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PHYLOGENETIC RELATIONSHIPS OF TANAIDACEA (EUMALACOSTRACA: PERACARIDA) INFERRED FROM THREE MOLECULAR LOCI

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ABSTRACT

Molecular phylogenetic analyses were conducted on species of some common tanaidacean families within the suborders Apseudomorpha and Tanaidomorpha based on partial DNA sequences for three genes: one mitochondrial (COI) and two nuclear (H3 and 28S). One nuclear gene (28S) resolved the two suborders as monophyletic groups while H3 and COI could only resolve Tanaidomorpha as monophyletic. The total evidence analysis (in-group taxa having at least two out of the three sequences) resolved both suborders as monophyletic, but only Tanaidomorpha showed strong support. All analyses support the monophyly of Kalliapseudidae (two out of three subfamilies represented) with the family clearly separated from the other apseudomorph families represented here. Relationships between and within the other apseudomorph families could not be resolved with strong support. Within Tanaidomorpha, most analyses supported a sister group relationship between the Tanaoidea (Tanaidae) and the Paratanaoidea. Results suggested that the monotypic *Hargeria* should be considered a junior synonym of *Leptochelia*, corroborating morphological evidence. Lack of resolution is likely due to inadequate taxon sampling, and differences in topology are largely due to weak support for relationships. This is the first attempt at using molecular data to determine phylogenetic relationships of tanaidaceans.

KEY WORDS: molecular phylogenetics, Peracarida, Tanaidacea

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INTRODUCTION

Tanaidacea is an order of free-living, benthic, peracarid eumalacostracans characterized by having a carapace that covers the first two thoracic somites, which are fused with the head, and having the second thoracopod modified as a cheliped. The group is composed of over 1000 species in three extant suborders, most of them being small (2-5 mm in length) (Drumm et al., 2006). Apseudomorpha (characterized by possession of a biramous antennula and a mandibular palp) appears to be less derived than the other suborders (Sieg, 1988). Tanaidomorpha (characterized by possession of a uniramous antennula, and lacking a mandibular palp) appears to be the most highly derived suborder (Sieg, 1988). Neotanaidomorpha is the smallest and best characterized of the three suborders and is represented by a single family (see Gardiner, 1975); its species (slope, abyssal, and hadal in habits) have morphological characteristics that are transitional between those of the two other extant suborders. Most tanaidaceans are found in marine and brackish water environments, but a few may be found in freshwater (Larsen, 2005 and references therein). They are the most diverse and abundant fauna in some deepwater environments. Although relatively few species are estuarine, they often reach very high population densities in such systems and thus are ecologically significant.

Phylogenetic analyses of Tanaidacea have been limited to four attempts based on morphological data (Sieg, 1983; Larsen and Wilson, 2002; Guerrero-Kommritz and Brandt, 2005; Bird and Larsen, 2009), and only three of these (Larsen and Wilson, 2002; Guerrero-Kommritz and Brandt, 2005; Bird and Larsen, 2009) were carried out using a computer-assisted cladistic approach at the subfamilial or superfamilial level. Larsen (2001) performed the only molecular study dealing solely with Tanaidacea; he used restriction fragment length polymorphisms (RFLP's) to identify cryptic species of *Paratanais* from a single microhabitat.

Molecular phylogenetic studies that included Tanaidacea have focused on resolving relationships within Peracarida, limiting representative taxa to one (Jarman et al., 2000), three (Spears et al., 2005), or five (Wilson, 2009) species. Wilson (2009) used two apseudomorphs and three tanaidomorphs (two in the superfamily Paratanaoidea and one in the superfamily Tanaoidea) and the analysis based on DNA sequence data (18S) resolved Tanaidomorpha as monophyletic, but Apseudomorpha was found to be polyphyletic. Within Tanaidomorpha, one of the paratanaoids resolved as the sister to the tanaoids.

I was interested in testing the hypotheses that the suborders Tanaidomorpha and Apseudomorpha and the superfamilies Paratanaoidea and Tanaoidea are monophyletic with a more extended coverage of representative taxa. I was also interested in testing the hypothesis that *Hargeria rapax* (Harger, 1879) is sister to *Leptochelia dubia* (Krøyer, 1842), as has been shown with morphological data (Bird and Larsen, 2009).

In this study, phylogenetic relationships of some common tanaidacean families within the suborders Apseudomorpha and Tanaidomorpha are inferred on the basis of partial sequences for three genes: one mitochondrial (COI), and two nuclear (H3 and 28S). Unfortunately, sequences for all of the species were not successfully amplified for the same gene during PCR so a total evidence analysis was conducted on those taxa (13) that have at least two sequences.

Table 1. Species used in analyses, including collection localities, suborder and family affiliations, voucher numbers, and GenBank accession numbers. Cloned sequences are marked with an asterisk.

Taxa	GCRL acc. # (voucher)	Locality	GenBank accession no.		
			COI	H3	28S
Suborder Apseudomorpha					
Family Apseudidae					
Apseudes cf. *bermudeus*	2963	St. Andrew Bay, FL	HM016216	HM016178	HM016191
Apseudes olimpiae	2949	St. Andrew Bay, FL	_	HM016179*	_
Hoplomachus propinquus	2960	Long Key, FL	HM016200	HM016182*	_
Family Metapseudidae					
Pseudoapseudomorpha sp.	2954	Belize	HM016208	HM016177	HM016194
Synapseudes sp.	2961	Belize	HM016207	_	_
Family Parapseudidae					
Parapseudes sp.	2952	Belize	HM016217	HM016176	HM016193
Parapseudes latifrons	2957	Japan	_	HM016174	_
Discapseudes sp.	2967	Panama	_	HM016181	HM016192
Family Kalliapseudidae					
Alokalliapseudes macsweenyi	2964	Florida	HM016211	HM016184	HM016189
Monokalliapseudes schubartii	2956	Brazil	HM016210	HM016186	HM016190
Psammokalliapseudes granulosus	2959	Ft. Lauderdale, FL	HM016209	_	_
Suborder Tanaidomorpha					
Superfamily Tanaoidea					
Family Tanaidae					
Tanais dulongii	2968	Dania Beach, FL	HM016204	_	_
Zeuxo normani	2965	Japan	HM016203	HM016171	HM016197
Superfamily Paratanaoidea					
Family Leptocheliidae					
Hargeria rapax	2966	Dania Beach, FL	HM016214	HM016183	HM016198
Leptochelia dubia	2955	Ft. Lauderdale, FL	HM016215	HM016187*	HM016199
Leptochelia forresti	2958	Dania Beach, FL	HM016206	_	_
Leptochelia longichelipedes	2953	Belize	HM016201	_	_
Pseudoleptochelia sp.	2962	Belize	HM016202	HM016173	_
Family Paratanaidae					
Paratanais sp.	2951	Belize	HM016205	HM016175	HM016195
Family Nototanaidae					
Nototanais sp.	2969	Belize	_	HM016188	HM016196
Family Pseudotanaidae					
Pseudotanais sp.	2950	Panama	_	HM016172	_
Outgroups					
Cumacea					
Diastylis crenellate			AF352298		
Gynodiastylis sp.			AF520447		
Oxyrostylis smithi			AF137512		
Isopoda					
Eurycope complanata					EU414395
Munnopsurus sp.					EF682340
Acanthocope galathea					EF682337

Materials and Methods

Taxa

For the cytochrome *c* oxidase subunit I (COI), 16 species of Tanaidacea from seven families in the suborders Apseudomorpha and Tanaidomorpha were successfully analyzed, and three species of Cumacea were taken from GenBank and for use as out-groups (Table 1). For the Histone 3 (H3), 17 species of Tanaidacea were successfully sequenced, and two species of Isopoda were taken from GenBank for use as the out-groups (Table 1). For the nuclear large subunit ribosomal DNA sequences (28S rRNA), 11 species of Tanaidacea were successfully examined, and three species of Isopoda were taken from GenBank for use as the out-group (Table 1). Previous analyses of molecular data have shown Tanaidacea to be closely related to Isopoda and Cumacea (Jarman et al., 2000; Spears et al., 2005; Meland and Willassen, 2007; Wilson, 2009) as has some morphological data (Siewing, 1956, 1963; Richter and Scholtz, 2001), although the sister group for these anatomical studies remains ambiguous.

Specimens were preserved in 95% ethanol for subsequent DNA extraction and kept at −20°C. Richard W. Heard (Gulf Coast Research Laboratory [GCRL]) identified species. Voucher specimens are archived as cataloged museum specimens at the GCRL (Table 1).

DNA Extraction, PCR Amplification, Cloning, and Sequencing

Total genomic DNA was extracted from whole specimens using DNeasy Tissue Kits (Qiagen). Ethanol was removed by placing specimens in sterile DI water prior to extraction. Extended incubation times were used to increase DNA yields. The elution step was done with 150 µl instead of 200 µl. A partial region of the COI gene (~ 680 bp) was amplified using the universal LCO1490 (5′ - GTCAACAAATCATAAAGATATTGG - 3′) and HCO2198 (5′ - TAAACTTCAGGGTGACCAAAAAATCA - 3′) primers (Folmer et al., 1994). A partial region of the 28S-rDNA gene (~ 800 bp) was amplified using the universal 28S-RD1.3f (5′- GGATTCCCTYAGTAAGKGCG - 3′) and 28S-rD4b (5′ - CCTTGGTCCGTGTTTCAAGAC - 3′) primers (Whiting, 2002). The histone 3 (H3) gene was amplified (~ 340 bp) using the universal H3AF (5′ - ATGGCTCTGACCAAGCAGACVGC - 3′) and H3AR (5′ - ATATCCTTRGGCATRATRGTGAC - 3′) primers (Colgan et al., 1998). PCR amplification of COI was carried out in 50 µl reactions using 0.2 µM of each primer, 1 U *Taq* polymerase, 25 µl MasterAmp™ 2X PCR Premix F (Epicentre® Biotechnologies), 5 µl template, and 17.75 µl ddH_2O. The thermal regime for PCR was: 3 min at 95°C, 35 cycles of 15 s at 94°C for denaturation, 1 min at 48°C for annealing, 3 min at 72°C for extension, and a final 5 min at 72°C. PCR products were subject to electrophoresis through 1-2%

agarose gel stained with ethidium bromide, and these were visualized under ultraviolet illumination to verify product band size. PCR products were purified with Qiaquick PCR Purification Kits (Qiagen). Purified PCR products were used in 5 µl sequencing reactions utilizing BigDye® Terminators v3.1 (Applied Biosystems, USA). Products of these reactions were furnished at the University of Illinois at Urbana Champaign for sequencing on an ABI 3730XL capillary sequencer. Fragments were sequenced in both directions using the same primers as in PCR amplification.

PCR amplification of H3 and 28S was carried out at Brigham Young University (BYU) in 28 µl reactions using 2.8 µl of each primer (10 mM), 1 unit of Hotmaster™ *Taq* DNA polymerase, 4.48 µl dNTP mix (10 mM), 2.8 µl 10 × PCR buffer (Hotmaster™ with Mg^{+}), 3 µl template, and 11.97 µl ddH_2O. The thermal regime for PCR was: 3 min at 95°C, 45 cycles of 30 s at 95°C for denaturation, 45 s at 50°C for annealing, 1 min at 72°C for extension, and a final 5 min at 72°C. PCR products were subject to electrophoresis through a 1% agarose gel stained with SYBR Green and visualized under ultraviolet illumination to verify product band size. PCR products were purified using Millipore plates. Sequence reactions were run with purified PCR products and ABI BigDye Ready Reaction kit. Reactions were cleaned using Millipore Multiscreen filter plates and sequenced on an ABI 3730XL automated sequencer. Three specimens [one each in *L. dubia*, *Hoplomachus propinquus* (Richardson, 1902), and *Apseudopsis olimpiae* (Guţu, 1986)] showed multiple chromatogram peaks on both strands of the H3 fragment. To distinguish the H3 genotypes carried by these specimens, PCR products were cloned into a plasmid vector using the TOPO TA Cloning kit (Invitrogen, Carlsbad, CA) that was then used to transform competent bacterial cells. Five positive colonies were harvested and used as templates to amplify and sequence the H3 marker. All sequences including cloned H3 genotypes were accessioned to GenBank (Table 1).

Sequence Alignment and Phylogenetic Analyses

Sequence contigs were assembled using Sequencher™ v. 4.7 (Gene Codes Corp., Ann Arbor, MI). COI and H3 sequences were manually aligned with the aid of amino acid composition in Se-Al (Rambaut, 2001), precluding ambiguous alignment. 28S sequences were aligned with ClustalX (Thompson et al., 1997) using default settings and refined manually in Se-Al. The 28S sequences were truncated to a 371 bp fragment because the rest of the fragment proved to be difficult to align between the suborders due to a hypervariable region. Identities of sequences were confirmed with BLAST searches in GenBank.

Substitutional saturation was examined in COI and H3 by plotting pairwise numbers of transitions and transversions at the first, second, and third codon positions against pairwise genetic distances in the program DAMBE (Xia and Xie, 2001). Base composition and heterogeneity were analysed in PAUP*. Pairwise sequence divergence was analysed using MEGA v.4.0 (Tamura et al., 2007).

Maximum parsimony analyses were performed using PAUP* 40b10 (Swofford, 2002). Heuristic searches were performed using 100 random addition sequences with the tree-bisection-reconnection (TBR) branch swapping algorithm. Bootstrap values were used to assess nodal support using 1000 pseudo-replicates. Gaps were treated as a fifth character state.

Maximum Likelihood (ML) analysis was conducted using PhyML v2.4.4 (Guindon and Gascuel, 2003). The model of sequence evolution for each gene was assessed using Modeltest 3.7 (Posada and Crandall, 1998). For COI, the AIC selected the K81uf + I + G model and the hLRT criterion selected GTR + I + G. Since the former is not an option in PhyML, the GTR + I + G model was chosen. For H3 and 28S + H3, the SYM + I + G model was selected and for 28S, the TrN + G model was selected (using the AIC criterion). The proportion of invariable sites and the gamma distribution parameter were estimated by the program, and default settings were used for the other parameters. A bootstrap analysis with 1000 replicates was performed.

Bayesian analyses were performed with MrBayes 3.1 (Ronquist and Huelsenbeck, 2003). Modeltest (Posada and Crandall, 1998) was used to choose the best fit model for each gene and partition, as in the ML analysis. Following a total evidence approach (Kluge, 1989) phylogenetic analyses were performed on the combined 28S + H3 + COI datasets. The combined dataset with all out-groups was partitioned so that the best fit models were applied separately to each gene region and each codon position for the two protein-coding genes (unlink command). All analyses were run with default priors. Two independent runs of four chains were started from a random tree for 5×10^6 generations, sampling every 100 generations with a temperature setting of 0.2. Analyses were repeated twice. The average standard deviation of split frequencies was used to check for convergence. The burn-in phase was estimated by examination of the log likelihood plots in Tracer v1.4.1 (Rambaut and Drummond, 2007). Stationarity occurred around 500,000 generations, thus the first 5000 trees were discarded as burn-in. A consensus with posterior probabilities was produced with the 'sumt' command.

Results

COI

The final alignment yielded a total of 618 sites for phylogenetic analysis, of which 363 were parsimony informative. The sequences contained some gaps and several non-synonymous substitutions, but no stop codons were detected and the gaps were in triplets so the reading frame was not interrupted, supporting that the functional mitochondrial COI gene was amplified rather than a nuclear pseudogene (see Buhay, 2009 for a discussion on the problems associated with COI). The parsimony analysis found a single tree of 1971 steps (CI = 0.45, RI = 0.48). The sequences had a high A + T content (mean = 66%). Significant differences in base composition were detected, even when out-group taxa were excluded ($X^2 = 164.5$, $P < 0.001$). Sequence divergence among families was high (30-46% uncorrected p-distances). The third codon positions showed saturation for both transitions and transversions, and the first codon position showed some indication of transitional saturation (data not shown), indicating homoplasy.

The MP and ML trees had identical topologies, with the ML tree generally having higher support values (Fig. 1A). The monophyly of Tanaidacea was supported with MP but not ML analyses. There was a metapseudid/apseudid/parapseudid clade but lacking support [< 50% bootstrap support (BS)], so the node was collapsed into a polytomy. The analysis was therefore not able to resolve relationships between the apseudomorph families. Tanaidomorpha was weakly supported (56% BS, both MP and ML). An analysis on inferred amino acids (data not shown) gave higher support for clades of Tanaidacea and Tanaidomorpha (88% and 75% BS, respectively) than the analysis based on nucleotide sequences. The monophyly of Kalliapseudidae was supported with ML (80% BS) and the clade of Kalliapseudinae was strongly supported (85% MP, 96% ML). Within the tanaidomorph clade, Tanaidae forms a sister group to a leptocheliid clade (including *Paratanais*) with only weak support (56% MP, ML). *Hargeria rapax* and *L. dubia* form a sister group relationship (62% MP, 87% ML). An unexpected result was the sister group relationship between *Paratanais* sp. and *Leptochelia forresti* (Stebbing, 1896).

The Bayesian analysis showed convergence well before 5 million generations (standard deviation of split frequencies = 0.003), and the tree topology (Fig. 1B) differed from the MP and ML trees. Tanaidomorpha does not emerge as a monophyletic group (the apseudids, metapseudids and parapseudid are nested within this clade). The two metapseudid species formed a sister group relationship (pp = 0.69). *Apseudes bermudeus* Băcescu, 1980 formed a

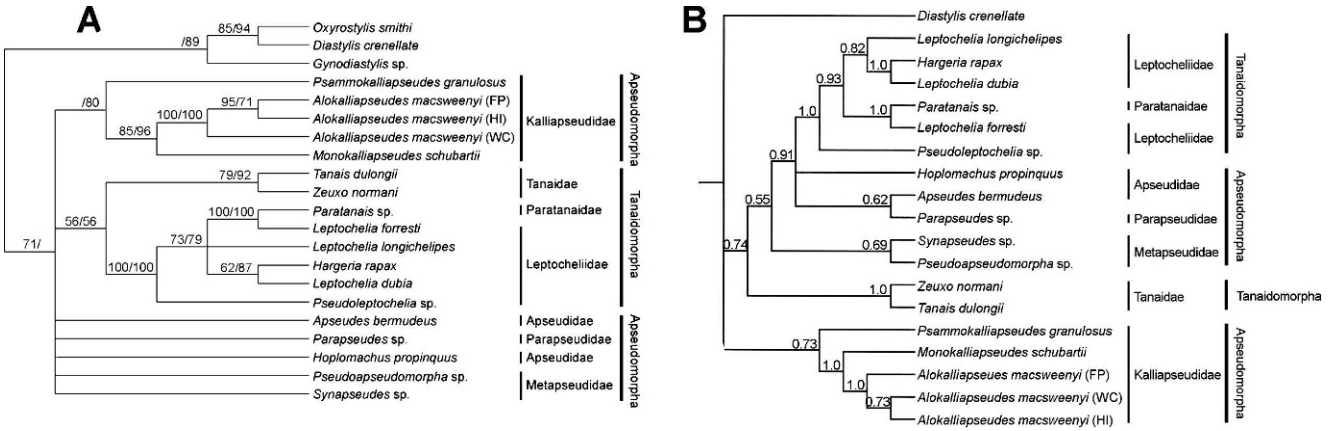

Fig. 1. A, Cladogram based on COI. Sidebars indicate higher taxa within Tanaidacea. Numbers above the branches indicate non-parametric bootstrap values > 50% (MP/ML). B, Bayesian phylogenetic analysis consensus based on COI. Sidebars indicate higher taxa within Tanaidacea. Numbers above the branches indicate Bayesian posterior probability (PP) values. Specimens of *Alokalliapseudes macsweenyi* were sampled from three locations in NW Atlantic and Gulf of Mexico: FP = Fort Pierce, Florida; HI = Horn Island, Mississippi; WC = Whiskey Creek, Dania Beach, Florida. The trees were rooted with three cumacean species (sequences from GenBank).

sister group relationship with *Parapseudes* sp. (pp = 0.62), rendering Apseudidae polyphyletic.

H3

The final alignment yielded a total of 323 sites for phylogenetic analysis, of which 130 were parsimony informative. The sequences contained no indels and only three non-synonymous substitutions (one each in *Pseudoleptochelia*, *Parapseudes* sp., and *Discapseudes*). No stop codons were detected, supporting that the functional H3 gene was amplified rather than a pseudogene. The parsimony analysis found a single tree of 546 steps (CI = 0.40, RI = 0.50). The base composition was: A = 26%, C = 28%, G = 24% and T = 22%. No significant differences in base composition were found ($X^2 = 51.2$, $P = 0.58$). Sequence divergence among families ranged from 15-28% uncorrected p-distances. The third codon positions showed saturation for both transitions and transversions (data not shown).

All three analyses gave largely congruent results (Fig. 2A). The Bayesian analysis showed convergence well before 5 million generations (standard deviation of split frequencies = 0.006). The kalliapseudid clade was resolved with strong support from ML and Bayesian analysis (82% and 1.0, respectively). The leptocheliid clade was supported, as was the sister group relationship between *L. dubia* and *H. rapax*. The apseudid clade was strongly supported (100% MP, 99% ML, 1.0 PP). The metapseudid *Pseudoapseudomorpha* sp. was resolved as the sister group to the apseudid clade with weak (52% MP, 61% ML) to strong (0.99 PP) support. Species in the other families (Parapseudidae, Tanaidae, Paratanaidae, Pseudotanaidae, and Nototanaidae) could not be resolved. A peculiar finding was one of the clones of *A. olimpiae* formed a sister group to *H. propinquus* and not to the other *A. olimpiae* clone.

28S

The final alignment yielded a total of 371 sites for phylogenetic analysis, of which 193 were parsimoniously informative. The parsimony analysis found a single tree of 607 steps (CI = 0.72, RI = 0.68). The base compositon was: A = 28%, C = 22%, G = 29% and T = 22%. No significant differences in base composition were found ($X^2 = 47.7$, $P = 0.06$).

All three analyses gave congruent results (Fig. 2B). The Bayesian analysis showed convergence well before 5 million generations (standard deviation of split frequencies = 0.002). All nodes that were resolved showed strong support: Tanaidomorpha, Apseudomorpha, Tanaidae + Paratanaoidea, Kalliapseudidae + Parapseudidae/Apseudidae, Leptocheliidae (*L. dubia* + *H. rapax*), Paratanaidae + Nototanaidae. Relationships within the parapseudid/apseudid clade could not be resolved.

28S + H3 + COI

The final alignment yielded a total of 1311 sites for phylogenetic analysis, of which 653 were parsimoniously informative. The MP analysis yielded a single most parsimonious tree of 2328 steps (CI = 0.610, RI = 0.521).

All three analyses gave similar results (Fig. 3). The Bayesian analysis showed convergence well before 5 million generations (standard deviation of split frequencies = 0.005). The isopod *Eurycope complanata* was used to root the tree, and the other two isopod out-groups grouped with it. The cumacean out-groups were the next to branch off in a polytomy next to a weakly supported Tanaidacea. Apseudomorpha and Tanaidomorpha both resolved as monophyletic, but only Tanaidomorpha showed strong support. Within Apseudomorpha, Kalliapseudidae (*Alokalliapseudes* + *Monokalliapseudes*) and Apseudidae (*Apseudes* + *Hoplomachus*) were resolved as monophyletic with strong support. ML and Bayesian but not MP analyses resolved Parapseudidae (*Discapseudes* + *Parapseudes*) as monophyletic with strong support. The kalliapseudid clade resolved as sister to an unresolved apseudid + parapseudid + metapseudid clade. Within Tanaidomorpha, the paratanaid (*Paratanais* sp.) resolved as sister to the nototanaid (*Nototanais* sp.) with strong support, and this clade was sister to a leptocheliid clade with strong support. The leptocheliid

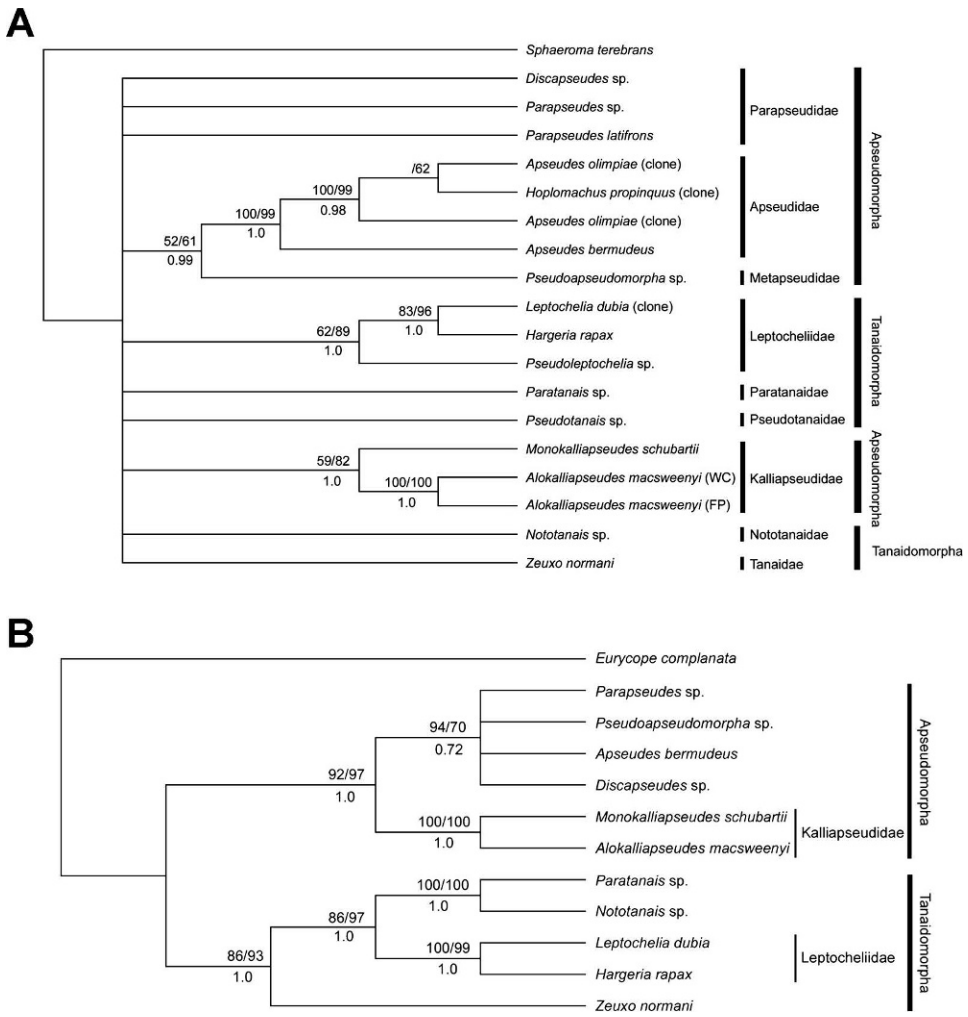

Fig. 2. A, Cladogram based on H3. B, Cladogram based on 28S. Sidebars indicate higher taxa within Tanaidacea. Numbers above the branches indicate non-parametric bootstrap values > 50% (MP/ML). Numbers below the branches indicate Bayesian posterior probability (PP) values. The trees were rooted with an isopod species (sequence from GenBank).

clade was only weakly supported with MP and Bayesian analyses. The tanaid *Zeuxo normani* (Richardson, 1905) resolved as sister to Paratanaidoidea with strong support.

Discussion

When multiple isopods and cumaceans were used as outgroups (total evidence analysis), Tanaidacea monophyly was only weakly supported. It must be noted that the isopods *Sphaeroma terebrans* (Genbank acc. # FJ656807) and *Asellus aquaticus* (GenBank acc.# AJ238321) grouped within the in-group when they were used as additional outgroups. Wilson (2009) also showed isopods grouping within Tanaidacea when he analyzed an 18S dataset.

The 28S and total evidence (28S + H3 + COI) datasets both resolved Apseudomorpha and Tanaidomorpha as monophyletic groups. MP and ML analysis of the COI dataset resolved Tanaidomorpha as monophyletic (with very weak support) but could not resolve Apseudomorpha. COI could not resolve the deeper divergences, and this was not unexpected since it is a fast-evolving protein coding gene that often shows significant levels of genetic diversity at the intraspecific level (Cox and Hebert, 2001). However, it has been used with some apparent success in higher-level phylogenetic studies of other peracaridean crustaceans such as Cumacea (Haye et al., 2004).

All analyses support the monophyly of Kalliapseudidae. Representatives from two of the three subfamilies were included in the COI dataset and supported *Psammokalliapseudes* (Tanapseudinae) as the sister group to Kalliapseudinae. In ongoing morphological analyses, a cladistic analysis based on morphological characteristics supported the monophyly of the family (Drumm, unpublished data). Another potential characteristic (in combination with the loss of the maxillula palp) uniting the family might be the presence of exopods on the last two pairs of pereiopods in the manca stages (post-marsupial instars with incompletely developed post-cephalic appendages), as this has been shown to occur in all three presently defined subfamilies. However, because mancas are rarely collected and/or examined, relatively few species have been confirmed for the presence of this character. The only other group confirmed to have this character is the subfamily Pseudosphyrapodinae in the family Sphyrapodidae Guţu, 1980, but

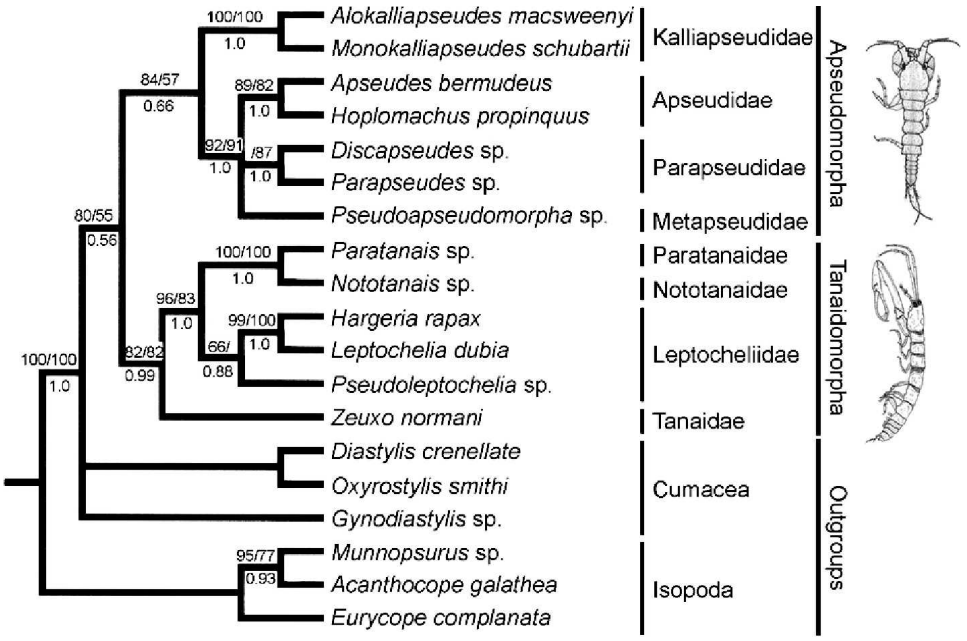

Fig. 3. Cladogram based on H3 + 28S + COI. Sidebars indicate higher taxa within Tanaidacea. Numbers above the branches indicate non-parametric bootstrap values > 50% (MP/ML). Numbers below the branches indicate Bayesian posterior probability (PP) values. Pictured are the apseudomorph *Hoplomachus propinquus* and the tanaidomorph *Hargeria rapax*, taken from Suárez-Morales et al. (2004).

no other potential synapomorphies have been identified between these two families that would suggest a close phylogenetic affinity. For undetermined reasons, the presence of exopods appears to have been independently retained within these two disparate groups. Sphyrapodid specimens were not available for molecular analysis. Another result from all analyses is the clear separation of Kalliapseudidae from the other apseudomorph families represented here.

The relationships between and within the other apseudomorph families represented in this study (Apseudidae, Parapseudidae, and Metapseudidae) could not be resolved with strong support. MP and ML analyses of the COI dataset collapsed them into a polytomy. Bayesian analysis of COI showed weak support for a metapseudid clade (*Synapseudes* + *Pseudoapseudomorpha*) and a sister group relationship between the apseudid, *Apseudes* cf. *bermudeus*, and the parapseudid, *Parapseudes* sp., rendering Apseudidae polyphyletic. The total evidence (28S + H3 + COI) and H3 datasets showed support for an apseudid clade. Guţu (2008) transferred the apseudid genus *Hoplomachus* to Metapseudidae, but the present study does not support that view. Apseudidae are a heterogenous assemblage, and phylogenetic relationships in this family must be examined in more detail. The present lack of resolution is likely due to inadequate taxon sampling, and differences in topology are largely due to weak support for relationships.

Within Tanaidomorpha, all analyses except the COI Bayesian and all H3 analyses supported a sister group relationship between Tanaoidea (Tanaidae) and the Paratanaoidea, which is in agreement with the current morphologically based classification. An expected result was the sister group relationship between *H. rapax* and *L. dubia*, since they are distinguished by only one quantitative morphological character confined to the male of *H. rapax* (the male of *H. rapax* has an anal plate, whereas that of *L. dubia* lacks one). Thus, it appears that *Hargeria* should be considered a junior synonym of *Leptochelia*, which confirms the cladistic analysis of morphological characters of Bird and Larsen (2009). One unexpected finding was the apparent sister group relationship between *Paratanais* sp. and *L. forresti* in the COI analysis. Unfortunately, neither 28S nor H3 sequences for *L. forresti* were obtained, so this sister group relationship could not be confirmed. In the 28S and total evidence analyses, *Nototanais* sp. emerged as a sister group relationship with *Paratanais* sp., and these formed the sister group to the leptocheliid clade. The H3 analysis supported a leptocheliid clade: (*Pseudoleptochelia* sp. (*L. dubia*, *H. rapax*)).

While a full understanding of Tanaidacea phylogeny remains a distant objective, the present analysis is intended to provide a framework for testing alternative hypotheses, i.e., monophyly of Paratanaidae and Apseudidae, and relationships among the apseudomorph families with more representative taxa. Constructing a robust molecular phylogeny for Tanaidacea will be a major challenge because many families are represented by only one or a few species, and/or are exclusive to deep-sea environments from where it is difficult to obtain fresh material (Neotanaidae, Agathotanaidae, Anarthruridae, etc.). Collaborative partnerships with deep-sea researchers will be essential to further studies.

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