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.
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; Mirolubov, 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 Thoecostraca, class Maxillopoda). The concept of the class Maxillopoda has since been abandoned, and here we consider Cirripedia as a subclass within the class Thoecostraca 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 autapomorphies for the families, but left the kentrogonid 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
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 kentrogonid 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     | Norway     |
| Cyphosaccus norvegicus     | MN650337*      | Galathea dispera       | 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 tyrrehna    | Croatia    |
| Pellogaster curvata        | MN650342*      | Pagurus prideaux       | Norway     |
| Pellogaster paguri         | DQ826570       | Pagurus bernhardus     | Sweden     |
| Pellogasterella sulphata   | DQ826572       | Pagurus cuanensis      | Sweden     |
| Polyacanthus planus        | AY265368       | Grapsus albo lineatus  | Taiwan     |
| Polyacanthus japonicus     | DQ826565       | Callianassa japonica   | Japan      |
| Sacculina carcinii         | AY265366       | Carcinus maenas        | Sweden     |
| Sacculina oblonga          | AY265367       | Cyclograpsus intermedius | Japan     |
| Septosaccus rodriquezii    | DQ826571       | Clibanarius erythropus | Sardinia   |
| Sylon hippolytes           | DQ826564       | Pandalus sp.           | Canada     |
| Thomoponita littoralis     | DQ826573       | Leptodiexaratus        | Singapore  |
| Tortugaster boschmai       | MN650341*      | Munida sarsi           | Norway     |
| Triangulus galatheae       | MN650340*      | Galathea intermedia    | Norway     |
| Triangulus munidae         | MN650339*      | Munida sarsi           | Norway     |
| Ibla quadrivalvis          | AYS206565      | –                      | Tasmania   |
| Poecilasma inaequilaterale | AYS20654       | –                      | Gulf of Mexico |
| Semibalanus balanoides     | AYS20626       | –                      | Denmark    |

The generic names are those before the revision carried out in the present paper.

*Accession numbers of new sequences.
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.

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.        |
| Myctomorphyida | 1                | 2                 | Caridea                |                  |
| Peltogastridae | 13               | 48                | Anomura                | Redefined        |
| Peltogasterellidae | 4               | 8                 | Thalassinoidea         |                  |
| Parthenopeidae | 1                | 2                 | Brachyura              | Redefined        |
| Sacculinidae   | 6                | 175               | Calianassoidae         |                  |
| Polyascidae    | 2                | 8                 | Brachyura              | fam. nov.        |
| Clitosaccidae  | 2                | 2                 | Gebiidea               |                  |
| Thompsoniidae  | 4                | 24                | Paguroidea             |                  |
|                |                  |                   | Caridea                |                  |
| Polysaccidae   | 1                | 2                 | Brachyura              |                  |
|                |                  |                   | Anomura                |                  |
| Chthamalophilidae | 3           | 4                 | Caridea                |                  |
| Duplorbidae    | 3                | 5                 | Balanomorpha           |                  |
|                |                  |                   | Isopoda                |                  |
| Pirusaccidae   | 1                | 1                 | Cumacea                |                  |
|                |                  |                   | 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.

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 Mycetomorphae, 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 3). This shows that the Akentrogonida is at least diphylectic 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.
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 nonmycetomorphid akentrogonids varies slightly between

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 Akentrogonoidea. 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.
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 vancouversensis on host; C, Pelagaster paguri; D, Lernaeodiscus posthumeus on host; E, Pelagasterella gracilis on host; F, Polysaccus 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 carinata.
The monophyly is assured by the lack of several crucial apomorphies for this family. Groups are close allies of Chthamalophilidae, but they 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), Peltopasterellidae (6, new family), Polysaccidae (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 colletic 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 LIIJEBORG, 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. Coleteric glands normally simple, sometimes subdivided tubes. Paired receptacles with straight or tortuous ducts. Disposition of coleteric 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.), Galatheasacus Boschma, 1929 (two spp.), Lernaeodiscus Müller, 1862 (eight spp.), Ommatogaster Yoshiida & 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 Reinhardt, 1948 (three spp.), Triangulopsis Guerin-Ganivet, 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 & GLENNEN GEN. NOV. (MONOTYPIC)

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

Type species: Paratриangulus (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 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* (Reischman, 1959), and we suggest that this 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.

**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,
From Figure 3, it follows that the amended characters can separate Sacculinidae and Polyascidae. There seems at present to be no possibility that such a character for Sacculinidae was given by Øksnebjerg (2000), 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 SACULINIDAE LILLJEBORG 1861, AMENDED**

**Type genus:** *Sacculina* Thompson, 1836.

**Type species:** *Sacculina carcinic* 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, Lützen & Takahashi (2003). 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 carcinic*, *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).

**POLYASCIDAIE HŒG & 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 polygeneus* (Lützen & Takahashi, 1997), *Polyascus gregarius* (Okada & Miyashita, 1935) and *Polyascus planus* (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-Ganivét, 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 POLYASCIDAE** **LÜTZEN & TAKAHASHI, 1996** (MONOGENERIC)

*Type genus:* *Polyascus* Høeg & Lützen, 1993.

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

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

*Host:* Callianassoidea.

*Genera:* *Polyascus* (two spp.).

*Remarks:* Both species have elongated, colonial externae. The family is represented here only by *Polyascus 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, *Polyascus japonicus* and *Polyascus 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; Øksnebjerg et al., 2019). The third segment of the antennule in *Polyascus japonicus* has a specialized pointed shape that suggests its use in antennular penetration. In contrast, the antennule in *Polyascus 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 larva 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.) and *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-Vedrine, 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 balan(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. Inspection of the mantle cavity of balanomorphan to be the ‘most highly morphologically specialized’. In general, the chthamalophilids can easily be argued to be the ‘most highly morphologically specialized’. 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.

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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 infecting 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 Crustacea, and the most basal taxa seem to have 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 Crustacea, and the most basal taxa seem to have 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 Crustacea, and the most basal taxa seem to have the original rhizocephalan hosts were anomuran Crustacea, and the most basal taxa seem to be confined largely to this group (Fig. 2).

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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 infected crustacean. Notably, most of the ‘akentrogonid’ species seem to have relaxed their effects on the host (Høeg, 1992; 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 akentrogonid mechanism (Fig. 1).
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 *Neocragon communis* (Rathbun, 1899). E, colonial externae of *Peltogasterella gracilis* on *Pugurus sp.* F, *Chthamalophilus delagei* on *Chthamalus stellatus* (Poli, 1791). G, colonial externae of *Thylacoplethus edwardsii* on *Synalpheus stimpsoni* (de Man, 1888). H, *Sylon hippolytes* Krøyer, 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 infestation 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), Pelogasterellidae, 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 externae 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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REFERENCES

Andersen ML, Bohn M, Høeg JT, Jensen PG. 1990. Cyprid ultrastructure and adult morphology in Ptychascus barnwelli, new species, and P. glaber (Cirripedia: Rhizocephala), parasites on semiterrestrial crabs. Journal of Crustacean Biology 10: 20–28.

Beckage NE, Thompson SN, Federici BA. 1990. Parasites and pathogens of insects, Vol. 1, parasites. San Diego: Academic Press.

Bocquet-Védrine J. 1961. Monographie de Chthamalophilus delagei J. Bocquet-Védrine, Rhizocéphale parasite de Chthamalus stellatus (Pol). Cahiers de Biologie Marine 2: 455–593.

Boschma H. 1933. The Rhizocephala in the collection of the British Museum. Journal of the Linnean Society of London, Zoology 38: 473–552, 1 pl.

Boschma H. 1955. The described species of the family Sacculinidae. Zoologische Verhandelingen 27: 1–76.

Boyko CB, Harvey AW. 2000. A review of the family Lernaeodiscidae (Cirripedia: Rhizocephala). I. The genus Lernaeodiscus Müller, 1862: new synonymy, hosts, range and the description of a new species. Journal of Crustacean Biology 20: 663–673.

Bresciani J, Høeg JT. 2001. Comparative ultrastructure of the root system in rhizocephalan barnacles (Cirrata: Cirripedia: Rhizocephala). Journal of Morphology 249: 9–42.

Brooks DR, McLennan DA. 1993. Parascript. Parascript. Parasites and the language of evolution. Washington: Smithsonian Institutional Press.

Brusca RC, Moore W, Shuster SM. 2017. Invertebrates. Oxford: Oxford University Press.

Castresana J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Molecular Biology and Evolution 17: 540–552.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9: 772–772.

Darwin C. 1854. A monograph on the sub-class Cirripedia, with figures of all the species. The Lepadidae; or, pedunculated cirripedes. London: Ray Society.

Darwin C. 1857. A monograph on the sub-class Cirripedia, with figures of all the species. The Verrucidae, etc., etc. London: Ray Society.

Delage Y. 1884. Evolution de la Sacculine (Sacculina carcini Thomps.). Crustacé endoparasite de l’ordre nouveau des Kentrogonides. Archives Zoologie Expérimentales et Générales Sér. 2: 417–736.

Edgar RC. 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics 5: 113.

Feuerborn H. 1931. Ein Rhizocephale und zwei Polychaeten aus dem Süßwasser von Java und Sumatra. Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie 5: 618–660.

Fleischer J, Grell M, Høeg JT, Olesen J. 1992. Morphology of grooming limbs in species of Petrolisthes and Pachycheles (Crustacea: Decapoda: Anomura: Porcellanidae): a scanning electron microscopy study. Marine Biology 113: 425–435.

Glenner H. 2001. Cypris metamorphosis, injection and earliest internal development of the Kentrogonid rhizocephalan Loxothylacus panopei (Gissler). Crustacea: Cirripedia: Rhizocephala: Sacculinidae. Journal of Morphology 249: 43–75.

Glenner H, Hebsgaard MB. 2006. Phylogeny and evolution of life history strategies of the parasitic barnacles (Crustacea, Cirripedia, Rhizocephala). Molecular Phylogenetics and Evolution 41: 528–538.

Glenner H, Høeg JT. 1994. Metamorphosis in the Cirripedia Rhizocephala and the homology of the Kentrogon and trichogon. Zoologica Scripta 23: 161–173.

Glenner H, Høeg JT, Klysner A, Brodin Larsen B. 1989. Cypris ultrastructure, metamorphosis and sex in seven families of parasitic barnacles (Cirrata: Cirripedia: Rhizocephala). Acta Zoologica (Stockholm) 70: 229–242.

Glenner H, Høeg JT, Rees DJ, Schubart CD. (2020). Chapter 2: Phylogenetic analyses suggest a single origin of freshwater barnacles. In: Rodgers C, Kawai T, eds. The crustacean invasion of freshwater. Crustacean Issues 20. Abingdon: Routledge, Taylor & Francis Group.

Glenner H, Høeg JT, Stenderup J, Rybakov AV. 2010. The monophyletic origin of a remarkable sexual system in akentrogonid rhizocephalan parasites is confirmed by molecular and larval structural data. Experimental Parasitology 125: 3–12.

Glenner H, Lützen J, Takahashi T. 2003. Molecular and morphological evidence for a monophyletic clade of asexually reproducing Rhizocephala: Polyascus, a new genus (Cirripedia). Journal of Crustacean Biology 23: 548–557.

Glenner H, Thompson PF, Rybakov AV, Galil BS, Høeg JT. 2008. The phylogeny of rhizocephalan parasites of the genus Heterosaccus using molecular and larval data (Cirripedia: Rhizocephala: Sacculinidae). Israel Journal of Ecology and Evolution 54: 223–238.

Goddard JHR, Torchin ME, Kuris AM, Lafferty KD. 2005. Host specificity of Sacculina carci, a potential biological control agent of introduced European green crab Carcinus maenas in California. Biological Invasions 7: 895–912.

Hickman C Jr, Keen S, Larson A, Eisenhour D, l’Anson H, Roberts L. 2017. Integrated principles of zoology, 17th edn. New York: McGraw Hill.
Høeg JT. 1990. “Akentrogonid” host invasion and an entirely new type of life cycle in the rhizocephalan parasite Clitostomus paguri (Cirripedia: Rhizocephala). Acta Zoologica (Stockholm) 66:1–45.

Høeg JT. 1991. Functional and evolutionary aspects of the sexual system in the Rhizocephala (Thecostraca: Cirripedia). In: Bauer RT, Martin JW, eds. Sexual system in the Rhizocephala (Thecostraca: Cirripedia). 37–52.

Høeg JT. 1992. Cypris Larvae in Polyascus mediterraneus and Myctetomorpha vancouverensis: their importance in analyzing the phylogeny and sexual evolution of parasitic barnacles (Crustacea: Cirripedia: Rhizocephala). Israël Journal of Ecology and Evolution 53:9–31.

Ichikawa A, Yanagimachi R. 1957. The sexual nature of a rhizocephalan, Pelagostella socialis. Journal of the Faculty of Science Hokkaido University Series 6, Zoology 13:1–4: 384–389.

Ichikawa A, Yanagimachi R. 1958. Studies on the sexual organization of the rhizocephala I. The nature of the “testes” of Pelagostella socialis Krüger. Annotationes Zoologicae Japonenses 31:82–96.

Jensen PG, Høeg JT, Bower S, Rybakov AV. 1994. Scanning electron microscopy of lattice organs in cyprids of the Rhizocephala Akentrogonida (Crustacea Cirripedia). Canadian Journal of Zoology 72:1018–1026.

Jensen AR, Høeg JT, Glenner H, Lützen J 2019. Variation in juvenile stages and success of male acquisition in Danish and French populations of the parasitic barnacle Sacculina carcini (Cirripedia: Rhizocephala) parasitizing the shore crab Carcinus maenas. Journal of Marine Research 15: 191–203.

Kobayashi M, Wong YH, Oghuro-Okano M, Dreyer N, Høeg JT, Yoshida R, Okano K. 2018. Identification, characterization, and larval biology of a rhizocephalan barnacle, Sacculina yatsui Boschma, 1936, from northwestern Japan (Crustacea: Sacculinidae). Journal of Crustacean Biology 38:329–340.

Kristensen T, Nielsen AI, Jørgensen AI, Mortensen KN, Glenner H, Christensen JT, Lützen J, Høeg JT. 2012. The selective advantage of host feminization: a case study of the green crab Carcinus maenas and the parasitic barnacle Sacculina carcini. Marine Biology 159:2015–2023.
Lafferty KD, Kuris AM. 1996. Biological control of marine pests. Ecology 77: 1989–2000.

Larsen MH, Høeg JT, Mouritsen KM. 2013. Influence of infection by Sacculina carpini (Rhizocephala) on consumption rate and prey size selection of the European green crab Carcinus maenas. Journal of Experimental Marine Biology and Ecology 446: 166–176.

Legendre P, Dessevices Y, Bazin E. 2002. A statistical test for host–parasite coevolution. Systematic Biology 51: 217–234.

Lin H-C, Høeg JT, Yusa Y, Chan BKK. 2015. The origins and evolution of dwarf males and habitat use in thoracican barnacles. Molecular Phylogenetics and Evolution 91: 1–11.

Lützen J. 1985. Rhizocephala (Crustacea: Cirripedia) from the deep sea. Galathea Reports 16: 99–112.

Lützen J, Glenner H, Loerz A-N. 2009. Parasitic barnacles (Cirripedia: Rhizocephala) from New Zealand offshore waters. New Zealand Journal of Marine and Freshwater Research 43: 613–621.

Lützen J, Itani G, Jespersen Å, Hong J-S, Rees D, Glenner H. 2016. On a new species of parasitic barnacle (Crustacea: Rhizocephala), Sacculina shiinoi sp. nov., parasitizing Japanese mud shrimps Upogebia spp. (Decapoda: Thalassinidae: Upogebiidae), including a description of a novel morphological structure in the Rhizocephala. Zoological Science 33: 204–212.

Martin JW, Davis GE. 2001. An updated classification of the recent Crustacea. Natural History Museum of Los Angeles County Science Series 39: 1–115.

Martin JW, Olesen J, Høeg JT, eds. 2014. Atlas of crustacean larvae. Baltimore: Johns Hopkins University Press.

Miroljubov AA. 2017. Muscular system in interna of Peltogaster paguri (Rhizocephala: Peltogastridae). 2017. Arthropod Structure and Development 46: 230–235.

Moorey M. 1974. Duplorbis, Cirripède parasite. Unpublished Doctoral Thesis, Faculté des Sciences, Université de Nancy.

Moorey M. 1991. Le genre Duplorbis, rhizocephale endoparasite. Morphologie et anatomie. Bulletin du Museum National d’Histoire Naturelle 4 sér. A 13: 61–72.

Mouritsen K, Geýtii S, Lützen J, Høeg JT, Glenner H. 2018. Population dynamics and development of the rhizocephalan, Sacculina carpini, parasitic on the shore crab Carcinus maenas. Diseases of Marine Organisms 131: 199–211.

Nagler C, Hornig MK, Haug JT, Noever C, Høeg, JT, Glenner H. 2017. The bigger, the better? Volume measurements of parasites and hosts: parasitic barnacles (Cirripedia, Rhizocephala) and their decapod hosts. PLoS ONE 12: e0179058.

Newman WA, Zullo VA, Withers TH. 1969. Cirripedia. In: Moore RC, ed. Treatise on invertebrate paleontology, part R, Arthropoda 4(1). Lawrence, Kansas/Geological Society of America, R206–R296.

O’Brien JJ. 1999. Parasites and reproduction. In: Knobil E, Niell J, eds. Encyclopedia of Reproduction, Vols 1–4. Oxford: Academic Press, Elsevier, 638–646.

Øksnebjerg B. 2000. The Rhizocephala (Crustacea: Cirripedia) of the Mediterranean and Black Seas: taxonomy, biogeography, and ecology. Israel Journal of Zoology 46: 1–102.

Pawlik JR. 1987. Bocquetia rosea, new genus, new species, an unusual rhizocephalan parasite of a sponge-inhabiting Membranobalanus osculti (Pilsbry) from California. Journal of Crustacean Biology 7: 265–273.

Pechenik J. 2015. Biology of the invertebrates, 7th edn. New York: McGraw Hill.

Pérez-Losada M, Harp M, Høeg JT, Achituv Y, Jones D, Watanabe H, Crandall KA. 2008. The tempo and mode of barnacle evolution. Molecular Phylogenetics and Evolution 46: 328–346.

Pérez-Losada M, Høeg JT, Crandall KA. 2004. Unravelling the evolutionary radiation of the thoracican barnacles using molecular and morphological evidence. Systematic Biology 53: 244–254.

Posada D, Crandall, KA. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817–818.

Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. 2018. Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. Systematic Biology 67: 901–904.

Reischman PG. 1959. Rhizocephala of the genus Peltagasterella from the coast of the state of Washington to the Bering Sea. Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen 62 C: 409–435.

Ritchie LE, Høeg JT. 1981. The life history of Lernaeodiscus porcellanea (Cirripedia: Rhizocephala) and co-evolution with its pericaridian host. Journal of Crustacean Biology 1: 334–347.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Hohna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542.

Rybakov AV, Høeg JT. 1992. The anatomy of Arctuchosaccus kussakini, new genus, new species (Rhizocephala: Akentrogonida) from the ultraabyssal isopod Antartacarus zenkewitchi Kussakini (Arctoridae). Journal of Crustacean Biology 12: 592–599.

Rybakov AV, Høeg JT. 2013. The taxonomic position of rhizocephalan crustaceans of the genus Parthenopea Kossman, 1874 (Cirripedia: Rhizocephala) with validation of a new family, Parthenoideidae fam. nov. Russian Journal of Marine Biology 39: 357–362.

Spear S, Abele LG, Applegate MA. 1994. A phylogenetic study of cirripeds and their relatives (Crustacea Thecostraca). Journal of Crustacean Biology 14: 641–656.

Thresher RE, Werner M, Høeg JT, Svane I, Glenner H, Murphy N, Wittwer C. 2000. Developing the options for managing marine pests: specificity trials on the parasitic castrator, Sacculina carpini, against the European crab, Carcinus maenas, and related species. Journal of Experimental Marine Biology and Ecology 254: 37–51.

Waser AM, Goedknecht MA, Dekker R, McSweeney N, Witte JIJ, Van der Meer J, Thieltges DW. 2016. Tidal elevation and parasitism: patterns of infection by the rhizocephalan parasite Sacculina carpini in shore crabs Carcinus maenas. Marine Ecology Progress Series 545: 1–28.

Weinstein SB, Kuris AM. 2016. Independent origins of parasitism in Animalia. Biology Letters 12: 20160324.

© 2019 The Linnean Society of London, Zoological Journal of the Linnean Society, 2020, 190, 632–653.
WoRMS. 2019. Rhizocephala. World Register of Marine Species. Available at: http://www.marinenspecies.org/aphia.php?p=taxdetails&id=1109

Yamaguchi S, Høeg JT, Iwasa Y. 2014. Evolution of sex determination and sexually dimorphic larval sizes in parasitic barnacles. Journal of Theoretical Biology 347: 7–16.

Yanagimachi R. 1960. The life cycle of Peltogasterella gracilis (Rhizocephala, Cirripedia). Bulletin of the Biological Station Asamushi 10: 109–110.

Yanagimachi R. 1961a. Studies on the sexual organization of the Rhizocephala. III. The mode of sex determination in Peltogasterella. Biological Bulletin (Woods Hole) 120: 272–283.

Yanagimachi R. 1961b. The life-cycle of Peltogasterella (Cirripedia, Rhizocephala). Crustaceana 2: 183–186.

Yoshida R, Hirose M, Hirose E. 2015. Peltogasterella sensuru n. sp (Crustacea: Cirripedia: Rhizocephala) from off Okinawa Island (Ryukyu Archipelago, Japan) with remarks on its single brood externae. Systematic Parasitology 92: 31–44.

Yoshida R, Osawa M, Hirose M, Hirose E. 2011. A new genus and two new species of Peltogastridae (Crustacea: Cirripedia: Rhizocephala) parasitizing hermit crabs from Okinawa Island (Ryukyu Archipelago, Japan), and their DNA-barcodes. Zoological Science 28: 853–862.

Yusa Y, Yoshikawa Y, Kitaura J, Kawane M, Ozaki Y, Yamato S, Høeg JT. 2012. Adaptive evolution of sexual systems in pedunculate barnacles. Proceedings of the Royal Society B: Biological Sciences 279: 959–966.