

PHYLOGENETIC ANALYSIS AND SYSTEMATIC REVISION OF REMIPEDIA (NECTIOPODA) FROM BAYESIAN ANALYSIS OF MOLECULAR DATA

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ABSTRACT

We performed a phylogenetic analysis of the crustacean class Remipedia. For this purpose, we generated sequences of three different molecular markers, 16S rRNA (16S), histone 3 (H3), and cytochrome c oxidase subunit I (COI). The analyses included sequences from 20 of the 27 recent species of Remipedia, plus four still-undescribed species. The data matrix was complemented with sequences from online databases (The European Molecular Biology Laboratory and GenBank®). *Campodea tillyardi* (Diplura), *Hutchinsoniella macracantha* (Cephalocarida), *Penaeus monodon* (Malacostraca) and *Branchinella occidentalis* (Branchiopoda) served as out-groups. In addition to the classic computer-based alignment methods used for protein-coding markers (H3 and COI), an alternative approach combining structural alignment and manual optimization was used for 16S. The results of our analyses uncovered several inconsistencies with the current taxonomic classification of Remipedia. Godzilliidae and the genera *Speleonectes* and *Lasionectes* are polyphyletic, while Speleonectidae emerges as a paraphyletic group. We discuss current taxonomic diagnoses based on morphologic characters, and suggest a taxonomic revision that accords with the topologies of the phylogenetic analyses. Three new families (Kumongidae, Pleomothridae, and Cryptocorynetidae) as well as three new genera (*Kumonga*, *Angirasu*, and *Xibalbanus*) are erected. The family Morlockiidae and the genus *Morlockia* are removed from synonymy and returned to separate status.

KEY WORDS: Bayesian phylogeny, molecular taxonomy, structural alignment

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INTRODUCTION

The comparatively small crustacean class Remipedia Yager, 1981 currently encompasses 27 described species. Nearly all the extant representatives of this taxon inhabit subterranean sections of anchialine caves in coastal regions. The only known species found in a fully marine, sub-seafloor cave is *Speleonectes kakuki* Daenekas et al., 2009. Another exception to the general ecological profile is *Speleonectes epilimnius* Yager and Carpenter, 1999, collected from the highly oxygenated surface water of an anchialine cave on San Salvador (Carpenter, 1999).

Remipedes live generally in salinity ranges from 18 to 35 ppt and temperature from 22 to 26°C. These crustaceans are thought to be scavengers and top predators in the ecosystems they inhabit, but evidence exists that they probably feed on detritus as well (Koenemann et al., 2007d). Although our knowledge of remipedes has increased greatly in the last dozen years, there are still large gaps in our understanding of their ecology, ethology, and evolutionary history.

The distinctive body plan of the class, composed of a head with six fused, appendage-bearing somites and an undivided, homonomously segmented trunk, cause remipedes to stand out among the disparity of crustacean shapes and forms. Because of the homonomous trunk, remipedes initially were regarded as “primitive” crustaceans (Fig. 1).

Early phylogenetic analyses based on morphological data sets supported a presumed basal position with different assemblages of crustaceans (Schram and Hof, 1997; Wills, 1997; Lange and Schram, 1999). However, the results of more recent studies of cerebral structures, DNA sequences, and larval embryology suggest that Remipedia represents a derived, rather than a primitive group (Fanenbruck et al., 2004; Harzsch, 2004; Koenemann et al., 2007b, 2009b; Ertas et al., 2009; Stemme et al., 2012). These hypotheses have variously placed remipedes as a sister group to:

- all other crustaceans (Schram, 1986; Wheeler et al., 2004);

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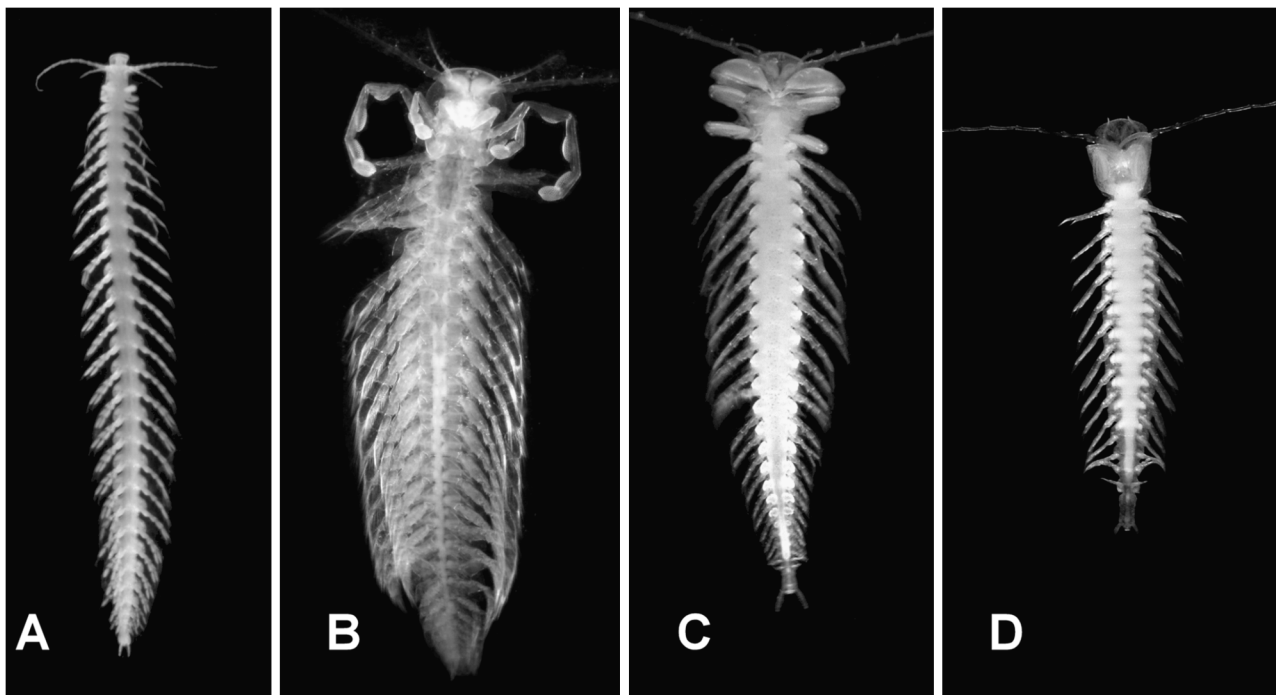


Fig. 1. Photographs of four species of Remipedia (not shown at same scale). A, *Speleonectes tanumekes* Koenemann et al., 2003 (dorsal view; photo by J. L. van der Ham); B, living specimen of *Cryptocorynetes elmorei* Hazerli et al., 2009 (ventral view; body length 11 mm; modified from Hazerli et al., 2009); C, living specimen of *Pleomothra fragilis* Koenemann et al., 2008 (body length not known; modified from Koenemann et al., 2008). D, *Godzillionomus frondosus* Yager, 1989 (body length approximately 8 mm). A: dorsal view, B-D: ventral views.

- cephalocarids (Spears and Abele, 1997; Giribet et al., 2001; Regier et al., 2005, 2010; Koenemann et al., 2010);
- cirripedes (Lavrov et al., 2004; Carapelli et al., 2007);
- malacostracans (Fanenbruck et al., 2004; Harzsch, 2004; Koenemann et al., 2010; Stemme et al., 2012).

In addition, several analyses also place remipedes, in some cases together with cephalocarids, in various relationships to hexapods, including collembolans (Cook et al., 2005), diplurans (Carapelli et al., 2007; Koenemann et al., 2010), and all Hexapoda (Regier et al., 2010; Stemme et al., 2012; von Reumont et al., 2012) (for a detailed overview see Jenner, 2010).

At present, Remipedia includes two orders. The extinct Enantiopoda Birshtein, 1960 contains the fossil species *Tesnusocaris goldichi* Brooks, 1955 and *Cryptocaris hootchi* Schram, 1974 (see Emerson and Schram, 1991; Neiber et al., 2012), while Nectiopoda Schram, 1986 encompasses all extant species divided up until now amongst three families: Speleonectidae Yager, 1981, Godzilliidae Schram et al., 1986, and Micropacteridae Koenemann et al., 2007a.

Extant remipedes exhibit a disjunct geographic distribution (Fig. 2). The highest abundance and diversity is in the greater Caribbean region including the Bahamas, the Yucatán Peninsula, Cuba, the Dominican Republic, and the Turks and Caicos Islands. As the center of biodiversity, the Bahamas and the Turks and Caicos Islands have two endemic families according to the currently accepted systematics of the group: (1) Godzilliidae, composed of three genera and six species (*Godzillius robustus* Schram et al., 1986; *Godzillius fuchsi* Gonzalez et al., 2013; *Godzillionomus*

frondosus Yager, 1989; *Godzillionomus schrami* Iliffe et al., 2010; *Pleomothra apletocheles* Yager, 1989; and *Pleomothra fragilis* Koenemann et al., 2008); and (2) the monotypic Micropacteridae with *Micropacter yagerae* Koenemann et al., 2007a from the Turks and Caicos.

Within Speleonectidae, *Cryptocorynetes* Yager, 1987a (*C. haptodiscus* Yager, 1987a; *C. longulus* Wollermann et al., 2007; and *C. elmorei* Hazerli et al., 2009) is known from the Bahamas Islands, while the monotypic genus *Kaloketos* Koenemann et al., 2004 occurs in the Turks and Caicos Islands. Of the remaining speleonectid genera, *Speleonectes* Yager, 1981 (as currently accepted) has an ampho-Atlantic distribution, with many species from the Caribbean area and two species known from Lanzarote (Canary Islands) in the eastern Atlantic; *Lasionectes* Yager and Schram, 1986 shows a greater disjunction, with *Lasionectes entrichoma* Yager and Schram, 1986 in the Bahamas and *Lasionectes exleyi* Yager and Humphreys, 1996 from Bundera Sinkhole in Western Australia.

We have yet to achieve a consensus on classification and phylogenetic relationships within Remipedia. Koenemann et al. (2007c) presented a morphological cladistic analysis of 17 extant species of Nectiopoda, in which different methodological approaches were applied. It supported monophyly of Godzilliidae, confirmed the familial status of Micropacteridae, and revealed Speleonectidae as paraphyletic. In the first comprehensive molecular analysis, based on COI from some 22 remipede taxa, Godzilliidae and Speleonectidae emerged as paraphyletic assemblages, but no molecular data were available at that time from Micropacteridae (Neiber et al., 2011).

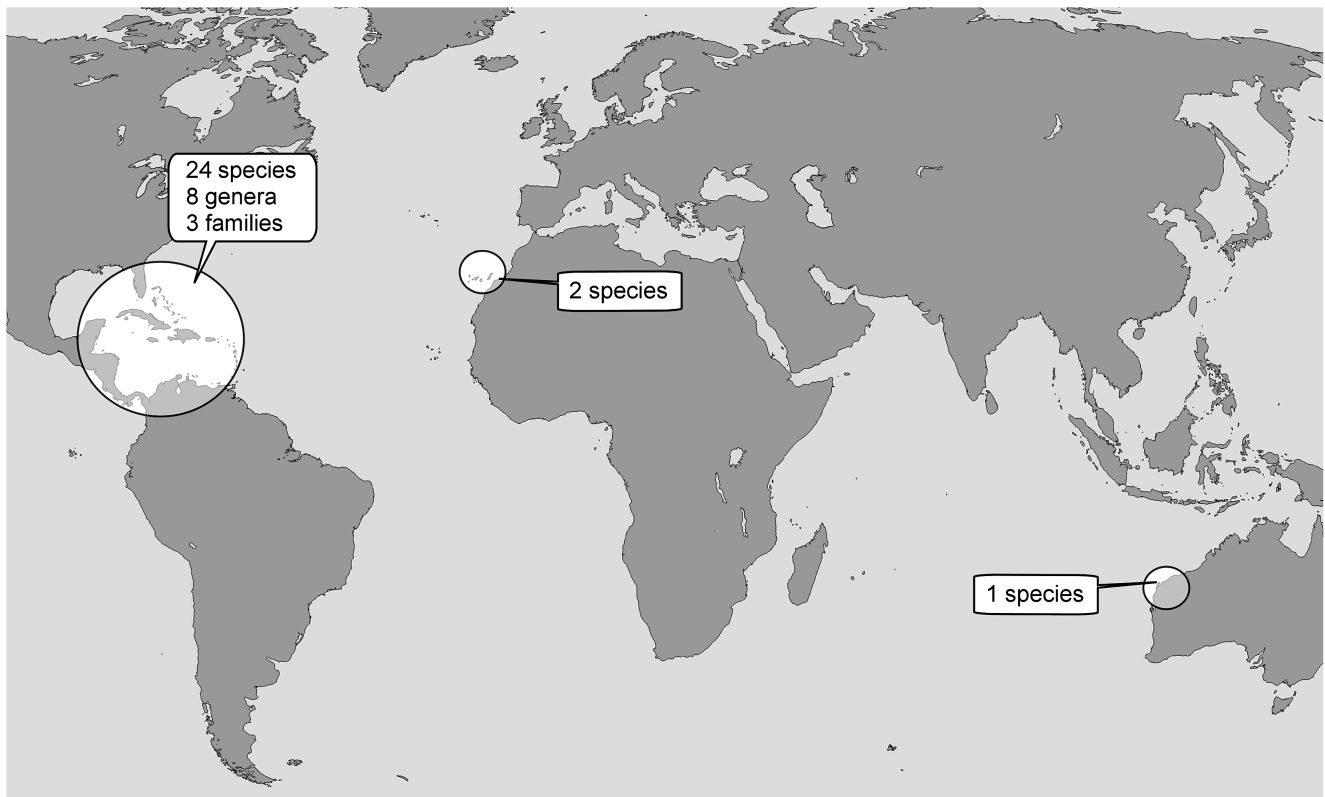


Fig. 2. Global distribution of extant Remipedia, including a total of 27 described species, 8 genera and 3 families (according to taxonomy prior to our revision).

In this study, we examine the phylogenetic relationships among Remipedia using molecular sequence data from COI, H3 and 16S.

MATERIAL AND METHODS

Taxon Sampling and Preservation

All animals were preserved in 70% to 96% ethanol immediately after collection; for DNA extraction, samples were stored at -20°C . See Table S1 in the online Supplementary material for a detailed list of sampled specimens.

Out-Group Selection

We selected four out-group taxa as representatives of crustacean and hexapod lineages to effectively polarize the data from Remipedia; these taxa include *Penaeus monodon* Fabricius, 1798 (Malacostraca), *Branchinella occidentalis* Dakin, 1914 (Branchiopoda), *Hutchinsoniella macracantha* Sanders, 1955 (Cephalocarida) and *Campodea tillyardi* Silvestri, 1931 (Hexapoda) (see Table S4 in the online Supplementary material).

Newly Generated Sequence Data

Total genomic DNA was extracted either from trunk segments or trunk limbs of our remipedes using the DNeasy[®] Blood & Tissue Kit (Qiagen, Hilden, Germany). To ensure success of the DNA extraction, purified isolates were run on 0.8% agarose gels, and stained with ethidium bromide. A fragment of 709 base pairs (bp) or 1096 bp including primers of the mitochondrial marker COI was amplified by polymerase chain reaction (PCR) with the universal primers LCO-1490 and HCO-2198 (Folmer et al., 1994) or the primers COI as and COI s, respectively (see Table S3 in the online Supplementary material). Using the universal primer pair 16Sar and 16Sbr (Palumbi, 1996) or the crustacean specific primer pair CRUST16SF and CRUST16SR (Ivey and Santos, 2007), fragments of 581 bp or 1047 bp including primers of the mitochondrial 16S rRNA gene were amplified. Partial sequences of the nuclear H3 gene (374 bp including primers) were

generated with the primer pair H3 AF and H3 AR (Folmer et al., 1994, Table S3 in the online Supplementary material).

PCRs were performed with 1 μl of each DNA sample in a 25 μl volume: 1 μl of 10 mM dNTP (deoxyribonucleoside triphosphate), 2 μl of 50 mM MgCl_2 , 2.5 μl of 10 \times NH_4 -Reaction-Buffer (Bioline, Luckenwalde, Germany), 1 μl of each primer (10 pM), 16.3 μl H_2O and 0.2 μl (=1 unit) of BIOTAQ[™] DNA polymerase (Bioline) in a Mastercycler[®] ep gradient thermal cycler (Eppendorf, Wesseling-Berzdorf, Germany). The reactions were initiated by a denaturation step of 2 minutes at 93°C , followed by 40 cycles of denaturation (45 seconds, 93°C), annealing (1 minute, primer pair-specific annealing temperature, see Table S3 in the online Supplementary material) and elongation (1 minute, 72°C). Cycling was terminated with a 7 minute elongation step at 72°C . Purification of PCR products was carried out using the NucleoSpin[®] Extract II kit (Macherey-Nagel, Düren, Germany). DNA sequences for the COI, 16S rRNA, and H3 gene fragments were obtained through Macrogen Sequencing Service (Macrogen, Seoul, South Korea), and assembled using the SeqMan[™] II software package (DNASTAR, Madison, WI, USA, Swindell and Plasterer, 1997). All new sequences were deposited in the GenBank[®] database after removing primers (see Table S2 in the online Supplementary material for GenBank[®] accession numbers). Additional remipede sequences were downloaded from GenBank[®], and added to our data sets.

Alignments

Prior to alignment, we carried out BLASTN and MEGABLAST (Altschul et al., 1990) searches for each sequence, including both newly generated and published (GenBank[®]) sequences, to identify possible contamination. Ambiguous sequences were excluded from the analyses. In addition, we verified that the COI data did not contain any nuclear copies of mitochondrial-derived genes (NUMTs) according to the procedure described in Buhay (2009).

The protein coding genes COI and H3 were aligned with MUSCLE (Edgar, 2004) using default parameters. For the 16S dataset, we followed an alternative approach by considering secondary structure information. First, the 16S sequence from *Artemia franciscana* Kellogg, 1906 was added

to the matrix to serve as a leading sequence for structural alignment. Secondly, we performed a pre-alignment with MUSCLE (Edgar, 2004) with default parameters. Subsequently, the pre-aligned 16S matrix was realigned manually based on the information for secondary structure of *A. franciscana* from the European ribosomal RNA database (Wuyts et al., 2004) (for descriptions and discussions of methods see Kjer, 1995; Kjer et al., 2007; Koenemann et al., 2010). Finally, the 16S sequence of *A. franciscana* was removed again from the alignment before analyzing the data. Both terminal regions were excluded from the pre-aligned data set, because these regions appeared to contain erroneous or doubtful sequence fragments for a number of taxa.

Data Sets

Because for some species DNA had been extracted from different samples, in some instances also from different locations, there was more than one sequence per taxon available. For taxa with multiple sequences, we chose a representative sequence for each taxon, based on the length and quality of the electropherograms. For our analyses we used a total of 37 COI sequences, 26 H3 sequences, and 30 16S sequences (for details see Supplementary on-line file, Tables 1 and 2). Since each of the three markers covered slightly different sets of taxa, we conducted separate runs for each marker, and an additional run with all three markers combined as follows: COI (Run 1), 16S (Run 2), H3 (Run 3), COI + 16S + H3 (Run 4). For Run 5, we included all COI sequences available, so that some species are represented by two or more specimens. The matrices of Runs 6 and 7 were identical with the matrix of Run 4, except for the following modifications: (1) in Run 6, the 16S sequence of *S. gironensis* Yager, 1994 was excluded, and (2) in Run 7, we omitted the 16S sequence of *S. gironensis* as well as the

COI sequence of *P. fragilis*. The 16S sequence of *S. gironensis* was excluded because it differed in some parts markedly from all other 16S sequences of Remipedia, and it therefore was difficult to align. The COI sequence of *P. fragilis* was excluded because it was almost identical to sequences of *P. aplocheles* indicating either a possible contamination or, e.g., a very young split between *P. aplocheles* and *P. fragilis* resulting in incomplete sorting of lineages.

When we began our analyses, *S. tulumensis* and *S. cf. tulumensis* were suspected of being different species, but recently Neiber et al. (2012) showed that *S. tulumensis* and *S. cf. tulumensis* are the same taxon.

Phylogenetic Analysis

Bayesian inferences (BI) of phylogeny were performed using MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). For each single marker, we conducted an extensive set of pre-tests on the preliminary data matrices in order to assess the effects of varying the analysis parameters. As a result, we assumed that different models of evolution apply for the first, second, and third codon positions in the COI data set, whereas the 16S and H3 data sets were not further subdivided into smaller partitions. Models of sequence evolution were selected using the Akaike information criterion (AIC, see Akaike, 1974; implemented in MrModeltest 2.3, Nylander, 2004) for each data set separately with a random neighbor-joining tree (Saitou and Nei, 1987) as starting tree, and model scores were computed in PAUP* Version 4.0b10 (Swofford, 2003). Two different types of evolution models were selected: (1) the Hasegawa-Kishino-Yano model (HKY, Hasegawa et al., 1985) for the second codon positions in the COI data set, and (2) the general time reversible model (GTR, Rodriguez et al., 1990, Yang, 1993) for the remaining partitions.

Table 1. Shape of sternal bars and sternal plates of remipede taxa.

Taxon	Sternal bars 1-13	Sternal bar 14	Sternal bars from segment 15 onwards	Sternal plates
Micropacteridae				
<i>Micropacter</i>	greatly reduced	convex flap	absent	rounded
Gozilliidae				
<i>Godzillius</i>	isomorphic	isomorphic	isomorphic	rounded
<i>Godzilliognomus</i>	isomorphic	isomorphic	isomorphic	rounded
Pleomothridae				
<i>Pleomothra</i>	isomorphic	isomorphic	isomorphic	rounded
Kumongidae				
<i>Kumonga</i>	long, straight	large, triangular	15 + 16 slightly convex, then becoming triangular	rounded
Speleonectidae				
<i>S. lucayensis</i>	narrow, sublinear	large, concave	15-23 slightly concave, 24-convex flaps	rounded
<i>S. kakuki</i>	narrow, sublinear	large, concave	15-20 sublinear, 21-30 triangular flaps	rounded
<i>S. minnsi</i>	isomorphic	isomorphic	isomorphic	rounded
<i>Lasionectes</i>	narrow, slightly concave	large, concave	distinctly concave	rounded
Morlockiidae				
<i>Morlockia</i>	isomorphic	isomorphic	isomorphic	rounded
Cryptocorynetidae				
<i>Cryptocorynetes</i>	concave	large, convex (triangular)	15: concave, then straight, 19: convex flap	with pointed corners
<i>Angirasu</i>	long, slightly concave	large, convex	convex flap	with pointed corners
<i>Kaloketos</i>	concave	large, convex	concave	with pointed corners
Incertae familiae				
<i>Xibalbanus</i>	isomorphic	isomorphic	isomorphic	rounded
Incertae familiae and incerti generis				
"S." <i>gironensis</i>	isomorphic	isomorphic	isomorphic	rounded
"S." <i>epilimnius</i>	isomorphic	isomorphic	isomorphic	rounded
"S." <i>tanumekes</i>	isomorphic	isomorphic	isomorphic	rounded

Table 2. Shape of terminal claw complexes of maxillae (Mx2) and maxillipeds (Mxp) of remipede taxa.

Taxon	Description of claw complexes
Micropacteridae <i>Micropacter</i>	Mx2 and Mxp different. Mx2: longfinger-type, 1 long denticle flanked by 4 shorter denticles; Mxp: horseshoe-type with 9-10 fused denticles.
Godzilliidae <i>Godzillius</i>	Mx2 + Mxp subequal or slightly different. Grappling hook-type: Mx2 with 7-10 prongs; Mxp with 10 prongs or with about 6 denticles flanked by 3 prongs on each side.
<i>Godzilliognomus</i>	Mx2 + Mxp subequal. Grappling hook-type with 5 prongs.
Pleomothridae <i>Pleomothra</i>	Mx2 + Mxp subequal. Longfinger-type: 1 long flanked by several shorter denticles.
Kumongidae <i>Kumonga</i>	Mx2 + Mxp subequal. Longfinger-type: 1 long spine flanked by 2 shorter stout spines and Posterior arc of 5-6 less robust spines.
Speleonectidae <i>Speleonectes</i>	Mx2 + Mxp subequal. Horseshoe-type: 1 separate arcuate spine and an arc of 7-15 smaller denticles flanked by 2 somewhat stouter denticles.
<i>Lasionectes</i>	Mx2 + Mxp subequal. Longfinger-type: trifold structure of three spines, the central one being the longest. Between the central and anterior spine is a comb-like row of about 5-6 short, delicate denticles.
Morlockiidae <i>Morlockia</i>	Mx2 + Mxp subequal. Horseshoe-type: 1 free arcuate spine and an arc of 7-14 denticles, the lateral ones slightly more robust.
Cryptocorynetidae <i>Cryptocorynetes</i>	Mx2 + Mxp subequal, small in relation to penultimate maxillary and maxillipedal segments. Horseshoe-type: composed of a free spine and an arc of 8-14 deeply incised denticles.
<i>Angirasu</i>	Mx2 + Mxp subequal. Horseshoe-type: arc of 7-8 small denticles flanked by 2 stronger, separate spines.
<i>Kaloketos</i>	Mx2 + Mxp subequal. Horseshoe-type: arc composed of 17-19 fine denticles flanked by stouter, somewhat more separated denticles on each side.
Incertae familiae <i>Xibalbanus</i>	Mx2 + Mxp subequal. Horseshoe-type: arc of small denticles flanked posteriorly by 1 robust, separate spine.
Incertae familiae and incerti generis "S." <i>gironensis</i>	Mx2 + Mxp subequal. Horseshoe-type: arc of about 13 denticles flanked by a very large anterior spine and a somewhat smaller separated spine.
"S." <i>epilimnius</i>	Mx2 + Mxp subequal. Horseshoe-type: 1 robust, subterminal anterior spine and posterior arch of numerous fused denticles, anteriormost denticle elongate, more robust.
"S." <i>tanumekes</i>	Mx2 + Mxp subequal. Horseshoe-type: arc of 17-20 fine denticles flanked by 2 stouter, separate denticles.

MrModeltest 2.3 analyses also indicated that among-site variation in the different partitions is best modeled assuming a proportion of invariable sites (I) in combination with a discrete gamma-distributed among-site variation of substitutions (G). For the combined data set comprising COI, 16S rDNA, and H3 sequences, the same models of sequence evolution as in the data sets for the single gene fragments were used. In an additional analysis, we applied a codon model (invertebrate mitochondrial genetic code) implemented in MrBayes 3.1.2, based on the formulations outlined in Goldman and Yang (1994) and Muse and Gaut (1994) in combination with the GTR + G + I model to all available COI sequences of Remipedia and the selected out-groups.

Metropolis-coupled Markov-chain Monte Carlo (MCMCMC) searches in MrBayes Version 3.1.2 were run with ten chains in two separate runs for 20 000 000 generations (Runs 1-4, 6 + 7), or with four chains and 10 000 000 generations in two separate runs (Run 5) with default priors, trees sampled every 1000th generation, and separate estimation of parameters for individual partitions.

In addition to plotting the negative logarithmic likelihoods against sampled generations, potential scale reduction factors (PSRF, Gelman and Rubin, 1992) provided in MrBayes 3.1.2, and the AWTY software (Nylander et al., 2008) were used to ensure that the MCMCMC searches had reached stability. The first 25% of the generations were discarded as

burn-in period. Majority-rule consensus trees with average branch lengths and posterior probabilities (pp) as node support values were calculated in MrBayes 3.1.2. Posterior probability values of more than 0.95 were considered as strong support.

RESULTS

The COI p-distances ranged from 0.000 to 0.234 in H3, 0.000 to 0.403 in COI and 0.000 to 0.379 in 16S among in-group species.

COI, H3, 16S

Remipedia emerges as a monophyletic group, although internal poly- and paraphyly is noted for formerly-recognized clades within Nectiopoda (Fig. 3). In this group, Micropacteridae is the most basal family of Remipedia, but the posterior probability value is low (0.62). Godzilliidae sensu lato are polyphyletic with a maximally supported clade (clade A), consisting only of *Godzillius robustus*, *Godzil-*

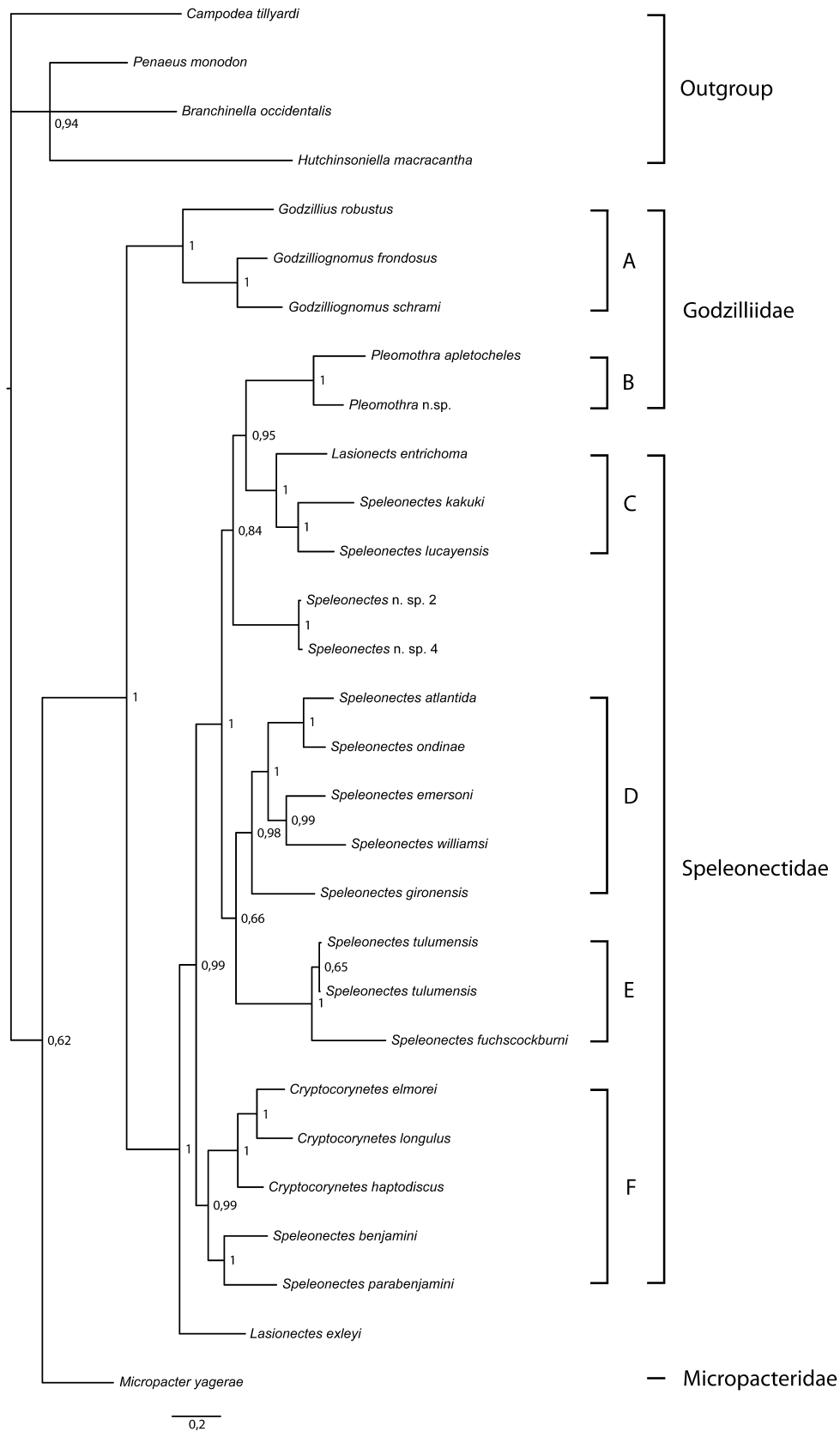


Fig. 3. Bayesian consensus tree of Run 7 (Combined analysis with all three markers COI, 16S and H3). Numbers on nodes represent posterior probability values. Neiber et al. (2012) concluded that the specimens from Cenote Crustacea, which were assumed to represent a new, undescribed species (*Speleonectes* cf. *tulumensis*) when starting the analyses, are conspecific with *Speleonectes tulumensis* Yager, 1987b. Therefore, two samples of *S. tulumensis* are present in our analyses.

lius fuchsi, *Godzilliognomus frondosus*, and *Godzilliognomus schrami* appear close to the base of the Remipedia clade, whereas the other three supposed godzilliids (*P. aplocheles*, *P. fragilis*, and a new, still undescribed, species of *Pleomothra* Yager, 1989) emerge among taxa that are currently classified as Speleonectidae.

The pattern of relationships we recovered for Speleonectidae shows several inconsistencies with the current taxonomy. *Lasionectes* is polyphyletic; *L. exleyi* has a singular position outside the other speleonectids, whereas *L. entrichoma* is a sister taxon to a clade containing *Speleonectes lucayensis* Yager, 1981 and *S. kakuki* nested deep inside the Speleonectidae sensu lato. The two Canary Island species are in a sister group relation to *S. emersoni* Lorentzen et al., 2007 and *S. williamsi* Hartke et al., 2011, while *S. gironensis* emerges as sister group to these taxa (clade D). The two species from the Yucatán Peninsula form a monophyletic group (clade E).

The genus *Cryptocorynetes* is monophyletic and forms a sister group to another species pair, *Speleonectes benjamini* Yager, 1987a and *Speleonectes parabenjamini* Koenemann et al., 2003. The clades of these clusters have high support values. Thus, the genus *Speleonectes* is polyphyletic, with *S. parabenjamini* and *S. benjamini* as sisters to *Cryptocorynetes*, while the remaining speleonectids form a large clade that also contains *L. entrichoma* and *Pleomothra*.

The clades A to F show high to very high pp-values, while some of the nodes basal to them have low support values. For example, the node giving rise to clade B + C and a small clade with the two undescribed speleonectids *S. n. sp. 2* and *S. n. sp. 4*, has a pp-value of 0.84. The lowest support values are found for the basal split into *M. yagerae* and all remaining Remipedia (0.66), and the split into clades D and E.

COI

In addition to the combined data matrix, we prepared a matrix with all available COI sequences, comprising specimens that represent species from additional, geographically separated localities (Run 5). The overall topology of its consensus tree is similar to the one of the combined analysis. However, there are some interesting differences. The position of *Speleonectes gironensis* shifts to become a sister taxon of the clade containing [*L. entrichoma*, [*S. kakuki*, *S. lucayensis*]] and the species of *Pleomothra* appear to emerge as a sister group of two still undescribed speleonectids (*S. n. sp. 2*, *S. n. sp. 4*). The posterior probability (pp) values are very high in the Remipedia clade, only the split between *Cryptocorynetes* and *S. benjamini* shows low pp-value (0.59). *Micropacter yagerae* and *S. parabenjamini* are not represented (Fig. 4).

16S

Although the topology of the 16S tree (not shown) resembles the topology of the combined tree (Fig. 3), some distinct differences exist. The most prominent one concerns the position of *L. exleyi* and its support values. *Lasionectes exleyi* is placed inside the clade comprising [*Cryptocorynetes*, [*S. benjamini*, *S. parabenjamini*]], but the pp value is very low. *Cryptocorynetes longulus*, *M. yagerae*, *S. fuchscockburni* Neiber et al., 2012, and *S. gironensis* are missing from the 16S data set.

DISCUSSION

Taken together, the results of our new molecular phylogenetic analyses stand in opposition to the current taxonomy of Remipedia. Speleonectidae, Godzilliidae, and the genera *Speleonectes* and *Lasionectes* are either para- or polyphyletic assemblages, whereas the genera *Cryptocorynetes*, *Pleomothra*, and *Godzilliognomus* Yager, 1989 appear as monophyletic groups in all analyses conducted (Fig. 5). The labeling of the clades in this section refers to Fig. 3.

Micropacteridae

In our analyses, *M. yagerae* is only represented by a single marker (H3); therefore, the support for its position at the base of the remipedian clade might be considered weak. While the phylogenetic position of Micropacteridae within Nectiopoda needs further evaluation, we nonetheless believe that their familial status is justified. Below, we provide a morphologic diagnosis of the family that includes several distinct autapomorphies.

Clade A (Godzilliidae)

In all analyses we performed, Godzilliidae, as previously understood, emerge as a polyphyletic group. While *Godzilliognomus* and *Godzillius* form a very highly supported monophyletic clade, the genus *Pleomothra* appears within the "speleonectids," likewise with high support values. Hence, the current structure of the godzilliids is not tenable.

Therefore, we redefine Godzilliidae sensu stricto to take into account this new information (see below).

Clade B (*Pleomothra*)

Yager (1989) assigned *P. aplocheles* to Godzilliidae (together with *Godzillius robustus* and *Godzilliognomus frondosus*) predominantly based on the morphology of the three post-mandibular prehensile limbs. Both species are equipped with robust sub-triangular third maxillary segments that lack endites, and highly similar modifications and setal patterns in maxillae and maxillipeds. Like in *Godzillius* and *Godzilliognomus*, the maxillary and maxillipedal segments in *Pleomothra* are long and narrow, and fused beyond the elbow. Based on the results of our analyses, however, it appears that these features evolved independently in *Pleomothra* and *Godzilliognomus*/*Godzillius*.

The genus *Pleomothra* possesses some remarkable autapomorphies. The most striking features are the morphology of the maxillules and the head shield. The massive fourth segments of the maxillules bear a prominent, presumably enditic process, forming a subchelate unit with the distal segments. Moreover, Koenemann et al. (2007c) suggest the presence of a secondarily derived joint between maxillary segments three and four. The maxillary termini are subchelate, i.e., not bearing the distinctive remipede fangs. The head shield of *Pleomothra* is equipped with unique posterolateral projections.

If we ignored the morphology of the maxillae and the maxillipeds, then an assignment of this genus to Speleonectidae might be conceivable. However, because of the very distinct autapomorphies of the second maxillae and the maxillipeds, we believe that it is necessary to erect a separate family for the genus *Pleomothra* (see below).

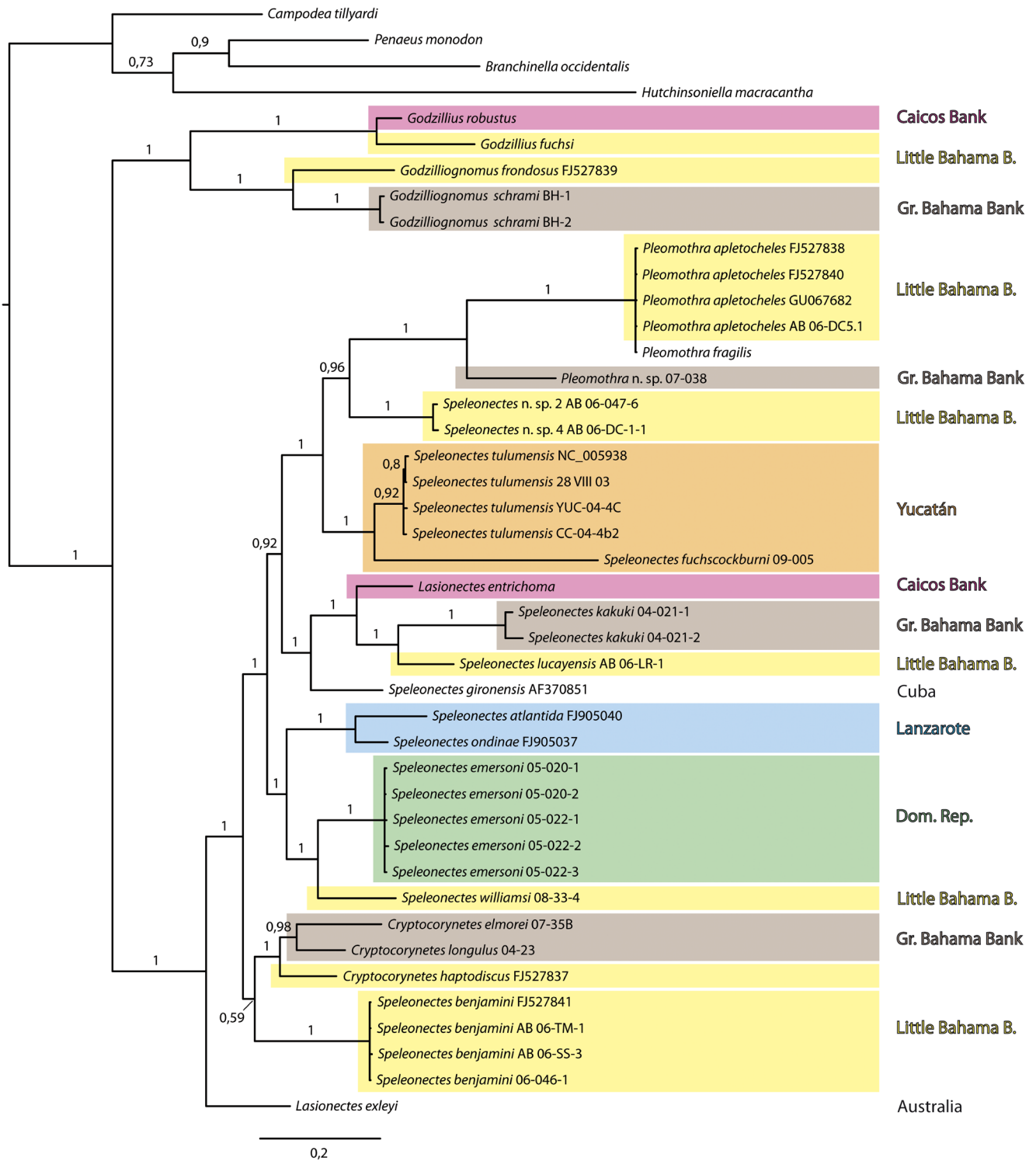


Fig. 4. Consensus tree of COI data set (Run 5). Numbers on nodes represent posterior probability values. The colored rectangles indicate the geographic region in which the samples were collected (Little Bahama B. = Little Bahama Bank; Gr. Bahama Bank = Great Bahama Bank; Yucatán = Yucatán Peninsula; Dom. Rep. = Dominican Republic).

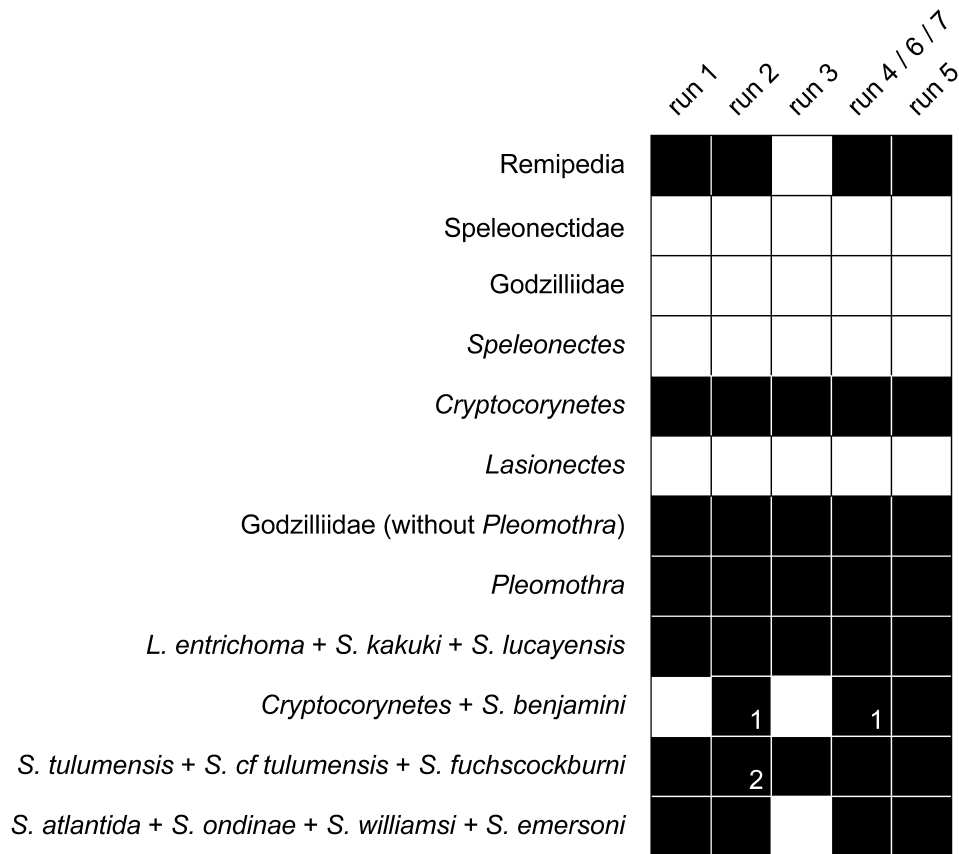


Fig. 5. Navajo rugs showing the distribution of Remipedia clades for all seven analyses presented in this study (top row). Black squares indicate monophyly, white squares non-monophyly. 1 = plus *S. parabenjamini* Koenemann et al., 2003; 2 = without *S. fuchscockburni* Neiber et al., 2012.

Clades B-F (*Speleonectidae sensu* Yager, 1981)

The large clade (B-F), including members until now placed in *Speleonectidae*, shows the most inconsistencies with the current taxonomy.

Clade C

The results of our analyses challenge the status of a monophyletic genus *Lasionectes*. It seems that *L. entrichoma* from the Caribbean and *L. exleyi* from Western Australia are not only separated from each other geographically, but phylogenetically as well. The isolated status of *L. exleyi* requires a separate genus for this species. We herein propose *Kumonga* n. gen. as the type genus for a new, monotypic Kumongidae n. fam. (see below).

The phylogenetic position of *Kumonga exleyi* close to the base of a “speleonectid” clade suggests an ancient split, and perhaps also a global distribution range of remipedes. We predict that there will indeed be more discoveries of remipedes between Australia and the Caribbean, supporting a Tethyan origin of Remipedia.

Our analyses retrieved *L. entrichoma* in a highly supported clade with *S. kakuki* and *S. lucayensis*. This clade, which we can conceive of as representing the core *Speleonectidae*, can be distinguished by some well-defined synapomorphies (Fig. 6) (see Systematics).

Clades D and E

Our analyses reveal ambiguous results concerning the position of the species from the Yucatán Peninsula, viz., *Speleonectes tulumensis* Yager, 1987b and *S. fuchscockburni*. In the COI tree, these three species are the sister group of the clade with [*Pleomothra* [*S. n. sp. 2*, *S. n. sp. 4*]]. In contrast to this arrangement, in the combined analysis, these three species appear as the sister group to the clade containing *S. ondinae* (García-Valdecasas, 1984); *S. atlantida* Koenemann et al., 2009a, *S. emersoni*, and *S. williamsi*. Hence, based on the molecular analyses alone, an unambiguous assignment of the Yucatán species is not possible at this time.

However, we recognize a number of synapomorphies for a clade consisting of the subclades [[*S. ondinae*, *S. atlantida*], [*S. emersoni*, *S. williamsi*]] and [[*S. tulumensis*, *S. cf. tulumensis*] *S. fuchscockburni*]]. These synapomorphies could serve to unite all these taxa into a single family: maxillary endites of segments 4 small and conical; maxillary and maxillipedal third segments weakly expanded, medial margins with few simple setae or small clusters with few setae; segments proximal and distal from elbows equally long; sternal bars isomorphic; caudal rami longer than anal segment (Fig. 7).

Of greater interest, however, is the clade with [[*S. ondinae*, *S. atlantida*], [*S. emersoni*, *S. williamsi*]]; the taxa appear morphologically clearly related, which harkens back to the original description of *S. ondinae* (see García-Valdecasas,

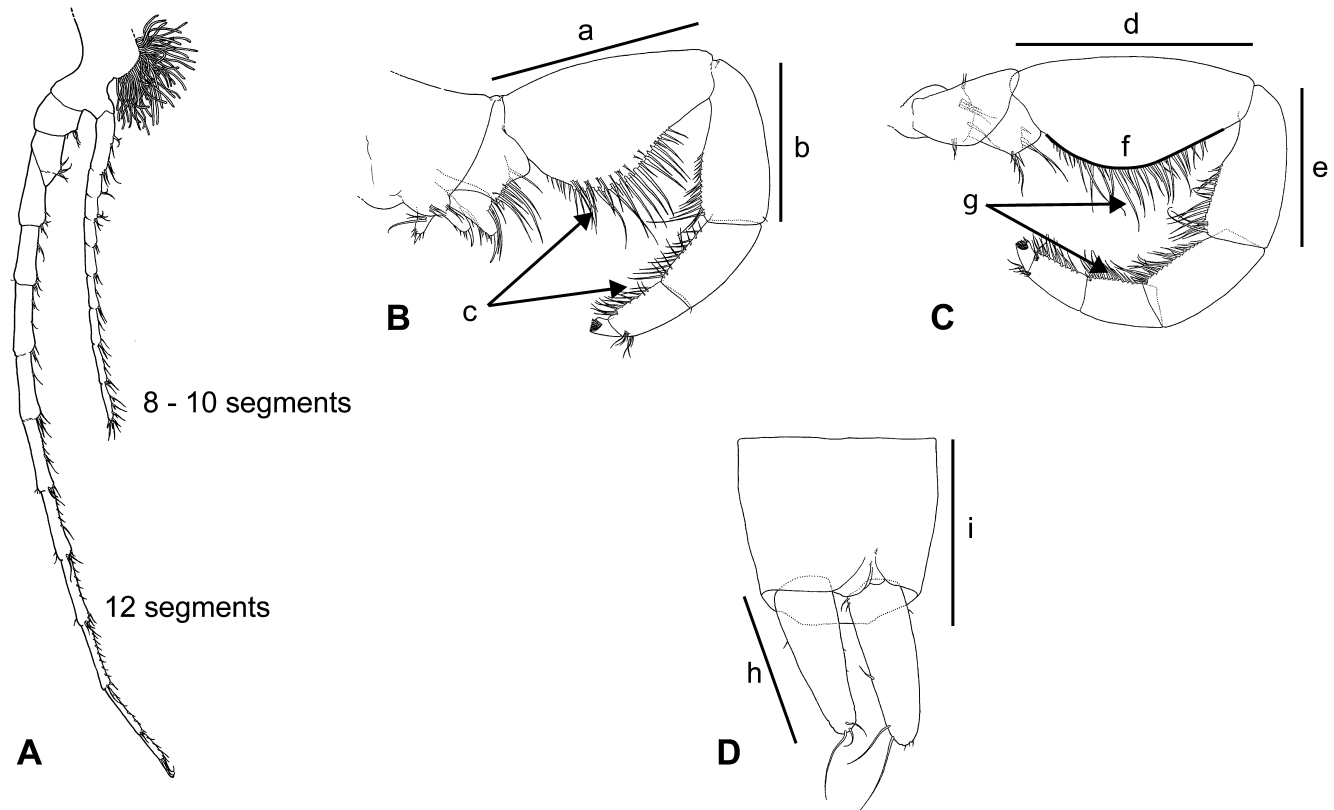


Fig. 6. Explanation of the diagnostic characters of clade C by taking the example of *S. kakuki* Daenekas et al., 2009. A = antennule, B = first maxillule, C = second maxilla, D = maxilliped, E = anal segment and caudal rami (modified from Daenekas et al., 2009). a = length of lacertus, b = length of fourth segment, c = number, shape and distribution of setae, d = length of lacertus, e = length of fifth segment, f = shape of endite, g = number, shape and distribution of setae, h = length of caudal rami, i = length of abdominal segment.

1984) at which time a separate genus was recognized, *Morlockia*, in its own family, Morlockiidae. Based on our molecular data and distinct morphological features, a return to this status is warranted.

Morphologically, clades D and E are quite separated from clade C, wherein lies the type species of *Speleonectes*. It is clear that also a new genus is required for the species in clade E (*S. tulumensis* and *S. fuchscockburni*), for which we propose the name *Xibalbanus* n. gen. (see below).

Clade F

In all of our analyses, the species of *Cryptocorynetes* form a clade with *S. benjamini* and *S. parabenjamini* that is distinctly separated from the clade C of “core speleonectids.” All representatives of this clade have several morphological features in common. These features support a separate familial group consisting of *Cryptocorynetes*, *S. parabenjamini* and *S. benjamini*, which in turn requires a new genus to accommodate *S. parabenjamini* and *S. benjamini*.

Taxa and Species of Uncertain Systematic Position

We have already noted above gaps in our current knowledge of molecular sequences for several species. The affiliation of these taxa can only be undertaken based on their morphological characters; we present some hypotheses for these affiliations here.

Kaloketos pilosus Koenemann et al., 2004

Kaloketos pilosus shares the pattern of sternal bars bearing posterolateral projections with *Cryptocorynetes*, *S. parabenjamini* and *S. benjamini*. Furthermore, the ventral rami of the antennules are very long and are composed of 18 segments. Therefore, we assign *K. pilosus* to clade F, i.e., as a separate genus in Cryptocorynetidae n. fam.

Speleonectes gironensis

The topologies we obtained reveal conflicting positions for *S. gironensis*. In Run 4 (see above), it is the sister taxon to the clade with [[*S. atlantida*, *S. ondinae*], [*S. emersoni*, *S. williamsi*]], while in Runs 1 and 5, it is sister to the clade [[*S. kakuki*, *S. lucayensis*], *L. entrichoma*].

On the one hand, the lacerti of maxillae and maxillipeds of *S. gironensis* are covered with setae almost entirely along the medial margin, and their lacerti are longer than the segments distal to the elbow. The endites of the lacerti of the maxillae and maxillipeds are elongated and expanded. These features suggest an assignment of *S. gironensis* to clade C. On the other hand, the ventral flagella of the antennules are composed of 10 segments, the sternal bars are isomorphic (Table 1), and the caudal rami are longer than the anal segment. The endites of segment 4 of the maxillules show characters of both clades, they are conical but also protracted. These characters would ally *S. gironensis* with members of clade D.

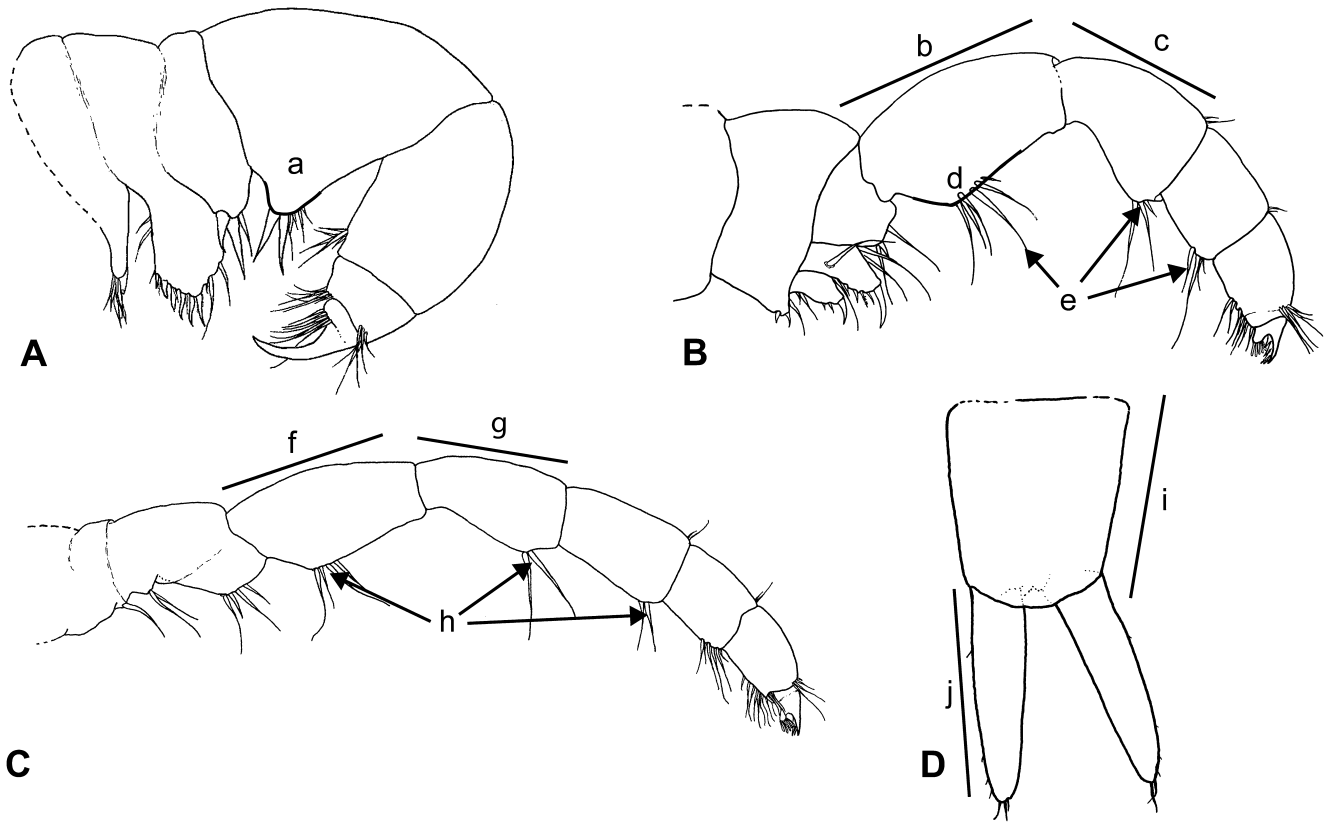


Fig. 7. Explanation of the diagnostic characters of clades D and E by taking the example of *S. emersoni* Lorentzen et al., 2007 (proposed new assignment: *Morlockia emersoni*). A = first maxillule, B = second maxilla, C = maxilliped, D = anal segment and caudal rami (modified from Lorentzen et al., 2007). a = shape of endite, b = length of lacertus, c = length of fourth segment, d = shape of endite, e = number, shape and distribution of setae, f = length of lacertus, g = length of fifth segment, h = number, shape and distribution of setae, i = length of abdominal segment, j = length of caudal rami.

Consequently, an unambiguous assignment of *S. gironensis* to a particular clade is not possible at this time, neither by molecular analyses nor morphologically, although in the combined analysis this species clearly fits in clade D.

Speleonectes minnsi Koenemann et al., 2003

An assignment of *S. minnsi* to [*L. entrichoma*, [*S. kakuki*, *S. lucayensis*]] is highly plausible, because it shares all described apomorphies of this clade.

Speleonectes epilimnius and *Speleonectes tanumekes*
Koenemann et al., 2003

Lorentzen et al. (2007) noted that *S. emersoni* is morphologically similar to the species *S. epilimnius* and *S. tanumekes*. Indeed, *S. epilimnius* and *S. tanumekes* could be assigned to clade D because they share the apomorphies of this group. However, *S. epilimnius* is equipped with setae of medium length on the maxillipeds, and has only a small number of trunk segments (21), which is more typical for the members of clade D, while *S. tanumekes*, on the other hand, has long setae on the segments of the maxillipeds and a high number of trunk segments (38-40), and therefore might fit better to clade E.

SYSTEMATICS

[M. Hoenemann, M. T. Neiber, F. R. Schram, and
S. Koenemann]

Micropacteridae Koenemann, Iliffe, and van der Ham, 2007

Diagnosis.—Cephalon long and narrow. Body very slender, no lateral epimeral extensions of trunk tergites; trunk composed of 16 segments. Sternal bars almost completely reduced. Frontal filaments with bifid, subterminal, accessory branches. Dorsal rami of antennules with 10 segments, ventral flagella short with 3 segments. Third endites of maxillules greatly reduced, lacerti deep and proximally spinose, brachia deep, margins naked except terminally. Maxillae and maxillipeds subequal in length, subchelate, lightly setose along median edges, brachial claws opposed to lacertal bases, lacerti relatively deep, brachia fused to 2 equally long segments (plus claws); claws different on each limb pair, terminal claw complexes of maxillae composed of 1 dominant denticle and 4 shorter denticles. Prominent denticle is almost twice as long as adjacent denticles, with pore-like opening on mid-proximal stem. Claw complexes of maxillipeds developed as horseshoe-like arcs of 9-10 fused denticles, with pore-like openings on distal (fused) part of each arc (see Table 2). Anal somite fused with trunk somites 16 and 15; caudal rami tiny.

Type genus.—*Micropacter* Koenemann, Iliffe, and van der Ham, 2007.

Remarks.—*Micropacter* is the only, monotypic genus of the family.

Godzilliidae Schram, Yager, and Emerson, 1986

Diagnosis.—Head shield not subrectangular, lateral margins with bulges (*Godzillognomus*) or slightly concave (*Godzillius*). Dorsal rami of antennules with 10-11 segments, ventral flagella tiny, two-segmented. Third endites of maxillules reduced and virtually naked; fangs long and stiletto-like. Maxillae and maxillipeds markedly subchelate; lacerti blade-like, armed with finely packed short setae; brachia very narrow, fused, with 1 (*Godzillognomus*) or 2 (*Godzillius*) faint sutures distally, brachia segments immobile to each other, armed with densely packed small scale-like setae; lacerti and brachia subequal on maxillae, brachia larger than lacerti on maxillipeds. Terminal claws of maxillae and maxillipeds pronged, grappling-hook-like. Caudal rami terminally setose.

Type Genus.—*Godzillius* Schram, Yager, and Emerson, 1986.

Included Genera.—*Godzillius*, *Godzillognomus* Yager, 1989.

Included Species.—*Godzillius robustus* Schram, Yager, and Emerson, 1986; *Godzillius fuchsi* Gonzalez, Singpiel, and Schlagner, 2013; *Godzillognomus frondosus*, Yager, 1989; *Godzillognomus schrami* Iliffe, Otten, and Koenemann, 2010.

Pleomothridae n. fam.

Diagnosis.—Head shield trapezoidal, with posterolateral corners developed as projections. Ventral rami of antennules short. Maxillules with terminal chelae, segments 3 without endites. Maxillae and maxillipeds subequal; brachia with fused segments, exhibiting one faint suture distally; lacerti and brachia subequal in length; lacertal and brachial margins with scale-like setae. Caudal rami as long as or longer than anal somite. Terminal claw complexes of maxillae and maxillipeds each composed of one prominent spine flanked by shorter spines.

Type Genus.—*Pleomothra* Yager, 1989.

Included Species.—*P. apletocheles* Yager, 1989; *P. fragilis* Koenemann, Ziegler, and Iliffe, 2008.

Kumongidae n. fam.

Diagnosis.—Dorsal rami of antennules with 13 segments, those of ventral flagella with 10-12 segments. Endites of maxillary segments 2 with dense clusters of long setae apically and subapically; endites of segments 3 bearing single seta with broad base and 5 spine-like setae of medium-length; expanded inner margins of segments 4 densely packed with long setae. Segments 3 of maxillae bulbous and expanded, endites strongly enlarged. Brachia of maxillae and maxillipeds longer than lacerti, equipped with dense rows of medium-sized to long plumose setae. Terminal claw complexes of maxillae and maxillipeds composed of one prominent spine flanked by shorter spines. Caudal rami longer than anal segment.

Type Genus.—*Kumonga* Hoenemann, Neiber, Schram, and Koenemann, n. gen. (type species: *Lasionectes exleyi* Yager and Humphreys, 1996).

Etymology.—*Kumonga*, a giant spider, is one of the legendary Japanese Kaiju monsters that battled with Godzilla.

Remarks.—At present, only one genus and species are recognized, for which the diagnoses remain the same as that of the family.

Included Species.—*K. exleyi* (Yager and Humphreys, 1996) n. comb.

Speleonectidae Yager, 1981

Diagnosis.—Head shield subrectangular to trapezoidal. Dorsal rami of antennules composed of 12 segments, ventral rami with 8-10 segments. Maxillary, third endites well developed, with two spine-like setae, but shorter than endites 1 and 2; lacerti and brachia distinctly expanded. Maxillae and maxillipeds with rows of long setae on distomedial margins of lacerti and brachia; lacerti expanded; maxillae smaller than maxillipeds; brachia longer than segments distal of elbow. Terminal claw complexes of maxillae and maxillipeds composed of densely spaced denticles arranged as horseshoe-shaped crests. Terminal claws trifold, longest denticle central (arcuate spine in *Speleonectes*, long finger-type in *Lasionectes*); pores on posterior surfaces of claws. Anal somite and caudal rami subequal in length, rami with terminal setal tufts.

Type Genus.—*Speleonectes* Yager, 1981.

Included Genera.—*Speleonectes*, *Lasionectes* Yager and Schram, 1986.

Included Species.—*S. kakuki* Daenekas, Yager, Iliffe and Koenemann, 2009; *S. lucayensis* Yager, 1981; *L. entrichoma* Yager and Schram, 1986.

Morlockiidae García-Valdecasas, 1984

Diagnosis.—Head shield subrectangular; frontal filaments dilated, accessory branches located distally, longer than main filaments. Trunk with 19 to 25 somites. Third endites of maxillules with 2 stout setae, opposed by generally 2 (3) similar setae on lacerti; these stout setae flanked by small number of simple setae; fangs long and curved at 90° to brachia axes. Maxillary and maxillipedal segments moderately slender, lacerti wider 1/2way along length, relatively few scattered simple setae at the widest points and not densely arrayed; first brachial segments inflated distally, with clusters of scattered setae located mediodistally on brachial segments. Terminal claw complexes of maxillae and maxillipeds subequal, horseshoe type composed of free arcuate spines and each arc of 7-14 denticles with lateral ones somewhat more robust. Anal somites and caudal rami subequal in length, rami virtually naked, with only a few scattered simple setae.

Type Genus.—*Morlockia* García-Valdecasas, 1984.

Morlockia García-Valdecasas, 1984

Type Species.—*Morlockia ondinae* García-Valdecasas, 1984.

Included Species.—*Morlockia ondinae* García-Valdecasas, 1984; *M. atlantida* (Koenemann et al., 2009a) n. comb., *M. williamsi* (Hartke et al., 2011 n. comb., and *M. emersoni* (Lorentzen et al., 2007) n. comb.

Cryptocorynetidae n. fam.

Diagnosis.—Accessory branches of frontal filaments arising basomedially, thumb-like, shorter than main filaments. Antennules with very long (50% of body length or longer) dorsal rami, composed of a minimum of 14 segments. Sternal plates with posterolateral projections; heteromorphic sternal bars on trunk segments 1 to 13 concave, those of trunk segment 14 large and convex. Maxillae and maxillipeds slender, maxillipeds distinctly longer than maxillae. Terminal claw complexes of maxillae and maxillipeds of horseshoe-type.

Type Genus.—*Cryptocorynetes* Yager, 1987a

Included Genera.—*Cryptocorynetes* Yager, 1987a; *Kaloketos* Koenemann, Iliffe, and Yager, 2004; *Angirasu* n. gen.

Included Species.—*C. haptodiscus* Yager, 1987a; *C. longulus* Wollermann, Koenemann, and Iliffe, 2007; *C. elmorei* Hazerli, Koenemann, and Iliffe, 2009; *K. pilosus* Koenemann, Iliffe, and Yager, 2004; *A. benjamini* (Yager, 1987a) n. comb.; *A. parabenjamini* (Koenemann, Iliffe, and van der Ham, 2003) n. comb.

Angirasu n. gen.

Diagnosis.—Fangs of maxillules very long, only moderately curved; stiletto-like. Maxillary and maxillipedal lacerti with barely or only slightly expanded margins; only with simple setae on antero- to midmedial margins; brachia with simple setae on distomedial margins, not equipped with feathered setae or discoid organs. Terminal claw complexes of terminal maxillae and maxillipeds subequal, horseshoe-type with 7-8 small denticles flanked by 2 stronger, separate spines. Anal segment slightly longer than wide; caudal rami with terminal clusters of setae and few medial and basomedial setae.

Etymology.—*Angirasu* is another of the legendary Japanese Kaiju monsters that battled with Godzilla.

Type Species.—*Speleonectes benjamini* Yager, 1987a.

Included Species.—*A. benjamini* (Yager, 1987a) n. comb.; *A. parabenjamini* (Koenemann et al., 2003) n. comb.

Incertae familiae *Xibalbanus* n. gen.

Diagnosis.—Head shield subrectangular. Frontal filaments dilated, accessory branches located mediolaterally, shorter than main filaments. Trunk segments up to 42, usually more than 30 in adults; sternal bars isomorphic. Third maxillary endites and lacerti with 2 opposing serrate setae each, accompanied by few simple setae. Maxillipeds distinctly longer than maxillae; maxillary and maxillipedal lacerti slightly expanded with few simple setae; first brachial segments with distinctly expanded distomedial margins, bearing few simple setae, the following brachial segments subrectangular with few simple setae at distomedial margins.

Terminal claw complexes of maxillae and maxillipeds subequal, horseshoe-type each with arc of 10 small denticles each flanked by 1 stronger, separate denticle. Caudal rami very long, at least 2.5 times longer than anal segment, with few scattered setae.

Type Species.—*Speleonectes tulumensis* Yager, 1987b.

Etymology.—Composed from Xibalba, the underworld in ancient Mayan mythology, and the Latin suffix -anus, meaning “originating from.”

Included Species.—*X. tulumensis* (Yager, 1987b) n. comb., *X. fuchscockburni* (Neiber et al., 2012) n. comb.

Remarks.—The position of this genus shifts on our trees depending on the molecules and taxa used: either on the clade with morlockiids, or on a clade with pleomothrids and some yet-to-be-described taxa. We suspect *Xibalbanus* belongs with the former (Fig. 8), but for the time being we leave its familial affiliations as uncertain.

CONCLUSIONS

The molecular data derived from 16S rDNA (16S), histone 3 (H3), and cytochrome c oxidase subunit I (COI) reveal several inconsistencies within the current taxonomic classification of Remipedia. Amongst the currently recognized families, Godzilliidae, as well as the genera *Speleonectes* and *Lasionectes* emerge as polyphyletic groups, while *Speleonectidae* appears to be paraphyletic. We discuss current taxonomic diagnoses based on morphologic characters, and suggest taxonomic changes that accord with the topologies of the phylogenetic analyses, which we summarize here for the extant remipedes:

Remipedia Yager, 1981

Nectiopoda Schram, 1986

Cryptocorynetidae n. fam.

Cryptocorynetes Yager, 1987a

C. haptodiscus, *C. elmorei*, *C. longulus*

Angirasu n. gen.

A. benjamini, *A. parabenjamini*

Kaloketos Koenemann, Iliffe, and Yager, 2004

K. pilosus

Godzilliidae Schram, Yager, and Emerson, 1986

Godzillius Schram, Yager, and Emerson, 1986

G. robustus, *G. fuchsi*

Godzillognomus Yager, 1989

G. frondosus, *G. schrami*

Kumongidae n. fam.

Kumonga n. gen.

K. exleyi

Micropacteridae Koenemann, Iliffe, and van der Ham, 2007

Micropacter Koenemann, Iliffe, and van der Ham, 2007

M. yagerae

Morlockiidae García-Valdecasas, 1984

Morlockia García-Valdecasas, 1984

M. ondinae, *M. atlantida*, *M. emersoni*, *M. williamsi*

[? “*S.*” *gironensis* Yager, 1994]

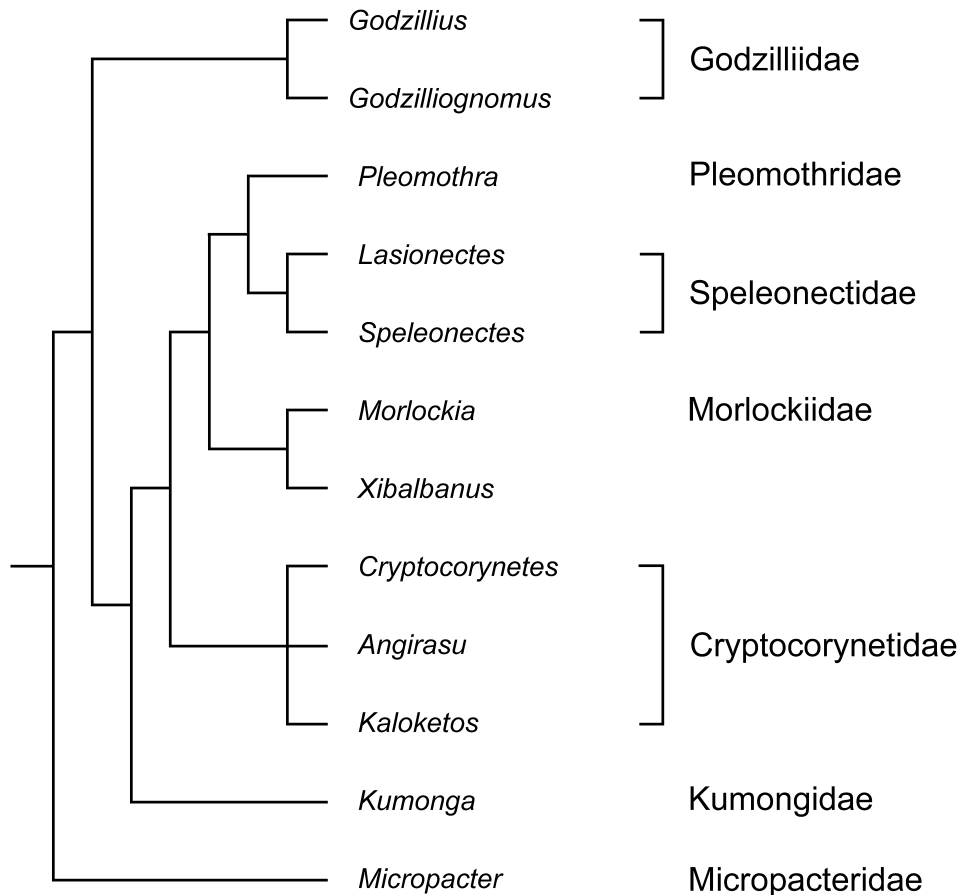


Fig. 8. Summary rendition of Fig. 3, with new taxonomic nomenclature proposed herein.

Pleomothridae Yager, 1989

Pleomothra Yager, 1989

P. aplocheles, *P. fragilis*

Speleonectidae Yager, 1981

Speleonectes Yager, 1981

S. lucayensis, *S. kakuki*, *S. minnsi*

Lasionectes Yager and Schram, 1986

L. entrichoma

Incertae familiae

Xibalbanus n. gen.

X. tulumensis, *X. fuchscockburni*

Incertae familiae and incerti generis

? "*S.*" *epilimnius*

? "*S.*" *tanumekes*

The descriptions of several new "speleonectids" are in preparation. Once these occur, and perhaps some new molecular data are generated, we may be able to clarify the taxonomic issues not resolved in this study, e.g., the family affiliation of *Xibalbanus* and the taxonomic position of "*S.*" *gironensis*, "*S.*" *tanumekes* and "*S.*" *epilimnius*.

It is significant to note that in our revision of Remipedia, while some formerly geographically widespread genera, e.g., *Speleonectes* and *Lasionectes*, are divided into separate, more geographically restricted taxa, the genus *Morlockia* is resurrected and, by now including three additional species, has an amphi-Atlantic distribution. Five of the seven revised remipede families (Micropacteridae, Pleomothridae, Godzil-

liidae, Cryptocorynetidae, and Speleonectidae) are represented only from the Bahamas archipelago, with the latter three families extending across this chain of islands from the Caicos Bank in the southeast, through the Great Bahama Bank, to the Little Bahama Bank in the northwest. Morlockiidae, currently with a single genus, includes species from the Canary Islands, Cuba, Dominican Republic and the Little Bahama Bank. Finally, Kumongidae is represented by a single species from Western Australia. Thus, speculation as to the timing and potential modes of open ocean dispersal of remipedes persists.

The shallow epicontinental waters comprising the Neo-Tethys were fragmented by the break-up of Pangaea. The developing rift through Pangaea resulted in the westward extension of the Tethys into the opening Atlantic in the Early Jurassic (200 Ma), at that time an epicontinental sea extending from the far eastern coastline of Pangaea continuously to the Yucatán Plate. By the Middle Jurassic (170 Ma), the widening rift between 'Africa' and the 'Americas' allowed for more than 6 km deep oceanic water (Thiede, 1979) to extend into the developing Atlantic (see <http://jan.ucc.nau.edu/rcb7/nat.html>, accessed 5 April 2013), while by the Early Cretaceous (120 Ma) extensive carbonate deposits extended from the Bahamas area westward through the Florida Plateau to the Yucatán Peninsula. These geological episodes and also the closure of the Tethys Seaway in the present day Mediterranean region (19-10 Ma; McQuarrie et al., 2003;

Harzhauser et al., 2007), provide a framework against which to test dispersal versus vicariant models of remipede evolution against future molecular clock data.

The crustacean composition of anchialine faunas is highly predictable, often even at the generic level, no matter how far apart in the world they occur (Wagner, 1994; Jaume et al., 2001). Remipedes are members of the core anchialine fauna suite along with other crustacean taxa such as *Danielopolina* Kornicker and Sohn, 1976 (thaumatocyridid Mydocopa), thermosbaenaceans of the genus *Halosbaena* Stock, 1976, and epacteriscid copepods (Boxshall and Iglkowska, 2012). The remipede distribution fits closely with the hypothesis that many widespread, disjunct, anchialine species have descended from marine species in the Tethys Ocean, whose disjuncture can be explained by sea floor spreading due to plate tectonic movement (Wägele, 1985; Humphreys, 1993; Wagner, 1994; Sanz and Platvoet, 1995; Jaume et al., 2001; Kano and Kase, 2004; Page et al., 2008). However, the origin and timing of remipede evolution remains enigmatic. A number of studies using morphological (Metacrangonyctidae: Jaume and Christenson, 2001) or molecular clock analyses (Atyidae: Page et al., 2008; Cirolanidae: Baratti et al., 2010; Metacrangonyctidae: Bauzá-Ribot et al., 2012; Gobiiformes: Chakrabarty et al., 2012) have recently presented results consistent with amphi-oceanic or inter-oceanic divergence. Conversely, oceanic dispersal within some of these higher anchialine taxa (*Danielopolina* and *Halosbaena*) is indicated by their presence on isolated seamounts (Humphreys and Danielopol, 2006; Humphreys et al., 2009; Shimomura, and Fujita, 2009).

Our remipede data proved unsuitable for molecular clock analysis. We were neither able to relate branching patterns in our phylogenetic trees to major vicariant events, nor to assumed evolutionary splits in our out-group taxa. This was also found by von Rintelen et al. (2012) in their molecular phylogeny of 32 of the 42 genera of Atyidae. These authors were unable to relate branching patterns in their phylogenetic trees to the opening of the Atlantic due to inconsistencies in the three calibration dates they used. Nevertheless, what we now have is a quantum leap in improvement in our understanding of the evolution of remipedes.

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SUPPLEMENTARY MATERIAL

Table S1. Newly generated Remipedia sequences. The numbers indicate the sequenced fragment in comparison to the reference sequences (COI and 16S = *S. tulumensis*; GenBank acc. no. NC_005938; H3 = *Homo sapiens*, GenBank acc. nr. NM_003493). The numbers in parentheses next to the gene name show the overall length (in base pairs) of the reference sequence; see Table 2 for full versions of abbreviated generic names.

Species	Voucher	Amplified region		
		COI (1539)	H3 (411)	16S (1333)
<i>G. robustus</i>	03-19	58-638	24-351	152-1216
<i>G. fuchsi</i>	AB-06-RS1	44-702	—	—
<i>Gn. frondosus</i>	06-048-4	63-656	—	232-1196
	06-047-8	—	24-351	—
	06-50	—	24-351	—
	AB06-SS-4.1	—	—	609-1196
<i>Gn. schrami</i>	07-048-2	85-702	—	662-1246
	07-49	—	24-351	—
	BH-1 Eleuthera	85-702	—	—
<i>P. aplocheles</i>	AB06-DC-5.1	20-1121	24-351	—
	AB06-RS-2	85-702	—	638-1250
	AB06-SS-2	90-661	—	697-1113
	AB-06-L2	88-681	—	—

Table S1. (Continued.)

Species	Voucher	Amplified region		
		COI (1539)	H3 (411)	16S (1333)
<i>P. fragilis</i>	BH_EC	126-702	–	–
<i>P. n. sp.</i>	07-038	44-702	24-351	662-1158
<i>C. elmorei</i>	07-035B	44-702	24-351	231-1196
<i>C. haptodiscus</i>	AB06-SS-1.1	20-674	24-351	130-1230
<i>C. longulus</i>	C3-04-23	69-667	24-351	–
	04-20	–	24-351	–
<i>L. entrichoma</i>	03-16	223-684	24-351	648-1229
<i>L. exleyi</i>	BES-10169	44-1066	24-351	136-1241
<i>S. atlantida</i>	LZ 2.3	95-702	–	169-1173
	LZ 2.1	–	24-351	169-1132
	Dzul 9999-GBIF	–	–	334-1132
	LZ 1.1	–	–	294-1123
<i>S. benjamini</i>	06-047-2	88-702	24-351	133-1209
	06-046-1	44-702	24-351	–
	04-23	–	–	606-1209
	AB06-SS-3	46-702	–	620-1209
	AB06-TM-1	44-1313	–	619-1209
<i>S. emersoni</i>	05-020-01	85-702	–	–
	05-020-02	44-702	–	–
	05-022-01	66-702	24-351	153-1229
	05-022-02	44-702	–	–
	05-022-03	88-702	–	–
<i>S. fuchscockburni</i>	09-005	85-702	24-351	–
<i>S. gironensis</i>		–	24-351	668-1210
<i>S. kakuki</i>	04-021-1	44-702	–	604-1234
	04-021-2	44-637	–	–
	BH-330	–	24-351	136-1234
<i>S. lucayensis</i>	AB06-LR-1	43-702	24-351	657-1215
<i>S. n. sp. 2</i>	AB-06-047-6	85-702	–	659-1082
<i>S. n. sp. 4</i>	AB06-DC-1.1	217-943	–	649-1235
<i>S. ondinae</i>	LZ 1.2	36-707	24-351	165-1171
<i>S. parabenjamini</i>	04-023_SK	–	24-351	661-1068
<i>S. tulumensis</i>		–	24-351	–
<i>S. cf. tulumensis</i>	YUC-04-4C	63-1266	–	–
	28 VIII 03	63-1266	–	–
	CC-044B2	62-1266	–	–
	06-41H	–	24-351	661-1084
<i>S. williamsi</i>	08-033-4	44-702	24-351	713-1196
<i>M. yagerae</i>	03-18	–	24-351	–

Table S2. List of taxa used for phylogenetic analysis, including GenBank accession numbers of COI, H3 and 16S sequences, and voucher numbers of specimens from the research collection of S. Koenemann; newly generated sequences are shown in boldface.

Species	Voucher	Sampling site	COI	H3	16S
Godzilliidae					
<i>Godzillius robustus</i>	03-19	North Caicos Island	JF332152	KC989960	KC990000
<i>Godzillius fuchsi</i>	AB-06-RS1	Great Abaco Island	JF332153	–	–

Table S2. (Continued.)

Species	Voucher	Sampling site	COI	H3	16S
<i>Godzillionomus frondosus</i>	06-048-4	Great Abaco Island	FJ527839	–	KC989998
	06-047-8	Great Abaco Island	–	KC989961	–
	06-50	Great Abaco Island	–	KC989983	–
	AB06-SS-4.1	Great Abaco Island	–	–	KC989999
<i>Godzillionomus schrami</i>	07-048-2	Eleuthera	JF332154	–	KC990013
	07-49	Eleuthera	–	KC989962	–
	BH-1	Eleuthera	KC989994	–	–
<i>Pleomothra apletocheles</i>	AB06-DC-5.1	Great Abaco Island	FJ527840	KC989963	KC990006
	AB06-RS-2	Great Abaco Island	KC989995	–	KC990004
	AB06-SS-2	Great Abaco Island	GU067682	–	KC990005
	AB-06-L2	Great Abaco Island	FJ527838	–	–
<i>Pleomothra fragilis</i>	BH_EC	Exuma Cays	KC989984	–	–
<i>Pleomothra</i> n. sp. E	07-038	Eleuthera	JF332155	KC989964	KC990014
Speleonectidae					
<i>Cryptocorynetes elmorei</i>	07-035B	Eleuthera	JF332156	KC989966	KC989996
<i>Cryptocorynetes haptodiscus</i>	AB06-SS-1.1	Great Abaco Island	FJ527837	KC989967	KC989997
<i>Cryptocorynetes longulus</i>	C3-04-23	Cat Island	JF332157	KC989968	–
<i>Lasionectes entrichoma</i>	03-16	North Caicos Island	JF332158	KC989969	KC990001
<i>Lasionectes exleyi</i>	BES-10169	Cape Range Penninsula	JF332159	KC989970	KC990002
<i>Speleonectes atlantida</i>	LZ 2.3	Lanzarote	FJ905040	–	FJ905034
	LZ 2.1	Lanzarote	–	KC989971	FJ905033
	Dzul 9999-GBIF	Lanzarote	–	–	FJ905031
	LZ 1.1	Lanzarote	–	–	FJ905032
<i>Speleonectes benjamini</i>	06-047-2	Great Abaco Island	FJ527841	KC989972	KC990007
	06-046-1	Great Abaco Island	KC989985	–	–
	04-23	Cat Island	–	–	–
	AB06-SS-3	Great Abaco Island	KC989986	–	KC990011
<i>Speleonectes emersoni</i>	AB06-TM-1	Great Abaco Island	KC989987	–	KC990012
	05-020-01	Dominican Republic	JF332161	–	–
	05-020-02	Dominican Republic	KC989988	–	–
	05-022-01	Dominican Republic	KC989989	KC989973	KC990008
	05-022-02	Dominican Republic	KC989990	–	–
<i>Speleonectes gironensis</i>	05-022-03	Dominican Republic	KC989991	–	–
<i>Speleonectes gironensis</i>		Cuba	AF370851	KC989974	AF370874
<i>Speleonectes kakuki</i>	04-021-1	Cat Island	JF332163	–	–
	04-021-2	Cat Island	KC989992	–	–
	BH-330	North Andros	–	KC989975	KC990009
<i>Speleonectes lucayensis</i>	AB06-LR-1	Great Abaco Island	JF332160	KC989976	KC990010
<i>Speleonectes</i> n. sp. 2	AB-06-047-6	Great Abaco Island	JF332164	–	KC990015
<i>Speleonectes</i> n. sp. 4	AB06-DC-1.1	Great Abaco Island	KC989993	–	KC990016
<i>Speleonectes ondinae</i>	LZ 1.2	Lanzarote	J905037	KC989977	FJ905035
<i>Speleonectes parabenjamini</i>	04-023_SK	Cat Island	–	KC989978	KC990017
<i>Speleonectes tulumensis</i>		Yucatán Pennisula	AY456190	KC989979	AY456190
	YUC-04-4C	Yucatán Pennisula	JF297645	–	–
	28 VIII 03	Yucatán Pennisula	JF297646	–	–
	CC-044B2	Yucatán Pennisula	JF297647	–	–
	06-41H	Yucatán Pennisula	–	KC989982	KC990019
<i>Speleonectes williamsi</i>	08-033-4	Grand Bahama Island	JF332162	KC989980	KC990018
<i>Speleonectes xibalba</i>	09-005	Yucatán Pennisula	JF297644	KC989981	–
Micropacteridae					
<i>Micropacter yagerae</i>	41698	Providenciales Island	–	KC989965	KC990003
Outgroup taxa					
<i>Branchinella occidentalis</i> (COI, H3)/ <i>B.</i> sp. (16S)		–	EF189664	EF189600	AF110871
<i>Hutchinsoniella macracantha</i>		–	AY456189	AY456189	AF110867
<i>Penaeus monodon</i> (COI, 16S)/ <i>P. semisulcatus</i> (H3)		–	AF217843	AF217843	DQ079698
<i>Campodea fragilis</i> (H3)/ <i>C. tillyardi</i> (COI, 16S)		–	AF370844	DQ529236	AF110860

Table S3. List of primers used in this study, including information on amplification direction, and annealing temperatures for PCRs.

Primer name	Direction	Sequence (5' → 3')	Annealing temperature (°C)
COI			
LCO1-1490	Forward	GGTCAACAAATCATAAAGATATTG	50
HCO1-2198	Reverse	TAAACTTCAGGGTGACCAAAAAATCA	50
COI s	Forward	T (AGT) AT (CT) GGAGGATT (CT) GG	50
COI as	Reverse	CGTCG (AT) GG (CT) AT (AT) CC	50
16S			
CRUST16SF	Forward	TAATTCAACATCGAGGTCGCAA	50
CRUST16SR	Reverse	TTTGTACCTTKTGTATTAGG	50
mt16S-ar	Forward	CGCCTGTTTATCAAAAACAT	57
mt16S-br	Reverse	CCGGTCTGAACTCAGATCACGT	57
H3			
H3 AF	Forward	ATGGCTCGTACCAAGCAGACVGC	50
H3 AR	Reverse	ATATCCTTRGGCATRATRGTGAC	50

Table S4. 16S alignment of Runs 2, 4, 6 and 7, dashes indicate gaps, and question marks missing nucleotide information.

Species code	Sequence
Campodea	TAATTTTAA-TAATTAAGCAAAAT-AAGGCTCGCTGTTTATCAAAAAACATGGTTTGTGA-----CTATAAAAAATTTGG-CCTGCTC
Penaeus	GTATAATTAAGGAACTCGGCAAAAT-ACTACTTTTGGCTGTTTATCAAAAAACATGCTATATGA-----TTGTATATATAAAGTCTAG-CCTGCCC
Branchinella	??
Hutchinsoniella	CTTAATTCAGGAACTCAGCAAAAATAACATCCCGCTGTTTAAFAAAAAACATGCTTCCGTG-----ACATAATA-GGAA-AGTCCAACTCTGCCC
G_robustus	TCATTCAGAAAGAACTTGTCAAAAT-ATAAATTCGCGCTGTTTATCAAAAAACATGGTCTCTTGT-----ATAAATAAGAGGCTGTA-CCTGCCC
Gn_fronodosus	TTATTTTAAAGAAATTTGGCAAAA-CAAGTTTCCGCAATGTTATCAAAAAACATGGCTTTTCGA-----ATAGAAAAATGTAAGTCTGA-CCTGCCC
Gn_schrami	??
P_apletocheles	??
P_fragilis	??
P_nsp	??
M_yagerae	??
C_elmorei	TCATCGTGAAGGAACTCGGCAAAAT-GTGGTTTCTGGCTGTTTAAACAAAAACATGGCTTTTGT-----GGATATAATAAGTTCGGG-CCTGCCC
C_haptodiscus	TTGTTTAAAGGAACTCGGCAAAAT-ATGGTTTCTGGCTGTTTAAACAAAAACATGGCTTTTGT-----GAAAAATAAAGTTCGGG-CCTGCCC
C_longulus	??
L_entrichoma	??
L_xelvi	ATGTTTAAAGGAACTCGGCAAAAT-AACTTCTCTGGCTGTTTAAACAAAAACATGGCTTTTGT-----TGGAGATAAGAGTCTTTTCTGCTC
S_antlantida	TTTTCAGTAAAGGAACTAGGCAAAA-ATAGCATCTGGCTGTTTAAFAAAAAACATGGCTTTTGT-----TATATATAATAAAGTCTGG-CCTGCTC
S_benjamini	GTGTTTAAAGGAACTCGGCAAAAT-ATGGTTTCTGGCTGTTTAAACAAAAACATGGCTTTTGT-----GTGGTTGATATAAAGTCTGG-CCTGCCC
S_emersoni	TTAAGTTAAAGGAACTAGGCAAAA-ATGATATCTGGCTGTTTAAFAAAAAACATGGCTTCTTGTATAGGTTTATAAATAAAGAGTCTAA-CCTGCCC
S_gironensis	??
S_kakuki	GTATATGAAGGAACTTGGCAAAAT-TTAAATGTTCTGGCTGTTTAAFAAAAAACATGGCTTTTGT-----TGATAAATAAAGTCTGA-CCTGCCC
S_lucayensis	??
S_nsp2	??
S_nsp4	??
S_ondinae	??
S_parabenjamini	TTTTTAA-TAAAGGAACTAGGCAAAAATAATGTTCTGGCTGTTTAAFAAAAAACATGGCTTTTGT-----TTAAATAATAAAGTCTGG-CCTGCCC
S_tulumensis	??
S_williamsi	TTTTACATAAAGGAACTCGGCAAAAT-AAACACTTCCACTGTTTAAFAAAAAACATGGCTCTTGT-----GCAGTTTGTATATAGGAGTTCGGA-CCTGCTC
S_xibalba	??
S_cf_tulumensis	??
Campodea	AATGA-----AAT-----TTAAATAGCTGCAGTAAAT-TAACTGTACAAAAGGTAGCATAAATCATAGTCTTTTAAATTTGTTGGCTGGAATGAATGGTTTTT
Penaeus	ACTGA-ATATATTT-----TTAAAGGGCCGGGTATAC-TGACCGTGCAGGAGGTAGCATAAATCATAGTCTTTTAAATGAAAGCTTGTATGAATGGTTTTGG
Branchinella	ACTGA-----GTA-----TTGAAGGCCGTTGGTATAC-TGACCAATGCAGGAGGTAGCATAAATCATTTGCCCTTAAATAGGGCTGGAATGAAGGTTTGG
Hutchinsoniella	ACTGA---AATAAT-----TTAAAGGCCGCGGTAAATTTTGGCCGTAAAGGTAGCATAAATATTTGCTCCTAAATTAGAGACTAGAATGAAAGTCAAA
G_robustus	ACTGA--TAGTAG-----TTGAAGGCCCGCAGTAAATCTGACTGTCTAAAGGTAGCATAAATCATTTGTCATTTTAAATTTGTTGAATGTAATGGTTTTG
Gn_fronodosus	ACTGA--TTTATAA-----TTGAAGGCCCGCGGTAAAT-TAACTGTCTAAAGGTAGCATAAATCATTTTCCAAATTAATTTGTTGGAATGGAATGAATGGTTTTG
Gn_schrami	ACTGA--TTAAATAA-----TTGAAGGCCCGCGGTAAAT-TAACTGTCTAAAGGTAGCATAAATCATTTTCCAAATTAATTTGTTGGAATGGAATGAATGGTTTTG
P_apletocheles	ACTGA--GGTTACAC-----TTAAAGAGCTGCAGTATACTGACTGTACGAAAGGTAGCATAAATTTGTCATTTAAATTTGTTAGACTTTGGATGAAATGGTTTTG
P_fragilis	??
P_nsp	ACTGA--GGTAAAAAC-----TTAAAGAGCTGCAGTATACTGACTGTACTAAAGGTAGCATAAATTTGTCATTTAAATTTGTTAGACTTTGTATGAATGGTTTTG
M_yagerae	??
C_elmorei	??
C_haptodiscus	GCTGT--TTAG-----ATGAAGGGCTGCGGTATACTTGACCGTACAAAAGGTAGCATAAATGGTTTGCCTATTAATTTGTTGGCTTGTATGAATGGCTTGG
C_longulus	ACTGT--TTGT-----ATGAAGGGCTGCGGTATACTTGACCGTACAAAAGGTAGCATAAATGGTTTGTCTATAATTTGTTGGCTTGTATGAATGGTCTGT
L_entrichoma	GCTGT--ATATAIT-----ATGAAGGGCTGCGGTATATTTAACTGTACGAAAGGTAGCATGATAATTTGTTCTATTTAAATTTGACTTGTATGAATGGTTTTG

Table S4. (Continued.)

Species code	Sequence
L_exleyi	GCTGC-TTTTGTAAA---GTAAGGGCTGCAGTATACTTGGCTGACGAAAGGTAGCATATAATAAATGTCCTATTGCAATTGTTGGCTGGTATGAATGGAATG
S_atlantida	TCTGA-TATCATATATA---TTGAAGAGCTGCAGTATACCTTGGCTGACCTACAAAAGGTAGCATAGTATGTTGGCTTCCCTATTAATATATGGCTTGTATGAATGGTTTTG
S_benjamini	GCTGT--AGGA-----ATGAATGGCTGCGGTATACCTTGGCTGACCTACGAAAGGTAGCATATAATTTGGCTTCCCTATTAATATGTTGGCTTGAATGAATGGTTTTG
S_emersoni	TCTGA-TATTTAGTAA---TTGAAGGGCTGCAGTATACTTGGCTGACCTACAAAAGGTAGCATATAATTTGGCTTCCCTATTAATATGTTGGCTTGTATGAATGGTTTTG
S_gironensis	TCTGATTCATAAATA---TTAAAGGGCTGCAGTACAACTAACCTGACAAAAGGTAGCATATAATACTTTGGCTTCCCTATTAATATGTTGGCTTGCATGAATGGCCCCC
S_kakuki	ACTGA-AAATGTTTGG---TTAAAGGGCTGCAGTATTTGGCTGACCTACAAAAGGTAGCATATAATTTGGCTTCCCTATTAATATGTTGGCTTGAATGAATGGTTTTG
S_lucayensis	GCTGA-ATGGATATGG---TTGAAGGGCTGCAGTATACTTGGCTGACCTACAAAAGGTAGCATATAATTTGGCTTCCCTATTAATATGTTGGCTTGCATGAATGGTTTTG
S_nsp2	GCTGA-TAAGTTTAA---TTAAAGGGCTGCAGTATACTTGGCTGACCTACAAAAGGTAGCATATAATTTGGCTTCCCTATTAATATGTTGGCTTGCATGAATGGTTTTG
S_nsp4	GCTGA-TAAGTTTAA---TTAAAGGGCTGCAGTATACTTGGCTGACCTACAAAAGGTAGCATATAATTTGGCTTCCCTATTAATATGTTGGCTTGCATGAATGGTTTTG
S_ondinae	TCTGA-TATTTGATAA---TTGAAGGGCTGCAGTATACTTGGCTGACCTACAAAAGGTAGCATATAATTTGGCTTCCCTATTAATATGTTGGCTTGTATGAATGGTTTTG
S_parabenjamini	GCTGC---TTTCG---GTGAAGGGCTGCGGTATACCTTGGCTGACCTACAAAAGGTAGCATATAATTTGGCTTCCCTATTAATATGTTGGCTTGTATGAATGGTTTTG
S_tulumensis	TCTGAAATGGTTTTAGGGTTTTAAAGGGCTGCAGTATACTTGGCTGACCTACAAAAGGTAGCATATAATTTGGCTTCCCTATTAATATGTTGGCTTGAATGAATGGTTTTG
S_williamsi	??
S_xibalba	??
S_cf_tulumensis	TCTGAAATGGTTTTAGGGTTTTAAAGGGCTGCAGTATACTTGGCTGACCTACAAAAGGTAGCATATAATTTGGCTTCCCTATTAATATGTTGGCTTGAATGAATGGTTTTG
Campodea	ACGGAGTGTATACTTTTAAATAAT---TAA-TTGAATTTTAA-TAAAT-TAG-TGAAAAAGCTTAAAGATATGGATTAAGGGACGATAAAGACCCCTATATAGA
Penaeus	ACAAAAAGTAACTGTCTCAGTTTATA---TAG-TTGAACCTTAACT---TT-AAG-TGAAAAGGCTTAAATACCTTTAAAGGGGACGATAAAGACCCCTATATAA
Branchinella	ACGAGAAACTAGCTCTCTCTCTATTT---AC-TTGAAGTTTAA-TTATTT-TGG-TGAAAAGGCTTAAATTTCTCTAGAGGACGATAAAGACCCCTATATAA
Hutchinsoniella	ACGGTGTATCTGTCTCCTAATAA---TAT-TAAAAATTTACT-TT-AAG-TGAAAAGGCTTAAATAAAATCAATAAG-ACGACAAGACCCCTGTAGA
G_robustus	ACGARAAGTTGAATGTCTCTGTGTG---TTTA-TTGAATTTTAA-TTTTTT-TAG-TGAAGATGCTTGAATGTTGCTGAGGGACGAGAAGACCCCTGTAGA
Gn_fronodosus	ACGAGAAATVGAATGCTTAAATAGTAT---TTAA-GTGAATTTTACT-TTCTCG-TGAGGAGGCTAGAAATTTTGTGAGGGACGAGAAGACCCCTGTAGA
Gn_schrami	ACGAGAAATVGAATGCTTAAATAGTAT---TTTAT-ATGAAATTTTACT-TT-TAG-TGAAGAGGCTTAAATTTTAAAGGGACGAGAAGACCCCTGTAGA
P_apletocheles	ACAAGTGAATCTGTCTCTCAAAA---AGGG-TTGAATTTTCA-TTTTTC-TAG-TGAAGAGCTTGAATGAATCCAAAGGGACGAGAAGACCCCTATATAGA
P_fragilis	ACAAGGAGGAACTGTCTTTTGA---AATG-TTGAATTTTCA-TTTTTT-TAG-TGAGGAGGCTTAAATTTTAAAGGGACGAGAAGACCCCTATATAGA
M_yagerae	??
C_elmorei	ACAAGAGATCGACTGTCTGTGTGA---TATA-TTGAATTTTACT-TT-TC-TGG-TGAGGAGGCTTAAATTTTCCAAAGGGACGAGAAGACCCCTGTAGA
C_haptodiscus	ACAAGAGATYGACTGTCTTATAATA---TTGA-TTGAATTTTACT-TT-CC-AGG-TGAGGAGGCTTGGAYTTTTGCCAAAGGGACGAGAAGACCCCTGTAGA
C_longulus	??
L_entrichoma	ACAAGATGTTAGCTGTCTGTATAA---TAAATTTTAA-TTTTTA-TAG-TGAGTAGGCTTGGTATGTTTCTAAGKGCAGAAAAGACCCCTATATAGA
L_exleyi	ACAAGGATTAACCTGTCTCCATAATA---TTTGTATTTGAATTTTCAAT-TT-TGG-TGAAAGGGCCTTAAATTTTCCARGGGACGATAAAGACCCCTATATAGA
S_atlantida	ACAAGA TGTGACTGTCTATTTGTA---GTATA-TTATAATTTTACTCTTAAAG-TGAAAGGCTTAGGATGGTCTGAGGGACGAAAAGACCCCTATATAGA
S_benjamini	ACAAGAGATCTACTGTCTATAATA---TATA-TTGAATTTTACT-TT-CC-TT-TAG-TGAAAGGCTTAGGATTTAGCCAAAGGGACGAGAAGACCCCTGTAGA
S_emersoni	ACAAGATATGACTGTCTGTATTTAT---TGAC-TT-TAAATTTTACTTTTCTTAAAG-TGAAAGGCTTGGAAATGTAATTTGAGGGACGAGAAGACCCCTATAGA
S_gironensis	ACGAGA TATAA CTGTCTACTACGA---TACAATTTGAACCTTCTCCAAAG-TGAAAGGCTTGGATACAACTCCGGGGACGAAAAGACCCCTATATAGA
S_kakuki	ACGAGATGTTAGCTGTCTCCATTTAAATTAATA---TTTAA-TTGAAGGCTTAAATTTTAAAGGGACGAGAAGACCCCTATATAGA
S_lucayensis	ACGAGATATTAACCTGTCTCCATAATA---TTTTT-TTGAATTTTAA-TTTTTT-TAG-TGAAAGGCTTAGGATGTTTAAAGGGACGAGAAGACCCCTATATAGA
S_nsp2	ACGTGATCTACTGTCTCTGGCAATAATTTG---TTGAAATTTTACT-TT-TAG-TGAAAGGCTTAAATTTTCCAAAGGGACGAAAAGACCCCTATATAGA
S_nsp4	ACGTGATCTACTGTCTCTGGCAATAATTTG---TTGAAATTTTACT-TT-TAG-TGAAAGGCTTAAATTTTCCAAAGGGACGAAAAGACCCCTATATAGA
S_ondinae	ACAAGATTTAACCTGTCTATTTATAT---AATG-TTGAATTTTACTCTTAAAG-TGAAAGGCTTAGGATGGTCTGAGGGACGAAAAGACCCCTATATAGA
S_parabenjamini	ACAAGAGATCTGTCTCAGTATA---TAG-TTGAATTTTCAATTTTC--AG-TGAGGAGGCTTGGATTTTAAAGGGACGAGAAGACCCCTATATAGA
S_tulumensis	ATGAGATGTGATCTGTCTGTGTATA---GATA-TAGATAATTTTCACT-TG-TGG-TGAA TAGGAGGCTTAAATTTTCCCGAGGGACGAAAAGACCCCTGTAGA
S_williamsi	ACRAGATGTTMMCTGTCTCCWTTGTAA---ATGA-TTGAMATWCMCTT-CCTTAMKTGAAGGCTTAGGATGTTTGTGGGACGAAAAGACCCCTATATAGA
S_xibalba	??
S_cf_tulumensis	ATGAGATGTTGATCTGTCTGTGTATA---GATA-TAGATAATTTTCACT-TG-TGG-TGAA TAGGCAATAATTTTCCCGAGGGACGAAAAGACCCCTATATAGA

Table S4. (Continued.)

Species code	Sequence
Campodea	GCTAAATAT-----TTAAATAGA-----TTTATA-TTTTA-G-GTG-AAATAA-----TTATTTG-TTAAAG--
Penaeus	ACTTAAACA-----TAAATTTGATTAAT-----ATAAATG-TTA-G-TATAACT--TGAT-----TTTAA-TTA-ATGT--
Branchinella	CTTTTAA-----AATTAATATAG-----TTTATA-G---A-TG-TTAT--C-----AAGTTAATA-GGTT
Hutchinsoniella	ACTTCAATAC-----AAAAATTT-----AATA-TTA-G-TT-----CTTATAAA-----AAATTT
G_robustus	GTTTTAGTG-T-----GTATTT-----CAAT-----AAT-----GTGTAGTGT-A--TG-GT-----TATGTATATATATTT
Gn_fronodosus	TTTTTAGTA--A-TG-TTGT-----TTTTTGTGT-ATTT-----TTTTTGTGTGACTT-----ATTGAA-----T
Gn_schrami	TTTTTAAATA-TA-G-TTTT-----TTTTTGTGTGACTT-----TTTTTGTGTGACTT-----ATTGAA-----T
P_apletocheles	TTTTTAGTA-----AATTTGATTTAT-GATAG-----TTAG-----TTAAATGTTT-AAATTTGATC-----GACGG-AGAAGT-TTAGGCTGTT
P_fragilis	??
M_yagerae	TTTTTAGTA-----AATTTGATTTAT-GATAG-----TTAG-----TTAG-----TTAAATGTTT-AAATTTGATC-----GACGG-AGAAGT-TTAGGCTGTT
C_elmorei	TTTTTAAATTTCTTA-G-TTTTGTGGG-----ATT-GG-----TATTTGTTT-GG-TTTTGTGTTTAA-TA-----GTTGA-----GTATTTGAGTCTGTT
C_haptodiscus	??
C_longulus	TTTTTAAATTTCTTA-G-TTTTGTGGG-----ATT-GG-----TATTTGTTT-GG-TTTTGTGTTTAA-TA-----GTTGA-----GTATTTGAGTCTGTT
L_entrichoma	TTTTTAAATTTCTTA-G-TTTTGTGGG-----ATT-GG-----TATTTGTTT-GG-TTTTGTGTTTAA-TA-----GTTGA-----GTATTTGAGTCTGTT
L_xilvi	TTTTTAAATTTCTTA-G-TTTTGTGGG-----ATT-GG-----TATTTGTTT-GG-TTTTGTGTTTAA-TA-----GTTGA-----GTATTTGAGTCTGTT
S_atlantida	TTTTTAAATTTCTTA-G-TTTTGTGGG-----ATT-GG-----TATTTGTTT-GG-TTTTGTGTTTAA-TA-----GTTGA-----GTATTTGAGTCTGTT
S_benjamini	TTTTTAAATTTCTTA-G-TTTTGTGGG-----ATT-GG-----TATTTGTTT-GG-TTTTGTGTTTAA-TA-----GTTGA-----GTATTTGAGTCTGTT
S_emersoni	TTTTTAAATTTCTTA-G-TTTTGTGGG-----ATT-GG-----TATTTGTTT-GG-TTTTGTGTTTAA-TA-----GTTGA-----GTATTTGAGTCTGTT
S_gironensis	TTTTTAAATTTCTTA-G-TTTTGTGGG-----ATT-GG-----TATTTGTTT-GG-TTTTGTGTTTAA-TA-----GTTGA-----GTATTTGAGTCTGTT
S_kakuki	TTTTTAAATTTCTTA-G-TTTTGTGGG-----ATT-GG-----TATTTGTTT-GG-TTTTGTGTTTAA-TA-----GTTGA-----GTATTTGAGTCTGTT
S_lucyavensis	TTTTTAAATTTCTTA-G-TTTTGTGGG-----ATT-GG-----TATTTGTTT-GG-TTTTGTGTTTAA-TA-----GTTGA-----GTATTTGAGTCTGTT
S_nsp2	TTTTTAAATTTCTTA-G-TTTTGTGGG-----ATT-GG-----TATTTGTTT-GG-TTTTGTGTTTAA-TA-----GTTGA-----GTATTTGAGTCTGTT
S_nsp4	TTTTTAAATTTCTTA-G-TTTTGTGGG-----ATT-GG-----TATTTGTTT-GG-TTTTGTGTTTAA-TA-----GTTGA-----GTATTTGAGTCTGTT
S_ondinae	TTTTTAAATTTCTTA-G-TTTTGTGGG-----ATT-GG-----TATTTGTTT-GG-TTTTGTGTTTAA-TA-----GTTGA-----GTATTTGAGTCTGTT
S_parabenjamini	TTTTTAAATTTCTTA-G-TTTTGTGGG-----ATT-GG-----TATTTGTTT-GG-TTTTGTGTTTAA-TA-----GTTGA-----GTATTTGAGTCTGTT
S_tulumensis	TTTTTAAATTTCTTA-G-TTTTGTGGG-----ATT-GG-----TATTTGTTT-GG-TTTTGTGTTTAA-TA-----GTTGA-----GTATTTGAGTCTGTT
S_williamsi	TTTTTAAATTTCTTA-G-TTTTGTGGG-----ATT-GG-----TATTTGTTT-GG-TTTTGTGTTTAA-TA-----GTTGA-----GTATTTGAGTCTGTT
S_xibalba	TTTTTAAATTTCTTA-G-TTTTGTGGG-----ATT-GG-----TATTTGTTT-GG-TTTTGTGTTTAA-TA-----GTTGA-----GTATTTGAGTCTGTT
S_cf_tulumensis	TTTTTAAATTTCTTA-G-TTTTGTGGG-----ATT-GG-----TATTTGTTT-GG-TTTTGTGTTTAA-TA-----GTTGA-----GTATTTGAGTCTGTT
Campodea	TAAGATTAC--TGGGGCG-----TATAGTTTTTT-----TATAAGTTTTTT-----CAAAATTAAT--TGATA-GTTATTTGATCATATA--
Penaeus	TTTG--TTGCGTTGGGCGACGGGATATAATAGTAACTGTTCTTAAATATTTTTATTA-----CAAGTATA-----TTGAAGAATAATGATCCTTTTAT-T
Branchinella	TTTTATTTCTTTGGGCGACGTTGAGGTTGAGATATCTCAAAATGTTTTTA-----CATTAATA-----ATG-----ATTTTGTGATCCTTTAG--
Hutchinsoniella	TTGTATTTCTTTGGGTTGACCAAT-TTTAAATAATAAACAACCTTATTTTTA-----CATATATA-----ATGAAACTTTTATTTGACCCAGATAT
G_robustus	AT--TTTGGTTGGGCGATTAAT-TAAGATACGATCTTAAATTTAGTTTTAAAT-----CAGTGTGG-----CTG-AAATTTTATAGATCCATTTAT-T
Gn_fronodosus	AC--TTTGGTTGGGCGATTTTA-AAAGAAAATATCTTTTGTTTTAAATGTTTTAT-----CAATTAATTT--TTG-----TTTTTATTTGATCCAGAAT-T
Gn_schrami	AT--TTTAAATTTGGGCGATTTTA-AAAGAAAATATCTTTTAAATGTTTTAT-----CAATTAATTT--TTG-----TTTTTATTTGATCCAGAAT-T
P_apletocheles	AT--TTTGTCTGGGCGGACGTT--GAAGAAGAAGTATCTTTTAAATGTTTTAAAT-----CAGTGTGG-----ATG-----AGATTTGATCCTTTAT-T
P_fragilis	??
P_nsp	AT--TTTTTGTGGGTTGGCAGTT--GAAGATAAGAAATCTTTTAAAGT-----CATTTAGG-----GTG-----AATTTGATCCTTTAT-T
M_yagerae	??
C_elmorei	GATGTTTTAGCTGGGCGGCTGTG--AGAGAGACTGATCTTTTATTTGTTTGG-----CAATTTAGG-----ATG-----TTTGTGATCCTTTAT-T
C_haptodiscus	GATGTTTTAGCTGGGCGGCTGTG--GGAGATGAATATCTCTGTTTGTATTTGG-----CAATTTAGG-----TTG-----TATTTGATCCTTTAT-T
C_longulus	??
L_entrichoma	AT--TTTTTGTGGGCGGACGTT--GAGGAAGAGAAATCTTTTAAAGT-----CATTTAGG-----GTG-----AATTTGATCCTTTAT-T

Table S4. (Continued.)

Species code	Sequence
L_exleyi	AA---TTTAGCTGGGCGGCTATT--GAAGATGAGGATCTTTCAGT--TGATTTTGGG-----CAGATTTGT--TTG-----TAGTGATCCTTTTAT-T
S_atlantida	A--G--TTTTACTGGGCGGTAGTT--AAAGATATGGATCTTAGTTTGTAGTAAATG-----CACTGATGG--GTG-----TAATGATCCTTTTAT-T
S_benjamini	AT---TTTTGCTGGGCTGGTGT--GAAGATGAGAACTTTCAGTTTATTTGGTTGG-----CATTTTGA--GTG-----TAATGATCCTTTTAT-T
S_emersoni	A---TTTTACTGGGCGGCTATT--GAAGATCCGGATCTTCAAATTT--ASTGATATTT-----CATTTGTGG--GTG-----TAATGATCCTTTTAT-T
S_gironensis	G-----ATTTACTGGGCGGCTGTCTGAAGAAAATACTTCAAGTTATTAATAAT--CACCAAATCA--GTG-----ATCTGATCCTTTTAT-T
S_kakuki	AT---TTTTGCTGGGCGGCGGAT--GAAGATGTGAATCTTCAAGTTTGTAGAAG-----CATTTTGG--GTG-----TGGTGATCCTTTGTGT-T
S_lucayensis	AT---TTTTGCTGGGCGGCGGTT--GAATGTAAATGATCTTCAAGTTTGTATTTG-----CATTAGTGA--GTG-----TAATGATCCTTTTAT-T
S_nsp2	-----TT--GCTGGGCGGCTGT--GAAGATAAGAACTTTCAGTTTGTGTTG-----CGAAATTAATTTGTTG-----TTTTGTTGATCCTTTGATTT
S_nsp4	-----TTTTGCTGGGCGGCTGTT--GAAGATAAGAACTTTCAGTTTGTGTTG-----CAATTAAT--GTTG-----TTTTGTTGATCCTTTGATTT
S_ondinae	GA-G--TTTTACTGGGCGGCTAGTT--AAAGATATAGATCTTTAGTTTATTTAATTT-----CACTTATAA--GTG-----TTGGTATCCTTTTAT-T
S_parabenjamini	AT---TTTTACTGGGCGGCTGTT--GAAGATGAGGATCTTTCAGTTTATTTAATTT-----CACCCTGG--GTG-----TTTTG????????
S_tulumensis	AG-GTTTTACTGGGCGGCTATT--GAAGAAGTAGATCTTTCAGATTTTAAATGAGATTTACACTCACTTGTGG--GTA--TTTTGTTTTGATCCTTATATAT
S_williamsi	G---TTTTGCTGGGCGGCAAT--GAGGATTTTTTATCTTTCAGTTATTTAAT-----CATCTATGG--ATGT-----AATGATCCTTTTAT-T
S_xibalba	??
S_cf_tulumensis	AG-GTTTTACTGGGCGGCTATCT--GAAGAAGTAGATCTTTCAGATTTTAAATGAGGTTTAAATGAGGTTTACACTCACTTGTGG--GTA--TTTTGTTTTGATCCTTATATAT
Campeoda	---ATAAAAATTAGTAAAGTTACCTTAGGGAATAACAGTATAATTAATTTTGGAGATACAAAATTGACAAAATAG--TTTTATAA CCTCGATGTTGAAATFAAA
Penaeus	AAAGATTAATAAGATT--AAGTTACTTTTAGGGAATAACAGCGTAATCTTTTGGAGATCTCATCGACAAGAGG--TTTTGCGACCTCGATGTTGAAATTAAG
Branchinella	--TGA--ATTAGGATAAAGTTACCTTAGGGAATAACAGCGTAATTTTCTTTGAGAGTCTTATCGACAGAAAAG--TTTTCGACCTCGATGTTGGTTTCAGG
Hutchinsoniella	TCTGAT--AAAAGRAAGTTACCTCGGGGAATAACAGCGTAACTTTTCAAAGGACACATACTTCAAAGGACCTCGATGTTGGACTAAG
Gn_robustus	ATTTGGTTAAAGATC--AAATTACCTCAGGGAATAACAGCGTAACTTTTATGATTTTAAAGATTTCTTATCGATAATAAG--TTTTGCGACCTCGATGTTGAAATTAAG
Gn_fronodosus	TTTTGATTAATAAGATT--AAAAATACCTCAGGGAATAACAGCGTAACTTTTAAAGATTTTAAAGATTTCTTATTTGAAATAAAG--TTTTCGACCTCGATGTTGAAATTAAG
Gn_schrami	TTTTGATTAATAAGATT--AAAAATACCTCAGGGAATAACAGCGTAACTTTTAAAGATTTTAAAGATTTCTTATTTGAAATAAAG--TTTTCGACCTCGATGTTGAAATFAAA
P_apletocheles	ATGGATAGAAAGATAAAAATACCTTAGGGAATAACAGCGTAACTTTTGGAGATTCATATCGACAAGAGAG--ATTTGCGACCTCGATGTTGAAATTAAG
P_fragilis	??
P_nsp	ATGGATATAGAGATG--AAAAATACCTTAGGGAATAACAGCGTAACTTTTGGAGATTTAAAGATTTTAAAGATTTCTTATTTGAAATAAAG--TTTTCGACCTCGATGTTGAAATFAAA
M_yagerae	??
M_elmorei	??
C_haptodiscus	ATGGAT--ATAAGAA--AAAAATACCTCAGGGAATAACAGCGTAACTTTTGGAGATTCGATATCGATAGAGAAG--TTTTGCGACCTCGATGTTGAAATFAAA
C_longulus	ATGGATATTAAGAAA--AAAAATACCTCAGGGAATAACAGCGTAACTTTTGGAGATTCATATCGATAGGAAAAG--TTTTCGACCTCGATGTTGAAATFAAA
L_entrichoma	??
L_exleyi	ATGGATAATAAGGAG--TAAAATACCTTAGGGAATAACAGCGTAACTTTTCTGAGAGTTCTAATATTTCTGAGAGTTCTAATCGACAGATTA--TTTTGCGACCTCGATGTTGAAATTAAG
S_atlantida	ATGGATAATAAGATT--AAAAATACCTTAGGGAATAACAGCGTAACTTTTCTGAGAGTTCTAATATTTCTGAGAGTTCTAATCGATAGGGAAG--TTTTCGACCTCGATGTTGAAATTAAG
S_benjamini	ATGGATAATAAGATT--TAAAATACCTTAGGGAATAACAGCGTAACTTTTCTGAGAGTTCTAATATTTCTGAGAGTTCTAATCGATAGGTTTTCGACCTCGATGTTGAAATTAAG
S_emersoni	ATGGATAATAAGATT--AAAAATACCTTAGGGAATAACAGCGTAACTTTTCTGAGAGTTCTAATATTTCTGAGAGTTCTAATCGATAGTAAAG--TTTTGCGACCTCGATGTTGAAATTAAG
S_gironensis	ATGGATAATAAGAAA--AAAAATACCTTAGGGAATAACAGCGTAACTTTTCTCAAGGCCGATCGAAGAAATGA--TTTTGCGACCTCGATGTTGAAATTAAG
S_kakuki	ATAAATTAAGAAGAAA--AAAAATACCTTAGGGAATAACAGCGTAACTTTTCTGAGAGTTCTAATATTTCTGAGAGTTCTAATCGACAGATTTG--TTTTGCGACCTCGATGTTGAAATTAAG
S_lucayensis	ATGGAAAATAAGAGG--AAAAATACCTTAGGGAATAACAGCGTAACTTTTCTGAGAGTTCTAATATTTCTGAGAGTTCTAATCGACGATTAAG--TTTTGCGACCTCGATGTTGAAATTAAG
S_nsp2	ATGG??
S_nsp4	ATGGATAATAAGATA--AAAAATACCTTAGGGAATAACAGCGTAACTTTTCTGAGAGTTCTAATATTTCTGAGAGTTCTAATCGACAGGAAAAG--TTTTGCGACCTCGATGTTGAAATTAAG

