



## A revision of the *Pyura stolonifera* species complex (Tunicata, Ascidiacea), with a description of a new species from Australia

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### Abstract

*Pyura stolonifera* is a large solitary ascidian found in Africa, Australasia and South America. The taxonomic status of different populations of this species is disputed, especially since there is evidence for several distinct morphological and genetic units that point towards the existence of multiple cryptic species. While some researchers still recognize *P. stolonifera* as a single species, others treat the different populations as distinct species. Here, we present a revision of the *P. stolonifera* species complex based on the examination of samples from all regions where there are reliable reports of this taxon. We recognize four species that are both morphologically and genetically distinct, one of which is new to science and is formally described here. This species is morphologically distinct from the other three members of the species complex in terms of the colour and texture of the tunic, the arrangement of the gonads within the gut and the shape of the dorsal tubercle, among other characters. We name the new species *Pyura dalbyi* after Dr. J.E. Dalby Jr., whose research on its ecology and distribution provided the incentive for examining this species more closely.

**Key words:** Pyuridae, *Pyura dalbyi* n. sp., ascidian, taxonomy, *Pyura herdmani*, *Pyura praeputialis*, disjunct distribution, southern Africa, Chile, Morocco

### Introduction

The ascidian genus *Pyura* (Suborder Stolidobranchia, Family Pyuridae) exclusively comprises solitary forms and its species can be found worldwide. One of the largest species in this genus is *Pyura stolonifera* (Heller, 1878), which forms extensive and dense aggregates that can dominate all available substrata from the mid-intertidal to the subtidal zone (Monniot & Bitar 1983; Kott 1985; Dalby 1995; Castilla *et al.* 2000; Rius *et al.* 2010). Like many other widespread ascidian species, *P. stolonifera* has a disjunct distribution that spans several continents, mostly in temperate latitudes. It is abundant in the southern hemisphere, particularly in southern Africa (where it is known as red bait) and Australia (where it is known as cunjevoi) (Kott 1985), but has also been reported in the northern hemisphere in north-west Africa (Monniot & Bitar 1983; Lafargue & Wahl 1986–1987). Recent reports from South America (Castilla *et al.* 2000) and New Zealand (Hayward & Morley 2009) suggest that the species has been introduced to these regions. The taxonomic status of the different populations is unresolved. Some researchers recognize only one species (Kott 1985; Dalby 1997a; Kott 2006), while yet others recognize more than one distinct species (Millar 1962; Monniot & Bitar 1983; Monniot *et al.* 2001; Castilla *et al.* 2002).

As *Pyura stolonifera* can be found mostly on land-masses that were part of the former Gondwanan supercontinent that split up during the Mesozoic, it has been suggested that the different populations are Gondwanan relics (Kott 1985, 2006). Notwithstanding the possibility that such an ancient origin would eventually have resulted in speciation of each of these isolated populations, Kott (2006) maintained that all are conspecifics, a claim that has been repeatedly challenged. For example, Monniot & Bitar (1983) recognized two different species (the Australasian *P. praeputialis*, Heller, 1878, and the African *P. stolonifera*) and suggested that the Chilean population had recently been introduced from Australia. Castilla *et al.* (2002) confirmed this on the basis of mitochondrial DNA

sequence data and recommended referring only to the southern African population as *P. stolonifera* and using *P. praeputialis* for the Australian and South American populations. Kott (2006) rejected both the idea that the Chilean population is a recent introduction and the claim that it is restricted to a single bay (Antofagasta, Castilla *et al.* 2000) on the basis of specimens having been collected elsewhere in South America during the 19<sup>th</sup> century. The record on which this assessment was based (Van Name 1945) actually included a description of a different species, namely *P. bradleyi* (Van Name 1931), and no mention of *P. stolonifera* could be found in this extensive monograph of American ascidians. As regular shipping traffic between Australia and South America has occurred since the 19<sup>th</sup> century, with Pacific trade intensifying from 1840 onwards (Bach 1976), the introduction of *P. praeputialis* to Chile is not unlikely. The African populations of *P. stolonifera* are often considered to be a single species (e.g. Branch *et al.* 2002), although it is now well established that this region is inhabited by two well-differentiated species (Monniot *et al.* 2001), namely *P. stolonifera* and *P. herdmani* (Drasche, 1884). Additional evidence for the existence of multiple species within what Kott (1985) considered to be *P. stolonifera* was reported by Dalby (1997a). In this study, two morphs were identified in south-east Australia (photographs of both are shown in Kott 1985, p. 420), one of which has a yellow test and was found exclusively in bays, and the other a brown test and a preference for settling on the open coast. It was also found that these morphs are reproductively isolated and that their breeding seasons differ (Dalby 1997b), that some of their endosymbionts are morph-specific (Dalby 1996) and that they have parapatric distributions (Dalby 1997a).

Overall, the existence of several distinct morphological and genetic units (Monniot & Bitar 1983; Dalby 1997b; Monniot *et al.* 2001; Castilla *et al.* 2002), as well as high levels of intraspecific morphological variation (Kott 2006), support the idea of cryptic speciation within what has traditionally been referred to as *P. stolonifera*. Hereafter, we use the term '*P. stolonifera* species complex' to refer to the group of morphologically similar species that includes *P. stolonifera*, *P. herdmani* and *P. praeputialis*.

We review the taxonomy of the *P. stolonifera* species complex by examining samples from most regions inhabited by its species and describing morphological variability among and within regions. Additionally, we describe the morphology and distribution of a new species of *Pyura* from Australia that has traditionally been referred to as *P. stolonifera*, despite strong evidence for its distinctiveness (Dalby 1997a,b). Finally, we provide an identification key for the species associated with the *P. stolonifera* species complex.

## Material and methods

We collected samples from all regions where species of the *P. stolonifera* species complex have been reported (Table 1, Fig. 1), except New Zealand and Tahiti. All specimens were preserved in 4% formalin solution upon collection, and taxonomic identification was done by removing the tunic, dissecting the body of the animal, and identifying the morphological characters. When necessary, specimens were stained with Nile blue sulphate. All specimens are deposited in the Iziko South African Museum, Cape Town.

## Revision of the group

### External and internal characters shared across all studied species of the *P. stolonifera* species complex

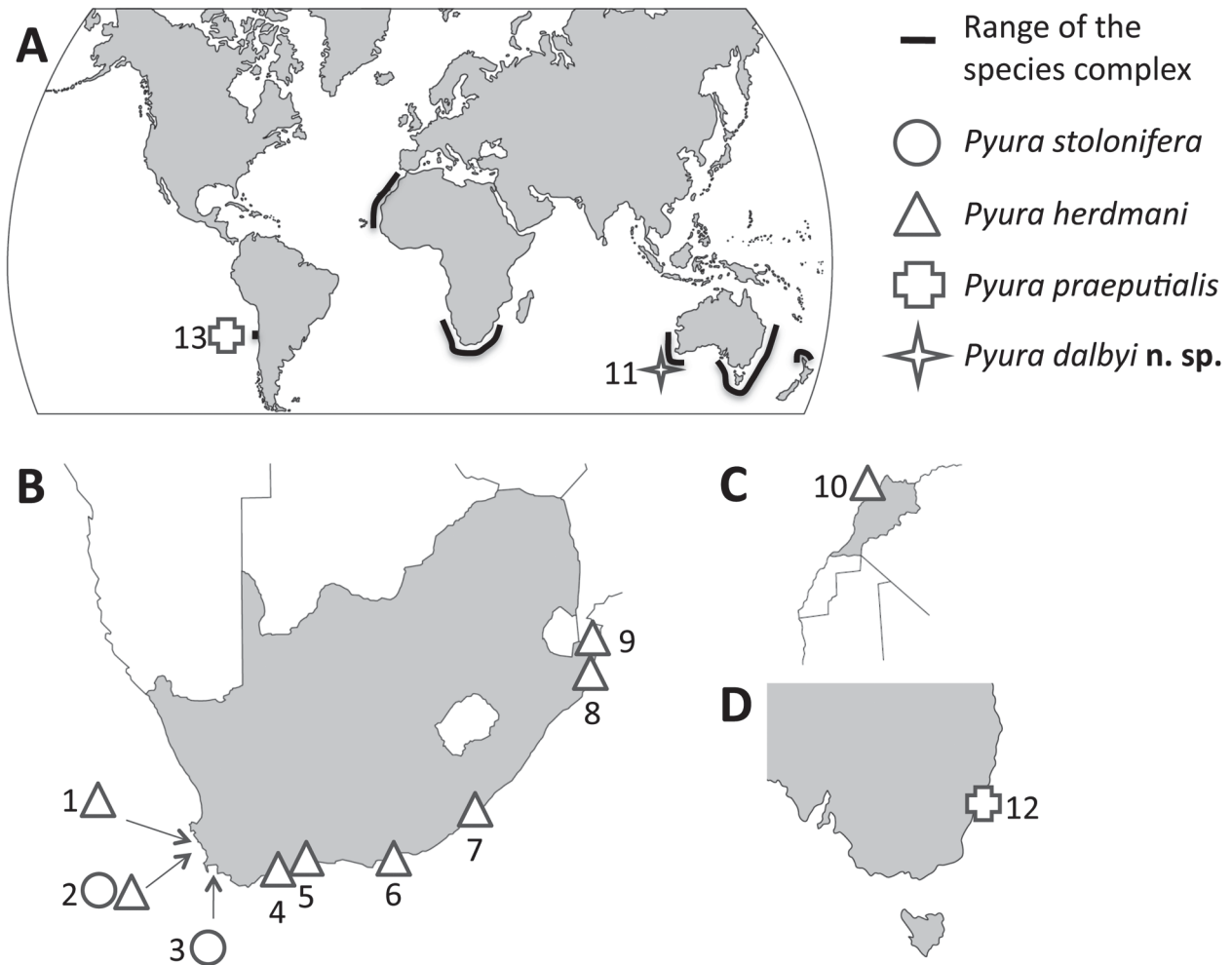
**External appearance.** Most species can grow to a large size, with the maximum length (when non-contracted) normally ranging between 100–150 mm, although South African and Australian individuals can reach 300–400 mm (Kott 1985; Branch *et al.* 2002). The tunic is generally thick and tough, both siphons are large, project from the rest of the body and are in close proximity to each other. The siphonal spines are generally cone-like and pointed.

**Internal appearance.** The body wall is soft and fleshy with marked muscular bands. The branchial tentacles are highly ramified (ramification complexity is always of third order branches), and the branchial sac generally has six folds on each side of the body. The dorsal lamina, when present, is normally short with languets. The gonads are block-shaped and attached to the body wall. The gut forms a loop around the left gonad. Endocarps can be present either on top of the gonads and gut, or between these organs. A large branched hepatic gland covers the stomach.

**TABLE 1.** Specimens of the *Pyura stolonifera* species complex examined in the present study. Sampling locations and their characteristics, collection date, morphological characters examined, species name and museum reference number are indicated.

Country / Sampling locality (No. in Fig. 1)	Geographic location (Latitude / Longitude)	Habitat	Collection date (DD/MM/YYYY)	Tunic colour	Tunic texture	Pointed papillae on the tunic	Body wall colour	Dorsal tubercle	Dorsal lamina	Number of rows on the right gonad	Species name	Iziko museum reference number
<b>South Africa</b>												
Langebaan (1)	33°01'07"S, 17°56'48"E	Subtidal, sheltered	21/09/2009	Pale brown and violet	Rough with epibionts	Yes	Yellow	Sponge- like	Short with long languets	Single	<i>P. herdmani</i>	SAM-A25994
Yzerfontein (2)	33°20'49"S, 18°09'06"E	Intertidal, exposed	31/08/2007 & 21/09/2009	Dark green	Smooth with few epibionts	No	Orange	Double spiral	Absent	Single	<i>P. stolonifera</i>	SAM-A25995
St. James, False Bay (3)	34°07'14"S, 18°27'31"E	Intertidal, exposed	21/09/2009	Brown	Rough with epibionts	Yes	Yellow	Sponge- like	Short with long languets	Single	<i>P. herdmani</i>	SAM-A25996
Mossel Bay (4)	34°10'42"S, 22°08'41"E	Subtidal, sheltered	19/09/2009	Dark green	Smooth with few epibionts	No	Orange	Double spiral	Absent	Single	<i>P. stolonifera</i>	SAM-A25997
Knysna (5)	34°03'17"S, 23°03'46"E	Subtidal, sheltered	15/09/2009	Brown	Rough with epibionts	Yes	Yellow- orange	Sponge- like	Medium with long languets	Single	<i>P. herdmani</i>	SAM-A25998
Port Elizabeth (6)	33°57'59"S, 25°38'04"E	Subtidal, sheltered	14/09/2009	Bright orange	Rough with epibionts	Yes	Yellow- orange	Sponge- like	Short with long languets	Single	<i>P. herdmani</i>	SAM-A25999
			05/07/2007	Brown	Rough with epibionts	Yes	Yellow- orange	Sponge- like	Short with long languets	Single	<i>P. herdmani</i>	SAM-A26000

Country / Sampling locality (No. in Fig. 1)	Geographic location (Latitude / Longitude)	Habitat	Collection date (DD/MM/YYYY)	Tunic colour	Tunic texture	Pointed papillae on the tunic	Body wall colour	Dorsal tubercle	Dorsal lamina	Number of rows on the right gonad	Species name	Iziko museum reference number
Morgan Bay (7)	32°42'39"S, 28°20'27"E	Intertidal, exposed	10/04/2009	Brown	Rough with epibionts & sand	Yes, but small	Yellow-orange	Sponge-like	Short with long languets	Single	<i>P. herdmani</i>	SAM-A26001
Mission Rocks (8)	28°15'41"S, 32°29'47"E	Intertidal, exposed	20/07/2009	Brown	Rough with epibionts	Yes, but small	Yellow	Sponge-like	Absent	Single	<i>P. herdmani</i>	SAM-A26002
<b>Mozambique</b>												
Ponta do Ouro (9)	26°50'40"S, 32°53'43"E	Intertidal, sheltered	02/03/2010	Brown	Rough with epibionts	Yes, but small	Yellow-orange	Sponge-like	Absent or short with small languets	Single	<i>P. herdmani</i>	SAM-A26003
<b>Morocco</b>												
La Madrague (10)	30°30'54"N, 9°44'48"W	Subtidal, exposed	22/03/2009	Brown	Rough with epibionts	Yes, but small	Yellow-orange	Sponge-like	Absent	Single	<i>P. herdmani</i>	SAM-A25993
<b>Australia</b>												
Albany harbour, Albany (11)	35°01'57"S, 117°53'25"E	Subtidal, sheltered	18/11/2009	Yellow	Smooth	No	Yellow-orange	Sponge-like but two bulges	Short with small languets	Double	<i>P. dalbyi</i> n.sp.	SAM-A25988 & SAM-A25989
Balmoral Beach, Sydney (12)	33°49'37"S, 151°15'22"E	Intertidal, sheltered	04/03/2008	Brown	Rough with sand grains	No	Orange-red	Double spiral cone	Absent	Double	<i>P. praeputialis</i>	SAM-A25990
<b>Chile</b>												
Antofagasta (13)	23°42'25"S, 70°25'51"W	Intertidal, exposed	10/10/2009	Brown	Rough with epibionts	No	Orange-red	Double spiral cone	Absent	Double	<i>P. praeputialis</i>	SAM-A25992



**FIGURE 1.** Distribution of the *Pyura stolonifera* species complex and localities where specimens were collected. A. Approximate distribution of the species complex; B. Southern African region with South Africa highlighted; C. Northwest coast of Africa with Morocco highlighted; D. South-east Australia and Tasmania. Numbers correspond to collection localities listed in Table 1, and symbols represent species.

### Morphological characters for discerning among species

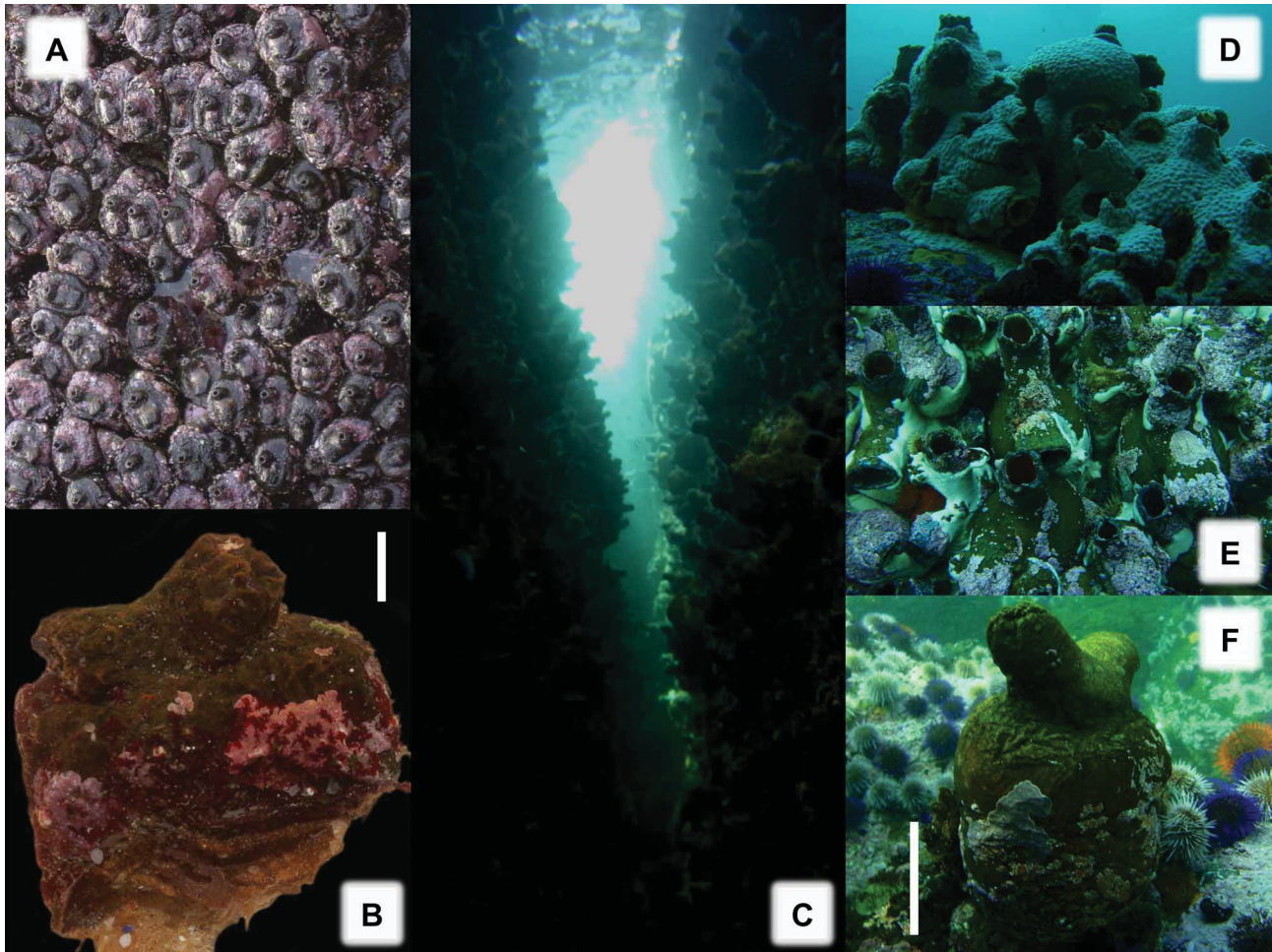
Based on the taxonomic literature (Millar 1955, 1962; Monniot & Bitar 1983; Kott 1985; Monniot *et al.* 2001) there are three species within the *P. stolonifera* species complex that can be distinguished on the basis of three morphological characters (Table 2).

**TABLE 2.** Morphological characters that discern the three taxa traditionally associated with the *Pyura stolonifera* species complex. The taxonomic references where a complete morphological description can be found are included.

Species	Pointed papillae on the tunic	Row of lobes on the right gonad	Dorsal tubercle	Morphological description
<i>Pyura stolonifera</i>	No	Single	Double spiral cone	Monniot <i>et al.</i> 2001
<i>Pyura herdmani</i>	Yes	Single	Sponge-like	Monniot <i>et al.</i> 2001
<i>Pyura praeputialis</i>	No	Double	Double spiral cone	Kott 1985

**Remarks concerning morphology and habitat.** *Pyura stolonifera* can form large aggregates that occupy all available substratum both in intertidal (Figs. 2A, B) and subtidal areas (Figs. 2C, D, E, F). This species can reach a large size (Fig. 2F) and thrives on exposed rocky shores, while it is generally very rare in sheltered environments.



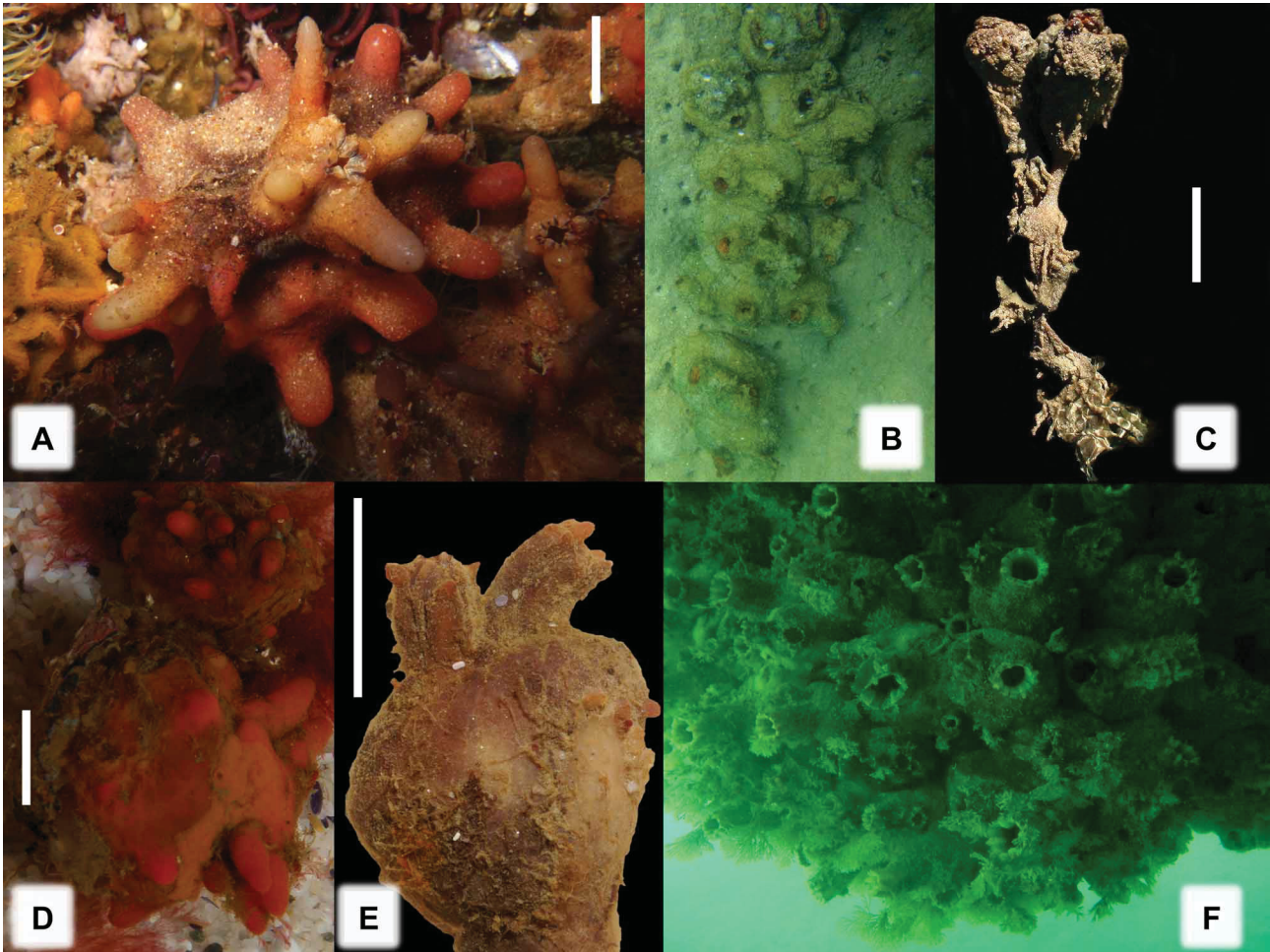


**FIGURE 2.** *Pyura stolonifera* from False Bay, South Africa. A. Large aggregate of *P. stolonifera* in the lower intertidal; B. Individual collected in the lower intertidal; C. Large aggregates covering vertical walls. Photograph: Matthew Melidonis; D. Aggregates of individuals with the tunic covered by colonial ascidian *Didemnum* sp., except around the siphons; E. Detail of a subtidal aggregate on a horizontal surface; F. Solitary individual, 7.4 m depth. Scale bars: B. 10 mm; F. 100 mm.

The dorsal lamina is always absent (Table 1). Although most examined specimens showed six branchial folds on both sides of the body, we found a few individuals with only five folds on the right side of the body.

*Pyura herdmani* is readily identifiable by its pointed papillae of the tunic (Figs. 3A, D). However, the tunic of *P. herdmani* can be highly variable in terms of its morphology (Figs. 3A, C, D, E). This species is found in a wide range of habitats, including intertidal and subtidal areas, rocky and sandy substrata, as well as sheltered (e.g. harbours or marinas, where they may grow on artificial substrata, Fig. 3F) and exposed sites. When growing on sandy bottoms (Fig. 3B), *P. herdmani* can grow a large peduncle to anchor itself into the sand (Fig. 3C). We found considerable variability in terms of tunic colour and texture, size of pointed papillae on the tunic and dorsal lamina across African locations (see Table 1 for variability of these characters).

Compared to other members of the species complex that have squat, rounded bodies, *P. praeputialis* is taller, cylindrical in shape and often has a greater diameter at the top than at the base (Figs. 4B, D). The tunic is thick, often embedded with sand, and the siphons are surrounded by a conspicuous ridge (Fig. 4C). The dorsal lamina is always absent (Table 1), and there are six branchial folds on each side of the body, although we observed seven folds on the left side of the body of a few specimens. There are two different morphs in Australia: Individuals found in central New South Wales and Victoria tend to be larger and taller (height up to 300 mm), whereas those in Tasmania, South Australia and Victoria are smaller and more squat (Kott 1985). The larger morph is common on the open coast and at the entrance of bays, whereas the smaller morph is commonly found in more sheltered sites. *Pyura praeputialis* shows a preference for rocky substrates and often forms dense aggregates (Figs. 4C, E, F), particularly on horizontal surfaces (Figs. 4A, C).



**FIGURE 3.** Morphological variability of *Pyura herdmani* in South Africa. A. Specimen with large pointed papillae on the tunic, wreck of the SS Clan Stuart, False Bay, 6.8 m depth. Photograph: Matthew Melidonis; B. Individuals attached to sandy bottom at 1.5 m depth under a jetty in the West Coast National Park, Langebaan Lagoon; C. Large pedunculated aggregate characteristic of the individuals living on sandy bottoms, Langebaan Lagoon; D. Specimen found on the underside of a jetty in a marina in the Knysna lagoon; E. Specimen collected from the undersides of a jetty in Langebaan Lagoon; and F. Underwater view of a large aggregate underneath a jetty in the West Coast National Park, Langebaan Lagoon. Scale bars: A. 10 mm; C. 50 mm; D. 10 mm; E. 50 mm.

## Description

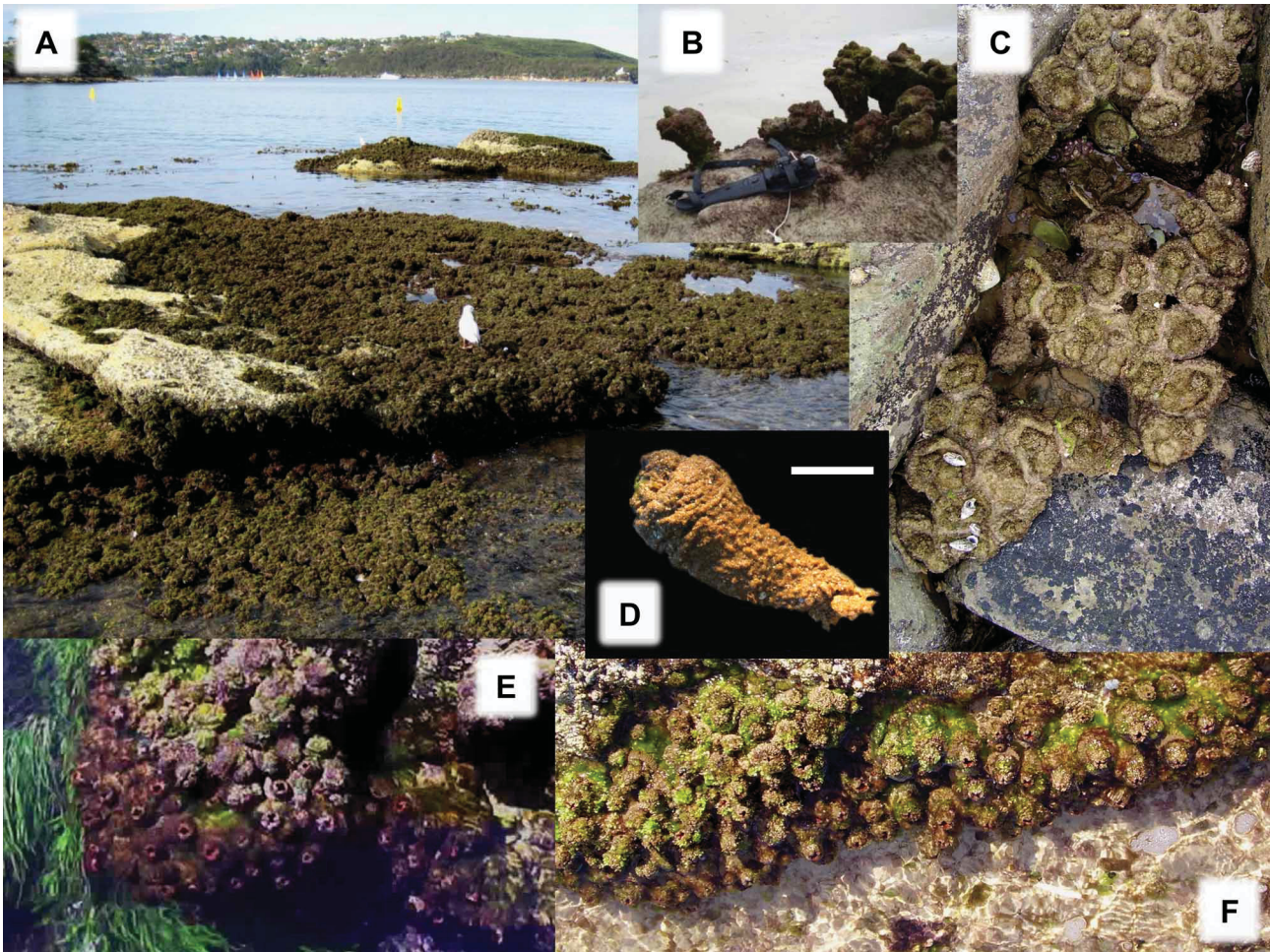
### *Pyura dalbyi* n. sp.

Figs. 5–8

**Material examined.** Specimens were found subtidally under a jetty in Albany harbour, Western Australia (Table 1). Holotype: SAM-A25988, Paratype: SAM-A25989.

**Description.** Individuals are squat with an extended base (Fig. 5A). Their maximum dorsal length ranges from 35 to 95 mm, their height (measured from the base to the top of the siphonal area) from 35 to 60 mm, and their width (measured in contracted individuals) from 25 to 50 mm. The tunic is smooth (no pointed papillae on the tunic) (Figs. 5A, B, C), with few epibionts, and not as thick and tough as that of the African representatives of the *P. stolonifera* species complex. The individuals examined were attached to one another, forming a dense aggregate. Siphonal spines are long and pointed, and their bases are slightly expanded (Figs. 6A, B). The inner half of the siphonal lining is iridescent when observed under a dissecting microscope (Fig. 6C).





**FIGURE 4.** A. A dense mat of *P. praeputialis* covering a rock platform in Balmoral Beach, Sydney; B. Individuals displaying the characteristic upright stance. Photograph: Craig Styan; C. An aggregate in the lower intertidal area at Point Cartwright, Queensland; D. An individual specimen collected at Balmoral Beach, Sydney, showing the elongated shape characteristic of *P. praeputialis* from the Australian east coast; E. and F. Aggregates growing in tidal pools in Newcastle, New South Wales, and Stradbroke Island, Queensland, respectively. Scale bar: D. 20 mm.

The animal has a fleshy body wall that is light orange in colour. The body size ranges in maximum length from 30 to 85 mm and in width from 20 to 45 mm. There are circular muscular bands around the siphons and the anterior longitudinal bands are present across the body wall and do not cross over each other.

There are 17 to 32 ramified oral tentacles of different sizes (alternating between large and small, with more tentacles in larger individuals than in smaller ones) (Figs. 6D, E), and the ramification complexity is of the third order branches. There are no atrial tentacles at the entrance of the exhalant siphon.

The branchial stigmata are straight (Fig. 7A) and between nine and 11 are present per mesh (i.e. between longitudinal vessels in the space between folds). There are six complete branchial folds (Fig. 7B), with around 20 vessels per fold. The branchial formula of two individuals is: R.E. 5 (14) 4 (19) 3 (20) 3 (22) 3 (22) 3 (22) 6 D.L. 7 (22) 4 (25) 3 (25) 3 (22) 3 (20) 3 (16) 8 E.L., and R.E. 11 (20) 3 (25) 4 (26) 4 (27) 4 (30) 4 (37) 7 D.L. 9 (29) 3 (31) 3 (30) 3 (28) 4 (22) 4 (19) 10 E.L. The large sponge-like dorsal tubercle is not arranged as a double spiral cone as in *P. praeputialis* and *P. stolonifera*, but instead is largely spherical with two distinct bulges (Figs. 7C, D). We found a less complex and convoluted dorsal tubercle in smaller individuals than in larger ones (Figs. 7C, D). The dorsal lamina, which is always present, is short and contains small languets (Figs. 7E, F).





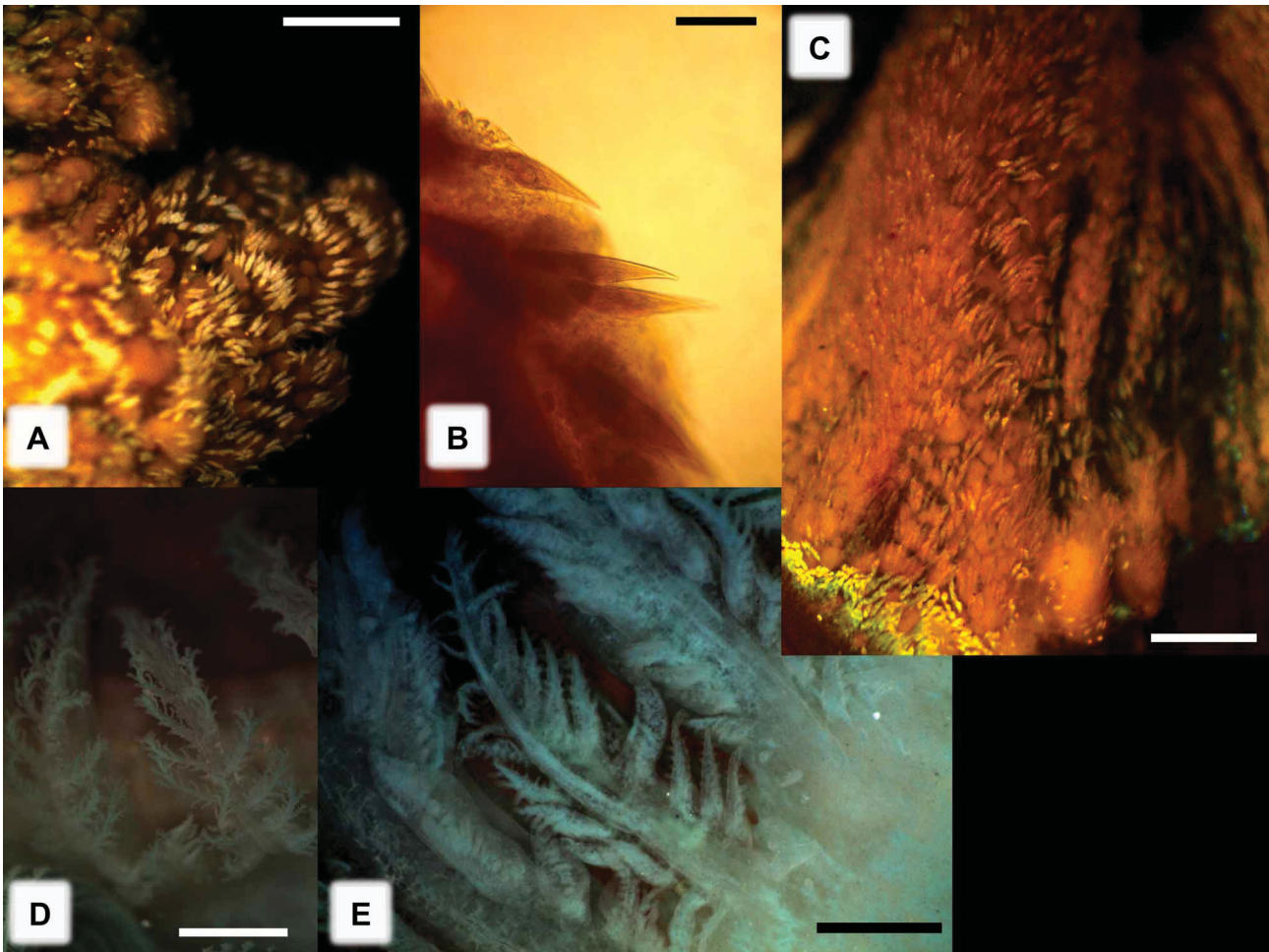
**FIGURE 5.** *Pyura dalbyi* n. sp. A. Lateral view of a preserved specimen; B. Top view of another preserved specimen; C. Aggregated and separated live specimens photographed immediately upon collection. Scale bars: A. 10 mm; B. 10 mm.

The gonads are attached to the body wall and form two rows of lobes on the right side of the body (Figs. 8A, C). The left gonad is inside the gut loop and also forms two rows of lobes (Figs. 8B, C). The gonoduct on the right side of the body is short (extending 3 mm away from the first gonadic block) and is located just below the gonad, whereas the one on the left side of the body is long (opening approximately 10 mm away from the first gonadic block), and it crosses below the gut and opens next to the anus (Fig. 8D). The gut forms a sharply curved loop on the left side of the body, followed by a secondary loop that is not as sharply curved (Figs. 8B, C). The hepatic gland is large and branched (Figs. 8B, C) and contains 3 or 4 hepatic lobes. Endocarps are absent both around and on top of the gonads and the gut. The anal border has 6 to 10 irregular, rounded lobes (Figs. 8D, E).

**Distribution.** This species has been found in several localities in Victoria, on the southeast coast of Australia (Dalby 1997a). It has also been found in Albany, Western Australia (this study). Unlike its South African and Australasian sister taxa, whose distributions extend from the subtidal to the lower intertidal, *P. dalbyi* has been found almost exclusively subtidally (Dalby 1997a).

**Etymology.** The species is named *Pyura dalbyi* after Dr. J. E. Dalby Jr. who reported distributional, morphometric and ecological differences between this species and *P. praeputialis*.

**Remarks.** The yellow and sand-free tunic easily differentiates specimens of *P. dalbyi* from the other species of the *P. stolonifera* species complex. Internally, the shape of the dorsal tubercle is one of the most conspicuous characteristics, as it is quite irregular when compared to the cone-like dorsal tubercle found in *P. praeputialis* and *P. stolonifera*. This is especially evident in smaller individuals of *P. dalbyi*. While the sponge-like shape of the dorsal tubercle in *P. herdmani* can be quite similar to that of *P. dalbyi*, it lacks the two distinct bulges of the latter. The double row of gonadic blocks on the left side of the body inside the gut is a unique character of this species. All African species and *P. praeputialis* have just one row of gonadic blocks surrounded by the gut. The long siphonal



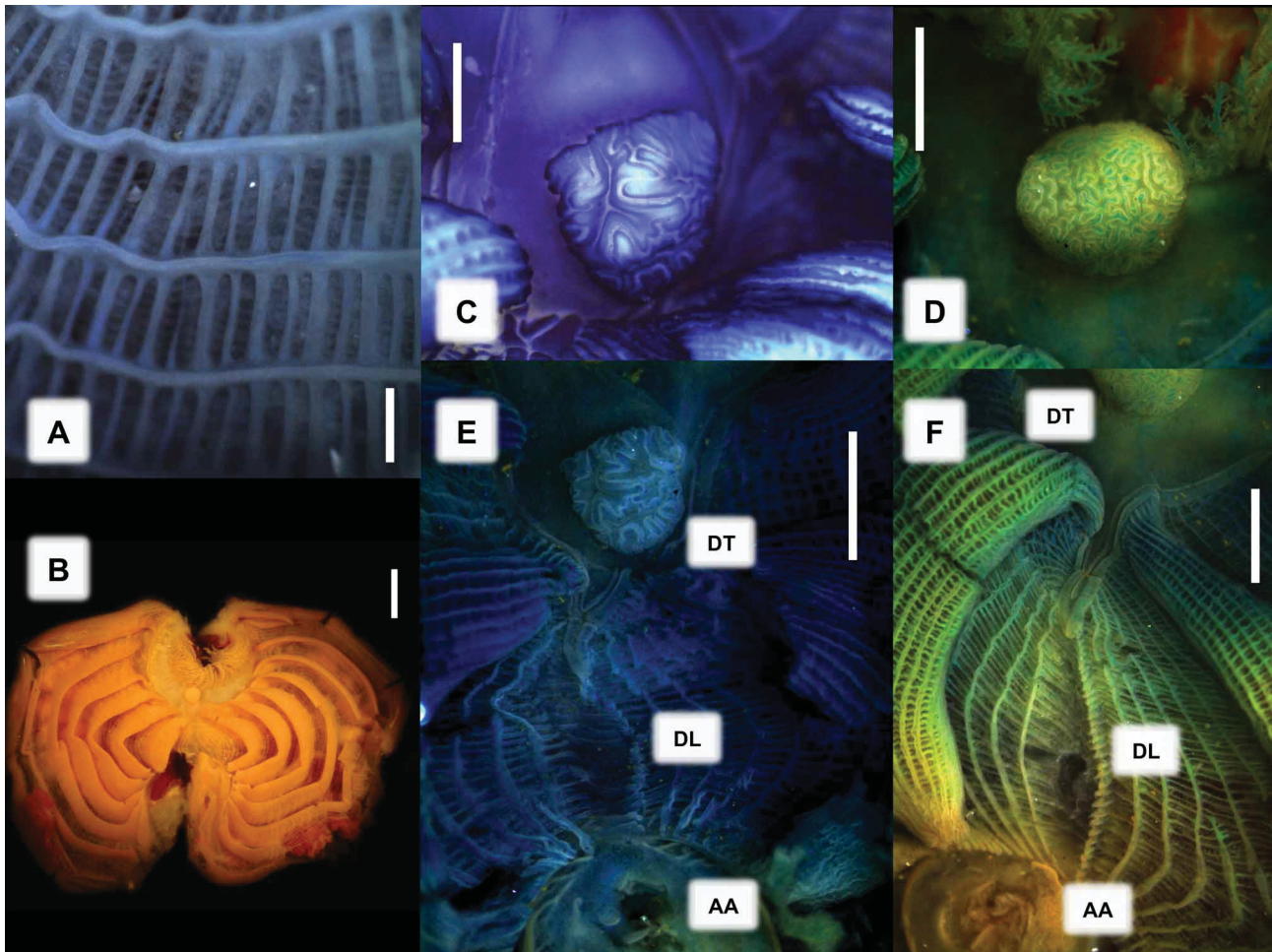
**FIGURE 6.** *Pyura dalbyi* n. sp. A. Siphonal spines as seen from the outer surface; B. Detail of the shape of the siphonal spines; C. Siphonal spines showing the entire siphonal lining, from the external surface (top) to near the base of the siphon (bottom), where it is iridescent. D. and E. Siphonal tentacles of two different specimens. Scale bars: A. 500 µm; B. 100 µm; C. 500 µm; D. 2 mm; E. 1 mm.

spines of *P. dalbyi* are also unique, because in the other species the spines are shorter or less pointy. *Pyura dalbyi* lacks endocarps on top of the gonads and gut, which is uncommon in this group. None of the descriptions of *Pyura* species included in the extensive monograph of Australian ascidians by Kott (1985) corresponds to *P. dalbyi*. The presence of a picture of a large aggregate of *P. dalbyi* identified as *P. stolonifera* (see Plate VIIIa, page 420) indicates that both species were included under the same name.

### Key to the species of the *Pyura stolonifera* species complex

1. A single row of gonadic lobes forms the right gonad (African species) ..... 2
- Two rows of gonadic lobes form the right gonad (Australasian species) ..... 3
2. Pointed papillae on the tunic, especially around the siphons; dorsal tubercle sponge-like ..... *P. herdmani*
- No papillae on the tunic; dorsal tubercle forms a double spiral cone ..... *P. stolonifera*
3. Brown tunic, often enmeshed with sand; dorsal tubercle forms a double spiral cone ..... *P. praeputialis*
- Tunic yellow and smooth, often with epibionts; dorsal tubercle sponge-like with two bulges ..... *P. dalbyi* n. sp.





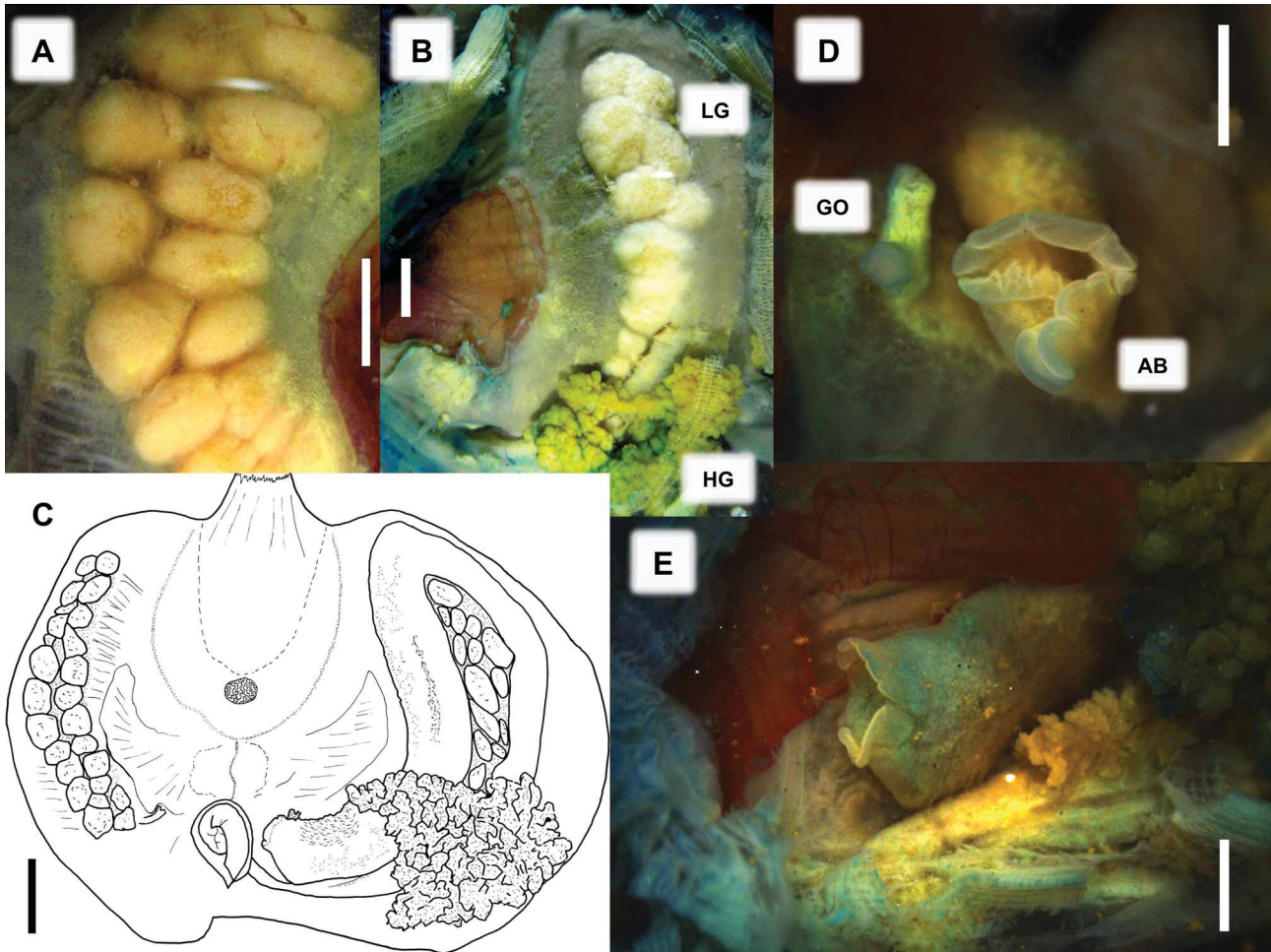
**FIGURE 7.** *Pyura dalbyi* n. sp. A. Branchial sac between folds, showing straight stigmata; B. Branchial sac with six marked branchial folds; C. and D. Dorsal tubercle in a small and large individual, respectively; E. and F. Dorsal lamina with languets in a small and a large individual, respectively; Abbreviations: DL—dorsal lamina, DT—dorsal tubercle, AA—atrial (excurrent) aperture. Scale bars: A. 500  $\mu$ m; B. 5 mm; C. 1 mm; D. 2 mm; E. 2 mm; F. 2 mm.

## Discussion

The ascidians often referred to in the literature as *Pyura stolonifera* (Monniot & Bitar 1983; Kott 1985; Lafargue & Wahl 1986–1987; Dalby 1997a; Monniot *et al.* 2001) constitute at least four morphologically distinguishable species. Two of these occur in Africa (*P. stolonifera* sensu stricto and *P. herdmani*) and the other two are native to Australia (*P. praeputialis* and the species described here, *P. dalbyi*), although one of them, *P. praeputialis*, has recently been introduced to New Zealand and South America. *Pyura dalbyi* is readily distinguishable from the other three species on the basis of some very distinctive morphological characters, and its status as a separate species is strongly supported by genetic data (PRT unpublished data).

The *P. stolonifera* species complex has a wide but disjunct distribution and is particularly common in temperate regions of the southern hemisphere. We collected specimens from all three continents where the species complex has been reported, but were unable to obtain specimens from New Zealand (Hayward & Morley 2009) and Tahiti (Kott 1985). The specimens from New Zealand have been identified as *P. praeputialis* (M. Page personal communication, National Institute of Water and Atmospheric Research, New Zealand), while the record of *P. stolonifera* from Tahiti (Kott 1985) is puzzling. Neither *P. stolonifera* nor *P. praeputialis* were reported from New Caledonia by Monniot *et al.* (1991) or Monniot (2007), and Kott (2006) no longer mentions Tahiti as one of the regions where *P. stolonifera* occurs.





**FIGURE 8.** *Pyura dalbyi* n. sp. A. Two rows of lobed gonadic sacs without endocarps on the right of the body; B. Gut forming a sharply curved first loop enclosing the two rows of gonadic sacs on the left side of the body; C. Internal structure detailing the position of the gonoducts, hepatic gland, dorsal tubercle, gonad on the right side of the body and the gut and gonad on the left side; D. Rectal opening (view from the top) showing the irregular, rounded lobes. The gonoduct openings of the left gonad can be observed next to the rectal opening; E. Rectal opening (view from the side). Abbreviations: LG—left gonad, HG—hepatic gland, GO—gonoduct opening, AB—anal border. Scale bars: A. 5 mm; B. 10 mm; C. 10 mm; D. 2 mm; E. 2 mm.

On the basis of a single character (the number of rows of gonadic lobes at the right gonad), we can distinguish between two major groups that live in allopatry, namely the African and Australasian forms that have previously been referred to as *P. stolonifera* and *P. praeputialis*, respectively (Monniot & Bitar 1983; Castilla *et al.* 2002). Each major group consists of at least two species with overlapping distributions. Below, we discuss the implications of our taxonomic revision for the species living in each region.

**Southern Africa.** The African species *P. stolonifera* and *P. herdmani* have overlapping distributions in temperate South Africa, where they often occur in sympatry. This has resulted in considerable taxonomic confusion. For example, Castilla *et al.* (2002) sequenced a fragment of the mitochondrial DNA cytochrome oxidase subunit I of *P. stolonifera* from False Bay, South Africa (sequence code: Py\_Safr). However, when this sequence was compared with other sequences of both *P. stolonifera* and *P. herdmani*, it clustered with individuals of *P. herdmani* from the temperate coast of South Africa (PRT unpublished data). Hence, while their conclusion that the Australian and southern African forms are different species was correct, it may have been based on an incorrectly identified African specimen. However, *P. stolonifera* and *P. herdmani* can cross-fertilize (MR unpublished data), and as the specimen used by Castilla *et al.* (2002) was collected in an area where the two species occur in sympatry and a uniparentally inherited genetic marker was used, it is possible that it may have been a hybrid.

**North-west Africa.** In previous studies, samples from Morocco and Senegal were identified as *P. stolonifera* (Monniot & Bitar 1983; Lafargue & Wahl 1986–1987; Monniot & Monniot 1994). Neither Lafargue & Wahl (1986–1987) nor Monniot & Monniot (1994) provided a formal description of the specimens, but the description

from Monniot & Bitar (1983) includes the sponge-like dorsal tubercle typical of *P. herdmani*. This, together with the findings from our study, suggests that *P. stolonifera* sensu stricto does not occur in north-west Africa and that it is endemic to the temperate coast of South Africa.

**Australasia.** Like their southern African congeners, the two Australasian species *P. praeputialis* and *P. dalbyi* have partially overlapping distributions. The two morphs identified by Dalby (1997a) as *P. stolonifera* had parapatric distributions in Victoria, Australia, with the brown morph being present on the open coast and at the entrance of bays, and the yellow morph in sheltered areas. The descriptions of the two morphs and their preferred habitats indicate that they represent *P. praeputialis* (which has a brown tunic and prefers more exposed habitats) and *P. dalbyi* (which has a yellow tunic and occurs in more sheltered areas).

**South America.** Kott (2006) argued that *Pyura bradleyi* (synonymised as *P. stolonifera*) was present both on the Peruvian and Chilean coasts and that it was part of the indigenous fauna. The specimens from Chile examined here were identified as *P. praeputialis* and shared only few characteristics with *P. bradleyi* described by Van Name (1931). Furthermore, the description of *P. bradleyi* given by Van Name (1945) includes two characters that serve to distinguish this species from the members of the *P. stolonifera* species complex: a different organization of the gonads, and a larger and longer atrial siphon compared to the oral siphon.

**Directions for future research.** We found a substantial amount of intra-regional morphological variability, which together with the presence of genetically distinct lineages within each species (PRT unpublished data), indicates that some of the species identified in the present study may comprise additional cryptic species. Firstly, *P. herdmani* shows high morphological variability in southern Africa and this may either be attributed to the existence of different species or to hybridization. Genetically, the species can be divided into temperate and subtropical/tropical lineages (PRT unpublished data) that occur in different marine biogeographic provinces. Morphologically similar but genetically distinct sister taxa that are found in the same provinces have been identified in other coastal invertebrates from this region (e.g. Ridgway *et al.* 1998; Edkins *et al.* 2007; Teske *et al.* 2008, 2009). Secondly, the small morph of *P. praeputialis* identified by Kott (1985) that has been reported in Tasmania, South Australia, Victoria and New Zealand seems to have different habitat preferences than the large morph occurring on the east coast of Australia and in Chile. Unlike the latter, which often forms large aggregates in exposed areas, the small morph either forms small, sporadic clumps in sheltered localities or shows a preference for settling on artificial structures (PRT personal observation). Bennett & Pope (1960) reported that in Tasmania, *P. praeputialis* occurs high on the shore, and the area on the lower shore that would be occupied by dense aggregations on the mainland (supposedly by the large morph) is somewhat bare. The evidence for differences in habitat preference, together with distribution ranges that suggest only limited overlap, supports the idea that the large and small morphs of *P. praeputialis* may each constitute a distinct species.

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