

Revision of *Platydoris angustipes* and description of a new species of *Platydoris* (Gastropoda: Nudibranchia) from southeastern Brazil based on comparative morphology

Patricia O. V. Lima¹, Luiz Ricardo L. Simone¹

1 Museu de Zoologia da Universidade de São Paulo, Cx. Postal 42494; 04299-970 São Paulo, SP, Brazil

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Corresponding author: Patricia O. V. Lima (patylima84@gmail.com)

Abstract

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Key Words

Discodorididae Platydoris angustipes Platydoris guarani new species Platydoris angustipes (Mörch, 1863) is a common nudibranch in the Western Atlantic, ranging from Florida, USA, to Rio de Janeiro, Brazil. In this study, we examined the anatomy of *P. angustipes* along its distribution, including its type material. Our analysis shows consistent differences between the Caribbean and Brazilian populations, mainly in the reproductive system, radular teeth and odontophore musculature. This strongly suggests that the two populations actually belong to distinct species. The Brazilian population is described herein as a new species, *Platydoris guarani* sp. n.

Introduction

The nudibranch *Platydoris angustipes* (Mörch, 1863) is presently considered a widespread species in the western Atlantic, ranging from the Caribbean to southern Brazil, and reaching Ascension Island to the east (Rosenberg et al. 2009; Padula et al. 2017). It is very commonly found in Brazilian waters (Marcus and Marcus 1967; García et al. 2002; Valdés et al. 2006; Padula et al. 2012; Alvim and Pimenta 2013; Galvão-Filho et al. 2015; Padula et al. 2017).

In their phylogenetic analysis of the genus *Platydoris*, Dorgan et al. (2002) reviewed the morphological data from the literature noting the distribution of *P. angustipes* in the Caribbean Sea, but not in Brazil. However, Alvim and Pimenta (2013), in a taxonomical revision of Discodorididae, list *P. angustipes* for Brazilian waters.

Since there is doubt whether the Caribbean and Brazilian populations of *Platydoris angustipes* are the same

taxa, in the present study we performed a detailed comparative anatomical survey of them. This included the holotype, from Saint Thomas (U.S. Virgin Islands), and specimens collected in Honduras, the Virgin Islands and Brazil (Rio de Janeiro state). We were able to recognize significant morphological differences that led us to the description of the Brazilian population as a new species.

Materials and methods

The studied material come from museum collections, consisting of specimens preserved in 70% ethanol. Dissections were performed under a stereomicroscope by standard techniques, with the specimens immersed in fixative (Simone 2004, 2011). The initial steps of the anatomical investigation were done through a longitudinal cut on the tissue covering the dorsal visceral mass. Digestive, circulatory, excretory, reproductive and central

nervous systems were investigated in detail. The terminology used for odontophore muscles was based on Ponder et al. (2008), Simone (2011) and Lima and Simone (2015). Digital photographs were taken at each dissection step. Illustrations were prepared with the aid of a camera lucida. Scanning electron microscopy (SEM) images of the radula were obtained at MZSP.

Institutions abbreviation: CAS – California Academy of Sciences (San Francisco, USA); ZMUC - Statens Naturhistoriske Museum (Copenhagen, Denmark); MZSP – Museu de Zoologia da Universidade de São Paulo (São Paulo, Brazil); UFBA – Universidade Federal da Bahia (Bahia, Brazil).

The following abbreviations are used herein: aa: anterior aorta; ab: afferent branchial vein; an: anus; am: ampulla; ap: posterior aorta; au: auricle; at: aortic trunk; bc: bursa copulatrix; bg: blood gland; bs: buccal sphincter; ce: cerebral ganglia; cp: pedal commissure; cu: caecum; dd: duct of digestive gland; dg: digestive gland; ds: salivary duct; es: oesophagus; ev: efferent branchial vein; ey: eye; fg: female gland; ft: foot; gb: buccal ganglia; gc: gill circle; ge: gonopore; gf: gill filament; gg: gastro-oesophageal ganglia; go: gonad; gp: pedal ganglia; hd: hermaphrodite duct; in: intestine; mo: mouth; m2 - m10: odontophore muscles; mt: oral tube muscle; ne: nephrostome; oc: odontophore cartilage; ot: oral tube; ov: oviduct; pa: papilla; pc: pericardium; pe: penis; pl: pleural ganglia; pr: prostate; ra: radula; rg: rhinophoral ganglia; ri: rhinophore; rm: retractor muscle gill; rp: reproductive system; rs: radular; sac; rv: renal vesicle; sg: salivary gland; st: stomach; sn: nervous system; sp: spine; sr: seminal receptacle; to: oral tentacle; ud: uterine duct; va: vagina; vd: vas deferent; ve: ventricle; vp: reproductive system vein; vn: nervous system vein: vs: radular sac vein.

Results

Family Discodorididae Bergh, 1891 Genus *Platydoris* Bergh, 1877

Type species. *Doris argo* Linnaeus, 1767, by subsequent designation (O'Donoghue 1929).

Platydoris angustipes (Mörch, 1863)

Figures 1-6

Doris (Argus) angustipes Mörch, 1863: 32

Platydoris angustipes var. alaleta Bergh, 1877: 505, pl. 58, figs. 13-18. Platydoris rubra White, 1952: 118, fig. 17, pl. 6, fig. 6.

Platydoris angustipes: Ev. Marcus and Er. Marcus 1967: 93, fig. 112;
Er. Marcus and Ev. Marcus 1970: 67, fig. 121; Meyer 1977: 301;
Humann 1992: 243; Dorgan et al. 2002: 282, figs. 1B, 2B, 11-13;
Valdés et al. 2006: 182; Debelius and Kuiter 2007: 245; Camacho-García et al. 2014: 121; Goodheart et al. 2016: 9, fig.4f.

Type locality. St. Thomas, U. S. Virgin Islands.

Redescription. External morphology (Figure 1A–E): Size ~25 mm length, ~20 mm width. Body dorsoventrally flattened and wide (Fig. 1A, B). Foot not exceeding notum (Fig. 1B). Rhinophores with ~19 transverse lamellae, rhinophoral sheaths with small lobes (Fig. 1C). Gill composed of six tripinnate branchial leaves, arranged in branched fashion with anus in middle of rachis; branchial sheaths also with small lobes (Fig. 1E). Mouth opens in anterior ventral region, between anterior region of notum and foot. Digitiform tentacles present. Anterior border of foot bilabiate, with longitudinal groove (Fig. 1B).

Haemocoel organs (Figure 2A–B): Pericardium and posterior half of visceral mass occupying ~15% of haemocoel volume. Buccal mass located anteriorly, occupying ~10% of haemocoel volume. Nervous system positioned dorsally in relation to buccal mass, covered by blood gland, occupying ~5% of haemocoel volume. Reproductive system on right side of animal, occupying ~25% of haemocoel volume. Stomach on left side of animal, intestine with small curve on its anterior portion; digestive gland system occupying ~45% of haemocoel volume.

Circulatory and excretory systems (Figures 1D, E; 2A-C): Pericardial cavity dorsal, located posteriorly to digestive gland and anteriorly to gill circle (Fig. 2A). Afferent and efferent branches located inside each gill filament, flowing from and to afferent and efferent branchial veins, respectively (Fig. 1D). Gill retractor muscle split, originating from base of gill circle, running longitudinally up to half of foot, inserting into dorsal surface of foot (Fig. 2B). Auricle funnel-like (wider anteriorly), with thin walls. Ventricle slightly taller than wide, with thick muscular walls (Fig. 2C). Aortic trunk branches very close to ventricle; anterior artery irrigates reproductive system, buccal mass, odontophore and nervous system posterior artery irrigates stomach and digestive gland (Fig. 2C). Renal vesicle located on dorsal right side of pericardium, near the base of auricle, connecting to inner surface of pericardium, $\sim 1/6$ of ventricle size (Fig. 2A). Renal chamber extending from dorsal to medial sinus, anteriorly connected to renal vesicle, extending posteriorly to center of gill circle (Fig. 1E). Nephrostome readily visible. Blood gland clearly divided in anterior and posterior portions (Fig. 2A).

Digestive system (Figures 1E; 2A–B, D–E; 3A–E; 6A–C): Oral tube composed of outer and inner lips, with thick transversal fold; **mt**, three long pairs of retractor muscles of buccal mass, originating on oral tube and running dorsally and ventrally along it; inserting in the side of the body; about four times as wide and three times as long as m10 (Fig. 2D, E). Odontophore oval, connected to oral tube by pair of ventral protractor muscles (**m10**); thin longitudinal dorsal and ventrolateral protractors of oral sphincter originating in anterior region of odontophore and inserted in posterior region of integument, close to oral tube (Figs 2E; 3C). Oral sphincter surrounding chitinous part of oral tube (Fig. 2D). Odontophore muscles: **m2**, pair of strong buccal mass retractor muscles, four times longer than wide, originating on anterior

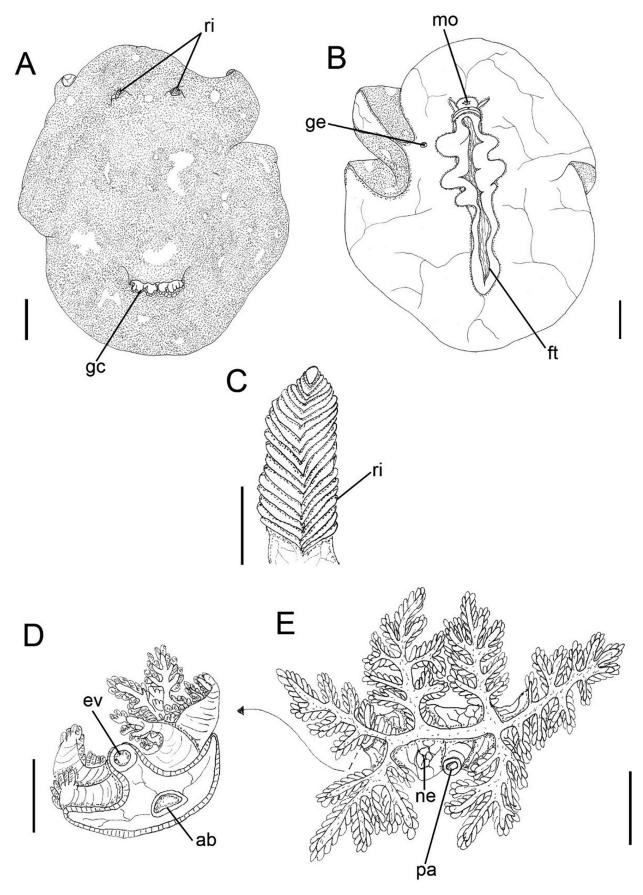


Figure 1. Platydoris angustipes, fixed animal **A** dorsal view **B** same, ventral view **C** rhinophore **D** gill filament with transversely sectioned showing afferent and efferent branchial ring **E** gill circle. Scale bars: 5 mm (**A**, **B**); 1 mm (**C**); 2 mm (**D**, **E**).

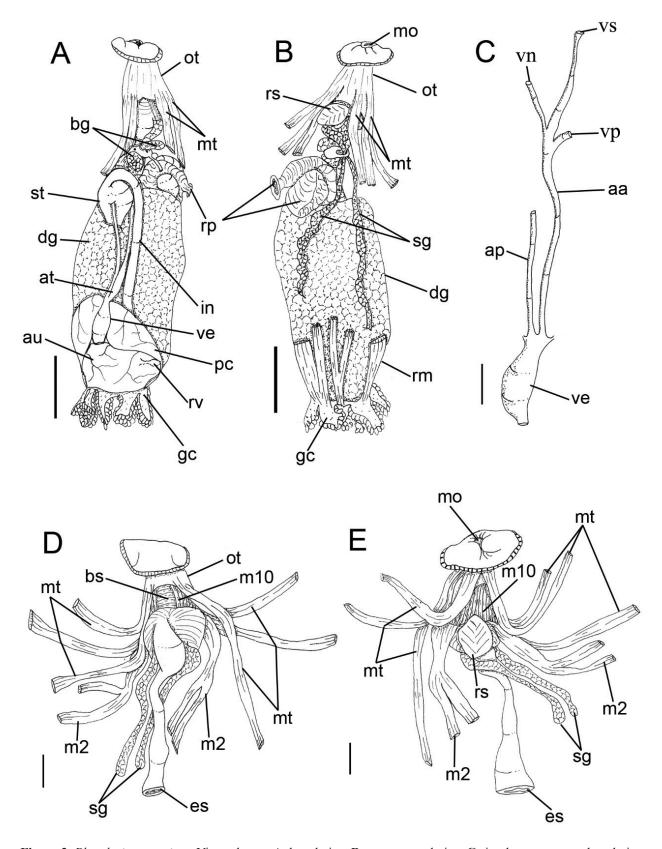


Figure 2. *Platydoris angustipes.* Visceral mass **A** dorsal view **B** same, ventral view **C** circulatory system, dorsal view. Anterior digestive system **D** dorsal view **E** same, ventral view. Scale bars: 5 mm (**A**, **B**); 2 mm (**C**–**E**).

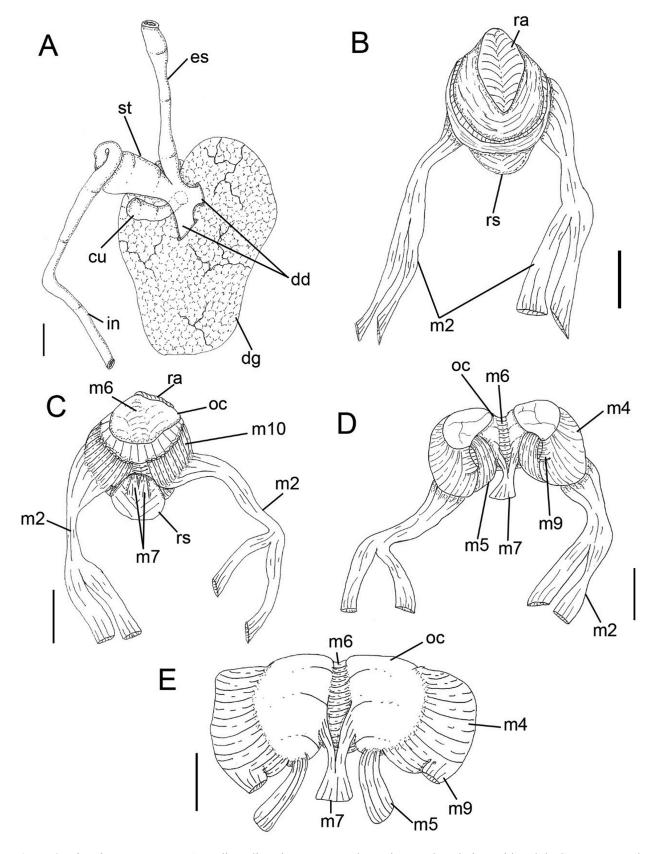


Figure 3. *Platydoris angustipes.* **A** medium digestive system. Odontophore **B** dorsal view with radula **C** same, ventral view **D** same, dorsal view with removed radula **E** same, dorsal view with folded down muscles. Scale bars: 2 mm.

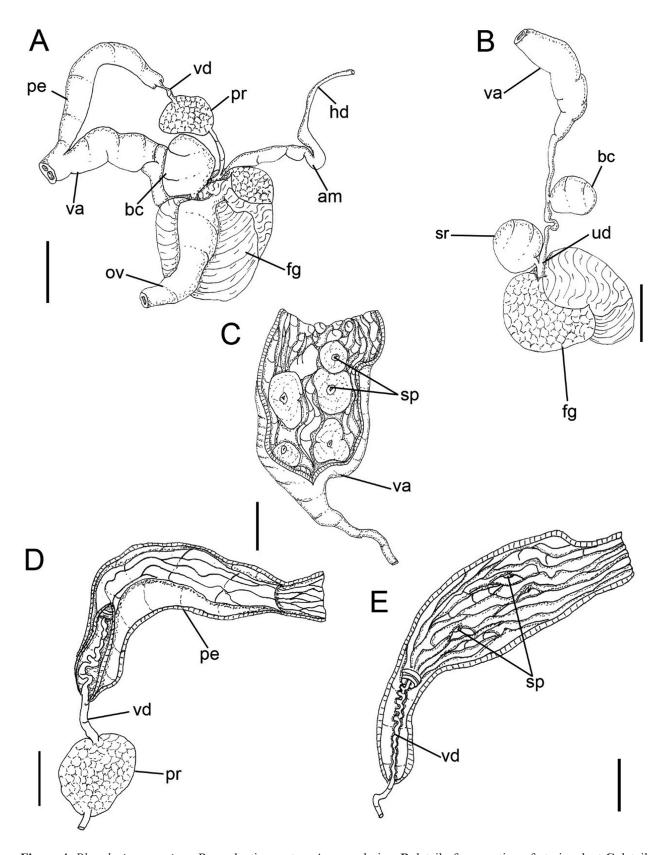


Figure 4. Platydoris angustipes. Reproductive system **A** general view **B** detail of connection of uterine duct **C** detail of vagina's spines **D** detail of internal part of insertion of vas deferens **E** detail of penis's spines. Scale bars: 2 mm (**A**, **B**); 1 mm (**C**-**E**).

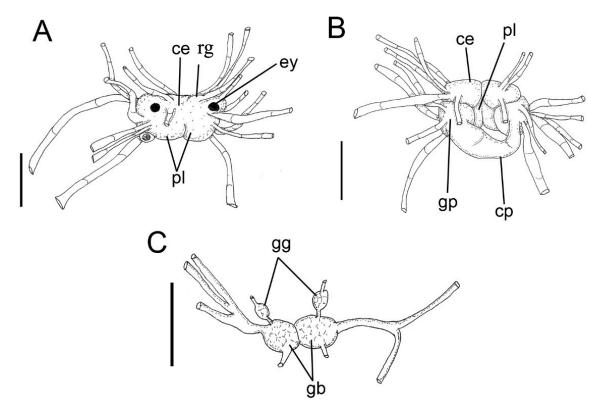


Figure 5. *Platydoris angustipes.* Nervous system **A** dorsal view **B** same, ventral view **C** buccal and gastroesophageal ganglia. Scale bars: 1 mm.

dorsal odontophore, running laterally to m4 and inserted ventrally in dorsal portion of foot, bifurcate on insertion (Fig. 3B-D); m4, pair of strong and broad dorsal tensor muscles, as long as wide, covering ~2/3 of cartilage, inserted in ventral portion of subradular membrane (Fig. 3D, E); **m5**, pair of dorsal auxiliary tensor muscles, twice as long as wide, originating on posteriormost region of odontophore cartilage, covering ~1/3 of posterior cavity of odontophore, as long as m4, but with $\sim 1/3$ of its width, inserting in ventral side of subradular membrane around radular sac (Fig. 3D, E); m6, unpaired horizontal muscle, with transversal fibers connecting to median surface of left and right odontophore cartilages, about same length and half as wide as m4, posterior portion as wide as anterior portion (Fig. 3D, E); m7, pair of thin and narrow muscles, originating on inner surface of odontophore cartilages, running together posteriorly, inserting into radular sac (Fig. 3D, E); m9, unpaired and horizontal muscle, originating on posterior portion of m4, connecting of the two components of the m4 pair (Fig. 3D, E). Pair of odontophore cartilages elliptical, occupying ~2/5 of odontophore volume (Fig. 3D, E). Subradular membrane thin, strong, translucent. Radular sac ~1/4 as large as odontophore. Radular teeth (Fig. 6A-C): rachidian teeth absent; formula 42 x 62.0.62 (in 25 mm long specimen, CASIZ 76667). Each lateral tooth with broad base, tapering towards apex, hook-shaped, with single terminal cusp; two outermost teeth spatulate. Pair of salivary

glands long, tubular; duct inserting in anterior region of esophagus, extending posteriorly to ventral middle region of digestive gland (Fig. 2B). Esophagus simple, originating dorsally to odontophore, inserting directly in anterior region of stomach; internal longitudinal folds with same diameter along entire length (Figs 2D, E; 3A). Stomach oval, occupying ~30% of visceral mass volume, with folds at the center of entire inner surface (Figs 2A; 3A). Common opening for esophagus and stomach located on digestive gland. Intestine with longitudinal folds along its entire length; diameter ~1/2 that of esophagus and more uniform than it (Fig. 3A). Digestive gland dark beige, cone-shaped; largest organ of visceral mass, occupying ~40% of its volume; anterior portion about twice as wide as posterior portion; inner surface of gland sponge-like, bearing two distinct main ducts (Fig. 3A). Anus opening into anal papilla in the center of gill circle, ~1/4 of gill filament length (Fig. 1E).

Genital system (Figure 1B; 2B; 4A–E): Located between buccal mass and digestive gland, mostly dorsally-positioned on right side of animal (Fig. 2B). Genital opening on ventral right side, on anterior third of animal, located between foot and notum (Fig. 1B). Gonad immersed in digestive gland, difficult to distinguish from it. Hermaphrodite duct thin, long. Ampulla located on female gland, elongated and tubular. Prostate rounded, glandular, connected with female gland duct, ~1/3 of ampulla length (Fig. 4A, D). Vas deferens short, ~1/5 of ampulla

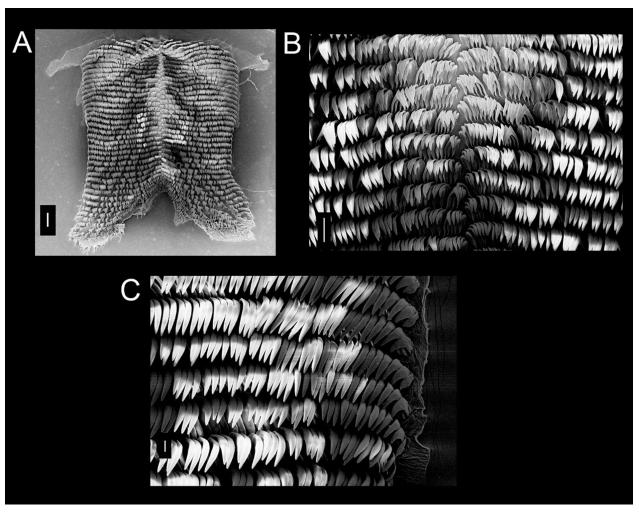


Figure 6. SEM of radula of *Platydoris angustipes*. **A** panoramic view **B** central region with rachidian tooth absent **C** lateral teeth. Scale bars: 300 μm (**A**); 100 μm (**B**); 30 μm (**C**).

(Fig. 4D). Penis' muscle absent. Penis muscular, cylindrical and elongated, about three times longer than prostate; thin folds on internal surface, with cartilage between them and three small tubercles each with a central spine (Fig. 4D, E). Female gland well-developed, rounded, occupying ~1/5% of reproductive system volume; divided into mucus gland (~2/3 of female gland, color beige) and albumen gland (~1/3 of anteriormost region, dilated, irregularly shaped, color dark brown) (Fig. 4B). Oviduct occupying ~1/2 of female gland's volume (Fig. 4A). Uterine duct thin, relatively short, $\sim 1/7$ of vagina length; located on the base of seminal receptacle, inserted in female gland near oviduct (Fig. 4B). Seminal receptacle rounded, as large as bursa copulatrix, $\sim 1/3$ of vagina length; connected to bursa copulatrix through stalk with ~1/2 of vagina length (Fig. 4B). Bursa copulatrix rounded, ~1/3 of vagina length, connected directly to vagina (Fig. 4B). Vagina cylindrical, elongated, approximately as long as and twice as wide as penis; internal surface with cartilage between folds and five large tubercles each with a central spine; located dorsally in relation to prostate and parallel to penis in genital opening (Fig. 4 A-C).

Nervous system (Figure 5A–C): Located dorsally in relation to odontophore, mostly covered by blood gland. Pair of cerebral and pleural ganglia fused with one another. Pedal ganglia ventrally fused with cerebral and pleural ganglia, but not fused between themselves. Pedal commissure simple, broad and short, surrounding esophagus and salivary glands (Fig. 5B). Buccal ganglia short, located ventrally to odontophore between radular sac and anterior portion of esophagus, connected to cerebral ganglia through long and slender connective tissue, united to gastro-esophageal ganglia by short connective tissue. Gastro-esophageal ganglia circular, ~1/4 of buccal ganglia length (Fig. 5C). Rhinophoral (olfactory) ganglia inconspicuous. Dorsal eyes located on cerebral ganglia (Fig. 5A).

Distribution. United States of America (Florida and Virgin Islands) (Marcus and Marcus 1967; Marcus and Marcus 1970); Cuba (Guanahacabibes Peninsula) (Espinosa et al. 2012); Cayman Islands (Hess et al. 1994); Panama (Bocas del Toro) (Goodheart et al. 2016); Jamaica (Marcus and Marcus 1970); Curaçao (Marcus and Marcus

1970); Trinidad & Tobago (White 1952); Ascension Island (Padula et al. 2017).

Habitat. On reefs, tide pools, from 0 to 73 m depth.

Material examined. CARRIBEAN SEA, West Indies, Lesser Antilles, Martinique, cliffs S of St. Pierre, CASIZ 76667, 1 specimen (William Liltved on "Gloriamaris", 28/ix/1986, 10-85ft – Liltved – West Indies Cruise 1986); HONDURAS, MZSP 75996, 1 specimen (Col. Marcus, P-938); Saint Thomas, ZMUC-GAS 1505, 1 specimen (Riise 152 – Holotype of *Doris angustipes*); Saint Croix, Virgin Island, ZMUC-GAS 2020, 1 specimen (Riise 1860 – Holotype of *Platydoris angustipes alaleta*).

Platydoris guarani sp. n.

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Platydoris angustipes Er. Marcus, 1957: 422, fig. 81-89; Ev. Marcus 1972: 79; García et al. 2002: 53, fig.2K; García et al. 2008: 148; Alvim and Pimenta 2013: 186, figs. 2C; 21–22; Padula et al. 2012: 3 (non Mörch 1863).

Type material. Holotype: BRAZIL, Rio de Janeiro, Ilha Grande, Angra dos Reis, MZSP 86082, 1 specimen (E.P. Gonçalves, L.R. Simone & P. Oristanio, coll., 24/ix/2006, 17m depth, Ponto 3, Pinguino Wreck). Paratype: BRAZIL, Rio de Janeiro, Ilha Grande, Angra dos Reis, MZSP 134877, 2 specimens (E.P. Gonçalves, L.R. Simone & P. Oristanio, coll., 24/ix/2006, 17m depth, Ponto 3, Pinguino Wreck).

Type locality. Brazil, Rio de Janeiro state, Angra dos Reis.

Etymology. The specific epithet is a noun in apposition, derived from the native Guarani indigenous people, some tribes of which still reside in Rio de Janeiro.

Diagnosis. Body of orange color, with a white ribbon on its edge and brown spots just above the ribbon (they can be seen both dorsally and ventrally). Radula with outermost teeth not spatulate, with apex hook-shaped; cusp simple and smooth. Presence of m4a and m7b odontophore muscles. Gonad readily visible. Absence of spines on internal surface of penis and vagina.

Description. External morphology (Figure 7A–C): Size ~60 mm length, ~40 mm width. Body color orange with white ribbon on its edge and brown spots just above the ribbon that can be seen both dorsally and ventrally (Fig. 7A–B). Body flattened and wide with small tubercles around dorsum. Rhinophores with ~25 transversal lamellae, very thin; color dark orange; rhinophoral sheaths with very small lobes (Fig. 7C). Gill composed of six white tripinnate branched branchial leaves, arranged in circular fashion surrounding anus; branchial sheaths also with very small tubercles (Fig. 7C). Mouth opens in anterior ventral region, between anterior region of notum

and foot. Digitiform tentacles present. Anterior border of foot bilabiate and longitudinally notched.

Haemocoel organs: Of similar proportions as *P. angustipes* (see above).

Circulatory and excretory systems (Figure 8A, B): Same as *P. angustipes*, but with renal vesicle very large, well-developed, of about same length and width as ventricle (Fig. 8B), extending from dorsal to medial sinus, anteriorly connected to renal vesicle, extending posteriorly to center of gill circle and opening in nephrostome (Fig. 8A). Nephrostome pore not readily apparent.

Digestive system (Figures 8C, D; 9A-D; 11A–C): Same pattern as *P. angustipes*, but with the following differences: m2, twice as long, not bifurcated on insertion (Fig. 9A, B); m4a, pair of thin muscles originating in posterior region of m4 and inserting in the middle of odontophore cartilages, dorsally connected to m7b (Fig. 9D); m7b, pair of thin and short muscles originating in posterior region of m6, with joint insertion with posterior part of m7 (Fig. 9C, D). Radular sac $\sim 1/5$ as large as odontophore (Fig. 9A, B). Radular teeth (Fig. 11A-C): anterior region broader than in P. angustipes; rachidian teeth also absent; formula 35 x 60.0.60 (in 60 mm long specimen, MZSP86082). Each lateral tooth with broad base, tapering towards apex, hook-shaped, with single terminal cusp; outermost teeth narrower than in P. angustipes, inner base width ~1/2 lateral teeth width, apex also hookshaped, cusp simple and smooth (Fig. 11C). Pair of salivary glands long, tubular, bulging in anterior portion and tapering posteriorly; duct inserting in anterior region of esophagus, extending posteriorly to ventral middle region of digestive gland (Fig. 8D). Esophagus simple, originating dorsally to odontophore, inserting directly in anterior region of stomach; longitudinal folds on inner surface with same diameter along esophagus' entire length. Stomach oval, with folds on the center of entire inner surface (Fig. 8C). Common opening for esophagus, stomach and caecum located on digestive gland. Intestine with longitudinal folds along its entire length; diameter similar to that of esophagus; anterior portion S-shaped, about twice longer than in P. angustipes (Fig. 8C). Caecum: short elongated sac, located ventrally to stomach, opening in anterior portion of stomach close to esophageal insertion; $\sim 1/12$ length and $\sim 1/5$ width of stomach (Fig. 8C). Digestive gland dark beige; largest organ of visceral mass; cone-shaped, anterior portion about twice as wide as posterior portion; inner surface of gland sponge-like, bearing distinct main duct. Anus opening into anal papilla on the center of gill circle, similar to P. angustipes.

Genital system (Figure 10A–B): Located between buccal mass and digestive gland, longitudinal on right side of animal. Genital opening on right side, on anterior third of animal, located between foot and notum. Gonad circling around all digestive gland, but easy to distinguish, unlike in *P. angustipes*. Hermaphrodite duct thin, long. Ampulla located on female gland, elongated and tubular. Prostate rounded, glandular, of same length as ampulla (Fig. 10A). Vas deferens about same length

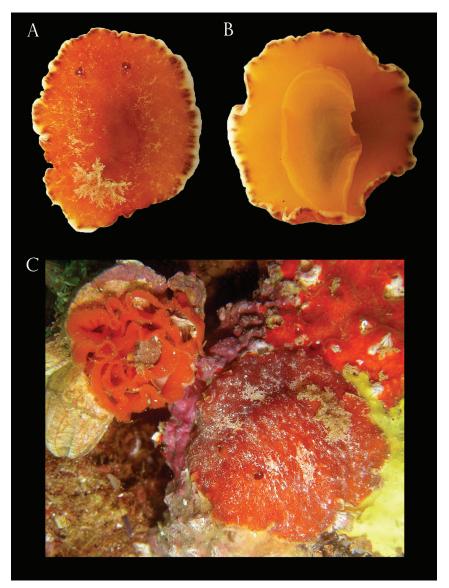


Figure 7. *Platydoris guarani*, living animals. **A.** Dorsal view, specimen from Papagaio, Cabo Frio, Rio de Janeiro (MZSP 97331). **B.** Same, ventral view. **C.** Living animal with spawning, specimen from Enseada da Meia Lua, Cabo Frio, Rio de Janeiro (MZSP 97625). All photograph by V. Padula.

as ampulla Penis muscular, cylindrical and very broad, \sim 1/2 length and three times wider than ampulla, without spines (Fig. 10A). Female gland well-developed, rounded, occupying ~20% of reproductive system volume; divided into mucus gland (~2/3 of female gland, color beige) and albumen gland ($\sim 1/3$ of anteriormost region, dilated, irregularly shaped, color dark brown). Oviduct occupying ~1/5 of female gland volume (Fig. 10A). Uterine duct thin, relatively short, length ~1/10 of vagina length, located on the base of seminal receptacle, inserted in female gland near oviduct (Fig. 10B). Seminal receptacle elongate, as long as bursa copulatrix and ~1/3 its width; connected to vagina through stalk with same length and ~1/2 width of vagina (Fig. 10B). Bursa copulatrix rounded, ~1/2 length of vagina, connected to vagina posteriorly to seminal receptacle (Fig. 10B). Vagina cylindrical, very broad, with wide and thick folds, without spines; approximately as long and as wide as penis;

positioned dorsally in relation to prostate and parallel to penis in genital opening (Fig. 10B).

Nervous system (Figure): Same as in *P. angustipes*.

Distribution. Brazil (Valdés et al. 2006). Pernambuco: Fernando de Noronha (García et al. 2002); Alagoas: Saco da Pedra (Padula et al. 2012); Bahia: Praia de Itapoã (García et al. 2008); Rio de Janeiro: Cabo Frio: Ilha Comprida; Arraial do Cabo: Prainha (Alvim and Pimenta 2014).

Habitat. Under stones, associated with sponges and ascidians (García et al. 2002), from 0 to 17 m depth.

Material examined. Types. Additional material: BRAZIL, Rio de Janeiro, Enseada da Meia Lua, Cabo Frio, MZSP 97625, 1 specimens (V. Padula, coll., 23/iv/2010); Ilha dos Papagaios, MZSP 97515, 1 specimen (V. Padul, coll., 17/x/2009).

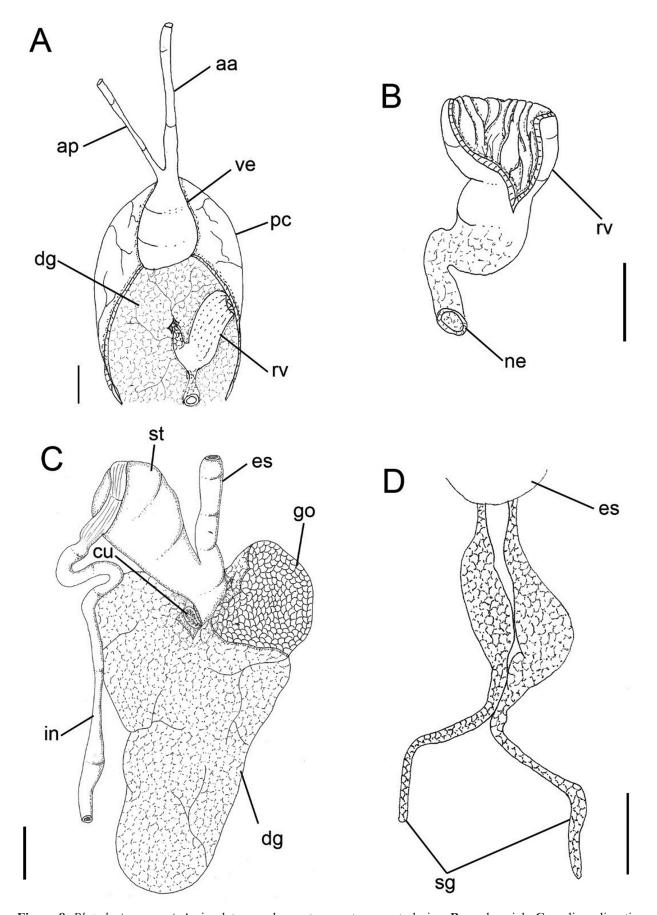


Figure 8. *Platydoris guarani*. **A** circulatory and excretory system, ventral view **B** renal vesicle **C** medium digestive system, dorsal view **D** detail of salivar glands. Scale bars: 2 mm (**A**, **B**, **D**); 5 mm (**C**).

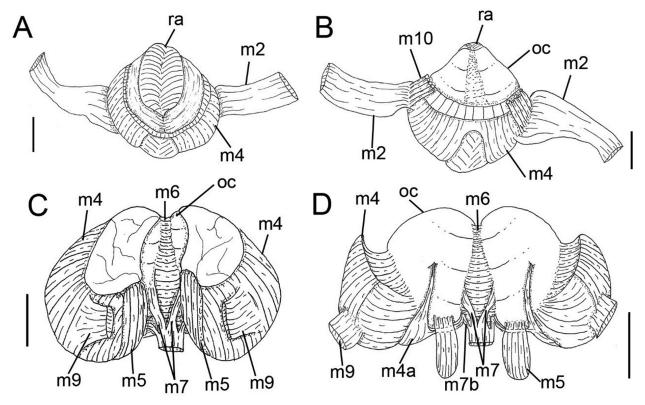


Figure 9. *Platydoris guarani*. Odontophore. **A** dorsal view with radula **B** same, ventral view **C** same, dorsal view with removed radula **D** same, dorsal view, with folded down muscles. Scale bars: 2 mm.

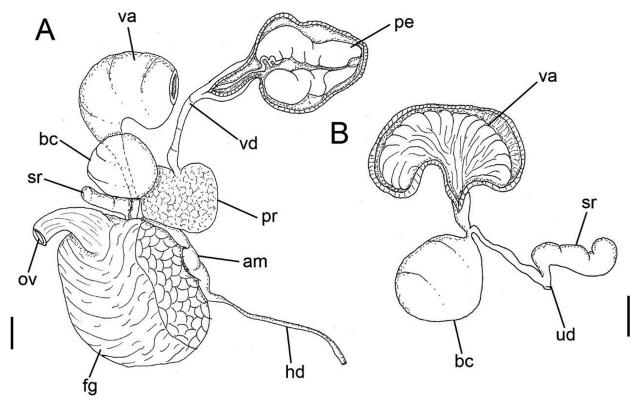


Figure 10. *Platydoris guarani*. Reproductive system **A** general ventral view **B** detail of Bursa copulatrix, seminal receptacle, uterine duct and vagina. Scale bars: 2 mm.

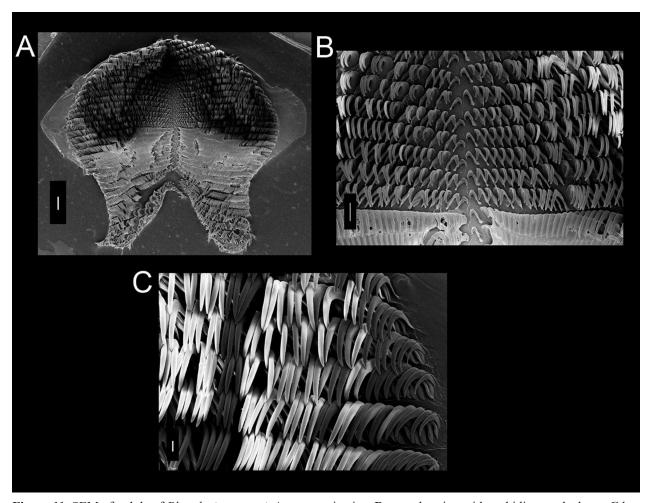


Figure 11. SEM of radula of *Platydoris guarani*. **A** panoramic view **B** central region with rachidian tooth absent C lateral teeth. Scale bars: 300 μm (**A**); 100 μm (**B**); 30 μm (**C**).

Discussion

In the previous anatomical and phylogenetic studies including *Platydoris angustipes* (Mörch 1863; Bergh 1877; Marcus and Marcus 1967; Dorgan et al. 2002) and P. guarani sp. n. (Marcus 1957; Padula et al. 2012; Alvim and Pimenta 2013), several external diagnostic features have been used to distinguish them, but no suggestions of separating them was ever made. Such features included: the rounded and dorsoventrally flattened body, the foot not exceeding notum, the dorsum covered with small caryophillidia, the digitiform oral tentacles, the bilabiate and longitudinally-notched anterior border of the foot, and the six tripinnate branchial leaves. Moreover, the color pattern of the body of P. guarani was deemed distinctive, with a white ribbon on its edge and dark spots on both dorsal and ventral parts of the notum (García et al. 2002; Alvim and Pimenta 2013).

Not all of the differences mentioned above are actually consistent, but the unique set of distinct features found in the present study between the Caribbean and Brazilian specimens, allow the separation in two distinct species. The main diagnostic features are discussed below.

The rhinophore of *P. angustipes* has circa 19 lamellae, while *P. guarani* has circa 25. However, Dorgan et al. (2002) reported 25 lamellae for *P. angustipes* and Alvim and Pimenta (2013) reported 19–25 lamellae for *P. guarani*. As such, this possible diagnostic feature should be studied on a larger sample.

Regarding the circulatory system, the renal vesicle of *P. angustipes* has approximately 1/6 of the ventricle size, while in *P. guarani* this structure is very large, of about the same size as the ventricle. Moreover, the nephrostome in *P. angustipes* is readily visible (Fig. 1E), whereas in *P. guarani* it is not.

Some differences were found in the anterior portion of the digestive system, particularly in the odontophore muscles: mt and m2 are very long in *P. angustipes* (Fig. 2D, E), while those of *P. guarani* are about half the size (Fig. 9A, B); m2 bifurcates on its insertion in *P. angustipes* (Fig. 3C), which is not the case in *P. guarani*. Two muscles occur only in *P. guarani*, namely m7b and m4a (Fig. 9C, D).

In addition, there are visible differences in the midgut. In *P. angustipes* the cecum is circa half the length of the midgut (Fig. 3A), while in *P. guarani* it is much shorter,

about 10% of the midgut (Fig. 8C). The anterior portion of the intestine of *P. guarani* is S-shaped (Fig. 8C), while that of *P. angustipes* is straight (Fig. 3A).

The posterior end of the radula of *P. guarani* (Fig. 11A) is wider than the same region of *P. angustipes* (Fig. 6A). In *P. guarani*, the two outermost lateral teeth are narrower than in *P. angustipes* (about half the width; Fig. 11C) and hook-shaped. The outermost lateral teeth of the radula of *P. angustipes* are wide (Fig. 6C), as observed by Dorgan et al. (2002: fig. 11C), with the two outermost teeth spatulate. The same occurs in *P. guarani* (Marcus and Marcus 1970), but the two outermost spatulate teeth have shorter cusps and several denticles (see fig. 121); this can also be observed in young rows of teeth.

There are also differences in the reproductive system. In *P. angustipes* it is very difficult to distinguish the gonad from the digestive gland, whereas in *P. guarani*, the gonad surrounds the digestive gland, being readily visible (Fig. 8C). In both species, we could not observe the accessory gland (*contra* Marcus 1957; Dorgan et al. 2002; Alvim and Pimenta 2013).

The most significant difference between *P. angusti*pes and *P. guarani* is the absence of spines and cuticle in both the vagina and penis of *P. guarani* (Fig. 10A, B). An additional diagnostic feature was given by Dorgan et al. (2002): *P. angustipes* has more spines on the inner surface of the penis (even more than what we observed, only three prominent spines; Fig. 4E).

Furthermore, *Platydoris guarani* is clearly distinguishable from other species of *Platydoris* by the absence of spines and cuticle in both vagina and penis, and absence of the accessory gland. *Platydoris guarani* resembles *P. carolynae* (Dorgan et al. 2002: fig. 29C) in the hookshape of outermost teeth.

In conclusion, the most characteristic differences between the two populations of *Platydoris*, Brazilian and Caribbean, reside in the digestive system, especially in the odontophore, radula and reproductive system. Based on these findings, *Platydoris angustipes* (Mörch, 1863) is here restricted as a Caribbean species, while the Brazilian population is described as a new species, *Platydoris guarani*. The analysis of additional specimens is still necessary to ascertain which species actually occurs on Ascension Island.

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