

A new polyclad species associated with *Bankia martensi* (Bivalvia) from the South Pacific coast of Chile, and its taxonomic position within Euplanidae (Platyhelminthes, Ilyplanioidea)

FRANCISCO BRUSA^{1,2} AND CRISTINA DAMBORENEA^{1,2}

¹División Zoología Invertebrados, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Paseo del Bosque s/nº, B1900FWA, La Plata, Argentina, ²Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

We describe a new genus and a new species of Polycladida, Namyhplana henriettae (Platyhelminthes, Euplanidae), which lives associated with the tubes of the bivalve Bankia martensi in the fjords of the South Pacific Ocean in Chile. This species is characterized by a ribbon-like body, light brown colour, anterior region devoid of pigment, few eyes, no tentacles, pharynx in the anterior region of the body with few folds, independent gonopores anterior to the medial body line, seminal vesicle strongly muscularized, digitiform penial papilla oriented posteroanteriorly, a curled vagina, and a large Lang's vesicle ventral to the intestine. A phylogenetic analysis of the Euplanidae, based on morphological characters, is presented. With the dataset used, the family Euplanidae does not seem to be a monophyletic group. The new species is closely related to Taenioplana teredini (also discussed here) which presents life-habit characteristics that are similar to those of the new species.

Keywords: Platyhelminthes, Polycladida, Acotylea, commensal, South Pacific Ocean, *Taenioplana teredini*, Euplanidae, phylogenetic analysis

Submitted 5 April 2013; accepted 5 August 2013; first published online 10 September 2013

INTRODUCTION

Polyclads are marine, benthonic flatworms that generally live in rocky substrates. Most of them are free living, while some live in intimate association with other invertebrates (e.g. corals, hermit crabs, echinoderms and molluscs), forming commensal relationships (Newman & Cannon, 2003). Several species of commensal polyclads live in the mantle cavity of gastropods and polyplacoforan molluscs (Faubel *et al.*, 2007). A few species have been reported as associated with boring bivalves. One of these, *Taenioplana teredini* Hyman, 1944 (Acotylea, Euplanidae), was first described as living in the tubes of *Teredo* spp. on the coast of Hawaii (Hyman, 1944). Later, *T. teredini* was found in association with another *Teredo* species, *Teredo furcifera* Martens in Semon, 1894, as well as with other bivalves including *Bankia fimbriatula* Moll & Roch, 1931, and *Lyrodus massa* (Lamy, 1923) (Riser, 1970, 1974). *Taenioplana teredini* was recently reported to be associated with different invasive bivalves in several continents (Carlton, 2009; Carlton & Eldredge, 2009, and citations therein).

Polyclads of the south-east Pacific coast are poorly studied. From the 19th Century to the early 20th Century, twelve species from central and southern Chile were described, including five from the Juan Fernández archipelago (Schmarda, 1859; Plehn, 1896; Ritter-Zahony, 1907; Bock, 1913, 1923). Marcus (1954) described three new species from southern Chile and reported another four, two of them already mentioned from the Juan Fernández archipelago and Central Chile (Peru–Chilean province *sensu* Boschi, 2000). Faubel (1983) and Baeza *et al.* (1997) described two new polyclads from North Chile. Bulnes (2009) recorded five polyclad species already known in Chile, but some of them were in fact misidentified (Brusa & Damborenea, 2011). More recently, during a study on the diversity of bivalve molluscs from southern Chile, we found an unidentified polyclad living in tubes bored in the wood of *Nothophagus* that fall into the sea when they die and sink to the bottom of the fjords, turning into the substrate for *Bankia martensis* (Stempell, 1899) (Teredinidae). This polyclad appeared morphologically similar to *T. teredini*, suggesting a very close relationship between the two taxa.

In the present study, we describe this unidentified polyclad as a new species belonging to a new genus. To clarify relationships of the new taxon within the family Euplanidae, we carry out a phylogenetic analysis based on morphological characters. In addition, we compare the new species with its putative close relative, *T. teredini* that we also discussed.

Corresponding author:
F. Brusa
Email: fbrusa@fcnym.unlp.edu.ar

MATERIALS AND METHODS

Sixteen specimens of the new polyclad taxon were collected on 15 February 2010 at the Fiordo Cahuelmo ($42^{\circ}15'24.9''S72^{\circ}24'10.9''W$) in southern Chile between 50 and 95 m deep. The specimens were extracted manually from tubes bored in the wood by *Bankia martensi* (Bivalvia, Terebridae). Collected specimens were deposited in the Helminthological Collection of the Museo de La Plata (MLP), Argentina; and in the Museo de Zoología of Universidad de Concepción (MZUC), Chile. For taxonomic identifications we used the Faubel (1983, 1984) classification system of acotyleans.

For morphological analysis, collected specimens were fixed in 10% formalin, then washed and preserved in 70% ethanol. After body measurements were taken, the fixed specimens were photographed. The body regions containing the pharynx and the reproductive structures of four specimens were removed and embedded in paraplast for serial microtome sectioning. Samples were sectioned sagittally at 8 μ m, stained using haematoxylin–eosin and Masson's trichrome and, finally, mounted in synthetic Canada balsam. Whole mounts were prepared by first dehydrating the fixed specimens of the new taxon through an ascending alcohol series, then clearing the specimens in xylene and, finally, mounting them in synthetic Canada balsam. In addition, one whole specimen was stained with acetic carmine and mounted in synthetic Canada balsam. Schematic reconstructions of the reproductive system were made, based on the sectioned specimens and whole mounts.

We studied the whole mounted holotype and sagittal sections of the paratype of *Taenioplana teredini* from collections of the National Museum of Natural History (USNM, USA). In addition, we sectioned and stained a paratype of *T. teredini* deposited at the Bishop Museum (BPBM, Hawaii).

For the phylogenetic analysis, we used 16 out of the 19 valid species of Euplanidae (superfamily Ilyplanoidea) (Faubel, 1983, 1984). *Aprostatum longipenis*, *Euplanoida concolor* and *E. pardalis* were excluded from analysis, because no complete information of these three species was available. In the absence of molecular and/or morphological phylogenetic analyses at the family level, six representative species of different families within Ilyplanoidea Faubel, 1984 were selected as outgroups (Anocellidae, *Anocellidus profundus* Quiroga, Bolaños & Litvaitis, 2006; Discoprosthidae, *Discoprosthides patagoniensis* Faubel, 1983; Ilyplanidae, *Ilyella gigas* (Schmarda, 1859) of uncertain position according to Doignon *et al.* (2003), *Euiyoida malagasensis* (Doignon, Artois & Deheyn, 2003) and *Postenterogonia orbicularis* (Schmarda, 1859); and Mucroplanidae, *Mucroplana caelata* Sopott-Ehlers & Schmidt, 1975).

For all taxa considered we coded 26 morphological characters (Table 1). Information about character states of the new species and of *T. teredini* was obtained from the specimens studied here; that of the remaining species was obtained from the literature (Plehn, 1896; Laidlaw, 1903a, b; Bock, 1913; Hyman, 1939, 1941, 1944, 1953, 1954, 1955; Marcus, 1947, 1954; Sopott-Ehlers & Schmidt, 1975; Faubel, 1983; Doignon *et al.*, 2003; Quiroga *et al.*, 2006; Faubel *et al.*, 2007; Holleman, 2007). Missing data were coded as '?', inapplicable character states as '-', and characters with multiple states in brackets (Table 2). Parsimony analysis was performed using the program TNT (Goloboff *et al.*, 2008). All characters were treated as unordered and weighted equally. A heuristic

Table 1. List of characters and respective character states.

1.	Mode of life: (0) free living; (1) ectosymbiont; (2) endosymbiont
2.	Body shape: (0) elongate; (1) oval
3.	Tentacles: (0) absent; (1) marginal; (2) nuchal
4.	Marginal eyes: (0) absent; (1) only in the anterior margin of the body; (2) in the whole body margin
5.	Tentacular eyes: (0) absent; (1) present
6.	Cerebral eyes: (0) absent; (1) a single cluster; (2) two clusters
7.	Eyes in the precerebral area: (0) absent; (1) present
8.	Pharynx: (0) ruffled with simple pharyngeal folds; (1) ruffled with complex pharyngeal folds; (2) ruffled separated in two functional parts, anterior and posterior
9.	Pharynx position: (0) on anterior third of body; (1) on middle or posterior third of body
10.	Gonopores: (0) close to each other; (1) far from each other; (2) common pore
11.	Orientation of male copulatory apparatus: (0) positioned anterior to male pore and directed backwards; (1) positioned posterior to male pore and directed forwards; (2) positioned laterally to male pore, directed medially
12.	Position of male copulatory apparatus: (0) in posterior half of body; (1) in anterior half of body or central
13.	Penis papilla: (0) absent; (1) armed penis papilla (stylet); (2) unarmed penis papilla
14.	Adenoids (muscular projections into the male atrium): (0) absent; (1) with prostatoid organs
15.	Prostate: (0) absent; (1) prostate vesicle; (2) prostatic glandular cells
16.	Seminal vesicle: (0) simple; (1) tripartite (anchor-shaped); (2) absent
17.	Morphology of the distal part of the sperm ducts: (0) not differentiated; (1) spermiducal vesicles; (2) spermiducal bulbs (strongly muscularized ends of the sperm ducts, next to the copulatory apparatus, accessory seminal vesicles after Lang 1884, Bock, 1913)
18.	Vagina: (0) short; (1) long
19.	Orientation of the vagina: (0) looping toward male complex; (1) directed posteriorly
20.	Lang's vesicle: (0) absent; (1) present
21.	Shape of Lang's vesicle: (0) bulbous; (1) enlarged; (2) extending to both sides
22.	Uterus: (0) paired; (1) a median sac
23.	Common uterine duct: (0) absent; (1) present
24.	Uterine canals: (0) extending posteriorly from vagina; (1) extending anteriorly from vagina
25.	Genito-intestinal duct: (0) absent; (1) present
26.	Connection between Lang's vesicle and the exterior: (0) absent; (1) present

search was performed by tree bisection–reconnection (TBR) branch swapping of 1000 series of random-addition sequence, saving 100 (most parsimonious) trees after each replicate. The option 'collapse tree' after search was selected. Bremer Support was calculated to assess branch support.

SYSTEMATICS

Order POLYCLADIDA Lang, 1881
 Suborder ACOTYLEA Lang, 1884
 Superfamily ILYPLANOIDEA Faubel, 1984
 Family EUPLANIDAE Faubel, 1983
Namyhplana gen. nov.

DIAGNOSIS

Euplanidae with pharynx anterior to mid-body; eye spots cerebral, pre-cerebral and anterior marginal; male copulatory

Table 2. Matrix of 22 taxa and 26 characters used in this analysis. Unknown states are represented by ‘?’, inapplicable characters by ‘–’ and characters with multiple states in brackets.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	
<i>Aprostatum clippertoni</i> (Hyman, 1939)	0	1	0	0	1	2	0	1	1	1	0	1	1	0	0	0	0	0	1	1	1	[12]	?	?	0	0	
<i>Aprostatum stiliferum</i> Bock, 1913	0	1	0	2	0	0	1	2	1	0	0	0	1	0	1	0	2	0	0	1	0	1	?	?	0	0	
<i>Diplopharyngeata filiformis</i> Plehn, 1896	?	1	0	0	0	2	1	3	0	0	0	?	2	0	0	0	0	0	1	0	–	1	0	1	0	0	
<i>Euplana carolinensis</i> Hyman, 1940	0	1	0	0	1	2	0	1	1	0	0	0	0	0	0	0	0	0	0	0	–	?	0	?	0	0	
<i>Euplana gracilis</i> Girard, 1853	0	0	0	0	1	2	0	1	0	0	0	0	0	0	0	0	1	0	0	0	–	1	0	?	0	0	
<i>Euplana hymanae</i> Marcus, 1947	0	1	0	0	1	2	0	2	1	0	0	0	0	0	0	0	1	0	0	0	–	1	1	1	0	0	
<i>Euplanina horrida</i> Sopott-Ehlers & Schmidt, 1975	0	0	0	0	0	2	1	1	1	0	0	1	0	0	0	2	1	0	1	1	0	1	1	1	0	1	
<i>Euplanoida elioti</i> (Laidlaw, 1903)	?	1	0	0	0	2	0	1	0	0	?	1	0	0	0	0	0	?	0	1	0	1	1	?	0	0	
<i>Euplanoida malayana</i> (Laidlaw, 1903)	?	?	0	0	1	2	0	?	1	0	0	?	2	0	0	0	0	1	1	1	0	1	1	?	0	0	
<i>Euplanoida pacificola</i> (Plehn, 1896)	?	1	0	0	1	2	0	2	1	0	0	0	2	0	0	0	0	0	0	1	1	1	1	1	0	0	
<i>Euplanoida penangensis</i> (Laidlaw, 1903)	?	1	0	1	1	2	0	?	1	0	?	0	2	0	0	0	?	?	?	1	0	1	1	?	0	0	
<i>Euplanoida tropicalis</i> (Hyman, 1954)	?	1	0	0	1	2	0	2	1	0	0	0	2	0	0	1	2	0	0	1	1	1	1	1	0	0	
<i>Namyhplana henriettae</i> sp. nov.	1	0	0	1	0	1	1	1	0	0	0	1	2	0	0	0	1	0	0	1	1	1	1	1	0	0	
<i>Paraprostatum echinolittorinae</i> Faubel & Sluys, 2007	2	1	0	0	1	2	0	2	0	0	0	1	2	0	0	0	1	1	0	0	–	1	0	1	0	0	
<i>Semonia maculata</i> Plehn, 1986	?	1	0	[12]	0	[12]	1	?	?	2	0	?	2	0	0	0	1	0	1	0	–	1	0	1	0	0	
<i>Taenioplana teredini</i> Hyman, 1944	1	0	0	1	0	2	0	1	0	0	0	1	2	0	0	0	1	0	0	0	–	1	1	1	0	0	
<i>Anocellidus profundus</i> Quiroga, Bolaños & Litvaitis, 2006	0	1	2	0	0	0	0	1	0	1	1	1	1	0	0	2	2	0	1	0	1	0	1	0	0	1	0
<i>Discoprosthides patagoniensis</i> Faubel, 1983	?	1	2	0	0	0	0	2	1	0	[02]	0	0	1	2	0	2	0	1	1	0	1	0	1	0	0	
<i>Ilyella gigas</i> (Schmarda, 1859)	0	0	0	?	?	?	?	1	?	1	0	?	2	0	2	2	2	1	1	1	2	1	1	?	0	0	
<i>Euilyoidea malagasensis</i> (Doignon, Artois & Deheyn, 2003)	2	0	0	0	1	2	0	1	0	1	0	1	2	0	2	2	0	1	0	1	0	1	0	0	0	0	
<i>Postenterogonia orbicularis</i> (Schmarda, 1859)	0	1	0	2	1	1	1	2	1	0	0	1	2	0	1	0	0	?	0	0	–	1	1	1	0	0	
<i>Mucroplana caelata</i> Sopott-Ehlers & Schmidt, 1975	?	1	0	0	0	0	1	–	[12]	?	0	1	?	2	0	2	2	0	?	0	1	0	2	–	1	1	

apparatus with unarmed penis papilla horizontally placed; female apparatus with Lang's vesicle.

TYPE SPECIES

Namyhplana henriettae sp. nov.

ETYMOLOGY

We dedicate the name of the genus to Libbie Henrietta Hyman (1888–1969) who contributed to the knowledge of the Platyhelminthes of South America at the beginning of the 20th Century. The first part of the name (*Namyh*) is an anagram of Hyman.

Namyhplana henriettae sp. nov.
(Figures 1–5)

ETYMOLOGY

We dedicate the name of the species to Libbie Henrietta Hyman (1888–1969).

TYPE MATERIAL

Holotype: sectioned specimen mounted on 16 slides (from tubes made in the wood by *Bankia martensi*, Fiordo Cahuelmó, Chile; coordinates: 42°15'22.4"S72°23'48.5"W; water depth: 50–95 m) (MLP 6682); coll. D. Zelaya, 15 February 2010.

Paratype: 15 specimens (from tubes made in the wood by *Bankia martensi*, Fiordo Cahuelmó, Chile; coordinates: 42°15'22.4"S72°23'48.5"W; water depth: 50–95 m); coll. D. Zelaya, 15 February 2010. Three of them are sectioned specimens mounted on a series of 56 slides (MLP 6683); two specimens are whole mounted (MLP 6683); and ten additional specimens are preserved in ethanol (MLP 6683, MZUC 40120).

COMPARATIVE MATERIAL EXAMINED

Taenioplana teredini Hyman, 1944. Holotype: whole-mounted specimen (USNM 20636): Oahu Island, Honolulu, Hawaii (coordinates: 21°30'N157°85'W); collected by C. Edmondson on 24 February 1944. Paratype: one specimen mounted on two slides (USNM 20637); two specimens, one sectioned and mounted on 16 slides (BPBM-F 115), and one preserved in ethanol (BPBM-F 117): Oahu Island, Honolulu, Hawaii (coordinates: 21°30'N157°85'W), collected by C. Edmondson on 24 February 1944.

DIAGNOSIS

Acotylea with a ribbon-like body. Body colour light brown, with two dorsal dark brown longitudinal bands. Few eyes, in the unpigmented anterior region. Tentacles absent. Pharynx with few folds. Gonopores independent, in the middle of the body. Seminal vesicle strongly muscularized connected to digitiform penis papilla oriented posteroanteriorly. Vagina curled. Lang's vesicle large, ventral to the intestine.

DESCRIPTION

Fixed specimens have a light brown dorsum with two dark brown, broad, dorsal longitudinal bands (Figure 1). At the brain level the central light band is broader. The ventral surface is beige. The body is ribbon-like with the anterior end slightly tapering and a blunt posterior end. Fixed adult specimens are 20–25 × 1.5 mm. Tentacles absent. The distance between the anterior margin of the body and the anterior end of the pharynx is 2.6 mm. The distance

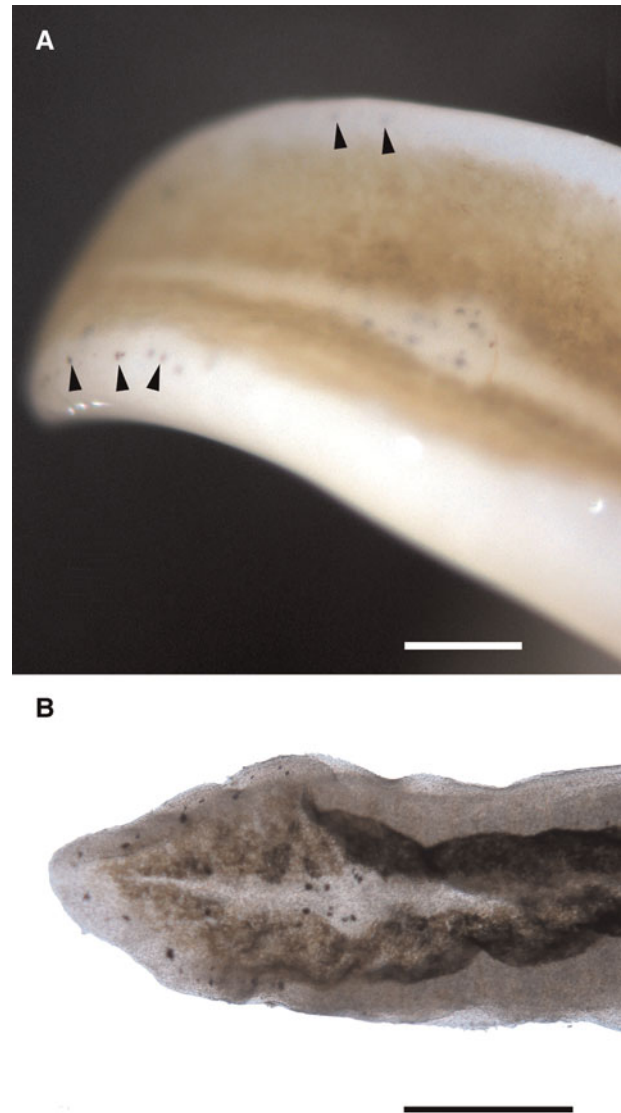


Fig. 1. Anterior end of *Namyhplana henriettae*: (A) fixed specimen under stereomicroscope; arrow heads show the marginal eyes; (B) whole-mounted diaphanized specimen in Canada balsam showing the cerebral and marginal eyes. Scale bars: A, 500 µm; B, 1000 µm.

between the anterior margin of the body and the mouth is 3 mm. The pharynx is 1.1 mm long. The distance between the rear end of the pharynx and the female gonopore is 1.8 mm. The distance between gonopores is 1 mm (Figures 2 & 3).

Few eyes occur on the anterior region of the body, some of which are dorsal to the brain in the unpigmented centrodorsal region, and others are located in the unpigmented pre-cerebral marginal region (Figures 1 & 4A). The musculature of the body wall consists of an outer longitudinal layer of one or two fibres in thickness, then a layer of circular muscles one fibre in thickness, and an inner longitudinal layer 4–5 fibres in thickness. The epidermis is ciliated, with the dorsal cilia longer than the ventral ones. The pharynx is located anteriorly to the medial region of the body and is folded, showing up to four folds (Figure 4B, C).

Separate gonopores are located in the middle third of the body (Figure 4B, D–F). Testes are located along the

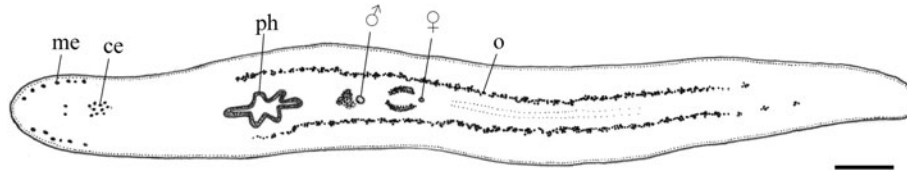


Fig. 2. Schematic representation of *Namyhlana henriettae* in dorsal view. Abbreviations: ce, cerebral eyes; me, marginal eyes; o, oviduct; ph, pharynx; ♀, female gonopore; ♂, male gonopore. Scale bar: 1000 μ m.

body, in dorsal position. A pair of well-developed spermiducal vesicles with thin muscularized walls, ventral in the anterior region, occupy the total height of the body, next to the copulatory apparatus (Figure 5). The spermiducal vesicles are charged with spermatozooids that flow independently into the seminal vesicle in front of the male gonopore (Figure 4D, E). The seminal vesicle is oval ($400 \times 200 \mu$ m) and strongly muscularized and contained spermatozooids in all studied specimens (Figure 4D, E). A very long and thin ejaculatory duct in horizontal position and dorsal to the male atrium connects the seminal vesicle to the penis papilla (Figure 4D, E). The penis papilla is digitiform, 1×0.1 mm, and is in horizontal position, with the distal end directed forward. The ejaculatory duct that runs along the penis papilla is sinuous. The male genital atrium is covered by a ciliate and glandular epithelium (Figure 4D, E).

The ovaries are dorsal and occupy a large part of the body. The dilated oviducts are full of oocytes (Figure 4B). They extend from the anterior region to beyond the level of the female gonopore, separately reaching the internal vagina that curves towards the dorsum, forms a curl, and continues into the median vagina surrounded by a large number of eosinophil glands (mucous *sensu* Faubel, 1983) (Figure 4D, F). The external vagina, positioned dorsoventrally, flows into the female gonopore, which presents an asymmetrical sphincter (Figure 4F). The external vagina is lined up with a high epithelium and shows a 'pocket'-like expansion towards the posterior region. A short canal connects the internal vagina to a large Lang's vesicle, that extends posteriorly for a long distance, ventrally to the intestine. Ventral evaginations of the intestine are intimately in contact with Lang's vesicle (Figure 5).

Taenioplana teredini Hyman, 1944
(Figures 6–8)

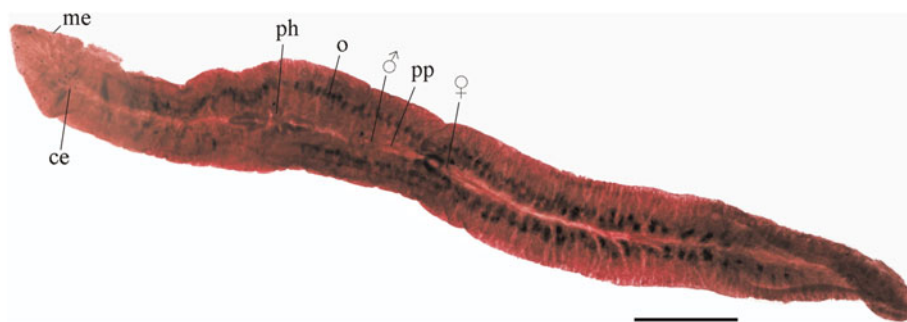


Fig. 3. Dorsal view of *Namyhlana henriettae*. Specimen stained with acetic carmine. Abbreviations: ce, cerebral eyes; me, marginal eyes; o, oviduct; ph, pharynx; pp, penis papilla; ♀, female gonopore; ♂, male gonopore. Scale bar: 1000 μ m.

MATERIAL EXAMINED

Holotype: USNM 20636. The specimen is from Oahu Island, Honolulu, Hawaii ($21^{\circ}30'N157^{\circ}85'W$); collected by C. Edmondson on 24 February 1944 and found in association with *Teredo* sp.

Paratypes: USNM 20637, BPBM- F 115, BPBM- F 117. All these specimens are from Oahu Island, Honolulu, Hawaii ($21^{\circ}30'N157^{\circ}85'W$); collected by C. Edmondson on 24 February 1944 and found in association with *Teredo* sp.

REMARKS

The study of these specimens allowed us to provide new details of some structures of *Taenioplana teredini*. The paratype sagittally sectioned in this study provided new details of some structures, especially of the female reproductive system, because the Bishop Museum paratype (BPBM-F 115) specimen is more mature (the eggs are mature, and such vaginal glands are well-developed) than Hyman's (USNM 20637).

The body wall is well-developed with long cilia on the dorsal and ventral epithelia. The ventral musculature has three layers: an external longitudinal, a middle circular, and an internal longitudinal layer. The dorsal musculature is thicker than the ventral one, has a longitudinal external layer and a circular internal layer from which dorso-ventral muscular fibres arise. An inner longitudinal layer in the dorsal musculature was not observed. Under the musculature of the dorsal region, a dark pigment forms stripes on different regions of the body, but mainly on the anterior region. This pigment is also observed externally in the holotype (Figure 6).

The brain is spherical and encapsulated in the anterior pre-pharyngeal region. The distribution of the eyes is the same that Hyman (1944) described. All eyes have a pigmented cup. The epithelium in this region is low and the cells are almost flat and unpigmented. The pharynx is folded; many folds occupying a large part of the first third of the body.

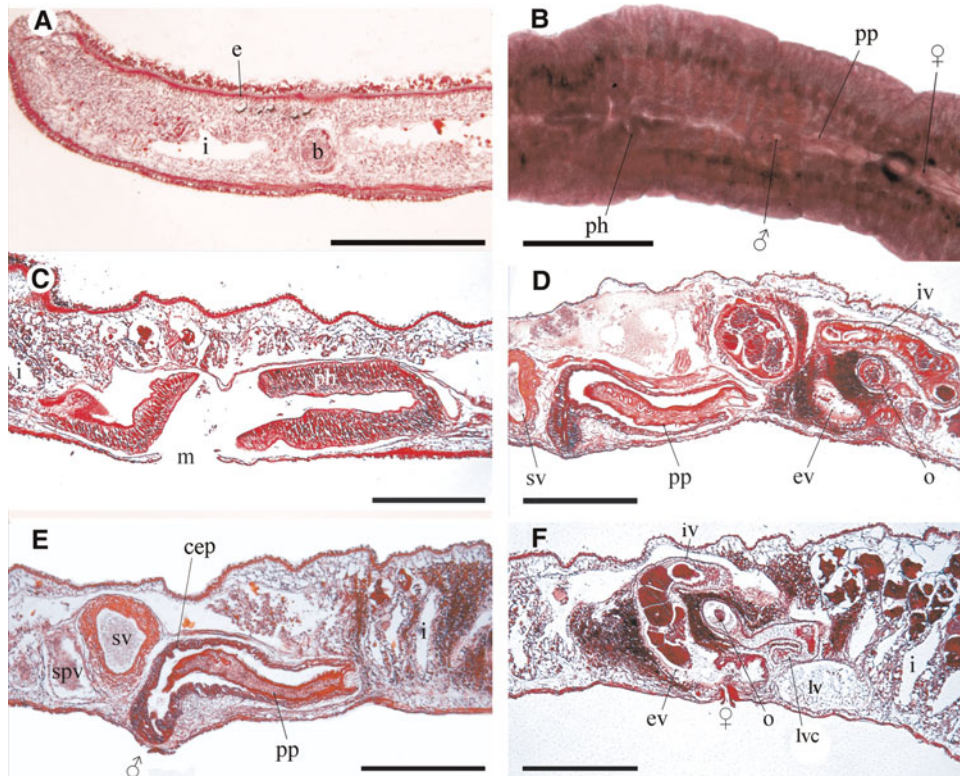


Fig. 4. Anatomical features of *Namyhlana henriettae*: (A) sagittal section of the cerebral region; (B) whole-mounted preparation of the pharyngeal region and gonopores; (C) sagittal section of the pharyngeal region; (D–F) sagittal section of the region of the copulatory system. Abbreviations: b, brain; cep, ciliated epithelium; e, eyes; ev, external vagina; i, intestine; iv, internal vagina; lv, Lang's vesicle; lvc, Lang's vesicle canal; m, mouth; o, oviduct; ph, pharynx; pp, penis papilla; spv, spermiducal vesicle; sv, seminal vesicle; ♀, female gonopore; ♂, male gonopore. Scale bars: A, C–F, 500 µm; B, 1000 µm.

The morphology of the male genital system coincides with the original description (Hyman, 1944). The testes are dorsal. In the female genital system the ciliated oviducts extend from the anterior region to beyond the level of the female gonopore. They flow into a common oviduct. The ciliated internal vagina that curves towards the dorsum, forms a curl, and continues forward (Figures 7 & 8). The external vagina, oriented vertically, with high, folded, and ciliated wall, surrounded by

numerous eosinophil glands, flows into the female gonopore without sphincter. A genito-intestinal duct originates in the curl of the internal vagina and goes backwards through a long section until it broadens, opening into an intestinal pocket. In the specimen studied here (BPBM-F 115), the genito-intestinal duct is longer than the one described by Hyman (1944) as a short duct communicating with a ventral intestine pocket.

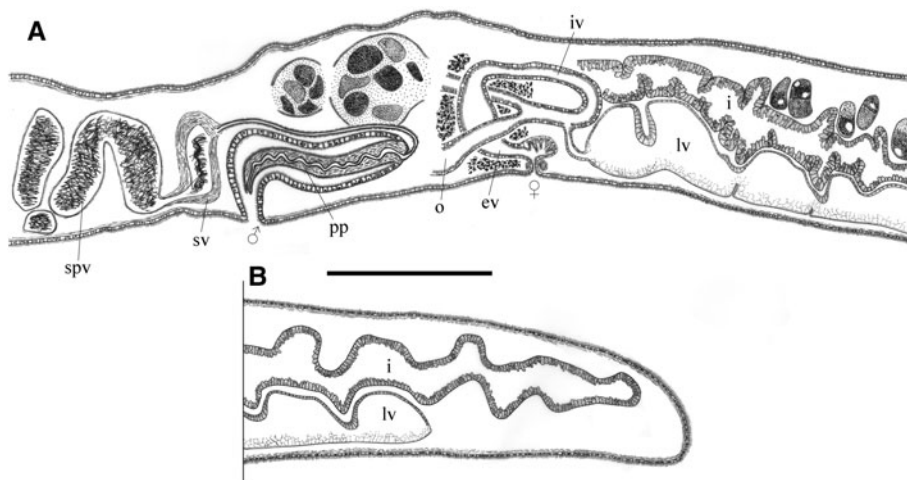


Fig. 5. Schematic reconstruction of the copulatory system of *Namyhlana henriettae*. Anterior end on the left: (A) sagittal reconstruction of the entire copulatory system; (B) sagittal reconstruction of the posterior region of the body. Abbreviations: ev, external vagina; i, intestine; iv, internal vagina; lv, Lang's vesicle; o, oviduct; pp, penis papilla; spv, spermiducal vesicle; sv, seminal vesicle; ♀, female gonopore; ♂, male gonopore. Scale bar: 1000 µm.



Fig. 6. Holotype of *Taenioplana teredini* (USNM 20636). Anterior end on the left. Scale bar: 5 mm.

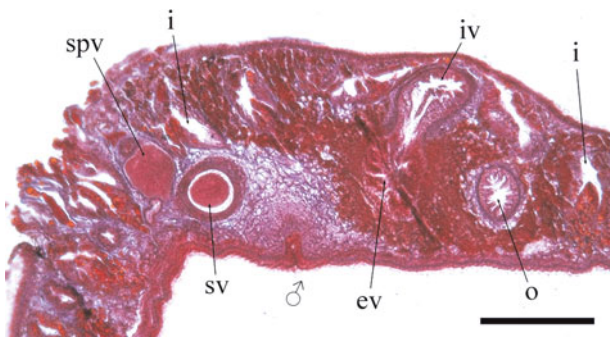


Fig. 7. Copulatory region of *Taenioplana teredini*. Sagittal section of the paratype specimen (BPBM-F 115). Abbreviations: ev, external vagina; i, intestine; iv, internal vagina; o, oviduct; sv, seminal vesicle; spv, spermiducal vesicle; ♂, male gonopore. Scale bar: 250 μ m.

PHYLOGENY

Parsimony analysis of the data matrix (Table 2) yielded one most parsimonious tree of length 88 (consistency index 0.41; retention index 0.53). Bremer support values are indicated on the nodes of the tree (Figure 9).

The phylogenetic analysis does not recover the family Euplanidae as a 'natural' group, since two Ilyplanidae species and *Anocellis profundus* (Anocellidae) are included in the Euplanidae ingroup. These species form a clade supported by the orientation of the uterine canals in relation to the position of the vagina (#24.0) and by a large distance

between the genital pores (#10.1). Moreover, *Aprostatum stiliferum* is outside the family Euplanidae in the analysis because of the presence of a prostatic vesicle (#15.1 see data matrix), and peculiarities in the distribution pattern of eyes, especially the marginal ones (#4.2 and #6.0, see data matrix). The absence of prostatic structures (#15.0), the presence of cerebral eyes in two clusters (#6.2) and the presence of tentacular eyes (#5.1, homoplastic) support the other species of the family Euplanidae and three outgroup species.

Namyhplana henriettae sp. nov. is inferred as sister of *Taenioplana teredini*, sharing the mode of life (#1.1) and the particular disposition of the marginal eyes (#4.1). However, *T. teredini* lacks Lang's vesicle (#20.0), a character shared instead with other family members. Also, it possesses a genito-intestinal duct (#25.1), a character shared with one of the outgroup species, *Mucroplana caelata*.

The new species presents unarmed penis papilla, Lang's vesicle and pharynx in the anterior region of the body. Although these characters are shared with most of the species included in the genus *Euplanoida* Faubel, 1983, the new species is inferred as distantly related to *Euplanoida*. It remains unclear, whether *Euplanoida* is a monophyletic genus, since relationships among its species are unresolved in our analysis.

DISCUSSION

The study of polyclad flatworms living in tubes of the bivalve *Bankia martensi* from southern Chile allowed us to describe a new species of a new genus, *Namyhplana henriettae*. This species displays a unique combination of characters, mainly of the reproductive system, including the Lang's vesicle ventral to the intestine, absence of a genito-intestinal duct and presence of a large, unarmed penis papilla placed horizontally with the distal end directed forward. Despite this lost feature, the whole copulatory apparatus of *N. henriettae* is directed backwards, like in *Taenioplana teredini*. The absence of prostatic structures places *N. henriettae* in the family Euplanidae. To understand the relationships of the new species within this family we carried out phylogenetic analysis based on morphological data, the first analysis of this kind at the family level.

Results suggest that *N. henriettae* is sister to *T. teredini*. The two species are indeed highly similar based on the general (external and internal) morphology (Figure 9). Characteristics shared between the two species include the short band of eyes along the anterior margin, an anterior

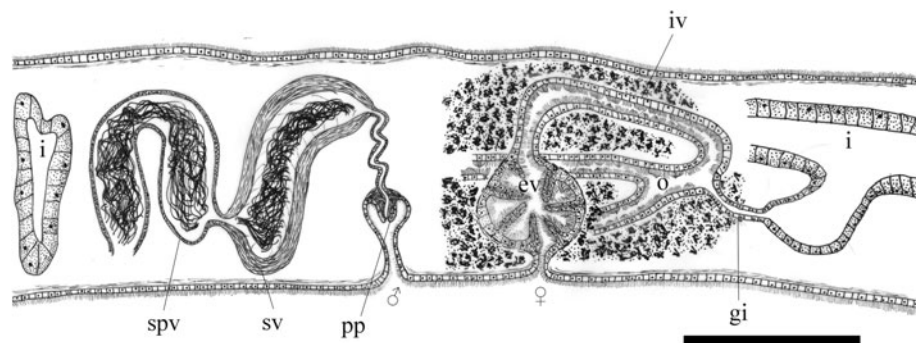


Fig. 8. Schematic reconstruction of the copulatory system of *Taenioplana teredini*. Abbreviations: ev, external vagina; gi, genito-intestinal duct; i, intestine; iv, internal vagina; sv, seminal vesicle; o, oviduct; pp, penis papilla; spv, spermiducal vesicle; ♀, female gonopore; ♂, male gonopore. Scale bar: 250 μ m.

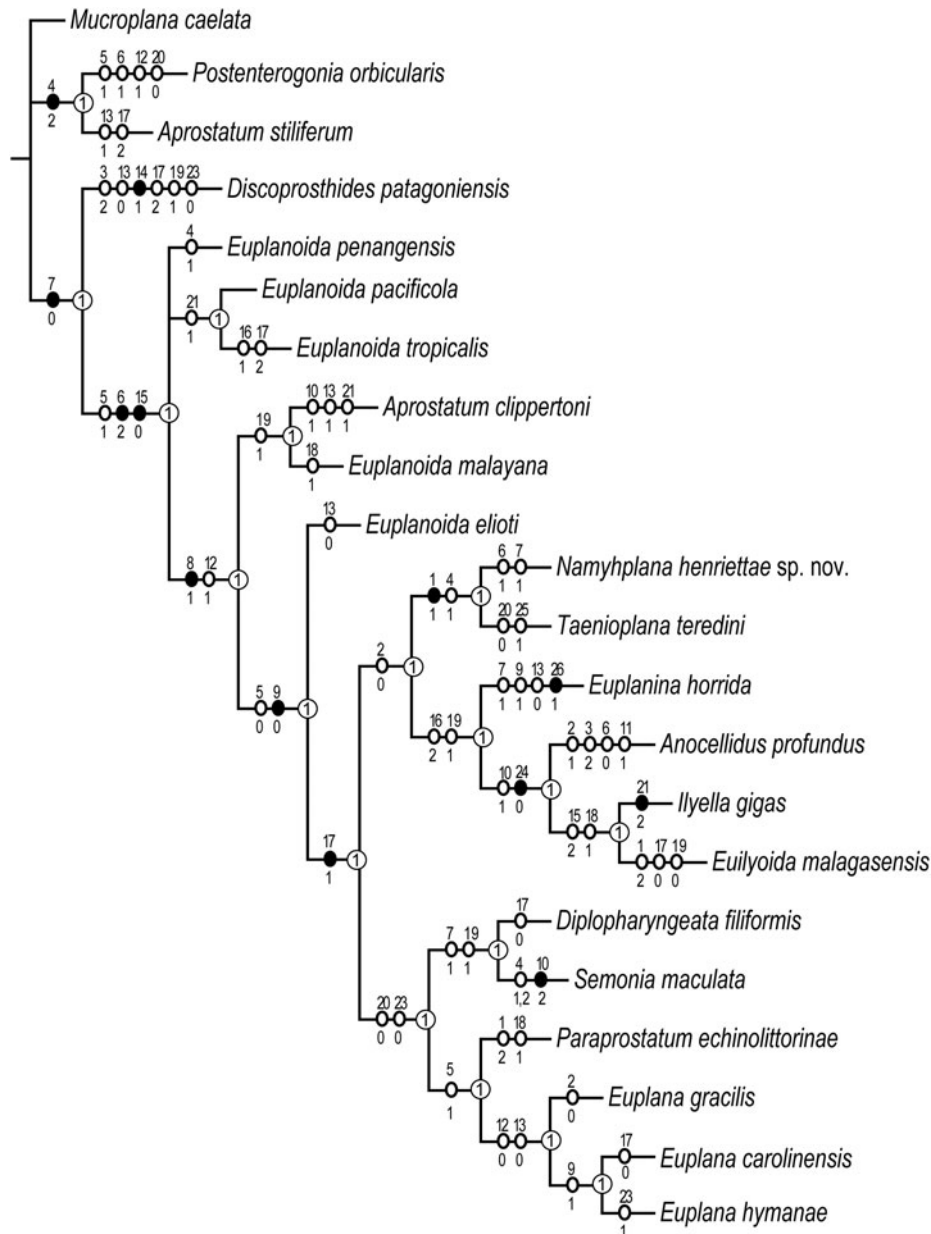


Fig. 9. Most parsimonious tree inferred with phylogenetic analysis of the morphological dataset of Euplanidae species (length 88 steps, consistency index = 0.41; retention index = 0.53). Numbers indicate characters as listed in Table 1. Apomorphies are in black, and homoplasies in white. Bremer support values are indicated on nodes.

pharynx and the copulatory apparatus in the anterior half of the body. External morphological differences between the two species include the cerebral eyes, fewer in number in *N. henriettae* than *T. teredini*, and an unpigmented region in the epidermis at the level of the brain, where cerebral eyes are present, only in the new species. The most important internal morphological difference is the presence of Lang's vesicle in the new species, absent in *Taenioplana*. This is currently considered a diagnostic feature at the generic level (Faubel, 1983; Faubel *et al.*, 2007). Bock (1927) and Hyman (1944) suggest a possible 'homology' among Lang's vesicle, the genito-intestinal duct and the vaginal duct. This hypothesis is supported by the residual products of the reproductive system, such as spermatozooids and yolk, found in Lang's vesicle of *N. henriettae*, and by the evaginations present in the intestine, touching Lang's vesicle.

Other differences between *N. henriettae* and its sister species, *T. teredini*, include the location of the pharynx, situated closer to the medial region of the body in the new species and smaller in size than in *T. teredini*. The penis papilla is large and placed horizontally with the distal end directed forward in *N. henriettae*, whereas it is small and placed dorso-ventrally in *T. teredini*. The ejaculatory extra-penial duct is straight in *N. henriettae*, whereas it is curled before entering the small penis papilla in *T. teredini*. The vagina of the new species is longer and more twisted than in *T. teredini*, and its distal portion has a posterior pocket with an epithelium of very long cilia, which is absent in *T. teredini*. The sphincter in the female gonopore of the new species is strong and more developed posteriorly, while it is absent in *T. teredini*.

When comparing the new species to other species within the family Euplanidae, *N. henriettae* shows similar characteristics to

some species of *Euplanoida* (unarmed penis papilla and Lang's vesicle). In this context, the generic assignment of other *Euplanoida* species should be reviewed, since *Euplanoida elioti* lacks the unarmed penis papilla considered a diagnostic characteristic of the genus.

Several studies have contributed to increasing our understanding of the systematics of the family Euplanidae (Faubel, 1983, 1984; Prudhoe 1985; Doignon *et al.*, 2003; Quiroga *et al.*, 2006). Some of these authors (Doignon *et al.*, 2003; Quiroga *et al.*, 2006), and results from our phylogenetic analysis as well, do not support monophyly of the family and of its genera *Aprostatum* and *Euplanoida*. Comprehensive phylogenetic analyses using more detailed anatomical and molecular information are necessary to disentangle relationships of species currently ascribed to Euplanidae.

Namyhplana henriettae lives associated with the tubes that *B. martensi* makes in the wood of *Nothofagus* submerged in the fjords from southern Chile. This is similar to the habitat of *T. teredini* intimately associated with *Teredo* sp. in different parts of the world. The current wide distribution of *T. teredini* is probably due to the commercial transportation of the wood used by *Teredo* sp. between ports (Hoagland & Turner, 1980; Carlton, 2009). Therefore, it is possible that the geographical distribution of *N. henriettae* will also expand in the future, and we advise monitoring of the possible introduction of this polyclad along with the transportation of the wood used by *Bankia*.

ACKNOWLEDGEMENTS

We thank Diego Zelaya (for the collection of the polyclads, the identification of *Bankia martensi*, and the information on the habitat where the specimens of *Namyhplana henriettae* were collected), the USNM and the Bishop Museum (for the loan of the type material of *Taenioplana teredini*) and the librarians of the Instituto Nacional de Desarrollo Pesquero (for the access to bibliographical material essential for this work).

FINANCIAL SUPPORT

This study was partially funded by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Universidad Nacional de La Plata.

REFERENCES

- Baeza J.A., Veliz D., Pardo L.M., Lohrmann K. and Guisado C. (1997) A new polyclad flatworm, *Tythosoceros inca* (Platyhelminthes: Polycladida: Cotylea: Pseudocerotidae), from Chilean coastal waters. *Proceedings of the Biological Society of Washington* 110, 476–482.
- Bock S. (1913) Studien über Polycladen. *Zoologiska Bidrag Uppsala* 2, 31–344.
- Bock S. (1923) Polycladen aus Juan Fernandez. In Skottsberg C. (ed.) *Natural history of Juan Fernandez and Easter Island*. Uppsala: Almqvist & Wiksell Tryckeri, AB, pp. 341–372.
- Bock S. (1927) Ductus genito-intestinalis in the Polyclads. *Arkiv für Zoologi* 19A, 1–15.
- Boschi E.E. (2000) Species of decapods crustaceans and their distribution in the American marine zoogeographic provinces. *Revista de Investigación y Desarrollo Pesquero* 13, 7–136.
- Brusa F. and Damborenea C. (2011) Polycladida Acotylea from Patagonia. Redescription of *Crassiplana albatrossi* (Pseudostylochidae), lectotype designation and first record of *Notocomplana palta* (Notoplanidae). *Zootaxa* 2903, 29–38.
- Bulnes V.N. (2009) Polycladida-Planarias Marinas. In Häussermann V. and Försterra G. (eds.) *Fauna marina Bentónica de la Patagonia Chilena*. Santiago: Nature in Focus, pp. 355–368.
- Carlton J.T. (2009) Deep invasion ecology and the assembly of communities in historical time. In Rilov G. and Crooks J.A. (eds) *Biological invasions in marine ecosystems*. Berlin, Heidelberg: Springer, pp. 13–56.
- Carlton J.T. and Eldredge L.G. (2009) Marine bioinvasions of Hawaii. The introduced and cryptogenic marine and estuarine animals and plants of the Hawaiian archipelago. *Bishop Museum Bulletin in Cultural and Environmental Studies* 4, 1–20.
- Doignon G., Artois T. and Deheyn D. (2003) *Discoplana malagasensis* sp. nov., a new turbellarian (Platyhelminthes: Polycladida: Leptoplanidae) symbiotic in an ophiuroid (Echinodermata), with a cladistic analysis of the *Discoplana/Euplana* species. *Zoological Science* 20, 357–369.
- Faubel A. (1983) The Polycladida, Turbellaria. Proposal and establishment of a new system. Part I. The Acotylea. *Mitteilungen aus dem Hamburgischen Zoologischen Museum und Institut* 80, 17–121.
- Faubel A. (1984) The Polycladida, Turbellaria. Proposal and establishment of a new system. Part II. The Cotylea. *Mitteilungen aus dem Hamburgischen Zoologischen Museum und Institut* 81, 189–259.
- Faubel A., Sluys R. and Reid D.G. (2007) A new genus and species of polyclad flatworm found in the mantle cavities of gastropod molluscs in the high-intertidal zone of the Pacific coast of Central America. *Journal of the Marine Biological Association of the United Kingdom* 87, 429–434.
- Goloboff P., Farris J. and Nixon K. (2008) TNT, a free program for phylogenetic analysis. *Cladistics* 24, 774–786.
- Hoagland K.E. and Turner R.D. (1980) Range extension of teredinids (shipworms) and polychaetes in the vicinity of a temperate-zone nuclear generating station. *Marine Biology* 58, 55–64.
- Holleman J.J. (2007). Some New Zealand polyclads (Platyhelminthes, Polycladida). *Zootaxa* 1560, 1–17.
- Hyman L.H. (1939) Some polyclads of the New England coast, especially of the Woods Hole region. *Biological Bulletin. Marine Biological Laboratory, Woods Hole* 76, 127–152.
- Hyman L.H. (1941) The polyclad flatworms of the Atlantic coast of the United States and Canada. *Proceedings of the United States National Museum* 89, 449–495.
- Hyman L.H. (1944) A new Hawaiian polyclad flatworm associated with *Teredo*. *Occasional Papers of the Bernice Pauahi Bishop Museum* 18(4), 73–75.
- Hyman L.H. (1953) The polyclad flatworms of the Pacific coast of North America. *Bulletin of the American Museum of Natural History* 100, 269–391.
- Hyman L.H. (1954) Some polyclad flatworms from the Hawaiian Islands. *Pacific Science* 8, 331–336.
- Hyman L.H. (1955) Some polyclad flatworms from Polynesia and Micronesia. *Proceedings of the United States National Museum* 105, 65–82.
- Laidlaw F.F. (1903a) On the marine fauna of Zanzibar and British East Africa, from collections made by Cyril Crossland in the Years 1901

- and 1902. Turbellaria Polycladida. I. The Acotylea. *Proceedings of the Zoological Society of London* 2, 99–113.
- Laidlaw F.F.** (1903b) On a collection of Turbellaria Polycladida from the Straits of Malacca. (Skeat Expedition 1899–1900). *Proceedings of the Zoological Society of London*, 301–318.
- Marcus E.** (1947) Turbellarios Marinhos do Brasil. *Zoologia. Universidade de São Paulo. Boletins da Faculdade de Filosofia, Ciências e Letras* 12, 99–215.
- Marcus E.** (1954) Reports of the Lund University Chile Expedition 1948–49. II. Turbellaria. *Lunds Universitets Arsskrift* 49, 3–115.
- Newman L.J. and Cannon L.R.G.** (2003) *Marine flatworms: the world of polyclads*. Collingwood: Csiro Publishing.
- Plehn M.** (1896) Neue Polycladen, gesammelt von G. Chierchia bei der Erdumschiffung der Korvette Vettor Pisani von Hern Prof. Dr Kükenthal im nördlichen Eismeer und von Semon in Java. *Jenaische Zeitschrift* 30, 137–176.
- Prudhoe S.** (1985) *A monograph on polyclad Turbellaria*. British Museum (Natural History). Oxford: Oxford University Press.
- Quiroga S.L., Bolaños D.M. and Litvaitis M.K.** (2006) First description of deep-sea polyclad flatworms from the North Pacific: *Anocellidus* n. gen. *profundus* n. sp. (Anocellidae, n. fam.) and *Oligocladus voightae* n. sp. (Euryleptidae). *Zootaxa* 1317, 1–19.
- Riser N.W.** (1970) Biological studies on *Taenioplana teredini* Hyman 1944. *American Zoologist* 10, 553.
- Riser N.W.** (1974) Epilogue. In Riser N.W. and Morse M.P. (eds) *Biology of the Turbellaria*. New York: McGraw-Hill, pp. 517–524.
- Ritter-Zahony R.** (1907) Turbellarien: Polycladiden. Ergebnisse der Hamburger Magalhaensische Sammelreise 1892/93. *Naturhistorisches Museum Hamburg* 3, 1–19.
- Schmarda L.K.** (1859) *Neue wirbellose Thiere beobachtet und gesammelt auf einer Reise um die Erde 1853 bis 1857. Bd. I: Turbellarien, Rotatorien und Anneliden. 1. Hälfte*. Leipzig: W. Engelmann, 66 pp.
- and
- Sopott-Ehlers B. and Schmidt P.** (1975) Interstitielle fauna von Galapagos. XIV. Polycladida (Turbellaria). *Mikrofauna des Meeresbodens* 54, 193–222.

Correspondence should be addressed to:

F. Brusa
División Zoología Invertebrados
Museo de la Plata
Paseo del Bosque s/n°. B1900FWA
La Plata, Argentina
Email: fbrusa@fcnym.unlp.edu.ar