

Comparative Morphology and Redescription of *Pleurobranchus* Species (Gastropoda, Pleurobranchoidea) from Brazil

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(Received 23 December 2014; Accepted 24 December 2015)

Juliana Alvim and Alexandre Dias Pimenta (2016) The taxonomy of Pleurobranchus from Brazil is little known because the internal anatomy and possible intraspecific variation are poorly understood. This study recognizes three valid species from Brazil: Pleurobranchus areolatus Mörch, 1863, P. iouspi Ev. Marcus, 1984 and P. reticulatus Rang, 1832. Pleurobranchus reticulatus and P. areolatus were misidentified for a long time because of their simple descriptions, without photographs of living animals. However, they are distinguished by: color of the rhinophores, P. reticulatus has a uniform color, while P. areolatus has the same pattern as the dorsum; P. reticulatus is larger and has more pinnae free from the body wall than P. areolatus; the prostate is tubular and highly convoluted in P. reticulatus, while in P. areolatus is elongated with the proximal portion rounded; and, the jaws of P. areolatus, the smaller species, have more rows and fewer platelets per row than P. reticulatus. Pleurobranchus emys and P. iouspi are considered synonyms based on the examination of portions of the types, mainly due the presence of a dense layer of spicules in the mantle and because their original descriptions do not differ significantly. A discussion about some possible synonymies was provided and their recognition were based mainly on the diameter of the tubercles of the mantle, the presence/absence of flaps that surrounds the gonopore and the development of a leaflet in the convex portion of the penis. The detailed anatomical study allowed recognition of a general pattern in the position and presence or absence of extrinsic and intrinsic odontophore muscles for Pleurobranchus. The circulatory and nervous systems do not provide useful characters for species delimitation.

Key words: Pleurobranchidae, Pleurobranchus reticulatus, P. areolatus, P. iouspi, Western Atlantic.

BACKGROUND

The Pleurobranchidae are marine epibenthic predators of sessile filter-feeding invertebrates (Martynov and Schrödl 2009). The family includes seven genera, of which *Pleurobranchus* has about 20 valid species, distributed in temperate and warm coastal waters. The genus is considered monophyletic, and a sister group of *Boreoberthella* (Martynov and Schrödl 2009).

In the review of the family Pleurobranchidae from the western Atlantic, Ev. Marcus (1984) pointed out that the classification was, in many cases, based on insufficiently described material. According to Ev. Marcus (1984), the flaps around the gonopore and the shape of the protruded penis are useful to distinguish species; however, these structures cannot be evaluated, in many cases, since so few original and subsequent descriptions include those characters.

Unfortunately, the morphology of the radula and jaw plates, the classical hard structures, are useful only for generic separation and hardly differ between species within a genus. The calcareous shell is often dissolved due to preservation or is lost as the specimen reaches old age (Ev. Marcus 1984). In summary, species of Pleurobranchidae in general, and *Pleurobranchus* in particular, must be described in detail to improve the morphological delimitation of species, enable re-evaluation of

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the traditional characters or uncover novel ones useful in taxonomy. Recently, Goodheart et al. (2015) provided a phylogenetic framework for the classification of *Pleurobranchus*, based on the mitochondrial genes *cytochrome c oxidase I* (*COI*) and 16S rDNA and the nuclear gene *histone* 3 (*H3*) using Bayesian and maximum likelihood approaches, trying to elucidate delimitation of species of *Pleurobranchus*. This paper concluded that many traditionally well-known species of *Pleurobranchus* are genetically identical and should be considered synonyms.

Knowledge of the taxonomy of *Pleurobranchus* from Brazil is limited, and the internal anatomy and possible intraspecific variation are little understood. The original descriptions are somewhat brief (*e.g.*, Ev. Marcus 1984), and subsequent records of species merely expanded their geographic distribution without adding new morphological information (*e.g.*, Ev. Marcus 1976a, 1977, 1984; García et al. 2002; Padula et al. 2012).

García et al. (2008) recognized five species of Pleurobranchus from Brazil: P. emys Ev. Marcus, 1984, Pleurobranchus iouspi Ev. Marcus, 1984, Pleurobranchus evelinae Thompson, 1977, P. atlanticus Abbott, 1949 and P. areolatus Mörch, 1863. Rios (2009) listed four species, including all those listed by García et al. (2008), except P. evelinae. Goodheart et al. (2015), based on molecular data, concluded that the morphotype traditionally identified as Pleurobranchus areolatus from Brazil is, in fact, P. reticulatus Rang, 1832, a species originally described from the Gulf of Guinea; and, the morphotype from Brazil determined as P. atlanticus should be named P. areolatus, once P. atlanticus is considered junior synonym of P. areolatus by them. Goodheart et al. (2015) also suggested that P. iouspi could be a synonymy of P. testudinarius Cantraine, 1835.

Misinterpretations and erroneous determinations are frequently present in the literature (e.g., García et al. 2008; Rios 2009). This paper presents a revision of the species of *Pleurobranchus* from Brazil, with re-descriptions of the valid species based on detailed anatomical data, with the aim of improving the taxonomical delimitation of these species.

MATERIALS AND METHODS

Most of the studied material was collect between 2006 and 2013 in Brazil by the authors

and collaborators. All material collected was deposited in the Malacological Collection of Museu Nacional / Universidade Federal do Rio de Janeiro (MNRJ). Additionally, material previously deposited were also examined in the following collections: Malacological Collection of Museu de Zoologia, Universidade de São Paulo, Brazil (MZSP); Malacological Collection of the Museu Nacional, Universidade Federal do Rio de Janeiro, Brazil (MNRJ); Zoological Collection of Seção de Assistência ao Ensino, Museu Nacional, Universidade Federal do Rio de Janeiro, Brazil (SAE ML); Malacological Collection Prof. Henry Ramos Matthews, Universidade Federal do Ceará, Brazil (CMPHRM); Museum of Victoria, Melbourne, Australia (MV); Muséum national d'Histoire naturelle, Paris, France (MNHN); Natural History Museum, London, United Kingdom (NHMUK).

Description of external morphology was based upon living and preserved specimens. Dissections and drawings of digestive system, including intrinsic and extrinsic muscles of odontophore, reproductive system, nervous system and circulatory system were performed under a stereomicroscope with a drawing tube.

The radula and jaw were cleaned in 10% potassium hydroxide, subsequently rinsed in water and mounted for examination in a JEOL JSM-6390LV scanning electron microscope (SEM).

The nomenclature used to name the portions of the gill follows Willan (1983) and the odontophore muscles follows Ponder et al. (2008: 350-351, figs. 13.9-13.10), with additional information added by suffixes (d: dorsal; v: ventral). In the nervous system, when the nerve bifurcates, the number of the nerve is followed by letters, *e.g.* "a", "b".

In the lists of examined material, the number inside brackets indicates the number of specimens in each lot, followed by the number of specimens dissected. Additionally, *Pleurobranchus testudinarius* Cantraine, 1835 (Museum Victoria F85659) were examined for comparison.

RESULTS

Superfamily Pleurobranchoidea Gray, 1827 Family Pleurobranchidae Gray, 1827 *Pleurobranchus* Cuvier, 1804

Pleurobranchus Cuvier, 1804: 163. Type species by monotypy: Pleurobranchus peronii Cuvier, 1804.

Oscanius Leach, 1852: 29. Type species by original

designation: Oscanius argentatus Leach, 1852 (= Lamellaria membranacea Montagu, 1815).

Susania Gray, 1857: 202. Type species by original designation: Pleurobranchus testudinarius Cantraine, 1835.

Oscaniella Bergh, 1897: 94. Type species by monotypy: Oscaniella purpurea Bergh, 1897.

Description: (adapted from Willan 1987; Thompson 1970; Ev. Marcus 1984; present study): Relatively large adults (up to 210 mm; cf. Willan 1987); cleft in anterior mantle border; mantle bearing tubercles varying shape, size, pointed to conical; gill rachis with double row of alternate tubercles; male opening surrounded by non-retractile external sheath; anal opening lies over the end of gill membrane; jaw elements with three or more denticles on either side of the main cuspid; radula teeth simple, occasionally which an extra denticles; odontophore muscles: mj, muscle surrounding jaws, and pair of m4, main dorsal tensor muscle of radula, well developed; m4 originating in lateral region of cartilages, surrounding them ventrally, inserting into subradular membrane; pair of m5, secondary dorsal tensor muscle of radula, covering median portions of cartilage, extending up to dorsal region and inserting laterally in mj; m7 absent; one pair of m10d originating in mo, inserting into m4; ventrally, pair of m10v, protractor muscle of odontophore, joined before connecting posterior portion of canal oral with ventral portion of m4: pair of strong retractor muscles originates in most posterior portion of m5; single auxiliary muscle m10a, ventral tensor muscle of radula, originating in anterior portion of oral canal, running in middle of buccal mass, inserting into radular sac.

Remarks: In view of the long taxonomic history of the genus *Pleurobranchus*, Willan (1987) believed that a relatively large number of apomorphic traits led some investigators to split *Pleurobranchus* into several genera (*Oscaniella*, *Oscanius*, *Susania*), based on one or a few of these traits. These generic names are currently considered as junior synonyms of *Pleurobranchus* (Willan 1987; Cervera et al. 2000; Martynov and Schrödl 2009).

Oscanius was mentioned by Gray (1847a: 163, 1847b: 268), without description. A few years later, Leach (1852) provided a brief description in a posthumous paper published by Gray with the permission of Leach's family, because when the work of Leach was being readied for printing the author was prevented from completing it by ill health in 1820. *Oscanius* Gray, 1847 is not available because it does not meet the

requirements of Article 12.1 of the International Code of Zoological Nomenclature (ICZN 1999), that in order to be available, every new name published before 1931 must be accompanied by a description or a definition of the taxon that it denotes, or by an indication. Therefore, the author of the genus *Oscanius* is Leach (1852).

Pleurobranchus reticulatus Rang, 1832 (Figs. 1A-B; 2-6)

Pleurobranchus reticulatus Rang, 1832: pl. 1; Pilsbry (1896: 216); Vayssière (1898: 354); Cervera et al. (1996: 154); Neves et al. (2007: 265, figs. 1-3); Goodheart et al. (2015: 338; figs. 7A-E).

Pleurobranchus areolatus auct. non Mörch, 1863: Edmunds (1968: 85, figs. 2-3); Ev. Marcus (1976a: 16; 1977: 9; 1984: 60, figs. 1, 34-43); Rios (1994: 206, pl. 69; 2009: 417); García et al. (2002: 50, fig. 2H); García et al. (2008: 94); Troncoso et al. (2009: 409, figs. 17.2h; 17.6); Padula et al. (2012: 3, fig. 5D); Pereira et al. (2014: 1); Padula et al. (2014: 3, fig. 1B).

Type material: Type material of *P. reticulatus* is not known to exist, not found at MNHN (Valdés and Héros, 1998; Goodheart et al. 2015).

Type locality: Gulf of Guinea.

Material examined: Brazil: Alagoas state: Recife da Jatiuca: MNRJ 13104, 22/xi/2003, M. Dorigo coll. [1; 1 dissected]; Saco de Pedra: MNRJ 12928, 12/i/2008, V. Padula coll. [3]; Bahia state: Guarapuá: Ilha de Tinharé: MNRJ 13290. xii/2008. P. M. S. Costa coll. [1]; Rio de Janeiro state: Cabo Frio: MZSP 75475, Marcus coll. [1]; MZSP 119938 [1 microscopic slide with radula and jaw platelets]; MZSP 119939 [1 microscopic slide with radula and jaw platelets]; Praia das Conchas: MZSP 50335, L. R. L. Simone coll. [1]; MZSP 97328, xii/2007, V. Padula coll. [2]; Enseada do norte: MNRJ 18223, 30/ii/2008, V. Padula coll. [1; 1 dissected]; Arraial do Cabo: Prainha: MNRJ 10844, ii/1984, G. Nunan & M. R. Sá coll. [4; 2 dissected]; MNRJ 14468, ii/1984, G. Nunan & M. R. Sá coll. [1, 1 dissected]; Praia do Forno: MNRJ 11710, 23/vi/2007, J. Alvim & V. Padula coll. [3]; MNRJ 12055, 20/x/2007, J. Alvim coll. [1; 1 dissected]; Angra dos Reis: MZSP 85984 [1]; Ilha Grande: MZSP 84228 ii/2007, L. R. L. Simone coll. [1]; São Paulo state: Laje de Santos: MZSP 90723, ii/2003, C. M. Cunha coll. [2].

Specimen records (Fig. 2): Ghana (Edmunds 1968); Gulf of Guinea (Rang 1832); English Bay and Soudan Bay, Ascension islands Padula et al. (2014); Brazil: Fernando de Noronha (García et al. 2002); Maranhão state (Ev. Marcus 1984); Alagoas state (Padula et al. 2012); Bahia state

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(present study); Rio de Janeiro state: Cabo Frio (Ev. Marcus 1977, 1984) and Arraial do Cabo (Ev. Marcus 1976a); São Paulo state: Laje de Santos (present study).

Description: External morphology (Figs. 1A-B; 3A): Living specimens yellowish-orange with tubercles of many colors: dark red, red, bright orange and pale orange; space inter-tubercles white; mantle edge orange; red rhinophores with white ring-shaped ornamentation; oral veil white with orange marks and orange edge; foot dorsally white with orange blotches and edge. Living specimens up to 80 mm in length; length of preserved specimens 42-57 mm; width 26-34 mm; length of foot 20-56 mm; width of foot 32-39 mm. Body subquadrate, slightly depressed. Mantle covered foot partially. Mantle surface covered by many large rounded/oval/hexagons tubercles (1-2 mm in diameter) of different sizes and irregularly arranged; generally bigger and more concentrated in middle of mantle than at mantle edge. Oral veil broad connected with head region; laterally, oral tentacles with deep notch over almost its length. Rhinophores rolled joined at their bases, up to one third of the length. Gill exposed laterally (Fig. 3A); 2/3 length of body; main rachis with rounded tubercles at point of origin of pinnae, forming zig-zag line (Fig. 3A); small rounded



Fig. 1. Living species of *Pleurobranchus* from Brazil. (A-B) *Pleurobranchus reticulatus* Rang, 1832. (A) adult (MNRJ 11710, 28 mm long alive). (B) juvenile (MNRJ 11710, 5 mm long alive). (C-D) *Pleurobranchus areolatus* Mörch, 1863 (MNRJ 18760, 27 mm long alive), photographs by V. Padula. (C) dorsal view. (D) ventral view. (E-F) *Pleurobranchus iouspi* Ev. Marcus, 1984. (E) bright yellow morphotype (MNRJ 33070, 99 mm length of preserved specimen), photograph by C. A. Rangel. (F) reddish morphotype (SAE ML 95, 75 mm length of preserved specimen), photograph by F. Moraes.

tubercles at base of pinnae; bipinnate pinnae; 18-30 pinnae; 12-16 pinnae free from the body wall, attached by branchial membrane. Anal opening lying over end of gill membrane (Fig. 3A). Prebranchial pore opening approximately beside main rachis (Fig. 3A). Nephropore under second pinnae. Genital aperture surrounded by anterior flaps (Fig. 3A). Penis semi-internal, never completely internal when retracted. Foot round at posterior end; foot sometimes projecting beyond notum. Metapodial gland elongated, 1/7 times foot length; anteriorly bilabiated, upper lip notched, smaller than lower one.

Mantle (Figs. 4A-C): Mantle covered by calcareous spicules. Two types of spicules in mantle: linear, rod-like (length: 70.0-200.0 μ m; thickness: 7.4-10.0 μ m) (Fig. 4A); stellate (ray length: 17.3-27.2 μ m; ray thickness: 4.2-5.5 μ m) (Fig. 4B). Additionally, some elongated, but irregular (a not well definite shape) spicules grouped in some parts of mantle (length, difficult to evaluated because of shape, this measure refers to the distance, straight, between two ends: 60.0-120.0 mm; thickness: 10.0 μ m) (Fig. 4C).

Shell (Figs. 5A-B): Yellowish-cream,

decalcified, flattened and fragile; two times longer than wide; brownish periostracum; not observed in some specimens; subquadrangular in outline. Length 4.6 mm, width 2.1 mm (in a preserved specimen with 52 mm in length); Length: 6.6 mm, width 3.3 mm (in a preserved specimen with 57 mm in length); and, length 3.9 mm, width 2.0 mm (in a preserved specimen with 42 mm in length). Spire has 1.5 whorls. Protoconch smooth. Lines of growth distinct; shell sculptured with longitudinal lines transverse to growth lines immediately after protoconch; anterior portion of last whorl only the lines of growth are recognizable. Shell above heart, on right side of blood gland.

Circulatory system: Pericardium well developed in anterior portion of body (near cerebro-pleural ganglia). Blood flowing into auricle from gills, kidney and venous sinuses. Efferent branchial vessel connecting the gill with auricle. Auricle on right side, ventricle on left; auricle with thin wall; ventricle muscular. Blood gland small, creamy covering left part of pericardium. Blood gland close or joined to aorta.

Digestive system (Figs. 5C-F; 6A-F): Transverse mouth in middle of snout tip. Oral



Fig. 2. Map of distribution of *Pleurobranchus reticulatus* Rang, 1832, *Pleurobranchus areolatus* Mörch, 1863 and *Pleurobranchus iouspi* Ev. Marcus, 1984. Type localities indicated by stars.



Fig. 3. *Pleurobranchus reticulatus* Rang, 1832. (A) lateral view, detail near gill (MNRJ 13104). (B-D) reproductive system. (B) (MNRJ 18223). (C) detail of connections of reproductive system (MNRJ 10844). (D) penis dissected longitudinally (MNRJ 18223). (E) nervous system (MNRJ 12055). Abbreviations: a, anus; am, ampulla; bc, buccal ganglion; bu, bursa copulatrix; ccpb, connective between buccal and cerebro-pleural ganglia; cp, nerves that leave from the cerebro-pleural ganglion; cpg, cerebro-pleural ganglion; cpp, commissure between the pedal ganglia; dd, deferent duct; e, eye; f, female opening; fg, female gland; gi, gill; gm, gill membrane; nb, nerves that leave from the buccal ganglion; ov, oviduct; p, penial papilla; pb, prebranchial aperture; pf, penial flap; pg, pedal ganglion; pr, prostate; rg, rhinophoral ganglion; rn, rhinophoral nerve; sr, seminal receptacle; tu, tubercles; va, vagina.

canal muscular just posterior to mouth (Fig. 6A). Muscle surrounding jaws (mj) strong, pair of large jaws located in its inner surface; mj originating in lateral and dorsal surfaces of oral canal, inserting into lateral and dorsal regions of buccal mass (Figs. 6A-C). Jaws amber, lighter posteriorly; jaw of two plates surrounding radula inside buccal cavity (Fig. 6D); elongated, reaching level of radula. Each jaw plate showing alternate rows formed by elongated elements with slight cruciform lateral expansion (preserved specimen measuring 42 mm in length: 39 elements transversally, 59 elements longitudinally) (Fig. 6C); elements consist on a main cusp with 2-6 denticles of different sizes in each side, usually five (Fig. 6C); anterior elements worn, denticles sometimes unclear. Pair of jugal muscle m1v inserting into m5. Pair m4, main dorsal tensor muscle of radula,



Fig. 4. *Pleurobranchus reticulatus* Rang, 1832, spicules (MNRJ 12928). (A) linear, rod-like. (B) stellate. (C) irregular spicules (a not well definite shape).

well developed, originating in lateral region of cartilages, surrounding them ventrally; inserting into subradular membrane (Figs. 6A-C). Pair m5, secondary dorsal tensor muscle of radula, covering median portions of cartilage, extending up to dorsal region; originating in posterior surface of cartilages; inserting laterally in mj (Figs. 6A-C). Pair m7 absent. Pair m10d, originating in posterior portion of oral canal; inserting into m4 (Figs. 6B-C). Pair m10v, protractor muscle of odontophore, connected posterior portion of canal oral with ventral portion of m4 (Fig. 6A). Single auxiliary muscle m10a, ventral tensor muscle of radula, originates in anterior portion of oral canal, running in middle of buccal mass and inserting into radular sac (Fig. 6A). Pair of strong retractor muscles originates in most posterior portion of m5 (Figs. 6A, C); it is separated in 3/4 its total length and jointed in its posterior portion, lying above anterior portion of digestive gland. Odontophore cartilage subguadrate in outline (Fig. 6F). Radula cream; formula 69 × 165.0.165 (from preserved specimen 42 mm length). Innermost lateral tooth with enlarged base and hook-shaped cusp curved towards to base of tooth (Fig. 5D). Lateral teeth hook-shaped, larger and more developed in center of rows (Fig. 5E). Outermost lateral teeth less developed (Fig. 5F). Aperture of acid gland located between jaw plates (Figs. 6D-E). Duct of acid gland thin (same width as salivary duct) (Figs. 6B-C), passing within nerve ring. Acid gland slightly ramified, restrict to anterior portion of digestive organs. Esophagus tube-like passing into voluminous stomach (Figs. 6A-B, E). Salivary gland small and in front of digestive gland. Ducts of salivary glands entering pharynx musculature laterally to esophagus, opening into base of pharyngeal cavity between radula and jaw plates (Fig. 6D); not convoluted; without a visible ampullae. Stomach elongated and with thickened wall, it could be in different states of expansion (Figs. 6A-B, E); internally, could have some folds, wrinkled wall; posteriorly smooth, except by the ventral furrow that leads to intestine (Fig. 6E); stomach passes ventrally into digestive gland. Intestine short opening under gill membrane into anus; thin wall; internally, longitudinal folds near its apertures. Salivary, digestive and hermaphrodite glands forming a single aggregate (Figs. 6A-B).

Reproductive system (Figs. 3B-D): Triaulic. Ampulla elongated, three times wider than deferent duct; convoluted. Spermoviduct branching into two ducts, shorter oviduct and other duct leading to prostate. Prostate tubular and extremely convoluted; eight times wider than deferent duct. Deferent duct long, thin and convoluted (Figs. 3B-C), narrowing into a non cuticular and semicontractile penis (Fig. 3C). Penis (in a preserved specimen with 42 mm length: length 8.5 mm; width 3.5 mm) (Figs. 3C-D); convex portion of penis with



Fig. 5. *Pleurobranchus reticulatus* Rang, 1832, SEM. (A-B) shell (MNRJ 12055). (B) detail near the protoconch. (C) elements of the jaw (MNRJ 18223). (D-F) radula (MNRJ 18223). (D) innermost lateral teeth. (E) lateral teeth. (F) outermost lateral teeth.



Fig. 6. *Pleurobranchus reticulatus* Rang, 1832, digestive system. (A) ventral view (MNRJ 18223). (B) dorsal view (MNRJ 18223). (C) dorsal view, detail of posterior portion with esophagus deflected (MNRJ 10884). (D-E) foregut sectioned longitudinally from ventral side (MNRJ 18223). (F) odontophoral cartilages (MNRJ 18223). Abbreviations: a, anus; ag, duct of acid gland; bc, buccal ganglion; ca, oral canal; dg, digestive gland; hg, hermaphrodite gland; j, jaw plates; in, intestine; m1, jugal protractor muscle of odontophore; m4: main dorsal tensor muscle of radula; m5, accessory dorsal tensor muscle of radula; m10, protractor muscle of odontophore; m10a, ventral tensor muscle of radula; mj, jaw muscle; mo, mouth; mr, retractor muscle; oa, opening of the duct of the acid gland; oe, esophagus; ra, radula; rs, radula sac; sd, salivary duct; sg, salivary gland; so, opening of the duct of the salivary gland in the oral membrane; st, stomach.

a wide leaflet (Figs. 3C-D); evident penial sheath, enlargement near gonopore (Fig. 3C). Vaginal duct slightly convoluted with two allosperm vesicles arranged semi-serially (Fig. 3C). Proximally, vagina divides into seminal receptacle and bursa copulatrix as a short duct, both allosperm receptacles lying close to each other, their stalks are crossed (Fig. 3C). Seminal receptacle shortstalked; elongated; highly convoluted (Fig. 3C). Bursa copulatrix rounded. Vaginal opening immediately ventral to penis. Vagina about three times wider than deferent duct; became wider near female aperture (Fig. 3C). Semimal receptacle approximately same wider than prostate. Genital aperture surrounded by anterior flaps, normally two (Fig. 3B).

Nervous system (Fig. 3E): Nerve ring above oral canal. Cerebral and pleural ganglia fused. Eyes located latero-centrally of cerebro-pleural complex; eyes borne upon very short optical nerves. Rhinophoral ganglia placed at bases of rhinophores, near cerebro-pleural ganglia; two main nerves leave from rhinophoral nerves, these runs until distal portion of rhinophores; rhinophoral nerves with many secondary nerves, perpendicular in relation of main nerves. Nerves leaving cerebro-pleural ganglia: cp1 inserting latero-ventrally, almost into foot; cp2 bifurcating near base, both cp2a and cp2b inserting dorsally into mantle; cp3 runs laterally until inserts into body wall, in right side nerve enters into mantle near the base of gonopore and, in left side nerve enters into mantle near anterior portion of digestive gland; cp4 runs until the most posterior portion of body; cp5 very short nerve, located in middle of ganglia, inserting dorsally into mantle; cp6 thin nerve, inserting into mantle dorsally; cp7 thin nerve, innervating latero-ventral side of body wall; cp8 innervates female gland mass. Connective between visceral and cerebro-pleural ganglia very short, almost imperceptible. Connective between buccal and cerebro-pleural ganglia leads from ventral view of cerebro-pleural ganglia, in the most posterior portion of ganglion. Nerves leaving buccal ganglia, in antero-posterior order: nb1 bifurcating near origin, both nb1a and nb1b inserts into esophagus; nb2 inserting into salivary ducts; connective cerebro-pleural-buccal shortly after nb2; nb3 bifurcating near origin, anterior branch (nb3a) inserting into m4; posterior branch (nb3b) leading to m5; nb4 inserting into radular sac. Connective cerebro-pleural-pedal extremely short and it can only be seen after carefully dissecting. Pedal commissure short and leaving from the most

anterior posterior of pedal ganglion. Pedal ganglia smaller than cerebro-pleural complex, in anteroposterior order: np1 inserting ventrally into oral veil; np2 inserting anteriorly into foot; np3 innervates foot; np4 inserting ventrally into foot and runs until the most posterior portion of body.

Pleurobranchus areolatus Mörch, 1863 (Figs. 1C-D; 2; 7-10)

- Pleurobranchus areolatus Mörch, 1863: 28; Pilsbry (1896: 199);
 Vayssière (1898: 338); Ev. Marcus and Er. Marcus (1962: 466, figs. 14-15; 1963: 25; 1967: 44; 1967: 163, fig. 19);
 Er. Marcus and Ev. Marcus (1970: 55); Keen (1971: 811, fig. 16, pl. 19); Abbott (1974: 347); Thompson (1977: 108, figs. 12c-d; 13a-b); Cervera et al. (2004: 32); Ardila and Rachello (2005: 60, fig. 2); Espinosa et al. (2005: 63);
 Valdés et al. (2006: 113); Redfern (2013: 295, figs. 812A-B); Goodheart et al. (2015: 343, figs. 18A-G, 19C-F, 20-22).
- Oscaniella areolata: Bergh (1897: 111, figs. 31-41, pls. 9).
- Pleurobranchus crossei Vayssière, 1897: 353, fig. 1; Vayssière (1898: 332, figs. 148-154); Valdés et al. (2006: 114); Redfern (2013: 295, figs. 813B-E); Ortea et al. (2014: 120, figs. 1B-C, 3A-B). Synonymized by Thompson (1977).
- Pleurobranchus (Susania) atlanticus Abbott, 1949: 73, figs. 1-10, pl. 5. Synonymized by Thompson (1977).
- Pleurobranchus atlanticus: Padula et al. (2012: 3, fig. 5E).
- Susania gardineri White, 1952: 106, figs. 2-5, pl. 6, fig. 1. Synonymized by Ev. Marcus (1984).
- *Pleurobranchus reesi* White, 1952: 107, figs. 3, 6, pl. 6, fig. 2. Synonymized by Goodheart et al. (2015).
- Pleuroranchus evelinae Thompson, 1977: 108, figs. 12E-F, 13C-E; Ev. Marcus (1984: 63, figs. 44-50, in part); Redfern (2001: 167, pl. 72, fig. 691E-F); Espinosa et al. (2005: 63); Valdés et al. (2006: 112); Ortea et al. (2014: 122, figs. 1D, 2). Synonymized by Goodheart et al. (2015).

Type material: Not located in Natural History Museum of Denmark and Natural History Museum.

Type locality: St. Thomas, United States Virgin Islands.

Material examined: Syntypes of *P. reesi*: NHMUK 1934.9.11.102-104, Bird Key reef, Dry Tortugas, G. Tandy and J.S. Colman leg. [3]. Holotype of *P. evelinae*: NHMUK 19773W, Discovery Bay, Jamaica, T.E. Thompson coll. [1]. Holotype of *P. crossei*: MNHN-IM-2000-30102, Caribbean Sea [1]. Syntypes of *P. gardineri*: NHMUK 1934.9.11.99-101, Dry Tortugas, G. Tandy and J.S. Colman leg. [3]. Colombia: MZSP 119937, K. Bandel leg. [1 microscopic slide with radula and jaw platelets]; Brazil: Alagoas state: Galés de Maragogi: MNRJ 18760, 29/xii/2007, V. Padula coll. [1; 1 dissected].

Specimen records (Fig. 2): Florida (Abbott 1949; White 1952; Ev. Marcus and Er. Marcus 1962; 1967); Puerto Rico (Er. Marcus and Ev.

Marcus 1970; Valdés et al. 2006); Virgin Islands: St. Thomas (Mörch 1863; Ev. Marcus and Er. Marcus 1962; Valdés et al. 2006); Mexico (Ev. Marcus and Er. Marcus 1967; Valdés et al. 2006); Bahamas (Redfern 2001, 2013; Valdés et al. 2006); Goodheart et al. 2015); Cuba (Espinosa et al. 2005, 2007), Guadeloupe and Martinique (Ortea et al. 2014); Jamaica (Thompson 1977; Valdés et al. 2006); Curaçao (Ev. Marcus and Er. Marcus 1963; Er. Marcus and Ev. Marcus 1970); Aruba, Costa Rica, Bermuda, St. Marteen/St. Martin, Venezuela (Valdés et al. 2006); Panama (Valdés et al. 2006; Goodheart et al. 2015); Colombia (Ardila and Rachello 2005); Brazil: Alagoas (Padula et al. 2012); Canary Islands (Ortea et al. 2014); Madeira (Cervera et al. 2004). Specimens recorded from Pacific Ocean (Ev. Marcus and Er. Marcus 1967; Bertsch and Smith 1973; Bertsch 1979) under the name *P. areolatus* may in fact be this species, but judgment must be reserved at present.



1mm

Fig. 7. *Pleurobranchus areolatus* Mörch, 1863 (MNRJ 18760). (A) dorsal view, dotted line indicates the position of the shell internally. (B) ventral view. (C) lateral view, detail near gill. (D) reproductive system. (E) nervous system. Abbreviations: a, anus; am, ampulla; bc, buccal ganglion; bu, bursa copulatrix; ccpb, connective between buccal and cerebro-pleural ganglia; cp, nerves that leave from the cerebro-pleural ganglion; cpg, cerebro-pleural ganglion; cpp, commissure between the pedal ganglia; dd, deferent duct; dg, digestive gland; e, eye; f, female opening; fg, female gland; fo, foot; gi, gill; go, gonopore; nb, nerves that leave from the buccal ganglion; np, nerves that leave from the pedal ganglion; ot, oral tentacle; ov, oviduct; mg, metapodial gland; ne, nephropore; pb, prebranchial aperture; pg, pedal ganglion; pr, prostate; rg, rhinophoral ganglion; rh, rhinophore; rn, rhinophoral nerve; se, shell; sr, seminal receptacle; sta, statocyst; va, vagina.

Description: External morphology (Figs. 1C-D; 7A-C): External morphology of *P. areolatus* is very similar to P. reticulatus as described above, with the following exceptions: Living specimens white with brownish-red blotches on dorsum; some tubercles with pale-orange tips; mantle edge orange; rhinophores with dark red, orange and white blotches; rhinophoral apex white; oral veil same color pattern of mantle; ventrally, foot translucent white with its edge with orange and white alternate pigment (Fig. 1D). Living specimen 27 mm in length. Preserved specimen: length 12 mm; width 8 mm; length of foot 11 mm; width of foot 6 mm. Mantle covered foot entirely. Mantle surface covered by pointed low tubercles (125-375 μ m in diameter); tubercles at mantle edge smaller in diameter, higher and more concentrated than middle of mantle; irregularly arranged. Gill 2/3 length of body; main rachis with tiny rounded



Fig. 8. Pleurobranchus areolatus Mörch, 1863 (MNRJ 18760), spicules. (A) linear, rod-like. (B-C) stellate.

tubercles at point of origin of pinnae, forming zigzag line; 19 pinnae; 9 pinnae free from body wall, attached by branchial membrane. Pre-branchial pore opening approximately beside main rachis (Fig. 7C). Nephropore lying above female aperture. Genital aperture surrounded by a thin fold. Penis not protruded (Fig. 7C). Foot posteriorly with small white semi-circled metapodial gland (Fig. 7B); metapodial gland 1/20 foot length.

Mantle (Figs. 8A-C): Mantle covered by sparse spicules, only in some parts of mantle it was found. Two types of spicules in mantle: linear, rod-like (length: 70.0 μ m -160.5 μ m, mainly between 70.0 μ m 90.0 μ m; thickness: 5.0 μ m) (Fig. 8A) and stellate with four or five irregular rays (ray length: 70.0 μ m- 112.5 μ m; ray thickness: 10.0 μ m) (Figs. 8B-C). Rod-like spicules calcareous; stellate spicules composed of an unidentified organic matrix.

Shell (Figs. 7A; 9A-B): White to brownish with light golden tones in some parts of shell, visible according to incidence of light; subquadrangular in outline. Length 3.4 mm, width 2.3 mm (in a specimen with 27 mm long alive); approximately 1.5 times longer than wide. Spire has 1.5 whorls. Protoconch smooth. Lines of growth distinct (Fig. 9B). Shell sculptured with longitudinal lines transverse to growth lines immediately after protoconch. Shell situated in middle portion of body, on the left side (Fig. 7A); partially above pericardium, blood gland and digestive gland.

Circulatory system: Circulatory system of *P. areolatus* identical to the *P. reticulatus* as described above.

Digestive system (Figs. 9C-F; 10A-G): Digestive system of P. areolatus very similar to P. reticulatus as described above, with the following exceptions: Each jaw plate showing alternate rows formed by elongated elements with cruciform lateral expansion (Fig. 9C); elements consist on a main cusp with 2-3 denticles in each side, sometimes asymmetrical (Fig. 9C). Pair of jugal muscle m1v absent. Pair m5, secondary dorsal tensor muscle of radula, large and broad, covering median portions of cartilage, extending up to dorsal region; originating in posterior surface of cartilages; inserting laterally in mj (Figs. 10A-C). Pair m10d (dorsal) absent. Pair of strong retractor muscles originates in most posterior portion of m5 (Figs. 10A, C, F); it is separated in almost its total length. Odontophore cartilage wider anteriorly than posteriorly (Fig. 10G). Radula translucent-white; formula 66 × 90.0.90 (in a specimen of 27 mm long alive). Outermost lateral teeth elongated, less developed (Fig. 10F). Aperture of acid gland located between jaw plates (Fig. 10E). Duct of acid gland thin (twice diameter of salivary duct)

and short (Figs. 10B-C); aperture located between jaw plates. Stomach elongated (Figs. 10A-B, E); internally, stomach wall thin without longitudinal



Fig. 9. Pleurobranchus areolatus Mörch, 1863 (MNRJ 18760), SEM. (A-B) shell; (B) detail near the protoconch. (C) elements of the jaw. (D-F) radula. (D) innermost lateral teeth; (E) lateral teeth. (F) outermost lateral teeth.



Fig. 10. *Pleurobranchus areolatus* Mörch, 1863 (MNRJ 18760), digestive system. (A) ventral view. (B-C) dorsal view. (C) detail of posterior portion with esophagus deflected. (D-E) foregut sectioned longitudinally from ventral side. (F) retractor muscle, which inserts in posterior portion of m5. (G) odontophoral cartilages. Abbreviations: a, anus; ag, duct of acid gland; bc, buccal ganglion; ca, oral canal; dg, digestive gland; hg, hermaphrodite gland; j, jaw plates; in, intestine; m4: main dorsal tensor muscle of radula; m5, accessory dorsal tensor muscle of radula; m10, protractor muscle of odontophore; m10a, ventral tensor muscle of radula; mj, jaw muscle; mo, mouth; mr, retractor muscle; oa, opening of the duct of the acid gland; oe, esophagus; ra, radula; rs, radula sac; sd, salivary duct; sg, salivary gland; so, opening of the duct of the salivary gland in the oral membrane; st, stomach.

folds; posteriorly smooth, except by a ventral furrow that leads to intestine (Fig. 10E); stomach passes ventrally into digestive gland. Intestine long opening under gill membrane into anus; thin wall; internally, longitudinal folds well-developed. Salivary, digestive and hermaphrodite glands forming a single aggregate (Figs. 10A-B).

Reproductive system (Fig. 7D): Triaulic. Ampulla elongated with same width as vas deferent and vagina; not convoluted. Spermoviduct branching into two ducts, oviduct other duct leading to prostate. Prostate tubular, sausage-like; proximal portion rounded; not convoluted; three times wider than deferent duct. Near gonopore without trace of any penial sheath. Penis not observed. Vaginal duct not convoluted with two allosperm vesicles arranged semiserially. Vaginal duct slightly convoluted with two allosperm vesicles arranged semiserially, both allosperm receptacles lying close to each other, their stalks are crossed. Seminal receptacle elongate; same wider than deferent duct. Bursa copulatrix bulky, rounded; connected with vagina approximately in its first 1/3. Vaginal opening immediately ventral to penis. Vagina about same diameter of deferent duct.

Nervous system (Fig. 7E): Nervous system of *P. areolatus* very similar to *P. reticulatus* as described above, except by the absence of cp6, cp7 and cp8.

Pleurobranchus iouspi Ev. Marcus, 1984 (Figs. 1E-F; 2; 11-17)

- Pleurobranchus iouspi Ev. Marcus (1984: 68, figs. 57-61); Rios (2009: 417).
- Oscanius (?) testudinarius auct. non Cantraine, 1835: Ev. Marcus (1970: 939, fig. 30).
- Pleurobranchus testudinarius auct. non Cantraine, 1835: Rios (1994: 206); Cunha et al. (2014: 47); Goodheart et al. (2015: 340, figs. 7H-I, in part).
- *Pleuroranchus evelinae* auct. non Abbott, 1949: Ev. Marcus (1984: 63, in part).
- Pleurobranchus (Susania) emys Ev. Marcus (1984: 70, figs. 57-61). syn. nov.
- Pleurobranchus emys: Rios (2009: 417).
- Pleurobranchus atlanticus auct. non Abbott, 1949: Rios (2009: 417).

Type material: Holotype, as five microscopic slides: MZSP 119936. Each microscopic slide presents one of the following features: radula (Fig. 11A); jaw (Fig. 11B); portions of mantle (Fig. 11C); unrecognizable parts of reproductive system (Fig. 11D); and penis (Figs. 11E-H).

Type locality: 24°47'S, 45°15'W; São Paulo State, Brazil.

Material examined: Holotype; Syntypes of P. emys: Colombia: Santa Marta: MZSP 119934 [one microscope slide with radula and jaw platelets]; MZSP 119935 [one histological microscope slide with sections of the mantle]. Brazil: Maranhão state: 2°05'S, 42°44'W: CMPHRM-A 693, 46 m; Rio de Janeiro state: Arraial do Cabo: Oratório: MNRJ 33242, 20/iii/2014, A. Kassuga coll. [2; 1 dissected]; Rio de Janeiro: Cagarras Archipelago: Redonda Island: MNRJ 10708, P. S. Young & C. S. Serejo coll. [1]; MNRJ 10707, P. S. Young & C. S. Serejo coll. [1]; SAE ML 95, 04/iv/2012, 15 m, F. Moraes coll. [1; 1 dissected]; Rasa Island: MNRJ 33069, 07/iii/2014, 7 m, F. Moraes coll. [1; 1 dissected]; Maricá: Maricás Islands: MNRJ 33070, 20/ii/2014, Carlos Rangel and Jéssica Pinho coll. [1; 1 dissected].

Specimen records (Fig. 2): Colombia: Santa Marta (Ev. Marcus 1984); Brazil: Maranhão state (Ev. Marcus 1970); Rio de Janeiro state (Goodheart et al. 2015; present study); São Paulo state (Ev. Marcus 1984); Santa Catarina state (Cunha et al. 2014).

Redescription: External morphology (Figs. 1E-F; 12A-C): Coloration of living specimens with highly variable, with two main types. First type completely pale orange or bright yellow (Fig. 1E), sometimes with white marks; some specimens with a bright pink ring surrounding pointed (high) tubercles; mantle edge completely orange or white; rhinophores, tentacles, gill and foot same color pattern of mantle. Second color type is reddish (red, orange, dark red) (Fig. 1F); space between tubercles white; pointed (high) tubercles usually with darker pigment (dark red), sometimes a bright pink ring surrounding it; mantle edge same pattern of rest of body or white; rhinophores, tentacles, gill and foot same color pattern of mantle. Length of preserved specimens 51-99 mm; width 46-73 mm; length of foot 34-74 mm; width of foot 16-58 mm. Body subquadrate, slightly depressed, narrows posteriorly. Mantle covered foot partially, sometimes projecting beyond notum. Mantle covered by medium to large tubercles (1-7 mm) irregularly arranged all around dorsum; tubercles shape can be rounded or pointed (high); usually at mantle edge is located tubercles with lower diameter; pointed tubercles usually located in middle of dorsum (Fig. 1F); tubercles can be contracted when specimen is disturbed. Oral veil sturdy and broad that connects with head region; laterally, oral tentacles with a deep notch over almost its length. Rhinophores rolled and joined at their bases, up to one quarter of its length.

Gill exposed laterally (Fig. 12A); 1/2 length of body; main rachis with rounded tubercles at point of origin of pinnae, forming zig-zag line; small

rounded tubercles at base of pinnules; tubercles composed by spicules; tripinnate pinnae; 18-24 pinnae; 7-12 pinnae free from the body wall,



Fig. 11. Microscopic slides of the holotype of *Pleurobranchus iouspi* Ev. Marcus, 1984 (MZSP 119936); for discussion about slides's label, see text. (A) radula. (B) jaw platelets. (C) portions of the mantle. (D) unrecognizable parts of reproductive system. (E) penis. (F-H) details of the penis. Abbreviations: bb, basal bulb; dd, deferent duct; p, penial papilla; pe, penis; pr, prostate.



Fig. 12. *Pleurobranchus iouspi* Ev. Marcus (1984). (A) lateral view, detail near gill (MNRJ 33070). (B-C) detail of gonopore. (B) view from the top (MNRJ 33069). (C) lateral view (SAE ML 95). (D-E) reproductive system deflected; (D) (MNRJ 33070). (E) (MNRJ 33069). (F) penis dissected longitudinally (SAE ML 95). (G) nervous system (SAE ML 95). Abbreviations: a, anus; am, ampulla; bc, buccal ganglion; bu, bursa copulatrix; ccpb, connective between buccal and cerebro-pleural ganglia; cp, nerves that leave from the cerebro-pleural ganglion; cpg, cerebro-pleural ganglion; cpp, commissure between the pedal ganglia; dd, deferent duct; e, eye; f, female opening; fg, female gland; gi, gill; go, gonopore; gm, gill membrane; nb, nerves that leave from the buccal ganglion; np, nerves that leave from the pedal ganglion; ov, oviduct; p, penial papilla; pb, prebranchial aperture; pf, penial flap; pg, pedal ganglion; pr, prostate; rg, rhinophoral ganglion; rh, rhinophore; rn, rhinophoral nerve; sr, seminal receptacle; tu, tubercles; va, vagina.

attached by branchial membrane. Anal opening lying over end of gill membrane. Pre-branchial pore opening approximately beside main rachis. Nephropore lying under/near first pinnae or between first and second pinnae. Elongated penis with 21.6 mm in length (in a preserved specimen with 51 mm in length) and 27 mm in length (in a preserved specimen with 75 mm in length); penis and female aperture surrounded by a very wide sheath (0.9 mm high) composed of three flaps (Figs. 12A-C). Posteriorly rounded foot with elongated metapodial gland, corresponding 1/7 to 1/4 of body length; anteriorly bilabiated, upper lip notched and smaller than the other one.

Mantle (Figs. 13A-D): Mantle, tubercles, rhinophores and dorsal portion of oral veil densely covered by spicules, which makes it seems coriaceous. Two types of spicules: linear, rod-like (length: 314.0-427.2 µm; thickness: 13.0-16.0 µm) (Fig. 13A); stellate spicules with three similar rays (ray length: 13.8-18.9 µm) (Fig. 13B) and with five rays, four of which are arranged in same plane (ray length: 45.4-102.2 µm; ray thickness: 22.7-34.0 µm) (Fig. 13C). Mantle densely covered by spicules with five rays (Fig. 13D); perpendicular ray usually smaller than others rays (ray length: 45.4-56.8 μ m). Rod-like and stellate spicules with three rays calcareous; spicules with five rays partially calcareous, but not entirely formed by calcium carbonate. As sodium hypochlorite dissolve it, also composed of an unidentified organic matrix.

Shell (Fig. 14A): White to brownish with light golden tones in some parts of shell, depending on incidence of light; fragile; brownish periostracum; not observed in some specimens; subquadrangular in outline. Shell approximately 1.5 times longer than wide; length 2.2 mm, width 1.6 mm (in a preserved specimen with 51 mm in length); and, length: 6.6 mm, width 3.7 mm (in a preserved specimen with 75 mm in length). Spire has 1.5 whorls. Protoconch smooth. Growth lines distinct; sculptured with longitudinal lines transverse to growth lines. Shell in anterior/left portion of body, immediately anterior to heart, partially above pericardium and blood gland.

Circulatory system: Circulatory system of *P. iouspi* identical to the *P. reticulatus* as described above.

Digestive system (Figs. 14B-F; 15-16): Digestive system of *P. iouspi* very similar to the *P. reticulatus* as described above, with the following exceptions: Each jaw plate showing alternate rows formed by elongated elements with slight cruciform lateral expansion (preserved specimen measuring 51 mm in length: 30 elements transversally, 50 elements longitudinally) (Figs. 14B-C) (Holotype: 43 elements transversally, 60 elements longitudinally); elements consist on a main cusp with 1-5 denticles in each side (Figs. 14B-C) (Holotype with 2-5 denticles in each side), which could be of different sizes and asymmetrical



Fig. 13. *Pleurobranchus iouspi* Ev. Marcus, 1984, spicules. (A) linear, rod-like (MZSP 119936). (B-D) stellate spicules (SAE ML 95). (B) three similar rays. (C) five rays, which four of them in the same plane. (D) transversal cut in the mantle.

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in relation to main cusp (Figs. 14B-C). Pair of m4, main dorsal tensor muscle of radula, short, originating in lateral region of cartilages, surrounding them ventrally, inserting into subradular membrane (Figs. 15A-C). Pair of m5, secondary dorsal tensor muscle of radula large



Fig. 14. Pleurobranchus iouspi Ev. Marcus (1984), SEM. (A) shell (MNRJ 33069). (B-C) elements of the jaw (SAE ML 95). (D-F) radula. (D) innermost lateral teeth (MNRJ 33069). (E) lateral teeth (SAE ML 95). (F) outermost lateral teeth (MNRJ 33069).



Fig. 15. *Pleurobranchus iouspi* Ev. Marcus (1984), digestive system. (A) dorsal view (MNRJ 33069). (B) dorsal view, detail of posterior portion with esophagus deflected (SAE ML 95). (C) vetral view (MNRJ 33069). (D) odontophoral cartilages (SAE ML 95). (E) foregut sectioned longitudinally from ventral side (SAE ML 95). Abbreviations: a, anus; ag, duct of acid gland; bc, buccal ganglion; ca, oral canal; dg, digestive gland; hg, hermaphrodite gland; j, jaw plates; in, intestine; m1, jugal protractor muscle of odontophore; m4: main dorsal tensor muscle of radula; m5, accessory dorsal tensor muscle of radula; m10, protractor muscle of odontophore; m10a, ventral tensor muscle of radula; mj, jaw muscle; mo, mouth; mr, retractor muscle; oa, opening of the duct of the acid gland; oe, esophagus; ra, radula; rs, radula sac; sd, salivary duct; sg, salivary gland; so, opening of the duct of the salivary gland in the oral membrane; st, stomach.

(A)

and broad, covering median portions of cartilage, extending up to dorsal region; originating in posterior surface of cartilages; inserting laterally in mj (Figs. 15A-C). Pair of strong retractor muscles that originates in most posterior portion of m5 (Figs. 15A, C) separated in almost its total length. Radula cream; formula 107 × 255.0.255 (holotype, specimen with 60 mm preserved length), 76 × 260.0.260 (from preserved specimen with 75 mm length) and 64 × 148.0.148 (from preserved specimen with 51 mm long). Duct of acid gland thin and short (same width as salivary duct) (Figs. 15A-B); not convoluted. Esophagus internally with thin longitudinal folds (Fig. 16A). Stomach voluminous and rounded filled with food (Fig. 16A); internally, anteriorly with thin and smooth wall, except by the ventral furrow; posteriorly with large flaps and a central-ventral furrow that leads to intestine (Fig. 16A); stomach passes ventrally into digestive gland. Intestine long and thin walled; internally, smooth, with thin longitudinal folds near anus.

Identifiable stomach contents: Small bivalves (in most part of the cases juveniles; e.g. Arcidae); small crustaceans (e.g., Tanaidacea: *Paratanais* coelhoi Araújo-Silva and Larsen, 2012; Ostracoda); arborescent bryozoans; algae; grains of sand.

Reproductive system (Figs. 12B-F): Triaulic. Ampulla elongated, same width as vas deferent and vagina; curved, but not convoluted. Spermoviduct branching into two ducts, shorter oviduct and other duct leading to prostate. Prostate tubular and convoluted (Figs. 12D-E); four times wider than deferent duct (Fig. 12F). Deferent duct thin, narrowing into a long and noncontractile penis; vas deferens forming loops inside penis (Fig. 12F). Penis (in a preserved specimen with 51 mm length: length 14 mm; width 1.5 mm) (in a preserved specimen with 75 mm length: length 20.8 mm; width 2.3 mm) (Figs. 12A-C; E). Basal penial lobe asymmetrical (Fig. 12B). Penis with some sparse rod-like spicules. Vaginal duct convoluted with two allosperm vesicles



Fig. 16. *Pleurobranchus iouspi* Ev. Marcus (1984), digestive system. (A) foregut sectioned longitudinally from ventral side (SAE ML 95). Abbreviations: a, anus; ca, oral canal; j, jaw plates; in, intestine; m5, accessory dorsal tensor muscle of radula; mj, jaw muscle; oa, opening of the duct of the acid gland; oe, esophagus; ra, radula; rs, radula sac; st, stomach.

arranged semi-serially. Proximally, vagina divides into seminal receptacle and bursa copulatrix as a short duct, both allosperm receptacles lie close to each other, their stalks are crossed (Fig. 12D). Seminal receptacle elongated; not convoluted (Fig. 12D). Bursa copulatrix rounded. Vaginal opening immediately ventral to penis (Fig. 12A). Vagina about same diameter of prostate. Genital aperture surrounded by three well-distinct large penial flaps; two of these flaps are bifurcated after their base (Figs. 12A-B).

Nervous system (Fig. 12G): Nervous system of *P. iouspi* very similar to *P. reticulatus* as described above, except for: the absence of cp6, cp7 and cp8; the connective between buccal and cerebro-pleural ganglia leaving the most anterior portion of cerebro-pleural ganglia in ventral area and inserts in buccal ganglia; and, np3 innervates laterally the body until enters into foot.

Remarks on the type material of Pleurobranchus iouspi (Figs. 11A-H): The histological slides of the type of *Pleurobranchus* iouspi mentioned in the original description were rediscovered in the Malacological Collection of the MZSP, as well as most of species dealt in Ev. Marcus (1984), they were all on the same slide collection. Although the slides are not appropriately labeled, all have the same handwritten symbol ("IO", probably referring to the name *iouspi*) (Figs. 11A-E). Furthermore, the original figure of the penis (Ev. Marcus 1984: 69, fig. 61) closely resembles the slide-mounted material (Figs. 11F-H), which has a peculiar anterior basal bulb, making it rather asymmetrical (Fig. 11G); a thin deferent duct, narrowing into a long and noncontractile penis (Fig. 11H); and a vas deferens forming loops inside the penis (Fig. 11H). Ev. Marcus (1984) described P. iouspi based on a single specimen, therefore, since most of species dealt in Ev. Marcus (1984) were on the same slide collection found and the fact that the original figure of the penis closely resembles the slide-mounted material lead us to believe that the remaining slides should also correspond to the holotype. The radula formula was found to be $107 \times 255.0.255$. although Ev. Marcus (1984) described it as 100 × 250.0.250. The probable difference in the number of rows is due to the slightly broken condition of the posterior-most portion of the radula.

Remarks on the type material of Pleurobranchus emys (Figs. 17A-D): The original material studied by Ev. Marcus (1976b, 1984) was searched for in the recently located slides belonging to the collection of Er. Marcus and Ev. Marcus, housed in the MZSP. It was find some histological slides of the syntypes of Pleurobranchus emys Ev. Marcus, 1984: MZSP 119934, syntype, Santa Marta, Colombia [one microscope slide with radula and jaw platelets]; MZSP 119935, probable syntype, Santa Marta, Colombia [one histological microscope slide with sections of the mantle]. Pleurobranchus emys Ev. Marcus, 1984 was described based on the material studied by Ev. Marcus (1976b) from Colombia (then named Oscanius testudinarius). Ev. Marcus (1984) clearly stated that the type of P. emys corresponds a microscope slide with radula and jaw platelets, and two color slides of living specimens. Part of the above-mentioned type series was rediscovered in the MZSP. One slide with radula and jaw (Fig. 17A) is clearly the microscope slide mentioned by Ev. Marcus (1984), and is herein considered as a syntype lot (MZSP 119934); one histological slide with sections of the mantle (Fig. 17B), not mentioned in original description, is herein considered a probable syntype (MZSP 119935). Evidence that these histological slides correspond to the material originally used to describe P. emys is: the slide with sections of mantle shows the dense layer of radiating spicules as described by Ev. Marcus (1984) (Fig. 17D), and is labeled as "Susania emys 1983", one year before publication, and as "Oscanius testudidarius", the name originally used by Ev. Marcus in 1976 (Fig. 17B); the slide with radula and jaw is labeled "Oscanius testudinarius", with the name "testudinarius" scratched over (Fig. 17A), indicating that this was the previous determination by Ev. Marcus (1976b), subsequently changed to P. emys; this slide also matches the description and drawings of Ev. Marcus (1984).

DISCUSSION

The three Brazilian species of *Pleurobranchus* recognized herein as valid have a confused and problematic nomenclatural history, mainly due to the brief original descriptions and because some of these species were differentiate based only on external coloration.

Historically, there are different points of view about the names *Pleurobranchus atlanticus*, *Pleurobranchus areolatus*, *Pleurobranchus crossei* and *Pleurobranchus evelinae* since many authors considered part of them as synonyms and the configuration of the synonymy among these species also changed through the years. In the most recent paper about *Pleurobranchus*, Goodheart et al. (2015) concluded that these four species names, including *Pleurobranchus gardineri* White, 1952, *Pleurobrachus reesi* White, 1952 and *Pleurobranchus emys*, refer to a same genetic lineage, and thus all are considered junior synonymies of *P. areolatus*. According to those authors, the specimens traditionally named as *P. areolatus* from Brazil, on the other hand, in fact represent *Pleurobranchus reticulatus* Rang, 1832, a species originally described from Gulf of Guinea.

In the present paper, almost all nomenclatural acts proposed by Goodheart et al. (2015) are followed, except the synonymy between *P. emys* and *P. areolatus* and between *P. iouspi* and *P. testudinarius* Cantraine, 1835; here *P. emys* is considered junior synonymy of *P. iouspi*, while *P. iouspi* and *P. testudinarius* are considered distinct species. Despite agreeing with almost all nomenclatural acts proposed by Goodheart et al. (2015) some morphological aspects need to be highlighted, which could point at possible synonymies, specially concern the diameter of the tubercles of the mantle, the presence/ absence of flaps that surrounds the gonopore and the development of a leaflet in the convex portion of the penis. These features could split Pleurobranchus species in two main groups: those with tubercles with 1-3 mm in diameter, in a coarser pattern, with raised areas of oblong hexagons, gonopore surrounded by flaps in a complex arrangement, sometimes in two flaps, and the presence of a well developed leaflet in the convex portion of the penis; and those that present low pointed tubercles in the mantle with 125-375 µm in diameter, gonopore never surrounded by flaps and the leaflet in the convex portion of the penis much less pronounced than the first group. The first group would represent the morphotype here named as P. reticulatus and its possible synonymies, P. areolatus sensu Mörch (1863), P. crossei and P. gardineri; and, the second group embrace the morphotype called P. areolatus by Goodheart et al. (2015), non Mörch (1863), with the names P. atlanticus, P. evelinae and P. reesi.

These potential synonymies are not properly proposed because these differences were based in the original or subsequent descriptions/illustrations

Fig. 17. Microscopic slides of the syntype of *Pleurobranchus emys* Ev. Marcus, 1984, for discussion about slide's label, see text. (A) radula and jaw platelets (MZSP 119934). (B) cuts of the mantle (MZSP 119935). (C) elements of the jaw (MZSP 119934). (D) detail of mantle cut, showing the dense layer of radiating spicules (MZSP 119935).

and when available in the type material, that most often refer to only one specimen, which enable us to understand the intraspecific variation of the species. It would be necessary a great sample of specimens from the Caribbean to conclude this discussion, once it is not possible in this moment analyze specimens from Caribbean, synonymyze *P. areolatus* with *P. reticulatus* and consequently change the name of the morphotype called as *P. areolatus* by Goodheart et al. (2015) is premature and nomenclaturaly disruptive at this moment.

Possible synonymy between *Pleurobranchus reticulatus* and *P. areolatus* sensu Mörch (1863)

Mörch (1863) described Pleurobranchus areolatus based on a brief description and the types are probably lost; however, Bergh (1897: 111-113, pl. 9, figs 31-41) dissected and illustrated two of Mörch's type specimens. Bergh's description matches with the description of P. reticulatus made by Neves et al. (2007) and with the morphotype called here as P. reticulatus mainly due the shape and size of tubercles, the two wide flaps, which surround gonopore, and the oval leaflet attached in the convex portion of the penis. Bergh (1897) described these tubercles as polygonal, mostly hexagonal, of different sizes up to 2.5 mm in length. This description is more closed resembling to P. reticulatus than P. areolatus of this study and sensu Goodheart et al. (2015) because the wellrelaxed specimen of P. areolatus of this study has low pointed tubercles with 125-375 µm in diameter (Fig. 1C), while P. reticulatus has tubercles with 1 -2 mm in diameter, in a coarser pattern, with raised areas of oblong hexagons (Figs. 1A-B) (Table 1).

Bergh (1897: pl. 9, fig. 33) did not describe the penis in detail or even the gonopore, but in its illustration, the gonopore is surrounded by two wide flaps and the penis has an oval leaflet attached in the convex portion of the penis as described by Neves et al. (2007). Pleurobranchus atlanticus and P. evelinae, which correspond P. areolatus of this study and sensu Goodheart et al. (2015), were also described with a flap-like ridge on its anterior face (Thompson 1977; Abbott 1949: fig. 6), however in the illustrations available and in the type material of P. evelinae (NHMUK 19773W) it is not as pronounced as in P. reticulatus. Furthermore, the gonopore surrounded by two wide flaps was never reported for P. atlanticus or P. evelinae and is not present in P. areolatus of this study. The comparison of the descriptions of Bergh (1897), Neves et al. (2007), the present study and

the assumption of Goodheart et al. (2015), that the specimens of Gulf of Guinea and Brazil correspond to a same genetic lineage, lead us to believe that possibly the name *P. areolatus* sensu Mörch (1863) could be a junior synonym of *P. reticulatus*.

Comparison between *Pleurobranchus reticulatus* from Brazil and Gulf of Guinea

The comparison between the results of the present study with the description of Neves et al. (2007) reveals that the specimens of P. reticulatus from Brazil and from the Gulf of Guinea are similar, mainly in relation to the reproductive system (e.g., shape of the penis with a wide leaflet, deferent duct long and highly convoluted and seminal receptacle elongated and convoluted). However, they show some intraespecific variations: in specimens from Gulf of Guinea the gonopore is surrounded by flaps clearly differentiate in two, in specimens from Brazil this arrangement is found (Fig. 3B), but also it can be observed a complex arrangement of the flaps, where the differentiation in two flaps is not clear; in the Brazilian specimens, the vagina is slightly convoluted, while in those from Gulf of Guinea, the vagina is very convoluted; spicules were found only in Brazilian specimens. The main difference is regarding the color of the mantle, specimens from Gulf of Guinea can be recognized by the thinner white reticulations on the mantle and the presence of dark tubercles inside the polygonal areas in the adults, while living specimens of the morphotype from Brazil have a yellowish-orange mantle with tubercles of many colors: dark red, red, bright orange, and pale orange with white spaces between the tubercles (reticulations). Despite such differences in mature specimens, juveniles are guite similar. In an early stage, the general color of the mantle is semi-transparent whitish with complex white reticulations; the tubercular spots are purple rose; and, the edge of mantle has a conspicuous yellow/orange line (Fig. 1B; Neves et al. 2007: fig. 1C).

Therefore, it is conclude that *P. reticulatus* from Brazil and Gulf of Guinea are the same morphotype mainly based on reproductive system, but also due the similarity of the general color pattern of the juveniles.

Edmunds (1968) recorded *P. areolatus* from Ghana, providing a description that matches *P. reticulatus*, mainly with respect to the oval leaflet attached to the penis edge and the general color pattern, which is a opaque white lines with large black spots in the center of many of the polygons surrounded by this network. This led Cervera et al. (1996) re-identified it as *P. reticulatus*. The unique difference observed between specimens

from Ghana and Brazil is the color of the foot in living specimens, described by Edmunds (1968) as maroon, while in the specimens studied here it is

Table 1.	Comparison among Pleurobranchus from Brazil	

	P. areolatus Mörch, 1863	<i>P. reticulatus</i> Rang, 1842	P. iouspi Ev. Marcus, 1984
Type-locality Geographic distribution in Brazil	Biscayne Bay, Florida Alagoas state	Gulf of Guinea Maranhão, Alagoas, Bahia, Rio de Janeiro and São Paulo states and Fernando de Noronha	São Paulo state, Brazil Maranhão, Rio de Janeiro, São Paulo and Santa Catarina states
Size (length) Rhinophores	up to 60 mm rhinophores with dark red, orange and white blotches, apex with white pigment	up to 120 mm completely with a unique color that could be dark orange, red, dark red, black); in Brazil usually red	51-99 mm (preserved specimens) in same color pattern of mantle: bright yellow or red with orange apex
Mantle (color)	white with brownish-red blotches on dorsum; some tubercles with pale- orange tips; mantle edge orange	yellowish-orange with tubercles of many colors: dark red, red, bright orange and pale orange; space inter-tubercles white; mantle edge orange	two types: completely bright yellow, sometimes with white marks; reddish (red, orange, dark red) with space inter- tubercles white; sometimes with a bright pink ring at base
Shape of tubercles Diameter of tubercles	pointed low tubercles 125-375 μm	rounded/oval/ hexagons 1-3 mm	rounded or pointed, elongated (very high) 1-7 mm
Mantle (tubercles)	tubercles at mantle edge smaller in diameter, higher and more concentrated than middle of mantle	generally bigger and more concentrated in middle of mantle than in mantle edge	generally pointed in middle of mantle; in mantle edge usually rounded
Spicules	linear, rod-like (length: 70-160.5 μm); stellate (ray length: 70-112.5 μm)	linear, rod-like (length: 70-200 μ m); stellate (ray length: 17.29-27.19 μ m). Additionally, some elongated, but irregular (a not well definite shape) spicules grouped in some parts of mantle 60-120 μ m)	linear, rod-like (length: 314-427.2 μm); stellate spicules with three similar rays (ray length: 13.8-18.9 μm) and with five rays, four of which are arranged in same plane (ray length: 45.4-102.2 μm. Mantle densely covered by spicules with five rays
Gill- pinnae number	19-22	18-30	18-24
Pinnae free from the body wall	9	12-16	7-12
Position of the Shell	partially above heart, blood gland and digestive gland	above the heart	immediately anterior to heart, partially above pericardium and blood gland
Shell	length 3.4-5.0 mm; width 2.3-2.4 mm	length 3.9-9.0 mm; width 2.0-4.5 mm	length 2.2-6.6 mm; width 1.6-3.7 mm
Jaw	35 elements transversally; 80 elements longitudinally	39-46 elements transversally; 59-70 elements longitudinally	30-43 elements transversally; 50-60 elements longitudinally
Elements of jaw	a main cusp with 1-6 denticles in each side, generally 1-3	main cusp with 2-6 denticles in each side	main cusp with 1-5 denticles in each side
Retractor muscles m1v	separated in almost its total length Absent	separated in 3/4 its total length present	separated in almost its total length present
Radula	75 × 190.0.190	58-70 × 160-186.0.160-1986	64-107 × 148-260.0.148-260
Stomach	thin without longitudinal folds;	some folds, wrinkled wall; posteriorly	anteriorly with thin and smooth wall, except
wall internal	posteriorly smooth, except by a ventral furrow that leads to intestine	smooth, except by the ventral furrow that leads to intestine	by the ventral furrow; posteriorly with large flaps and a central-ventral furrow that leads to intestine
Gonopore	surrounded by a unique fold	surrounded by anterior flaps, usually two	surrounded by three well-distinct large penial flaps; two of these flaps are bifurcated after their base
Penis	penis has a flap-like ridge on its	8.5 mm in length; basal penial lobe	51-75 mm in length; basal penial lobe
	anterior face	symmetrical; convex portion of penis with a wide leaflet	asymmetrical
Cerebro-pleural ganglia	cp1; cp2; cp3; cp4; cp5	cp1; cp2; cp3; cp4; cp5; cp6; cp7; cp8	cp1; cp2; cp3; cp4; cp5
References	Abbott (1949); Thompson (1977); present study	Vayssière (1898); White (1952); Ev. Marcus (1984); Neves et al. (2007); present study	Ev. Marcus (1984), present study

white with orange blotches.

Possible synonymy between *Pleurobranchus reticulatus* and *P. crossei*

Pleurobranchus crossei Vayssière, 1897 was described based on a single preserved specimen without color information, which resulted in inconclusive inferences regarding possible intraspecific variation. Pleurobranchus crossei, species originally described from the Caribbean Sea, was considered a synonym of either P. areolatus or P. atlanticus. The not detailed description probably led Thompson (1977) to consider P. crossei as a junior synonym of P. areolatus, a position followed by Ev. Marcus (1984). On the other hand, Valdés et al. (2006) considered P. crossei as a valid species, with P. atlanticus as its junior synonym. Recently, Ortea et al. (2014) mentioned that P. crossei is abundant in the Caribbean islands and recorded it from the Canary Islands, considering thus, an amphi-Atlantic distribution. Furthermore, Ortea et al. (2014) clearly stated that P. evelinae is distinct from P. crossei, but did not present a clear differentiation between these two species and did not describe the genital aperture and penis of them.

Vayssière (1897, 1898) described the mantle tubercles of P. crossei as having irregular polygonal edges and the penis with a membranous expansion in the anterior edge (Vayssière 1898: 332, fig. 150), which matches the description of Neves et al. (2007) for P. reticulatus, rather than the low pointed tubercles found in P. areolatus of this study and sensu Goodheart et al. (2015); the genital opening is surrounded by a wide membrane, subsequently subdivided into three lobes in P. crossei, which was not observed in P. areolatus of this study. The examination of the holotype of P. crossei (MNHN-IM-2000-30102) revealed that Vayssière (1898) was referring to the two wide flaps in a complex arrangement described by Neves et al. (2007) for P. reticulatus. Furthermore, it was observed the oval leaflet attached in the convex portion of the penis of the holotype.

Therefore, *Pleurobranchus crossei* is considered a possible synonym of *P. reticulatus* due to the presence of the most remarkable features of *P. reticulatus*, the flaps surrounding the gonopore and the presence of a well developed leaflet in the convex portion of the penis.

Possible synonymy between *Pleurobranchus reticulatus* and *P. gardineri*

White (1952) described the mantle covered by oval papillae, most of which 3 mm in diameter. In the illustration of the dorsal view of the mantle, the oval leaflet attached in the convex portion of the penis is folded and is clearly recognized (White 1952: 108, fig. 2), as shown in here for *P. reticulatus* (Figs. 1A; 3C-D). The well-developed oval leaflet attached in the convex portion of the penis is also found in the syntypes of *P. gardineri* (NHMUK 1934.9.11.99-101), which corroborate our hypothesis.

Distiction between *Pleurobranchus reticulatus* and *P. areolatus* of the present study and sensu Goodheart et al. (2015)

Pleurobranchus reticulatus and P. areolatus were misidentified for a long time because of their simple descriptions, without photographs of living animals. However, living specimens are clearly distinguished by the color of the rhinophores (Table 1), at least in specimens from Brazil. In mature or juvenile specimens of P. reticulatus, the color of the rhinophores is variable (dark orange, red, dark red or black), but with a single color in each specimen (Figs. 1A-B); while in P. areolatus, the color has the same pattern as the dorsum (with white blotches) (Fig. 1C). Moreover, P. reticulatus is larger (up to 120 mm long) and has more pinnae (12-16) free from the body wall than P. areolatus (up to 50 mm long, with 9 pinnae). The diameter of the mantle tubercles is quite different in P. areolatus (125-375 μm, Fig. 1C) and *P. reticulatus* (1-3 mm, Fig. 1A-B). Additionally, the penis of P. reticulatus has a more developed leaflet attached in the convex portion of the penis (Figs. 3C-D) in comparison to P. areolatus; the gonopore is surrounded by two wide flaps in P. reticulatus, which is absent in *P. areolatus*; the prostate is tubular and highly convoluted in P. reticulatus, while in P. areolatus is elongated with the proximal portion rounded, seems detached circle (Fig. 7D); and, the jaws of P. areolatus, the smaller species, have more rows and fewer platelets per row (80 transverse rows and 35 platelets per row) than P. reticulatus (59-70 transverse rows and 39-46 platelets per row) (Table 1). In Brazil, P. reticulatus is a common species, while *P. areolatus* is rare and until now was only recorded from northeastern Brazil (Padula et al. 2012).

Possible synonymy among *Pleurobranchus* areolatus sensu Goodheart et al. (2015), *P. atlanticus* and *P. evelinae*

Pleurobranchus evelinae, species originally described from Jamaica, was considered junior synonym of *P. atlanticus*, species from Florida, by Rudman (2006). The original description of P. evelinae and P. atlanticus matches perfectly with P. areolatus of the present study in all external features, especially the following features: the tiny tubercles covering the dorsum (Fig. 1C); the pale-brown coloration of the dorsum (Fig. 1C); the dark maroon and white blotches on the dorsum (Fig. 1C); an orange border around the mantle (Fig. 1C); and the dense white pigment on the tips of the rhinophores and oral lobes (Fig. 1C). Furthermore, the examination of the holotype of P. evelinae (NHMUK 19773W) revealed that there is no flaps surrounding the gonopore and the penis presents a flap-like ridge on its anterior face like in P. atlanticus (Abbott 1949: 79, fig. 6), not as pronounced as in *P. reticulatus*.

The specimen illustrated by Valdés et al. (2006: 112) and by Ortea et al (2014: 146, fig. 1D) as P. evelinae is different with respect to the general color pattern from the specimens of P. areolatus that occurs in Brazil, since P. evelinae has the dorsum orange or dark brown without brownish-red blotches, and the mantle edge not orange. The specimen of Goodheart et al. (2015: 344, fig. 18C) from Panama has the same color pattern as the specimens illustrated by Valdés et al. (2006: 112) and Ortea et al. (2014: 146, fig. 1D) and was considered as the same species of P. areolatus sensu Goodheart et al. (2015) based on molecular data. The specimens illustrated by Valdés et al. (2006: 114) and by Ortea et al. (2014: 146, figs. 1B-C) determined as P. crossei, is herein considered as P. areolatus of this study.

Synonymy between *Pleurobranchus areolatus* of the present study and *P. reesi*

The species *Pleurobranchus reesi* has a general description, mainly with data of external morphology, but without any data of reproductive system. Goodheart et al. (2015) did not examine the syntypes of *Pleurobranchus reesi* White, 1952, however they considered *P. reesi* a junior synonym of *P. areolatus* without adding a discussion about this synonymy. The penis of the types is not protruded out of the gonopore, thus could not be observed in detail (NHMUK 1934.9.11.102-

104). The description made by White (1952) is too general to identify the species. The most powerful argument is based on the morphology of jaw elements and the retract ability of penis. White (1952) described the jaw elements with only one denticle of each side of main cusp, this low number of denticles is unusual in P. reticulatus, which when it presents two denticles is only in some elements and not in the entire jaw, on the other hand Thompson (1977) described 1-2 denticles to P. evelinae, which is considered here a possible synonym of P. areolatus of this study. The penis is semi-contractile in all P. reticulatus examined in this study and it is completely contractile in P. areolatus of this study. The types of P. reesi do not present part of the penis exposed, and therefore probably correspond P. areolatus of this study.

The record of Pleurobranchus emys from Brazil

The remaining two species recorded from Brazil, *P. iouspi* and *P. emys*, also have a confused taxonomic history. The records of *P. emys* in the catalogues of García et al. (2008) and Rios (2009) are due to misinterpretations of the articles by Ev. Marcus (1970, 1976b, 1984), who successively recorded *Oscanius testudinarius*, *P. evelinae*, and *P. emys* from the western Atlantic.

First, we examine the records of P. testudinarius from the western Atlantic. Pleurobranchus testudinarius Cantraine, 1835, originally from the Mediterranean, was recorded from Maranhão state (Brazil) by Ev. Marcus (1970) as "Oscanius (?) testudinarius", based on an incomplete specimen; later, Ev. Marcus (1976b) expanded its distribution to Colombia. Only the specimen from Colombia was re-identified and described as P. emys Ev. Marcus (1984); therefore, P. emys was never recorded from Brazil. The record from Maranhão state (Brazil) have been erroneously referred to P. evelinae (Ev. Marcus 1976b; Thompson 1977). The Maranhão specimen has large flaps on the genital aperture only found in P. iouspi among species occurring in the Western Atlantic (Ev. Marcus 1984; present study).

Pleurobranchus iouspi and *P. testudinarius* are distinct species

Pleurobranchus iouspi is currently considered a potential synonym of *P. testudinarius* (Goodheart et al. 2015). The configuration of the penial flaps is similar in both *P. testudinarius* and *P. iouspi*, however, they are not identical. According to the original description and illustrations and the redescription in the present study of *P. iouspi* and the specimen from Maranhão state (Ev. Marcus 1970), the genital aperture, including the penis, is surrounded by three distinct large penial flaps (Figs. 12A-C). In contrast, *P. testudinarius* has several penial flaps laterally and ventrally to the penis in *P. testudinarius* (Martynov and Schrödl 2009: 60, fig. 5A; this study, *P. testudinarius* from Bay of Naples, Italy [1]: MV F85659). Therefore, the penial flaps do not surround the penis in *P. testudinarius* as is the case in *P. iouspi* (Figs. 12A-B), which leads us to believe that the specimen from Maranhão state (Ev. Marcus 1970) actually corresponds to *P. iouspi*.

Pleurobranchus iouspi Ev. Marcus, 1984 was described based on a single specimen from the coast of São Paulo state, and only recorded as "Oscanius (?) testudinarius" based on specimen from Maranhão state. Recently collected specimens and the rediscovery of the slides of the holotype in the MZSP allowed us to re-describe this species. The distinguishing features mentioned in the original description, which allowed the determination of the specimens in this study are: the size of the tubercles on the mantle, genital apertures surrounded by three large flaps (Figs. 12A-C), deferent duct spiraling into the smooth muscular conical penis, the non-contractile penis (Figs. 11 F-H; 12F), and the asymmetrical basal penial lobe (Figs. 11G; 12B-C, E-F). Goodheart et al. (2015) analyzed only nuclear data (histone 3, H3) of specimens from Brazil and Mediterranean Sea and suggest that P. iouspi and P. testudinarius are possibly synonyms. We believe that it is better to keep P. testudinarius and P. iouspi as distinct due the fact that the nuclear data alone of few specimens is not a strong evidence of conspecificity because it is a conservative data, the fragment studied by Goodheart et al. (2015) is very short resulting in a limited data problem, and mostly due the diagnostic differences in the flaps on the gonopore. The detailed anatomical study provided in the present paper permit that future studies compare anatomically this description with material from Mediterranean Sea.

Synonymy between *Pleurobranchus iouspi* and *P. emys*

The spicule layer in the mantle suggest that *Pleurobranchus emys* and *P. iouspi* are potentially synonyms. A dense layer of radiating spicules was observed in the specimens analyzed here. Such

a layer was previously described only for P. emys in the western Atlantic (Dall and Simpson 1901; Verrill 1901; Thompson 1977; Ev. Marcus 1984). The analysis of transverse cuts of the mantle of the holotype of P. iouspi and P. emys showed that there is a dense layer of stellate spicules (Figs. 17D, 13B-D). Beneath it is another less dense layer of linear spicules (Fig. 13A). Ev. Marcus (1984) believed that the jaw platelets of P. emys differ sharply from other pleurobranchines in their narrow anterior part and the much wider posterior part; however, the type material (MZSP 119934) shows this difference only in a few parts of the jaw. This could be an artifact of preservation, because most of the jaw follows the same pattern as the genus, with 2-5 denticles on each side of the main cuspid (Fig. 17C). The radula teeth are all hook-shaped, and as stated above, the mantle is composed by stellate spicules (Fig. 17D). Since living specimens of P. iouspi vary widely in coloration (Figs. 1E-F), and the descriptions of P. emys and P. iouspi do not differ significantly (Ev. Marcus 1970, 1976b, 1984), it is considered that P. emys and P. iouspi in the present study.

The placement of *P. emys* into synonymy under *P. areolatus* made by Goodheart et al. (2015) seems arbitrary since the types were not examined not even specimens from the type locality, Colombia, and the authors did not provide a discussion about this synonymy. If *P. emys* is synonym of other species is *P. iouspi* due the similarities discussed above. *Pleurobranchus areolatus* differs from *P. emys* in relation to the general color of the body (Table 1); the size and shape of the tubercles of the dorsum, in *P. emys* it is large and pointed, while in *P. areolatus* is low; and, due the dense layer of stellate spicules found in *P. emys*.

CONCLUSIONS

Historically, the classification of members of *Pleurobranchus* was largely based on preserved and/or insufficiently described specimens (*e.g.*, Vayssière 1897; Ev. Marcus 1984), which resulted in many synonyms, once the delimitation of species was so confused.

Features of the external morphology that characterize the genus *Pleurobranchus* are the cleft in the anterior mantle border (Figs. 1A-C, E-F), and the tuberculate gill rachis (Figs. 3A, 12A), which are not useful to differentiate species. In mature individuals, the flaps around the gonopore (Figs. 3B; 12A-B) help to distinguish species.

The reproductive systems of the three species studied here show the same general pattern, with minor differences in the proportions of ducts and organs, except for the penis (Figs. 3C-D; 11F; 12B, F). The penis is undoubtedly the most striking character for species differentiation, *e.g.*, the general shape, symmetrical or asymmetrical basal penial lobe (Figs. 3C-D; 11F; 12B, F), and retraction of the penis (permanently external, semi-internal or fully internal while retracted) (Fig. 3C, 12E). Unfortunately, the penis was insufficiently described in the older descriptions (Cantraine 1835; Mörch 1863).

The circulatory and nervous systems do not show useful characters for species differentiation. The only difference found in the nervous system were the nerves that extend from the cerebropleural ganglia, cp6, cp7 and cp8, which are present in *P. reticulatus* but not in *P. iouspi* and *P. areolatus* (Figs. 3E; 7E; 12G).

The radula and jaw elements, when mentioned, are the unique features of the digestive system described in older descriptions. The detailed anatomical study of *P. reticulatus*, *P. areolatus* and *P. iouspi* allowed us recognize a general pattern in the position and presence/ absence of extrinsic and intrinsic odontophore muscles (see description of the genus *Pleurobranchus*). As already observed for *Pleurobranchaea* (Alvim et al. 2014), *Berthella* and *Berthellina* (Alvim and Pimenta 2015), the odontophoric muscles are useful characters, particularly to distinguish among genera, and in some cases species, and we believe that they should be considered in future descriptions.

The increase in knowledge about *Pleurobranchus* morphology as well the intraspecific variation of each species allowed the recognition of three species for the Brazilian coast: *P. areolatus*, *P. iouspi* and *P. reticulatus*.

Acknowledgments: We are grateful to Dr. Fernando Moraes (project "Ilhas do Rio"), Dr. Carlos A. Rangel (project "Ilhas do Rio") and Jessica Rodrigues de Pinho (MNRJ), for collecting specimens of *Pleurobranchus iouspi* and for the great color photos; Dr. Vinicius Padula (Zoologische Staatssammlung München), for collecting several specimens and for the great photographs of *Pleurobranchus areolatus*; Dr. Guilherme Muricy (MNRJ), for helping with the photos of spicules; Chris Rowley (Museum Victoria), for sending excellent photos of Pleurorobranchus testudinarius; Dr. Manuel Caballer Gutiérrez (MNHN) for excellent images of the type of Pleurobranchus crossei; Dr. John Taylor and Andreia Salvador (NHMUK) for some information about the penis of the types of Pleurobranchus reesi, Susania gardineri and Pleurobranchus evelinae; Harry Taylor (NHMUK Photographic Unit), for excellent images of the types of Pleurobranchus reesi, Susania gardineri and Pleurobranchus evelinae; MSc. Patrícia O. V. Lima (MZSP), Bárbara Romera (MZSP) and Dr. Carlo M. Cunha (The Academy of Natural Sciences of Drexel University, Philadelphia), for helping with information about Marcus's collection in MZSP; Dr. Luiz Simone (MZSP) for loan of material. MSc. Amanda Garcez da Veiga (UFRJ), for the help with SEM operation. Some photos of this study are part of the project "Ilhas do Rio", held at the Institute "Mar Adentro" and sponsorship of Petrobras through Petrobras Environmental Program. This work was supported by Conselho de Aperfeiçoamento de Ensino Superior (CAPES) (J.A., PhD's scholarship) and FAPERJ (Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro), through project E-26/110.325/2014 and project E-26/110.068/2014.

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