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A new molecular phylogeny-based taxonomy of parasitic barnacles (Crustacea: Cirripedia: Rhizocephala)

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Rhizocephalans are abundant members of marine ecosystems and are important regulators of crustacean host populations. Morphological and ecological variation makes them an attractive system for evolutionary studies of advanced parasitism. Such studies have been impeded by a largely formalistic taxonomy, because rhizocephalan morphology offers no characters for a robust phylogenetic analysis. We use DNA sequence data to estimate a new phylogeny for 43 species and use this to develop a revised taxonomy for all Rhizocephala. Our taxonomy accepts 13 new or redefined monophyletic families. The traditional subdivision into the suborders Kentrogonida and Akentrogonida is abandoned, because both are polyphyletic. The three 'classical' kentrogonid families are also polyphyletic, including the species-rich Sacculinidae, which is split into a redefined and a new family. Most species of large families remain to be studied based on molecular evidence and are therefore still assigned to their current genus and family by default. We caution against undue generalizations from studies on model species until a more stable species-level taxonomy is also available, which requires more extensive genus- and species-level sampling with molecular tools. We briefly discuss the most promising future studies that will be facilitated by this new phylogeny-based taxonomy.

ADDITIONAL KEYWORDS: development – host–parasite systems – larval transfer – molecular phylogeny – parasitism – taxonomy.

INTRODUCTION

Rhizocephalan barnacles are one of the most advanced groups of parasites in the Metazoa (Brusca *et al.*, 2017) and can occur with high prevalence on their mostly decapod hosts, where the prevalence can reach high levels. Owing to their profound influence on the infested animals, they can be important regulators of the affected populations (Høeg, 1995; Høeg & Lützen, 1995; Lafferty & Kuris, 1996; O'Brien, 1999; Thresher *et al.*, 2000; Waser *et al.*, 2016; Mouritsen *et al.*, 2018). The study of rhizocephalan biology also offers insight into adaptation to advanced parasitism in terms of, for example, sexual biology (Høeg, 1991; Yamaguchi *et al.*, 2014), larval strategies (Høeg, 1995; Martin *et al.*, 2014; Jensen *et al.*, 2019) and infestation and control of the host (Høeg, 1985, 1990; Glenner, 2001; Goddard *et al.*, 2005; Kristensen *et al.*, 2012; Larsen *et al.*, 2013). Rhizocephala comprise ~300 species and thus amount to ~18% of all cirripedes. Moreover, morphology, ontogeny and effects on the host vary extensively within rhizocephalans. Thus, they offer an opportunity to study evolutionary diversification within a highly successful group of parasites.

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To study host–parasite diversification, it is essential to have a phylogenetic framework (Brooks & McLennan, 1993; Legendre *et al.*, 2002). Unfortunately, phylogenetic hypotheses for Rhizocephala have been lacking owing to the highly reduced morphology of these parasites (Høeg *et al.*, 2009; Høeg *et al.*, 2019). Until settlement of the cypris larva on the potential host, rhizocephalans have a similar development to other cirripedes, but the subsequent parasitic stages differ fundamentally by lacking segmentation, appendages, sensory structures and almost all internal organs other than those concerned with reproduction (Høeg, 1992, 1995; Høeg & Lützen, 1995; Høeg & Møller, 2006). This leaves few characters of use for taxonomic purposes and virtually none that can be compared with other cirripedes. Thus, a morphology-based phylogenetic analysis is near impossible. After infection, the parasite enters an internal developmental phase, wherein it develops a ramified root system that infiltrates the host and serves as a nutritional uptake feeding device (Bresciani & Høeg, 2001; Miroliubov, 2017; Nagler *et al.*, 2017). Eventually, it erupts on the surface of the host with an external reproductive part, called the externa (Fig. 1). The characters traditionally used for intrinsic rhizocephalan taxonomy have almost exclusively been those relating to the externa, which offers few morphological features, resulting in an essentially formalistic system (Høeg & Lützen, 1985, 1996; Øksnebjerg, 2000). Larval ultrastructure, especially in the cyprid, does offer a reliable means for analysis and direct comparison with other cirripedes and can even be used to delineate monophyletic units formally (Høeg & Rybakov, 1992, 1996a, 2007; Glenner *et al.*, 2008, 2010; Martin *et al.*, 2014). But for the majority of taxa, the use of larval characters is impeded by a lack of data.

With few exceptions, the existing taxonomy of Rhizocephala is therefore not founded on any attempt at a phylogenetic approach. Not surprisingly, Rhizocephala was one of the first targets for using molecular methods in phylogenetic analysis (Spears *et al.*, 1994), and the few existing studies indicate that polyphyly is rampant at all systematic levels, whence the existing taxonomy provides little biological insight or is even directly misleading (Glenner & Hebsgaard, 2006; Glenner *et al.*, 2010). In this study, we offer a fundamentally revised taxonomy of the Cirripedia Rhizocephala based on molecular data and using the phylogeny presented herein combined with previous molecular phylogenetic studies (Glenner & Hebsgaard, 2006; Glenner *et al.*, 2010, 2020; Lützen *et al.*, 2016; Høeg *et al.*, 2019).

Recent taxonomic and phylogenetic studies

The only recent taxonomic reviews of all Rhizocephala are by Høeg & Lützen (1996) and Høeg *et al.* (2019), but also Høeg & Lützen (1985) and especially Øksnebjerg (2000) offer much valuable taxonomic information on regional faunas. All these studies are traditional in that they do not attempt to establish monophyletic taxa.

According to Martin & Davis (2001), Rhizocephala is a superorder within the infraclass Cirripedia (subclass Thecostraca, class Maxillopoda). The concept of the class Maxillopoda has since been abandoned, and here we consider Cirripedia as a subclass within the class Thecostraca and Rhizocephala as an order of the superorder Cirripedia. Rhizocephala is presently subdivided into two suborders: Kentrogonida with four families and Akentrogonida with six families (Fig. 2; Høeg *et al.*, 2019). Originally, Akentrogonida were viewed as the most 'primitive' rhizocephalans owing to the absence of the so-called kentrogon stage during infection of the host (Bocquet-Vedrine, 1961; Newman *et al.*, 1969). Although not backed by direct observation, the akentrogonid forms were therefore believed to infest *in situ*, without passing through the entirely endoparasitic phase first postulated by Delage (1884) and much later verified experimentally (Ritchie & Høeg, 1981; Glenner, 2001). If Bocquet-Vedrine's (1961) claim were true, the akentrogonid forms would constitute a paraphyletic assemblage, whereas the apomorphic presence of a kentrogon producing the internal phase would characterize a monophyletic Kentrogonida, but such cladistic arguments were not used at the time. Høeg (1990) showed that in Clistosaccidae, the cyprid infests the host by using an antennule to penetrate the integument and inject an endoparasitic stage without formation of a kentrogon stage (Fig. 1). Subsequently, Glenner & Høeg (1994) used, for the first time, a cladistic approach and discussed the potential autapomorphies for Rhizocephala as a taxon. They suggested that the replacement of a kentrogon with infection by antennular penetration is apomorphic and defines a monophyletic Akentrogonida (Fig. 1), whereas Kentrogonida were predicted to constitute a paraphyletic assemblage. Following this view, Høeg & Rybakov (1992) revised the Akentrogonida and listed potential apomorphies for the families, but left the kentrogonidan ones unchanged.

The advent of molecular techniques enabled a broad-based phylogenetic analysis, for the first time, using other barnacles as outgroups. Glenner & Hebsgaard (2006) performed the first analysis of intrinsic rhizocephalan relationships, which indicated that both the suborder Kentrogonida and several of its families and genera are polyphyletic. Most notably, it appeared that akentrogonid forms were nested within Sacculinidae (173 spp.), which was therefore rendered polyphyletic. Glenner *et al.* (2010) pursued this question further and showed that the akentrogonid forms at their disposition formed a monophyletic clade, but their analysis crucially lacked

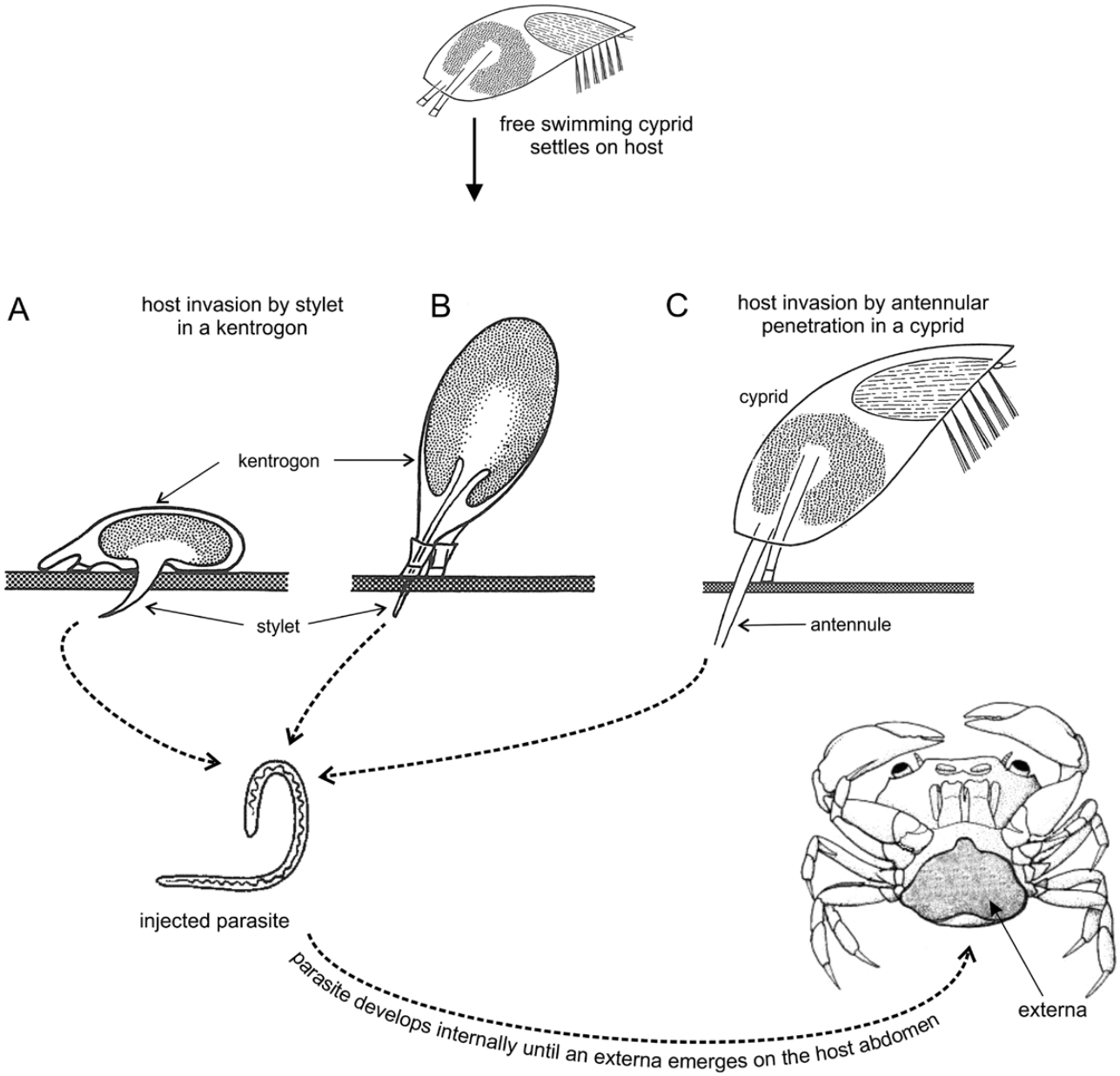

Figure 1. Generalized representation of host invasion in the Cirripedia Rhizocephala. A free-swimming cyprid settles on the prospective crustacean host. In the kentrogonid mode, the cyprid first moults into a kentrogon stage (A, B), which then uses a stylet to penetrate through the integument and inject the internal parasitic stage; two different kentrogon morphologies are illustrated (see Glenner & Høeg, 1994). C, in the akentrogonid mode, the cyprid does not form a kentrogon but penetrates into the host using one of its antennules. After injection, the parasite grows internally until a reproductive sac (externa) emerges on the host.

the monogeneric family Mycetomorphidae, which morphologically seems to combine traits from both rhizocephalan suborders (Glenner *et al.*, 1989; Høeg & Rybakov, 1996a, b, 2007). Høeg *et al.* (2019) recently addressed this problem in an analysis that included a wide range of rhizocephalan species. They found *Mycetomorpha* to be nested among kentrogonidan forms, and this entails that the Akentrogonida also become polyphyletic (at least diphyletic) in its present definition. In a few additional studies, Glenner *et al.* (2008), Lützen *et al.* (2016) and Glenner *et al.* (2020) addressed specific issues in Sacculinidae,

whereas Hiller *et al.* (2015) addressed species of the akentrogonid family Thompsoniidae. Most of the cited and molecularly based studies have shown that several existing taxa are not monophyletic, but none has taken taxonomic steps to reorganize taxonomy around this new phylogenetic framework. Rhizocephalan taxonomy therefore remains much at odds with the increasingly detailed phylogeny based on molecular analyses.

In the present study, we present a new molecularly based phylogeny of all Rhizocephala, including some kentrogonid species that have not been analysed before. Combining this phylogenetic estimate with insights from our previous studies (Glenner & Hebsgaard, 2006; Glenner *et al.*, 2010, 2020; Lützen *et al.*, 2016; Høeg *et al.*, 2019), we present a 'composite' phylogeny based on 43 rhizocephalan species and covering all but two of the existing rhizocephalan families. We then use this composite phylogeny to present a totally revised and phylogenetically based taxonomy of all Rhizocephala.

MATERIAL AND METHODS

Molecular analysis

For this new analysis, DNA was extracted from eight rhizocephalan species. Sequences from an additional 15 rhizocephalan species and three thoracican species used as outgroup were downloaded from GenBank (Table 1). Total genomic DNA was extracted using the Qiagen DNeasy Blood and Tissue kit (QIAGEN Inc., Valencia, CA, USA), following the manufacturer's protocols. Partial 18S gene sequences were amplified using polymerase chain reaction (PCR) carried out on a Bio-Rad C1000 Thermal Cycler using Takara polymerase in 25 µL reactions using the primer pair 18S-329 (CAGCMGCCGCGGTAATWC) and 18S-328 (CCTGGTTGATCCTGCCAG) (Spears *et al.*, 1994). The PCR products were sequenced in both directions using the PCR primers and two internal primers [18S-A– (CAGCMGCCGCGGTAATWC) and 18S-B+ (ATTCCCCGTTACCCG)] to obtain the entire ~1800 bp PCR product.

Table 1. Taxa included in the phylogenetic analyses of the 18S gene, with list of GenBank accession numbers, host species and sample location

Species	GenBank number	Host	Region
Briarosaccus auratum	MN650344*	*Lithodes aequispinus*	Alaska
Chthamalophilus delagei	GU190696	*Balanus improvisus*	France
Clistosaccus paguri	GU190697	*Pagurus bernhardus*	Sweden
Cyphosaccus norvegicus	MN650337*	*Munidopsis serricornis*	Norway
Galatheascus striatus	MN650343*	*Galathea dispera*	Norway
Heterosaccus californicus	AY265359	*Loxorhynchus grandis*	California
Lernaeodiscus ingolfi	MN650338*	*Munida sarsi*	Norway
Lernaeodiscus porcellanae	DQ826569	*Petrolisthes cabrilloi*	California
Loxothylacus texanus	L26517	*Callinectes sapidus*	Gulf of Mexico
Parthenopea subterranea	DQ826566	*Callianassa tyrrhena*	Croatia
Peltogaster curvata	MN650342*	*Pagurus prideauxi*	Norway
Peltogaster paguri	DQ826570	*Pagurus bernhardus*	Sweden
Peltogasterella sulcata	DQ826572	*Pagurus cuanensis*	Sweden
Polyascus planus	AY265368	*Grapsus albolineatus*	Taiwan
Polysaccus japonicus	DQ826565	*Callianassa japonica*	Japan
Sacculina carcini	AY265366	*Carcinus maenas*	Sweden
Sacculina oblonga	AY265367	*Cyclograpsus intermedius*	Japan
Septosaccus rodriguezii	DQ826571	*Clibanarius erythropus*	Sardinia
Sylon hippolytes	DQ826564	*Pandalus* sp.	Canada
Thompsonia littoralis	DQ826573	*Leptodius exaratus*	Singapore
Tortugaster boschmai	MN650341*	*Munida sarsi*	Norway
Triangulus galatheae	MN650340*	*Galathea intermedia*	Norway
Triangulus munidae	MN650339*	*Munida sarsi*	Norway
Ibla quadrivalvis	AY520655	–	Tasmania
Poecilasma inaequilaterale	AY520654	–	Gulf of Mexico
Semibalanus balanoides	AY520626	–	Denmark

The generic names are those before the revision carried out in the present paper.
*Accession numbers of new sequences.

Table 2. The families of Rhizocephala accepted in this study

Family	Number of genera	Number of species	Hosts	Comment
Triangulidae	1	4	Anomura	**fam. nov.**
Mycetomorphidae	1	2	Caridea	
Peltogastridae	13	48	Anomura	Redefined
Peltogasterellidae	4	8	Anomura	**fam. nov.**
Parthenopeidae	1	2	Thalassinidea	–
Sacculinidae	6	175	Brachyura Calianassoidea	Redefined
Polyascidae	2	8	Brachyura Gebiidea	**fam. nov.**
Clistosaccidae	2	2	Paguroidea Caridea	–
Thompsoniidae	4	24	Brachyura Anomura Caridea Stomatopoda	–
Polysaccidae	1	2	Callianassidae	–
Chthamalophilidae	3	4	Balanomorpha	–
Duplorbidae	3	5	Isopoda Cumacea	–
Pirusaccidae	1	1	Galatheidae	**fam. nov.**

All the families are monophyletic and, except for Duplorbidae and Pirusaccidae, they have been defined or confirmed using molecularly based phylogenetic analysis. Species numbers have been compiled from WoRMS (2019). Especially for Sacculinidae and Polyascidae, assignment of presently recognized species to genus and family will be subject to future molecularly based analysis.

DNA sequences were aligned in GENEIOUS R11 using the Muscle algorithm (Edgar, 2004), and ambiguous regions were excluded using GBLOCKS (Castresana, 2000) under less stringent settings. A phylogenetic analysis of the dataset was conducted using Bayesian inference of phylogeny. JModelTest2 (Darriba *et al.*, 2012) selected GTR+I+G as the best-fitting nucleotide substitution model (Posada & Crandall, 1998). Bayesian inference was conducted in MrBayes v.3.2.2 (Ronquist *et al.*, 2012), with priors set according to the suggested model. Two independent runs, using four Metropolis-coupled Markov chain Monte Carlo analyses, were performed. The chains were run for ten million generations and sampled every 500 generations. The first 25% of generations were discarded as burn-in, and a 50% majority-rule consensus tree was obtained from the remaining saved trees. Nodal confidence was measured with posterior probabilities calculated from the post burn-in tree distribution. The average standard deviation of split frequencies was checked for convergence towards zero, and MrBayes parameter files were examined in TRACER v.1.6 (Rambaut *et al.*, 2018) to assess whether runs had reached a stationary phase and converged on model parameters.

The composite phylogeny

Our revised taxonomy is based on the composite tree, constructed manually from our 18S phylogenetic estimate, including our new data and the trees provided by Høeg *et al.* (2019), Glenner *et al.* (2020) and Lützen *et al.* (2016). The latter three studies were all based on multiple genes, whereas the new tree is based on the 18S gene. The new analysis is nevertheless used here, because it includes some important species that have not been sequenced before and provides crucial information, especially at the base of the rhizocephalan tree. All four analyses used for our 'composite phylogeny' agree in all major aspects. Where minor differences exist, we follow the topology in the three multigene studies. We also collapse some topologies with low support. We emphasize that none of the few disagreements between the analyses used to construct the composite tree affects the monophyly of the revised families. Terminologically, we do not distinguish between polyphyly and paraphyly, but instead occasionally use the term 'not monophyletic' for such taxa.

Taxonomic revision

In our revision, we recognize only monophyletic families and only when they have high support in our new analysis or the comprehensive trees in the studies by Glenner *et al.* (2010, 2020) and Høeg *et al.* (2019). The families recognized here are principally based on the molecular analysis (Table 2). For future studies,

this implies that if molecular data show that a species is nested in one of the families listed here, it should be included in that taxon. The same applies if it is shown to be the sister group to a particular existing family, unless compelling morphological distinctness argues for the retention or erection of a separate family, as is presently the case for the Mycetomorphidae, for example. Fortunately, the majority of the new or revised families can be identified both by molecular evidence and by putative morphological apomorphies, and for two families where molecular data is lacking we diagnose by morphological evidence alone. A few families are still not easy to diagnose and separate based on morphology. This is not different from the existing taxonomy (Høeg & Lützen, 1996; Øksnebjerg, 2000), and future studies should attempt to find morphologically based apomorphies for these. Detailed use of histology of the externa, as described by Yoshida *et al.* (2011, 2015), and evidence from the ultrastructure of the larvae, as described by Glenner *et al.* (2010), will most probably assist in this venture.

Our tree contains considerable structure above the family level. But both the structure of the tree and some weakly supported nodes convinced us that it is impractical and unwise to maintain or create suprafamilial categories. At the genus level, more species need to be sequenced and analysed in order to arrive at a more phylogenetically based taxonomy. Therefore, we revise existing genera only if the species are contained in more than one of the redefined or new families, forcing us to take steps. Accordingly, we also accept some paraphyletic genera, as long as all species are contained within a monophyletic family. Finally, by the same conservatism, we keep within their present genera all species that have not yet been sequenced, a solution pending future analyses.

RESULTS

We present the new analysis of all Rhizocephala together with the present generic, family and suborder assignments of the analysed species in Figure 2. The monophyly of Rhizocephala (Fig. 2 node a) is well established based on multiple studies (Pérez-Losada *et al.,* 2004, 2008; Glenner & Hebsgaard, 2006). Both these molecular analyses and the larval morphological evidence presented by Høeg & Kolbasov (2002) also agree that the Rhizocephala and Thoracica are sister groups, with the burrowing Acrothoracica being the earliest branch on the tree of the Cirripedia. The monophyly of the Rhizocephala is supported by several morphological characters (Glenner & Høeg, 1994), especially with respect to the ontogeny of the earliest internal stages. Our 'composite tree' agrees with the monophyly of the Rhizocephala and provides a revised genus and family scheme for the group (Fig. 3).

The demise of Kentrogonida and Akentrogonida

Akentrogonidan forms are nested within the Kentrogonida, rendering the latter polyphyletic (Fig. 2). Furthermore, none of the three traditional families of the Kentrogonida (Peltogastridae, Lernaeodiscidae and Sacculinidae) is monophyletic, and the same is true for the genera *Triangulus* and *Sacculina* within these families. This necessitates a fundamental revision of rhizocephalan taxonomy, not least because the family Sacculinidae and the genus *Sacculina* are by far the most species-rich groups of the order.

The representatives of Akentrogonida, marked 'A', are not directly polyphyletic (Fig. 2). But Høeg *et al.* (2019) used molecular methods to show that the akentrogonid genus *Mycetomorpha* (family Mycetomorphidae) is not recovered with the remaining species of that group and instead is situated in a cluster of peltogastrids (Fig. 3, node 5). This shows that the Akentrogonida is at least diphyletic in its current definition (Høeg & Lützen, 1996; Glenner *et al.*, 2010), and the tree in Figure 2 even suggests that the akentrogonids might have evolved several times. On the available evidence, we conclude that the Akentrogonida evolved homoplastically at least twice, and we therefore choose to abandon both Akentrogonida and Kentrogonida as formal taxa. The terms 'kentrogonid' and 'akentrogonid' are still useful, but henceforth should be used only to indicate the morphological type of metamorphosis without any taxonomic implications (Fig. 1).

The rhizocephalan families

At the base of the tree, the lernaeodiscid species *Triangulus munidae* Smith, 1906 is, with high support, sister to all the remaining Rhizocephalans (Fig. 2, node b). This interesting result alone renders Lernaeodiscidae polyphyletic, but also the remaining lernaeodiscids are nested in two different positions within a clade that also includes several peltogastrid taxa (Fig. 2, node c). The peltogastrid genera *Peltogasterella* and *Cyphosaccus* do not group with this peltogastrid–lernaeodiscid cluster. Instead, these two genera sit together (Fig. 2, node e) within another, strongly supported clade (Fig. 2, node d) that also comprises two separate sacculinid clusters (Fig. 2, nodes f and g) and all akentrogonid forms used in this analysis. Likewise, Glenner & Hebsgaard (2006) found that *Peltogasterella* was separated from other peltogastrid species.

Our analysis clearly shows that *Triangulus munidae* (Fig. 2, node b) warrants recognition as a new family.

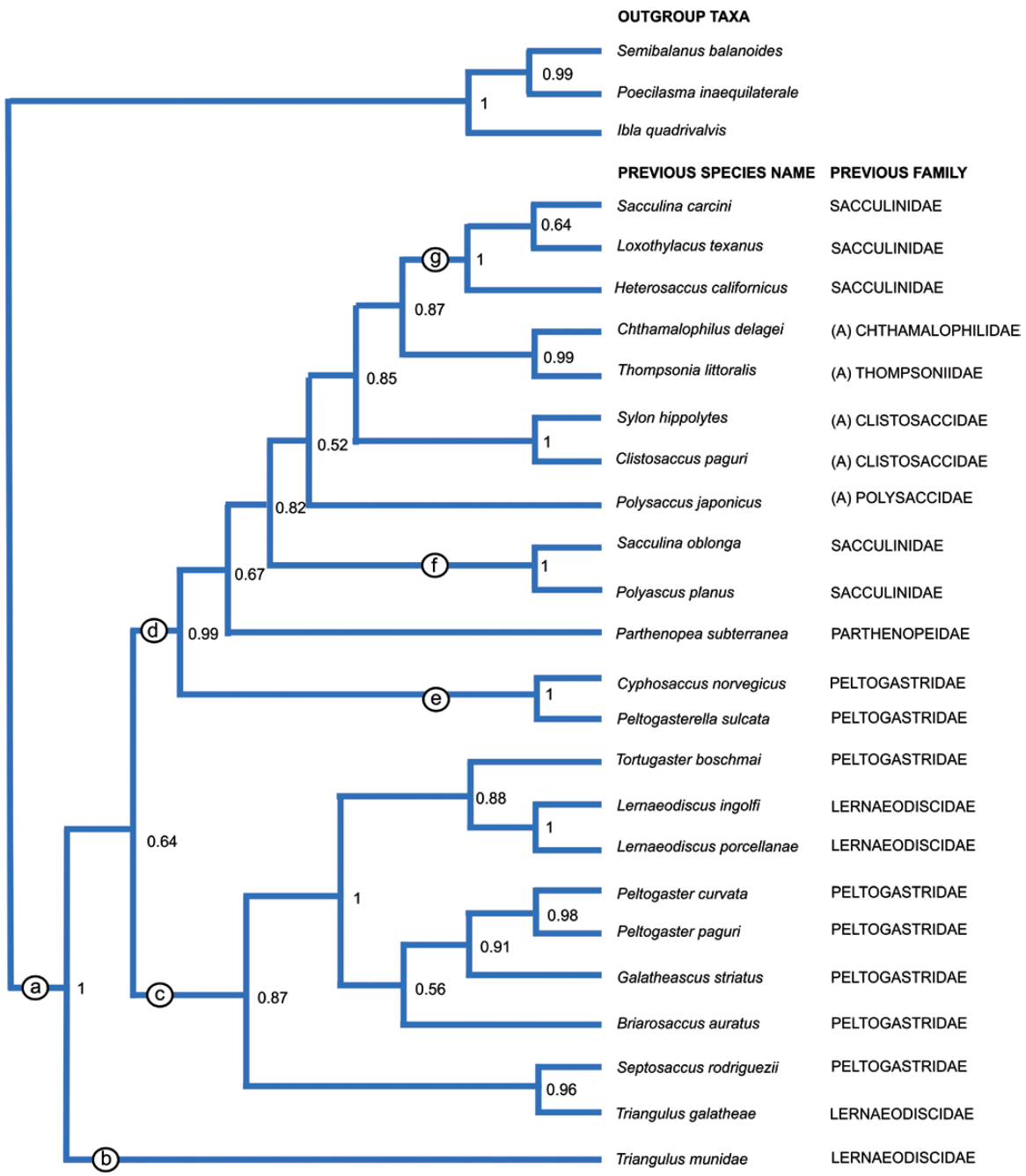

Figure 2. Molecular-based phylogenetic analysis of all Rhizocephala. The numbers show posterior probabilities for the nodes. Nodes marked by letters are discussed in the text. The columns on the right indicate the current species names and their affiliation to families before the present revision. '(A)' indicates a family until now relegated to the Akentrogonida. None of the families Peltogastridae, Lernaeodiscidae and Sacculinidae is monophyletic, and the same is true for the genera *Triangulus* and *Sacculina*. See Figure 3 for an extended phylogeny with revised family and genus names.

Lernaeodiscidae must be abandoned as a result of being polyphyletic. Instead, the most reasonable taxonomic step at present is to redefine Peltogastridae to include the numerous peltogastrids and former lernaeodiscids collected under Figure 2, node c, even if this clade is somewhat weakly supported. A new family must be erected for the former peltogastrid genera *Peltogasterella* and *Cyphosaccus* (Fig. 2, node e). Within the Figure 2, node d cluster, the exact position of the non-mycetomorphid akentrogonids varies slightly between

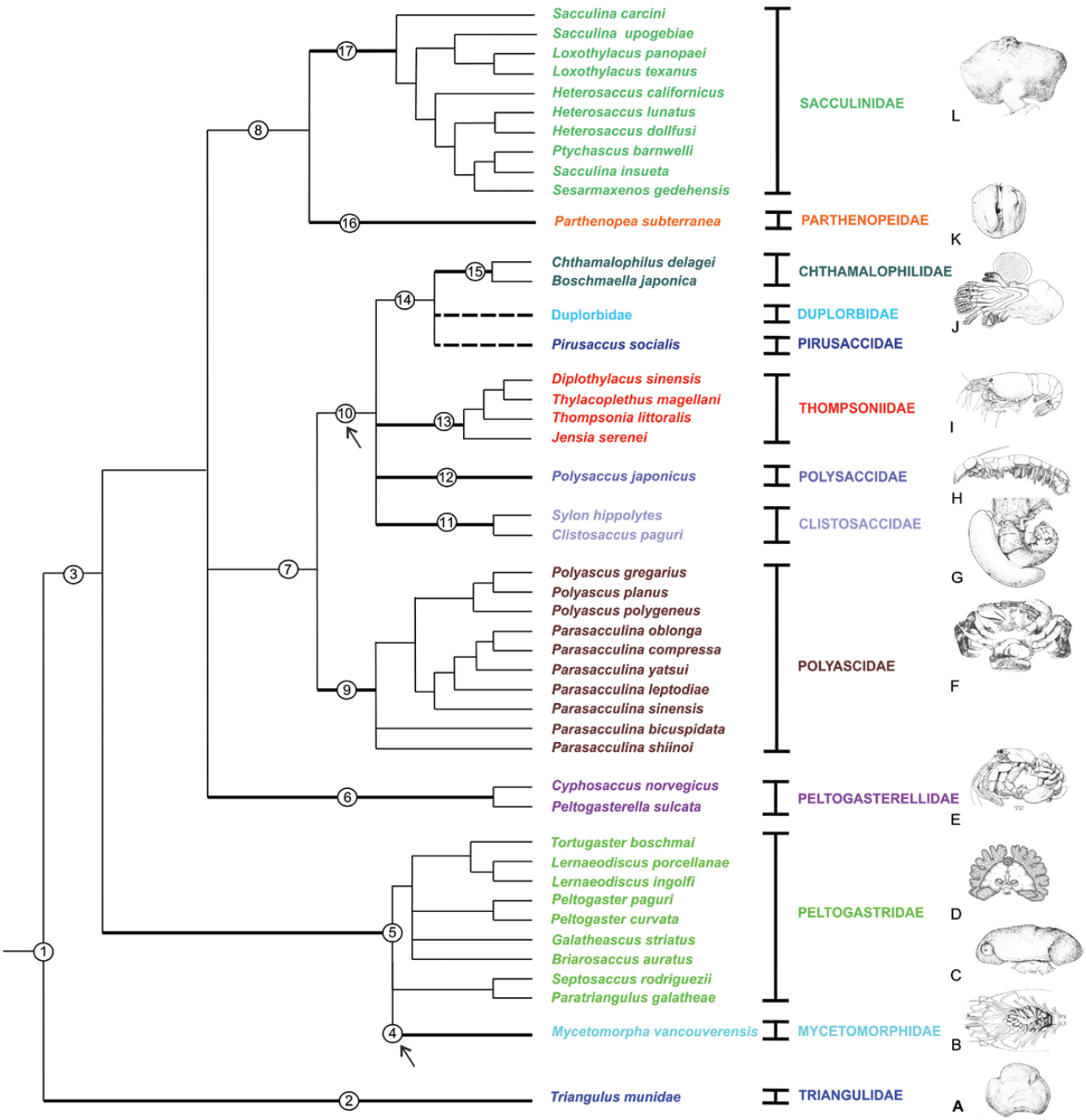

Figure 3. The revised taxonomy of Rhizocephala projected onto a composite phylogeny based on the tree in Figure 2 and extended with the analyses by Glenner *et al.* (2010; 2020), Lützen *et al.* (2016) and Høeg *et al.* (2019). Numbers represent the nodes and branches discussed in the text. Thick lines lead to family-level taxa. Triangulidae and Pirusaccidae are monotypic families. Each of the families Parthenopeidae, Mycetomorphidae and Polysaccidae is monogeneric and contains only two species each. *Mycetomorpha* is a close relative of peltogastrid species, but its precise position in that clade is unsettled. Families under node 10 represent the former 'akentrogonids' exclusive of Mycetomorphidae. Duplorbidae and Pirusaccidae (dashed lines) have not yet been subjected to molecular analysis, but there is solid morphological data for a close affinity with Chthamalophilidae. Arrows point to the origin of akentrogonid host invasion. Thumbnail drawings show externae of selected species: A, *Triangulus munidae*; B, *Mycetomorpha vancouverensis* on host; C, *Peltogaster paguri*; D, *Lernaeodiscus porcellanae* on host; E, *Peltogasterella gracilis* on host; F, *Polyascus polygeneus* on host; G, *Clistosaccus paguri* on host; H, *Polysaccus japonicus* Høeg & Lützen, 1993 on host; I, *Thylacoplethus edwardsii* Coutière, 1902; J, *Chthamalophilus delagei* on host; K, *Parthenopea mediterranea* Kossman, 1874; L, *Sacculina carcini*.

the several analyses used here, but they all agree that Sacculinidae as presently defined forms two clades that are not closely related (Fig. 2, nodes g and f). Therefore, Sacculinidae must be redefined to comprise all forms under node f, whereas the other sacculinid clade (node g) should be treated as a new family. These two clusters of 'sacculinid' species had slightly variable sister-group relationships in the analyses available until now, but they form a monophyletic unit. The remaining families can stay unchanged in terms of taxon composition, but for some we offer amended diagnoses below.

As shown in our composite tree (Fig. 3) and based on molecular evidence, we recognize the following new or redefined families of the Rhizocephala, with the numbers indicating nodes or branches: Triangulidae (2, new family), Mycetomorphidae (4), Peltogastridae (5, amended), Peltogasterellidae (6, new family), Polyascidae (9, new family), Clistosaccidae (11), Polysaccidae (12), Thompsoniidae (13), Chthamalophilidae (15), Parthenopeidae (16) and Sacculinidae (17, amended). As discussed below, only Mycetomorphidae and Polysaccidae have a somewhat uncertain status. Although not yet analysed by molecular methods, we also maintain Duplorbidae and create a new family to comprise the genus *Pirusaccus*. There is good morphological evidence that these two groups are close allies of Chthamalophilidae, but they lack several crucial apomorphies for this family.

SYSTEMATIC REVISION

ORDER RHIZOCEPHALA MÜLLER, 1862

Diagnosis (amended): The monophyly is assured by the molecularly based analyses of Glenner & Hebsgaard (2006) and Pérez-Losada *et al.* (2008). In addition, the following morphology-based apomorphies apply. All stages, including the nauplii and cyprids, lack any trace of an alimentary canal. The cyprids carry an aesthetasc seta and four additional setae on the fourth antennular segment. The male cyprids also carry a similar, but longer aesthetasc proximally on the third antennular segment. These are all parasites on Crustacea with a life cycle involving an initial and completely internal phase. This starts as a stage featuring an epithelium-enveloped bladder (primordium) containing a small lump of cells (nucleus) that represent the prospective visceral sac with the ovary. The adult parasite consists of an internal ramifying and nutrient-absorbing root system and an external sac (externa) housing the reproductive organs. All parasitic stages are without any segmentation or appendages.

Remarks: The morphological diagnosis is derived from Glenner & Høeg (1994) and Høeg & Rybakov (1992). The setation pattern of the antennules is subject to modification and loss within the order (Høeg & Rybakov, 1996a). A detailed account of the biology and morphology is given by Høeg (1995), Høeg & Lützen (1995) and Høeg *et al.* (2005). Larval morphology and development are treated by Høeg (1985, 1987, 1990, 1992), Martin *et al.* (2014), Glenner *et al.* (1989, 2010) and Glenner (2001). The existing general taxonomy is treated by Høeg & Lützen (1985, 1996), Høeg & Rybakov (1992), Øksnebjerg (2000) and Høeg *et al.* (2019).

FAMILY **TRIANGULIDAE** HØEG & GLENNER **FAM. NOV.** (MONOGENERIC)

Type genus: Triangulus Smith, 1906.

Type species: Triangulus munidae Smith, 1906.

LSID: urn:lsid:zoobank.org:act:A57984C8-7B8A-420F-AD6C-45E62B5445C8

Diagnosis: By the molecular data. Externa asymmetrical or symmetrical. Dorsal mesentery generally broad, ventral mesentery narrower and shorter. Receptacles, receptacle ducts and colleteric glands arranged asymmetrically within visceral mass. Receptacle ducts not ending in small papillae.

Hosts: Anomura, Galatheoidea.

Genera: Triangulus (four spp.). The two species of *Triangulus* included here (*Triangulus munidae* and '*Triangulus*' *galatheae*) do not form a monophyletic group. '*Triangulus*' *galatheae* is positioned within our redefined Peltogastridae, and we therefore (see p. 10) transfer it to a new genus. The type species, *Triangulus munidae*, is placed with high support as sister to all other Rhizocephala. Owing to its basal position, *Triangulus munidae* and other assured members (see below) of this new family will be crucial for tracing character evolution within all Rhizocephala. The World Register of Marine Species (WoRMS, 2019) lists four additional species of *Triangulus*. Of these, *Tortugaster boschmai* (Brinkmann, 1936) was already transferred from *Triangulus* to that genus in Peltogastridae (in its old definition) by Høeg & Lützen (1985); this view was seconded by Øksnebjerg (2000) and is upheld here. The remaining three species are *Triangulus bilobatus* (Boschma, 1925), *Triangulus cornutus* (Boschma, 1935) and *Triangulus papilio* (Kossman, 1872), which were transferred from *Lernaeodiscus* to *Triangulus* by Van Baal (1937). They are here by default left in *Triangulus* in Triangulidae, but with confirmation of this position subject to a future analysis. A new record of *Triangulus*

cf. *munidae* recently sampled from New Zealand might well prove to represent a new species in the family.

Remarks: The new family is based principally on molecular evidence. The morphological diagnosis is amended from that given for the genus *Triangulus* by Øksnebjerg (2000). A future analysis should search for a more robust diagnosis based on morphological apomorphies.

Family Peltogastridae Lilljeborg, 1861, amended

Type genus: Peltogaster Rathke, 1842.

Type species: Peltogaster paguri Rathke, 1842.

Diagnosis: By the molecular data. Externae never colonial; externa shape variable, elongated and sometimes tortuous, spherical to ovoid or more or less compressed dorsoventrally. Stalk issuing from between middle of dorsal side to near posterior extremity. Mantle opening at other end in elongate forms; in compressed forms, situated either in the midline or distinctly displaced to the left or right side. Visceral mass normally fused broadly with mantle. Colleteric glands normally simple, sometimes subdivided tubes. Paired receptacles with straight or tortuous ducts. Disposition of colleteric glands and receptacles either bilaterally symmetrical or asymmetrical.

Hosts: Anomura, Galatheoidea and Paguroidea; Gebiidea; Caridea.

Genera: Briarosaccus Boschma, 1930 (four spp.), *Dipterosaccus* Van Kampen & Boschma, 1925 (two spp.), *Galatheascus* Boschma, 1929 (two spp.), *Lernaeodiscus* Müller, 1862 (eight spp.), *Ommatogaster* Yoshida & Osawa, 2011 (one sp.), *Paratriangulus* gen. nov. (one sp.), *Peltogaster* (16 spp.), *Pterogaster* Van Baal, 1937 (two spp.), *Septodiscus* Van Baal, 1937 (one sp.), *Septosaccus* Duboscq, 1912 (four spp.), *Temnascus* Boschma, 1951 (one sp.), *Tortugaster* Reinhard, 1948 (three spp.), *Triangulopsis* Guerin-Ganivèt, 1911 (one sp.) and *Trachelosaccus* Boschma, 1928 (one sp.).

Remarks: The family comprises the taxa that originate at Figure 3, node 5. The diagnosis is adapted from Øksnebjerg (2000) to accommodate also former members of Lernaeodiscidae now included in the redefined family. Monophyly of Peltogastridae as here conceived is supported by the molecular analysis, but it is clearly the morphologically most variable taxon in Rhizocephala. Lilljeborg, who erected the family, published his taxonomic papers on rhizocephalans in identical form in several journals and languages, including private reprints (see, e.g. Høeg 1982). The citations used here are the most recently updated ones from the WoRMS (2019) database, as recommended by C. Boyko. The redefined family includes all former peltogastrids and lernaeodiscids, except for those transferred to the new families Triangulidae and Peltogasterellidae. These are parasitic on Anomura and Caridea (only *Trachelosaccus*), but never on Brachyura. Although not included in our analysis (Fig. 1), Yoshida *et al.* (2011) used molecular methods to show that *Ommatogaster* and *Dipterosaccus* are sister groups, and this clade is, in turn, sister to a species of *Peltogaster*. This indicates that both genera belong in Peltogastridae as presently defined. *Pterogaster*, *Septodiscus*, *Temnascus* and *Triangulopsis* have not yet been subjected to a molecularly based phylogenetic analysis but are retained in the redefined family pending future analysis. These genera are morphologically rather "close" to the peltogastrid forms included in the present analysis. The monotypic *Trachelosaccus* is also retained in the family, although this little-known form has a somewhat unusual morphology and differs, like Mycetomorphidae, in parasitizing Caridea (Høeg & Lützen, 1985).

Peltogasterella and *Cyphosaccus* are positioned higher in the tree and are therefore transferred to a new family (defined on p. 11). The monotypic genus *Angulosaccus*, formerly in Peltogastridae, is also transferred to this new family. Except for *Triangulus munidae*, species of the former Lernaeodiscidae as listed by Boyko & Harvey (2000) are included in our redefined Peltogastridae (viz. *Tortugaster*, *Lernaeodiscus*, *Paratriangulus* syn. *Triangulus*, *Triangulopsis* and *Septodiscus*. It seems clear that the diagnostic characters for the former Lernaeodiscidae (see Boyko & Harvey, 2000; Øksnebjerg, 2000) arose by convergence. A forthcoming phylogenetic analysis by molecular methods will include many members of the large genus *Peltogaster* and also the king crab (Lithodidae)-infesting genus *Briarosaccus*. This will entail changes at both species and generic levels, but the family as here defined will remain monophyletic (C. Noever, in preparation).

Genus *Paratriangulus* Høeg & Glenner **gen. nov.** (monotypic)

LSID: urn:lsid:zoobank.org:act:2F5CAA41-49D1-4D26-9EC4-F33D31537E0C

Type species: Paratriangulus (syn. *Triangulus*) *galatheae* (Norman & Scott, 1906) comb. nov.

Diagnosis: By the molecular data. Morphological definition as provided by Øksnebjerg (2000) for the type species

Remarks: The creation of this genus is required, because the two molecularly analysed species of *Triangulus* now fall into two separate families: Triangulidae and Peltogastridae. As explained above, the three remaining species of *Triangulus* are by default left in that genus and within the new family Triangulidae, pending future analysis.

Family Mycetomorphidae Høeg & Rybakov, 1992 (monogeneric)

Type genus: *Mycetomorpha* Potts, 1912.

Type species: *Mycetomorpha vancouverensis* Potts, 1912.

Diagnosis: Morphological diagnosis as provided by Høeg & Rybakov (1992), amended here by the following apomorphies: disc-shaped externa with numerous marginal branches; mantle aperture as a mesenteric canal, but remaining closed until oviposition; cyprids carry a terminally sited and quadrifid seta on the fourth antennular segment; dwarf males injected through the integument of the female, where they increase in size and secrete a distinct cuticle-like sheath around their body.

Hosts: Caridea.

Genera: *Mycetomorpha* (two spp.).

Remarks: The genus contains only two species, of which *Mycetomorpha albatrossi* Høeg & Rybakov, 1996b is poorly known, but the shape of the externa in this genus is unique (Høeg & Rybakov, 1996b). The family was previously placed in the now abandoned Akentrogonida, but Høeg *et al.* (2019) clearly placed *Mycetomorpha vancouverensis* as sister group to *Peltogaster paguri* and *Lernaeodiscus porcellanae* Müller, 1862. Compared with our analysis, the position in the study by Høeg *et al.* (2019) therefore shows that *Mycetomorpha* is located somewhere among the taxa clustered under node 4 in Figure 3. Until a more refined analysis including more former peltogastrids and lernaeodiscids is available, we choose to retain this morphologically unusual genus as a separate family.

Family **Peltogasterellidae** Høeg & Glenner **fam. nov.**

Type genus: *Peltogasterella* Krüger, 1912.

Type species: *Peltogasterella sulcata* (Lilljeborg, 1859).

LSID: urn:lsid:zoobank.org:act:43B04CD0-B6CF-4FAA-89DA-E8A0CD422EFC

Diagnosis: By the molecular data. Cylindrical or semicylindrical colonial externae all connected to a common internal root system; externae produce only one or two broods (Høeg & Lützen, 1985; Yoshida *et al.*, 2015) and are then replaced by a new generation of externae that do not develop in a one-to-one relationship to the preceding ones.

Host: Paguridae, Galatheidae.

Genera: *Angulosaccus* Reinhard, 1944 (one sp.), *Boschmaia* Reinhard, 1958 (one sp.), *Cyphosaccus* Reinhard, 1958 (four spp.), *Peltogasterella* (three spp.).

Remarks: The family comprises the taxa that originate at node 6 in Figure 3, and also *Boschmaia* and *Angulosaccus*. There are no molecular data for *Boschmaia*, but its close morphological similarity to *Cyphosaccus* argues for its placement here. The same argument applies to *Angulosaccus*. With this composition, the Peltogasterellidae contain all former peltogastrid species with colonial externae (Reischman, 1959), and we suggest that this represents an apomorphy for the new family. Colonial externae evolved elsewhere in Rhizocephala, but not with the morphological characteristics found here. It is noteworthy that Lützen *et al.* (2009) found *Boschmaia munidicola* Reinhard, 1958 in New Zealand waters, far removed from the type locality in the Caribbean. It is therefore questionable whether more than one species exists in this deep-water genus, exactly as these authors concluded for *Parthenopea* Kossmann, 1874. Colonial externae are not confined to Peltogasterellidae (see Discussion), but it still represents an apomorphy for the family at this level. Owing to the ingenious studies of Ryuzu Yanagimachi, *Peltogasterella gracilis* (Boschma, 1927) was the first rhizocephalan in which the presence of separate sexes and cryptic dwarf males was demonstrated. These findings entered all invertebrate and parasitological text books (Ichikawa & Yanagimachi, 1957, 1958; Yanagimachi, 1960, 1961a, b) and inaugurated modern research on the order.

Family Parthenopeidae Rybakov & Høeg, 2013 (monogeneric)

Type species: *Parthenopea subterranea* Kossmann, 1874.

Diagnosis: Morphological diagnosis as by Rybakov & Høeg (2013)

Host: Calianassidae.

Genera: *Parthenopea* Kossmann, 1874 (two spp.).

Remarks: At present, the genus contains only two similar species, *Parthenopea subterranea* Kossmann,

1874 and *Parthenopea australis* Lützen *et al.*, 2009, but these are widely separated geographically (Lützen *et al.*, 2009). *Parthenopea* was formerly included in Peltogastridae, but was moved to a new family by Rybakov & Høeg (2013) owing to morphological peculiarities. The present molecular analysis clearly supports the family status, because *Parthenopea* is placed separate from both our redefined Peltogastridae and the new family Peltogasterellidae.

Family Sacculinidae Lilljeborg 1861, amended

Type genus: *Sacculina* Thompson, 1836.

Type species: *Sacculina carcini* Thompson, 1836.

Diagnosis: By the molecular data. Externa laterally compressed. Mantle opening more or less opposite stalk, situated on the anterior margin or displaced to the left side. Thin dorsal mesentery only, extending from the stalk to the mantle opening, but often shorter or absent. Colleteric glands with a number of branched tubes, situated in the central part of the lateral surface of the visceral mass. Receptacles placed either dorsally or far posteriorly in the visceral mass, or in the basal region of the stalk (from Øksnebjerg, 2000).

Hosts: Brachyura and Calianassoidea.

Genera: *Drepanorchis* Boschma, 1927 (five spp.), *Heterosaccus* Smith, 1906 (15 spp.), *Loxothylacus* Boschma, 1928 (29 spp.), *Ptychascus* Boschma, 1933 (two spp.), *Sacculina* (167 spp., see below); *Sesarmaxenos* Annandale, 1911 (two spp.).

Remarks: The family comprises taxa that originate at node 17 in Figure 3. The high support values for both node f and g in Figure 2 show that the Sacculinidae as defined until now is polyphyletic and must be split into two monophyletic taxa, viz. an amended Sacculinidae and the new family Polyascidae. These two species clusters appeared already in the study by Glenner & Hebsgaard (2006) and have been confirmed with high support by all subsequent analyses of the 'sacculinid' species concerned (Glenner *et al.*, 2010; 2020; Høeg *et al.*, 2019). A morphology-based diagnosis for Sacculinidae was given by Øksnebjerg (2000), but there seems at present to be no possibility that such characters can separate Sacculinidae and Polyascidae. From Figure 3, it follows that the amended Sacculinidae should with certainty comprise the type species *Sacculina carcini*, *Sacculina upogebiae* Shiino, 1943 and the species of *Heterosaccus*, *Loxothylacus*, *Ptychascus* and *Sesarmaxenos*. *Loxothylacus* is monophyletic, as already shown by Glenner *et al.* (2008). Before our revision, the genus *Sacculina* contained 172 recognized species (WoRMS, 2019). By default, we include all species of that genus (167) in Sacculinidae, except the five (see p. 13) that have been shown specifically by molecular data to belong in the new family Polyascidae. Molecular data are still not available for the monotypic genus *Drepanorchis*. Both *Ptychascus* and *Sesarmaxenos* infest freshwater-inhabiting hosts (Feuerborn, 1931; Boschma, 1933; Andersen *et al.*, 1990) and in Figure 3 are nested in *Heterosaccus*, but further analysis must clarify whether these remarkable genera should be subsumed in the latter genus (Glenner et al., 2020).

Polyascidae Høeg & Glenner **fam. nov.**

Type genus: *Polyascus* Glenner, Lützen & Takahashi, 2003.

Type species: *Polyascus* (syn. *Sacculina*) *polygeneus* (Lützen & Takahashi, 1997).

LSID: urn:lsid:zoobank.org:act:A733BE00-3B81-4C57-BC0D-2A7DE85BDF89

Diagnosis: By the molecular data. External morphology resembling that in the Sacculinidae, but external cuticle smooth or almost smooth, normally without excrescences. One or two globular receptacles located at base of stalk, outside ovary. Two receptacle ducts with short, slightly sinuous tubes lined by heavy cuticle. Multiple externae can form by budding from the root system, but are only obligatory in species of *Polyascus*.

Hosts: Brachyura and Gebiidea.

Genera: *Parasacculina*, gen. nov. (five spp.) and *Polyascus* Glenner, Lützen & Takahashi, 2003 (three spp.)

Remarks: The family comprises the taxa that originate at node 9 in Figure 3 and is well defined by the molecular analysis. External morphology is similar to that seen in Sacculinidae, but a possible apomorphy for Polyascidae is the tendency to form multiple externae (Glenner *et al.*, 2003), which has become obligatory in *Polyascus*. Multiple externae at a frequency higher than by chance are extremely rare in Sacculinidae. The family consists of *Polyascus* (three spp.) and the new genus *Parasacculina* (five spp., see p. 13). All these species were formerly placed in Sacculinidae. In Figure 3, the genus *Polyascus* is monophyletic and united by the apomorphy of having colonial externae that are

replaced in successive generations. Compared with all earlier uses, the species names of *Polyascus polygenea* (Lützen & Takahashi, 1997), *Polyascus gregaria* (Okada & Miyashita, 1935) and *Polyascus plana* (Boschma, 1933) have been altered here and in WoRMS (2019) to comply with the masculine gender of the genus name. About 160 '*Sacculina*' species remain to be analysed by molecular data. The species concerned will, in all likelihood, be allocated to both Sacculinidae and Polyascidae, and this will undoubtedly also require additional generic level changes in both families. Glenner *et al.* (2003) listed a number of *Sacculina* species, where multiple externae seem to occur more frequently than by chance, although not obligatorily, but no formal taxonomic steps were taken. A future analysis is needed to decide whether these species should be transferred to Polyascidae, perhaps as members of *Parasacculina*.

Polyascus Glenner, Lützen & Takahashi, 2003

Type species: Polyascus polygeneus (Lützen & Takahashi, 1997).

Diagnosis (amended): By the molecular data. Externa morphology as for the family, but with obligatory multiple (colonial) externae that are replaced in successive generations

Species: Polyascus gregarius (Okada & Miyashita, 1935), *Polyascus planus* (Boschma, 1933) and *Polyascus polygeneus* (Lützen & Takahashi, 1997).

Remarks: The diagnosis is amended from Glenner *et al.* (2003) by specifying also the obligatory presence of multiple externae, which separates it from the new genus *Parasacculina* defined below.

Parasacculina Høeg & Glenner **gen. nov.**

Type species: Polyascus (syn. *Sacculina*) *shiinoi* (Lützen *et al.*, 2016).

LSID: urn:lsid:zoobank.org:act:EE2FD3C8-F9C1-49EE-AAE5-80176863160B

Diagnosis: As for the family, but, unlike *Polyascus*, without obligatory colonial externae.

Etymology: The name is chosen to indicate the close morphological similarity to species of *Sacculina*.

Species: Parasacculina leptodiae (Guerin-Ganivèt, 1911), *Parasacculina oblonga* (Lützen & Yamaguchi, 1999), *Parasacculina shiinoi* (Lützen *et al.*, 2016), *Parasacculina sinensis* (Boschma, 1933) and *Parasacculina yatsui* (Boschma, 1936).

Remarks: The genus is paraphyletic, and this is at present the only practical solution. It consists of species formerly placed in *Sacculina* that by molecular data do not belong in the redefined Sacculinidae, but form a monophyletic unit together with, but not within, the monophyletic genus *Polyascus*. The type species has recently been investigated by both molecular and morphological methods, and type specimens are available and in good condition. *Parasacculina* (syn. *Sacculina*) *yatsui* has previously (e.g. Glenner *et al.*, 2010) been identified erroneously as *Sacculina confragrosa* Boschma, 1933 (see Kobayashi *et al.*, 2018). A molecular analysis of the true *Sacculina confragrosa* has not yet been published.

Family Polysaccidae Lützen & Takahashi, 1996 (monogeneric)

Type genus: Polysaccus Høeg & Lützen, 1993.

Type species: Polysaccus (syn. *Thompsonia*) *mediterraneus* (Caroli, 1929).

Diagnosis: By morphology as in the study by Øksnebjerg (2000). The molecular analysis clearly separates the only analysed species, *Polysaccus japonicus* Høeg & Lützen, 1993, from all other families recognized here.

Host: Callianassoidea.

Genera: Polysaccus (two spp.).

Remarks: Both species have elongated, colonial externae. The family is represented here only by *Polysaccus japonicus* and is situated in a cluster (Fig. 3, node 7) that also contains some former akentrogonid families: Polyascidae and Sacculinidae. The closest ally may be Clistosaccidae (Glenner *et al.*, 2010), but the precise position is uncertain, explaining the polytomy at node 7 in Figure 3. It further complicates the situation that the only two species, *Polysaccus japonicus* and *Polysaccus mediterraneus*, deviate in larval morphology to an extent that puts doubt on the monophyly of the genus, hence also the family (Glenner *et al.*, 2010; Høeg *et al.*, 2019). The third segment of the antennule in *Polysaccus japonicus* has a specialized pointed shape that suggests its use in antennular penetration. In contrast, the antennule in *Polysaccus mediterraneus* is similar to

that found among peltogastrid species. Unfortunately, no molecular data are available for the type species, *Polysaccus mediterraneus*, meaning that a solution must await future additional data.

Family Clistosaccidae Høeg & Rybakov, 1992

Type genus: Clistosaccus Lilljeborg, 1861.

Type species: Clistosaccus paguri Lilljeborg, 1861.

Diagnosis (amended): By molecular data. Morphology-based apomorphies are a cypris larvae with the fourth antennular segment carrying only a single bifurcate seta situated at the apex. A reduced, near-identical pattern of setation on the cypris carapace is depicted by Jensen *et al.* (1994) and not found elsewhere in rhizocephalans. The second pair of lattice organs (LO2) in the cyprids is shaped like a hairpin, another morphology unknown elsewhere in rhizocephalans.

Host: Paguroidea and Caridea.

Genera: Clistosaccus (one sp.) and *Sylon* Kröyer, 1855 (one sp.).

Remarks: The family comprises taxa that originate at node 11 in Figure 3. Høeg & Rybakov (1992) subsumed Sylonidae into Clistosaccidae, and this is well supported by both molecular data and the virtually identical cypris larvae of the two species (Glenner *et al.*, 1989, 2010; Høeg *et al.*, 2019). Both genera are monotypic at present, but there is emerging molecular evidence that *Sylon* comprises several cryptic species (H. Glenner, unpublished data). The same might well be true for *Clistosaccus*, considering its wide circumpolar distribution and multiple paguroid hosts (Høeg, 1982; Høeg & Lützen, 1985).

Family Thompsoniidae Høeg & Rybakov, 1992

Type genus: Thompsonia Kossmann, 1874.

Type species: Thompsonia globosa Kossmann, 1874.

Diagnosis (amended): By molecular data. Morphology-based characters are numerous globular or club-shaped colonial externae without receptacles that emerge simultaneously from the host. and each releases only a single brood. These externae are replaced through several generations of new externae that often appear in increasing numbers. The cyprids carry one bifurcated seta and some smaller setae and structures apically on the fourth segment. The second pair of lattice organs is not different from the remaining four pairs.

Hosts: Anomura, Brachyura, Caridea and Stomatopoda.

Genera: Diplothylacus Høeg & Lützen, 1993 (four spp.), *Jensia* Boyko & Williams, 2015 (two spp.), *Thompsonia* (five spp.) amd *Thylacoplethus* Coutière, 1902 (13 spp.).

Remarks: The family comprises taxa that originate at node 13 in Figure 3. The family is monophyletic in all relevant analyses based on molecular data. *Jensia* was formerly named '*Pottsia*' Høeg & Lützen, 1993, but this was a preoccupied name (see Hiller *et al.*, 2015). Høeg & Lützen (1993) gave an extensive account of the systematics, morphology and phylogeny of this family, and to this is added the paper by Hiller *et al.* (2015). Among all Rhizocephala, Thompsoniidae have the widest taxonomic range of host animals.

Family Chthamalophilidae Bocquet-Vedrine, 1961

Type genus: Chthamalophilus Bocquet-Vedrine, 1957.

Type species: Chthamalophilus delagei Bocquet-Vedrine, 1957.

Diagnosis (amended): By molecular data. Morphology-based diagnosis amended from Høeg & Rybakov (1992) is cypris larvae being minute (< 100 µm long) and completely lacking a thorax, whence they can move only by walking on the antennules. The fourth segment is reduced to a mere rudiment. The cypris carapace is furnished with only four long setae located posteriorly. The externa is always surrounded by a double layer of cuticle separated by a fluid filled space, rendering the externa surface distinctly refringent. The male organs are invaginated from the mantle into the mantle cavity as free-floating bodies enveloped in cuticle. These 'primary spermatogenic islets' later split into several 'secondary islets' devoid of cuticle and in which spermatogenesis proceeds (Høeg *et al.*, 1990, 2019).

Hosts: Balanomorpha.

Genera: Bocquetia Pawlik, 1987 (one sp.), *Boschmaella* Bocquet-Védrine, 1967 (two spp.) and *Chthamalophilus* (one sp.).

Remarks: The family comprises taxa that originate at node 15 in Figure 3. The single species within

Bocquetia has not been analysed with molecular data, but shares the unique externa morphology of the family (Pawlik, 1987). The chthamalophilid species are unique in parasitizing balanomorphan barnacles. *Chthamalophilus* and *Boschmaella* both occur in intertidal barnacles, but *Bocquetia rosea* Pawlik, 1987 parasitizes a deeper water sponge-inhabiting host. Chthamalophilids have been reported from only six locations, all close to marine biological stations. *Boschmaella japonica* Deichmann & Høeg, 1990 occurs near the Misaki and Seto marine stations in Japan, *Boschmaella balani*(Bocquet-Védrine, 1967) near Arcachon, France, *Chthamalophilus delagei* Bocquet-Védrine, 1957 near Station Biologique de Roscoff, France and *Bocquetia rosea* off the Scripps Institution of Oceanography in Southern California. An undescribed Brazilian record (P. Young, personal communication) probably represents a new species. Within rhizocephalans, and therefore within cirripedes in general, the chthamalophilids can easily be argued to be the 'most highly morphologically specialized'. Inspection of the mantle cavity of balanomorphan barnacles is rarely done, but the scattered records indicate a near -cosmopolitan, if patchy, distribution of the family, which may (as evidenced by *Bocquetia*) extend to almost every habitat where balanomorphan barnacles are found.

Family Duplorbidae Høeg & Rybakov, 1992

Type genus: Duplorbis Smith, 1906.

Type species: Duplorbis calathurae Smith, 1906.

Diagnosis (amended): The cyprids have long and thick frontal filaments. Additional characters are externae with a mesenteric canal, as in chthamalophilids but lacking the double cuticle and refringent appearance of this family. The male organs are spermatogenic islets that originate as in the Chthamalophilidae, but they continue to be sheathed in a cuticle that continues to increase in thickness, and spermatogenesis proceeds without any formation of secondary islets (Mourey, 1974; Høeg & Rybakov, 1992).

Hosts: Isopoda (including Epicaridea) and Cumacea.

Genera: Arcturosaccus Rybakov & Høeg, 1992 (one sp.), *Cryptogaster* Bocquet-Védrine & Bourdon, 1984 (one sp.) and *Duplorbis* (three spp.).

Remarks: The family is rarely sampled, and no species are available for molecular analysis. Only *Arcturosaccus kussakinni* Rybakov & Høeg, 1992 has been well described. The externa morphology indicates a close relationship to Chthamalophilidae, but none of the autapomorphies listed for the externae and cyprids of this family is present in the duplorbids. A kind of mesenteric canal is also present in Mycetomorphidae but is apparently not homologous to the one in duplorbids and chthamalophilids. *Cryptogaster* occurs on a cumacean host, whereas two of the three species of *Duplorbis* are hyperparasitic on bopyrids, which is unique for Rhizocephala. In an unpublished thesis, Mourey (1974) offered a detailed histological account of what might be a new species of *Duplorbis* parasitizing bopyrids. Unfortunately, her published paper (Mourey, 1991) provides only a few of these morphological details and takes no taxonomic steps.

Family **Pirusaccidae** Høeg & Glenner **fam. nov.**

Type genus: Pirusaccus Lützen, 1985.

Type species: Pirusaccus socialis Lützen, 1985 (by monotypy).

LSID: urn:lsid:zoobank.org:act:E13A86B8-C88E-4709-8A7A-599CC6B58C6E

Diagnosis: Colonial externae of an elongated club shape. The male organs are bodies ('spermatogenic islets') floating free in the mantle cavity. Mesentery present; mesenteric canal absent.

Hosts: On the galatheoid species *Galacantha* (syn. *Munidopsis*) *rostrata* A. Milne Edwards, 1880.

Genera: Pirusaccus (one sp.).

Remarks: Until now, this monotypic genus was the only rhizocephalan not assigned to a family. It differs morphologically from both 'akentrogonids' with colonial externae (Polysaccidae and Thompsoniidae) and from the two other families (Chthamalophilidae and Duplorbidae) with spermatogenic islets. This argues for erection of a new family to recognize its uncertain position and unique character combination. *Pirusaccus* is a deep-sea form sampled only once; therefore, prospects are poor for obtaining material for molecular analysis. The larvae might offer valuable information, but the specimens described by Lützen (1985) were unfortunately not berried. In our composite phylogeny (Fig. 3), we suggest that the unique sexual system, with spermatogenic islets, is a synapomorphy for a clade comprising Pirusaccidae, Duplorbidae and Chthamalophilidae.

DISCUSSION

Unlike their normal presentation in textbooks (Pechenik, 2015; Hickman *et al.*, 2017), rhizocephalan barnacles are indeed biologically highly variable (Høeg & Lützen, 1995). The mode of invading the host, parasite morphology, sexual biology and host taxon vary extensively, and our updated phylogeny enables us to map these characters for the first time and attempt to analyse the underlying evolutionary processes that shaped this diversity. Such a venture will be the subject of future accounts; here, we pinpoint only some of the more important results and their perspectives.

Evolution of parasite–host relationship

Rhizocephalans are often loosely characterized as infesting decapod crustaceans and especially brachyuran ('true') crabs. Our phylogeny indicates that the original rhizocephalan hosts were anomuran Crustacea, and the most basal taxa seem to be confined largely to this group (Fig. 2). Higher in the rhizocephalan phylogeny, there seem to have been multiple shifts to new and sometimes widely different hosts. This is exemplified by the Thompsoniidae occurring on virtually all decapod groups and even on the distantly related stomatopods, and by chthamalophilids and duplorbids infesting barnacles and peracaridans. Many rhizocephalans exert deep control over the morphology, physiology and behaviour of their hosts (Høeg, 1995; Lafferty & Kuris, 1996; O'Brien, 1999). Thus, in shifting to another species, these processes must adapt to the new situation. Indeed, not all rhizocephalans have the classical full control over their hosts, such as arresting the moult cycle, complete castration and feminization of infested males (Høeg & Lützen, 1995). For example, in *Sacculina* there is evidence for an abundant amount of variation upon which selection could act to drive species diversification and host specificity and control (Kristensen *et al.*, 2012). A future analysis may show that shifts in host taxon might cause difficulty in maintaining extensive control of the infested crustacean. Notably, most of the 'akentrogonid' species seem to have relaxed their effects on the host (Høeg, 1982; Høeg *et al.*, 1990; Høeg & Lützen, 1995). A full understanding will require molecular studies of the underlying interactions between parasite and host and how this varies across the rhizocephalan phylogeny.

Generalizations from 'model species'

The former Sacculinidae and *Sacculina* were by far the most species-rich taxa within the Rhizocephala, comprising more than two-thirds of the recognized species (Boschma, 1955; and see WoRMS, 2019). Furthermore, most studies on rhizocephalan biology, such as effects on the host, population biology, biological control, sexual biology and larval biology, have been on the model species *Sacculina carcini* and, to some extent, on a few species of *Heterosaccus* and *Loxothylacus* (Høeg & Lützen, 1995). It is therefore highly significant that species of the former Sacculinidae are distributed into two distinct clades (Fig. 3, nodes 9 and 17), here each recognized as families. Any generalizations from penetrating studies on single species must accordingly take the new phylogenetically based taxonomy into account. Until many more species have been analysed by molecular methods, extreme caution must also be exercised with respect to generic level systematics. As discussed in detail by Høeg *et al.* (2019), many species are poorly described and of unreliable status, and in addition, the generic level systematics remains largely formalistic. The genus *Sacculina* (Sacculinidae) remains by far the largest within Rhizocephala, and it contains, by default, all 167 species not specifically shown to be situated in the new family Polyascidae. At present, this is the only workable solution. We predict that both Sacculinidae and Polyascidae will remain as useful taxa, but with accumulating molecular evidence we also expect changes at both species and generic levels within and between the two families, gradually evolving into a phylogeny-based taxonomy to species level.

The mechanism of host infection

Our composite phylogeny (Fig. 3) shows that akentrogonid forms are nested within kentrogonids and that they form two separate lineages, viz. Mycetomorphidae (Fig. 3, node 4) and the remaining forms (Fig. 3, node 10). A main result from the present study is therefore the abandonment of a formal subdivision into kentrogonid and akentrogonid rhizocephalans. The phylogeny clearly confirms that the kentrogonid mode of host invasion is original, whereas the akentrogonid mode is derived, as already argued by Glenner & Høeg (1994). It remains to be explained how the kentrogon evolved from non-parasitic barnacles and how this mode of host invasion was secondarily modified at least twice into an akentrogonid mechanism (Fig. 1).

Penetration through the integument of a host to become internal is one of the most challenging tasks for a parasite. In rhizocephalans, the classical means is the formation from the settled cyprid of the unique kentrogon stage (Fig. 1A, B) that accomplishes the injection of the parasite into the haemocoelic system of the host (Delage, 1884; Høeg, 1985, 1987; Glenner, 2001). Høeg (1990) was the first to demonstrate an entirely different mechanism, whereby a kentrogon

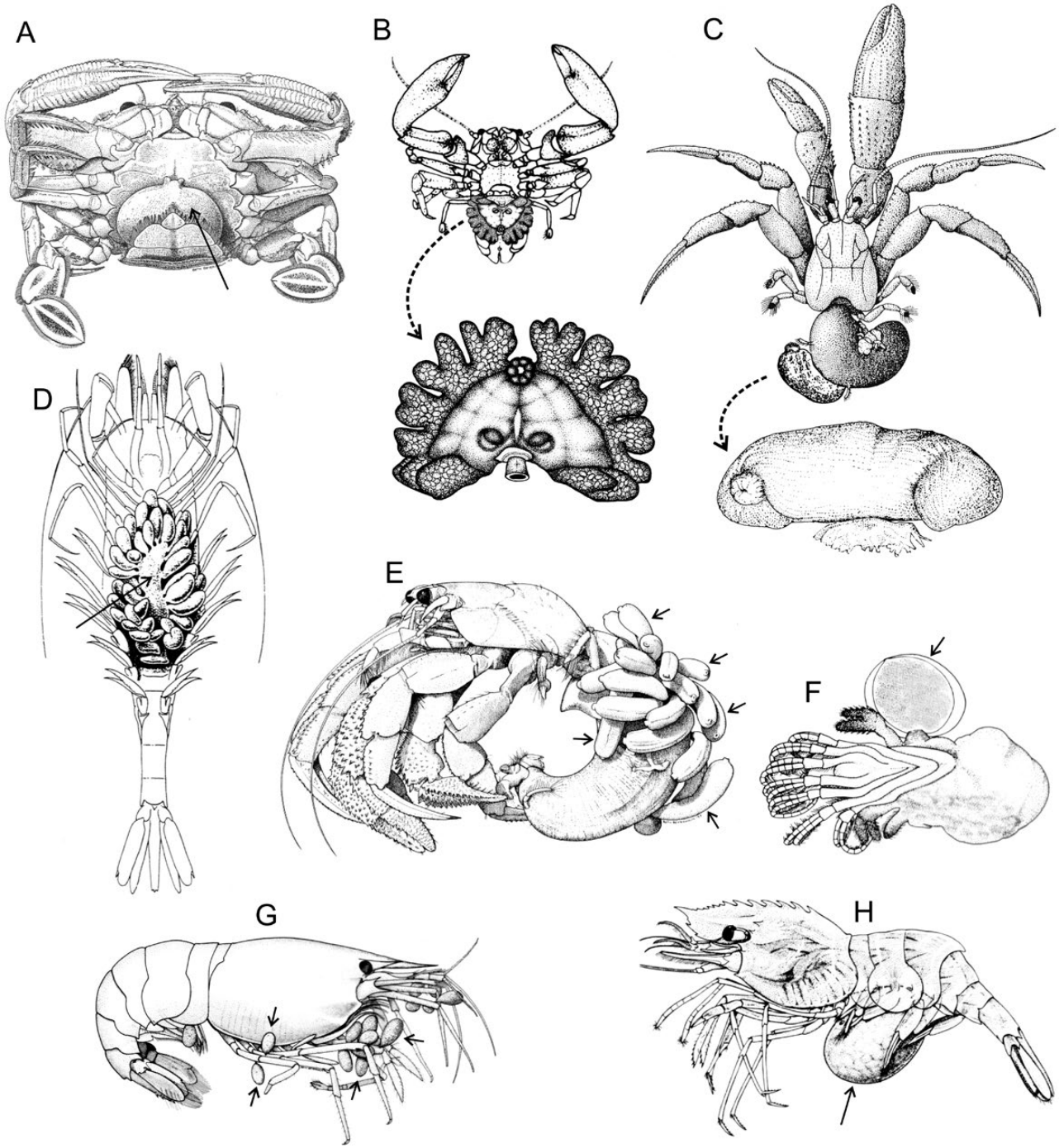

Figure 4. Diversity of rhizocephalan external parasites with their new family assignments. A, *Heterosaccus dollfusi* Boschma, 1960 (Sacculinidae) on *Charybdis longicollis* Leene, 1938 (see WoRMS). B, *Lernaeodiscus porcellanae* (Peltogastridae) on *Petrolisthes cabrilloi* Glassell, 1945. C, *Peltogaster paguri* (Peltogastridae) on *Pagurus bernhardus* Linnaeus, 1758. D, *Mycetomorpha vancouverensis* on *Neocrangon communis* (Rathbun, 1899). E, colonial externae of *Peltogasterella gracilis* on *Pagurus* sp. F, *Chthamalophilus delagei* on *Chthamalus stellatus* (Poli, 1791). G, colonial externae of *Thylacoplethus edwardsii* on *Synalpheus stimpsoni* (de Man, 1888). H, *Sylon hippolytes* Kröyer, 1855 on *Spirontocharis lilljeborgi* (Danielssen, 1859).

stage is absent and the cypris gains access to the host by using one of the cypris antennules to penetrate through the integument (Fig. 1C). This so-called akentrogonid mechanism was long discussed before it was observed and used to create a subdivision into two suborders. Originally, the akentrogonid mode of host infection was considered to be primitive (Bocquet-Vedrine, 1961; see Glenner *et al.*, 2010), but opposed to this Glenner & Høeg (1994) used Høeg's (1990) study to argue that presence of a kentrogon is, in fact, the plesiomorphic state, whereas its replacement with infection by the cyprid alone evolved later in the phylogeny of Rhizocephala. The analysis of Glenner *et al.* (2010) and the analysis presented here fully confirm this view, but also that the loss of a kentrogon may have occurred homoplastically within the taxon. All this calls into question what evolutionary forces shaped both the evolution and later loss of a kentrogon stage. Several studies suggest that the presence of a kentrogon serves as a defence against highly effective grooming defences of the potential host (Ritchie & Høeg, 1981; Høeg, 1985; Fleischer *et al.*, 1992; Høeg *et al.*, 2005). It is therefore possible that both the considerable variation in kentrogon morphology and function (Høeg, 1985; Glenner, 2001) and the eventual loss of this stage and replacement by cyprid antennular penetration (Høeg, 1990) should be seen in the perspective of the parasite evolving its infestation mechanism and facing host defensive mechanisms.

Parasites with multiple externae

Most rhizocephalans have only a single reproductive body, situated externally on the host and normally underneath the abdomen (Fig. 4). This so-called externa is female and contains the ovary and the male organs in the form of one or several dwarf males that have been implanted into the virginal female parasite (Ichikawa & Yanagimachi, 1957, 1958; Høeg, 1991; Høeg & Lützen, 1995). A spectacular situation is the presence of colonial externae, where multiple such bodies occur on the same host and are connected to the same internal root system, whence they represent one single parasitic individual (Fig. 4E, G). Such so-called colonial externae are found in all species of *Polyascus* (Polyascidae), Peltogasterellidae, Pirusaccidae and Thompsoniidae. Furthermore, it seems that such multiple externae are always cast off after producing a single brood, but are then replaced by a new generation of externae that need another invasion of cyprid males in order to reproduce (Høeg & Lützen, 1995). Our phylogeny clearly shows that such colonialism has evolved several times in parasites from a wide spectrum of hosts and, notably, within both kentrogonid and akentrogonid forms. This makes it an interesting venture to explain the adaptive significance of this system. Furthermore, the presence of colonial externa amounts to an asexual budding mechanism that is virtually unknown elsewhere in the Arthropoda, except rarely in insects (Beckage *et al.*, 1990).

Evolution of reproductive systems

Darwin (1851, 1854) identified cirripedes as a prime platform for studying the evolution of reproductive systems in animals. Although cirripedes in general have a variety of sexual systems (Yusa *et al.*, 2012), all rhizocephalans have separate sexes, where dwarf males in a highly reduced form are hosted by the female parasite (Høeg, 1991; Høeg & Lützen, 1995). The classic textbook system is two males hosted within a pair of male receptacles in the female parasite, but in reality the reproductive system varies extensively across taxa. One such example concerns the numbers of males per female and when these are recruited. In species with colonial externae, each single externa needs to recruit at least one male, and this must be repeated for every successive externa generation. Given that the number of externae can range from a few to many hundreds, this obviously has profound effects upon the reproductive biology, such as the numbers of males fertilizing each brood by the female. Yamaguchi *et al.* (2014) have presented a mathematical model for the evolution of this variation, but the new phylogeny offers a much improved platform for such a venture, similar to that recently done for thoracican barnacles (Yusa *et al.*, 2012; Lin *et al.*, 2015).

Conclusions

Although monophyly is strongly supported, the rhizocephalan barnacles vary extensively in terms of development, host taxon and control, parasite morphology and reproductive system. Furthermore, comprising only a few hundred species, they are nevertheless common in marine habitats and have profound effects on the crustacean populations infested. According to Weinstein *et al.* (2016), evolution into parasitism has been truly successful only a few times in the Metazoa, and the Cirripedia Rhizocephala is one such example. Our resulting phylogeny will enable the use of rhizocephalans as a model to study biological evolution within a highly specialized and biologically successful and diverse taxon of parasites.

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