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Ascidia subterranea sp. nov. (Phlebobranchia: Ascidiidae), a new tunicate belonging to the A. sydneiensis Stimpson, 1855 group, found as burrow associate of Axiopsis serratifrons A. Milne-Edwards, 1873 (Decapoda: Axiidae) on Derawan Island, Indonesia

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Abstract

A new tunicate, Ascidia subterranea sp. nov., was found in burrows of the axiid crustacean Axiopsis serratifrons on Derawan Island, Indonesia. It differs from other ascidians in its habitat as well as numerous morphological peculiarities which are described in detail. The shrimp Rostronia stylirostris Holthuis, 1952 was found inside A. subterranea sp. nov., and 4 species of bivalves, 3 species of polychaetes, 1 gastropod, 1 polyplacophoran and 1 sponge species were found as burrow associates besides the ascidian.

Key words: Tunicata, Phlebobranchia, Ascidiidae, Ascidia sydneiensis group, shrimp burrows, burrow associates, Indonesia

Introduction

Decapod burrows frequently harbor associated macrofauna. The nonsymbiotic mutualism between alpheid shrimp and their associated gobiiid fish is well documented (Karplus 1987). Burrows of axiidean and gebiidean shrimp (formerly grouped together as “Thalassinidea”, Robles at al. 2009, De Grave et al. 2009, Dworschak et al. 2012) have been found to accommodate invertebrates such as turbellarians, nemerteans, polychaetes, echiurans, bryozoans, gastropods, bivalves, phoronids and other crustaceans (copepods, cephalocarids, carideans, brachyurans and amphipods) (see recent summary in Dworschak et al. 2012 and references therein, but also MacGinitie 1934, Pohl 1946, Farrow 1971, Felder & Rodrigues 1993, O’Reilly 2000, Itani 2004, Kneer et al. 2008 a, Komai 2009), as well as vertebrates (gobiid fishes) (see recent summary in Dworschak et al. 2012 and references therein, but also MacGinitie & MacGinitie 1968, Hoffman 1981, Atkinson & Taylor 1991, Itani & Tanase 1996, Senou 2004, Suzuki et al. 2006, Kinoshita et al. 2010). If burrows are sufficiently spacious and stable, multiple associated species can coexist; amphipods, palaemonid shrimp, one sabellid and one spirorbid polychaete species, the bivalve Barrassymia cunningii A. Adams, 1856 and the goby Austrolethops wardi Whitley, 1935 were all found sharing burrows of the strahlaxiid shrimp Neaixus acahthus A. Milne-Edwards, 1878 in the Spermonde Archipelago, Indonesia (Kneer et al. 2008 a, Kneer et al. 2008 b). So far, tunicates have not been reported to inhabit crustacean burrows.

The axiid shrimp Axiopsis serratifrons A. Milne-Edwards, 1873 has a circumtropical distribution (Kensley 1981). It was found to construct relatively spacious burrows (diameter 40 * 80 mm, much larger than the shrimp with a maximum length of 60 mm) in a study by Dworschak & Ott (1993) in the Caribbean (Belize), but no
associated macrofauna was reported. Wirtz (2008) observed “an undescribed species of the family Gobiidae” sharing burrows of *A. serratifrons* in the Gulf of Guinea, Atlantic Ocean (Sao Tome and Principe). This “gobiid fish” later turned out to be two species: *Didogobius amicuscaridis* Schliewen & Kovacic, 2008 and *D. wirtzi* Schliewen & Kovacic, 2008.

*Axiopsis serratifrons* has been reported throughout the Indonesian Archipelago (Kensley 1981). Its burrows are a common feature on sand-covered intertidal to shallow subtidal reef flats on Derawan Island (East Kalimantant), and less common on Bone Batang Island (Spermonde Archipelago) (pers. obs. D. K.). For the present study, burrows of *A. serratifrons* on Derawan Island were dug up, and shrimp as well as associated animals were collected and identified.

**Material and methods**

Derawan Island is located in the Sulawesi Sea, 16 km off the coast of East Kalimantant, Indonesia. It is central in a barrier reef system (with Pulau Panjang to its North and Pulau Samama to its South) running parallel to the mainland coast, and consists of an intertidal to shallow subtidal reef flat crowed by a coral cay. All burrows were located in calcareous sediments (gravelly coarse sand, D50 = 591±30 µm) vegetated by the narrow leafed form of the seagrass *Halodule uninervis*. The vegetation was heavily grazed upon by green turtles *Chelonia mydas* Linnaeus, 1758 (Testudines: Cheloniidae), which depleted aboveground seagrass biomass by removing 100 % of the daily leaf production (Christianen et al. 2012) and tended to remove the whole plants including rhizomes and roots after flushing away the sediment with their front flippers. This behavior, which is unique to the turtles foraging on Derawan Island (pers. obs. D.K. & M.J.A.C.), together with small moving sand waves creates a spatial mosaic of unvegetated patches in the seagrass meadow.

Five burrows were dug up on the Derawan reef flat in January 2010 by carefully flushing away the sand around burrow openings by hand. Large coherent parts of the consolidated burrow walls with attached fauna could then be lifted up and transferred into plastic bottles. This was continued until the end of the burrow structure was reached. All fauna was fixed and preserved in ethanol. The ascidians which were removed from the tunic were stained with hemalum, and parts of them were dehydrated and mounted on slides in a plastic medium. For SEM, specimens were dissected, dehydrated in a graded series of ethanol, and subsequently critical point dried in a CPD 030 (Balzers Union, Liechtenstein). Dried specimens were sputter-coated with gold in a SCD 040 (Balzers Union, Liechtenstein) and viewed with a Fei Quantum 200 scanning electron microscope at 15 kV (FEI Co, The Netherlands).

Type specimens are deposited in the Museum für Naturkunde, Berlin, Germany (ZMB) and the Muséum National d'Histoire Naturelle, Paris, France (MNHN).

**Results**

While digging up the burrows their general architecture could be studied: two round openings, diameter ca. 1–1.5 cm, located ca. 5–10 cm apart, converge into a single tunnel ca. 10 cm below the sediment surface. The angle of this tunnel is first steep, but then becomes gradually more horizontal while its diameter expands to reach a maximum of over 5 cm at ca. 30 cm sediment depth, and ca. 30 cm away horizontally from the openings (Fig. 1C).

In addition to a pair of the shrimp *Axiopsis serratifrons* (Dworschak 2004), several more macrofaunal species were found in the examined burrows (Fig 1C). The most conspicuous burrow associate, found attached to the roof of all burrows individually or, in one case, as two individuals attached to each other, was a new tunicate species which is described below. A total of four individuals of the small shrimp *Rostronia stylirostris* Holthuis, 1952 (Decapoda: Palaemonidae) were found inside the tunicates (in one case in the branchial sac). Each burrow also harbored multiple specimens of sabellid, serpulid and spirorbid polychaetes. Some burrows contained one or two individuals of the bivalves *Booneostrea subucula* Jousseaume in Lamy, 1925 (Ostreoida: Ostreidae), a juvenile of *Barbatia amygdalustustum* Röding, 1798 and adults of *Calloarca tenella* Reeve, 1844 (Arcoida: Arcidae), and two individuals of *Corbula* (Anisocorbula) *taitensis* Lamarck, 1818 (Myoida: Corbulidae). The gastropod *Cheilea equestris* Linnaeus, 1758 (Littorinimorpha: Hippocinidae) and the polyplacophoran *Leptochiton* sp.
(Lepidopleurida: Leptochitonidae) were also found. Small colonies of the sponge *Tethya* sp. (Hadromerida: Tethyidae) were attached to the burrow walls as well.

**Taxonomy**

*Ascidia subterranea* sp. nov.

**Etymology.** The species name refers to the unusual habitat below the sediment surface, in burrows excavated by the shrimp *Axiopsis serratifrons*.

**Type locality.** Derawan Island, off East Kalimantan in the Sulawesi Sea, Indonesia, 2°17'12''N, 118°14'53''E.

**Syntypes.** Six specimens (1–6 as listed below):

- Naturkundemuseum, Berlin:
  - ZMB Tun 4015 (Specimen 1): Animal used for SEM preparations by T.S., remains: complete tunic with most of the coral gravel removed, part of oral siphon, part of branchial basket, larger piece of intestine with small part of branchial basket, small part of intestine, body parts not stained, gonads present. SEM stubs: 1. part of the dorsal lamina from the posterior third with a larger area of the right and a smaller area of the left branchial basket; 2. Oral tentacles and dorsal tubercle; 3. Oral tentacle and anteriormost part of endostyle; 4. Endostyle with a larger area of the left and a smaller area of the right branchial basket.
  - ZMB Tun 4016 (Specimen 2): Animal in its tunic, in very poor condition, not stained, gonads present.
  - ZMB Tun 4017: traces of empty tunic belonging to Specimen 2, encrusting coral gravel preserved.
  - ZMB Tun 4018 (Specimen 4): Animal used for SEM preparations by T.S., remains: tunic, body without dorsal part of the oral siphon and anterior part of the ventral branchial basket, not stained. SEM stubs: 5. oral tentacle, dorsal tubercle, and part of anterior branchial basket; 6. part of the dorsal lamina from the anterior third with a larger area of the left and a smaller area of the right branchial basket.

- Muséum National d'Histoire Naturelle, Paris:
  - MNHN P5 ASC.A 416/1 (Specimen 3): Animal in its tunic, in very poor condition, not stained, beginning of oral siphon and tentacles preserved.
  - MNHN P5 ASC.A 416/2 (Specimen 5): Tunic removed, posterior part of the body in good condition, not stained, tentacle area missing, internal part of the torn oral siphon stained in search of muscles, dissected by F.M., parts mounted on a slide for light microscopy.
  - MNHN P5 ASC.A 416/3 (Specimen 6): Tunic removed, body in good condition but opened along the body side length, stained, part of the branchial sac mounted on a slide for light microscopy by F.M., oral siphon missing.
  - MNHN P5 ASC.A 416/4: empty tunic belonging to Specimen 6, encrusting coral gravel preserved.

**Description.** Six specimens of a phlebobranch ascidian have been extracted from burrows of *Axiopsis serratifrons*. The ascidian specimens are more or less damaged, as they are soft and almost entirely covered with large coral debris, shells and diverse coarse particles. The tunic was strongly adhering to the wall of the shrimp tunnel (expect, possibly, for the part around the distal end of the oral siphon which was always missing). All individuals have the same elongate shape. The mineral coating is deeply incrusted into the tunic, which is irregular in thickness, cartilaginous or paper-like in few parts. When the tunic is removed, the general body shape is oval about 4.5 cm long prolonged by a tubular oral siphon at least as long as the body itself, but in all specimens torn at its extremity. In few places, where the tunic is free from included gravel or shells the tunic surface wears soft spiny papillae 0.6 to 0.8 mm long (Fig. 1B). The oral siphon is a long thin walled tube about 8 mm in diameter. It extends far above the circle of simple oral tentacles, and its rim is always missing, either torn in the process of excavating the burrows during sampling, or perhaps cut off by the shrimp. The atrial siphon, sessile in life, is shortly protruding from the dorsal side of the body when it is removed from the tunic (Fig. 2), at two thirds of the body length. Depending on contraction, the rim of the atrial siphon is smooth or undulating in 6 low lobes. On the body the mantle is extremely thin and transparent except at the muscular belt. The siphon sphincters are weak. The oral one is limited to the tentacle area. Anterior to the tentacle ring, its wall is completely devoid of the longitudinal muscles which are always present at the siphons of all other species in the genus *Ascidia*, a clearly unique,
FIGURE 1. A Opening of the burrow of Axiopsis serratifrons, with anterior end of the shrimp visible, surrounded by the narrow leafed morph of Halodule uninervis. B Tunic papillae (tpa) of Ascidia subterranea sp. nov. (MNHN P5 ASC.A 416/2). C Schematic representation of the habitat of Ascidia subterranea sp. nov., showing the position of the living tunicate on the burrow roof. Also shown are the pair of shrimps, and associated bivalves, gastropods and polychaetes. Three alternative (all of them speculative) positions for the siphon are depicted.
FIGURE 2. A, B Ascidia subterranea sp. nov. (MNHN P5 ASC.A 416/2), tunic removed seen from the left (A) and right (B) side respectively. C, D Corresponding drawings to the photographs in A and B. an—anus, as—atrial siphon, gd—gonadal ducts, ht(?)—enlarged blood vessel, probably the heart, in—in—testine, mb—muscle belt, ov—ovary, re—rectum, sto—stomach
distinguishing and very surprising character. The atrial sphincter is limited to the short tube of the siphon with thin fibres; it does not spread on the body sides. The left body side distended by the gut is totally devoid of muscular fibres (Fig. 2). On the right side the body musculature forms a belt made of a narrow ribbon of transverse thick and short fibres encircling the body outline (Fig. 2). Its course follows the ventral outline, curving at the end of the body to reach the dorsal midline. It is only interrupted at the aperture of the atrial siphon. Both extremities of this ribbon terminate at the level of the oral tentacles at the narrow oral sphincter. The oral tentacles, of which there are approximately one hundred in three orders of sizes, are simple, long, very close and pressed to each other, and planted on a thick rod (Fig. 3A, B). The peripharyngeal groove lies between two low ridges indented in a deep V. The dorsal tubercle opens in a simple U-shaped slit or a U with horns slightly rolled (Fig. 3A). The neural ganglion is located immediately behind the dorsal tubercle. The dorsal lamina is doubled above the neural ganglion only. It has a smooth rim at its beginning but it is fringed by triangular languets more posteriorly (Fig. 3C); it is sustained on its left side by strong ribs (Fig. 3C); the transverse vessels form similar ribs along the unperforated band of branchial tissue located on the right side of the dorsal lamina. The oesophagus entrance opens clearly before the end of the branchial sac and is circled by the dorsal lamina on its left (Fig. 3E). There is no accessory opening of the branchial sac. The branchial tissue is made of regular square meshes slightly pleated. The longitudinal vessels are thin and low (Fig. 3). At the crossing of the longitudinal and transverse vessels the branchial papillae are particularly short, in buttons, often two-lobed. There are four to eight longitudinal stigmata per mesh. The branchial sac extends far below the level of the gut down to the body end (Fig. 2). The gut occupies a large part of the left body side. The stomach is narrow, extends horizontally, and is not well delimited, prolonged in a vertical intestine, which curves in a narrow loop before entering a very large distended rectum of a brown colour (Fig. 2). This part of the gut is the only pigmented tissue of the entire body. The anus, widely open, has a smooth rim close to the siphon aperture. The top of the gut loop is anterior to the anus level. The gonads, when present, have the ordinary shape of the genus, with the ovary located inside the primary gut loop and the testis lobes scattered on the internal side of the gut. The genital ducts follow the rectum and end at the anus level. In one specimen a large vessel, probably the heart, was obvious in the thicker portion of the left body wall, posterior and parallel to the stomach.

Remarks. *Ascidia subterranea sp. nov.* belongs to the *Ascidia sydneiensis* Stimpson, 1855 species group. All of them are characterized by an elongate and laterally flattened body, a convoluted dorsal tubercle, an inflated rectum and a body musculature essentially consisting of a belt of transverse fibres running along the outline of the right side. Since *A. sydneiensis* is variable, very common and present world-wide a large synonymy has been accumulated and discussed by numerous authors (Kott 1985, Monniot C. 1987, Monniot C. & F. 1987, Nishikawa 1991); it certainly represents several species.

The distinctive characters of *Ascidia subterranea sp. nov.* are the long oral siphon in a tube devoid of muscles, a dorsal tubercle which is simple or c-shaped instead of convoluted, a branchial sac extending far below the gut, the anus with a smooth rim and long spiny papillae at the tunic surface scattered between the incrusted material. The ribbon of transverse muscles is more clearly limited and considerably narrower compared to all other specimens of *Ascidia sydneiensis* described so far. A similar design of the right body side musculature is also present in the species *Ascidia munda* Sluiter, 1898 but the gut shape is different, the dorsal tubercle is convoluted and the longitudinal muscles are in bands on both long siphons as in *A. sydneiensis*. The habitat inside the burrow of an axiid shrimp is very uncommon for an ascidian and allied to the peculiarity of morphological characters justify to create a new species.

Discussion

Unlike other axiidan shrimp which construct a burrow lining out of fine sediment particles and sticky mucus secreted from appendages (Dworschak 1998), *Axiopsis serratifrons* stabilizes its burrow walls and roofs by moving pieces of coral rubble (some of them considerably larger than the shrimp itself), placing them in an interlocking pattern and “vibrating” these pieces until firmly positioned (Kensley 1981, Dworschak & Ott 1993, pers. obs. D.K.). Consequently, the walls and roof represent a more or less continuous area of hard substratum (“masonry”). Tunicates have never been reported inhabiting crustacean burrows, the presence of *Ascidia subterranea sp. nov.* in burrows of *Axiopsis serratifrons* represents an new habitat for ascidians. All tunicates were found attached to the roof of the burrows with their tunica about half way between the entrance and the deepest part, and their long oral siphon extending along the roof of the burrows towards the entrance. Unfortunately the distal end of all oral...
siphons was missing. That could have either happened during the process of digging up the burrows, or they might have been bitten off by sea turtles or cut off by the shrimp earlier on. In living animals, the siphon might end inside the burrow just below the opening, where the burrow structure narrows (Fig. 1C/left). There is a close fit between the diameter of the burrow and the diameter of the shrimp at the burrow opening, and the shrimp might perceive any structures in this area as foreign objects and react by either cutting them away or relocating the burrow opening, unless the ascidian retracts when touched. We do not recall seeing siphons in or near the burrow openings but it is possible that they were retracted at that time. If the siphon was tolerated by the shrimp, another possibility is that it permanently ends at the sediment surface in one of the burrow entrances (Fig. 1C/middle), and was somehow overlooked. It might also exit through an individual opening, with a “turnoff” into the sediment below the opening (Fig. 1C/right). An immobile siphon could only be assumed to remain intact throughout the life of the tunicate if it was never “found” by the shrimp at some point (openings might be frequently relocated following disturbance of the uppermost sediment layer) or accidentally bitten off by a turtle.

The relationship between the host shrimp and most of the associates can be assumed to be largely mutualistic. The shrimp provides a stable area of “hard substratum”, and the tunicate, the sabellid and serpulid worms, the sponge *Tethya* sp. as well as the ostreids further promote stability by cementing the building blocks (pieces of coral gravel) of the burrow wall together, thereby reducing the time the shrimp has to spend on burrow maintenance and allowing a better circulation of water in it. Although *Axiopsis serratifrons* had a clear herbivorous signature in a food web analysis (Abed-Navandi & Dworschak 2005), it and the associated shrimp *Rostronia stylirostris* might opportunistically feed on the eggs of *Ascidia subterranea* sp. nov. The shrimp *Rostronia stylirostris* has previously been found inside a “black ascidian” (which was not living in a burrow) in the Red Sea (Fransen 2002). In the present study both male and (ovigerous) female *Rostronia stylirostris* were found. Unfortunately some of the shrimp had fallen out of damaged tunicates so it could not be determined if there was more than one specimen of shrimp per tunicate and if they live in pairs.

The bivalve *Calloarca tenella* was found to be able to climb up vertical walls, just like *Barrimysia cumingii* which had been found in burrows of *Neaxius acanthus* (see Kneer et al. 2008 a). These bivalves probably do not live in or permanently attached to the burrow walls, but might move about freely in the burrow.

A shrimp species closely related to *Axiopsis serratifrons*, the axiid *Callocaris macandreae* Bell, 1853, was assumed to live up to 10 years by Buchanan (1963). According to Bosley & Dumbauld (2011), the more distantly related axiid *Neotrypaea californiensis* Dana, 1854, might live to ten years or more. *Axiopsis serratifrons* may be similarly long-lived, and due to the fact that the species lives in pairs, it probably inhabits the same burrow over its entire post larval life span. While the entrances might be frequently damaged by waves and grazing sea turtles, then having to be repaired and possibly relocated in the process, the lower parts of the burrow structures represent “islands of stability” in an otherwise dynamic sedimentary habitat. Over 70 mollusk species were counted in an extensive survey of the seagrass macrobenthos of Derawan Island (Kneer unpublished data), but none of the species found inside the burrow were found anywhere else in the sediment. It is therefore concluded that the activity of the shrimp promotes species diversity within the seagrass meadows by providing a habitat for a variety of animals which are more typical of hard substrates. The burrow habitat may be attractive because competition for space, exposure (to e.g. waves) and predation are considerably less severe compared to the coral reef or other alternative hard bottom habitats typical of a tropical reef island.

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