

Systematics and Biodiversity

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/tsab20>

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Published online: 16 Dec 2013.

To cite this article: Christopher B. Boyko, Jessica Moss, Jason D. Williams & Jeffrey D. Shields (2013) A molecular phylogeny of Bopyroidea and Cryptoniscoidea (Crustacea: Isopoda), Systematics and Biodiversity, 11:4, 495-506, DOI: [10.1080/14772000.2013.865679](https://doi.org/10.1080/14772000.2013.865679)

To link to this article: <http://dx.doi.org/10.1080/14772000.2013.865679>

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Research Article

A molecular phylogeny of Bopyroidea and Cryptoniscoidea (Crustacea: Isopoda)

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(Received 4 May 2013; revised 31 October 2013; accepted 11 November 2013; first published online 11 December 2013)

Epicaridean isopods are parasitic on other crustaceans. They represent a diverse group of highly derived taxa in two superfamilies and 10 families. Little work has been done on the phylogeny of these parasites because of the difficulty in defining homologous characters for adults above the genus level. The females exhibit morphological reduction of characters and the males have few distinguishing characters. Moreover, epicarideans have only rarely been included in past studies of isopod phylogeny. Our objective was to derive a phylogeny of epicaridean taxa based on 18S rDNA, then use that phylogeny to examine the relationships of the bopyrid subfamilies, bopyroid families and epicarideans to cymothoid isopods. We tested the monophyly of the Epicaridea, evaluated hypotheses on relationships among epicaridean families and subfamilies, examined the evolution of the abdominal mode of infestation on caridean, gebiidean, axiidean and anomuran hosts and examined coevolution between epicarideans and their crustacean hosts. The molecular phylogeny indicated that Epicaridea were monophyletic with respect to Cymothooidea. Bopyroidea formed a monophyletic group without Dajidae and Entophilinae (now as Entophilidae). Both latter taxa grouped with Cryptoniscoidea, and this group was the sister taxon to the redefined Bopyroidea in all trees. The bopyrid subfamily Ioninae is the sister taxon to the other bopyrid subfamilies (except Entophilidae). Ioninae was elevated to family status but found not to be monophyletic; a new subfamily, Keponinae, was erected for all genera formerly placed in Ioninae except the type genus. The abdominal mode of parasitism appears to have evolved independently among the subfamilies. Coevolution between host and parasite phylogenies showed extensive incongruence, indicating frequent host-switching as a general pattern in Epicaridea.

<http://www.zoobank.org/urn:lsid:zoobank.org:pub:30ECFB13-2795-494E-AABE-6B5F84A57A67>

Keywords: Bopyridae, coevolution, Decapoda, Epicaridea, Isopoda, parasite

Introduction

Bopyroidea and Cryptoniscoidea, commonly referred to as epicarideans, are obligate parasitic isopods that infest crustacean hosts. Among Isopoda, epicarideans are unique in that their mouthparts are highly modified to form a suctional cone for feeding on the hemolymph of their crustacean hosts. Unlike most other isopods, which have direct development, all epicarideans have three to four larval stages (epicaridium, cryptoniscium, microniscus and bopyridium, if the latter is considered a separate larval stage) and a life cycle involving two hosts. Larvae use

calanoid copepod hosts, adult bopyroids use decapod hosts, and adult cryptoniscoids use decapods and a variety of other crustaceans as hosts. At present, there are two superfamilies in Epicaridea: Bopyroidea (three families) and Cryptoniscoidea (seven families) (Trilles, 1999; Martin & Davis, 2001; Williams & Boyko, 2012), with the bulk of species (696 of 795) contained within the globally distributed Bopyroidea. Epicarideans are a diverse, populous group, representing approximately 7.7% of described isopods (Williams & Boyko, 2012). Undoubtedly, there are still many hundreds of undescribed species, due in large part to lack of study and their cryptic lifestyles as ecto- and endoparasites. Areas of the Indo-West Pacific likely hold the greatest number of undescribed species. In fact, there is evidence that the bopyrid fauna of the Indo-West Pacific

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may be double the number presently known from that region (Markham, 1986) and recent studies in China (e.g. An *et al.*, 2010, 2012) support these predictions.

The three families of Bopyroidea are Bopyridae, Dajidae and Entoniscidae. The Bopyridae are by far the largest family and arguably the second largest family within Isopoda (Schotte *et al.*, 2013). It is currently divided into nine subfamilies comprising 605 described species (Williams & Boyko, 2012). Pseudioninae is the largest subfamily (232 spp.) and is considered the basal taxon within Bopyridae, both by virtue of morphology, which is closest to the free-living isopod bauplan and by host selection, which is broadly based. The next largest subfamilies are Bopyrinae (118 spp.), known from caridean shrimp, and Ioninae (105 spp.), known from brachyuran crabs, axioid and gebiidean shrimp, and palinuran lobster hosts. However, the boundaries between Ioninae and Pseudioninae are not clearly defined (Markham & Boyko, 2003), and purported 'transitory' forms with mixtures of subfamilial characters have been noted (Markham, 1986). The smaller subfamilies are Orbioninae (38 spp., on penaeoid shrimp), Hemiarthrinae (55 spp., on caridean shrimp), Athelginae (41 spp., on paguroid anomuran crabs), Argeiinae (12 spp., on caridean and stenopodid shrimp), Entophilinae (two spp., internally within anomuran and axioid hosts) and Phylodurinae (one sp., on gebiidean shrimps).

To complicate matters, there are three modes of host infestation in Bopyridae that may have taxonomic importance. Argeiinae, Bopyrinae, Orbioninae, Pseudioninae and most Ioninae, reside within the branchial chamber of their hosts. Athelginae, Hemiarthrinae, Phylodurinae and *Rhopalione* (historically within Ioninae) are found on the abdomens of their hosts and Entophilinae are found within the visceral cavity of their hosts. This raises the question of which mode is the ancestral state and whether the modes of infestation reflect monophyletic groupings of subfamilies or whether they arose independently among taxa.

Dajidae are comprised of 50 described species in 18 genera (Williams & Boyko, 2012). They occur in all oceans and adult female dajids are ectoparasitic on euphausiids, mysids, and penaeoid and caridean shrimp. They are found attached to the carapace, abdomen or thorax of their hosts. Dajids are thought to be a sister taxon to bopyrids, or perhaps having evolved from within Bopyroidea (Kensley, 1979).

Entoniscidae are comprised of 37 described species in 16 genera (Williams & Boyko, 2012). Entoniscids are internal parasites of anomuran and brachyuran crabs and, apparently rarely, in caridean and gebiidean shrimp. Female entoniscids have few distinguishing characters and, although placed in Bopyroidea, are so highly modified that they are often not recognized as isopods by the casual observer. Unlike most crustaceans, they do not moult to grow; that, coupled with the fact that their marsupium is grossly inflated as a hood that can extend dorsally over the bulbous bilobed cephalon,

gives them a bizarre worm-like appearance. Dwarf male and larval entoniscids retain the isopod bauplan, and males, epicaridium and cryptoniscium larvae often co-occur within the marsupium of a single female. Unlike in Bopyridae and Dajidae, the motile, fully developed epicaridium larvae possess a distinct, species-specific chelate process on the 6th pereopod (Shields & Earley, 1993).

There are approximately 100 described cryptoniscoid species but undescribed species may number in the hundreds (Williams & Boyko, 2012; Bourdon, pers. comm.), making cryptoniscoids the least understood group of epicarideans. Cryptoniscoidea females occur primarily within the brood chamber of isopods and other peracarids (either as ecto- or mesoparasites), although ostracods and occasionally decapods are also infested. A few cryptoniscoid species are endoparasitic. Females are highly modified as adults, being reduced to sac-like bodies with only faint segmentation. Males are immediately recognizable as isopods, albeit they exhibit neoteny, and resemble cryptoniscium larvae. In fact, the cryptoniscium larvae, functional males, and immature females are all indistinguishable with light microscopy (Hosie, 2008). The current taxonomic framework at the family level is unresolved and based largely on host choice rather than on morphological or molecular data. In contrast, genera are defined by the gross morphology of the females, and species by characters of cryptoniscium larvae.

Although some epicarideans have been studied using morphological methods, such as dissections of adults and brooded larvae, and histological and SEM studies, little has been done regarding the phylogeny of these highly derived parasites. This is primarily due to the difficulty of defining homologous characters and synapomorphies, especially above the subfamily level, and morphological reduction of characters in female epicarideans due to their parasitic mode of life. The males are much less modified, but they also have few morphological characters useful to distinguish among taxa. Molecular techniques may offer an alternative approach to elucidating their higher-level relationships and, potentially, their relationships to the free-living isopods. Molecular studies involving sequence analyses of several regions (12S and 16S LSU mitochondrial rDNA, COI mitochondrial DNA, and 18S SSU rDNA) have proven useful in elucidating other isopod phylogenies (Held, 2000; Mattern & Schlegel, 2001; Wetzer, 2001, 2002; Raupach *et al.*, 2004; Wetzer *et al.*, 2013), but few examples of epicarideans have been included in past studies.

The phylogenetic position of Epicaridea is highly variable and somewhat contentious. Based on morphological studies, Monod (1922), Strömberg (1972), Schmalfuss (1989), Wägele (1989), Brusca & Wilson (1991) and Brandt & Poore (2003) all offered different hypotheses on the relationships among epicarideans and other isopods, chiefly gnathiids, cymothoids and valviferans. Using a combination of molecular (SSU rDNA) and morphological

characters, Dreyer & Wägele (2001, 2002) found epicarideans to be derived from fish parasites in Cymothoidea. Their phylogeny suggested that predatory isopods (cirratanids) were basal, from which temporary ectoparasites of fishes (corallanids) were derived, leading to the evolution of permanent ectoparasites represented by the obligate parasites of fishes (cymothoids) and crustaceans (bopyrids) (Wägele, 1989; Dreyer & Wägele, 2001, 2002).

Whereas Dreyer & Wägele (2001) concluded that Epicaridea should be treated as a cymothoid family under the name Bopyridae, Brandt & Poore (2003, p. 916) indicated that elimination of epicaridean families is not necessary 'because they might be sister taxa of the Cymothoidea or another family-level taxon'. Indeed, the limited sampling of epicaridean ingroup taxa remained a major weakness of these and other analyses of Isopoda (e.g. Wetzer, 2002). For example, Dreyer & Wägele (2001) used only two bopyrid species to represent all epicarideans and Brandt & Poore (2003) used only the epicaridium larval stage of a single bopyrid species. Given the diversity of epicarideans, clearly more taxa are needed to fully evaluate the hypothesis that epicarideans evolved from a cymothoid-like ancestor. Such studies are complicated by the fact that cymothoid sequences are highly modified with many deletions and substitutions (Dreyer & Wägele, 2002), and the number of taxa sampled to date has been low for both epicarideans (5 spp.) and cymothoids (2 spp.).

Phylogenetic relationships among epicaridean families are even less clear, and no cladistic analyses have been completed on this group. Some authors have proposed hypotheses on the evolutionary relationships of bopyrid subfamilies but these were based on body plan morphologies or host phylogenies and did not consist of cladistic analyses based on homologous characters (Shiino, 1952, 1965; Markham, 1986; reviewed in Boyko & Williams, 2009). In fact, there remains confusion regarding homology of characters within epicarideans due to the marked reduction in female morphology, resulting from their parasitic mode of life. Even the characters of males, especially in Bopyroidea, are of dubious utility to derive phylogeny as they differ little from each other and most species can only be placed to subfamily by use of autapomorphic female characters. Larval characters (epicaridium and cryptoniscium) may be useful in testing phylogenetic hypotheses using morphological data, but for most taxa they are unknown or poorly described. Because of the difficulty in identifying even autapomorphic morphological characters, the validity of certain subfamilies such as Bopyrophrixinae and Argeiinae have been questioned (Adkison *et al.*, 1982; Bourdon & Boyko, 2005), and in the case of Bopyrophryxinae, invalidated based on morphological data alone (Bourdon & Boyko, 2005).

The goal of the present study was to survey epicarideans using 18S rDNA on a broader scale than has been done

previously. The objectives were to (1) test the monophyly of Epicaridea and (2) evaluate hypotheses on relationships among epicaridean families and subfamilies. Other evolutionary questions that were addressed include whether host switching occurred in the subfamilies of abdominally attached bopyrids (Athelginae, Hemiarthrinae and Phyllo-durinae) and whether these taxa were convergent in their abdominal mode of infestation on caridean, gebiidean and anomuran hosts (Markham, 1986). In addition, we examined the hypothesis that the bopyrids infesting axiidean and gebiidean shrimp represent a link between Pseudioninae and Ioninae (the 'thalassinidean transition' *sensu* Markham & Dworschak 2005). Finally, we provided a coevolutionary analysis between epicarideans and their crustacean hosts.

Materials and methods

We obtained tissue samples from at least one species in each of the subfamilies of Bopyridae (excepting Orbioninae where samples were procured but did not yield amplifiable DNA), Dajidae, Entoniscidae and one cryptoniscoid (cf. Cyproniscidae based on host choice) (Appendix 1, see online supplementary material, which is available from the article's Taylor & Francis Online page at <http://dx.doi.org/10.1080/14772000.2013.865679>). Some were preserved previously in 100% ethanol or had been formalin fixed and were later transferred to 70% ethanol for storage. When possible, an approximately 3 mm³ piece of tissue was excised using a sterile scalpel and placed into 1 mL of sterile distilled water, although in some cases eggs and/or embryos from the marsupium of a specimen were used. After 10 minutes the water was decanted and genomic DNA was extracted from tissues using a Qiagen DnEasy Tissue Kit (Valencia, CA) using the manufacturer's protocol.

Molecular analysis

The small subunit ribosomal DNA (SSU) was amplified using 'universal' SSU primers, nSSU-A and nSSU-B, modified from Medlin *et al.* (1988), 'universal' primers 18a1 and 1800 (Dreyer & Wägele, 2002) and internal SSU primers published in Dreyer & Wägele (2001). From an initial alignment of isopod SSU sequences, some modifications were made to the published primer sequences as inconsistencies across taxa were observed during an initial alignment of isopod SSU sequences at the primer binding region. Primer sequences are listed in Table 1. Annealing temperatures for primer pairs were optimized (Table 1). Reagent concentrations for all PCR reactions were the following: 1 × PCR buffer (20mM Tris-HCl (pH 8.4), 50mM KCl), 0.2 mg/mL bovine serum albumin (BSA), 2.0 mM MgCl₂, 0.2 mM dNTPs, 0.5 uM each primer and 0.25U unit *Taq* polymerase. Total reaction volume was 20 µL.

Table 1. List of primer pairs and PCR annealing temperatures used to amplify 18S rDNA. All primer sequences, indicated in parentheses, are written 5' to 3'.

| Primer pair | PCR annealing temperature °C |
|--|------------------------------|
| nSSUA (AACCTGGRTTGATCCTGCCAGT)/ 600R (GCARCTAACTTTAATATACG) | 45 |
| 700F (GTCTGGTGCCAGCMGCS)/ 1155R (CCGTCAATTCCTTTAAGTTTCAS) | 60 |
| 1000F (CGATCAGATACCGCCCTAGTTC)/ 1250R (CGCTCCACCAACTAAGAACGG) | 57 |
| 1155F (STGAACTTAAAGGAATTGACGG)/ nSSUB (GATCCTTCCGCAGGTTACCTAC) | 45 |
| 18a1 (CCTAYCTGGTTGATCCTGCCAGT)/ 1800 (TAATGATCCTTCCGCAGGT) | 53 |
| 100F (CCGCGAATGGCTCATTAATCAG)/ 700R (CGCGGCTGCTGGCACCAGCAC) | 50 |
| 400F (ACGGGTAACGGGGAATCAGGG)/ 1250R (CGCTCCACCAACTAAGAACGG) | 56 |
| 18a1 (CCTAYCTGGTTGATCCTGCCAGT)/ 600R (GCARCTAACTTTAATATACG) | 53 |
| 1155F (STGAACTTAAAGGAATTGACGG)/ 1800 (TAATGATCCTTCCGCAGGT) | 53 |
| 1155F (STGAACTTAAAGGAATTGACGG)/ reverse TCACACAGGAAACAGCTATGAC) | 56 |

(0.5 μ L of which was genomic DNA). The thermocycling parameters were an initial denaturation step at 95 °C for 4 minutes followed by 40 cycles of 95 °C for 1 minute, a primer pair specific annealing temperature for 1 minute, 68 °C for 2 minute, all followed by a final elongation step at 68 °C for 10 minutes.

Aliquots, 15 μ L, of PCR product were electrophoresed on a 2% agarose gel (w/v), stained with ethidium bromide and examined under UV light. Bands were excised from the gel using a sterile scalpel and DNA was purified from samples using a QiaQuick Gel Extraction Kit (Qiagen, Valencia, CA). Due to the length of the SSU region, four separate fragments were amplified from each sample; these were either bidirectionally sequenced or cloned using a TOPO-TA cloning kit and then bidirectionally sequenced using Applied Biosystem reagents and an ABI 3100 sequencer. Vector trimming and consensus files were created using CodonCode Aligner version 3.7.1.1. Sequences were aligned with other isopod SSU sequences found in GenBank and with the SSU sequence for *Tanais dulongii* (outgroup) for a total of 19 taxa (23 specimens) using the Clustal W algorithm and the MUSCLE algorithm in MacVector version 12.5 (default settings were used). The resulting Clustal W alignment was 3288 bp in length after sequences were trimmed to a uniform length. Resulting sequences have been deposited in GenBank (Accession Nos. KF765760–KF765774).

The Clustal W alignment was imported into M-Coffee (www.tcoffee.org), a program that aligns DNA, RNA or protein sequences based on predicted secondary structure by combining the output of multiple alignment programs. The default settings were used for the multiple alignment

methods. Multiple alignment methods included PCMA (profile consistency multiple alignment; Pei *et al.*, 2002), MAFFT (multiple alignment using fast fourier transform; Katoh *et al.*, 2002), Clustal W (Thompson *et al.*, 1994), DALIGN-TX (Subramanian *et al.*, 2008), POA (partial order alignment; Lee *et al.*, 2001), MUSCLE (multiple sequence comparison by log expectation; Edgar, 2004), PROBCONS (probabilistic consistency-based multiple alignment of amino acid sequences; Do *et al.*, 2005) and T-Coffee (Notredame *et al.*, 2000). The resulting alignment from M-Coffee was 3309 bp in length. All sequence data were retained for further phylogenetic analysis. Parsimony analysis was performed on the complete alignment in Geneious v.5.4.6 using PAUP v4.0b10 (Swofford, 2001). Non-parametric bootstrap support of clades was assessed by 100 bootstrap replications with 10 random stepwise additions, using the tree-bisection reconnection branch-swapping algorithm, and gaps were treated as informative. The tanaid *Tanais dulongii* was selected as the outgroup because tanaids and isopods, although not always considered sister taxa, are closely related peracarids (e.g. Poore, 2005; Wilson, 2009).

Maximum likelihood analysis was performed also using PAUP. Maximum likelihood was performed using 100 bootstrap replicates and the GTR+I+G (generalized time reversible model with a proportion of invariant sites and gamma-distributed rate variation across sites) as determined in Modeltest (Posada & Crandall, 1998). Bayesian inference (Rannala & Yang, 1996) was used to search for trees based on their posterior probability using MrBayes (Huelsenbeck & Ronquist, 2001). Rate variation was set to gamma, the substitution model used was GTR with

four gamma categories and the outgroup was designated as *Tanais dulongii*. For the Markov chain Monte Carlo settings, the chain length was set to 1 000 000 with four heated chains at a temperature of 0.2. The subsample frequency was set to 1000, the burn in length was set to 100 000 with a random seed of 22 512 (default).

Results of a co-phyletic analysis of crustacean hosts and isopod parasites are shown as a tangle-gram constructed using the program Jane (Conow *et al.*, 2010).

Results

From two simultaneous, independent analyses, 2002 final trees were produced using MrBayes. The standard deviation between the two runs was 0.22 with an estimated sample size of 650.249. A bootstrap consensus tree was generated from those trees (Fig. 1). Additional analyses

using Maximum likelihood (Fig. 2) and Maximum parsimony (not shown) methods produced nearly identical trees, differing only in the presence of polytomies in the cymothoid relationships (Maximum likelihood) and those of the Pseudioninae + Phylloporinae clade within Bopyridae (Maximum parsimony). Based on the Bayesian analysis, we recovered a monophyletic Epicaridea (72% posterior probability) with respect to the cymothoid taxa. Bopyroidea forms a monophyletic group (100% posterior probability) without Dajidae and Entophilinae. Instead, the latter two taxa group with Cryptoniscoidea (98% posterior probability) and this group appears as the sister taxon to Bopyroidea in our trees. Three clades were identified within Bopyroidea: (1) the basal clade of Entoniscidae, (2) *Ione* (type genus of Ioninae) and (3) all other Bopyridae except Entophilinae (100% posterior probability). Within Bopyridae there were two clades: one containing Pseudioninae, Phylloporinae, Bopyrinae and *Allokepon* (a taxon currently placed in Ioninae but not

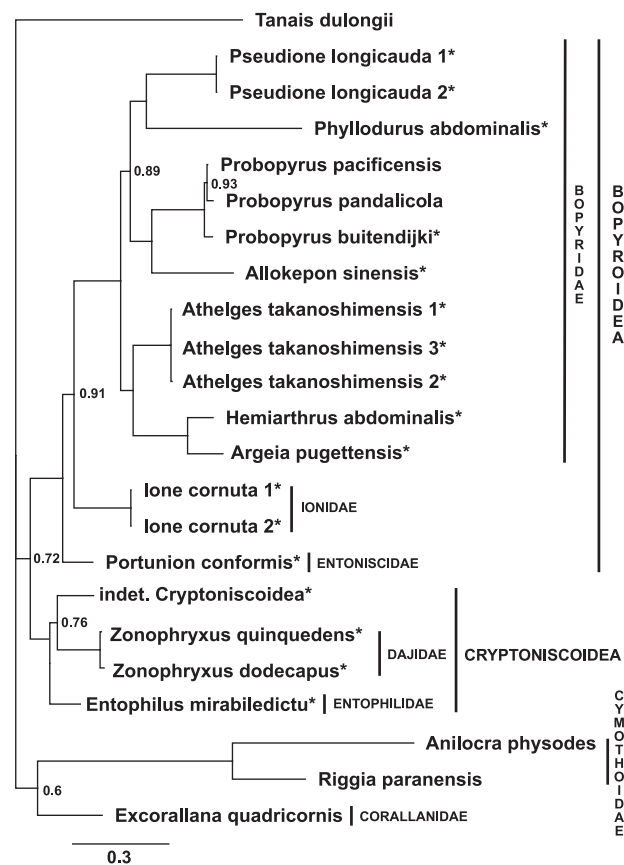


Fig. 1. Bayesian best tree of evolutionary relationships of 18 ingroup isopod taxa (representing Bopyridae, Dajidae, Entoniscidae and Cryptoniscoidea as well as three species of Cymothoidea: 2 Cymothoidae and 1 Corallanidae) inferred from an analysis of 18S rDNA data. Posterior probabilities are indicated near nodes. All branches without posterior probabilities indicated were supported by values >95%. * = new sequences added from this study; all other sequences from GenBank (Appendix 1, see supplementary material online).

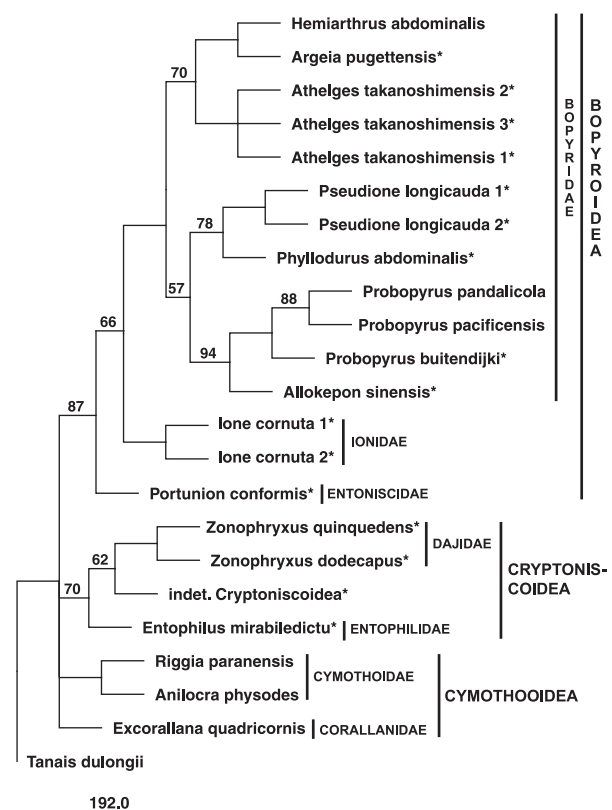


Fig. 2. Maximum likelihood best tree of evolutionary relationships of 18 ingroup isopod taxa (representing Bopyridae, Dajidae, Entoniscidae and Cryptoniscoidea as well as three species of Cymothoidea: 2 Cymothoidae and 1 Corallanidae) inferred from an analysis of 18S rDNA data. Support values are indicated near nodes. All branches without values indicated = 100% support. * = new sequences added from this study; all other sequences from GenBank (Appendix 1, see supplementary material online).

grouping with the type genus *Ione*) (89% posterior probability) and the other containing three subfamilies (Argeiinae, Athelginae and Hemiarthrinae). Within the group containing *Allokepon* there were two clades, one with the abdominal Phyllozurinae and branchial Pseudioninae (98% posterior probability) and another with the branchial Bopyrinae and *Allokepon* (100% posterior probability). Within the other major bopyrid clade were two subclades: one with the abdominal Athelginae and the other with the abdominal Hemiarthrinae and branchial Argeiinae (100% posterior probability). Within Cryptoniscoidea, Entophilinae was sister to the ostracod-parasitizing Cryptoniscoidea and Dajidae (98% and 76% posterior probabilities, respectively).

The Cymothooidea were monophyletic, but with only 60% posterior probability in this analysis, and were not within the monophyletic Epicaridea.

Discussion

The phylogenetic trees based on 18s rDNA analysis (Figs 1, 2) show very high support values for most branches. The aim of our study was focused on the relationships among the epicaridean taxa (i.e. those isopods that parasitize other crustaceans and have a complex life cycle with three or four larval stages and indirect development). Therefore, it is possible that our choice of gene, or gene region, did not reflect the overall relationships of parasitic isopods in general. With that caveat, we diligently worked with the sequences from all epicaridean specimens and within GenBank. Indeed, only tissue DNA from a single target species did not amplify for sequence analysis, that from a species of Orbioninae. Thus, because of our selectivity in using non-epicaridean datasets (two Cymothoidae and one Corallanidae), our analysis produced remarkably consistent patterns of relationships among all the included taxa.

Although the primary goal of this study focused on epicaridean phylogeny, we note that the epicarideans appear distinct from a monophyletic Cymothooidea (Corallanidae + Cymothoidae), where Corallanidae and Cymothoidae are more closely related to each other than either is to epicarideans. This contrasts with the results of Dreyer & Wägele (2001) where Cymothoidae and Epicaridea are sister taxa, but the presence of multiple polytomies in the earlier work and the limited sampling of Cymothooidea in the present one suggest that the position of epicarideans with respect to cymothooideans is not well resolved.

For epicaridean taxa, the data indicate that the current concept of Bopyroidea is not monophyletic, and that Dajidae and Entophilinae must be moved to Cryptoniscoidea in order for Bopyroidea to be monophyletic. Within the redefined Bopyroidea, there are still three families but they are now Bopyridae, Entoniscidae and Ionidae (new

status). Because *Ione* does not group with *Allokepon* and, by inference, all other 'ionine-like' genera which are similar to *Allokepon*, we conclude that all but *Ione* form a monophyletic group, which we have named Keponinae n. subfam. (see taxonomic section below). The position of Entoniscidae is in agreement with the conclusions of Adkison (1990) who noted that entoniscids were very different from both bopyrids and dajids and he even suggested that this 'may warrant separation at the superfamily level'. The method of brood pouch (marsupium) formation is very different in the three families and, indeed, even within Entoniscidae there are two methods to brood pouch formation (see Adkison, 1990).

Within Bopyridae *sensu stricto*, there are two major clades: (1) Pseudioninae (branchial) + Phyllozurinae (abdominal) and Bopyrinae (branchial) + *Allokepon* (branchial) and (2) Athelginae (abdominal) and Hemiarthrinae (abdominal) + Argeiinae (branchial). Based on the sister group relationship between Ionidae (branchial) and Bopyridae, as well as the presence of branchial parasites in both bopyrid subclades, we conclude that branchial parasitism is the ancestral state and abdominal parasitism evolved two times, with the branchial parasitism of Argeiinae being a reversal. Alternatively, Phyllozurinae and Hemiarthrinae could each have evolved abdominal parasitism independently (this does not consider the aberrant keponine *Rhopalione*, an abdominal parasite in an otherwise all-branchial parasitic subfamily). The evolution of branchial to abdominal parasitism correlates with developmental data showing that the cryptoniscids of abdominal parasitizing species in both Athelginae and Hemiarthrinae first lodge in the branchial chamber and subsequently move back to the abdomen (Pike, 1961).

Our analysis supports a monophyletic Cryptoniscoidea composed of the seven or eight families traditionally placed there, plus Dajidae and Entophilinae (now as Entophilidae) formerly in Bopyroidea. However, our analysis had only a single example of a 'traditional' cryptoniscoid, and that sample came from a parasitized ostracod which is now lost. The morphology of neotenous males in traditional cryptoniscoid families strongly suggests that these taxa form a monophyletic unit, but additional samples from each of the families is needed to test this hypothesis. At present, we can only state that the data support a relationship of Entophilidae as sister taxon to Dajidae + putative cyproniscid.

Our results can lead to new analyses of host and parasite phylogenies and hypotheses on their coevolutionary history. Unfortunately, coevolutionary analyses are limited by the fact that there is little consensus on the phylogeny of the hosts, especially within Malacostraca (Jenner *et al.*, 2009; Koenemann *et al.*, 2010). Because of this, we used several current molecular phylogenies on crustacean hosts (Jenner *et al.*, 2009; Koenemann *et al.*, 2010; Regier *et al.*, 2010; Lin *et al.*, 2012; Shen *et al.*, 2012), and

derived a tangle-gram to show host associations with parasitic isopods (Fig. 3). Extensive lack of agreement (incongruence) is found between hosts and parasites, regardless of the host phylogeny used, indicating that the parasites have made many host switches and there has been loss of parasites within host lineages. Similar levels of incongruence have been found in other groups of parasites such as monogeneans associated with teleost hosts (Desdevises *et al.*, 2002) and eucestodes associated with elasmobranch hosts (Caira & Jensen, 2001). Reviews of co-phyletic analyses in marine groups show a range of incongruence (e.g. host switching, extinction and duplication) and congruence (cospeciation) within their evolutionary histories (Hoberg & Klassen, 2002; Lanterbecq *et al.*, 2010). Some of the incongruence within our analysis reflects the fact that we examined the relationships at higher taxonomic levels (subfamily or higher); there could be more evidence for co-speciation within families restricted to certain hosts (e.g. athelgines as exclusive parasites of paguroids).

Aside from the degree of incongruence, there are other findings to report, including the fact that Cryptoniscoidea is the only group to parasitize basal crustacean hosts (Fig. 3). Species in one grouping of three genera (*Danalia*, *Faba* and *Zeuxokoma*) parasitize decapod hosts, although they may have derived this relationship through initial parasitism of rhizocephalan barnacles on decapods (Boyko, unpubl. data). More extensive sampling of cryptoniscoids is needed to determine whether some of the families within this group are restricted to specific host groups, as is evidenced by current knowledge of their host associations (Williams & Boyko, 2012). Branchial parasitism is likely the ancestral condition for bopyrids

(possibly a pseudionine-type morphology) and abdominal parasitism has evolved at least two times (being found on anomuran, brachyuran, caridean and gebiidean hosts) (Fig. 4). Outside of the traditional Cryptoniscoidea, endoparasitism is found in only two groups, Entophilidae and Entoniscidae, both of which are restricted to decapod hosts (see Williams & Boyko, 2012 for review of their diversity), an interesting result in that the two taxa are not closely related. The most parsimonious conclusion based on the molecular data is that the ancestral condition for parasitic isopods of crustacean hosts is endoparasitic (Fig. 4) but the highly derived and reduced morphologies of the endoparasites appear to contradict this. Addition of more cryptoniscoid taxa could help to clarify the position of endoparasitic taxa within the Epicaridea.

Some host groups such as Achelata, Astacida and Stenopodidea are only parasitized by one bopyrid subfamily each and the parasites are very restricted within these groups (e.g. within Astacida only homarids are documented to be parasitized). In contrast, caridean shrimp are parasitized by six groups (Dajidae, Entoniscidae, Argeiinae, Bopyrinae, Hemiarthrinae and Pseudioninae) that span a wide range of epicarideans. The most derived host groups (anomurans and brachyurans) are also parasitized by a range of parasites from two bopyrid subfamilies each (Fig. 3). As indicated by Boyko & Williams (2009), there may be some eco-physiological similarities of hosts that have facilitated the switch of the parasites between these host groups. Intermediate host associations could also play an important role in shaping coevolutionary patterns, but very limited data exist on use of copepod intermediate

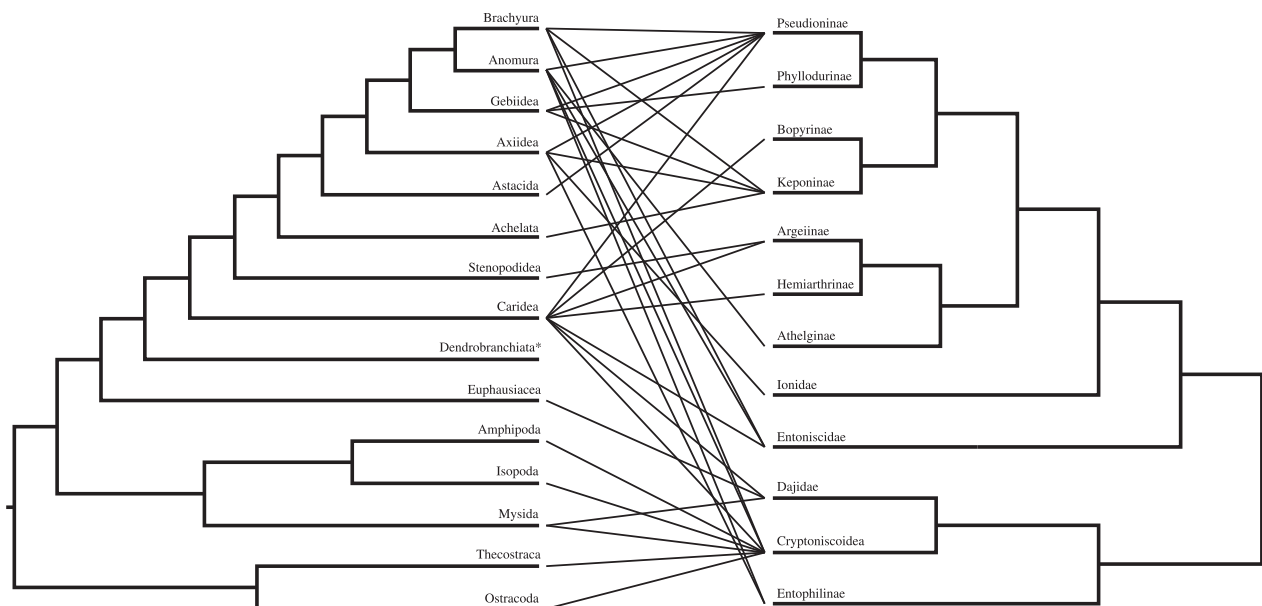


Fig. 3. Co-phyletic analysis of crustacean hosts and their isopod parasites. *Dendrobranchiata are parasitized by the bopyrid subfamily Orbioninae (not included in this analysis).

hosts (see Cribb *et al.* 2001, for one of the few reviews that consider coevolution of parasites with their intermediate and definitive hosts).

Finally, our phylogeny does not support the ‘thalassinidean transition’ as proposed by Markham (1986) that suggests bopyrids infesting callinassid and upogebiid shrimp represent a link between Pseudioninae and Ioninae *sensu lato*. Rather, Pseudioninae (branchial parasites) are sister to Phyllozurinae (abdominal parasites) with Keponinae n. subfam. (branchial parasites) as sister to Bopyrinae (branchial parasites). Ionidae *sensu stricto* (branchial parasites) are sister to Bopyridae *sensu stricto* (Fig. 3). Our phylogeny also does not support Markham’s (1986) hypothesis that Phyllozurinae gave rise to Athelginae (both abdominal parasitic groups), as these two taxa are in different subclades of Bopyridae (Fig. 4).

Further work is still needed to clarify the relationships within Cryptoniscoidea, especially with those families having neotenous males. Additionally, the placement of Orbioninae is unclear, as we were unable to obtain sequences, although from a morphological perspective this subfamily is closer to the Pseudioninae/Phyllozurinae/Bopyrinae/Keponinae clade than the Athelginae/Hemiarthrinae/Argeiinae clade. Molecular data for Orbioninae would allow testing of Markham’s (1986 hotlink?) hypothesis that the group is of relatively recent origin. Addition of further taxa from families with high morphological diversity suggesting multiple lineages (e.g. Entoniscidae and Dajidae) is highly desirable.

A summary of the current taxonomy of Bopyroidea and Cryptoniscoidea is presented next and includes modifications that are warranted based on our analysis and with consideration that one subfamily of Bopyridae (Orbioninae) and most Cryptoniscoidea are not represented in the present analysis. Higher level taxa within the parasitic and highly derived Epicaridea have traditionally been defined on autapomorphies, usually of females; however, researchers have been unable to provide convincing morphological synapomorphies for taxa above the family level (e.g. Markham, 1986). Based on study of the larval stages within Cryptoniscoidea, we believe that the best source of morphological synapomorphies at the family level and above is within these larval stages, which are currently unknown, or poorly described, for most epicaridean taxa.

Taxonomy

The following is a taxonomic summary of Bopyroidea and Cryptoniscoidea taxa based on the results of the present study, with a key to families and subfamilies of Bopyroidea.

BOPYROIDEA Rafinesque, 1815

Bopyridae Rafinesque, 1815

Argeiinae Markham, 1977

Athelginae Codreanu & Codreanu, 1956

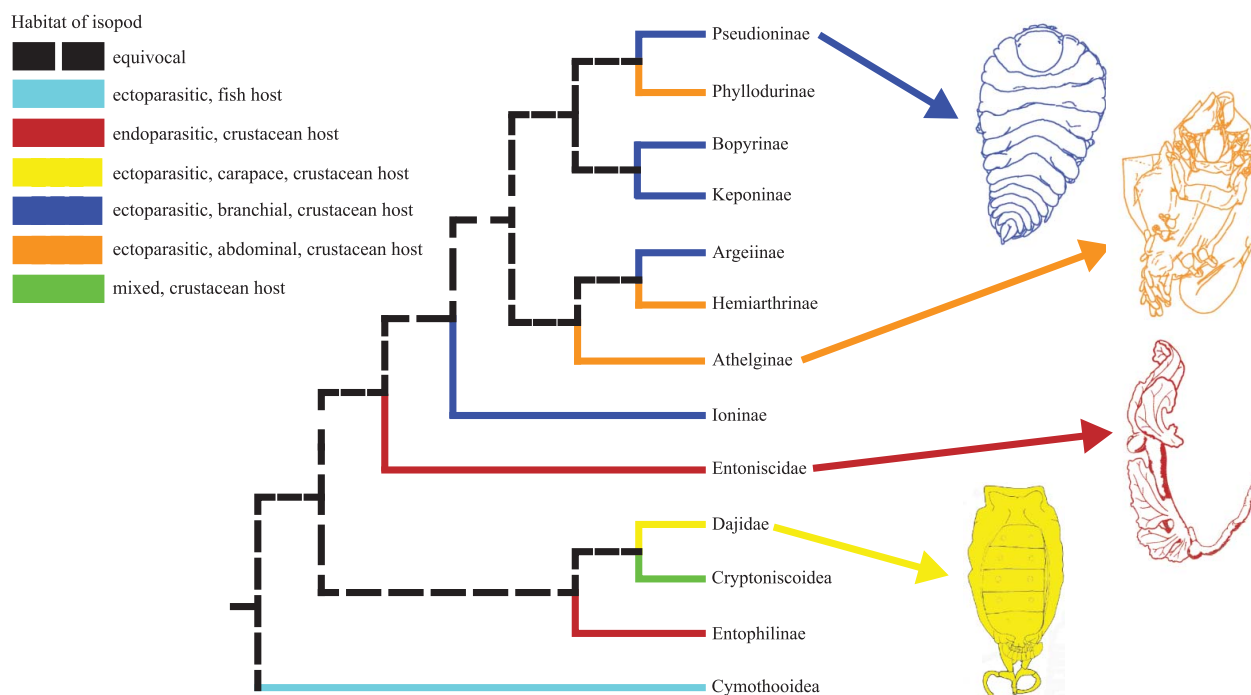


Fig. 4. The habitat of all family-level and higher isopod taxa mapped onto the phylogeny derived from the 18S rDNA analysis. Mixed = examples of ecto-, endo- and mesoparasitism in the taxon.

Bopyrinae Rafinesque, 1815

Hemiarthrinae Markham, 1972

Keponinae subfamily nov.

(type genus: *Kepon* Duvernoy, 1840)

Diagnosis: Females with 7 pereomeres and 5 pleomeres plus pleotelson; frontal lamina weakly to moderately developed, larger lamina recurved and surrounding cephalon; marsupium completely closed; mediodorsal tapered projections sometimes present on posterior pereomeres; 5 pairs of lateral plates with weakly tuberculate to filamentous marginal projections; 5 pairs typically uniramous pleopods; uniramous lamellar uropods. Males with 7 pereomeres and 5 pleomeres plus pleotelson; midventral tubercles often present; 5 pairs uniramous pleopods often present; pleotelson posteriorly bifurcated with posterolateral corners produced into weakly to very elongate extensions (often interpreted as uropods).

Included genera: *Allokepon* Markham, 1982 (5 spp.), *Anacepon* Nierstrasz & Brender à Brandis, 1931 (1 sp.), *Apocepon* Nierstrasz & Brender à Brandis, 1930 (3 spp.), *Atypocepon* Nierstrasz & Brender à Brandis, 1931 (1 sp.), *Cancricepon* Giard & Bonnier, 1887 (7 spp., see Remarks), *Carcinione* Bourdon, 1983 (1 sp.), *Cardiocepon* Nobili, 1906 (1 sp.), *Castrione* Brail Lima, 1980 (2 spp.), *Coxalione* Bourdon, 1977 (1 sp.), *Dactyokepon* Stebbing, 1910 (11 spp.), *Epicepon* Nierstrasz & Brender à Brandis, 1931 (2 spp.), *Ergyne* Risso, 1816 (1 sp.), *Grapsicepon* Giard & Bonnier, 1887 (6 spp.), *Hemicepon* Lemos de Castro & Brasil Lima, 1980 (1 sp.), *Heterocepon* Shiino, 1936 (1 sp.), *Hypercepon* Danforth, 1972 (1 sp.), *Hypocepon* Nierstrasz & Brender à Brandis, 1930 (2 spp.), *Kepon* Duvernoy, 1840 (3 spp.), *Leidya* Cornalia & Panceri, 1861 (4 spp.), *Lobocepon* Nobili, 1905 (1 sp.), *Megacepon* George, 1947 (6 spp.), *Mesocepon* Shiino, 1951 (1 sp.), *Metacepon* Nierstrasz & Brender à Brandis, 1931 (2 spp.), *Metathelges* Nierstrasz & Brender à Brandis, 1923 (1 sp.), *Onkocepon* An, Yu & Li, 2006 (2 spp.), *Onychocepon* Pérez, 1921 (3 spp.), *Paracepon* Nierstrasz & Brender à Brandis, 1931 (2 spp.), *Procepon* Shiino, 1937 (3 spp.), *Rhopalione* Pérez, 1920 (4 spp.), *Scyracepon* Tattersall, 1905 (6 spp.), *Trapezicepon* Bonnier, 1900 (3 spp.), *Tylokepon* Stebbing, 1904 (3 spp.).

Remarks: The distinction between *Ione* and other genera in Ioninae based on morphological characters (see below) has been recognized by the authors for some time. Duvernoy & Lereboullet (1841) were the first to recognize *Kepon* as the type of a taxon distinct from *Ione* at the family level. However, they used the vernacular term Képoniens, which was never subsequently Latinized or even mentioned by other authors. Therefore, the family-level name Keponinae dates from the present paper.

Females of species in Keponinae all possess pleomeres with extended, digitate or tuberculate lateral plates and pleopods, usually directed anterolaterally from pleon. The closely related Bopyrinae have females with flap-like pleopods and short (if present) non-pedunculate non-tuberculate lateral plates.

In addition to *Ione*, only four other genera of Ioninae occur on gebiid and/or axiid hosts and show morphological similarities to *Ione*: *Castrione* Brasil Lima, 1980 (2 spp.), *Coxalione* Bourdon, 1977 (1 sp.), *Hemicepon* Lemos de Castro & Brasil Lima, 1980 (1 sp.), and *Procepon* Shiino, 1937 (3 spp.). The males of *Coxalione* and *Hemicepon* lack filamentous pleopods and/or lateral plates, while the females of all four genera lack the abundant filamentous rami that extend from the lateral plates as seen in species of *Ione*. None of these genera are morphologically close enough to *Ione* to justify their inclusion in the narrowly defined Ionidae and all are tentatively included in the newly erected Keponinae pending further study; their characters are not included in the diagnosis of the subfamily. Males of *Rhopalione* Pérez, 1920 are very similar to other males in Keponinae but the females, while similar, present some differences in pleopod structure that make the inclusion of this genus in Keponinae questionable. Additionally, all Keponinae species are branchial parasites, except *Rhopalione* that are abdominal on pinnotherids.

Markham (1982) excluded *Ergyne savignyi* Stebbing, 1910 from *Ergyne* Risso, 1816, but did not place it in another genus. It appears very close to *Cancricepon xanthi* (Richardson, 1910), perhaps even identical with that species. We herein formally place it in *Cancricepon* as *C. savignyi* (Stebbing, 1910) **new combination**, giving *Cancricepon* seven total species.

Orbioninae Codreanu, 1967

Phyllodurinae Markham, 1977

Pseudioninae Codreanu, 1967

Entoniscidae Kossmann, 1881

Ionidae H. Milne Edwards, 1840 new status

(type genus: *Ione* Latreille, 1818)

Diagnosis: Females with 7 pereomeres, 5 pleomers plus pleotelson, wide frontal lamina extending laterally beyond margins of cephalon; coxal plates typically enlarged; typically 5 pairs of biramous pleopods; 6 pairs elongate lateral plates with numerous filamentous projections. Marsupium completely closed. Uropod uniramous, elongate and tubular. Male with 7 distinct pereomeres and 5 typically fused pleomeres, midventral tubercles lacking; pleopods lacking; 5 pairs of elongate simple lanceolate lateral plates and uniramous elongate uropods resembling lateral plates.

Included genera: *Ione* Latreille, 1818 (8 spp.).

Remarks: We consider species in this genus to occur only on hosts in the Axiidea, and not in Gebiidea and we agree with Bourdon (1968, p. 82) that the record of *Ione thoracica* on *Upogebia stellata* (Montagu, 1808) reported by Fraisse (1878; the sole record from any species of gebiidean) is dubious.

CRYPTONISCOIDEA

Remarks: The relationships among the cryptoniscoid families are poorly understood and most of the families have been based as much, or more, on host choice as on morphological characters. All of the families are in need of extensive study of all stages of their life cycles. There are putative cryptoniscoids reported from nebaliceans, cumaceans and ascothoracicans, but none of these have been placed to family (e.g. Grygier, 1981).

- Asconiscidae Bonnier, 1900
- Cabiropidae Giard & Bonnier, 1887
- Crinoniscidae Bonnier, 1900
- Cryptoniscidae Kossmann, 1880
- Cryptothiridae Sars, 1882 (*nomen dubium*)
- Cyproniscidae Giard & Bonnier, 1887
- Dajidae Giard & Bonnier, 1887
- Entophilidae Richardson, 1903 new status
- Hemioniscidae Bonnier, 1900
- Podasconidae Giard & Bonnier, 1895

INCERTAE SEDIS

- Colypuridae Richardson, 1905
- Rhabdochiridae Richardson, 1905

Remarks: Both of these families were erected on the basis of a single genus and species that appear to be either a male bopyroid (Colypuridae) or a larval type (Rhabdochiridae). Until additional material can be obtained, neither can be placed easily within the Isopoda.

Key to families and subfamilies of Bopyroidea

Key to families and subfamilies of Bopyroidea based on female characters (modified from Markham, 1985).

- 1a. Vermiform, pereopods and antennae lacking (endoparasitic). **Entoniscidae**
- 1b. Not vermiform; modified isopod body with pereopods and antennae present (ectoparasitic). 2
- 2a. Uropods with lateral plates; numerous filamentous rami extending from lateral plates. **Ionidae** (branchial)
- 2b. Uropods without lateral plates. 3
- 3a. Pleomeres with extended, digitate or tuberculate lateral

- plates, usually directed anterolaterally from pleon.
- **Keponinae** subfam. nov. (branchial)
- 3b. Pleomeres with short, simple, non-digitate or tuberculate lateral plates, usually directed laterally, or lateral plates lacking. 4
- 4a. Marsupium not much enlarged beyond margins of pereon, composed of five pairs of loosely fitting subequal oostegites; pleopods, when present, not pedunculate. 5
- 4b. Marsupium enlarged beyond at least one side of pereon, formed of close-fitting oostegites, usually fewer than five pairs of oostegites, unequal in size; pleopods and lateral plates, when present, pedunculate. 8
- 5a. Coxal plates and frontal lamina of cephalon greatly enlarged, giving anterior portion of body a semicircular aspect. **Orbioninae** (branchial)
- 5b. Coxal plates and frontal lamina not greatly enlarged, body more linear in appearance. 6
- 6a. Marsupium completely closed by oostegites. **Pseudioninae** (branchial)
- 6b. Marsupium with variably sized median exposed space between oostegites. 7
- 7a. Head oval or fusiform, never fused with pereon; lateral plates pedunculate; pleopods knob-like, uniramous. **Argeiinae** (branchial)
- 7b. Head subrectangular or subtriangular, often fully or partially fused with pereon; lateral plates, if present, not pedunculate; pleopods flap-like, usually biramous. **Bopyrinae** (branchial)
- 8a. Body symmetrical to slightly asymmetrical; brood pouch symmetrical to slightly asymmetrical (if asymmetrical then expanded on one side at posterior margin), formed by oostegites from both sides of body. 9
- 8b. Body highly asymmetrical; brood pouch greatly expanded and formed by oostegites from one side of body. **Hemiarthrinae** (abdominal)
- 9a. Body symmetrical, lateral margins of cephalon not overlapped by forward curved lateral portions of posterior pereomeres; lateral plates and pleopods falcate. **Phyllodurinae** (abdominal)
- 9b. Body asymmetrical, lateral margins of cephalon overlapped by forward curved lateral portions of posterior pereomeres; lateral plates and pleopods variably foliose (thin elongate to very broad). **Athelginae** (abdominal)

Acknowledgements

Thanks to Leopoldo Moro Abad (Canary Islands), Annette Brockerhoff (University of Canterbury, New Zealand), Joe Choong (National Fish Health Research Centre, Malaysia), Peter Dworschak (Naturhistorisches Museum, Wien), Darryl Felder (University of Louisiana, Lafayette), Todd Oakley (University of California-Santa Barbara), Gyo Itani (Kochi University) and Patricia Sadeghian (Santa Barbara Museum of Natural History) for specimen

donations and loans. Jan McDowall (VIMS) provided assistance with genetic analyses. Two anonymous reviewers provided many helpful comments and suggestions that improved the manuscript. This paper is Contribution No. 3319 of the Virginia Institute of Marine Science, College of William & Mary.

Supplementary material

Supplemental data for this article can be accessed here.

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Associate Editor: Polly Hayes