

Re-description of a female *Pontohedyle brasiliensis* (Rankin, 1979), a junior synonym of the Mediterranean *P. milaschewitchii* (Kowalevsky, 1901) (Acochlidia, Gastropoda)*

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Abstract. Currently 27 species are considered to be valid in the still enigmatic opisthobranch group of the Acochlidia. The taxonomic status of the acochlidian species, *Pontohedyle brasiliensis* (Rankin, 1979), remained unclear due to a lack of primary data. The present study provides the first structural and some histological data on a female *P. brasiliensis* from northern Brazil. The female genital system is reconstructed 3-dimensionally from serial semithin sections using AMIRA software. Our new results are compared with published data on a male *P. brasiliensis* from southern Brazil and on *P. milaschewitchii* (Kowalevsky, 1901) from the Mediterranean and Black Sea; in absence of morphological differences we consider *P. brasiliensis* as a junior synonym. The genus *Pontohedyle* thus comprises two valid species, the Atlantic/Mediterranean *P. milaschewitchii* and the tropical Indopacific *P. verrucosa* (Challis, 1970). Both possess unique bow-shaped, flattened oral tentacles, which are diagnostic for the genus and, thus, a probable autapomorphy.

Keywords. Mollusca, Opisthobranchia, taxonomy, morphology, anatomy, 3D reconstruction.

1. INTRODUCTION

Currently, 27 nominal acochlidian species are considered to be valid (SOMMERFELDT & SCHRÖDL 2005) which were conventionally classified into 12 different genera in 6 families (WAWRA 1987). All acochlidians have a characteristic shell-less body with a head-foot complex that can be at least partly retracted into a more or less elongate visceral hump. Most species belong to tiny members of worldwide coastal mesopsammic communities, while others are inhabitants of brackish waters or even limnic (see NEUSSER & SCHRÖDL 2007). Uniquely within the usually hermaphroditic opisthobranchs, microhedylid acochlidian species have separate sexes. While most acochlidians have two pairs of cephalic tentacles, a few gonochoristic species lack any rhinophores, i.e. of the genera *Ganitus* Marcus, 1953 and *Pontohedyle* Golikov & Starobogatov, 1972. There are three nominal *Pontohedyle* species: the tropical Indopacific *P. verrucosa* (Challis, 1970), the Atlantic/Mediterranean species *P. milaschewitchii* (Kowalevsky, 1901), and the Atlantic *P. brasiliensis* (Rankin, 1979) with uncertain taxonomic status.

Pontohedyle milaschewitchii was originally described from the Black Sea (KOWALEVSKY 1901) and later found throughout the Mediterranean (see HADL et al. 1969; JÖRGER et al. in press; POIZAT 1984; WAWRA 1986). Additionally, MARCUS & MARCUS (1954) described one single male

specimen of *P. milaschewitchii* from Ilhabela (São Paulo State), the coast of southern Brazil. Solely based on that literature information, RANKIN (1979) established the new genus and species *Gastrohedyle brasiliensis* and separated it from the Mediterranean *P. milaschewitchii* (as *Mancohedyle*); her diagnosis of *P. milaschewitchii* then was limited to the original description by KOWALEVSKY (1901). ARNAUD et al. (1986) listed *Gastrohedyle brasiliensis* as *Pontohedyle brasiliensis* with a question mark, and WAWRA (1987) regarded it as a probable synonym of *P. milaschewitchii*, however without giving any discussion on an entire set of putative external and internal morphological differences that were raised by RANKIN (1979).

Unfortunately, anatomical information of *P. brasiliensis* is restricted to a single male specimen. This type specimen of *P. brasiliensis* has not been discovered in the Marcus' collection of the Museu de Zoologia da Universidade de São Paulo (C. Magenta, São Paulo, pers. comm. 2006), and thus appears to be lost. Specimens of *Pontohedyle* from Brazil remain very rare. Even after exhaustive search at the original location, MARCUS & MARCUS (1954) were not able to rediscover further specimens. We conducted collections at Ilhabela, the type locality of *P. brasiliensis*, along the coast of Santa Catarina, Paraná and São Paulo State, southern Brasil, and at many sites in Pernambuco

and Paraíba, northern Brazil. This search only resulted in two specimens, one of them usable for histological analysis.

The present study provides the first structural and histological data on a female *Pontohedyle* from northern Brazil. The taxonomy of *P. brasiliensis* is revised by critically comparing our results with the published data on *P. brasiliensis* from Brazil and *P. milaschewitchii* from the Mediterranean.

2. MATERIAL AND METHODS

Two *Pontohedyle* specimens were extracted from sand samples (see SCHRÖDL 2006 for method of extraction), collected by seuba diving on the northern coast of Brazil (approx. 5 km off Porto de Galinhas, at 20 m depth) in January 2004. One retracted and damaged specimen was used for molecular analysis. The posterior part of the viscerol hump of the second specimen was also damaged. The specimens were slowly anaesthetised using 7 % isotonic $MgCl_2$ solution and fixed in 75 % ethanol. The preserved specimen used for histological analysis in the present study was transferred into Bouin solution for decalcification and afterwards stained with 0.5 % safranin. Then it was dehydrated by a graded acetone series and embedded in Spurr's low viscosity epoxy resin (SPURR 1969) for sectioning. The epoxy resin block was cut at 1.5 μm with a rotation-microtome (Microtom HM 360; Zeiss), using glass knives and contact cement at the lower cutting edge (HENRY 1977) to receive ribboned serial sections. The sections were stained with methylene blue-azure II (see RICHARDSON et al. 1960). Computer based 3D reconstruc-

tion of the female genital system was performed with the software AMIRA 3.0 (TGS Template Graphics Software, Inc., USA). The section series was deposited in the Zoologische Staatssammlung München (ZSM), Mollusca Section (ZSM Mol 20041037). For morphological and anatomical comparison, serial sections and 3D reconstructions of five individuals of *P. milaschewitchii* from the Mediterranean were used (ZSM Mol 20060522-20060525).

3. RESULTS

3.1. External morphology and spicules

Our examined living Brazilian specimen used for structural analysis, showed the usual body shape of marine interstitial aeolohidians, with a cylindrical anterior head-foot complex that is completely retractable into a broadened and elongated viscerol hump. The crawling individual measured approximately 2 mm, but the viscerol hump was damaged. The overall body coloration was whitish, with the brownish digestive gland shining through the tissue. The oral tentacles were bow-shaped and curved, rhinophores were lacking (see Fig. 3A). The eiliated foot was short, i.e. there was no free tail extending behind the head-foot complex, and its posterior edge was rounded. Monoaxone (i.e. needle shaped) spicules (about 25 μm length) were found randomly distributed over the head-foot complex and viscerol hump. Additionally, an accumulation of parallel orientated monoaxone spicules was detected between the oral tentacles. Light microscopic investigation of the sectioned head region indicates cilia on the anterior border of the head and oral tentacles.

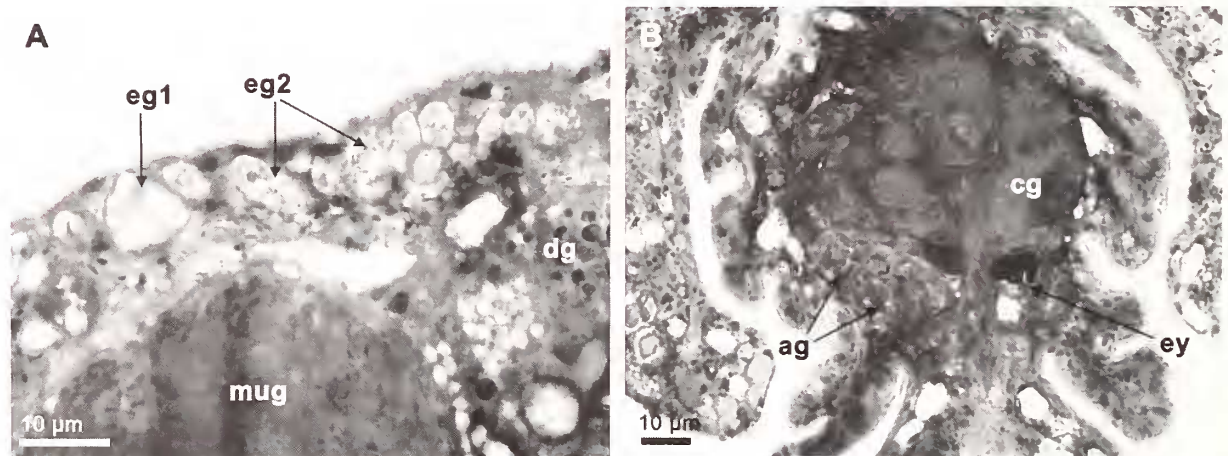


Fig. 1. Semithin sections of the female *Pontohedyle* from Brazil. (A) Cross-section of the visceral hump, showing the epidermis and the epidermal gland cells. (B) Horizontal section of the central nervous system. Abbreviations: ag accessory ganglia, cg cerebral ganglia, dg digestive gland, eg1 epidermal gland type I, eg2 epidermal gland type II, ey eyes, mug mucous gland.

3.2. Microanatomy

The bad condition of the only Brazilian specimen available for structural analysis did not allow detailed histological investigations of all major organ systems. Nevertheless, a brief treatise is given on the recognisable organs, focusing on the female genital system which could be reconstructed from serial sections.

3.2.1. Epidermal glands

The epidermis contains large spherical glandular cells (5–10 µm in diameter). They form a sub-epidermal sac, which is more or less filled with a homogenous whitish secretion (= epidermal glands type I, see Fig. 1A). Smaller vacuoles could be detected in the epidermis containing pinkish to violet stained granular material. These vacuoles occur in large numbers (= epidermal glands type II, see Fig. 1A).

3.2.2. Central nervous system

Praepharyngeal, large oval cerebral ganglia (approximately 50 µm), smaller pedal ganglia (approximately 30 µm) and groups of accessory ganglia could be detected. The cerebral ganglia are connected by a very strong and short commissure. A pair of dark pigmented eyes (diameter about 12 µm) nestles on the anterior side of the cerebral ganglia (see Fig. 1B). Groups of accessory ganglia are located anteriorly and laterally of the cerebral ganglia. Different from true ganglia true ganglia, accessory ganglia are well defined cell groups with a homogenous distribution of nuclei and without subdivision into cortex and medulla (NEUSSER et al. 2006). Here the accessory ganglia are more or less spherically shaped and grouped together like pearls on a chain (Fig. 1B).

3.2.3. Digestive system

The mouth opening is located subterminally between the oral tentacles. The thin walled oral tube is collapsed. The muscular pharynx extends in the posterodorsal part of the head-foot complex and contains the radula in its posterior region. The salivary glands form one mass on the left side of the head-foot complex, slightly extending into the visceral sac. The cells of the salivary glands contain dark blue stained granules. The tube-like oesophagus leaves the pharynx posterodorsally and connects to the digestive gland in the anterior region of the visceral hump. There is no histologically or anatomically detectable stomach. The digestive gland extends over the length of the remaining visceral hump and extrudes through the ruptured epidermis. It is sac-like in shape and its cells contain small dark blue and red stained granules. The epithelium of the digestive gland bears a series of small whitish and oval

vacuoles. Neither the intestine nor the anal opening could be detected due to the bad condition of the animal.

3.2.4. Excretory and circulatory systems

Only the kidney could be detected. It is triangular in shape and squeezed in between the digestive gland and the body wall on the right side of the anterior region of the visceral hump. The epithelium of the kidney is characterized by its usual vacuolated structure.

3.2.5. Female genital system

The examined individual is a mature female, recognisable by the presence of vitellogenic oocytes in the ovary. The female genital system is composed of the ovary, the nidamental glands and the oviduct (Fig. 2A, B). The ovary extruded through the ruptured epidermis of the visceral sac and was partially falling apart. Nevertheless, seven large vitellogenic oocytes are still *in situ* (Fig. 2F). The oocytes are comprised of a nucleus containing one nucleolus and yolk (characterised by dense aggregations of blue stained granules). The oocytes reach a diameter of about 50–60 µm. The albumen gland is tube-like in shape and its secretory cells are stained dark blue to dark violet (Fig. 2E). The secretory cells are alternated by supporting cells, which bear cilia. The membrane gland is comparably large and tube-like in shape. Its secretory cells are stained pinkish with glandular appearance and containing vacuoles (Fig. 2D). The supporting cells bear cilia. The long tube-like mucous gland runs parallel to the digestive gland in the anterior region of the visceral sac. The supporting cells of the mucous gland are also ciliated and the secretory cells are stained dark violet. The three nidamental glands connect directly to each other (i.e. without any defined proximal oviduct or adhesive region, see Fig. 2A). The distal ciliated oviduct (Fig. 2C) ventrally passes the digestive gland and leads to the right anterior region of the visceral hump. The genital opening is located on the right side of the body, at the transition from the head-foot complex to the visceral hump. A short ciliated band originates at the genital opening. It has a diameter of about 10 µm and runs anteriorly along the right side of the head-foot complex.

4. DISCUSSION

According to WAWRA (1987), acochlidians belonging to the genus *Pontohedyle* share microhedylid features such as having separate sexes and lacking copulatory organs. *Pontohedyle* species were characterized by the absence of rhinophores and a radula formula of 1.1.1. The combination of these features is unique among acochlidians, but may refer to plesiomorphies. The special shape of

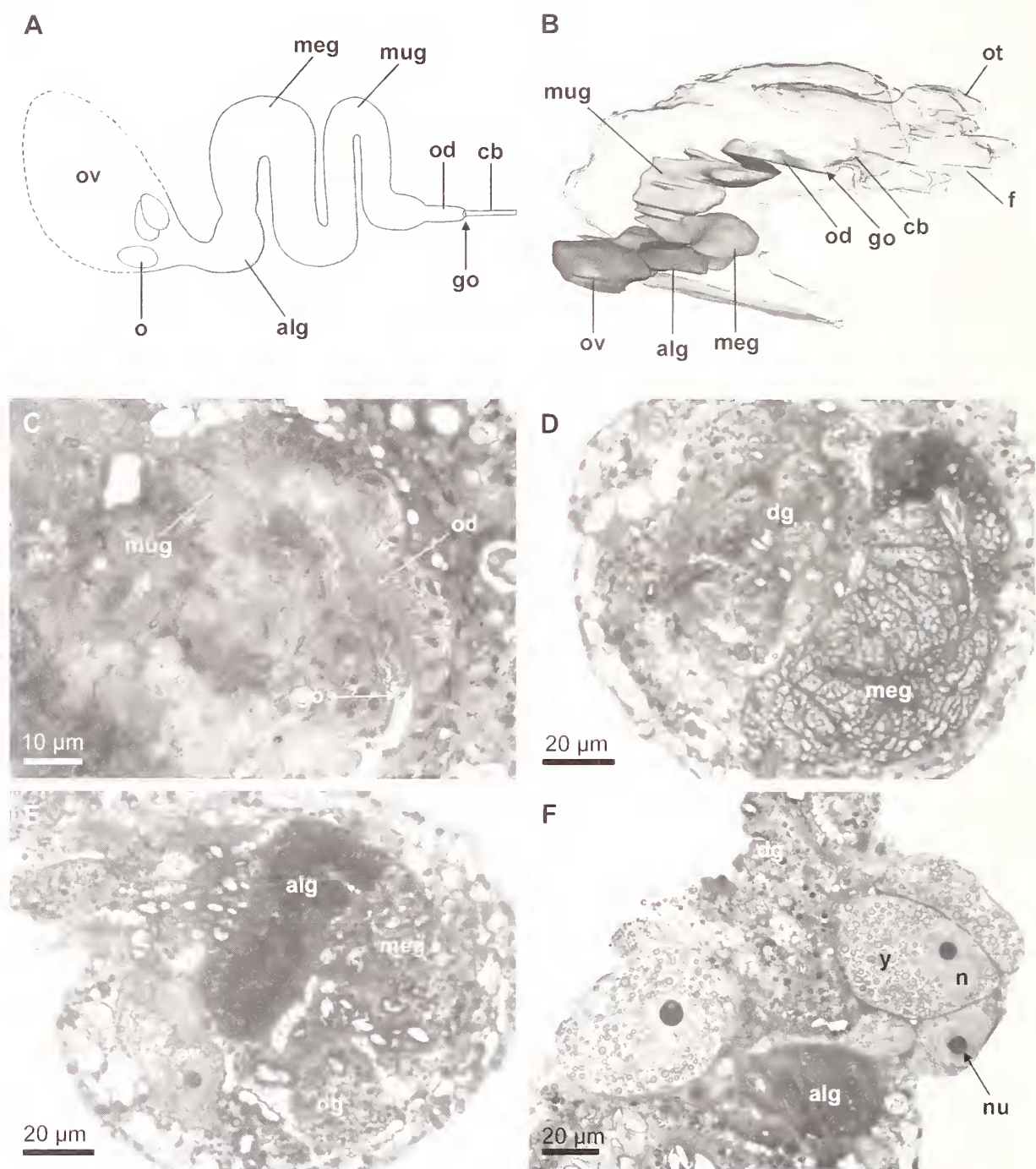


Fig. 2. Female genital system of the *Pontohedyle* from Brazil. (A) Schematic overview, lateral view. (B) 3D reconstruction, lateral right view, specimen retracted, approximately posterior half of viscerol sae and gonad missing (ruptured and therefore not reconstructed). (C) Semithin cross-section of oviduct and genital opening. (D) Semithin cross-section of membrane gland. (E) Semithin cross-section of the transition from albumen gland to membrane gland. (F) Semithin cross-section of mature oocytes. Abbreviations: alg albumen gland, cb eiliary band, dg digestive gland, f foot, go genital opening, meg membrane gland, mug mucous gland, n nucleus, nu nucleolus, o oocyte, od oviduct, ot oral tentacle, ov ovary, y yolk.

acoelid oral tentacles may provide more phylogenetic information. Apart from species of the genus *Pontohedyle*, flat oral tentacles only occur in the genera *Hedylopsis* and *Ganitus*. While the oral tentacles of the *Hedylopsis* are much broader than those of *Pontohedyle* (see Fig. 3F), the ones of *Ganitus* appear similar. *Ganitus* can be differentiated since the oral tentacles are never tapered towards the end (see Fig. 3E). In fact, the flat, elongated to bow-shaped oral tentacles of *Pontohedyle*, which are tapered towards the end (see Fig. 3A-D), are unique and diagnostic, and thus, a probable autapomorphy of *Pontohedyle*.

WAWRA (1987) regarded two *Pontohedyle* species as being valid, the tropical Indopacific *P. verrucosa* (Challis, 1970) and the temperate *P. milaschewitchii* (Kowalevsky, 1901). RANKIN (1979) however, established an additional species *P. brasiliensis* on the basis of a literature description of a single male specimen from Brazil. Table 1 compares potential distinguishing features of all three nominal *Pontohedyle* species, including the results of the present study on the female Brazilian specimen and the specimens of *P. milaschewitchii* from the Mediterranean used for comparison.

4.1. External morphology and spicules

Externally, the investigated specimen from Brazil confirms with the general acoelid characters (e.g. viscerol hump in which the head-foot complex can be at least partially retracted; see WAWRA 1987) and those of the genus *Pontohedyle* (lack of rhinophores). Using external characters, RANKIN (1979) differentiated *P. brasiliensis* from *P. milaschewitchii* by referring to the flat triangular versus bow-shaped oral tentacles, and the absence or presence of cilia on head and oral tentacles. However, the shape of the oral tentacles is variable within specimens of Mediterranean *P. milaschewitchii* (see JÖRGER et al. in press). They vary from bow-shaped to elongated triangular, including the flat and triangular form described by MARCUS & MARCUS (1954) for the Brazilian specimen (see Fig. 3B). Already MARCUS & MARCUS (1954) illustrated that the tentacles can have a more rounded tip (see fig. 13, 14). This character clearly varies for one individual, depending on the contraction of the animal (see Fig. 3D: *P. verrucosa* with supposedly slightly retracted tentacles). The variability of this character between individuals is underlined by the observation of our northern Brazilian specimen that had bow-shaped oral tentacles in living condition (see Fig. 3A).

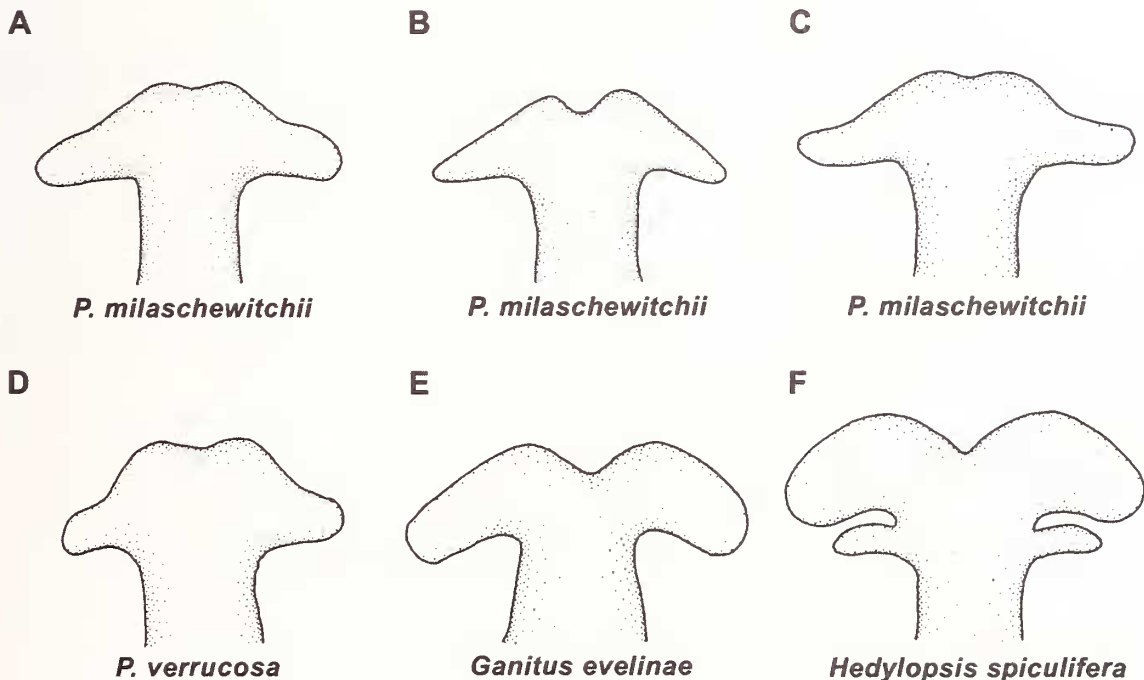


Fig. 3. Different types of flattened oral tentacles in acoelidians. (A) *Pontohedyle* from northern Brazil (present study), as *P. milaschewitchii*. (B) *P. milaschewitchii* from southern Brazil after MARCUS & MARCUS (1954; fig. 10). (C) *P. milaschewitchii* from the Mediterranean after KOWALEVSKY (1901; fig. 46). (D) *P. verrucosa* after CHALLIS (1970; fig. 5A). (E) *Ganitus evelinae* after MARCUS & MARCUS (1954; fig. 19). (F) *Hedylopsis spiculifera* (juvenile) after KOWALEVSKY (1901; fig. 49).

Table 1 . Comparison of characters previously used for species delineation in the genus *Pontohedyle*. ? = no data available; * MARCUS & MARCUS (1954) originally described the radula as 44 (2.1.2) misinterpreting the central denticle of the lateral plate as incomplete cleavage (CHALLIS 1970; JÖRGER et al. in press).

	<i>P. milaschewitchii</i> (Kowalevsky, 1901)	<i>P. brasiliensis</i> (Rankin, 1979)	<i>P. verrucosa</i> (Challis, 1970)
Data source	KOWALEVSKY (1901) WAWRA (1986) JÖRGER et al. (in press)	MARCUS & MARCUS (1954) present study	CHALLIS (1970)
Collecting site	Sebastopol (Black Sea) Princess Islands, Lesbos Island, Istria (Mediterranean Sea)	São Paulo, Porto de Galinhas, Brazil (Atlantic Ocean)	Maraunibina Island, Solomon Islands (Pacific Ocean)
Collecting habitat	coarse and fine sands, sublittoral (2–9m)	coarse and shell sands, intertidal and 20m depth	coarse, shell sand, intertidal
Foot	very short, posterior tip rounded	very short, posterior tip rounded	very short, posterior tip free and pointed
Oral tentacles	bow-shaped to triangular/elongated	bow-shaped to triangular/elongated	bow-shaped
Spicules	- aggregation of needle-shaped parallel orientated spicules between the tentacles - randomly distributed needle-shaped spicules throughout the body	- aggregation of needle-shaped parallel orientated spicules between the tentacles - randomly distributed needle-shaped spicules throughout the body	absent (?)
Eyes	present	present	absent
Radula	- 41–54 (1.1.1) - rhachidian tooth with 1 central cusp and 3 lateral denticles - lateral plate with 1 central denticle	- 44 (1.1.1)* - rhachidian tooth with 1 central cusp and 3 lateral denticles - lateral plate with 1 central denticle	- 43 (1.1.1) - rhachidian tooth with 1 central cusp and 3 lateral denticles - lateral plate without denticle
Digestive system	- no stomach detectable - salivary glands form one mass on the left side of the body, discharging into the oesophagus close to the transition of the pharynx	- “large, spherical stomach” according to MARCUS & MARCUS (1954), but no stomach detectable in the present study - salivary glands form one mass on the left side of the body	- no stomach described - salivary glands paired, discharging into the oesophagus “near its posterior end”
Male genital system	ciliated vas deferens, extending to the level anterior to the oral tentacles, genital opening dorsal to the mouth opening	genital opening on the posterior end of the head-foot complex	?
Female genital system	ciliary band extending from the genital pore to about one third of the head-foot complex	short ciliary band extending from the genital pore (present study)	?

RANKIN (1979) claimed cilia to be absent from the head and oral tentacles of *P. milaschewitchii*, in contrast to *P. brasiliensis*. However, a constant pattern of cilia could be detected on the oral tentacles of Mediterranean *P. milaschewitchii* (see JÖRGER et al. in press). Similar cilia were described for the Brazilian specimen by MARCUS & MARCUS (1954) and were also observed for the northern Brazilian specimen herein. Therefore, these external characters cannot be further used for separating species. In contrast, the Brazilian specimen described by MARCUS & MARCUS (1954) and the one studied herein resemble specimens of *P. milaschewitchii* (see JÖRGER et al. in press; KOWALEVSKY 1901) in all examined details, e.g. 1) body size and coloration, 2) shape of oral tentacles, 3) foot (short, posterior end rounded), 4) type (monoaxonic) and position (accumulation between oral tentacles and randomly distributed all over the body) of spicules, and 4) presence of cilia on head and oral tentacles.

4.2. Microanatomy

Anatomically, RANKIN (1979) saw differences between *P. milaschewitchii* and *P. brasiliensis* regarding fused versus separated cerebral and pleural ganglia, the radula formula, the presence/absence of a well developed stomach, and the development of the salivary glands.

4.2.1. Central nervous system

Probably based on small semi-schematic drawings of an entire specimen of *P. milaschewitchii* by KOWALEVSKY (1901; fig. 46, 48), RANKIN (1979) claimed the cerebral ganglia to be fused with the pleural ganglia in *P. milaschewitchii*, while they were described to be separated in *P. brasiliensis*. We could not clearly detect pleural ganglia in our damaged northern Brazilian specimen. However, JÖRGER et al. (in press) showed that the cerebral and pleural ganglia in Mediterranean *P. milaschewitchii* specimens are clearly separated, as usual for Acochlidia (HUBER 1993; SOMMERFELDT & SCHRÖDL 2005; WAWRA 1987).

4.2.2. Digestive system

MARCUS & MARCUS (1954) described a radula formula of 44 x 2.1.2 for their Brazilian specimen. However, CHALLIS (1970) suggested that the denticle in the lateral tooth might have been misinterpreted as a gap that appears to separate one broad lateral tooth into two. This explanation was accepted by WAWRA (1987) and is indeed very convincing. The radula of Mediterranean *P. milaschewitchii* closely resembles the one described by MARCUS & MARCUS (1954) for the Brazilian specimen: there is a triangular rhachidian tooth with one central cusp that is bordered by three lateral denticles, and just one broad

lateral plate on each side with one central denticle, thus with the formula 1.1.1 (JÖRGER et al. in press).

MARCUS & MARCUS (1954) saw an unusual large, spherical stomach in their Brazilian specimen, which was reflected in Rankin's generic name *Gastrohedyfe*. No special stomach was detected in our Brazilian specimen, but an oesophagus passing into a moderately developed digestive gland cavity which was filled with particles. This reflects the normal condition found in Mediterranean *P. milaschewitchii* (see JÖRGER et al. in press), and all other marine acochlidians. The large "stomach" described by MARCUS & MARCUS (1954) maybe easily explained as referring to a digestive gland cavity filled with particles or artificially swollen by gases due to decomposition.

RANKIN (1979) declared the salivary glands of *P. milaschewitchii* as "paired, well separated, long, thin, and tapering" in contrast to the large spherical salivary glands of *P. brasiliensis* forming one mass on the left side of the body. However, the salivary glands in Mediterranean *P. milaschewitchii* are just like those described by MARCUS & MARCUS (1954) for the Brazilian specimen and also those observed herein (JÖRGER et al. in press).

4.2.3. Genital system

MARCUS & MARCUS (1954) described their male Brazilian specimen as having a genital opening located on the right side of the head-foot complex close to the transition to the visceral hump. This is the usual position for the female genital pore *P. milaschewitchii* and of other male and female genital pores in microhedylid acochlidians. However, Mediterranean male *P. milaschewitchii* show a male genital pore in an unusual cephalic position dorsal to the mouth opening (JÖRGER et al. in press; WAWRA 1986). MARCUS & MARCUS (1954) used the anterior part of the head-foot complex of their specimen for radula preparation and were therefore unable to detect a male genital opening in an anterior position. The putative posterior opening in the male Brazilian specimen maybe thus explained by generalization and misinterpretation or maybe due to different ontogenetic stages. If additional male Brazilian specimens in different ontogenetic stages did not show any ciliated duct leading anterior to a cephalic male genital opening but a posterior genital opening, this would be the first serious indication for a specific separation of *P. brasiliensis* from *P. milaschewitchii*.

The female genital system of our Brazilian specimen closely resembles the one observed for *P. milaschewitchii* (JÖRGER et al. in press; WAWRA 1986) in 1) presence of a ciliary band originating from the genital opening; 2) position of the genital opening; 3) development and histo-

logy of the nidamental glands; 4) comparably small size of mature oocytes (around 60 µm). No differentiating features between the Brazilian specimen and its Mediterranean counterparts could be detected concerning the female genital system.

4.3. Taxonomy

All the differences between *P. milaschewitchii* and *P. brasiliensis* claimed by RANKIN (1979) are non-existent (cilia pattern, radula formula, shape of salivary glands, fusion of cerebral and pleural ganglia), variable (shape of oral tentacles) or can be easily explained by biological factors and artefacts (presence of large "stomach"). Morphological knowledge available at present (Table 1) strongly supports WAWRA (1987) in considering *P. brasiliensis* as a junior synonym of *P. milaschewitchii*. However, the considerable geographical distance between the Mediterranean and the northern and southern Brazilian populations of an interstitial species and the hydrographic differences between warm temperate and tropical waters require molecular investigation as soon as abundant Brazilian populations can be found.

Pontohedyle milaschewitchii as defined above is a Mediterranean and Atlantic species, while *P. verrucosa* was described from the Solomon Islands in the tropical Indopacific (CHALLIS 1970). Main differences to *P. milaschewitchii* are the absence of spicules, eyes and lateral radula denticles (Table 1). However, at least the lack of spicules might be due to a preservation artefact; *P. verrucosa* urgently needs redescription and comparison with some other potentially undescribed *Pontohedyle* species found in the tropical Indopacific (see SCHRÖDL et al. 2003).

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