would be more useful in providing an independent gene tree for comparison to the *18S* tree. Sequencing effort would be well-spent pursuing a nuclear protein-coding gene that has evolved slowly and would lend itself to resolving relationships deeper in the tree. Expressed sequence tags have proven useful in providing insight into other deep phylogenetic questions (Delsuc *et al*. 2006; Dunn *et al*. 2008) and may be promising for resolving the deep relationships within the Munnopsidae as well. It will be challenging to get uncontaminated sequences from many Munnopsidae because of the nature of munnopsid tissue, size, and the difficulty recovering live specimens from the deep sea, thus special care should be taken during the screening process to identify appropriate markers. Additionally, it has been many years since a cohesive effort has been put toward a morphological analysis of the group, thus this may prove helpful if undertaken.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Fig. S1 The 90% majority rule tree from the combined data set including only taxa for which all three genes (*18S*, *28S* and *COI*) were available from the Bayesian analysis of the Munnopsidae showing. Support values given are posterior probabilities then bootstrap values from the maximum parsimony analysis. Asterisks indicate 1.0 posterior probability. All unsupported branches were collapsed and support values below 0.90 posterior probability are not shown.

Fig. S2 The 80% majority rule tree from the combined data set including only taxa for which at least two of the three genes (*18S*, *28S* and *COI*) were available from the Bayesian analysis of the Munnopsidae showing. Support values given are posterior probabilities then bootstrap values from the maximum parsimony analysis. Asterisks indicate 1.0 posterior probability. All unsupported branches were collapsed and support values below 0.90 posterior probability are not shown.

Fig. S3 The 80% majority rule tree from an *18S* analysis containing the non-overlapping sequences (grey species names) made available during final revision of this manuscript from Raupach *et al.* (2009). Analysis parameters were the same as described for the *18S* analyses in the manuscript with the exception that it was run for only 10 million generation due to time constraints. Support values given are posterior probabilities; asterisks indicate 1.0 posterior probability. All unsupported branches are collapsed and support values below 0.80 posterior probability are not shown. Identical sequences (or very near) pairs are found between several of their sequences that were not noted in their paper and include: *Disconectes* sp. 2*/D. antarcticus*, *Dubinectes acutitelson/ D. nodosus*, and *Haplomesus* sp.*/H. insignis*.

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