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***Briarella doliaris* spec. nov.,
a new philoblennid copepod parasite from Australia:
a potential link to the Splanchnotrophidae**

(Copepoda, Poecilostomatoida)

Andrea Salmen, Roland Anton, Nerida G. Wilson & Michael Schrödl

Salmen, A., Anton, R., Wilson, N. G. & Schrödl, M. 2010. *Briarella doliaris* spec. nov., a new philoblennid copepod parasite from Australia: a potential link to the Splanchnotrophidae (Copepoda, Poecilostomatoida). Spixiana 33(1): 19–26.

Members of the quite common and diverse copepod family Splanchnotrophidae are specialised endoparasites of shell-less opisthobranch gastropod hosts. Another less well-known group of endoparasites also infesting opisthobranch sea slugs is the genus *Briarella* Bergh, 1876 that is currently placed within the Philoblennidae.

A new species of *Briarella* from Queensland, Australia, infesting the chromodorid nudibranch *Ceratosoma trilobatum* Gray, 1827 is described using scanning electron microscopy (SEM). The new species differs from the four currently known species *Briarella microcephala* Bergh, 1876, *Briarella risbeci* Monod, 1928, *Briarella disphaerocephala* Monod & Dollfus, 1932, and the unnamed *Briarella* sp. Bergh, 1876, by having a stocky rather than a vermiform body and longer lateral processes. Of all the members of this genus, *Briarella doliaris* most resembles splanchnotrophids due to the stocky body. It is thus possible, that *Briarella* and the Splanchnotrophidae share a common ancestor which switched to an endoparasitic lifestyle. If so, *Briarella doliaris* could represent the most basal offshoot of a clade of secondarily vermiform *Briarella* species, or it could be a direct sister taxon to splanchnotrophids, rendering the genus *Briarella* paraphyletic.

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Introduction

Traditionally, all endoparasitic copepods parasitizing in opisthobranch gastropods were considered to belong to the family Splanchnotrophidae Norman & Scott, 1906 (see review by Jensen 1987). Revising the family, Huys (2001) only recognised five genera, *Splanchnotrophus* Hancock & Norman, 1863 (4 species), *Ismaila* Bergh, 1867 (11 species), *Lomanoticola* Scott & Scott, 1895 (2 species), *Ceratosomicola* Huys, 2001 (4 species), and *Arthurius* Huys, 2001 (2 species), all of them highly modified endoparasites

in shell-less sea slugs (Schrödl 2002; Haumayr & Schrödl 2003; Schrödl 2003; Marshall & Hayward 2006; Salmen et al. 2008a,b).

Since the genus *Briarella* was first established, the gross-morphological similarity to the Splanchnotrophidae was emphasised (Bergh 1876; Jensen 1987; Huys 2001). The systematic placement of *Briarella*, however, was in a state of flux: Bergh (1876) claimed a relationship to the phyllichthyids, but did not integrate it there. His original descriptions unfortunately are quite inadequate and lack any information on mouthpart morphology (Huys 2001). First, *Briarella*

was placed within the Chondracanthidae (see Monod 1928), then it was included into the Splanchnotrophidae (see Monod & Dollfus 1932). In 1964, the genus *Briarella* was removed from the Splanchnotrophidae due to the presence of maxillipeds (Laubier 1964). Together with the genus *Philoblenna* Izawa, 1976, it was placed into the newly established Philoblennidae Izawa, 1976, because of obvious similarities such as two strong claws on the distal margin of the antenna, a long blade of the mandible and the maxilla displaying a subapical element on the allobasis (Izawa 1976).

Philoblenna, however, comprises ectoparasites that are attached to the gills of prosobranch gastropods, including littorinids and cowries (Izawa 1976; Ho 1981; Avdeev et al. 1986; Ho & Kim 1992; Huys 2001). Recently, both genera *Briarella* and *Philoblenna*, i.e. the Philoblennidae, were transferred to the Lichomolgidae considering several similarities in mouthpart morphology of the copepodite I of *Philoblenna* and *Critomolgus* (see Kim et al. 2004), but were later separated again (Boxshall & Huys 2007).

Herein, the Philoblennidae thus are treated as an independent family. Based on mouthpart morphology, it includes the genera *Briarella* and *Philoblenna*. Although mouthparts are unknown yet, Huys provisionally also included the poorly described genus *Chondrocarpus* into the Philoblennidae due to general body facies such as the presence of four pairs of lobate processes (Bassett-Smith 1903; Huys 2001). In contrast to the endoparasitic *Briarella* and *Chondrocarpus*, all members of *Philoblenna* possess swimming-legs and are considered to be more "primitive" (Huys 2001). Phylogenetic studies on splanchnotrophids and Philoblennidae still are impeded by the absence of suitable material for molecular analysis. Morphological knowledge on many species is restricted to old and inadequate original descriptions of a single or a few female specimens. Especially information concerning the mouthparts is often missing in older descriptions and thus, the taxonomy is unclear.

The genus *Briarella* currently consists of four species (Huys 2001). *Briarella microcephala* Bergh, 1876 parasitizes *Ceratosoma trilobatum* Gray, 1828 (see Bergh 1876; Hecht 1893; Jensen 1987). Monod (1928) found *Briarella risbeci* Monod, 1928 in *Hexabranchnus sanguineus* (Rüppell & Leuckart, 1828) (as *Hexabranchnus marginatus*), *Briarella disphaerocephala* Monod & Dollfus, 1932 utilises the host slugs *Platydorhis cruenta* (Quoy & Gaimard, 1832) and *Kentrodoris infraculata* (Abraham, 1877) (as *Doris infraculata*) (Monod & Dollfus 1932; Jensen 1987), and *Briarella* sp. Bergh, 1876 was found in *Chromodoris elisabethina* Bergh, 1877 and in *Asteronotus cespitosus* (van Hasselt, 1824) (see Bergh 1876; Jensen 1987). Thus far, all *Briarella* species are exclusively known as infesting dorid

nudibranchs in the Indo-Pacific (Huys 2001). It is unclear whether the similarity of *Briarella* species with other species in the genus *Splanchnotrophus* is due to common ancestry or, as implied by their classification in different families by more recent studies (e.g. Huys 2001; Kim et al. 2004), evolved convergently by adaptations to similar hosts.

In order to gain supplementary data for an analysis of relationships within the Poecilostomatoida, an additional, endoparasitic copepod species from the dorid nudibranch *Ceratosoma trilobatum* is described here using scanning electron microscopy (SEM) and is assigned to the genus *Briarella*. Based on even greater structural similarity than previously known from congeners, the new species is discussed as a potential link to splanchnotrophids.

Material and methods

Infection with female splanchnotrophids can usually be recognised due to the presence of external egg sacs, and sometimes endoparasites can be seen shining through host integument. In this case, no external signs were noted, and the parasites were discovered during routine dissection.

The infected sea slug analysed in this study was collected at Amity, North Stradbroke Island, Moreton Bay, Queensland, Australia and determined by N. Wilson. The host slug was deposited in the South Australian Museum (SAMD 19256).

The two female parasite specimens were relaxed in an isotonic MgCl₂ solution, the body was preserved in 75 % and the egg sacs in 90 % ethanol and given to the Zoologische Staatssammlung München (ZSM). Photographs of parasites were taken with a "Jenoptic ProgRes C12 plus" camera connected with an Olympus SZX 12 binocular. For SEM examination the copepods were dehydrated in an acetone series and critical-point dried in a BAL-TEC CPD 030 device. They were mounted on SEM stubs and coated with gold in a POLARON SEM COATING SYSTEM for 120 seconds. A LEO1430 VP scanning electron microscope was used for ultra-structural analysis and digital documentation.

The descriptive terminology used herein is adopted from Huys & Boxshall (1991), Gruner (1993), Huys (2001) and Haumayr & Schrödl (2003). The following terms are used to describe body segmentation: Cephalothorax (five head segments fused with a variable number of thorax segments), thorax and abdomen. In all postlarval Splanchnotrophidae the first pair of thoracopods is reduced (Huys 2001). The counting of thoracopods is adopted from Haumayr & Schrödl (2003).

The SEM is suitable to identify and document very fine and tiny structures. However, it is hardly possible to examine each sample from all sides. Due to the delicate nature of the parasites, host tissue and dirt cannot always be removed completely and may cover certain parasite structures.

Taxonomy

Class Copepoda H. M. Edwards, 1840
Order Poecilostomatoida Thorell, 1859
Family Philoblennidae Izawa, 1976

Genus *Briarella* Bergh, 1878

Briarella doliaris spec. nov.

Material. Holotype (♀, ZSMA20092004 mounted on SEM stub) and paratypes (1♀ ZSMA20092005, mounted on SEM stub and 1♀ ZSMA20092006 in ethanol) partly damaged, collected together by Nerida Wilson, 9m, Amity, North Stradbroke Island, Moreton Bay, Queensland, Australia, 27°24'13.81"S, 153°26'11.49"E, 07 December 2002. Host: *Ceratosoma trilobatum* Gray, 1828. 2♀♀ examined by SEM.

Etymology. The Latin species name *doliaris* refers to the barrel-shaped body.

Description (Figs 1-3)

Female. Body length 3.0-4.7 mm, (measurements were made from the anterior end of the cephalothorax to the posterior end of the abdomen, including the caudal rami and excluding antennae and the setae on caudal rami), width 1.1-1.4 mm, body stocky. Ratio of length to width about 1.71:1. Parasites whitish, slightly translucent (Fig. 1A). Cephalothorax distinctly set off from trunk; thorax enlarged with five pairs of lateral processes; abdomen long and slender (Fig. 1B). Segmentation of all body parts unclear.

Cephalothorax consisting of head with five pairs of cephalic appendages and first thoracic segment bearing maxillipeds (Fig. 1C, 3F). Antennule (Fig. 1D) long and unbranched, indistinctly 4-segmented; first segment long, bearing nine setae, five short ones and four long ones; second segment with three long setae and one short one; third segment with two long setae; fourth segment with six long setae at apex. Antenna (Fig. 1C, 3A) unbranched, 3-segmented; first and second segment with small spine on proximal edge; third segment with at least five minute spines, apex with two subequal strong claws. Labrum (Fig. 1C) well developed, bilobate; lobes very long. Mandible (Fig. 1E, 3B) with broad and thick base, tapering into long and flat blade with thorns on both edges like a saw blade. Mandible palp very thick with blunt tip (Fig. 1E, 3E). Maxillule (Fig. 1F, 3C) thick, bearing two small spines at apex and a triangular bulge laterally. Maxilla (Fig. 2A, 3D) 2-segmented; first segment enlarged, second segment biramous, longer ramus with two apical elements. Labium tongue-shaped. Maxilliped posterior to maxilla (Fig. 1C, 3F).

Second thoracopod biramous, located on second thoracic segment, close to cephalothorax (Fig. 1B). Exopodite indistinctly 2-segmented with one strong spine at proximal edge of first segment; second segment with 4 strong spines increasing in size distally, one seta at level of longest spine, one seta at base of thoracopod (Fig. 2B). Endopodite about as long as exopodite, blunt apex bearing one seta. Third thoracopod biramous. Exopodite as in second thoracopod; endopodite longer than exopodite, apex split in two short elements; one seta at base of third thoracopod (Fig. 2C). No further thoracopods detected.

Thorax with deep transversal furrows demarcating four pairs of lateral processes. Processes shorter than whole body; stout with round tip. Fifth pair of lateral processes shorter than all others and more slender, situated posterior to enlarged part of thorax, slightly bent medially.

Abdomen long and slender with four indistinct constrictions; genital openings not detected; egg sacs slender, slightly bent with pointed tip. Caudal rami long and stout; each ramus with two pinnate setae laterally and four pinnate ones at apex, latter with small bulge bearing one long pinnate seta (Fig. 2D).

Male. Not found.

Biology

For the present study, no biological information on *B. doliaris* was available, e.g. on the specimens' positions inside the host, or the colour of the egg sacs. Both parasites were damaged (see Fig. 1A,B) during their incidental discovery; egg sacs were removed, fixed in ethanol, and given to the ZSM separately. No males were found, despite considerable effort dissecting the host specimen.

Remarks

The females resemble each other regarding the size and shape of the body. The morphology of mouthparts is nearly identical; differences only exist with regard to number and position of setae. Thoracopods were only detectable in one specimen, in the second one they were covered with host tissue. Genital openings could not be detected in both specimens, but it is likely that they are situated on the first slightly swollen abdominal segment as it is usual for copepods (Gruner et al. 1993).

The specimens examined herein are members of the genus *Briarella* Bergh, 1876. Diagnostic features refer to the morphology of the mouthparts, especially the long mandible, the two claws on the third segment of the antenna (see Fig. 3A) and the shape of the maxilla, the five pairs of lateral processes on

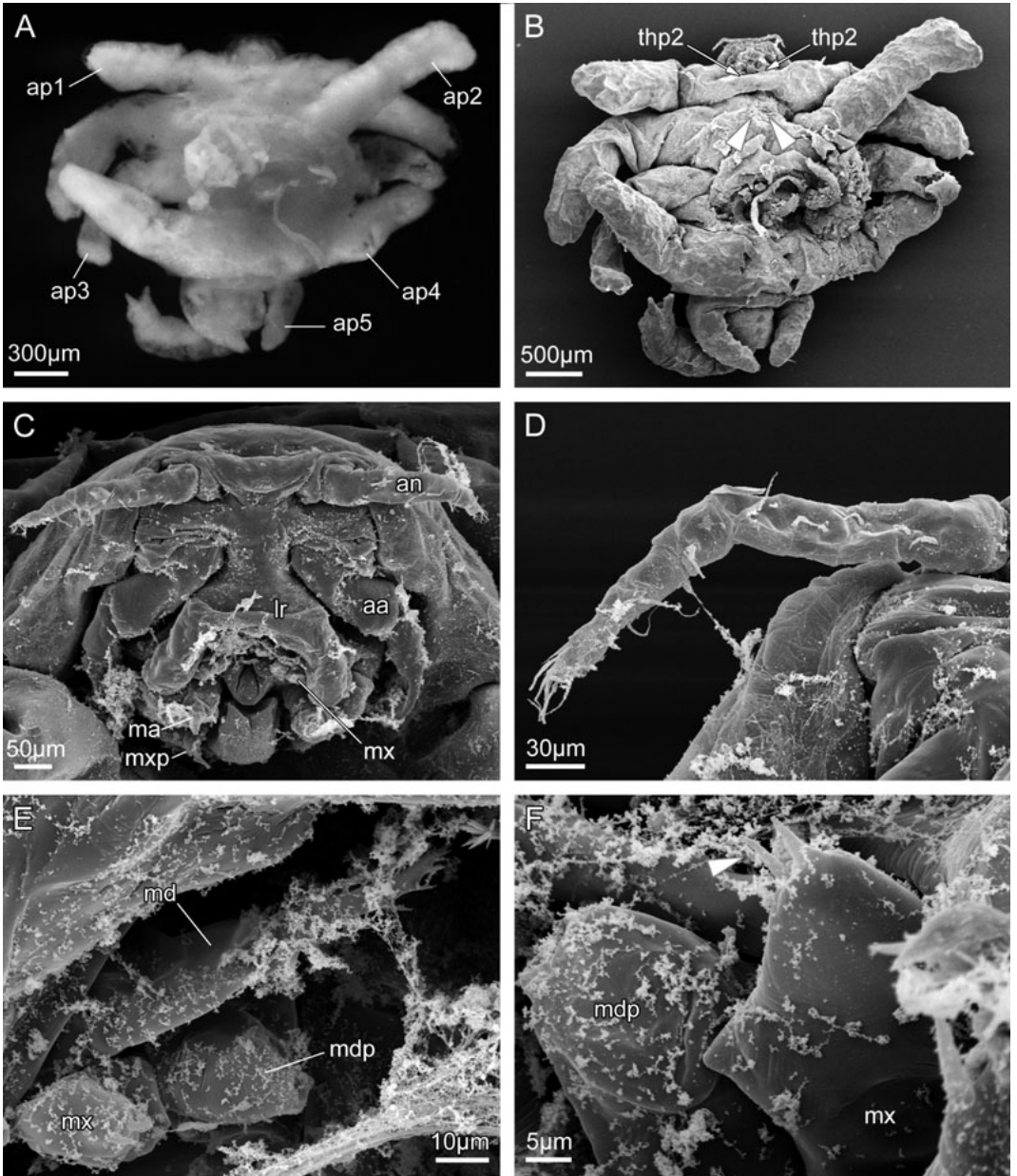


Fig. 1. *Briarella doliaris*, ♀. **A.** Habitus, ventral view (light microscope picture). **B-F.** SEM-micrographs. **B.** Habitus, ventral view. Position of 3rd thoracopods (arrows). **C.** Cephalic appendages. **D.** Antennule (right). **E.** Oral area (right side), mandible blade and palp, maxillule. **F.** Maxillule (right) with apical spines (arrow), mandible palp. Abbreviations: **aa**, antenna; **an**, antennule; **ap1-5**, appendages 1-5; **lr**, labrum; **md**, mandible; **mdp**, mandible palp; **mx**, maxillule; **ma**, maxilla; **mxp**, maxilliped; **thp2**, thoracopod 2.

the thorax and the presence of only two pairs of thoracopods, i.e., second and third ones (Monod 1928; Huys 2001). According to Huys (2001), four other species belong to this genus: *B. microcephala*

(type species), *B. risbeci*, *B. disphaerocephala* and an unnamed *Briarella* sp. (see also Monod 1928). *Briarella risbeci* has a very elongate body with four pairs of short lateral processes (“lobes” according to Monod

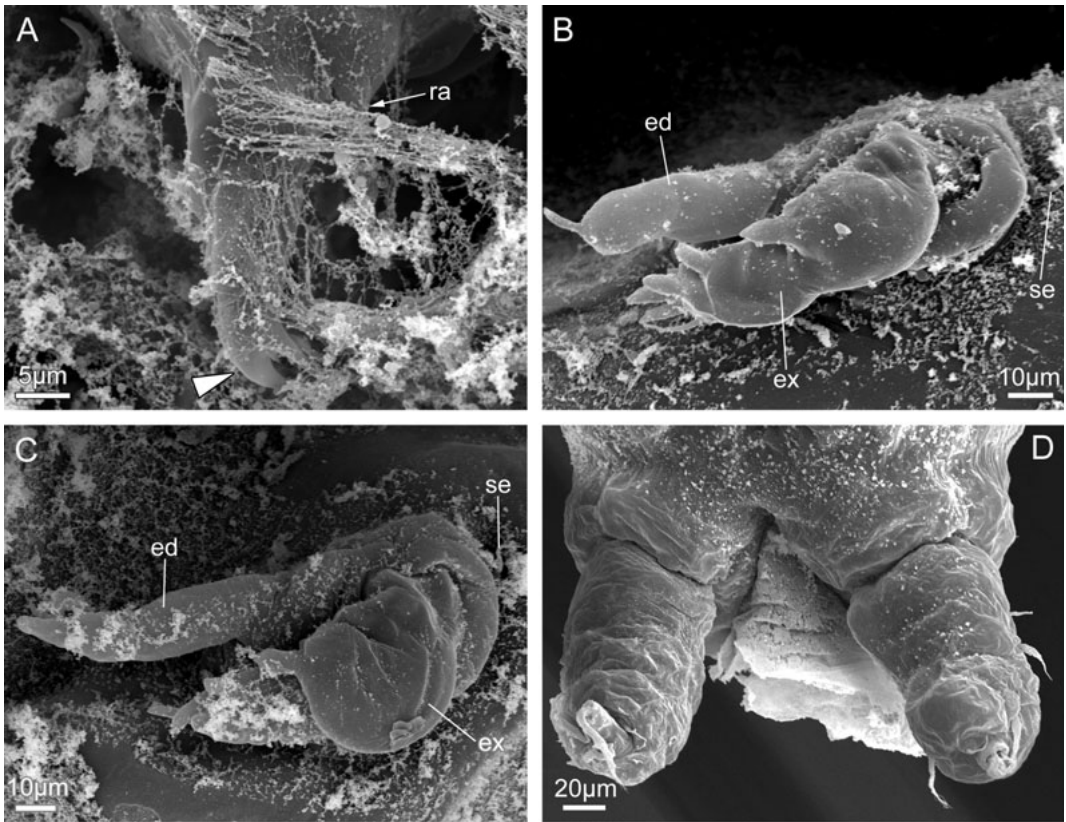


Fig. 2. *Briarella doliaris*, ♀. SEM-micrographs. **A.** Maxilla (left) with two apical elements (arrow). **B.** 2nd thoracopod (left). **C.** 3rd thoracopod (left). **D.** Caudal rami. Abbreviations: **ed**, endopodite; **ex**, exopodite; **ra**, ramus; **se**, seta.

1928), while *B. doliaris* shows a stocky body with an enlarged thorax and a slender abdomen, and five pairs of longer lateral processes. Furthermore, *B. risbeci* possesses three setae on the maxillule (Huys 2001), whereas *B. doliaris* has only two spines at the apex of the maxillule. Monod labelled a mandibular palp for *B. risbeci* (Monod 1928). Huys re-examined *B. risbeci* and *B. disphaerocephala*. In his drawings he reproduced a structure similar to the mandibular palp of Monod, but did not mention it in the text (Huys 2001). Nevertheless the presence of a mandibular palp can be confirmed in this study (Fig. 1E). In the specimens examined herein the antennule is indistinctly 4-segmented, while in *B. risbeci* it shows 5-6 segments (Monod 1928). Further differences concern the thoracopods. In *B. doliaris* both pairs of thoracopods are biramous, with a 2-segmented exopodite bearing 5 strong spines, whereas in *B. risbeci* the thoracopods are uniramous, with the second thoracopod bearing 5 spines and the third thoracopod bearing none (Monod 1928). In contrast to *B. risbeci*, which has egg sacs longer than the whole

body, *B. doliaris* has short egg sacs. *Briarella* thus far was exclusively found in dorid nudibranchs (Huys 2001), what also applies for *B. doliaris*, which infests *Ceratosoma trilobatum*. The latter is already known as host for *B. microcephala* (see Monod 1928).

Monod (1928) described *B. microcephala* with five pairs of lateral lobes, but with a very vermiform body shape; this stands in clear contrast to the stocky body of *B. doliaris*. *Briarella disphaerocephala* is considered to be similar to *B. risbeci* (see Monod 1928; Monod & Dollfus 1932; Huys 2001). In *B. disphaerocephala* the maxillule possesses three setae like in *B. risbeci* (see Huys 2001) and *B. disphaerocephala* possesses two more pairs of lateral lobes. One pair is situated on the sides of the head and one pair is located in the pregenital area (Monod & Dollfus 1932). Thus *B. disphaerocephala* is also different to *B. doliaris*.

Bergh's unnamed *Briarella* sp. (see illustration in Monod & Dollfus 1932: fig. 17E) externally is very similar to *B. microcephala* (see Bergh 1876) and to *B. risbeci* (see Monod 1928), and thus differs from the stocky body shape of *B. doliaris*. The egg sacs of

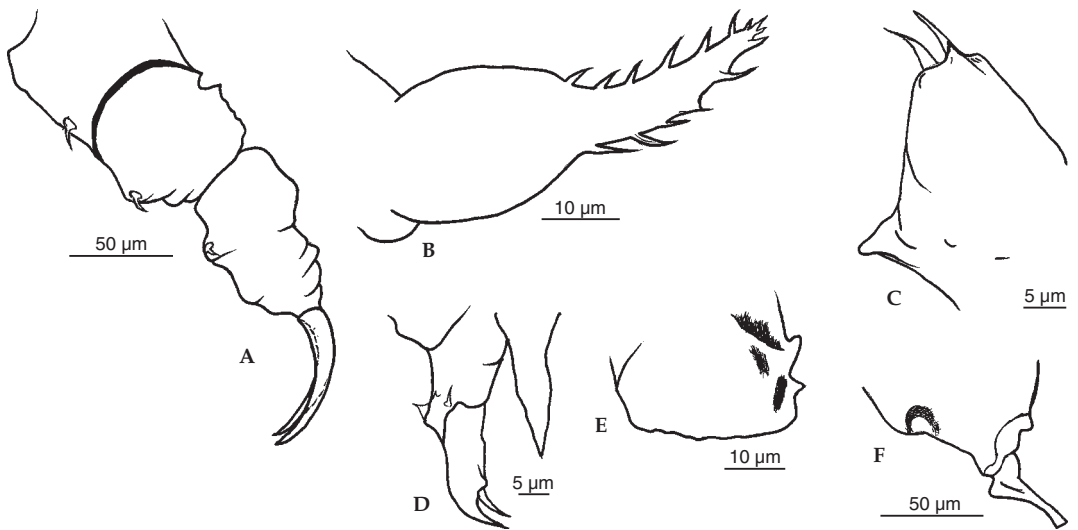


Fig. 3. *Briarella doliaris*, ♀. Cephalic appendages. A. Antenna. B. Mandible. C. Maxillule. D. Maxilla. E. Mandible palp. F. Maxilliped.

Briarella sp. are only half as long as the whole body, and thus more similar to those of *B. doliaris*. Bergh (1876) also describes the antennule of *Briarella* sp. as 5-segmented, while in *B. doliaris* it is indistinctly 4-segmented. *Briarella* sp. is only known from the Philippines, where it infests *Chromodoris elisabethina* and *A. cespitosus* as hosts (Monod 1928).

Our material examined thus differs from all known congeners, and the new species *B. doliaris* is established.

Discussion

On the one hand, according to our results, there is no doubt that *Briarella doliaris* spec. nov. belongs to the genus *Briarella*. The cephalic appendages of the new species *B. doliaris* fit exactly with the general description of *Briarella* and *Philoblenna* mouthparts by Huys (2001), supporting the common placement within the Philoblennidae (see Izawa 1976; Ho 1981; Huys 2001). On the other hand *B. doliaris* shows several novel features observed for the genus *Briarella* such as a maxillule possessing two instead of three setae (Fig. 2F), and the second and third thoracopods being biramous (Fig. 3B,C) instead of uniramous as described by Monod (1928) for *B. risbeci*. Furthermore, *Briarella doliaris* has a stocky body with four pairs of long and one pair of short lateral processes, whereas all other four *Briarella* species are vermiform with a varying number of short lateral processes (Monod 1928). More than other congeners, adult

B. doliaris thus resemble female splanchnotrophids, in particular the genus *Splanchnotrophus*, concerning the shape of the body and egg sacs and the biramous thoracopods (Huys 2001). The lateral processes are, concerning their length, also in a stage between *B. risbeci* and *Splanchnotrophus angulatus* Hecht, 1893 (see Monod 1928; Huys 2001). The fifth short lateral process of *B. doliaris* is similar to the lateral outgrowth of *S. angulatus* as described by Huys (2001). It is thus possible, that *B. doliaris* represents a “missing link” between the two genera *Briarella* and *Splanchnotrophus*. However, there are some major differences between *B. doliaris* and *S. angulatus*. One is the presence of maxillipeds and of a mandibular palp in *Briarella* which are generally missing in Splanchnotrophidae (see Huys 2001). Also, in *Briarella* the head is distinctly set off from the thorax, whereas in *Splanchnotrophus* there is no such distinct border (Huys 2001). Another difference is the presence of a very reduced fourth pair of thoracopods in splanchnotrophids like *S. angulatus* (see Huys 2001). Unfortunately such appendages could not be found in *B. doliaris*, possibly due to remainders of host tissue covering that particular area. This last point will need further investigation as soon as more material is available.

Accepting that *Briarella*, *Chondrocarpus* and *Philoblenna* belong to a monophyletic group (Izawa 1976; Ho 1981; Huys 2001), the strong similarity between *Briarella* and *Splanchnotrophus* may be explained by common ancestry. In this scenario, the Philoblennidae would include a plesiomorphic ectoparasitic

genus *Philoblenna* retaining features such as swimming legs (Izawa 1976; Ho 1981; Avdeev et al. 1986; Ho & Kim 1992; Huys 2001). The common ancestor of *Chondrocarpus*, *Briarella* and splanchnotrophids switched to an endoparasitic life in sea slug hosts, reducing swimming legs and evolving a stocky body with long lateral processes and evolving dwarf males.

If *Briarella* is monophyletic, then a stocky body with long lateral processes has evolved in the common ancestor with splanchnotrophids (and possibly *Chondrocarpus*), and *B. doliaris* would represent the most basal offshoot of a clade of secondarily vermiform *Briarella* species. If *Briarella doliaris* is the direct sister to splanchnotrophids (and perhaps *Chondrocarpus*), rendering the genus *Briarella* paraphyletic; potential synapomorphies of such a clade include the stocky body shape, reduced body size, the possession of a fifth lateral appendage (only four in *Chondrocarpus* and several splanchnotrophids), and a successive reduction of antennule segments.

Although the herein described *B. doliaris* is more similar to *Splanchnotrophus* than any of its congeners, such similarities still may reflect convergent adaptations to an endoparasitic mode of life in the same group of hosts. Available morphology-based phylogenetic analyses are not conclusive yet. Analyses by Ho (1991) resulted in the Splanchnotrophidae (in the old, much broader sense; current usage applies to Huys 2001) as sister to Shiinoidae, a group of ectoparasites on fish that is highly dissimilar to endoparasitic Splanchnotrophidae in the modern, strict sense.

In conclusion, morphological studies on more material including males are necessary. Future phylogenetic studies should explore whether *Briarella* (or a subset thereof) is the sister group to Splanchnotrophidae and/or *Chondrocarpus*. The traditional inclusion of all endoparasitic copepods of sea slugs in the Splanchnotrophidae may ultimately remain the preferred arrangement (Monod & Dollfus 1932; Jensen 1987).

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