**Pyura** (Tunicata: Asciidiacea: Pyuridae) on the coasts of Panama

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**Abstract**

Recent efforts have been taken to survey and describe the ascidian fauna of the Pacific and Atlantic coasts of Panama; however, the genus Pyura still remains poorly known. Sampling events have been occurring since 2003. In this research, we describe two new species from the Atlantic coast of Panama: *P. longispina* sp. nov., *P. lopezlegentilae* sp. nov., and two new species from the Pacific Coast of Panama: *P. carmanae* sp. nov., and *P. imesa* sp. nov. We also supply a tabular key for all the Pyura species found in Panama.

**Key words:** ascidian taxonomy, biodiversity, Stolidobranchia, Central America

**Introduction**

*Pyura* is currently known by 100 species (Shenkar et al. 2018) from polar to tropical waters and from shallow coasts to the deep sea. In the Caribbean Sea there are only seven known species: *P. antillarum* Van Name, 1921, *P. discrepans* (Sluiter, 1898), *P. munita* (Van Name, 1902), *P. ocellata* Monniot, 2016, *P. torpida* (Sluiter, 1898), *P. vannamei* Monniot C., 1994 and *P. vittata* (Stimpson, 1852) (Rocha et al. 2005), while on the Pacific side of Central America we can find *P. lignosa* Michaelsen, 1908, *P. cf. vannamei*: Monniot 1994, *Pyura* sp. aff. *vittata*: Tokioka 1972.

The first survey of ascidians in Bocas del Toro region revealed three undescribed species (Rocha et al. 2005), of which *Pyura beta* has been recently described (Skinner et al. 2019) and the other two will be described in this study together with two new species from the Pacific side of Panama. Among the list of Caribbean species, only *P. vannamei* and *P. vittata* were also found on the Atlantic side and *P. lignosa* on the Pacific side of Panama.

**Materials and methods**

Specimens were collected in many occasions between 2003–2014 from artificial and natural substrates in Bocas del Toro, in the Atlantic side of Panama. Most of the specimens were collected by snorkeling in very shallow water. In the Pacific side the tide has a wide range and most species occur deeper than 5 m in low tide, thus SCUBA diving was necessary to collect. Specimens from the Pacific coast of Panama were collected in January 2009, during a week-long survey.

Specimens were relaxed in menthol and preserved in 4% formalin following routine procedures. Holotypes were deposited in the Zoology Museum of Universidade de São Paulo (MZUSP) and paratypes were deposited in the Asciidiacea collection of Universidade Federal do Paraná (DZUP).

A tabular key of the *Pyura* spp. in Panama is supplied after the descriptions (Table 1)
Taxonomy

**Pyura carmanae sp. nov.**
(Figures 1, 2)
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**Materials examined:** Holotype: MZUSP553 one individual, Isla Canales de Tierra, Shark Point, 7º44'18"N 81º34'46.8"W, leg. R. M. Rocha, 10.01.2009.
Paratype: DZUP PYU-127 seven individuals, Isla Canales, Shark Point, 7º44'18"N 81º34'46.8"W, leg. R. M. Rocha, 10.01.2009.

**Etymology.** The name of the species is a homage to Mary Carman from Woods Hole Oceanographic Institution who coordinated the expedition that found the species and for her important work concerning the study and management of invasive ascidians.

**Description.** Living specimens are yellow and wrinkled (Fig. 1A, D); often found under rocks attached by the posterior region of the left side. The animals are usually 3–4 cm long but can reach 5.5 cm by 2 cm and are very flat (the holotype 3.5 cm long and 2.5 cm in diameter). The siphons are bumpy, apical and can be close together with little space separating the two or a little separated with the atrial siphon more lateral. The oral siphon is typically longer than the atrial siphon, but length varies among individuals (Fig. 1D), and none of the siphons have spinules on the border or on their internal lining. After fixation, animals still retain the yellow coloration, but the tunic is white inside and presents many bumps and horizontal wrinkles; notably in the anterior half of the animal. The rough tunic is relatively thick (1–3 mm), and can show a tessellate pattern in the posterior half (Fig. 1E). The tunic is usually clean of encrustations, but some bryozoans and entoprocts sparsely cover the surface.

The body wall coloration varies. In fresh specimens, red coloration can be seen on the siphons. After fixation, it is often white or a faint yellow. The musculature of the siphons is strong. Circular muscles are heavily dense around the siphons, making them contracted. Longitudinal fibers placed tightly together radiate from the siphons down the body resembling a continuous sheet of muscles, however, the U-shaped right gonad is often seen through the body wall (Fig. 1B, C).

There are 22 laterally flattened oral tentacles in the holotype on a ring of strong circular muscles embedded in the body wall, but the number ranges from 20 to 30 among samples (Fig. 2C). Tentacles are in one or two size orders but some specimens the tentacles mainly show one size, with a few smaller in between. Tentacles are thin, have a larger base that tapers off towards the end, and mostly have second order ramifications only; however, third order ramifications were viewed in some samples. The prepharyngeal groove is made up of two equal size lamellae, very close to the line of tentacles, forming a shallow V around the dorsal tubercle, which can be completely hidden under the tentacles. The aperture of the dorsal tubercle is U-shaped. The dorsal lamina does not begin immediately after the dorsal tubercle; instead, a small distance separates the two. The dorsal lamina is composed of long languets; 80 in the holotype. The pharynx is attached to the body wall by slender vessels.

The pharynx is uncolored with six folds per side (Fig. 2A). In some cases, the right side folds are higher than the left side, and the ventral folds are reduced. Although high, the folds are separated from each other and do not overlap. The holotype has 231 longitudinal vessels and the formula is (from right to left side):

E 3 (8) 2 (13) 2 (16) 3 (19) 2 (20) 4 (21) 4 DL 1 (24) 3 (18) 0 (20) 3 (16) 3 (10) 8 (7) 6 E

While another smaller (2.5 cm) exemplar had the following formula (177 vessels in total):

E 3 (6) 4 (9) 4 (13) 3 (12) 3 (11) 3 (19) 5 DL 1 (16) 2 (14) 3 (15) 3 (14) 3 (10) 2 (6) 3 E

Parastigmatic vessels are present in many regions of the pharynx. There are four to six stigmata per mesh in between folds, and four or five on folds (Fig. 2F).

Dense, irregular shaped endocarps are present on both sides of the animal. Those on the right are attached to the body wall dorsal and anterior to the right gonad, but absent between the right gonad and endostyle. Those on
the left are attached to the body wall dorsal and anterior to the gut loop and there are also a few endocarps inside the primary intestinal loop (Fig. 2B, E). Endocarps usually not present on the gut although in some specimens one or two small endocarps are on the descending intestinal wall. Endocarps are also present on the most anterior gonadal lobes. On several specimens, a few endocarps anteriorly placed looked to be storing an unknown substance.

The primary intestinal loop is large, with the ascending intestine running along the endostyle, but does not extending up to the prepharyngeal groove, although it surpass the level of the anus; the descending intestine is short and forms a shallow secondary loop with the rectum extending all the way inside the atrial siphon in specimens with siphons closer to each other (Fig. 2B). In specimens with siphons a little bit more spaced, the primary loop is more narrow and the descending intestine longer, forming a more posterior secondary loop (Fig. 2E). The intestine is isodiametric, transparent, and there can be a constriction before the anus, which has a smooth rim, sometimes enrolled outwards (Fig. 2G). The digestive gland lies along the esophagus and stomach, with many small portions followed by a large and elongated mass of tubular projections, often yellow or green, with one connection with the stomach wall (Fig. 2D).

**FIGURE 1.** *Pyura carmanae* sp. nov. A. External view of holotype. B. Tunic removed, right side view. C. Tunic removed, left side view. D. Two animals with long and separate siphons. E. Surface of tunic showing tessellate pattern. Scale bar = 1 cm.

The left gonad is spread inside the primary intestinal loop, the lobes have tubular connections with the main duct and are separated from each other (Fig. 2B). The left gonad has 18–35 lobes. The lobes vary in size, are irregularly shaped, although tend to be oval. The right gonad is ventral and forms a deep U in the posterior half of the animal, with 28–35 lobes (Fig. 2B). Oviduct and sperm duct are slightly lobed and equally long, opening right at the base of the atrial siphon (Fig. 2G).
Remarks. Tokioka (1972) described 12 individuals of this species from Playas del Coco, Costa Rica as *Pyura aff. vittata* and he argued that character variation in *P. vittata* was not enough known at that time for the separation of that population as a new species. Indeed, the right gonad of *P. carmanae* has the same shape as in *P. vittata*, but the dilated rectum, presence of endocarps on the intestine and gonads, larger number of longitudinal vessels and presence of spinules in the siphons in *P. vittata* distinguishes this species from *P. carmanae*. Further, in Central America, *P. vittata* is only found in the Atlantic coast both on mangrove prop roots, coral reefs and artificial substrates (Rocha et al. 2005; Carman et al. 2011), while *P. carmanae* is only found under rocks in the Pacific coast. Thus, the known geographical distribution of this species extends north at least until Costa Rica. *Pyura discrepans* (Sluiter, 1898) known from Colombia and Martinique is similar to *P. carmanae*. Main differences are the brownish-red tunic, irregular stigmata in the pharynx, presence of abundant endocarps on the descending intestine in the holotype but not in the specimen from Martinique and gonadal lobes not well attached to the body wall in *P. discrepans* (see Sluiter 1898, Monniot 1983, Monniot 2018). *Pyura ocellata* Monniot, 2016 recently described from French Guiana is also similar to *P. carmanae*. Main differences are the smaller size, short siphons, smaller number of stigmata per mesh, larger digestive track, larger number of gonadal lobes in *P. ocellata* (see Monniot, 2016). Among the samples in the Smithsonian Museum collection, USMN 014621 and USMN 014666 collected from Venado Beach, Panama and identified as *P. vittata*, actually belong in this species.

FIGURE 2. *Pyura carmanae* sp. nov. A. Dissected animal showing the pharynx (stained). B. Dissected animal with the pharynx removed, showing the digestive and reproductive systems. C. Anterior region with oral tentacles and dorsal tubercle (stained). D. Detail of the digestive gland. E. Dissected animal with separate siphons showing different intestine shape and the irregular endocarps (stained). F. Detail of the stigmata and parastigmatic vessels (stained pharynx). G. Atrial siphon showing the gonoducts and anus (stained).
**Pyura imesa** sp. nov.

(Figures 3, 4)

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*Not* *Pyura (Halocynthia) lignosa* Michaelsen, 1908: 256.

**Materials examined:** Holotype: MZUSP554 Isla Canales de Tierra, Shark Point, Pacific coast of Panama, 7°44'18"N 81°34'46.8"W, leg. R. M. Rocha, 10.01.2009.

Paratype: DZUP PYU-126 Isla Canales de Tierra, Shark Point, Pacific coast of Panama, 7°44'18"N 81°34'46.8"W, leg. R. M. Rocha, 10.01.2009.

**Etymology.** The name is a homage to the Integrated Molecular Ecology of Sponges and Ascidians lab at The University of North Carolina Wilmington for their contributions to ascidian molecular ecology.

**Description.** Only two specimens of this species were found in the collection. They were found under rocks, attached by their left or posterior region. Living specimens are orange even in preserved samples (Fig. 3A), and pearly white on the inside. The tunic is tough, 1–2 mm thick, and divided into small patches on the surface. The siphons are distant from each other with many tubercles around them, but no spinules or scales on their rim or internal lining. The holotype is 4 cm at its longest length and it is 2 cm high including the tunic, and it is slightly laterally flat. The siphons are both 0.9 cm long without the tunic.

The body wall is cream colored after fixation. Thin circular muscle fibers are densely packed around the siphons. Longitudinal muscles radiate from the siphons and are placed so tightly together in wide bands that they form a continuous sheet, resulting in the body wall being opaque in the anterior half of each side (Fig. 3B, C). There is a large velum in the oral siphon.

The tentacles are at the level of a strong band of circular muscles. There are 16 total in the holotype, the largest are more prevalent, with just a few small tentacles occasionally in-between. The right side of the specimen presents more tentacles than the left side where the tentacles are very spaced (1.0 mm apart from each other). The paratype has 12 oral tentacles. They are short, laterally flattened, with slightly large base. They have long second order ramifications lined along the middle of each side or displaced posteriorly, and minute second order ramifications.

**FIGURE 3.** *Pyura imesa* sp. nov. A. External view of animal. B. Right side of the holotype. C. Left side of the paratype. Scale bar = 1 cm.
A small distance, 0.05 mm, separates the tentacles and the prepharyngeal groove which is made up of two equal size lamellae. The lamellae form a deep V around the distinctive V-shaped dorsal tubercle (Fig. 4B).

The dorsal lamina does not start immediately after the prepharyngeal groove; instead, it starts after a short distance and it is divided in 90 languets (holotype) that are often found facing toward the right side. The length of the languets increases posteriorly and each languet lines up with the transverse vessels of the pharynx. The pharynx presents six folds per side. The vessel formula of the holotype is (from right to left, for a total of 200):

E 3 (10) 3 (14) 2 (15) 2 (16) 3 (13) 3 (16) 3 DL 2 (13) 2 (14) 2 (15) 2 (14) 2 (14) 2 (12) 3 E

Parastigmatic vessels are frequent but not in all meshes and there are usually 5 or 6 stigmata per mesh between folds and 4 or 5 on folds (Fig. 4E).

The intestine is isodiametric with a long narrow primary loop that occupies ¾ of the ventral mid-line of the body and without a secondary loop, ending in a bilabiated smooth anus (Fig. 4A). The atrial siphon base is very large with a wide and waving velum with the two dorsal waves larger than the others, but no filiform projections
There are endocarps on the body wall along outer side of the gut, the ones between the ascending intestine and the endostyle are laminar while the ones close to the descending limb are more dense, fleshy and folded (Fig. 4G). There are no endocarps attached to the body wall inside the intestinal loop. There are also a few endocarps on the body wall lining the dorsal margin of the right gonad, the two most anterior very small, and the two half the way down, larger and folded (Fig. 4A). The paratype have the two small anterior endocarps only. Endocarps are also found on the top of some of the gonadal lobes.

The left gonad lies in the primary intestinal loop, occupying all the space. The gonad lobes are irregularly shaped but tend to be elongated and positioned in pairs along the duct (Fig. 4A, G). The right gonad is ventrally placed close to the endostyle and it is longer than the left gonad. There are 37 lobes on the right side and 30 lobes on the left side in the holotype, and 33 on the right and 24 lobes of the left in the paratype. Male follicles tend to ramify when well developed. The gonoducts are equally short, wide and lobed (Fig. 4C).

Remarks. Tokioka (1972) described three animals from Playas del Coco, Costa Rica, under the name *P. lignosa*, but we believe that those animals belong in *P. imesa*. Although smaller, his exemplars have larger number of oral tentacles and gonadal lobes, and have small spinules around the siphons that we could not find in ours. Otherwise, the tessellated surface of the tunic and presence of tubercles around the siphons, ventral position of the gonads and disposition of endocarps in the body wall agree with our samples. The small animals described by Van Name (1945) under the name *P. lignosa* and collected in Gulf of California also seem to belong to *P. imesa*, extending its distribution to the whole Pacific coast of Central America and Mexico. As noted by Monniot (1994), this species is similar to *P. vannamei*, but we believe that the lack of large endocarps in the right side of the body wall, lack of dilated hind gut and lobes at the margin of the anus, and the geographical distribution justifies naming the Pacific population as a new species. Differences from *P. beta* Skinner, Rocha & Counts, 2019 are the absence of a second intestinal loop, absence of endocarps inside the primary loop, smaller size and, accordingly, smaller number of oral tentacles, longitudinal vessels and gonadal lobes in *P. imesa* (Skinner et al. 2019). *Pyura imesa* lives underneath rocks similarly to *P. carmanae*, however, the color and shape of the animal (distance between siphons), as well as the gonad shape, endocard abundance and position, distinguish those species.
The dorsal lamina is divided in short triangular languets, 101 in total, many of which are not aligned (two or three were seen side by side). The languets are very delicate and break easily when touched.

The pharynx has six folds in each side and they are high but almost do not overlap. The folds on the left side are more curved than the ones on the right side (Fig. 6A). A total of 445 longitudinal vessels are distributed according the following formula (right to left):

\[ E \ 4 \ (18) \ 11 \ (25) \ 12 \ (30) \ 15 \ (34) \ 8 \ (30) \ 10 \ DL \ 20 \ (27) \ 10 \ (33) \ 9 \ (35) \ 10 \ (32) \ 13 \ (28) \ 10 \ (16) \ 5 \ E \]

There is a large amount of short vessels linking the pharynx with the body wall or with other organs, meaning that the atrial cavity is small. Also we confirm the thick tissue and projections described by Monniot (1994) on the atrial side of the pharynx, along the dorsal region (Fig. 6E).

The alimentary canal forms a wide first loop occupying the whole left side, and a short and close secondary loop (Fig. 6B). There are a few large endocarps on the intestinal wall and also on the gonads. The green digestive gland forms one large portion. The anus contains a typhlosole, but the margin is smooth. The gonads are large and occupy most of the space of the atrial cavity in each side. They do not form well individualized lobes.

**Remarks.** Except by small differences in the number of tentacles and longitudinal vessels, this specimen agrees well with the description of *P. lignosa* by Monniot (1994). The individuals from Gulf of California and Nicaragua studied by Van Name (1945) and reported under the name *P. lignosa* do not seem to belong to this species and should be reviewed to have their identity correctly determined.

**Pyura longispina** sp. nov.
(Figures 7, 8)

**Materials Examined:** Holotype: MZUSP555 Mangrove Island, Bocas del Toro, 9°19'53"N 82°15'00"W, leg. R. M. Rocha, 21.12.2008

**Other material:** USNM 1132564 pilings in Bocas del Toro town, 9°20′15″N 82°14′26″W, leg. R. M. Rocha, 06.08.2008; USNM 1132853 pilings in Bocas del Toro town, 9°20′08″N 82°14′32″W, leg. R. M. Rocha, 18.08.2008.

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**Etymology.** The name reflects the strong and long spinules in the siphons also visible in living animals.

**Description.** Living specimens are usually heavily covered by epibionts and can be spotted in shallow waters both on mangrove roots and in coral habitats only because of the colorful wide siphons (Fig. 7A, B). The holotype is 4.0 cm at the longest length with the tunic, and most other samples are about this size. The tunic has a very
irregular and rough surface, and tubercles around the border of the siphons covered by spinules (Fig. 7D). It is 1–2 mm thick in the holotype, but can be 5 mm in some regions of contracted animals. After fixation the tunic presents a gradient of yellow brown while the inner layer is a yellow soft membrane.

Siphons lining present a variation of red and pink that can be seen in living animals when siphons are open. A wide zone of dense spinules pointing outwards occurs in both siphons (Fig. 7C, G, H). When located close to the margin of the siphon, spinules have an oval plate buried in the tissue from which a curved and pointed projection emerges (~85 µm, Fig. 7F), but they are more elongated and without the basal plate when inside the siphon (~140 µm, Fig. 7E). Many individuals had Entoprocta attached to the border of the oral siphon both externally and internally. Posterior to this spiny zone, the internal lining of the siphons show discs of white iridescent material which is not calcareous (Fig. C, H).

**FIGURE 7.** *Pyura longispina* sp. nov. A–B. Animals in the field. C. Lining of the oral siphon with spinules. D. External view of siphons with lobes and spinules in a preserved animal. E. Internal long spinules. F. Short spinules form the border of the siphon. G. Detail of oral siphon lining with spinules. H. Internal lining of the oral siphon. I. Internal lining of the oral siphon with white structures. Scale bars = 50 µm.

Without the tunic the holotype is 2.1 cm at the longest length. The body wall is almost opaque because of the dense musculature. Longitudinal muscle bands radiate from the siphons down the body wall. In some animals those fibers end before the gonadal region making this region transparent through the body wall. There are 24 longitudinal muscle bands on the right side and 25 on the left side in the holotype. Dense circular muscles surround
the siphons extending to their base, and a net of thin muscle fibers running in different directions lye over the longitudinal fibers and cover the whole body wall, but are more dense in the anterior region (Fig. 8A, B, C). There are minute spicule-like structures on the body wall that cannot be dissolved by hydrochloric acid (Fig. 8C). The siphons are 0.5 cm long in the holotype, but can be even longer in other specimens, and maintain the tinge of red even if long preserved.

FIGURE 8. *Pyura longispina* sp. nov. A. Right side of the animal without the tunic. B. Left side of the animal without the tunic. C. Detail of the body wall showing musculature patterns and minute concretions. D. Anterior region (stained). E. Dissected animal showing the pharynx. F. Oral tentacle. G. Detail of the pharynx tissue. H. Rectum with endocarps and anus; left gonad and gonoduct. I. Dissected animal without the pharynx, showing the reproductive and digestive systems. J. Detail of the digestive gland. Scale bar = 1 cm.
Some oral tentacles have a variation of brown and orange in preserved specimens. There are three size orders, with one or two exceptionally long tentacle, and the total number of tentacles is 22 in the holotype, 18–30 total variation. The tentacles are rather thick, show little ramifications. First order ramifications are long and form a line along the middle of the lateral side of each tentacle. Second order ramifications are minute or nonexistent in many tentacles (Fig. 8D, F). The prepharyngeal groove is made up of two equal size membranes that make a V that surround the dorsal tubercle which is round, protruding, with U-opening with horns slightly or very enrolled (Fig. 8D). There is very little space between the tentacle line and the prepharyngeal groove. The dorsal lamina is divided into thin and long languets; 62 in the holotype.

The pharynx has six high folds per side that bends dorsally on themselves or slightly overlap each other in some individuals. The ventral and dorsal folds are smaller (Fig. 8E). There are 348–450 total longitudinal vessels. The longitudinal vessel formula in the holotype and two other samples are (from right to left):

E 6 (23) 6 (26) 6 (26) 7 (28) 6 (30) 3 (28) 4 DL 2 (28) 4 (28) 5 (32) 4 (28) 6 (20) 8 (22) 6 E
E 5 (19) 6 (30) 8 (29) 6 (22) 10 (23) 5 (20) 7 DL 6 (18) 6 (27) 6 (27) 8 (26) 8 (23) 5 (23) 0 E
E 10 (20) 14 (26) 14 (27) 9 (36) 9 (25) 9 (16) 9 DL 9 (23) 7 (27) 8 (30) 9 (26) 9 (29) 13 (19) 6 E

There are many parastigmatic vessels and meshes between folds have 4–6 stigmata (Fig. 8G). Longitudinal vessels on the right side fray posteriorly forming long, thin languets around the esophagus aperture in both sides.

Endocarps are not present on the body wall on either side of the animal, but they are present along the primary intestinal loop, attached directly to the intestine wall of the ascending portion inside the loop (less numerous and flat), but outside the loop along the descending portion (numerous, larger, flat and irregularly lobed). There are also many irregular endocarps on each gonadal lobe.

The gut is large occupying 2/3 or 3/4 of the left side, with an open primary loop that does not extend up to the prepharyngeal groove (Fig. 8I). There is almost no secondary loop and the rectum is either horizontal with a constriction or bent anteriorly before the multilobed anus (Fig. 8H, I). The ascending limb of the intestine is also wrinkled in transverse ridges. The bulky digestive gland has one attachment to the stomach wall, and a small portion of tubules can be seen on the right side of the esophagus. It is formed by green flat tubules in preserved animals (Fig. 8J).

The gonads are very large: the left one occupies all the space inside the intestinal loop and the right one occupy almost all this side (Fig. 8I). They are formed by large and irregularly-shaped hermaphrodite lobes, further divided in other lobes so densely packed that it is not possible to see the gonducts, unless by their distal ends. Some animals have gonads with more regular undivided oval lobes. There are 12–21 main lobes on the right side and 12–16 on the left side. The testis form small white patches scattered over the ovary. The gonducts are tubular, with the sperm duct thinner and with variable length, sometimes slightly shorter than the oviduct, and sometimes slightly longer.

Remarks. This species is similar to *Pyura gangelion* (Savigny, 1816) known from Martinique (Monniot C. 1983, Monniot F. 2018) and Brazil (Skinner et al. 2019) where it has probably been introduced from either the Red Sea, tropical Indian or Pacific ocean (Monniot 1973, Monniot & Monniot 2001, Kott 2004). Main similarities are the presence of strong spines on the siphons, the white structures inside the siphons lining, and the general shape of the alimentary canal. However, *P. longispina* sp. nov. has shorter spinules of different shape, larger right gonad, lobed anus and it does not has the internal velum of the atrial siphon divided into four wide lobes, neither the line of endocarps along the heart which is very characteristic of *P. gangelion*. The heart tube is never close to the right gonad, being more displaced to the left side of the animal close to the ascending limb of the intestine.

*Pyura lopezlegentilae* sp. nov.
(Figures 9–11)

**Material examined:** Holotype: MZUSP556 Crawl Key, Bocas del Toro, 9°14’38”N 82°08’25”W, *leg.* R. M. Rocha, 25.08.2006
Paratypes: DZUP PYU-87, Punta Caracol, Isla Colon, Bocas del Toro, 9°22’37.6”N 82°18’7”W, *leg.* R. Collin, 05.08.2003; DZUP PYU-88, Solarte, Bocas del Toro, 9°17’30”N 82°10’20”W, *leg.* R. M. Rocha,

**FIGURE 9.** *Pyura lopezlegendilae* sp. nov. A–D. Animals in the field. In B and C it is possible to see the yellow flap formed by the atrial siphon vellum.

**Etymology.** The name of the species is in homage to Susanna López-Legentil for her important research contributions on ascidian genetics and systematics.

**Description.** Living specimens inhabit piers, mangroves, and coral reefs. Animals are oval shaped and reach up to 4 cm. Fresh specimens frequently display red and yellow siphons; however, in some samples, the siphons show only red coloration (Fig. 9). A distinctive membrane, resembling a flap and often yellow in color, is viewed through the atrial siphon and is part of the vellum that line that siphon (Fig 9B, C). Epibionts cover the outside of the tunic. After fixation, the tunic displays yellow and brown and is 1–3 mm thick. The inside of the tunic is white.
The holotype is 2.2 cm in height and 3.0 cm wide; brown in color when preserved. The siphons are extremely contracted from fixation. The tunic is rough with bumps densely concentrated around the siphons. Without the tunic, the holotype is 1.3 cm in height and 2.1 cm wide, discounting the siphons. Both the oral and atrial siphons are 4 mm long. The continuation of the tunic inside the siphons form an iridescent vellum, containing many short and parrot-beak-shaped spines (40–50 µm) with a red spot in each (Fig. 10C).

The body wall has no coloration; however, when fresh, the siphons are red. The red coloration dissipates the longer the length of fixation. A small round projection of the body wall is always viewed at the base of the oral siphon, on the ventral margin at the level of the anterior end of the endostyle (Fig. 10A). Dense circular muscles surround the siphons and extend a slight distance onto the body wall. Longitudinal muscles form muscle bands that radiate from the base of the siphons slowly disbanding toward the gonadal and intestinal region. The gonadal and intestinal region is transparent and the large, spherical gonad lobes are easily seen through the body wall (Figs 10A, B, D).

The holotype contains 29 laterally flattened tentacles with three orders of sizes, the largest 3–4 mm long. The number of tentacles ranges from 29 to 40. Several specimens show a higher degree of ramification than others. There is little separation between the tentacles and the prepharyngeal groove; which is made up of two equal sized membranes. The shape of the dorsal tubercle varies among samples. In the holotype, the dorsal tubercle is a thick U-shape situated in a deep V constructed by the prepharyngeal groove. The ends can be coiled. In one sample, the dorsal tubercle was offset to the right of the neural ganglion and significantly smaller than the other samples (Fig 11B, C). The dorsal lamina begins a few millimeters posterior to the V-shaped peritubercle region. Long languets are densely placed down the entire length of the dorsal lamina; 150 in the holotype.

The pharynx is uncolored with six high folds per side. Folds commonly being higher the closer to the dorsal lamina. Overlap of folds occurs; more prevalent on the left side (Fig. 11A). The holotype has a total of 369 longitudinal vessels; number of vessels range from 335 to 369.

FIGURE 10. Pyura lopezlegentilae sp. nov. A. Left side of the animal without the tunic. B. Right side of the animal without the tunic. C. Siphonal spinules. D. Detail of body wall musculature over the left gonad. Scale bars A = 1 cm, C = 50 µm.
The longitudinal vessel formula of the holotype is (from right to left):

E 4 (20) 4 (24) 6 (29) 3 (30) 4 (30) 2 (28) 4 DL 2 (29) 2 (27) 2 (29) 3 (30) 3 (30) 3 (20) 1 E

Longitudinal vessels fray toward the base of the animal, making languets around the esophageal opening. Parastigmatic vessels are present and the number of stigmata range from five to six per mesh.

Endocarps line the descending limb of the intestine; attached partially to the body wall and partially to the intestine. The endocarps are not fleshy, instead, they resemble parallel extensions of the intestine. They are not present in any other region of the body wall, neither on the gonadal lobes.

The alimentary canal occupies the entire left side of the animal (Fig. 11D). The digestive gland is large, occupying a majority of the ascending limb of the intestine. The isodiametric intestine primary loop is close to the prepharyngeal groove. The anus is lobed and lies at the base atrial siphon (Fig. 11F). There is a thick membrane around the atrial siphon that always form four long projections, two of them anterior and two posterior. This membrane appears to have two different layers: an iridescent tissue that lies on a thicker tissue that makes up most of the membrane. The iridescent tissue does not have spinules and it is not present in some samples, likely due to the long length of fixation. In one sample, the flap also contained numerous calcareous spicules in the upper layer. Calcareous spicules in the shape of antlers were also seen on the wall of blood vessels and intestine of some specimens (Fig 11E).

**FIGURE 11.** *Pyura lopezlegentilae* sp. nov. A. Dissected view of the animal with the pharynx. B. Oral tentacles (stained). C. Anterior region with oral tentacles and dorsal tubercle (stained). D. Dissected view of the animal without the pharynx, showing the reproductive and digestive systems. E. Close up view of a blood vessel with antler-like spicules. F. Atrial siphon, anus and gonoduct.

Gonad lobes are large and spherical; decreasing in size posteriorly. The number of lobes ranges from 12 to 22 on the left side. The right side of the animal is almost completely occupied by the large right gonad with 12 to 20 lobes (Fig. 11d). Gonoducts are long and open at the same level of the anus (Fig. 11F).

**Remarks.** In the field, *Pyura lopezlegentilae* can be confused with *P. longispina* due to the same size, round shape and red coloration of the siphons. However, no other *Pyura* species is known to have the conspicuous yellow flap that is visible through the atrial siphon of *P. lopezlegentilae*, and the internal view of the atrial velum show that its margin is indeed large and form long projections. Further, living *P. longispina* usually have a wider oral siphon than *P. lopezlegentilae*. Internally, the structure of the gonads is very different as well as the size and shape of
spinules. *Pyura lopezlegentilae* has also the gut shape, gonad shape and presence of calcareous spicules in common with *P. munita* but *P. munita* is usually smaller (max diameter 3 cm), covered by sand, has fewer longitudinal vessels (216–265) and does not have endocarps (Monniot 1972, 1983). Monniot (1972) mentioned a divided atrial vellum but did not comment anything special about it and the atrial vellum of *P. lopezlegentilae* is a very regular and noticeable structure. We studied two individuals collected in Belize and identified by I. Goodbody as *P. munita* (USNM 1092832 and USNM 17600). They were smaller animals and the vellum covering the siphons were iridescent but when observed under the compound microscope no spinules were found, but instead, we observed a layer of regular structures like small tiles (Fig. 12). Thus we believe that *P. lopezlegentilae* and *P. munita* are two different species.

**FIGURE 12.** *Pyura munita* (Van Name, 1902), USNM 1092832. A. Dissected anterior region showing iridescent vellum (arrows). B. microscopic view of vellum showing tile-like scales.

*Pyura vannamei* Monniot C., 1994
(Figures 13, 14)

*Pyura vannamei* Monniot C., 1994: 47, fig.2; Monniot C., 1983: 1032, fig. 4; Monniot F., 2018: 419, figs 19, 20
not *Pyura lignosa*: Tokioka, 1972: 403, fig. 10, 65–70 (= *P. imesa*)


**Description.** Living specimens are purple colored with epibionts sparsely covering the tunic but after preservation, the tunic turns red (Fig. 13). The tunic is wrinkled. The animals attach on corals by the ventral margin and live in a vertical position with both siphons facing up, 1–3 m deep. The siphons are distant from each other with the oral siphon being slightly larger than the atrial, both lined with tubercles. Animals are commonly 2–3 cm in diameter and can reach up to 11.5 cm at maximum length including the tunic, but most specimens are 6–8 cm long. The thickness of the tunic is 1–3 mm. It has a white, reddish or purple interior. The body wall is uncolored and transparent around the gonadal and intestinal region, but when the material is freshly preserved, a red layer of tunic can be difficult to detach of the body wall. The siphons are red and densely surrounded by circular muscles. Longitudinal muscles organized into broad bands and radiate from the siphons down to the beginning of the gut on the left side and the gonad, on the right side, thus the enlarged intestinal pouch and long gonads are visible through the body wall (Fig. 13B, C).

The lining of the siphons is red, without spinules, but with round white iridescent structures. The number of oral tentacles ranges from 18 to 33 (without counting very small ones), of four sizes. One large (11 cm long)
individual had two very long ventral tentacles (1 cm), ~30 large and medium sizes and ~10 very thin and small tentacles. They are laterally flat, with a very large base. First order branches are long and placed along the posterior margin, with small second order ramifications, and occasionally minute third order branches (Fig. 14A).

**FIGURE 13.** *Pyura vannamei* Monniot C., 1994. A. Preserved animal. B. Right side of the animal with the tunic removed. C. Left side of the animal with the tunic removed. D. Dissected animal showing the pharynx. D. Dissected animal with the pharynx removed, showing the digestive and reproductive systems. Scale bar = 1 cm.

The peritubercle region is a deep V with a long dorsal tubercle with narrow U-shaped aperture (Fig. 14B). The first dorsal folds in each side begin posterior to the peritubercle region and the languets of the dorsal lamina appear posterior to those folds. The languets are thin and long, 55–98. The length of the languets increases posteriorly and each languet lines up with the transverse vessels of the pharynx. The pharynx is white and presents six high folds per side with no fold overlap, the dorsal ones not so high as the others (Fig. 13D). The vessel formula of a 4 cm long individual was (from right to left side—352 total):

\[ E \ 6 \ (24) \ 4 \ (25) \ 5 \ (25) \ 4 \ (28) \ 4 \ (25) \ 4 \ (20) \ 3 \ DL \ 4 \ (22) \ 7 \ (24) \ 4 \ (29) \ 4 \ (28) \ 6 \ (22) \ 6 \ (17) \ 2 \ E. \]

and in an 11 cm long individual was (449 total):

\[ E \ 9 \ (25) \ 9 \ (29) \ 8 \ (29) \ 7 \ (35) \ 11 \ (25) \ 12 \ (25) \ 6 \ DL \ 5 \ (26) \ 10 \ (34) \ 7 \ (30) \ 8 \ (30) \ 10 \ (26) \ 7 \ (24) \ 2 \ E. \]
Longitudinal vessels fray toward the base of the animal, making languets around the esophageal opening. Parastigmatic vessels are present and there are 4–7 stigmata per mesh (Fig. 14F).

Fleshy large endocarps line both ascending and descending intestine, attached both to the body wall and intestine wall, but inside the secondary loop they are attached to the body wall only. The endocarps lining the ascending intestine are smaller and flatter. Very long laminar and irregular endocarps are observed on the top of each lobe on the left gonad but much smaller endocarps are observed on the right gonad. Four or five large fleshy endocarps, orange in recently preserved samples, are on the body wall, closely placed dorsally to the right gonad or much more centered along the middle longitudinal line (Fig. 13E, 14C).

The gut loop occupies the 2/3 of the left side of the body and is entirely attached to the body wall (Fig. 13E). The intestine forms a closed primary loop, followed by an open secondary loop. The intestine is not isodiametric; a large pouch occupies the descending intestine and rectum. The anus is lobed (Fig. 14D). The digestive gland is green or brown and form a main portion with two connections to the stomach and small extensions connect to the long esophagus (Fig 14E).

Both gonads are elongated and formed by a sequence of lined lobes which are not well defined in some sections. Testis follicles are finger-like and ramified, laying on the atrial side of the lobes. The left gonad is sometimes hidden by the large hind intestine and have around 30 lobes. The right gonad is ventral and lies extremely close to the endostyle, but forms a closed curve in the posterior end towards the atrial siphon. There are also around 30 lobes (Fig. 14C). Larger specimens do not have more gonadal lobes than smaller ones. The sperm duct is slightly shorter than the oviduct and open in the base of the atrial siphon (Fig. 14E).

**Remarks.** Externally, this species resembles *Pyura beta* in the field due to coloration and distant siphons. Internally, the most significant difference between *P. beta* and *P. vannamei* is the enlarged intestine present only in *P. vannamei*, but the lobed anus, endocarps attached to the intestine wall and the large fleshy endocarps that lie
dorsally next to the right gonad are also indicative of *P. vannamei*. Our specimens agree well with the description by Monniot (1994), but his Fig. 2A shows a very shallow peritubercle region which is usually very deep and narrow in Panamanian specimens.

**Pyura vittata** (Stimpson, 1852)
(Figures 15–17)

*Pyura vittata*: Van Name, 1945 (part): 321, fig 213 (upper figures); Monniot C., 1983: 1024, fig. 2, and synonymy; Monniot F., 2018: 423, fig 21–23; not Monniot F., 2016: 237, fig. 29 (= *P. beta*).

**FIGURE 15.** *Pyura vittata* (Stimpson 1852). A–D. Animals in the field. Scale bar = 1 cm.

Animals can reach 5.5 cm at the longest length. The tunic is leathery and rough with numerous organisms encrusting the brown or light brown surface (Fig. 15). The tunic is white inside and has a yellowish soft membrane. In the field, the siphons show four small triangular lobes, the oral siphon is usually apical and the atrial more lateral. There are long spines (~160 µm) lining both siphons with a very distinctive shape: narrow with a round enlargement in the middle and at the posterior extremity (Fig. 16C, D). Iridescent spots of blue, green or yellow color caused by the reflection of light by the enlarged areas of the spines are seen against a brown or reddish background (Fig. 15). After long fixation, the tunic turns light brown. Often, a tinge of red can still be seen around the siphons.

The body wall has many longitudinal muscles radiating from the siphons; they form thin bands that cross each other making a musculature net. Circular muscles densely surround both siphons. The U-shaped right gonad and the enlarged secondary loop of the alimentary canal on the left side are visible through the transparent body wall (Fig. 16A, B).

The tentacles project at the level of a strong muscular sphincter, the number ranging between 16–29. They are flat, very wide at the base and ramifying two or three times, with primary ramifications projecting along the posterior margin (Fig. 16E, F). The third order ramifications are minute and only appear in the largest tentacles that can reach 7 mm in length. The peritubercle region forms a deep V with the dorsal tubercle has U- or C-shaped aperture with enrolled ends. The dorsal lamina is divided in numerous thin and long densely packed languets.

The pharynx has six folds per side and is orange when fresh (Fig. 16G), but quickly loses coloration after fixation. The number of longitudinal vessels range from 305 to 410. Longitudinal vessels fray toward the base of the animal, making languets around the esophageal opening. Parastigmatic vessels are present.

Endocarps are present along the intestine, especially along the descending limb (Fig. 17B). Both gonads have large endocarps on each lobe, particularly the distal ones (Fig. 17C).
Identification Key

This tabular key includes all of the *Pyura* spp. that are known from the shallow waters on the Pacific and Atlantic sides of Panama. The table is based on observed and literature characteristics.

1. **Distribution**: A. Atlantic; P. Pacific
2. **Maximum length of specimen including tunic**
3. **Color in living specimen (tunic or siphons)**: B. Brown; Dr. Dark Red; O. Orange; P. Pink; Pu. Purple; R. Red; Y. Yellow; W. Wine color
4. **Color inside of the tunic**: B. Brown; O. Orange; R. Red; W. White; Y. Yellow
5. **Presence of spinules**: P. present; A. absent
6. **Maximum length of spinules (μm)**
7. **Position of the siphons**: C. Close to each other; D. Distant from each other (opposite sides); I. Intermediate distance (atrial siphon in half the distance between oral and posterior region)
8. **Total number of longitudinal vessels**
9. **Number of oral tentacles**
10. **Degree of tentacle ramification**: F. First order; S. Second order; T. Third order
11. **Number of gonad lobes on the right side**
12. **Number of gonad lobes on the left side**
13. **Margin of the anus**: L. Lobed; S. Smooth
14. **Presence of endocarps**: B. Body wall; G. Gonads; I. Intestine
15. **Peculiar characteristics**: E. numerous endocarps on the body wall; F. Enlarged siphon vellum forming a flap in atrial siphon; I. Enlarged intestinal pouch; T. Extremely thick tunic; V. Ventral right gonad.

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¹ character variation includes information in Monniot (1994), ² character variation includes information in Monniot (1983) and Monniot (2018); ³ character variation includes information in Tokioka (1972)
The alimentary canal occupies 2/3 of the left side. The primary loop does not reach the peripheraryngeal groove, forms a close curve with a vertical descending limb that forms another close second loop with the ascending rectum (Fig. 17A). The intestine is not isodiametric; the secondary loop and rectum are enlarged. The anus is lobed. The digestive gland is dark green and forms lobes connected by long tubes as in a cauliflower. It has two main connections to the stomach. On the left side, the gonad completely fill the space within the primary intestinal loop, the number of gonad lobes ranging from 30–47. The right side of the animal is occupied by a large characteristic U-shaped gonad with 26 to 42 gonadal lobes. The gonoducts are long, the oviduct slightly longer than the sperm duct, both opening at the level of the anus.

**Remarks.** This is one of the most common species both in mangrove and reefs around Bocas del Toro province (Rocha *et al.* 2005) and also found in Colon region but it has not been found on a survey of the Pacific coast (Carman *et al.* 2011). The specimens from Panama agree well with the description of *P. vittata* from Guadeloupe and Martinique (Monniot, C. 1983; Monniot, F. 2018). We believe that *P. vittata* reported by F. Monniot (2016) from French Guiana is actually *P. beta* Skinner, Rocha & Counts, 2019.

**FIGURE 17.** *Pyura vittata* (Stimpson 1852). A. Dissected view, showing the digestive tract (enlarged intestinal pouch) and left gonad. B. Detail of primary intestinal loop with endocarps. E. Detail of the left gonadal lobes with endocarps.

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**References**


