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Arthurius bunakenensis, a new tropical Indo-Pacific species of endoparasitic copepods from a sacoglossan opisthobranch host

(Crustacea, Copepoda, Poecilostomatoida, Splanchnotrophidae)

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Within the framework of our worldwide revision of splanchnotrophid copepod endoparasites we herein describe *Arthurius bunakenensis* spec. nov., the second species of the previously monotypic genus *Arthurius* Huys, 2001. During our joint International Bunaken National Park Diving Expedition 2003 we found female and male specimens parasitizing within the sacoglossan *Elysia pusilla* Bergh, 1872 at Gangga Island, off northern Sulawesi, Indonesia. The parasites are described using Scanning Electron Microscopy (SEM). As in *Arthurius elysiae* (Jensen, 1990), mandibles and maxillules are absent in both sexes which is diagnostic for the genus. While males are very similar to each other, females of *Arthurius bunakenensis* n. sp. clearly differ from *A. elysiae* by the number, shape and position of body processes. An unnamed putative congener described by Risbec (1930) shows similar body processes but was inadequately described for detailed comparison.

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Introduction

Most shell-less opisthobranchs are well-known for having bioactive compounds protecting them efficiently from potential predators (see Wägele et al. 2006). Parasites such as the bizarrely shaped endoparasitic copepods of the family Splanchnotrophidae, however, obviously overcome such chemical defence. Recent studies showed there is an extraordinarily high splanchnotrophid diversity and prevalence in temperate waters off central Chile, where over 20 % of shell-less opisthobranch species are already known as hosts for an array of probably

host-specific Ismailia species (e.g. Schrödl 2002, 2003, Haumayr & Schrödl 2003). In contrast, tropical Indo-Pacific waters count with much higher opisthobranch diversity but surprisingly low splanchnotrophid parasite diversity (3–4 different species according to Huys 2001). Our recent investigations added three new and also apparently host specific *Ceratosomicola* Huys, 2001 species to the fauna of Sulawesi (Salmen et al. 2008). In the present paper we contribute with another new and remarkable splanchnotrophid species; thus, as a whole, doubling the previously known species diversity of Indo-Pacific splanchnotrophids.

The monotypic splanchnotrophid genus *Arthurius* Huys, 2001 was established to accommodate the aberrant *Splanchnotrophus elysiae* Jensen, 1990. Together with the Caribbean *Ismaila jenseniana* Haumayr & Schrödl, 2003 and the Patagonian *I. magellanica* Haumayr & Schrödl, 2003, *A. elysiae* was the only nominal splanchnotrophid species infecting sacoglossan hosts (see Jensen, 1990; Huys, 2001; Haumayr & Schrödl, 2003). An unnamed *Arthurius*-like species was found parasitizing in *Elysia ornata* (Swainson, 1840) but was only superficially described by Risbec (1930). The present study adds a new *Arthurius* species found living as an endoparasite in *Elysiella pusilla* Bergh, 1872 off northern Sulawesi. The morphology of female and male parasites is analyzed by SEM. The new species is compared with congeners and the genus *Arthurius* is discussed.

Material and Methods

All available Opisthobranchia have been collected during the International Bunaken Marine Park Diving Expedition 2003 (see Burghardt et al. 2006) and examined externally for parasites (see Schrödl 2002). The only specimens infested with splanchnotrophid endoparasites belong to the sacoglossan *Elysiella pusilla*. After taking photographs of living hosts, the specimens were anaesthetized in isotonic $MgCl_2$ solution and fixed in 70 % ethanol. The parasites were dissected out of the preserved hosts and examined with an Olympus SZX 12 binocular equipped with a "Jenoptic ProgRes C12" camera. For SEM-examination the copepods were dehydrated in a graded acetone series. The parasites were 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 ultrastructural analysis and digital documentation. Descriptive terms used herein are adopted from Gruner et al. (1993), Huys (2001) and Haumayr & Schrödl (2003). For details see Salmen et al. (2008).

Taxonomy

Class Copepoda H. M. Edwards, 1840

Order Poecilostomatoida Thorell, 1859

Family Splanchnotrophidae Norman & Scott, 1906

Genus *Arthurius* Huys, 2001

Arthurius bunakenensis spec. nov.

(Figs 1-3)

Material. Holotype (SEM mounted ♀, Zoologische Staatssammlung München-ZSM 20071015), 2 paratypes (SEM mounted: 1♀, ZSM 20071016, 1♂, ZSM 20071017; all partly damaged), collected by Michael Schrödl, Gangga Island, Bunaken National Park, N-Sulawesi, Indonesia, 23 July 2003. Host: *Elysiella pusilla* Bergh, 1872 (ZSM Mol 20033712). 2♀, 1♂ examined by SEM.

Etymology. The species name refers to our successful international inventory of the marine life of the Bunaken National Park, in the northern outskirts of which the new species was found.

Description

Female (Figs. 1-2). Body compact, about as wide as long, measuring 0.62-0.64 mm in length (without processes). Segmentation of thorax and cephalothorax not detectable, abdomen 2-segmented. Parasites with orange tissue shining through thin, translucent cuticle (Fig. 1B). Thorax with six pairs of appendages; pairs 1, 3, 5 and 6 situated laterally, 1 and 3 directed towards anterior, 5 and 6 posterior. Appendages 2 and 4 situated more ventrally, with varying direction. All appendages, except for pair 2, with about same length (up to 1.1 mm); cone-shaped or ending in terminal bulb. Second pair of appendages shorter and more slender.

Cephalothorax displaced to ventral side, hardly demarcated from trunk. Antennule (Fig. 1F) 1-segmented and short with one pinnate seta at apex. Antenna (Fig. 1E) strong, long, probably 2-segmented. No further mouthparts detectable.

First thoracopod absent. Second thoracopod (Fig. 2A) vestigial, biramous; arising from ventral swelling. Endopod smaller than exopod, both spinous with pointed tip. No further thoracopods detectable (due to dirt covering specimens).

Abdomen indistinctly 2-segmented (Fig. 2B), with several integumental pores on surface. First segment with genital openings represented by large lateral slits. Second segment very small, bearing short and stout caudal rami. Each ramus with one minute spine mediolaterally and at least one at apex. Egg sacs banana-shaped and slightly greenish in living animal (Fig. 2F).

Male (Fig. 4). Body cyclopiform, measuring 252 µm in length. Cephalothorax (head and fused first tho-

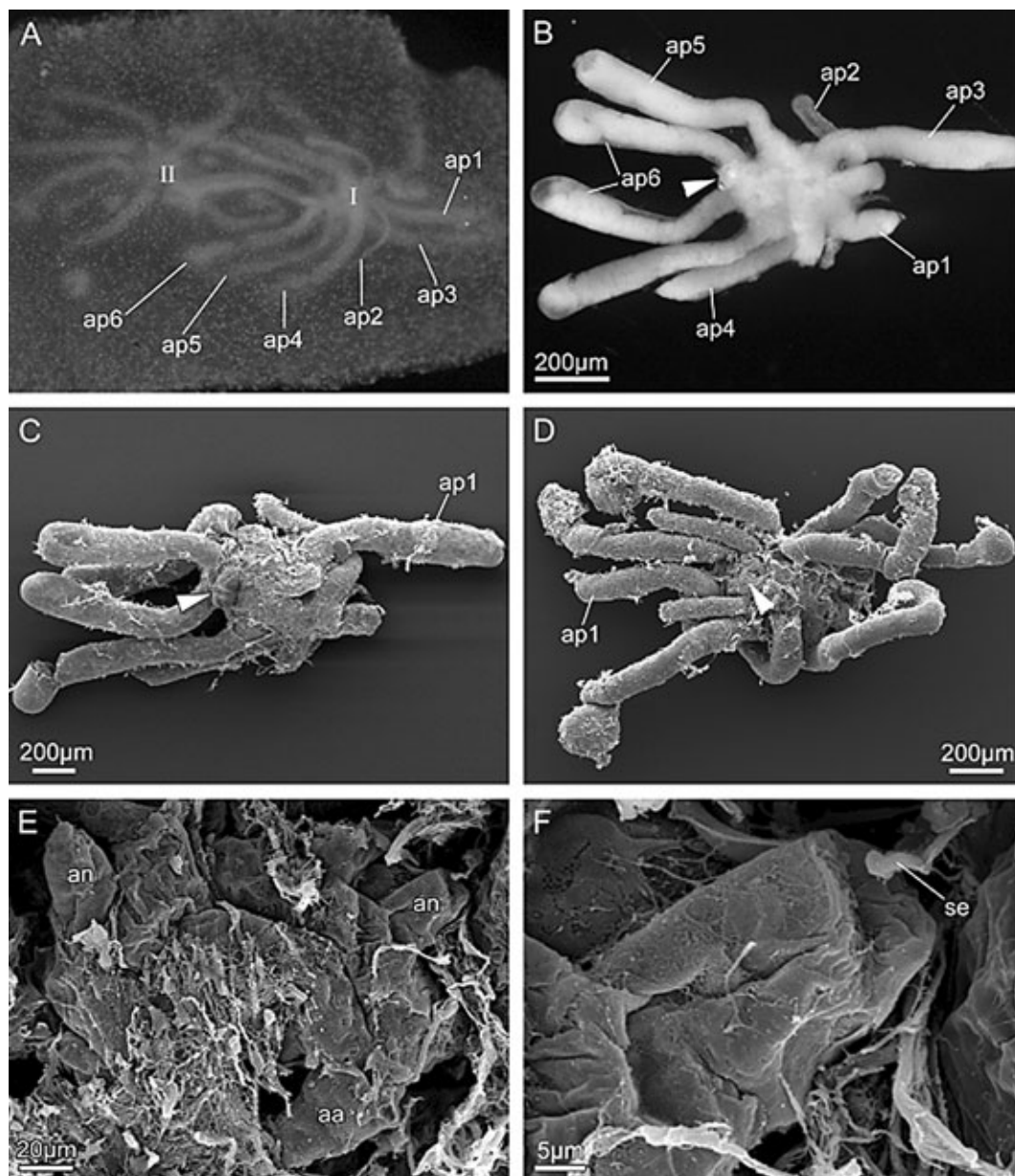


Fig. 1. *Arthurius bunakenensis* spec. nov., ♀. **A, B.** light microscope pictures. **A.** Parasites shining through host integument, ventral view. All six pairs of appendages visible. **B.** Habitus female I, dorsal view. Appendages 1-4 damaged. Abdomen (arrow). **C-F.** SEM-micrographs. **C.** Habitus female I, dorsal view. Abdomen (arrow). **D.** Habitus female II, ventral view. Cephalothorax (arrow). **E.** Cephalic appendages; antennules, antenna (left). **F.** Antennule (left). – **I**, female one; **II**, female two; **aa**, antennule; **an**, antenna; **ap1-6**, appendages 1-6; **se**, seta.

racic segment) large, distinctly set off from 5-segmented thorax; abdomen short, segmentation not detectable (Fig. 3A).

Cephalothorax and cephalic appendages (Fig. 3B)

large compared to body size. Antennule (Fig. 3C) short, 1-segmented; with at least six vestigial setae at apex. Antenna (Fig. 3B) very large, 2-segmented; first segment with one minute, blunt spine mediolater-

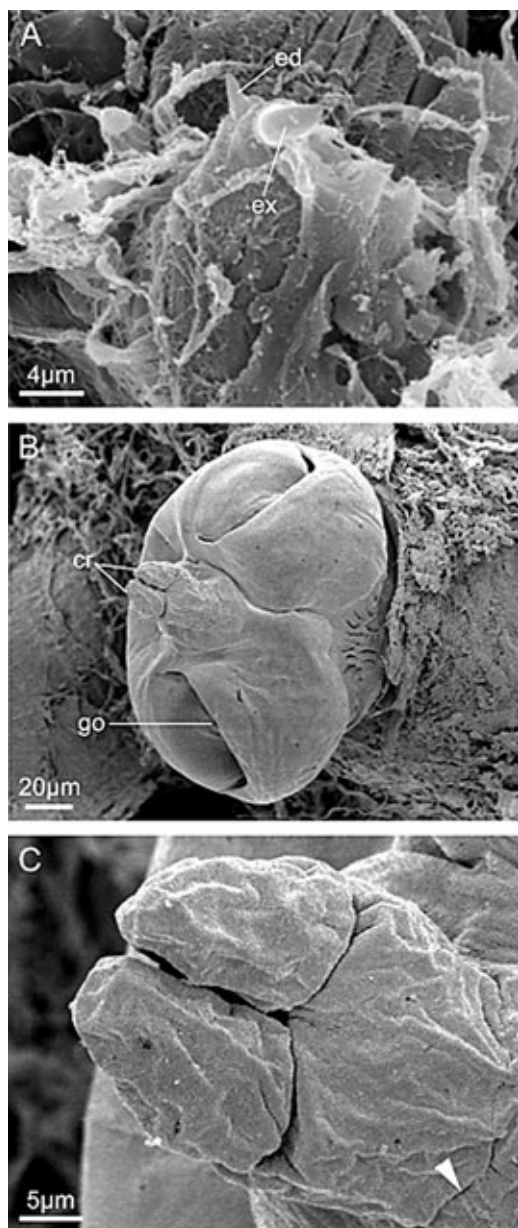


Fig. 2. *Arthurius bunakenensis* spec. nov., ♀. SEM-micrographs. A. 2nd thoracopod (right). B. Abdomen. C. Caudal rami; anal somite (arrow marks segment border). – cr, caudal rami; ed, endopod; ex, exopod; go, genital opening.

ally; second segment drawn out into strong claw. Labrum (Fig. 3D) rudimentary, with paired spinous projections. Mandible and maxillule absent. Maxilla not detectable. Labium medially incised (Fig. 3D).

Thorax without any processes, enlarged second and third segment fused, bearing second and third thoracopods; thoracic segments four to six slender (Fig. 3A).

First thoracopod absent. Second and third thoracopod very large compared to body size. Second thoracopod biramous (Fig. 3E), consisting of protopodit (coxa and basis indistinctly demarcated), 1-segmented endo- and exopod. Exopod with three spines laterally and one long and strong spine distally. Endopod with two strong apical spines, one twice as long as the other. Endo- and exopod with spinular pattern on surface. Third thoracopod biramous (Fig. 3E); comprising protopodit (coxa and basis), 1-segmented endo- and exopod. Exopod shorter than that of second thoracopod, with two lateral spines, distal spine shorter. Endopod with two terminal spines, both with about same length. Proximal edge of basis with three minute spines laterally. Endo- and exopod with spinular pattern on surface. Fourth thoracopod (Fig. 3F) slender, uniramous process with pointed tip; situated laterally at posterior edge of fourth thoracic segment.

One abdominal segment detectable from external (second one possibly retracted). Caudal rami long and strong. (Fig. 3A)

Biology. The single host specimen, i.e. the sacglossan *Elysiella pusilla*, was infested with two parasites of each sex. The females were laying one behind the other inside the host, with their heads pointing towards the host's head. Their abdomens were protruding through the host integument dorsally between the parapodia. One female beared egg sacs. The males were laying freely in the body cavity.

Remarks. The parasites investigated herein differ from all other splanchnotrophids, except for the thus far monotypic genus *Arthurius*, by the absence of mandible and maxillule, the special morphology of second and third thoracopods, the female cephalothorax displaced to the ventral side, and the female body processes having a terminal bulb (see Huys, 2001). Further features in common with *Arthurius elysiae* are the considerably reduced extremities, 1-segmented antennules, antennae and (male) maxillae, and the rudimentary labrum and labium. Uniquely among Splanchnotrophidae, *Arthurius elysiae* shows sexual dimorphism concerning cephalic appendages (Huys 2001, present study), i.e. the maxilla is 2-segmented in females and 1-segmented in males. It is unclear whether or not dimorphism applies to the mouthparts of the herein examined specimens as well, because the mouthparts of females are not clean enough to detect details. But it seems as if the antennules of male and female are different

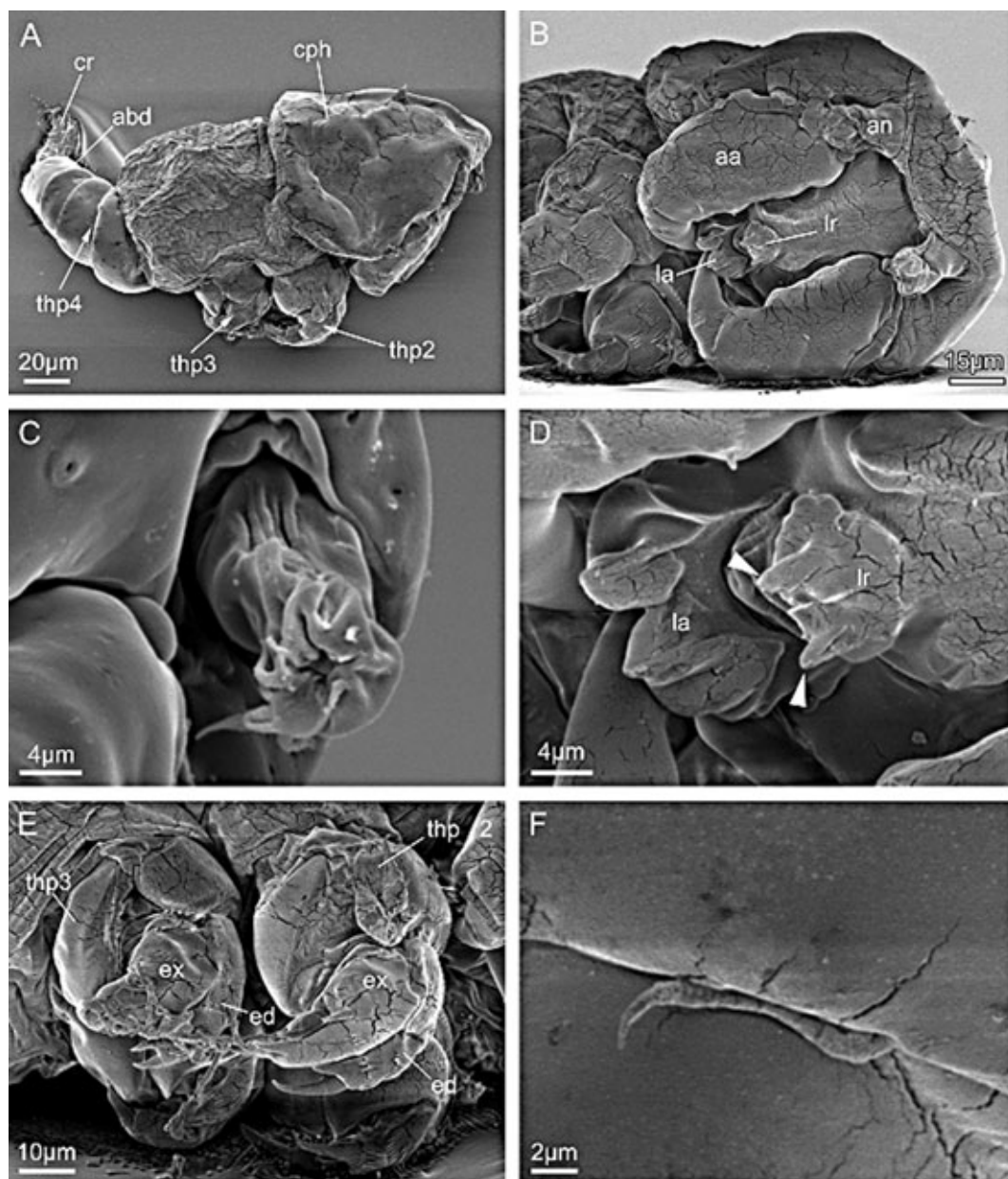


Fig. 3. *Arthurius bunakenensis* sp. nov., ♂. SEM-micrographs. **A.** Habitus, lateral view. Position of thoracopods 2-4. Abdomen, caudal rami. **B.** Cephalic appendages. **C.** Antennule (left). **D.** Oral area. Labium, labrum with paired spinous projections (arrows). **E.** 2nd and 3rd thoracopods. **F.** 4th thoracopod. – aa, antenna; abd, abdomen; an, antennule; cph, cephalothorax; cr, caudal rami; ed, endopodit; ex, exopodit; la, labium; lr, labrum; thp2-4, thoracopods 2-4.

regarding the armature of the apex. More individuals have to be examined to determine the degree of dimorphism and intraspecific variation. At least, the two females studied are similar regarding size

and shape of the body. The number and position of the lateral processes are equal in both individuals, only the terminal bulbs of the processes are more marked in female II.

There is thus no doubt that our specimens can be placed into the genus *Arthurius* which contained the type species *A. elysiae*, and an unnamed species illustrated by Risbec (1930) only. The males of *Arthurius bunakenensis* spec. nov., and those of *A. elysiae* show a high resemblance. This applies to the body shape, the cephalothorax that is not fused with second and third thoracic segments, the number of thorax segments, and the morphology of second and third thoracopod and of some of the mouthparts. Different is the number of thoracopods. *Arthurius elysiae* has two pairs of thoracopods ("leg 1 and 2" according to Huys 2001), while *Arthurius bunakenensis* spec. nov. has an additional third pair, i.e. thoracopod number four. In *A. elysiae* there are two spine-like projections anterior to the labrum (Huys 2001), whereas these projections in *Arthurius bunakenensis* spec. nov. are situated on the surface of the labrum (Fig. 2D). The abdominal segmentation of *Arthurius bunakenensis* spec. nov. is unclear, but it seems there are two segments (second one retracted) as in *A. elysiae* (Huys 2001).

The females of both species are similar regarding the shape of the body, of abdomen and egg sacs, and of second thoracopods. Evident differences refer to the number, shape and position of the body processes. *Arthurius elysiae* has three pairs of robust processes laterally and one pair of smaller processes ventrally (Huys 2001), slightly tapering towards distal and ending in a bulb. All processes of *A. elysiae* are conical with a broad base and just about as long as the whole body, i.e. female specimens look stout and massive. The second thoracopod of *A. elysiae* ("leg 1") is associated with the anteroventral process (Huys 2001). In contrast, *Arthurius bunakenensis* spec. nov. has four pairs of slender, more or less cylindrical processes situated laterally and two ventrally; the processes successively widen towards distally and at least some have a distinct terminal bulb. All processes, except the second one, are about twice as long as the whole body; female *Arthurius bunakenensis* spec. nov. look slender and delicate (Fig. 1A). The second thoracopod is not associated with any process.

Arthurius bunakenensis spec. nov., and Risbec's unnamed species show a high resemblance concerning the body shape, i.e. they possess the same number of processes in the same position (four lateral, two ventral). The unnamed species has a terminal bulb on every process (Risbec 1930), whereas in *Arthurius bunakenensis* spec. nov. only some of the processes end with bulbs. In both species the abdomen is dislocated dorsally. No details of cephalic appendages and of thoracopods are described for the unnamed

species, for this reason no further comparisons can be made.

Arthurius bunakenensis spec. nov. occurs, like its two congeners, in a sacoglossan host, but not in the same species. *Arthurius elysiae* is known from *Elysia australis*, Risbec's unnamed species from *Elysia ornata* and *Arthurius bunakenensis* spec. nov. from *Elysiella pusilla*. For the facts mentioned above the parasites examined herein are regarded as belonging to a distinct and new species.

Endoparasitic splanchnotrophids are known to occur in nudibranch and sacoglossan opisthobranchs. *Ismaila* species infect Nudibranchia and, with *Ismaila magellanica*, also Sacoglossa (Haumayr & Schrödl 2003). *Arthurius* infects only sacoglossan hosts, while *Splanchnotrophus*, *Lomanotricula* and *Ceratosomicola* are limited to nudibranch hosts. As all *Ismaila* and *Ceratosomicola* species, also the currently known *Arthurius* species appear to be host-specific; our knowledge on *Arthurius* is, however, still limited to very few infested host specimens. In contrast to *Ismaila* species, where hosts belong to several different nudibranch families and one sacoglossan family, hosts of *Ceratosomicola* all belong to one nudibranch family, the Chromodorididae (Salmen et al. 2008). The same applies to the three host species of *Arthurius*, which all are sacoglossan Plakobranchidae. As in the case of *Ceratosomicola*, radiation through switching to closely related host species and later speciation is likely.

The female *Arthurius* specimens examined herein were not in contact with gonads or other organs of their host, and they possibly lack anal slits. The complete loss of mandible and maxilla also points towards a highly specialized endoparasitic life as hemolymph suckers.

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