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Deep-sea ascidians from Papua New Guinea

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Abstract

Four deep-sea ascidian species collected during the KAVIENG 2014 expedition in Papua New Guinea are described, including additional characteristics not reported previously. *Fimbrora calsubia* is classified within the family Ascidiidae, *Dicopia fimbriata* and *Octacnemus bythius* within Octacnemidae, and *Culeolus recumbens* within Pyuridae. Anatomical observations confirmed previous descriptions for these four species collected elsewhere. Here, we describe additional morphological features for these species and provide the first barcode DNA sequences (based on a fragment of the mitochondrial gene Cytochrome Oxidase I) for *D. fimbriata* and *C. recumbens.*

Key words: Ascidians, deep-sea, Papua New Guinea, Fimbrora, Octacnemus, Culeolus

Introduction

The KAVIENG 2014 expedition (Principal Investigators: Philippe Bouchet, Jeff Kinch, Claude Payri) was part of the '*Our Planet Reviewed'* expeditions organized jointly by Muséum National d'Histoire Naturelle (MNHN; France), Pro-Natura International (PNI; created in Brazil in 1985 and currently with offices in Paris, France) and 'Institut de Recherche pour le Développement' (IRD; France), with support from Papua New Guinea's National Fisheries Authority. Deep-sea research was part of the '*Tropical Deep-Sea Benthos'* component of the expedition and took place on board *R.V. Alis* (France).

Four deep-sea ascidian species (Chordata; Tunicata) were recovered during this expedition in Papua New Guinea: *Fimbrora calsubia, Dicopia fimbriata, Octacnemus bythius* and *Culeolus recumbens*. *F. calsubia* Monniot & Monniot 1991 was first described in New Caledonia based on morphological observations of three specimens collected at 1,865 m. These animals were classified within the family Ascidiidae and a new genus (*Fimbrora*) was created to account for the unique morphological characteristics of the species, notably the presence of muscular blood vessels in the body wall (Monniot & Monniot 1991a). In addition, *F. calsubia* appeared to be capable of both microphagous and macrophagous feeding, which until then had only been observed for species within the family Octacnemidae (Monniot & Monniot 1991a).

D. fimbriata Sluiter 1905 and *O. bythius* Moseley 1877 are both classified within the family Octacnemidae. *D. fimbriata* was first collected at 1,788 m near Indonesia (Sluiter 1905) and further described by Monniot & Monniot (1991b) based on four individuals collected from around New Caledonia at depths ranging from 1,175 m to 1,410 m. More recently, another individual was collected in the Tasman Sea at 1,210 m, although the specimen collected was too damaged to provide a detailed description (Sanamyan & Sanamyan 1999). *O. bythius* was first described from the Schouten Islands (Tasmania) based on observations of a damaged individual collected by trawling at 1,957 m (Moseley 1877). This species was later observed by Herdman (1988) off the west coast of South America, Ritter (1906) off the coast of Ecuador, Millar (1959) in the Kermadec Trench (New Zealand), and Monniot & Monniot (1991b) in New Caledonia. All specimens were collected at depths greater than 1,100 m (Moseley 1877, Herdman 1988, Ritter 1906, Millar 1959, Monniot & Monniot 1991b). Species within the Octacnemidae family are notorious for their capacity to capture small prey like copepods with a highly muscularized and modified oral siphon, which allows them to thrive in deep-sea environments (Monniot 1998).

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Finally, the species *C. recumbens* Herdman 1881 is classified within the family Pyuridae, which is characterized by pedunculated morphology and a branchial sac lacking stigmata (Herdman 1881). This species was first described from a station located between the Cape of Good Hope and Kerguelen Island at 2,514 m (Herdman 1881) and since then it has been reported in other Sub-Antarctic waters, including Southern and Northern New Zealand, and the Indo-West Pacific region (reviewed in Primo & Vázquez 2007).

Although descriptions exist for the four species mentioned above, deep-sea specimens are rare and difficult to obtain undamaged. Moreover, until this study, no information existed on the deep-sea ascidian species inhabiting Papua New Guinea. Here, we obtained eight additional individuals of *F. calsubia*, one of *D. fimbriata*, one of *O. bythius* and one of *C. recumbens* from Papua New Guinea in depths from 672 to 1,220 meters. Specimen preservation allowed for confirmation of species identity based on previous observation as well as additional descriptions of previously unreported morphological characteristics. In addition, we were able to obtain the first barcoding sequences for the two deep-sea ascidians *D. fimbriata* and *C. recumbens*.

Material and methods

Sampling was performed in August and September of 2014 from four stations located around Papua New Guinea (Table 1) using a beam trawl aboard the *R.V. Alis* (IMO number 8806761; France). All specimens were immediately sorted on board and fixed in 95% ethanol for further morphological analysis at the Muséum national d'Histoire naturelle (MNHN) and molecular analysis (barcoding) at the University of North Carolina Wilmington (UNCW). The utilized preservation method did not damage the animals, which arrived at MNHN in good condition despite their soft and brittle tissues. Photos of the fixed specimens were taken in the laboratory. After dissection, some parts of the animals were stained with Masson's haemalum to allow better visualization of key morphological characters. The specimens are registered in the MNHN collection (see descriptions).

TABLE 1. Stations sampled in Papua New Guinea, including sampling code, date, GPS position and depth range in meters.

Station code	Sampling Date	GPS position	Depth range
CP 4433	August 30, 2014	02°16 S–150° 48'E	1056–1200
CP 4435	August 30, 2014	02°17'S–150° 51'E	1218–1220
CP 4436	August 30, 2014	02°16'S–150° 45'E	1128–1135
CP 4480	September 4, 2014	02°48'S–150° 42'E	672–1150

Ascidian barcoding. DNA extractions were performed on branchial or mantel tissue of *D. fimbriata* and *C. recumbens* to obtain a 610 bp fragment of the mitochondrial gene Cytochrome *c* Oxidase I (COI) corresponding to the standard barcoding partition (Hebert *et al.* 2003, Bucklin *et al.* 2011). DNA was extracted using the DNeasy Blood & Tissue kit following manufacturer's instructions (Qiagen). For COI amplification, two sets of primer combinations were used: the LCO1490 and HCO2198 (Folmer *et al.* 1994) pair for *D. fimbriata* and the Tun_forward (Stefaniak *et al.* 2009) and HCO2198 (Folmer *et al.* 1994) combination for *C. recumbens.* Amplification was performed with 1 µL of each primer (10 µM), 12 µL (0.5 units) of MyTaqTM HS Red Mix (Bioline) containing MyTaq HS DNA Polymerase and a buffer with dNTPs and $MgCl_2$, 10 to 20 µg/mL of DNA, and PCR water to a total-reaction volume of 25 µL. The PCR program included initial denaturing at 95ºC for 1 min, followed by 40 amplification cycles of 95ºC for 15 sec; annealing at 42ºC for 15 sec; and extension at 72ºC for 10 sec. A final extension step was carried out at 72ºC for 1 min. Sequencing reactions were carried out with the BigDye™ terminator v. 3.1 (Applied Biosystems) and the same primer set used during the amplification step. Sequences were obtained on an ABI prism 3500 genetic analyser (Applied Biosystems) available at UNCW Center for Marine Science. Consensus sequences from forward and reverse reads for each sample were created using Geneious v. 8 (Kearse *et al.* 2012). Both sequences have been deposited in GenBank under accession numbers KY882283 for *D. fimbriata* and KY882284 for *C. recumbens*.

Descriptions

Fimbrora calsubia Monniot C & Monniot F, 1991

Monniot C & Monniot F, 1991, New Caledonia
Monniot C 1993, Indonesia
Stations CP 4433, 2 specimens (MNHN P5 FIM 8); CP 4435, 3 specimens (P5 FIM 5 – P5 FIM 6); CP 4436, 3 specimens (P5 FIM 7)

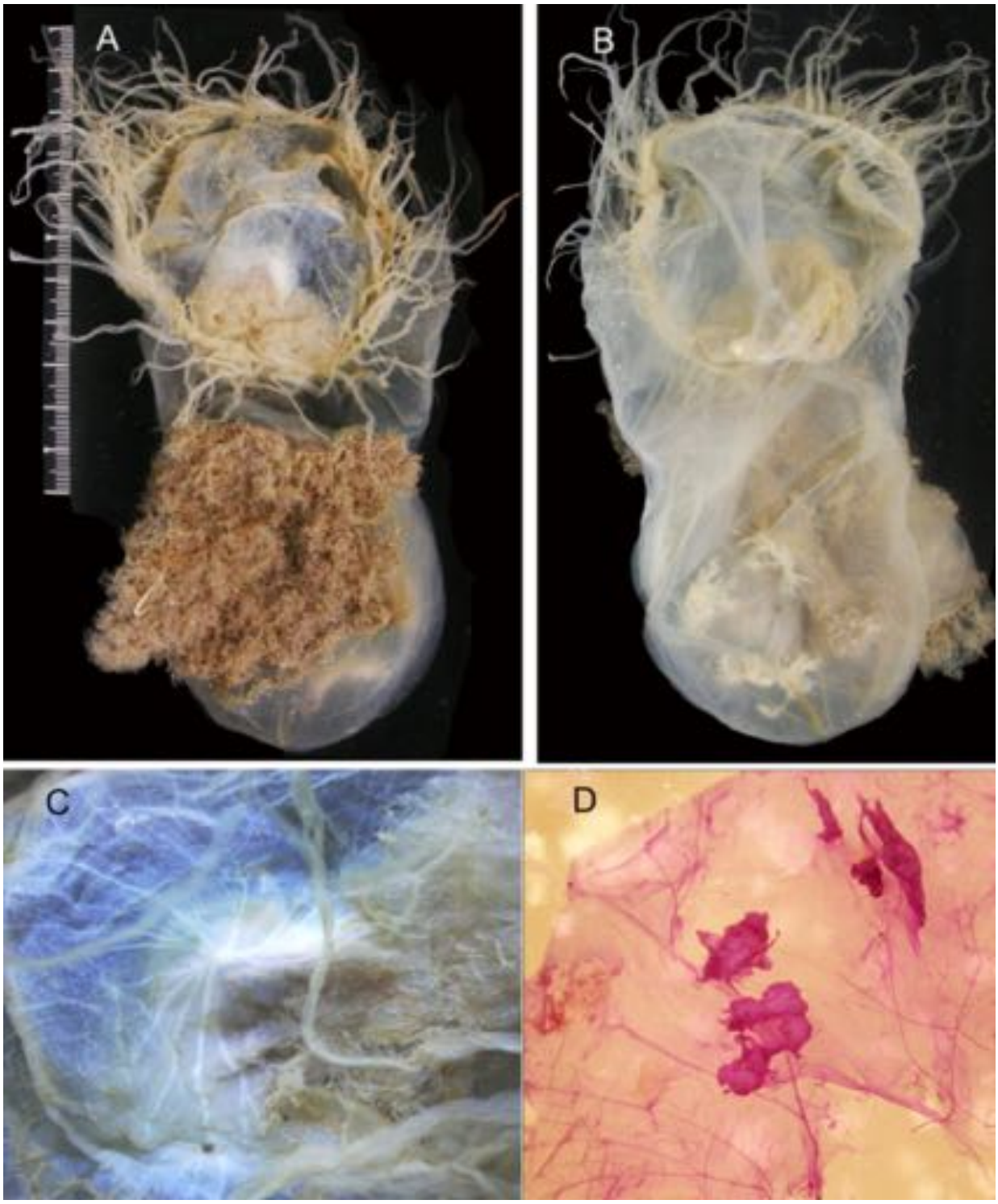

FIGURE 1. *Fimbrora calsubia*. A and B, ventral and dorsal sides of a specimen. C, neural area. D, detail of the ramified tissues of the posterior part of the abdomen stained with hemalum.

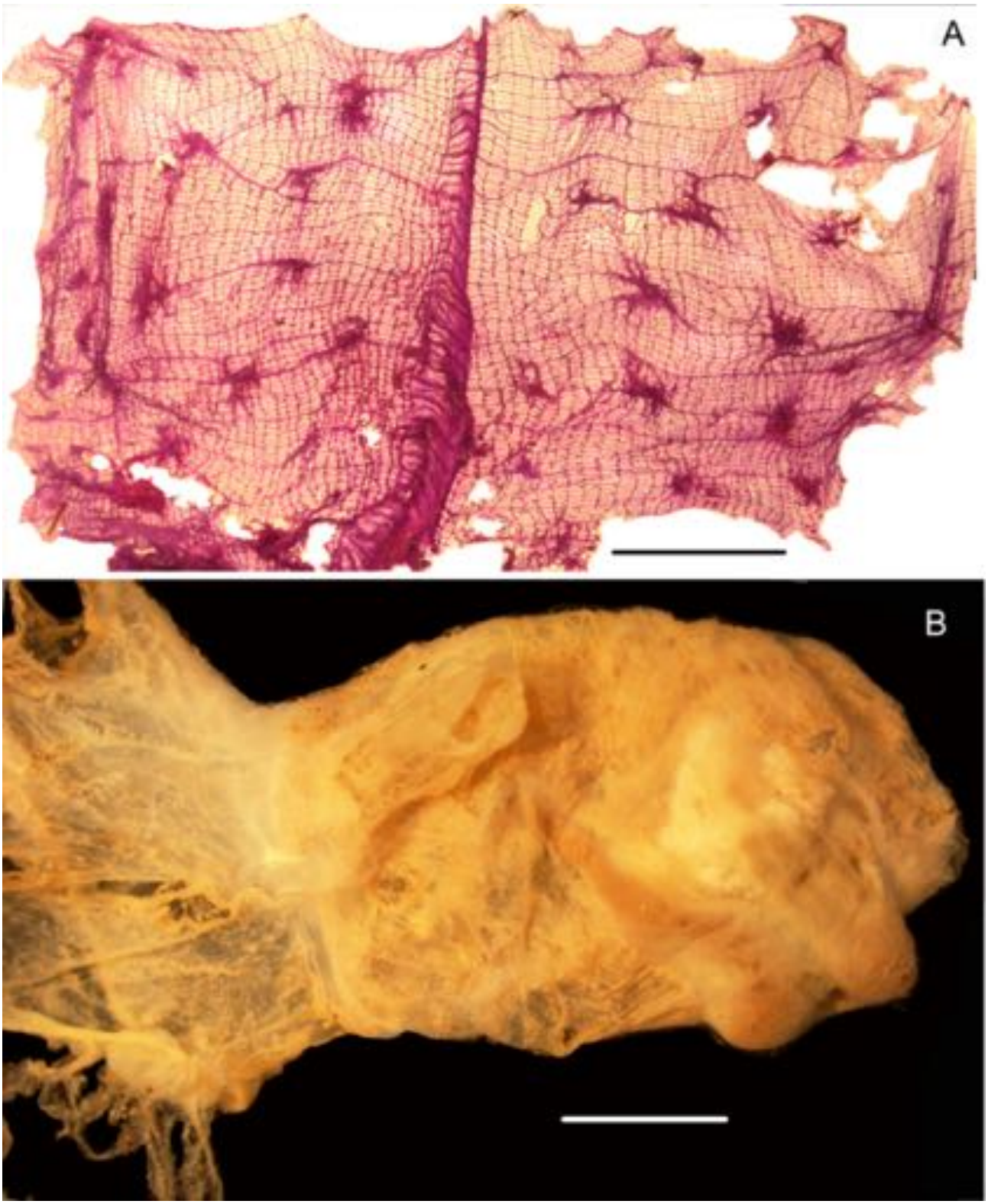

FIGURE 2. *Fimbrora calsubia*. A, branchial tissue stained with haemalum. B, body without tunic showing the atrial aperture. Scale bars 1cm.

The genus *Fimbrora* clearly belongs to the family Ascidiacea, exhibiting a branchial sac well developed with longitudinal vessels and true stigmata, although it may be confused with an Octacnemidae at first look. The newly collected specimens corresponded well with previous observations reported for the single species described for the genus *Fimbrora calsubia*, and previously collected near New Caledonia (Monniot & Monniot 1991a) and in Indonesia (Monniot 1993).

These large ascidians were attached to stones by a wide basal disk covered with dense filaments (Fig. 1A). The tunic is transparent and thin on the oral lobes but thicker basally. The total body length reaches 15 cm. The oral aperture is a wide funnel with the free edge prolonged by numerous thread-like lobes (Fig. 1A, B). A complex system of blood vessels can be seen through the transparent oral siphon as previously described and depicted (Monniot & Monniot 1991a). The tunic adheres to the body wall on the oral siphon but not on the abdominal region where a large cavity appears between the abdomen and the base of the body attached to the substrate. This cavity seems empty, but this may be due to strong contraction of the specimens fixed in ethanol. On the inner side of this posterior sac-like tunic protrude some ramified opaque tissue growth with no differentiated structure (Fig.1B) but stainable with haemalum (Fig. 1D); three to four of these structures are present in all specimens. The atrial siphon opens as a simple hole with a smooth rim on the dorsal side (Fig. 2B) near the neural area, slightly on the right side.

The musculature on the oral funnel comprises annular fibres at the edge of the thread-like lobes, and radial fibres crossing this ring become thinner when prolonged on the sides of the siphon. A strong sphincter surrounds the mouth aperture. Crossed muscular fibres enclose the abdominal part. Numerous short filiform tentacles arise from the mouth entrance. The neural ganglion (Fig. 1C) and the small neural gland are enclosed together in a small "V" of the prepharyngeal band. From the point of this "V" start two rapheal crests uniting posteriorly in a single lamina that increases in height to reach the bottom of the branchial sac (Fig. 2A). The branchial tissue is thin and flat (Fig. 2A) and is attached to the body wall by a few, thick strong bridles. There is an average of 45 to 50 longitudinal vessels on each side. The transverse vessels are different sizes with an irregular pattern (Fig. 2A). Ten to twelve elongated stigmata can be counted between two successive longitudinal vessels. No stained cells that would suggest the presence of ciliae appeared around the stigmata.

The different parts of the gut loop formed a dense mass and were not individualized. The gonad is massive and attached to the gut and no gonoducts were observed. The morphology of this newly collected species corresponded well to previous observations and does not vary across distant geographic locations from Indonesia to New Caledonia.

Dicopia fimbriata Sluiter, 1905

Sluiter, 1905: 05°46'S–134°0'E, 1788 m, Indonesia
Monniot C & Monniot F, 1991b, Loyalty Basin, New Caledonia
Sanamyan K & Sanamyan N, 1999, Tasman Sea
Station CP 4480, 1 specimen (MNHN P6 DIC13)
GenBank Accession Number: KY882283

The body measures 4.2 cm in height and 3 cm across the oral lobes (Fig. 3A, B). It was attached to the substrate by numerous thread-like filaments. The tunic is vitreous with a smooth surface, thin on the lips and with a cartilaginous consistency around the abdomen. The margin of the oral lobes is smooth and not rolled. The atrial aperture is a simple hole (Fig. 3D) that opens on the ventral side behind the ventral oral lobe. The oral musculature contains thin circular fibres that are crossed by thin short fibres at the top of the ventral lobe. Strong longitudinal muscular fibres lie on the dorsal oral lobe (Fig. 3C) with muscular bundles concentrated at the corners of the lips (Fig. 3A, B, C). Short longitudinal muscles are also present dorsally and ventrally to the mouth opening. The mouth is encircled with a sphincter. There is a ring of short filiform oral tentacles close to a prepharyngeal band with papillae. The branchial tissue is made of a few polygonal meshes without ciliae and there is a short dorsal lamina. The abdomen forms a compact mass with a closed intestinal loop and a hermaphroditic gonad. No morphological differences were noted when compare to previous descriptions of this species (Monniot & Monniot 1991b, Sanamyan & Sanamyan 1999).

There are only two other species with the genus *Dicopia: D. antirrhinum* Monniot, 1972 from the Atlantic Ocean has a tunic covered with papillae, giving it a velvet aspect and a different organization of the musculature. *Dicopia japonica* Oka, 1913 was described from Japan and is characterized by the presence of papillae on the tunic and a musculature on the oral lobes that is more developed than the one reported here.

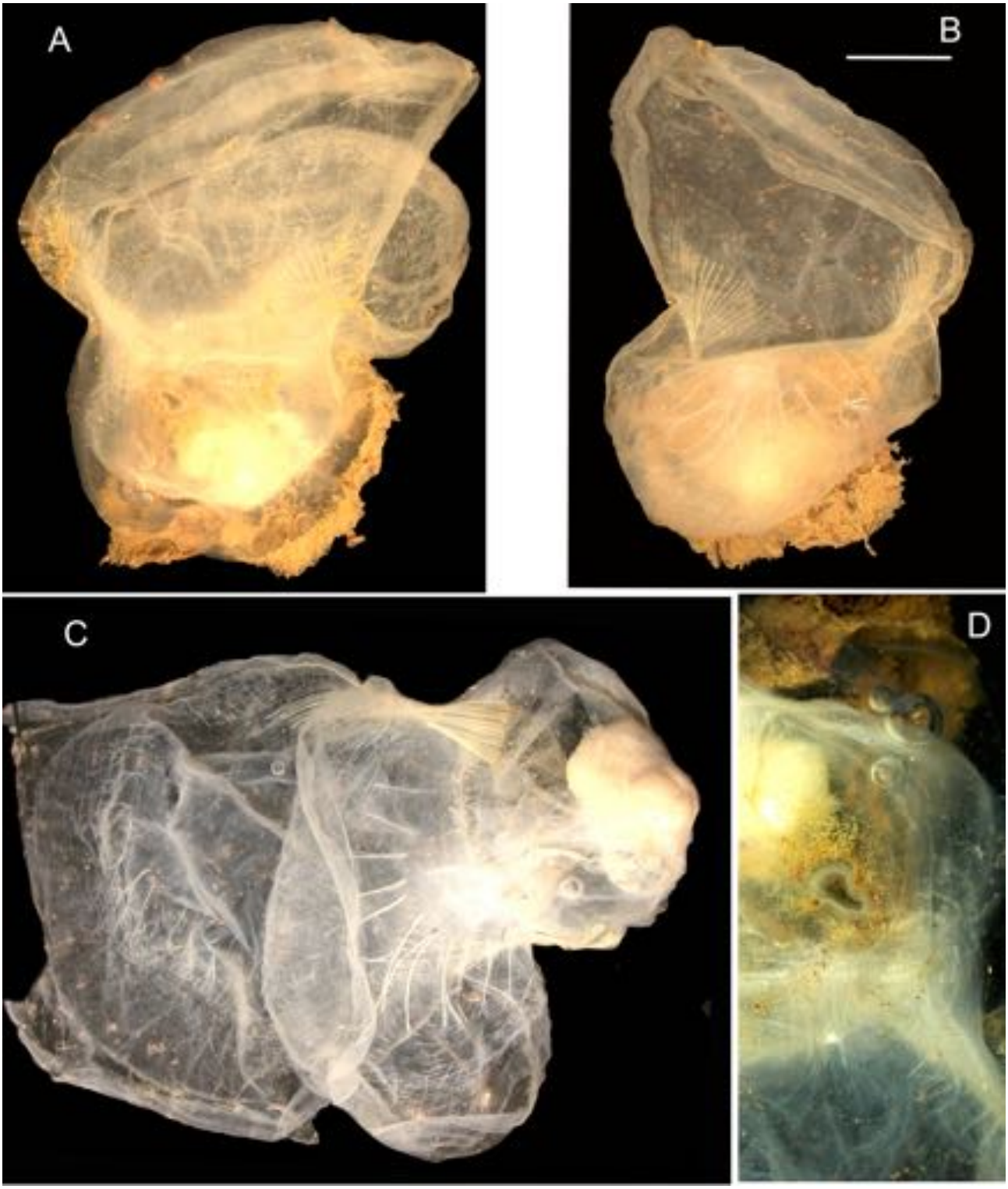

FIGURE 3. *Dicopia fimbriata*. A,B both sides of a specimens with tunic, scale bar 1cm. C, specimen without tunic. D, atrial siphon aperture.

Octacnemus bythius Moseley, 1876

Moseley, 1876, 2°33'S–144°4'E
Herdman, 1888
Millar, 1959 and synonymy

Monniot C & Monniot F, 1991b and synonymy
Station CP 4435, 1 specimen (MNHN P6 OCT 19)

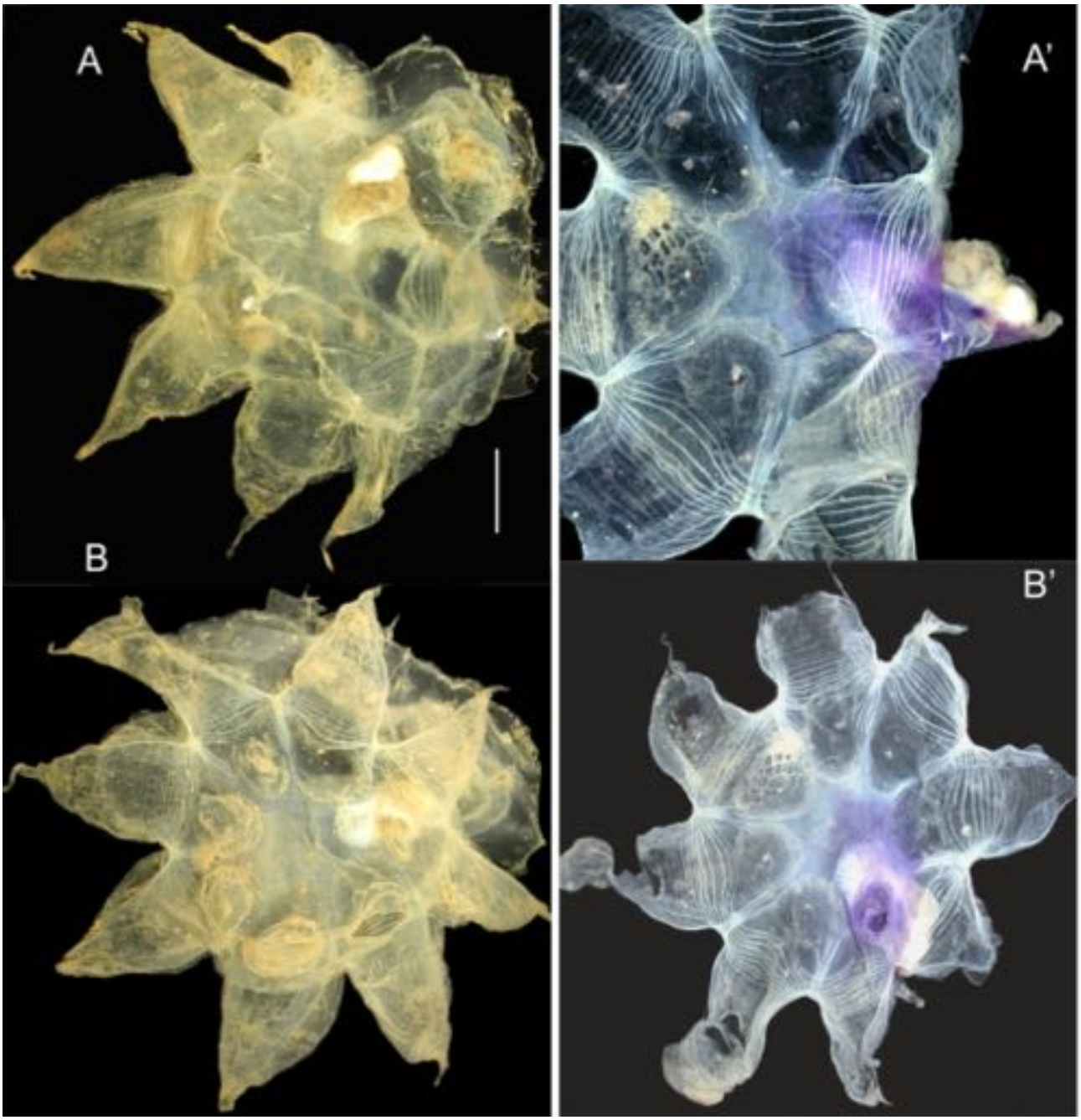

FIGURE 4. *Octacnemus bythius*. A, A' body with and without tunic, dorsal side. B, B' oral side with and without tunic. Scale bar 1cm.

The single specimen collected during the KAVIENG expedition has the same star-like morphology (Fig. 4) as the specimen-type collected from the same region. The distance between opposite oral lobes measures 6 cm. The vitreous tunic is very thin and smooth. The oral lobes are triangular with a pointed tip and no lateral denticles. At the base of each lobe on the oral side there are round vesicles (Fig. 4B). These vesicles appear to be prolongations of the tunic since no trace of them remains when the tunic is removed (Fig. 4B'). The musculature is typical of the genus, with circular fibres around the base of the oral lobes gathered by radial ribbons at regular intervals. These ribbons spread laterally and internally drawing arcs that connect with each other (Fig.4A', B'). A strong, fan-shaped muscle lies at the posterior side of the mouth and some muscular bundles are also present at the dorsal side of the aperture, behind the neural ganglion. A sphincter closes the mouth opening. The neural ganglion is close to

the oral sphincter, giving two lateral nerves on each side. A small neural gland is associated with the ganglion. There is no branchial sac. The pharyngeal pouch is lined by a wrinkled tissue pierced in the bottom right by a simple small hole. The pharyngeal tissue is raised forming a ridge close to the ganglion and may either be a dorsal lamina or a contraction artefact. The oesophagus opens at the left side of the pharynx pouch. The shape of the gut is indistinct. Male and female gonads are tightly attached to the gut. The body wall around the abdomen is particularly thin and apparently devoid of muscles. A narrow atrial cavity along the rectum opens into a hole close to the posterior part of the gut.

Octacnemus bythius differs from the Atlantic *O. zarcoi* Monniot C & Monniot F, 1984 in that *O. zarcoi* has unequal oral lobes and a muscular oral siphon. *O. alatus* Monniot C & Monniot F, 1985 from the southern Indian Ocean has two large oral lobes that look like wings. *O. vinogradovae* Sanamyan & Sanamyan, 1999 from Macquarie Island has the same albeit smaller oral lobes than *O. bythius*. However, *O. bythius* has a pharynx with longitudinal vessels and branchial perforations. *O. kottae* Sanamyan & Sanamyan, 2002 has pinnate oral lobes, a pedunculate abdomen and a pharynx that is entirely perforated. *O. ingolfi* Madsen, 1947 from the northern Atlantic has pinnate oral lobes and a long posterior appendage. The branchial sac of this species is also entirely perforated. All members of the genus *Octacnemus* are deep-sea species.

Culeolus recumbens Herdman, 1881

Herdman, 1881
Herdman, 1882
Monniot C & Monniot F, 1982, Antarctic; 1991, New Caledonia Figs. 31D–33 A, B
Monniot F & Monniot C, 2003 Figs. 34–49B
Sanamyan K & Sanamyan N, 1999, Tasman Sea
Station CP 4435, 1 specimen (MNHN S2 CUL 60)
GenBank Accession Number: KY882284

The body measures 2 cm in length and is attached to the substratum by a thin peduncle with a tuft of rhizoids measuring 10.5 cm. The peduncle is not sclerified but has sediment and foraminifers attached to it. The body tunic is soft and thin with a characteristic ring of triangular papillae at some distance around the atrial aperture (Fig. 5A). Some sand is present only on the oral siphon and at the twisted beginning of the peduncle. The body musculature is made of sphincters around the siphon apertures and of strong, spaced ribbons along the body (Fig. 5B). One of these muscles originates from the atrial siphon, is thicker than the rest and crosses each body side through the middle. The oral tentacles are long with few primary ramifications. The prepharyngeal band is slightly bent dorsally to enclose the dorsal tubercle, which opens into a simple hole. The neural ganglion is at mid-distance between the siphons. The branchial sac is damaged and eviscerated through the atrial aperture, with about 14 to 15 longitudinal vessels on each side making no distinct folds. The branchial meshes are large, square and without ciliae. The dorsal languets have a sharp tip. The gut (Fig. 5C) forms an open loop with a long narrow stomach covered with several successive hepatic lobes. The anus is fringed with numerous lobes. Two round gonads are located near the cloacal aperture on the right side and only one gonad is set within the gut loop. There is a long endocarp parallel to the endostyle on the right body side and a small vesicle at the top of the gut loop (Fig. 5C). No spicules were observed.

C. recumbens differs from all other species in the genus by having a peduncle that is not sclerified, few round gonads and endocarps. *C. recumbens* was known to be widely distributed in the southern regions of the Indian and Pacific oceans and its distribution now includes the equatorial waters around Papua New Guinea.

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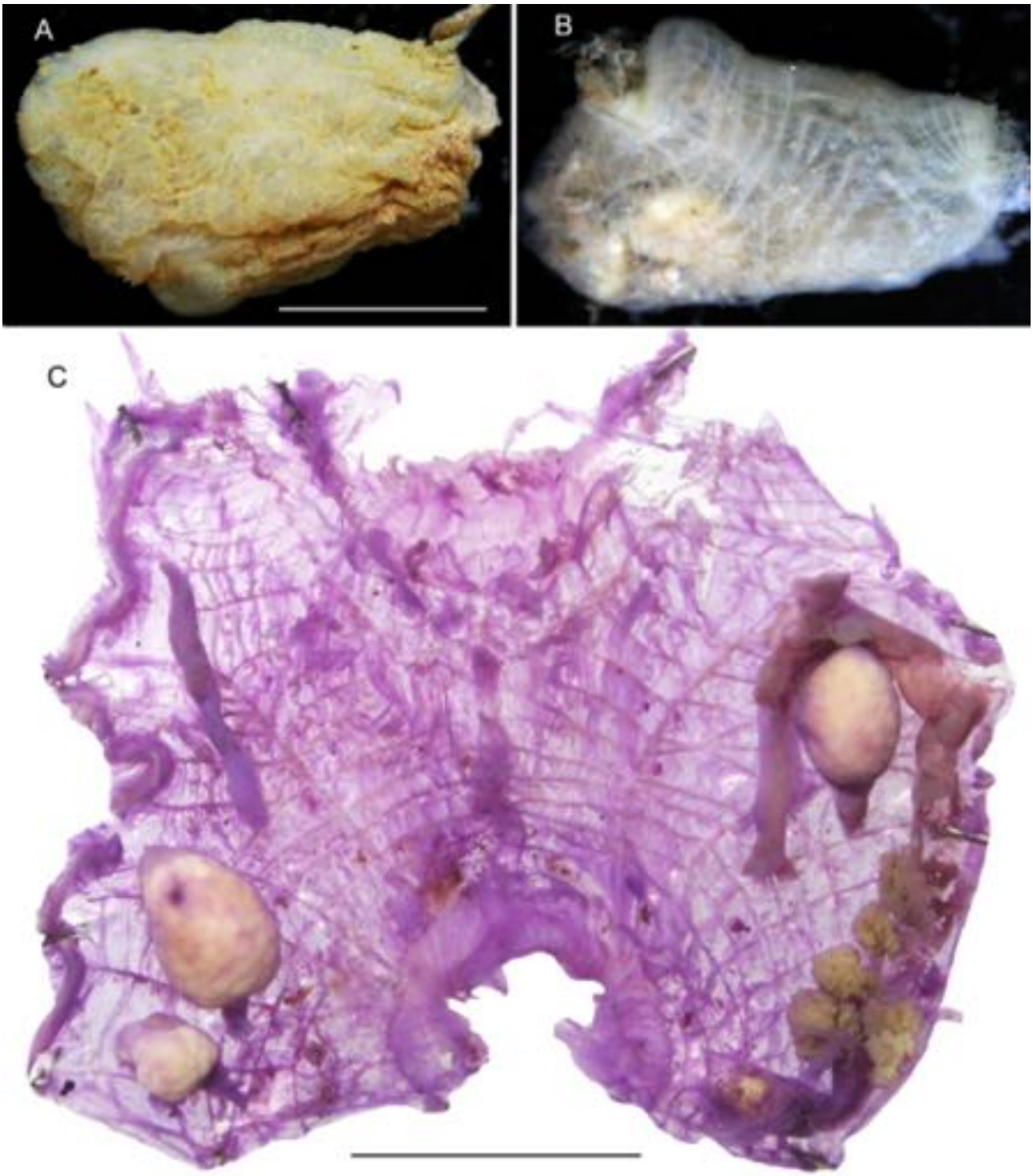

FIGURE 5. *Culeolus recumbens*. A, body with tunic, B, same than A without the tunic. C, body opened along the ventral line, branchial sac removed, stained with Masson's haemalum. Scale bars: A: 1cm; C: 5mm.

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