ISSN : 0082 - 6340
E-ISSN : 2337 - 876X
Accredited : 30/E/KPT/2018

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Vol. 47, no. 2, pp. 77–154 **December 2020**

Published by

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TREUBIA
RESEARCH CENTER FOR BIOLOGY - INDONESIAN INSTITUTE OF SCIENCES (LIPI)
Jl. Raya Jakarta-Bogor Km. 46, Cibinong, Bogor 16911, Indonesia
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http://e-journal.biologi.lipi.go.id/index.php/treubia

VOL. 47, NO. 2, DECEMBER 2020

CONTENT

TREUBIA

(A JOURNAL ON ZOOLOGY OF THE INDO-AUSTRALIAN ARCHIPELAGO)

ISSN : 0082 - 6340
E-ISSN : 2337 - 876X

Date of issue: 30 DECEMBER 2020

UDC: 593.4:595.35(594)

Pipit Pitriana

Exploring sponge-inhabiting barnacles of eastern Indonesia using micro-CT scanning

TREUBIA, December 2020, Vol. 47, No. 2, pp. 77–98.

We present a morphological study of Indonesian sponge-inhabiting barnacles using standard light microscopy in combination with micro-CT scanning and computer-aided 3D-reconstruction of the external shell morphology. A taxonomic analysis of the material detected four different genera of sponges inhabited by five different species of balanomorph barnacles, two of which are undescribed. Together with conventional morphological examination by dissection, we provide modern non-destructive imaging methods, using micro-CT scanning to enhance our knowledge of the morphological characters of sponge-inhabiting barnacles from eastern Indonesia. Although there were some methodological limitations regarding the contrast-enhancing technique, this study demonstrates micro-CT as a useful non-destructive technique of integrative taxonomy, for the examination of sponge-inhabiting barnacles.

(Pipit Pitriana, Andreas Wessel, Tina Aschenbach, and Kristina von Rintelen)

Keywords: Cirripedia, Indonesian biodiversity, integrative taxonomy, micro-computer tomography, shell morphology

UDC: 598.89:577.2

Jarulis

Characters of mitochondrial DNA D-loop hypervariable III fragments of Indonesian Rhinoceros Hornbill (*Buceros rhinoceros*) (Aves: Bucerotidae)

TREUBIA, December 2020, Vol. 47, No. 2, pp. 99–110.

The Rhinoceros Hornbill (*Buceros rhinoceros*) genetic characteristics consist of nucleotide polymorphisms, haplotypes, genetic distances, and relationships which are important for their conservation effort in Indonesia. We sequenced mitochondrial DNA D-loop hypervariable III fragments from five Rhinoceros Hornbill individuals at Safari Park Indonesia I and Ragunan Zoo, which were isolated using Dneasy® Blood and Tissue Kit Spin-Column Protocol, Qiagen. D-loop fragment replication was done by PCR technique using DLBuce_F (5'-TGGCCTTTCTCCAAGGTCTA-3') and DLBuce_R (5'-TGAAGG AGT TCATGGGCTTAG-3') primer. Thirty SNP sites were found in 788 bp D-loop sequences of five Rhinoceros Hornbill individuals and each individual had a different haplotype. The average genetic distance between individuals was 3.09% and all individuals were categorized into two groups (Group I: EC6TS, EC1RG, EC2TS and Group II: EC9TS, EC10TS) with a genetic distance of 3.99%. This result indicated that the two groups were distinct subspecies. The genetic distance between Indonesian and Thai Rhinoceros Hornbills was 10.76%. Five Indonesian Rhinoceros Hornbill individuals at Safari Park Indonesia I and Ragunan Zoo probably came from different populations, ancestors, and two different islands. This study can be of use for management consideration in captive breeding effort at both zoos. The D-loop sequence obtained is a

useful character to distinguish three Rhinoceros Hornbill subspecies in Indonesia.

(Jarulis, Choirul Muslim, Dedy Duryadi Solihin, Ani Mardiastuti, and Lilik Budi Prasetyo)

Keywords: Bucerotidae, control region, phylogenetic, Rhinoceros Hornbill conservation, zoo

UDC: 595.773.4(594.59)

Eka Kartika Arum Puspita Sari

Diversity of fruit flies (Tephritidae: *Bactrocera* spp.) in campus C of Airlangga University, Surabaya, Indonesia

TREUBIA, December 2020, Vol. 47, No. 2, pp. 111–122.

This research aims to get information about the species of host plants and fruit flies, composition and structure of community, distribution pattern, and impact of environmental factors to fruit flies in Campus C, Airlangga University. Research was conducted from August to November 2019. A modification of Steiner trap with methyl eugenol 1.5 ml bait was installed in nine sites. Each Steiner trap was placed on a mango tree 1-2 meters above ground level. Trapped fruit fly specimens were collected after one week. Four replications were made, with intervals between two periods of installation. As many as 682 host plants of the fruit flies were found at the study site consisting of 25 species from 15 families. Results showed that 1121 individuals of *Bactrocera* fruit flies were found, consisting of 5 species, namely *B. carambolae*, *B. dorsalis*, *B. minuscula*, *B. occipitalis*, and *B. musae*. The most abundant species was *B. carambolae* (62.8%), followed by *B. dorsalis* (27.3%), *B. minuscula* (8.4%), *B. occipitalis* (1%), and the lowest was *B. musae* (0.5%). *B. occipitalis* has an even distribution pattern, while four other species have aggregated distribution patterns. The diversity index at nine locations ranged from 0.772 (low) to 1.151 (moderate). *B. carambolae* and *B. dorsalis* were the dominant species. The presence of fruit flies was influenced by environmental (humidity, temperature, sunlight intensity, wind) and host plant factors.

(Eka Kartika Arum Puspita Sari, Moch. Affandi, and Sucipto Hariyanto)

Keywords: Dacinae, diversity, fruit flies, methyl eugenol, Steiner trap

UDC: 595.799:598.836:591.5(594.5)

Sih Kahono

First report on hunting behavior of migratory Oriental Honey-buzzard (*Pernis ptilorhynchus orientalis*) towards migratory giant honeybee (*Apis dorsata dorsata*) (Hymenoptera: Apidae) on Java Island, Indonesia

TREUBIA, December 2020, Vol. 47, No. 2, pp. 123–132.

Both migratory Oriental Honey-buzzard (*Pernis ptilorhynchus orientalis*) and migratory giant honeybee (*Apis dorsata dorsata*) can be found in South-east Asia. The Oriental Honey-buzzard is the main predator of the giant honeybee, prey upon its honeycomb, larvae, and honey. Its existence always follows the migration of the giant honeybee. They stay on Java island during the migratory season. The giant honeybee lives in a large colony and has a powerful sting that is useful for defence against its predators. The bee is among the most dangerous animals since its threatening defensive behavior causes severe impact on the eagle and is even frequently fatal for human beings. Data collections on hunting behavior of the Oriental Honey-buzzard were based on irregular observations and interviews between the year 2003 to 2019. We categorized five hunting behaviors during data collections: flying orientation around the bee's nest, attack on living nest, failure to collect the living nest, preying upon the newly empty nest, and transferring attack of the angry bee to people nearby. The safest hunting for the Oriental Honey-buzzard is to prey upon newly empty nest left by the honeybee. When the nest was still occupied by the bee colonies, the eagle should develop a strategy to avoid and reduce the risk of being attacked. It sometimes transfers the attack to people nearby.

(Sih Kahono, Dewi M. Prawiradilaga, Djunijanti Peggie, Erniwati, and Eko Sulistyadi)

Keywords: hunting behavior, Java, migratory giant honeybee, Oriental Honey-buzzard

UDC: 595.798:57.06(594.4)

Fuki Saito-Morooka

Taxonomic notes on the hover wasp genus *Eustenogaster* (Vespidae, Stenogastrinae), with description of two new species from Sumatra Island, Indonesia

TREUBIA, December 2020, Vol. 47, No. 2, pp. 133–154.

Wasps of the genus *Eustenogaster* van der Vecht, 1969, with 17 species currently recognized, are distributed from the Indian subcontinent in the west to the Philippines, Sulawesi Island and Java Island in the east. Two new species of hover wasp genus *Eustenogaster* (*E. multifolia* **sp. nov.**, *E. sumatraensis* **sp. nov.**) are described from specimens collected in Sumatra Island. The female of *E. vietnamensis* occurring in Vietnam are described for the first time. The lectotypes of *Paravespa eva* Bell, 1936 and *Ischnogaster ornatifrons* Cameron, 1902 are designated. The new taxonomic status is proposed for *Stenogaster eximioides* Dover and Rao, 1922 as a good (=valid) species of *Eustenogaster*. The synonymy of *Ischnogaster ornatifrons* Cameron, 1902 with *Eustenogaster micans* (de Saussure, 1852) has been confirmed. A revised key to species and a taxonomic and distributional checklist of all the species of *Eustenogaster* are provided.

(**Fuki Saito-Morooka, Hari Nugroho, Alan Handru, and Jun-ichi Kojima**)

Keywords: distributional checklist, lectotype, new status, revised key, synonym

Treubia, 47(2): 77–98, December 2020

DOI: 10.14203/treubia.v47i2.3968

EXPLORING SPONGE-INHABITING BARNACLES OF EASTERN INDONESIA USING MICRO-CT SCANNING

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Received: 10 September 2020; Accepted: 01 December 2020

ABSTRACT

We present a morphological study of Indonesian sponge-inhabiting barnacles using standard light microscopy in combination with micro-CT scanning and computer-aided 3D-reconstruction of the external shell morphology. A taxonomic analysis of the material detected four different genera of sponges inhabited by five different species of balanomorph barnacles, two of which are undescribed. Together with conventional morphological examination by dissection, we provide modern non-destructive imaging methods, using micro-CT scanning to enhance our knowledge of the morphological characters of sponge-inhabiting barnacles from eastern Indonesia. Although there were some methodological limitations regarding the contrast-enhancing technique, this study demonstrates micro-CT as a useful non-destructive technique of integrative taxonomy, for the examination of sponge-inhabiting barnacles.

Keywords: Cirripedia, Indonesian biodiversity, integrative taxonomy, micro-computer tomography, shell morphology

ABSTRAK

Kami menyajikan studi tentang morfologi teritip Indonesia yang hidup di dalam spons dengan menggunakan mikroskop cahaya yang dikombinasikan dengan pemindaian menggunakan micro-CT dan komputer untuk merekonstruksi gambar 3D dari morfologi cangkang luar. Hasil analisis taksonomi dari material sampel mendeteksi adanya empat genus berbeda dari spons yang dihuni oleh lima spesies berbeda dari teritip balanomorpha, dua diantaranya adalah spesies yang belum pernah dideskripsikan sebelumnya. Bersama dengan teknik pengamatan konvensional melalui pembedahan, kami juga melakukan metoda pengamatan modern tanpa merusak sampel dengan pemindaian menggunakan micro-CT untuk memperluas pengetahuan kita tentang karakter morfologi teritip penghuni spons dari Indonesia kawasan timur. Walaupun terdapat keterbatasan metodologi yang berhubungan dengan teknik penajaman kontras gambar, studi ini menunjukkan bahwa pemindaian dengan menggunakan micro-CT adalah teknik tanpa perusakan sampel yang berguna dalam taksonomi integratif untuk mengamati teritip yang hidup di dalam spons.

Kata kunci: Cirripedia, biodiversitas Indonesia, taksonomi integratif, tomografi *micro-computer*, morfologi cangkang

INTRODUCTION

Barnacles (Cirripedia) are sessile crustaceans where the adult forms are structurally and biologically diverse, and permanently attached to hard substrata or to other living organisms (Newman & Abbott, 1980; Høeg et al*.,* 2015). It comprises three orders, the Thoracica or 'true barnacles' (including Sessilia and Pedunculata), the Acrothoracica (burrowing barnacles), and the Rhizocephala (parasitic barnacles) (Høeg et al*.,* 2015). Some members of the suborder Balanomorpha selectively choose invertebrates or vertebrates as their hosts, ranging from sponges to whales (Foster, 1978). Many epizoic barnacles burrow into, or are overgrown, by the host's tissue (Anderson, 1994). Members of subfamily Acastinae and genus *Membranobalanus* are examples of epibiotic barnacles. Acastinae are obligate symbionts of Porifera and Cnidaria, but also be found in association with gorgonians, alcyonarians, or antipatharians; while *Membranobalanus* are obligate symbiont of Porifera (Kolbasov, 1993; Van Syoc et al., 2015; Yu et al., 2017).

Most species of Acastinae have spines or projections of various lengths on the external surface of the parietes, penetrating as anchors into the sponge tissue (Kolbasov, 1993). In addition, according to Van Syoc et al. (2015) the Acastinae with membranous bases may often be distinguished from *Membranobalanus* spp. by the presence of these calcareous projections on the shell wall. These calcareous projections can be used to differentiate members of the Acastinae and *Membranobalanus*. However, the spines often break away from the shell wall when specimens are removed from their host sponge, leaving only "pores" in the shell wall marking the site of attachment (Van Syoc et al., 2015).

The Acastinae of eastern Indonesia have been studied by Hoek (1913) and Broch (1931–1932) and both noted calcareous projections on the surface of the shell wall. For *Acasta conica,* Hoek (1913: 235) stated "the surface of the parietes is smooth in the lower half, covered in the upper half with rounded scale-like patches, the exact nature of which I have not been able to discover", and likewise Broch (1931–1932: 98) for *Acasta foraminifera*, "the white shell is anchored in the sponge tissue by rather large spines; it is, however, almost absolutely impossible to prepare away the tissue of the sponge without removing also every such spine, so intimate is the connection with the extremely hard tissue of the sponge".

Modern non-destructive imaging methods, such as X-ray micro-computed tomography (micro-CT), are particularly well suited to overcome the problems of traditional challenges associated with the size and fragility of small invertebrates (Faulwetter et al., 2013; Semple et al., 2018). Studies on invertebrates utilising 3D-imaging tools now encompass a wide range of applications, including for comparative, developmental and functional morphological research (Metscher, 2009a; Semple et al., 2018). However, the widespread application of micro-CT imaging has only been used in a few studies of taxonomy of barnacles to date. For example, Noever et al. (2015) studied the rhizocephalan root system of parasitic barnacles in their crustacean host. Hosie et al. (2019) described a new species of sponge-inhabiting barnacle (*Membranobalanus porphyrophilus*) from Australia and presented an interactive, 3D micro-CT derived volume reconstruction of the designated holotype.

In this study, we present the use of micro-CT scanning and 3D reconstruction as a non-destructive method to visualize and count sponge-inhabiting barnacles inside their host.

Secondly, we provide a conventional morphological study of the same barnacle specimens extracted from their host, using light microscopy. Our aim is to demonstrate the potentials use of micro-CT imaging in barnacle research, and to develop an approach for a practicable combination of different morphological techniques.

MATERIALS AND METHODS

Material

Sponge and barnacle specimens examined in this study were collected by the first author using snorkelling collecting techniques between April 2016 and September 2017 at Saparua Island and South East Sulawesi, Indonesia (Table 1). All sponges were photographed using a digital camera (DSLR Nikon D200). Specimens were then fixed and stored in 96% ethanol and transferred into 75% ethanol for long-term preservation. In total, 14 lots containing four genera of sponge inhabiting barnacles were collected from seven localities.

Specimen preparation, micro-CT, and 3D reconstruction

Specimen preparation

To prepare the samples for micro-CT scanning, sponge specimens were removed from ethanol and put into dry containers. To avoid movement, the sponges were held in place using cotton. The cotton was soaked in a small amount of ethanol to prevent specimens from drying and thereby shrinkage, which can occur if wet objects are left in a dry environment for a certain amount of time (Metscher, 2009b; Buytaert et al., 2014).

Staining method to enhanced micro-CT imaging of soft body of barnacle

Lugol’s solution (one part iodine and two parts potassium iodide in an aqueous solution) was used to stain the soft bodies of the barnacle specimens. To produce a concentration of 0.3%, 0.1% (w/v) iodine and 0.2% (w/v) potassium iodide were dissolved in double-distilled water (Heimel et al. 2019). To scan the soft body, one barnacle for each species was removed from its host sponge. Barnacle specimens were soaked in Lugol’s solution for seven days, then thoroughly rinsed in tap water and put into a dry tube for the scanning process.

Micro computer tomography (micro-CT)

Complete sponge specimens were scanned using X-ray imaging at the Museum für Naturkunde Berlin, Germany, using a Scanner FF35 (Yxlon, Hamburg). Raw files were produced using the software VGStudio Max (Volume Graphics, Heidelberg, Germany). Scanning occurred with the following parameters: 80 kV, 15 W, 16 μm voxel size, 2000 projections/turn helical CT.

3D reconstruction of the barnacle inside the sponge

The software Amira 6.0.0 was used for the 3D reconstruction of the raw files. After creating a new project, the raw files were imported into the workspace. To visualize the dataset, the "Volren" tool, a volume renderer, was applied. In segmentation mode, the magic wand tool was used to segment the barnacle. Subsequently, every segmented barnacle was assigned a different "label", making it possible to differentiate between single barnacles by assigning different colours to individual labels. Switching back to project mode, using the "Volren" volume renderer, the opacity of the surrounding medium was adjusted, so that the barnacle was clearly visible. For better visualization purposes, "Specular" was used for shading, and the "Ortho" tool was applied to crop the volume and remove part of the plastic container and surrounding matrix.

Specimen direct morphological examination

After specimens were scanned using micro-CT, one to three barnacle specimens were removed from each sponge. Based on the overview scans showing all barnacle specimens situated in each sponge, we could estimate which parts of the sponge to dissect to obtain a specific barnacle specimen. Morphological characters of the hard body parts (parietes and opercular plates) and soft body parts (mouthparts, six pairs of cirri, and penis) were examined as in Pitriana et al. (2020). Species were identified based on Lamarck (1818), Darwin (1854), Krüger (1911), Hoek (1913), Broch (1931), Rosell (1991), and Kolbasov (1993).

Hard body parts were separated from soft body parts using a scalpel. Shell plates were separated and cleaned with a bleach solution to remove any organic material, rinsed with fresh water, dried and observed under a stereo microscope (Leica M125) and photographed with a digital camera (Leica Microsystems M205C).

Mouthparts (labrum, palps, maxillae, maxillules and mandible) were dissected using a scalpel, and each entity mounted on a glass slide. Cirri were separated into couples of cirri I-VI and the penis, before being mounted on glass slides. All elements were then examined using a light microscope (Carl Zeiss Axioskop 20).

RESULTS

Four different genera of sponges inhabited by five species of barnacles, two of which are undescribed, were determined (Table 1). Only one host sponge, *Axynissa* sp. was inhabited by two barnacle species, the others only by one barnacle species. Four of the sponge barnacles have a calcareous basis and spines or calcareous projections on the external surface of the parietes. Only the barnacle species *Membranobalanus longirostrum* had a membranous basis and the external surface of the parietes without spines or calcareous projections. The number of specimens found in each host sponge varied between a minimum of six (*Euacasta* sp. 1 in *Axynissa* sp.) and a maximum of 23 barnacle specimens (*Membranobalanus longirostrum* in *Agelas* sp.) (Table 1).

Acasta cf *sulcata* Lamarck, 1818

The sponge *Spongia* sp. (Fig. 1a, b), found at Saparua Island, was inhabited by *Acasta sulcata* (Fig. 1c-e). In the micro-CT overview scan, 13 barnacle specimens were identified inside and near the surface layer of the sponge (Fig. 1c). Parietes externally smooth, with calcareous spines attached in some areas (Fig. 1d), ribbed internally (Fig. 1d, e). Basis cup-shaped, pointed at central umbo (Fig. 1e).

Color of shell whitish to yellowish, reddish patches towards apex; orifice small, toothed (Fig. 2a–l). Parietes externally smooth, with horizontal growth ridges and calcareous projections; internally, longitudinal ribs below sheath; radii and alae with horizontal ridges (Fig. 2a–e). Basis cup-shaped, basal rim crenated; shallow radial furrows extending from center to crenate basal rim (Fig. 2f). Details of shell plates (Fig. 2g, h). Scutum with horizontal growth ridges; basal margin slightly concave in mid-line; occludental margin toothed, tooth size increasing gradually from apex to base (Fig. 2i, j). Tergum semitransparent, apex beaked, tinged reddish; internally scutal margin also with reddish patches in upper part; depressor muscle crests absent; spur broad, spur furrow shallow; carinal margin perpendicular to basal margin (Fig. 2k, l).

Cirrus I with outer ramus 80% of inner ramus; posterior margin with serrulate and plumose setae; distal margins of terminal annulus of anterior and posterior rami with serrulate setae (Fig. 3a). Cirrus II with rami unequal; posterodistal angle of segments of both rami with thick, serrulate setae; distal margins of terminal segment of anterior and posterior rami with serrulate setae (Fig. 3b). Cirrus IV with rami unequal; hooks on frontal margins of segments of anterior ramus and basis (Fig. 3c). Cirrus V with two small teeth at frontal edge of proximal segments of anterior ramus (Fig. 3d). Cirri V and VI with rami subequal,

Table 1. Barnacle species and the host sponges from eastern Indonesia

Barnacles species	Host sponges species	Locality
Acasta sulcata Lamarck, 1818	*Spongia* sp.	Porto, Kulur, and Ihamahu (Saparua Island); water depth: app. 0-3 m
Euacasta dofleini (Krüger, 1911)	*Axynissa* sp.	Teluk Haria, Porto, and Desa Ihamahu (Saparua Island); water depth: app. 0-3 m
Euacasta sp. 1	*Axynissa* sp.	Pantai Marina (Southeast Sulawesi); water depth: app. 0-5 m
Euacasta sp. 2	*Cinacyrella* sp.	Desa Pia (Saparua Island); water depth: app. 0-3 m
Membranobalanus longirostrum (Hoek, 1913)	*Agelas* sp.	Teluk Haria (Saparua Island); water depth: app. 0-3 m

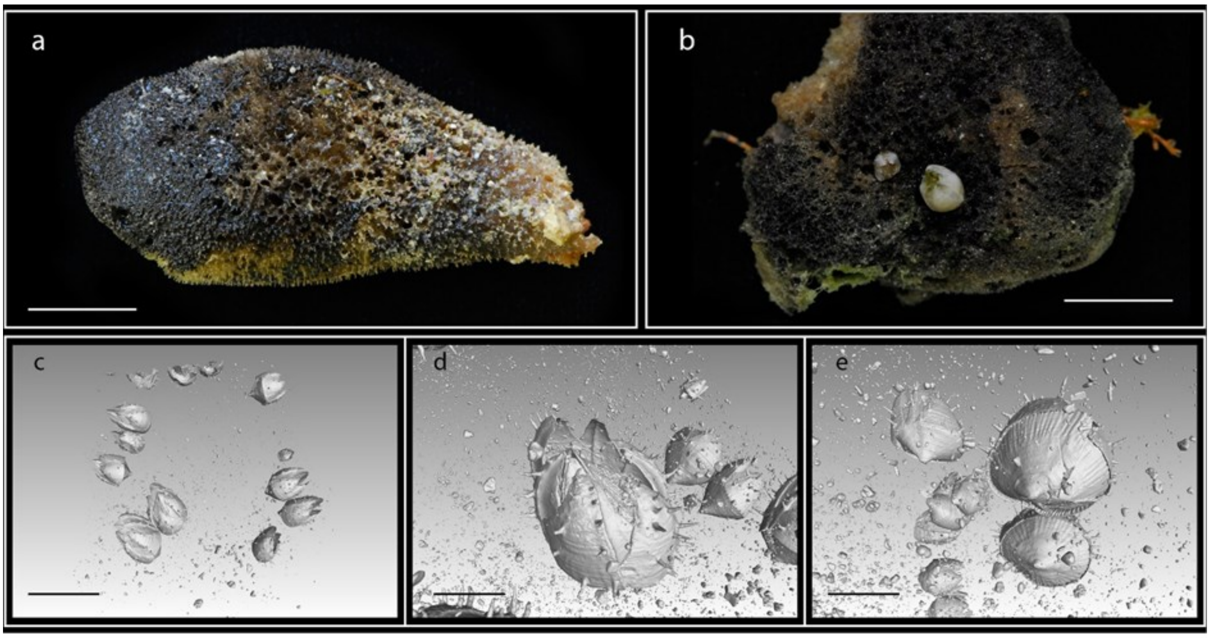

Figure 1. *Acasta sulcata* and its host sponge: **a)** sponge of *Spongia* sp.; **b)** *Spongia* sp. inhabited by *A. sulcata*; **c)** position of *A. sulcata* in the host sponge; **d)** projections calcareous in the external surface parietes of *A. sulcata*; **e)** bases of *A. sulcata*. Scale bars: 2 cm (**a**); 1 cm (**b, c**); 1 mm (**d, e**).

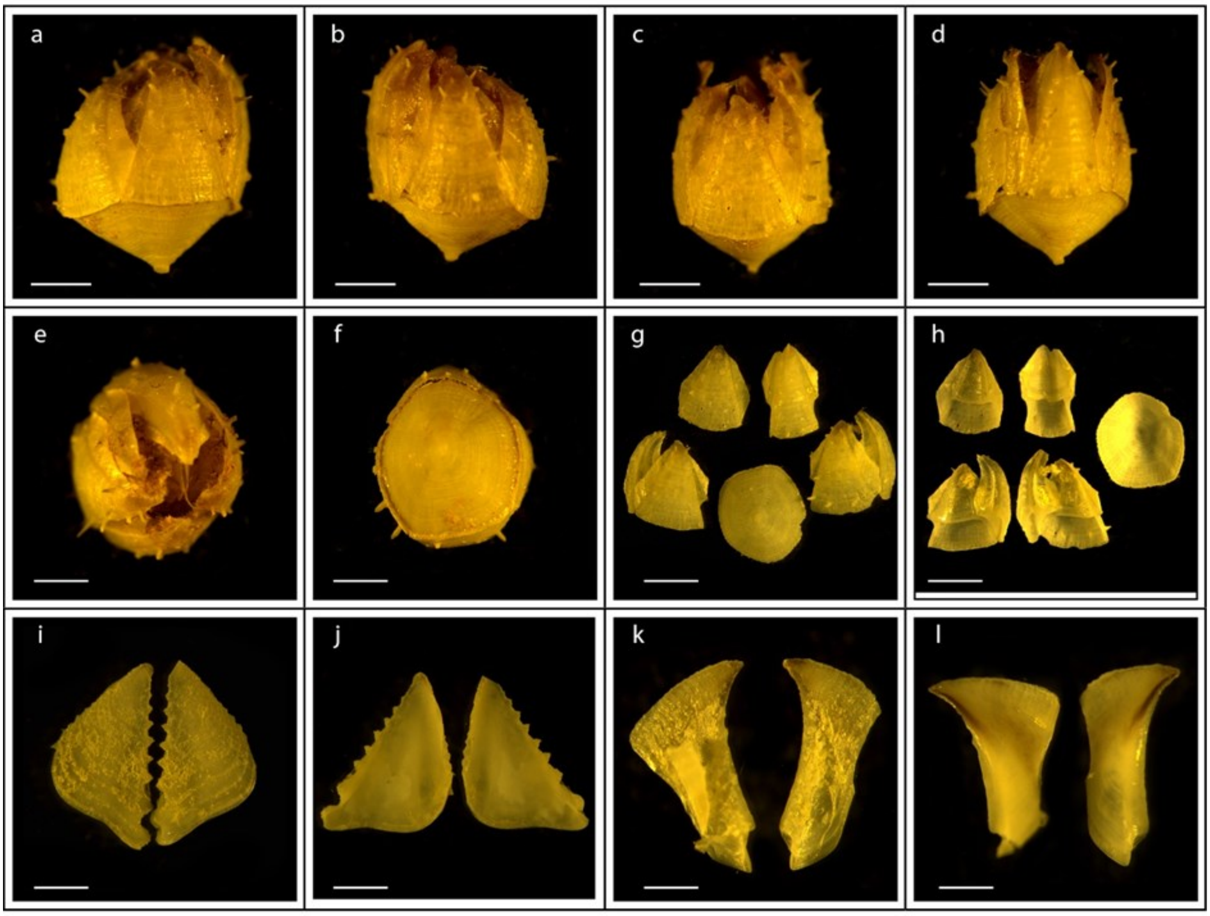

Figure 2. Shell and shell compartments of *Acasta sulcata:* **a)** right side view; **b)** left side view; **c)** rostral view; **d)** carinal view; **e)** upper view; **f)** lower view; **g, h)** external and internal view of shell plates and basis; **i, j)** external and internal view of scuta; **k, l)** external and internal view of terga.
Scale bars: 1 mm (**a–f**); 0.5mm (**g–h**); 0.25 mm (**i–l**).

terminal segments of anterior and posterior rami with serrulate setae. Maxillule cutting edge straight, without notch, upper and lower margins with numerous simple setae (Fig. 3e); mandible with five teeth, second to fourth teeth bifid, inferior angle with six denticles and simple setae, upper margin of lateral side with simple setae (Fig. 3f); labrum bilobed, separated by V-shaped deep notch, with three small teeth on each side of crest (Fig. 3g).

***Euacasta* cf *dofleini* (Krüger, 1911)**

The sponge *Axynissa* sp. (Fig. 4a), found at Saparua Island (Table 1), was inhabited by *Euacasta dofleini* (Krüger, 1911) (Fig. 4a). In the micro-CT overview scan of *Axynissa* sp. we found 12 specimens with their opercula facing in the same direction towards the surface of their sponge hosts (Fig. 4b). Basis quadrangular, with four indistinct, shallow radial furrows extending from center to crenate basal rim (Fig. 4c, d); calcareous projections attached on external surfaces of plates (Fig. 4d, e).

Color of shell yellowish, orifice toothed; wall plates with long and worm-shaped of calcareous projections on external surface; basis saucer-shaped, basal rim crenated (Fig. 5a–f). Scutum with radial striae, occludental margin toothed; tergum beaked, apex pinkish tinged (Fig. 5g–j). Carinolaterals with rudimentary parietes, represented by a narrow strip separating radii and ala (Fig. 5k, l).

Cirrus I with rami unequal, posterior margin with serrulate and plumose setae (Fig. 6a). Cirrus II with rami unequal, terminal segments of anterior and posterior rami with serrulate setae (Fig. 6b). Cirral armature of cirrus IV well developed, curved hooks on frontal margin of segments of anterior ramus and basis (Fig. 6c). Cirri V and VI long, slender, rami equal

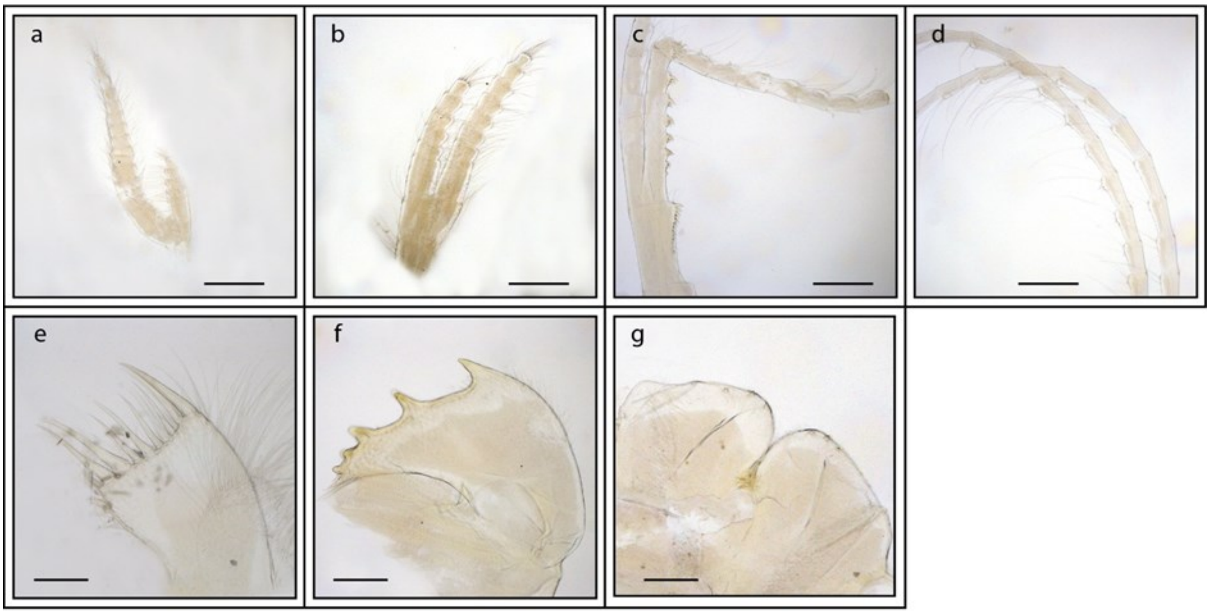

Figure 3. Parts of soft body of *Acasta sulcata:* **a)** cirrus I; **b)** cirrus II; **c)** cirrus IV; **d)** cirrus V; **e)** maxillule; **f)** mandible; **g)** labrum. Scale bars: 200 µm (**a–d**); 100 µm (**e–g**).

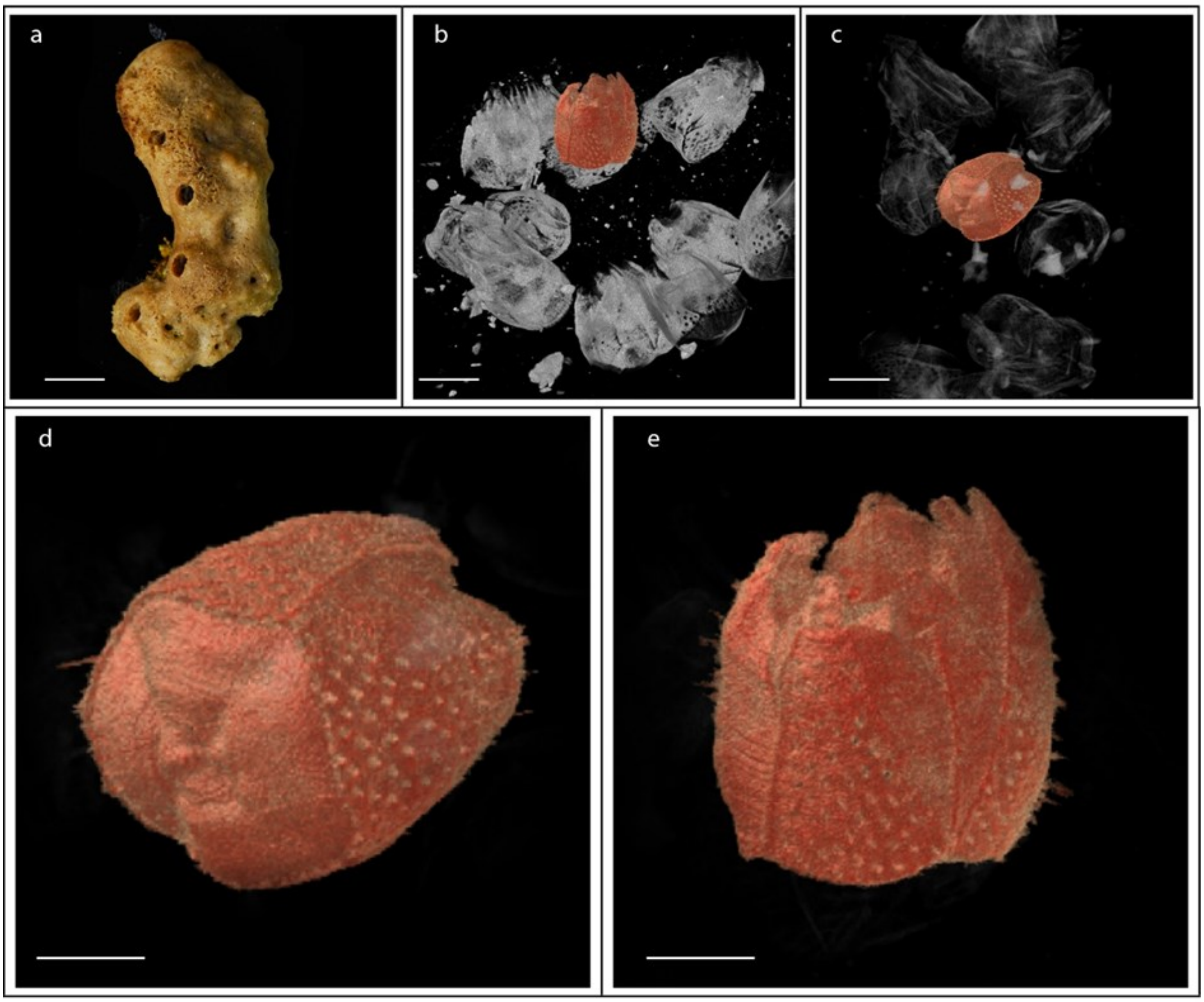

Figure 4. *Euacasta dofleini* and its host sponge: **a)** sponge *Axynissa* sp.; **b)** position of *E. dofleini* in the host sponge; **c)** base of *E. dofleini*; **d)** close-up of basis; **e)** parietes with calcareous projections on external surface. Scale bars: 3 cm (**a**); 1 mm (**b–e**).

(Fig. 6d, e). Penis annulate, basidorsal point absent, gradually tapering distally (Fig. 6f). Maxillule with nine large, cuspidate setae, upper and lower pairs largest; cutting margin straight, without notch (Fig. 6g). Mandible with five teeth, second bifid, inferior angle with three denticles and simple setae, lower margin bearing simple setae (Fig. 6h). Labrum bilobed with V-shaped deep notch, three teeth on each crest (Fig. 6i).

***Euacasta* sp. 1**

The sponge *Axynissa* sp., found in SE Sulawesi (Table 1), was inhabited by *Euacasta* sp. 1 (Fig. 7a, b). In the micro-CT overview scan of *Axynissa* sp. six barnacle specimens were detected inside the sponge. Barnacles were located near the surface layer of their sponge host (Fig. 7c). The operculum looming high over the parietes (Fig. 7d).

Color of shell yellowish, operculum soaring almost as high as parietes; orifice wide; plates externally with many calcareous projections (Fig. 8a–i). Basis quadrangular, growth

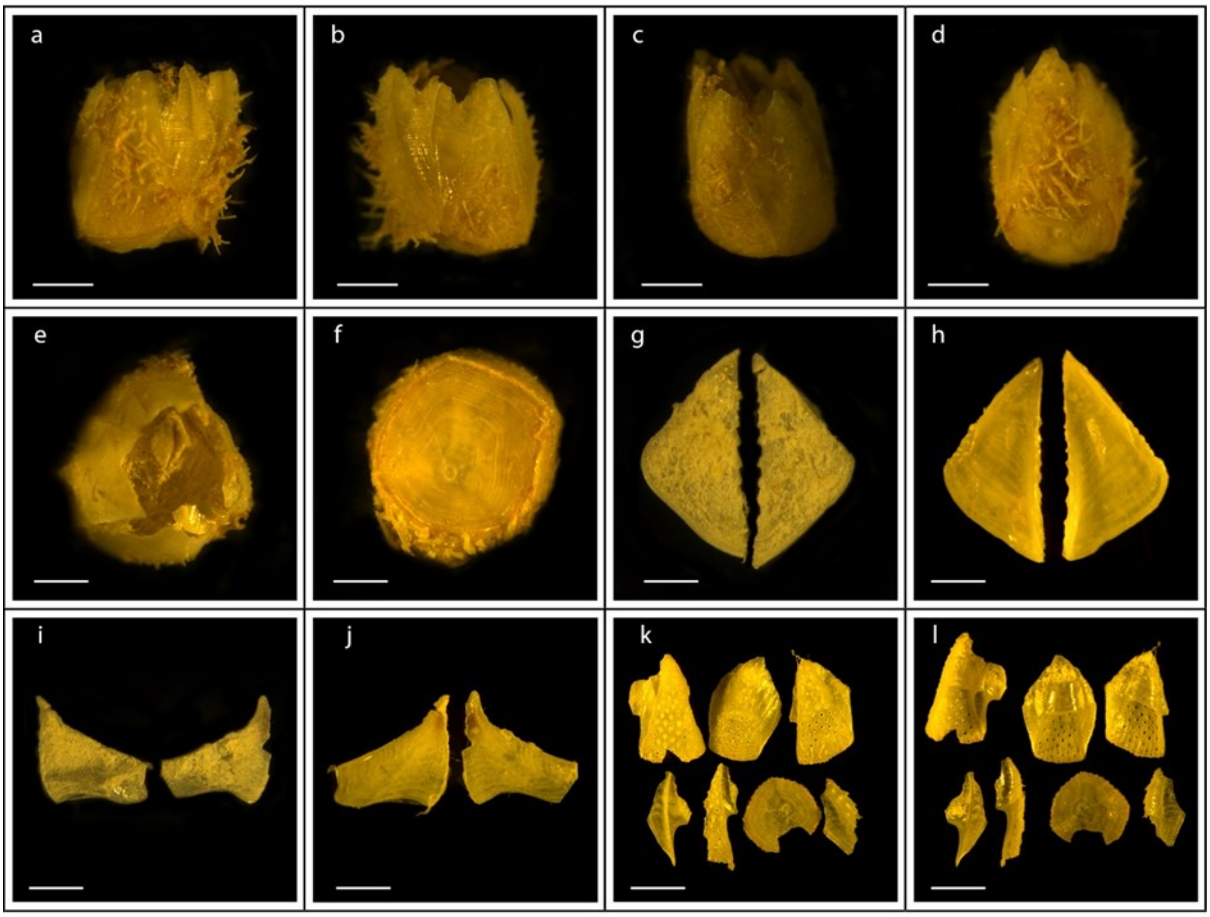

Figure 5. Shell and shell compartments of *Euacasta dofleini*: **a)** right side view; **b)** left side view; **c)** lateral plates view; **d)** rostral view; **e)** upper view; **f)** lower view; **g, h)** external and internal view of scuta; **i, j)** external and internal view of terga; **k, l)** external and internal view of shell plates and basis.
Scale bars: 1 mm (**a–f**); 0.25 mm (**g–h**); 0.50 mm (**i–l**).

ridges horizontal, basal rim crenated (Fig. 8e–g). Scutum with horizontal growth ridges, occludental margin toothed (Fig. 8h, i). Tergum with apex beaked, horizontal growth ridges present, spur broad (Fig. 8h, i).

Cirrus I with outer ramus 80% of inner ramus, posterior margin with serrulate and plumose setae, distal margins of terminal annuli of anterior and posterior rami with serrulate and bifid setae (Fig. 9a). Cirrus II with rami slightly unequal; posterodistal angle of segments of both rami with serrulate setae; distal margins of terminal segments of anterior and posterior rami with serrulate and bifid setae (Fig. 9b). Cirrus IV with rami unequal; hooks on frontal margin of segments of anterior ramus and basis (Fig. 9c-e). Cirrus V with three small teeth at frontal edge of proximal segments of anterior ramus (Fig. 9f, g). Cirrus VI long, slender, with rami equal in length (Fig. 9h). Penis annulate, gradually tapering distally, with very low basidorsal point (Fig. 9i). Maxillule cutting edge straight, notch absent, upper and lower margins with two large, stout, simple setae (Fig. 9j); mandible with five teeth, inferior angle with five denticles, lower margin of lateral angle with simple setae (Fig. 9k); labrum with V-shaped notch, three teeth on each side of crest (Fig. 9l).

Figure 6. Soft parts of *Euacasta dofleini*: **a)** cirrus I; **b)** cirrus II; **c)** proximal part of cirrus IV; **d)** cirrus V; **e)** cirrus VI; **f)** penis; **g)** maxillule; **h)** mandible; **i)** labrum. Scale bars: 200 µm (**a–b, d–f**); 100 µm (**c**, **g–i**).

Euacasta sp. 2

The sponge *Cinacyrella* sp., found at Saparua Island, was inhabited by *Euacasta* sp. 2 (Fig. 10a). In the micro-CT overview scan of *Cynacyrella* sp., nine specimens of *Euacasta* sp. 2 were located inside the sponge, living in a group near the surface layer of their host, with their orifices all facing in the same direction, towards the surface of the sponge (Fig. 10b, c). Pattern of holes all over parietes, except on operculum (Fig. 10d); opercular plates with slightly horizontal growth ridges (Fig. 10e).

We carefully tried to remove and dissect the barnacles from the host to verify the barnacle species, but the barnacles and the sponge were strongly adherent, making it very difficult to separate them without breakage. After several attempts, we separated the barnacle from the sponge host, but a large amount of sponge tissue was still attached to the barnacle shell (Fig. 11a). The barnacle was immersed in 2% bleach to digest the sponge tissue, but the bleach also destroyed the shell of the barnacle (Fig. 11c). The barnacle was immediately rinsed with tap water five times and air-dried for species identification. Basis of

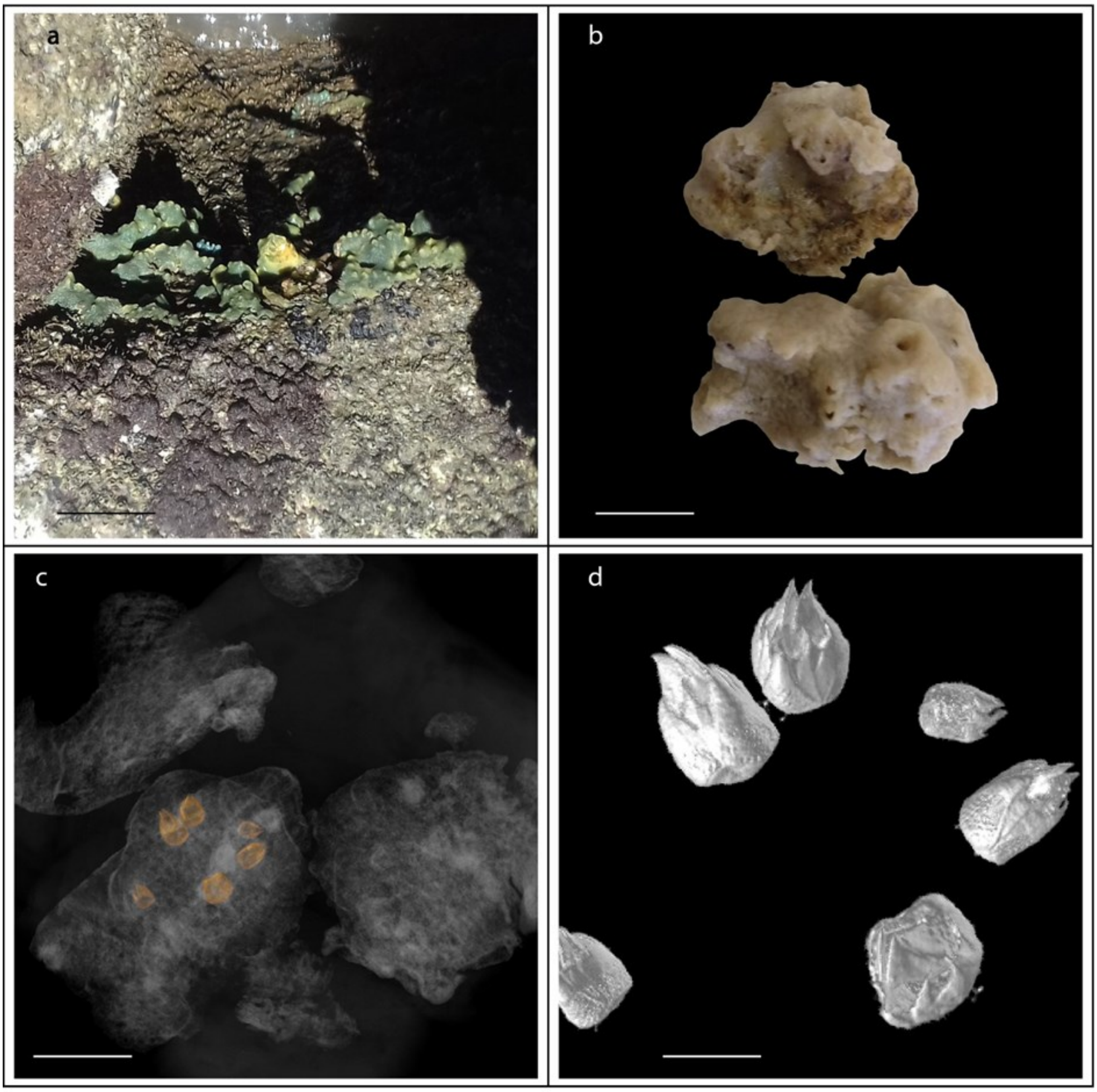

Figure 7. *Euacasta* sp. 1 and its host sponge: **a)** sponge *Agelas* sp. in the field sampling ; **b)** close-up of sponge *Agelas* sp. in the laboratory; **c)** position of *Euacasta* sp. 1 in host sponge; **d)** shell view of *Euacasta* sp. 1. Scale bars: 5 cm (**a**); 1 cm (**b**); 1 mm (**c–d**).

four separate plates (Fig. 11b), growth ridges horizontal, radial furrows shallow (Fig. 11d, e), crenate at basal rim (Fig. 11f). Parietes externally with many holes marking attachment of calcareous projections; internally with longitudinal ribs extending from upper to lower part; radii and alae with horizontal ridges (Fig. 11g, h); scutum semi-transparent, with horizontal growth ridges, tinged reddish in upper part, occludental margin slightly toothed; (Fig. 11i, j). Tergum semi-transparent, with horizontal growth ridges, apex beaked, tinged reddish (Fig. 11i, j).

Cirrus I with outer ramus 80% of inner ramus, anterior and posterior margin with thick serrulate and plumose setae, distal margin of terminal segments of both rami with serrulate

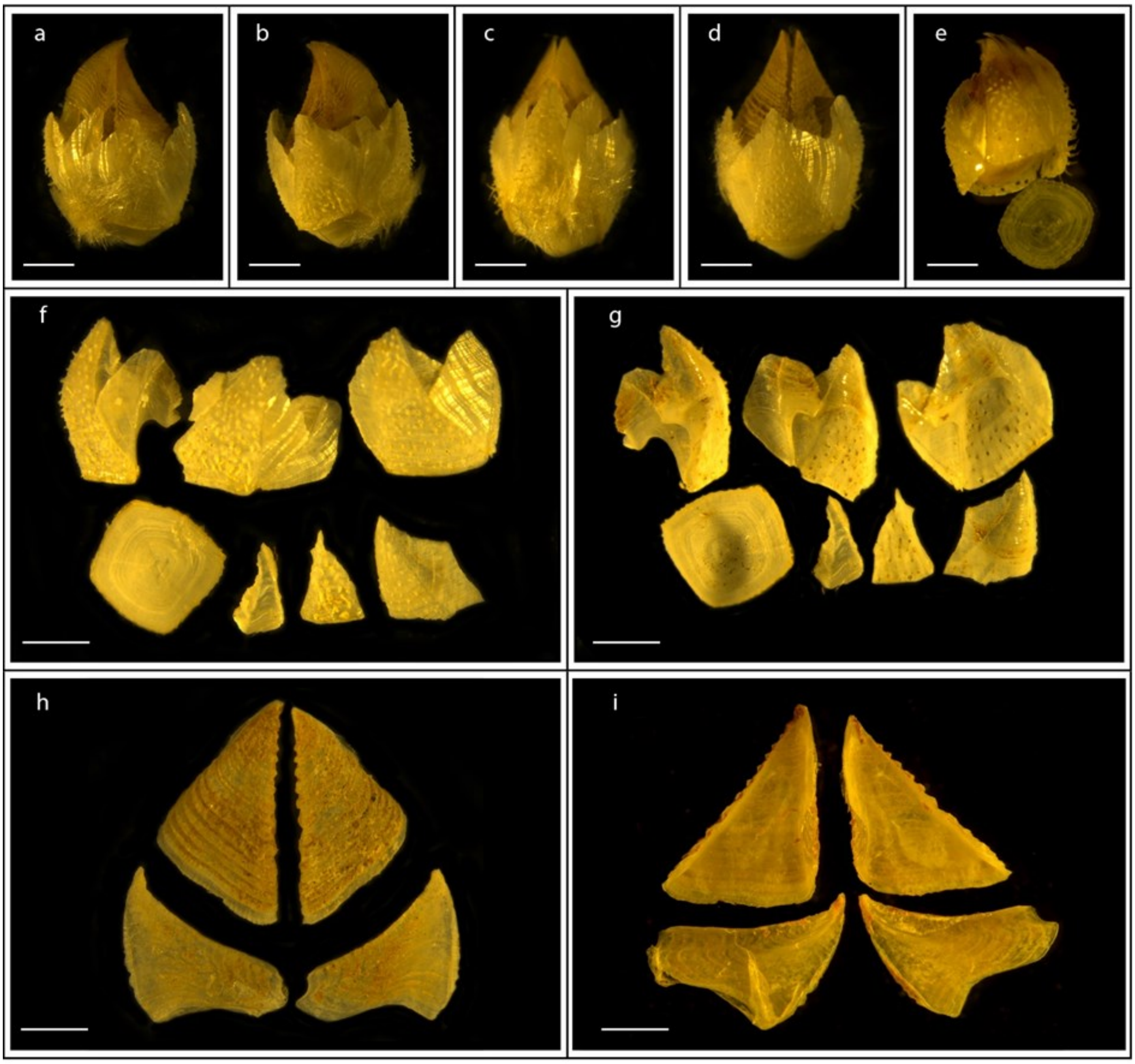

Figure 8. Shell and shell compartments of *Euacasta* sp. 1: **a)** shell, left lateral view; **b)** shell, right lateral view; **c)** carinal view; **d)** rostral view; **e)** lateral plates view with disattached basis; **f, g)** external and internal view of shell plates and basis; **h, i)** external and internal view of scuta and terga.
Scale bars: 1 mm (**a–e**); 0.5 mm (**f–g**); 0.25 mm (**h–i**).

setae (Fig. 12a). Cirrus II with rami unequal, posterodistal angle of segments of both rami with serrulate setae, distal margin of terminal segments of both rami with serrulate setae (Fig. 12b). Cirrus III with rami slightly unequal, similar to cirrus II but slender and longer (Fig. 12c). Cirrus IV with hooks on frontal margin of segments of anterior ramus and basis; two small teeth at frontal edge of proximal segments of anterior ramus (Fig. 12d, e). Penis with basidorsal point, annulate, sparse hair distally, gradually tapering distally (Fig. 12f). Maxillule cutting edge straight without notch, upper and lower margins with two large, stout, simple setae (Fig. 12g); mandible with five teeth, inferior angle with two stout denticles, lower margin of lateral side with simple setae (Fig. 12h); mandibular palp with dense setae on upper region (Fig. 12i); labrum with V-shaped notch, two small teeth on each side of crest (Fig. 12j).

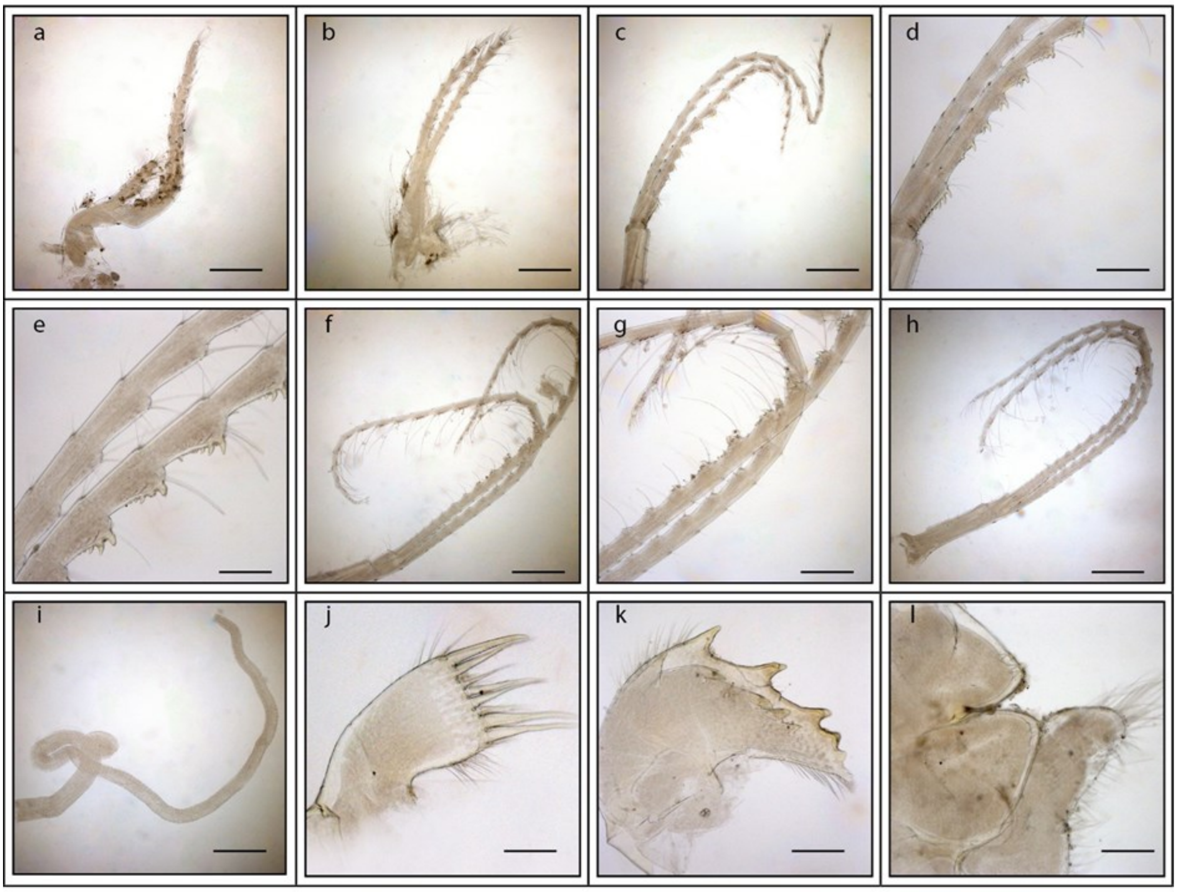

Figure 9. Soft body of *Eucasta* sp. 1: **a)** cirrus I; **b)** cirrus II; **c)** cirrus IV; **d)** close-up view of cirrus IV; **e)** close-up view of hooks of cirrus IV; **f)** cirrus V; **g)** close-up view of cirrus V; **h)** cirrus VI; **i)** penis; **j)** maxillule; **k)** mandible; **l)** labrum. Scale bars: 200 µm (**a–c, f, h, i**); 100 µm (**d, e, g, j–l**).

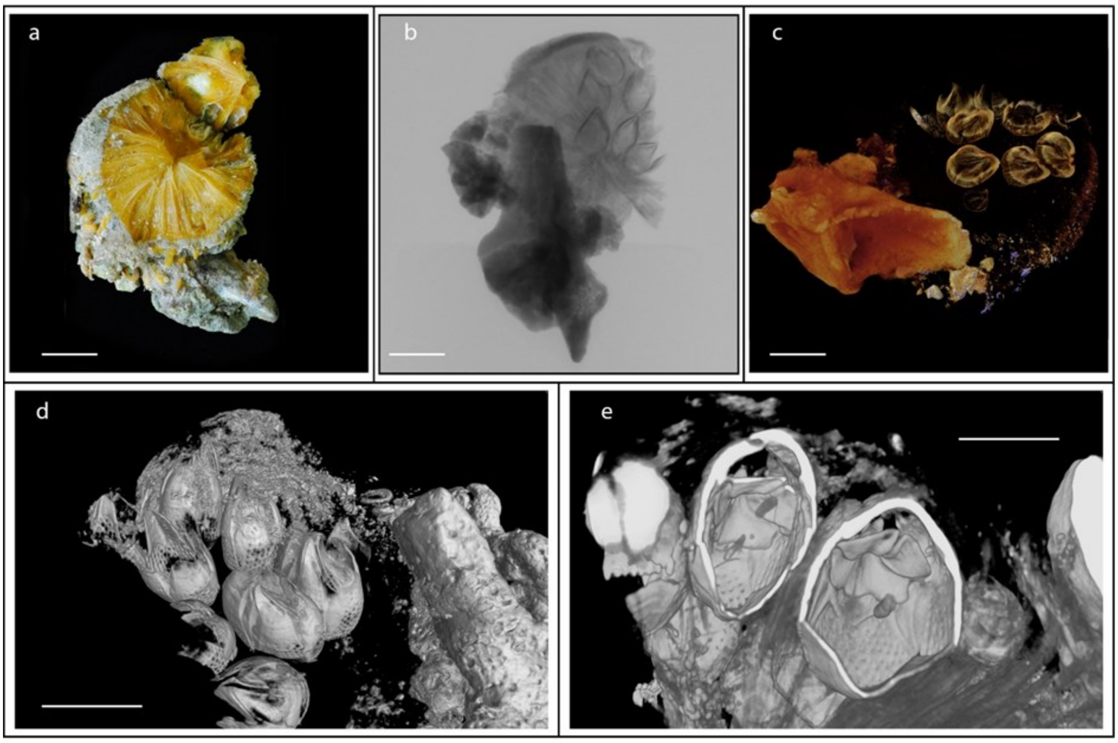

Figure 10. *Euacasta* sp. 2 and its host sponge: **a)** sponge of *Cinacyrella* sp.; **b)** position of *Eacasta* sp.2 in the host sponge; **c)** orifices facing in the same direction in the host sponge; **d)** shell view of *Euacasta* sp. 2; **e)** internal view of shell plates and operculum. Scale bars: 2 cm (**a**); 1 cm (**b, c**); 1 mm (**d, e**).

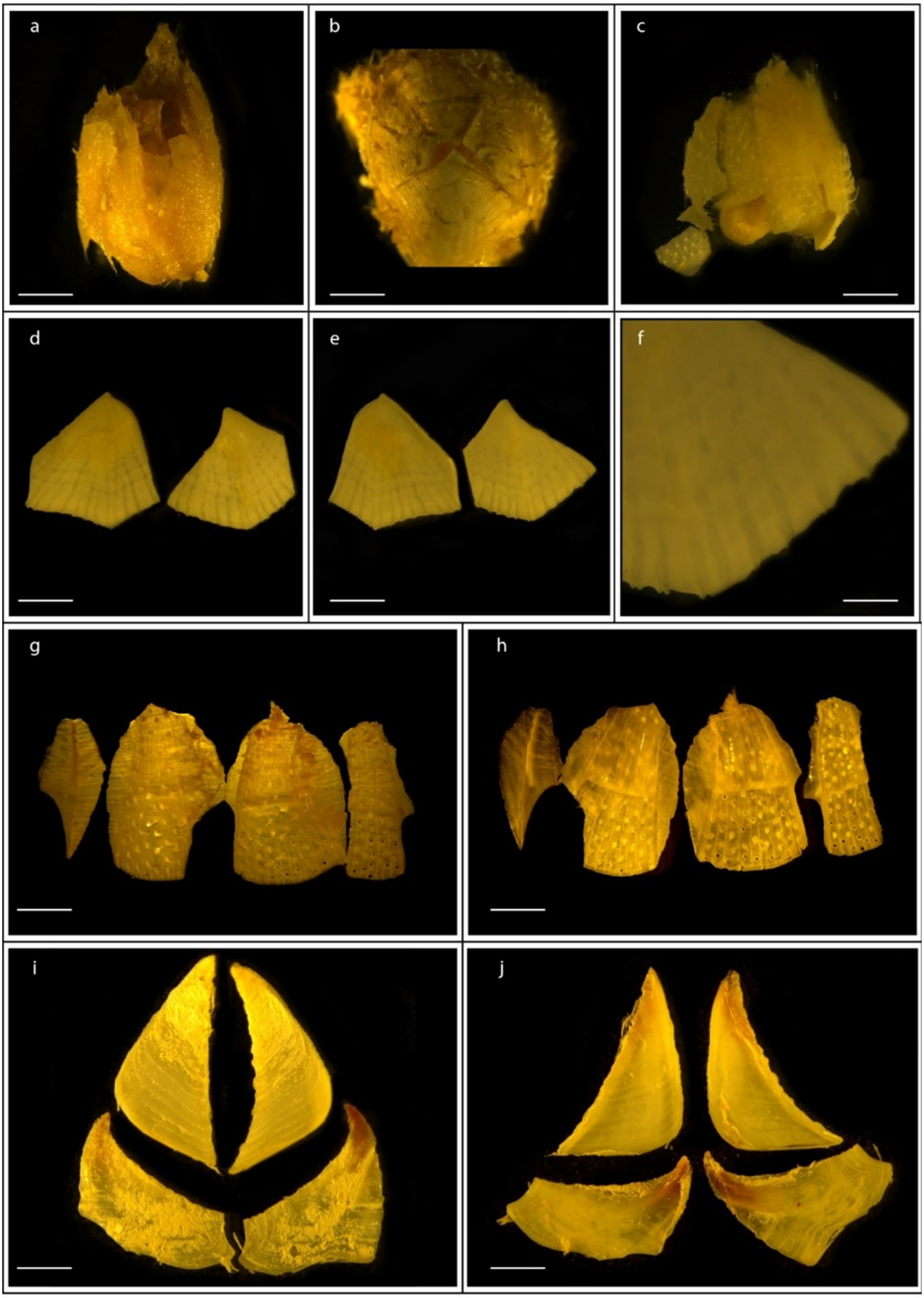

Figure 11. Shell and shell compartments of *Euacasta* sp. 2: **a)** shell, lateral view; **b)** shell, basal view; **c)** shell view after immersed in 2% bleach; **d, e)** external and internal view of broken basis; **f)** close-up view of basal rim; **g, h)** external and internal view of shell plates (partially broken); **i, j)** external and internal view of scuta and terga. Scale bars: 1 mm (**a–c**); 0.5 mm (**d, e, g–h**); 0.25 mm (**f, i–l**).

Membranobalanus longirostrum **(Hoek, 1913)**

The sponge *Agelas* sp. found at Saparua Island was inhabited by *Membranobalanus longirostrum* (Fig. 13a, b). In the micro-CT overview scan of *Agelas* sp. we found 23 specimens of barnacle inside the sponge. We discovered that only hard parts of shell can be seen through micro-CT scan (Fig. 13c, d).

Shell of barnacle thin, very brittle, smooth without calcareous projections; orifice large, irregularly toothed; basis membranous (Fig. 14a–e). Scutum with horizontal growth ridges, occludental margin toothed in lower part, adductor ridge absent (Fig. 14b, c). Tergum beaked, horizontal growth ridges present; spur moderately broad, close to basi-scutal angle (Fig. 14b, c). Carina and rostrum bowed; rostrum longest plate, basal part extending far beyond basal margin of other compartments (Fig. 14d, e).

Cirrus I with outer ramus 80% of inner ramus, terminal segments of anterior and posterior rami with serrulate setae (Fig. 15a). Cirrus II with rami slightly unequal, setae plumose and serrulate; distal segment of posterior ramus with serrulate setae (Fig. 15b).

Figure 12. Parts of soft body of *Euacasta* sp. 2: **a)** cirrus I; **b)** cirrus II; **c)** cirrus III; **d)** cirrus IV; **e)** close-up view of hooks of cirrus IV; **f)** cirrus V; **g)** maxillule; **k)** mandible; **i)** mandibular palp; **j)** labrum. Scale bars: 200 µm (**a–d, f**); 100 µm (**e, g–j**).

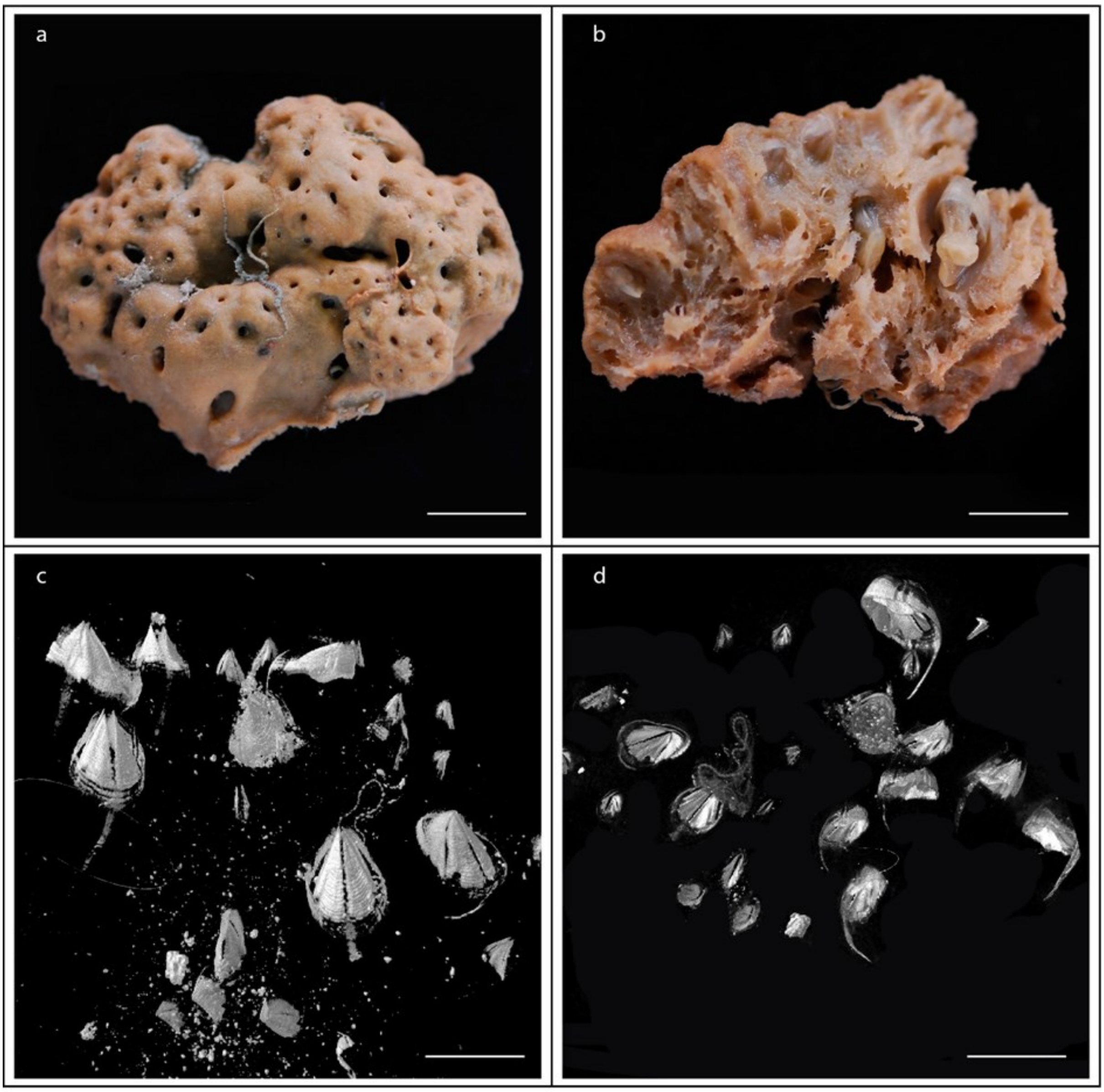

Figure 13. *Membranobalanus longirostrum* and its host sponge: **a)** sponge of *Agelas* sp.; **b)** *M. longirostrum* in the host sponge; **c)** orifice view of *M. longirostrum*; **d)** rostral lateral view of *M. longirostrum.* Scale bars: 2 cm (**a–b,**); 1 mm (**c–d**).

Cirrus III with rami equal (Fig. 15c). Rami of cirrus IV slightly unequal; triangular, spine-like teeth forming transverse comb-like rows on both segments of pedicel (Fig. 15d). Cirrus V and VI long, slender (Fig. 15e, f). Penis very long, much longer than cirri (Fig. 15g). Maxillule cutting margin straight, notch absent, with 11 large, cuspidate setae, lower pairs largest (Fig. 15h). Mandible with five teeth, second bifid, third small; inferior angle with three denticles and simple setae, lower margin bearing simple setae (Fig. 15i). Labrum bilobed with wide, not very deep notch, two teeth on each crest (Fig. 15j).

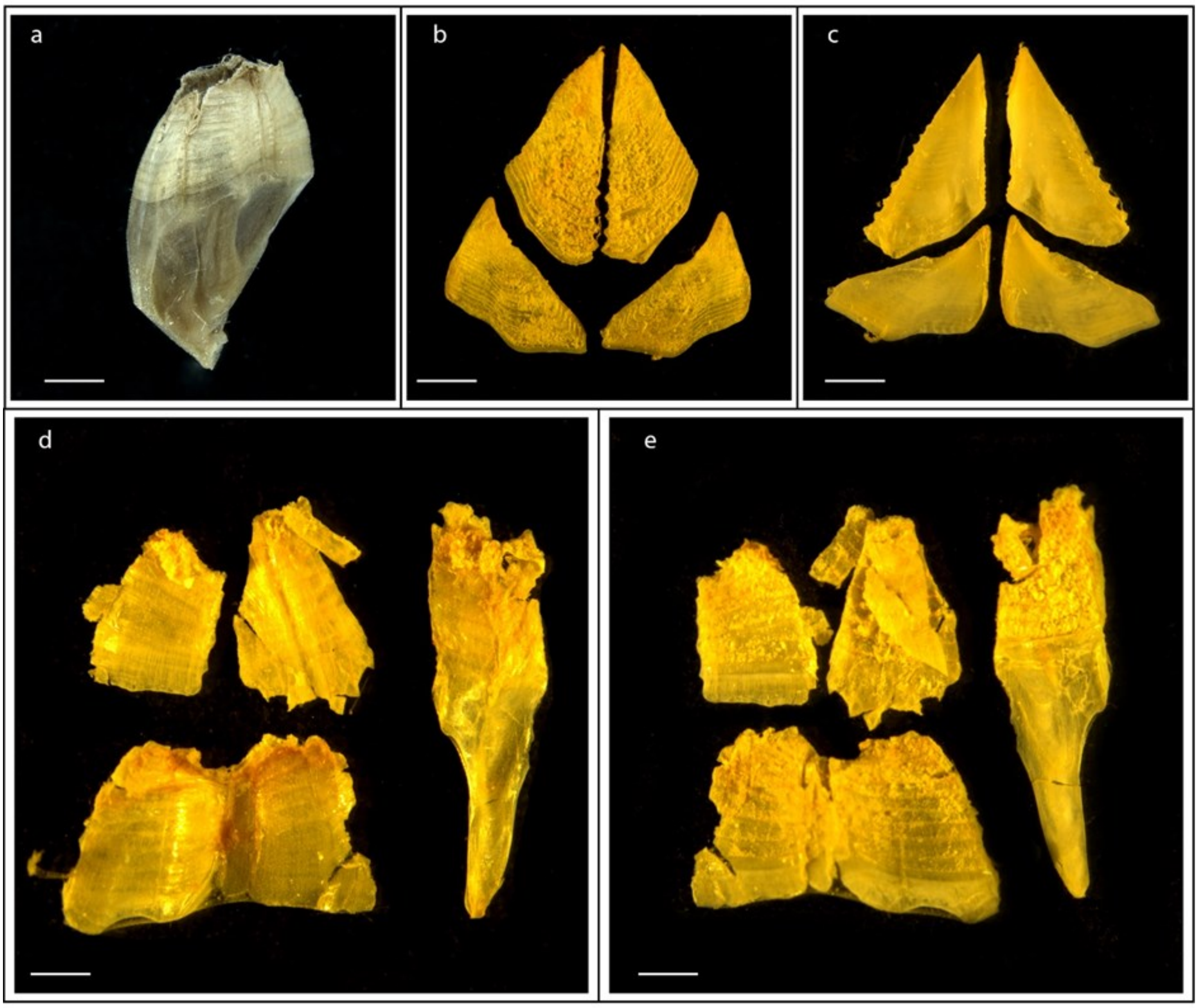

Figure 14. Shell and shell compartments of *Membranobalanus longirostrum*: **a)** shell, lateral view; **b, c)** external and internal view of scuta and terga; **d, e)** internal and external view of plates.
Scale bars: 1 mm (**a–f**); 0.25 mm (**g–h**); 0.50 mm (**i–l**).

DISCUSSION

The results from this morphological study using an innovative approach with micro-CT scanning and 3D reconstruction support the results of Anderson (1994) who ascertained that epizoic balanoid adaptations are related to the problem of overgrowth and burial, a reason why barnacles need to maintain their orifices at the surface of the sponge host. In our study, all barnacle specimens were facing the surface of their sponge hosts. Ilan et al. (1999) also stated that a sponge-inhabiting barnacle settles on the location of the opening to avoid overgrowth. They also found that the barnacle *Neoacasta laevigata* had a clumped distribution on its host sponge, *Carteriospongia foliascens*. According to Lewis (1992), this clump distribution related to their adaptation for reproduction. Since they are sessile hermaphrodites, the key factor in the pattern distribution of barnacles on a substrate is their distance from the nearest neighbor.

Figure 15. Soft body parts of *Membranobalanus longirostrum*: **a)** cirrus I; **b)** cirrus II; **c)** cirrus III; **d)** cirrus IV; **e)** cirrus V; **f)** cirrus VI; **g)** penis; **h)** maxillule; **i)** mandible; **j)** labrum.
Scale bars: 200 µm (**a–g**); 100 µm (**h–j**).

Yu et al. (2020) observed that the arrangement of barnacles in sponges is not random and that they mostly accumulate on the inhalant side, to make food capture easier. Sponges are important benthic suspension feeders. They are believed to slow water flow and raise water turbulence, resulting in an increased residence time of possible nutrient particles (Gili & Coma, 1998). Barnacles as epibionts can take advantage of such slowed water currents caused by sponges (Nagler et al., 2017). All the above statements might explain the reason why in this study the majority of barnacles found were arranged in groups near the surface of the host sponge.

In this study, barnacles of the Acastinae were found inhabiting the sponges *Spongia* sp., *Axynissa* sp., and *Cynacyrella* sp., which have also been reported previously as host sponges from research on sponge barnacle from America, the Red Sea, Indonesia and worldwide (Van Syoc & Winther, 1999; Ilan et al., 1999; Wibowo et al., 2011; Sulistiono et al., 2014; Van Syoc et al., 2015). According to Van Syoc (1988) and Hosie et al. (2019), *Membranobalanus* is restricted to inhabiting sponges species in the Clionaidae. In the present study, we found the species *M. longirostrum* inhabiting the sponge *Agelas* sp. of the family Agelasidae.

By using micro-CT, we can see the pattern of calcareous projections on the surface of the shell wall by looking at the pattern of circular holes on the shell, as these are the attachment sites of the calcareous projections. These calcareous projections or spines are believed to function as anchors into the surrounding sponge tissue. They are well developed in species that inhabit crumbly sponges, but are reduced among species hosted by elastic sponges (Kolbasov, 1993). The calcareous projections on the plates wall also can be used to distinguish Acastinae from members of the genus *Membranobalanus* (Van Syoc et al., 2015). Because of this important role, in the future there was a possibility that the pattern of these circular holes could also be used as an important character for species identification. This study also confirms the advantage of using micro-CT imaging when we examined *Euacasta* sp. 2, which was anchored so tightly in the sponge tissue that removing the specimen turned out to be impossible without breaking it.

Invertebrate studies utilising micro-CT scanning provide new ways of looking at the external and internal morphology of organisms in the µm to mm size range (Friedrich & Beutel, 2008). Although micro-CT scanning has great potential in morphological studies of barnacles by enhancing the visualization of external and internal anatomy, this study also had some methodological limitations. In *M. longirostrum*, the contrast in the 3D images was too low to see the membranous basis. This problem could have been caused because the membrane was packed too tightly to the sponge's tissue resulting in a low level of contrast between the membrane and surrounding host tissue. Although we applied a staining method to visualize the membrane and soft body parts, the contrast of the images was generally too low and we could not fully reconstruct the soft body parts. According to Metscher (2009a), by using a few simple contrast stains, micro-CT can provide versatile, high-contrast, quantitative 3D images of animal soft tissues. The methods can be used on a wide variety of animal specimens fixed and preserved by the most common methods. The most broadly useful contrast stains tested so far are inorganic iodine and phosphotungstic acid (PTA) (Metscher, 2009b). These staining methods, including iodine-based dyes such as Lugol's solution, pose a simple, cost-effective, and nontoxic option for contrast enhancement of soft tissues (Heimel et al., 2019). The use of this staining method has a broad range of different concentrations of iodine and staining durations depends on the type of tissue (Gignac et al., 2016). However, marine organisms exhibit a large diversity of tissue types, chemical components and material combinations, making it almost impossible to predict the effect of a certain contrast-enhancing technique (Faulwetter et al., 2013). A future approach could be to find the most appropriate staining method to obtain a higher and sufficinet resolution of the barnacles' soft body.

In conclusion, this study demonstrates that micro-CT scanning is a very useful modern technique for morphological and taxonomic examination of the sponge-inhabiting barnacles.

ACKNOWLEDGMENTS

The first author would like to thank Prof. Frank Riedel from the Freie Universität Berlin for his general support, Dr. André Beerlink (Xylon Hamburg) for his cooperation and introduction of Xylon Hamburg and Tri Aryono Hadi (LIPI) for identifying the host sponges. Daniel Josep Tala (LIPI) supported the sampling of barnacles in Indonesia. Bani Wahyudi and Abdul Basit helped improve the illustrations. Thanks to Kristin Mahlow and Prof. Johannes Müller for supporting the micro-CT work progress at the Museum für Naturkunde Berlin. We appreciate the considerable time and effort given by Diana Jones and one anonymous reviewer for comments and suggestions that helped us improve the original manuscript.

This study was funded by the Ministry of Research, Technology and Higher Education, the Republic of Indonesia within the Program for Research and Innovation in Science and Technology (RISET-Pro), World Bank Loan No. 8245-ID. Barnacle samples were collected by the first author with permit numbers as follows: 737/IPK.3/AP/IX/2016 for field work at Saparua Island dan B-671/IPK.3/KP.01.06/IX72017 for field work at South Sulawesi.

REFERENCES

Anderson, D.T. 1994. *Barnacles: Structure, Function, Development and Evolution*. London: Chapman & Hall: 357 pp.

Broch, H. 1931. Indo-Malayan Cirripedia. Papers from Dr Th. Mortensen's Pacific Expedition 1914–1916. *Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening (Copenhagen)*, 91: 1–146.

Buytaert, J., Goyens, J., De Greef, D., Aerts, P. & Dirckx, J. 2014. Volume shrinkage of bone, brain and muscle tissue in sample preparation for micro-CT and light sheet fluorescence microscopy (LSFM). *Microscopy and Microanalysis*, 20(4): 1208–1217. https://doi.org/10.1017/S1431927614001329

Darwin, C. 1854. *A Monograph on the Subclass Cirripedia with Figures of All the Species. The Balanidae, the Verrucidae, etc.* London: Ray Society: 684 pp, pls. 30. (1854 published 1855).

Faulwetter, S., Dailianis, T., Vasileiadou, A. & Arvanitidis, C. 2013. Contrast enhancing techniques for the application of micro-CT in marine biodiversity studies. *Microscopy and Analysis*, 27(2): S4-S7.

Foster, B.A. 1978. The Marine fauna of New Zealand: Barnacles (Cirripedia: Thoracica). *New Zealand Oceanographic Institute Memoir*, 69: 1–160.

Friedrich, F. & Beutel, R. G. 2008. *Micro-computer tomography and a renaissance of insect morphology*. Paper presented at the Proceeding SPIE 7078, Developments in X-Ray Tomography VI, San Diego, California.

Gignac, P.M., Kley, N.J. & Clarke, J.A. 2016. Diffusible iodine based contrast-enhanced computed tomography (diceCT): an emerging tool for rapid, high-resolution, 3-D imaging of metazoan soft tissues. *Journal of Anatomy*, 228(6): 889–909.

Gili, J.M. & Coma, R. 1998. Benthic suspension feeders: their paramount role in littoral marine food webs. *Trends in Ecology and Evolution*, 13: 316–321.

Heimel, P., Swiadek, N.V., Slezak, P., Kerbl, M., Schneider, C., Nurnberger, S., Redl, H., Teuschl, A.H. & Hercher, D. 2019. Iodine-enhanced micro-CT imaging of soft tissue on the example of peripheral nerve regeneration. *Contrast Media & Molecular Imaging*, 2019: 15. https://doi.org/10.1155/2019/7483745

Hoek, P.P.C. 1913. The Cirripedia of the Siboga Expedition. B. Cirripedia Sessilia. *Siboga Expeditie Monographe XXXIb.* Leiden: E.J. Brill: 129–275. https://doi.org/10.5962/bhl.ti-tle.12959

Hosie, A.M., Fromont, J., Munyard, K. & Jones, D.S. 2019. Description of a new species of *Membranobalanus* (Crustacea, Cirripedia) from southern Australia. *ZooKeys*, 873: 25–42. https://doi.org/10.3897/zookeys.873.35421

Høeg, J.T., Deutsch, J., Chan, B.K.K. & Semmler Le, H. 2015. "Crustacea": Cirripedia. In: A. Wanninger, ed. *Evolutionary Developmental Biology of Invertebrates 4: Ecdysozoa II: Crustacea*. Vienna: pp. 154–181. https://doi.org/10.1007/978-3-7091-1853-5_5

Ilan, M., Loya, Y., Brickner, I. & Kolbasov, G.A. 1999. Sponge-inhabiting barnacles on Red Sea coral reefs. *Marine Biology*, 133: 709–716. https://doi.org/10.1007/s002270050512

Kolbasov, G.A. 1993. Revision of the genus *Acasta* Leach (Cirripedia: Balanoidea). *Zoological Journal of the Linnean Society*, 109: 395–427. https://doi.org/10.1111/j.1096-3642.1993.tb00307.x

Krüger, P. 1911. Beiträge zur Cirripedienfauna Ostasiens. In: F. Doflein, ed. *Beiträge zur Naturgeschichte Ostasiens. Kongelige Bayerische Akademie der Wissenschaften, Munich Mathematische-physikalische Klasse, Abhandlungen Supplement-Band*, 2 (6): 1–72.

Lamarck, J.B.P.A. 1818. *Histoire naturelle des animaux sans vertèbres*. Tome 5. Paris: Deterville: 612 pp.

Lewis, J.B. 1992. Recruitment, growth and mortality of a coral-inhabiting barnacle *Megabalanus stultus* (Darwin) upon the hydrocoral *Millepora complanata* Lamarck. *Journal Experiment Marine Biology Ecology*, 162: 51–64.

Metscher, B. D. 2009a. MicroCT for comparative morphology: simple staining methods allow high-contrast 3D imaging of diverse non-mineralized animal tissues. *BMC Physiology*, 9: 11.

Metscher, B. D. 2009b. MicroCT for developmental biology: a versatile tool for high-contrast 3D imaging at histological resolutions. *Developmental Dynamics (American Association of Anatomists)*, 238: 632–640. https://doi.org/10.1002/dvdy.21857

Nagler, C., Haug, J.T., Glenner, H. & Buckeridge, J. 2017. *Litholepas klausreschi* gen. et sp. nov., a new neolepadine barnacle (Cirripedia, Thoracica) on a sponge from the Upper Jurassic lithographic limestones of southern Germany. *Neues Jahrbuch für Geologie und Paläontologie Abhandlungen*, 284(1): 29–42. http//doi.org//: 10.1127/njgpa/2017/0648.

Newman, W.A. & Abbott, D.P. 1980. Cirripedia: the barnacles. *Intertidal Invertebrates of California*, 504–535. http://decapoda.arthroinfo.org/pdfs/31753/31753.pdf.

Noever, C., Keiler, J. & Glenner, H. 2015. First 3D reconstruction of the rhizocephalan root system using MicroCT. *Journal of Sea Research*, 1–7. http://dx.doi.org/10.1016/j.seares.2015.08.002

Pitriana, P., Valente, L., von Rintelen, T., Jones, D.S., Prabowo, R.E. & von Rintelen, K. 2020. An annotated checklist and integrative biodiversity discovery of barnacles (Crustacea, Cirripedia) from the Moluccas, East Indonesia. *ZooKeys*, 945: 17–83. https://doi.org/10.3897/zookeys.945.39044.

Rosell, N.C. 1991. Crustacea Cirripedia Thoracica: MUSORSTOM 3 Philippines collection. In: A. Crosnier, ed. Résultats des Campagnes MUSORSTOM, Volume 9. *Mémoires du Muséum national d'Histoire naturelle* (A), 152: 9–61.

Semple, T.L., Peakall, R. & Tatarnic, N.J. 2018. A comprehensive and user-friendly framework for 3D data visualisation in invertebrates and other organisms. *Journal of Morphology*, 280(2): 223–231. https://doi.org/10.1002/jmor.20938

Sulistiono, S., Kawaroe, M., Madduppa, H. & Prabowo, R.E. 2014. Karakteristik morfologi teritip spons Indonesia. *Depik,* 3(2): 178–186. https://doi.org/10.13170/depik.3.2.1553

Van Syoc, R.J. 1988. Description of *Membranobalanus robinae*, a new species of sponge barnacle (Cirripedia, Archaeobalanidae) from Baja California, with a key to the genus. *Proceedings of the Biological Society of Washington*, 101: 832–837. https://www.jstor.org/stable/20106147

Van Syoc, R.J. & Winther, R. 1999. Sponge-inhabiting barnacles of the Americas: a new species of *Acasta* (Cirripedia, Archaeobalanidae), first record from the Eastern Pacific, including discussion of the evolution of cirral morphology. *Crustaceana*, 72: 467–486. https://doi.org/10.1163/156854099503528.

Van Syoc, R.J., Van Soest, R.W., Xavier, J.R. & Hooper, J.N. 2015. A phylogenetic overview of sponge-inhabiting barnacles and their host specificity (Crustacea, Cirripedia). *Proceedings of the California Academy of Sciences (Series 4)*, 62: 331–357.

Wibowo, R.A., Prabowo, R.E. & Nuryanto, A. 2011. Biodiversitas teritip yang hidup pada spons di Perairan Pantai Kepulauan Karimunjawa. In: *Prosiding Kongres dan Seminar Masyarakat Taksonomi Kelautan Indonesia*, Jakarta 20–22 September 2011: 219–235.

Yu, M.C., Kolbasov, G.A., Hosie, A.M., Lee, T-M. & Chan, B.K.K. 2017. Descriptions of four new sponge-inhabiting barnacles (Thoracica: Archaeobalanidae: Acastinae). *Zootaxa*, 4277(2): 151–198. https://doi.org/10.11646/zootaxa.4277.2.1.

Yu, M.C., Dreyer, N., Kolbasov, G.A., Høeg, J.T. & Chan, B.K.K. 2020. Sponge symbiosis is facilitated by adaptive evolution of larval sensory and attachment structures in barnacles. *Proceedings Royal Society B*, 287: 20200300. http://dx.doi.org/10.1098/rspb.2020.0300.

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Concise writing is recommended. All numbers under 10 and any number forming the first word of a sentence must be spelled out, except in the Materials and Methods section of taxonomic papers. Year should be completely written. Names of genera and species should be in italic type. It is recommended to use metric measurements in abbreviation (for examples: kg, cm, ml). Please consult and refer to a recent issue of TREUBIA for an acceptable format. Please note that starting in 2018, we adopt Mendeley reference management application, with Harvard referencing style.

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Acknowledgments. Acknowledgments of grants, assistance and other matters can be written in one paragraph.

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For example:

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LaSalle, J. & Schauff, M.E. 1994. Systematics of the tribe Euderomphalini (Hymenoptera: Eulophidae): parasitoids of whiteflies (Homoptera: Aleyrodidae). *Systematic Entomology*, 19: 235–258.

MacKinnon, J. & Phillips, K. 1993. *Field Guide to the Birds of Borneo, Sumatra, Java and Bali*. Oxford: Oxford University Press: 491 pp.

Natural History Museum 2013. Wallace100 - celebrating Alfred Russel Wallace's life and legacy. http://www.nhm.ac.uk/nature-online/science-of-natural-history/wallace/index.html 11 October 2013.

Higgins, P., Christidis, L., Ford, H. & Bonan, A. 2017. Honeyeaters (Meliphagidae). In: J. del Hoyo, A. Elliott, J. Sargatal, D.A. Christie & E. de Juana, eds. *Handbook of the Birds of the World Alive*. Barcelona: Lynx Edicions. http://www.hbw.com.

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