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A phylogenetic analysis of *Stenostomum* and its neotropical congeners, with a description of a new species from the Peruvian Amazon Basin

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Abstract. This study reports Peruvian freshwater species of *Stenostomum* (Catenulida) from the upper Yurúa River of the Amazon Basin. A total of 11 species were found. One of them, *Stenostomum ashanika*, sp. nov., is described. It can be distinguished by: a pre-pharyngeal constriction; a small cauda; a small, rounded, mobile and ventrally directed pharynx; and a tri-radiate mouth. The remaining 10 species are reviewed and illustrated; eight are first records for Peru. A preliminary phylogenetic analysis of neotropical stenostomid species based on morphological data is undertaken, including all *Rhynchoscolex*, *Stenostomum* and *Myostenostomum* species described for the area. The *Rhynchoscolex* species are basal and paraphyletic; the species of *Myostenostomum* are monophyletic, within the *Stenostomum* clade, supported by the presence of a muscular gizzard in the anterior region of the intestine. Ciliated pits and rhabdoids are synapomorphies of the *Stenostomum* + *Myostenostomum* clade. This is the first morphological study of the relationships between stenostomid species. The most relevant characters in the phylogenetic analysis are those related to cerebral brain lobes. The results also show that many of the morphological characteristics of the species under study are poorly known and should be studied in more depth.

Introduction

Stenostomidae is the most species-rich family among Catenulida, including ~65 freshwater species (Tyler *et al.* 2006–2010), 36 of them being either endemic to the Neotropics or mentioned for this region. Most species have been described from Brazil (Marcus 1945*a*, 1945*b*, 1949; Gamo and Leal-Zanchet 2004), but a few have also been recorded from Surinam (Van der Land 1970), Argentina (Noreña-Janssen 1995; Noreña *et al.* 2005) and Peru (Noreña *et al.* 2006). *Stenostomum*, with 52 species, is the most diverse genus within the Catenulida. Members of the genus are morphologically simple, generally lack a developed reproductive system, and predominantly reproduce asexually. Identification of species is best done when specimens are alive (Noreña *et al.* 2005; Larsson *et al.* 2008; Larsson and Willems 2010). Authors give differing importance to diagnostic morphological characters, making comparison of some species from different geographic regions difficult. In addition, some species descriptions are based on cultured organisms, whereas others are based on specimens collected in natural habitats. All these factors make stenostomids a difficult group for morphological studies.

The relationship of Catenulida with the remaining groups of Platyhelminthes has been controversial. Most studies based on their morphology (e.g. lobed cerebrum, unpaired protonephridium with particularly organised cyrtocytes, special type of pharynx simplex and aberrant aciliated spermatozoa) place them as a basal linage of Platyhelminthes (Karling 1974; Ehlers 1985, 1986). Molecular studies, carried out with a variety of markers, place Catenulida as the sister group to Rabdithophora (Peterson and Eernisse 2001; Jondelius *et al.* 2002; Telford *et al.* 2003; Larsson and Jondelius 2008) or as an early branching clade and sister group of the rest of Bilateria (Carranza *et al.* 1997). While these studies attempted to determine the relationships of Catenulida within Platyhelminthes, there has been little investigation of relationships between the catenulid families or between species.

Larsson *et al.* (2008) studied 16 species of four families of Catenulida from Sweden, using four molecular markers, to reconstruct the phylogeny of Catenulida, to identify cryptic diversity at the species level, and to establish the relationships among them. The analysis included 11 *Stenostomum* species. Their results showed that the genus *Anokkostenostomum* Noreña, Damborenea & Brusa, 2005 should be a junior synonym of *Stenostomum* and that *Suomina* Zacharias, 1902 should be treated as junior synonym of *Catenula* Dugès, 1832. However, this molecular study failed to resolve relationships among the *Stenostomum* species included in the analysis.

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The present work deals with 11 species of *Stenostomum* from the upper Yurúa River basin, Ucayali, Peru (Peruvian Amazon Basin). We describe a new species, *Stenostomum ashanika*, sp. nov., and we compare the morphological traits of the remaining species with those known from different regions. To analyse the relationships among *Stenostomum* species with morphological information, and assess the phylogenetic relevance of the main characters used in taxonomic identification, we performed a preliminary phylogenetic analysis on species of *Stenostomum* with morphological data. This included the known neotropical species and four other species recently described from other regions as well as representatives of other genera of Stenostomidae found in the neotropical region.

Materials and methods

Collection of material

The material was collected in July and August 2008 during an expedition to the upper Yurúa River, Ucayali department, Peru (Fig. 1). The area has suffered low anthropogenic impact, so communities of organisms can be considered pristine. The samples were collected during the low water level period, from aquatic vegetation in lagoons or abandoned river meanders ('*cochas*'), and from small tributary streams ('*quebradas*') of the Yurúa River, using 125 and 160-µm mesh nets (Table 1). The plants (roots and leaves) were rinsed within the net and returned to the water. Small fast-flowing channels and waterfalls were sampled by scraping the rock surface or fallen submerged tree trunks and then filtering the water with 125-µm mesh. The material was kept in plastic container and transported to the field laboratory, where the water of the samples was extracted in small dishes and observed under a stereo-microscope. Sample concentration methods were not applied.

Taxonomic descriptions

The specimens were individually collected and flattened on a slide (under a cover slip), studied under a light microscope, drawn free-hand and their relevant features were described. Measurements were taken from live specimens. Some specimens were fixed in Bouin's solution and preserved in 70% alcohol. Studied specimens were lodged in the Invertebrate Collection of the Museo de La Plata, Argentina (MLP) and in the Helminthological and Related Invertebrates Collection of the Museo de Historia Natural of the UNMSM, Lima, Peru (MUSM).

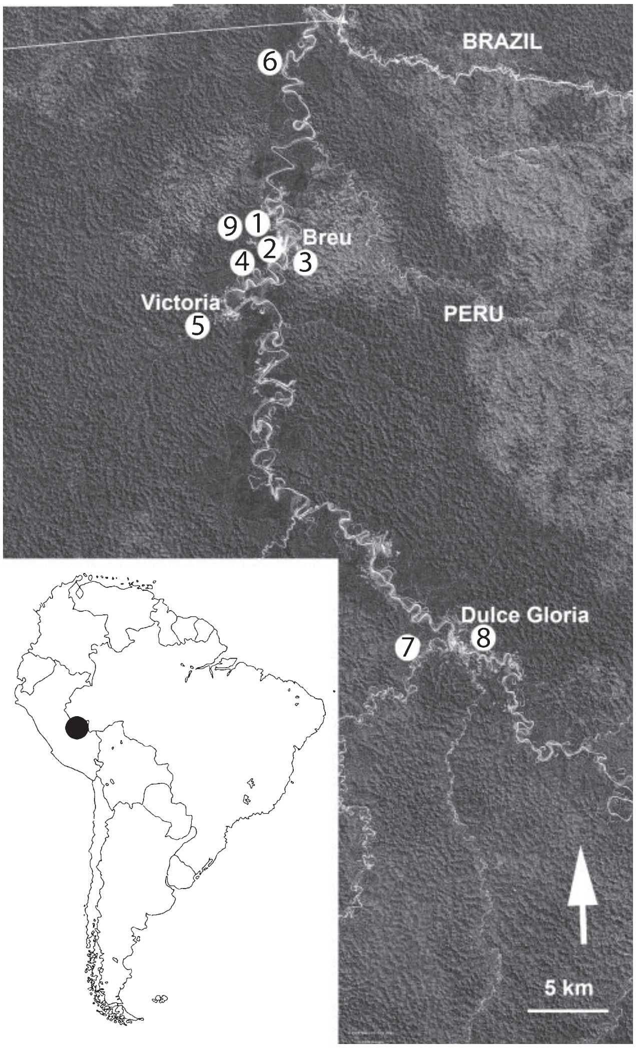

Fig. 1. Aerial photograph of the research area. The white spots are the sample sites (numbers correspond to the sampling localities mentioned in Table 1).

Table 1. Sampling sites in the upper Yurúa River, Ucayali, Peru

Site	Description	Latitude/longitude	Date
1. Quebrada Dos y medio, Breu	Small channel, soft bottom with *Poligonium* sp. and *Pistia* sp.	9°31.174′S 72°45.760′W	20 July 2008
2. Cocha Aeropuerto	Abandoned bend of the river with *Azolla* sp., *Pistia* sp., *Salvinia* sp. and *Paspalum* sp.	9°31.735′S 72°45.321′W	31 July 2008
3. Cocha in Breu	Abandoned bend of the river, with *Azolla* sp. and *Poligonium* sp.	9°32.225′S 72°45.020′W	21 July 2008 02 August 2008
4. Cocha Segunda	Abandoned bend of the river, with *Pistia* sp.	9°32.800′S 72°46.423′W	24 July 2008
5. Quebrada Sabotari, Victoria	Small channel	9°34.375′S 72°48.146′W	22 July 2008
6. Yurúa River	Mouth of a small channel, lateral area of the river with small falls	9°25.921′S 72°44.564′W	25 July 2008
7. Huacapishtea River, Dulce Gloria	Rapids	9°46.109′S 72°42.191′W	28 July 2008
8. Cocha Lopona, Dulce Gloria	Abandoned bend of the river	9°45.828′S 72°39.952′W	28 July 2008
9. Quebrada Antonino	Small channel, with dead leaves	9°31.236′S 72°46.828′W	30 July 2008

Phylogenetic analysis

Taxa included

The study included the known neotropical *Stenostomum* species and four recently described species from Sweden. Some *Stenostomum* species were recorded during fieldwork in the upper Yurúa River, Ucayali department, Peru. Other species from Argentina were studied previously by Noreña-Janssen (1995) and Noreña *et al*. (2005). The information for the remaining neotropical species was taken from the literature (Marcus 1945*a*, 1945*b*, 1949; Van der Land 1970). The new species described in this paper was also included in the analysis. *Stenostomum pegephilum* Nuttycombe & Waters, 1938 was not included in the analysis owing to discrepancies between the features described by Marcus and those from the original descriptions of specimens from the US, so many of the characters used in our study could not be coded.

The inclusion of *Stenostomum gotlandense* Larsson & Willems, 2010, *S. handoelense* Larsson & Willems, 2010, *S. heebuktense* Larsson & Willems, 2010 and *S. steveoi* Larsson & Willems, 2010, recently described from Sweden, allowed for an assessment of the monophyly of the neotropical *Stenostomum* species.

Seven other neotropical species of Stenostomidae were also included in the dataset: five *Rhynchoscolex* Leidy, 1851 and two *Myostenostomum* Luther, 1960 (Table 2). These species were included in the analysis to test the hypothesis of monophyly of *Stenostomum* species. *Chordarium evelinae* Marcus, 1945 (Chordariidae) was used to root the tree. This species provided more morphological features to compare with the species in the

Table 2. Matrix of 37 taxa and 41 characters used in this analysis
Unknown states are represented by '?' and inapplicable characters by '–'

Taxa	Characters								
	00000	00001	11111	11112	22222	22223	33333	33334	4
	12345	67890	12345	67890	12345	67890	12345	67890	1
S. amphotum Marcus, 1945	01211	10011	00111	00110	00101	11220	00100	01001	1
S. anatirostrum Marcus, 1945	11000	00110	00112	1110-	-0000	01140	00100	01000	1
S. arevaloi Gieysztor, 1931	01201	11110	01111	1-111	10101	01020	01100	00001	1
S. ashanika, sp. nov.	11110	10?10	00111	1110-	-20?1	00-0	00100	01001	1
S. bicaudatum Kennel, 1889	01201	20110	00111	10111	10201	01021	00100	00001	1
S. ciliatum Kepner & Carter, 1931	11101	10011	00111	00110	10001	01210	00100	01001	1
S. corderoi Marcus, 1945	11200	01010	00112	1110-	-22?1	01020	10100	01000	1
S. cryptops Nuttycombe & Waters, 1935	21010	01010	00112	02111	11001	11210	00110	01000	1
S. evelinae Marcus, 1945	01200	01011	00110	1110-	-11?1	11020	00110	01000	1
S. glandulosum Kepner & Carter, 1931	21110	000??	01111	11111	10100	01120	00100	01000	1
S. grande Child, 1902	01111	11010	00111	01110	00101	11020	00100	01001	1
S. gotlandense Larsson & Willems, 2010	11200	00?1?	00111	1010-	-10?0	0???0	00100	01000	1
S. handoelense Larsson & Willems, 2010	11200	0000?	00112	0010-	-01??	01120	00100	01000	1
S. heebuktense Larsson & Willems, 2010	11100	00?1?	00111	0210-	-02?1	10-0	00100	00000	1
S. hemisphericum Nassonov, 1924	11101	01010	00112	01110	10201	11100	00100	01001	1
S. leucops (Dugès, 1828) O. Schmidt, 1848	11200	01010	00111	01110	00100	11220	00100	01001	1
S. matarazzoi Marcus, 1949	01211	11011	00111	00110	02000	01020	00100	00001	1
S. membranosum Kepner & Carter, 1931	21210	0000-	00112	1210-	-1010	00-0	00100	000?0	1
S. paraguayense (Martin, 1908) Luther, 1908	01211	20011	01111	00111	12000	01020	01100	10001	1
S. pseudoacetabulum Nuttycombe & Waters, 1935	11201	11110	00111	1110-	-0001	11010	00100	00011	1
S. rosulatum Marcus, 1945	11001	11110	00111	01110	10000	11100	00100	01111	1
S. saliens Kepner & Carter, 1931	21000	01010	00112	1210-	-10?0	10-0	00100	00000	1
S. simplex Kepner & Carter, 1931	21010	01110	00112	02110	21110	010?0	00100	00000	1
S. steveoi Larsson & Willems, 2010	11200	00?0?	00112	0010-	-11?0	00-0	00100	01000	1
S. tenuicauda Graff, 1911	01211	11011	00111	00110	00001	11010	00100	01001	1
S. tuberculosum Nuttycombe & Waters, 1938	11411	11110	00112	1110-	-20?0	01110	00100	00000	1
S. uronephrium Nuttycombe, 1931	01411	11110	00110	01110	10101	11010	00100	01011	1
S. ventronephrium Nuttycombe, 1932	11110	01011	10111	1110-	-0100	01100	00100	00000	1
S. virginianum Nuttycombe, 1931	11100	01010	00111	12110	10100	11010	00100	01001	1
Rhynchoscolex evelinae Marcus, 1945	20-11	1100-	00112	0000-	-0001	01120	00-00	00001	0
R. nanus Marcus, 1945	20-10	0000-	00112	0010-	-10?1	01120	00-00	01000	0
R. platypus Marcus, 1945	20-10	0000-	00112	0100-	-10?1	01010	00-00	01000	0
R. simplex Leidy, 1851	20-10	0000-	00112	0000-	-20?1	01020	00-00	01001	0
R. pusillus Marcus, 1945	20-11	1000-	00112	0010-	-00?1	01120	00-00	01001	0
Myostenostomum marcusi Rogozin, 1992	11100	01010	00112	0110-	-10?1	01040	00101	01000	1
M. vanderlandi Rogozin, 1992	11101	01???	00112	0110-	-00?1	0???0	00101	0100?	1
Chordarium evelinae Marcus, 1945	10-10	0100-	0000-	-00-	-2011	01100	00000	01001	1

data matrix and generated less missing entries than other taxa from other families of Catenulida.

Characters

The analysis was performed using the 41 morphological characters listed below. Character states were assigned based on observations of *in vivo* material and the analysis of literature (Kepner and Carter 1931; Nuttycombe and Waters 1938; Marcus 1945*a*, 1945*b*, 1949; Luther 1960; Borkott 1970; Kolasa and Young 1974*a*, 1974*b*; Young and Kolasa 1974*a*, 1974*b*; Rogozin 1992; Noreña-Janssen 1995; Noreña *et al*. 2005). Character states that had not been previously described or were not observed in the material studied were coded using a question mark ('?'), while non-applicable character states were coded by a dash ('–').

For species not collected, we used the figures published by Marcus (1945*a*, 1945*b*, 1949) for the neotropical species and by Larsson and Willems (2010) for the species from Sweden, to avoid discrepancies caused by diverse interpretations and figures of specimens. For *Stenostomum leucops* we used figures published by Borkott (1970) and the original description for *Myostenostomum vanderlandi* Rogozin, 1992.

All characters were treated as unordered and equally weighted; 31 characters were binary (Table 2). Some character states used in the analysis are explained in Fig. 2, or are indicated in the figures of the species descriptions (Figs 3–5). For some character states a reference is given in the list below.

List of characters and states

(1) Relationship between the length from base of the posterior brain lobe to anterior end (Fig. 2*a*; AL) and width at the posterior end of the posterior brain lobes (Fig. 2*a*; AW): <1 (0); between 1 and 2 (1); >2 (2).
(2) Ciliated pits: absent (0); present (1).
(3) Angle of ciliated pits with respect to axial plane (Fig. 2*c*): smaller than 10° (0); between 10° and 20° (1); between 20° and 30° (2); between 30° and 40° (3); bigger than 40° (4).
(4) Body constriction behind the posterior brain lobes: absent (0); present (1).
(5) Development of the intestine in the posterior end (Fig. 2*b*, *c*): reaching the end (0); not reaching the end, leaving an intestine-free portion (1).
(6) Cauda (Fig. 2*a*): absent (0); single (1); double (2).
(7) Ciliated epidermis: homogeneous (0); heterogeneous (with conspicuous sensory cilia of greater length) (1).
(8) Length of epithelial cilia in relation to epithelial width: smaller or equal (0); greater (1).
(9) Rhabdoids: absent (0); present (1).
(10) Rhabdoid arrangement: homogenous (0); heterogeneous (1).
(11) Suprapharyngeal rhabdoid glands: absent (0); present (1) (see Marcus 1945*a*: 133, fig. 62).
(12) Epithelial refringent cells: absent (0); present (1).
(13) Ciliated groove separating the anterior end of the body: present (0); absent (1).
(14) Brain: compact (0); lobular (1).
(15) Anterior brain lobes: smooth (0); dentate (1) (Fig. 2*a*); with ganglia (2) (Fig. 2*b*).
(16) Posterior brain lobes: with lateral finger-like prolongations (0) (see Marcus 1945*b*: fig. 17*b*); saddle-like without lateral prolongations (1) (see Marcus 1945*b*: fig. 15*b*).
(17) Basal portion of anterior brain lobes (Fig. 2*b*; bp): not developed (0) (see Marcus 1945*b*: fig. 17); short and rounded, shorter than brain commissure (1) (see Marcus 1945*b*: fig. 26); longer than brain commissure (2) (see Marcus 1945*b*; fig. 20).
(18) Statocyst: present (0); absent (1).
(19) Light-refracting bodies: absent (0); present (1).
(20) Number of light-refracting bodies: one pair (0); more than one pair (1).
(21) Type of light-refracting bodies: type one, disc shaped with a large number of small spheres (0); type two, bowl shaped (1); type three, according to Nuttycombe and Waters' classification (1938: 224, fig. 3) (2).
(22) Mouth: circular (0); axially elongated (1); angular (2).
(23) Pharynx length (Fig. 2*b*; PL) as percentage of total body length: less than <15% (0); 15–25% (1); >25% (2).
(24) Pharyngeal lumen: ciliate (0); naked (1).
(25) Sphincter between pharynx and intestine: absent (0); present (1).
(26) Radial pharyngeal musculature: absent (0); present (1).
(27) Pharyngeal glands: absent (0); present (1).
(28) Type of pharyngeal glands: rounded (0); elongated clavulate (1); both types (2).
(29) Opening of pharyngeal glands: at the transition between mouth and pharynx (0); uniform in the whole pharynx (1); at the anterior region of the pharynx (2); at the posterior region of the pharynx (3); lateral to the pharynx (4).
(30) Tentacular pharyngeal organ ('claw organ'): absent (0); present (1).
(31) Pharynx divided into two regions (see Marcus 1945*b*: fig. 34*a*): absent (0); present (1).
(32) Rows of cells in pharynx (see Marcus 1945*b*: fig. 17): absent (0); present (1).
(33) Intestine: discontinuous in the zooid chain (0); continuous in the zooid chain (1).
(34) Intestinal caecum: absent (0); present (1).
(35) Muscular 'gizzard' in the intestine: absent (0); present (1).
(36) Intestine with rows of refractive acidophilus cells: absent (0); present (1) (see Marcus 1945*b*: fig. 17).
(37) Excretophores: absent (0); present (1).
(38) Anterior portion of the intestine contractile, with vacuolated cells: absent (0); present (1).
(39) Development of the protonephridium at the posterior end: straight (0); sinuous (1).
(40) Position of the nephridiopore: terminal (0); subterminal (1).
(41) Paratomy in adults: absent (0); present (1).

Analysis. Tree searches were conducted in TNT (Goloboff *et al*. 2008). Heuristic searches were performed by tree bisection–reconnection (TBR) branch swapping on 1000 series of random-addition sequence replicates. Support for nodes was assessed by calculation of Bremer support values. The WinClada version 1.00.08 (Nixon 2002) was used as a graphical interface.

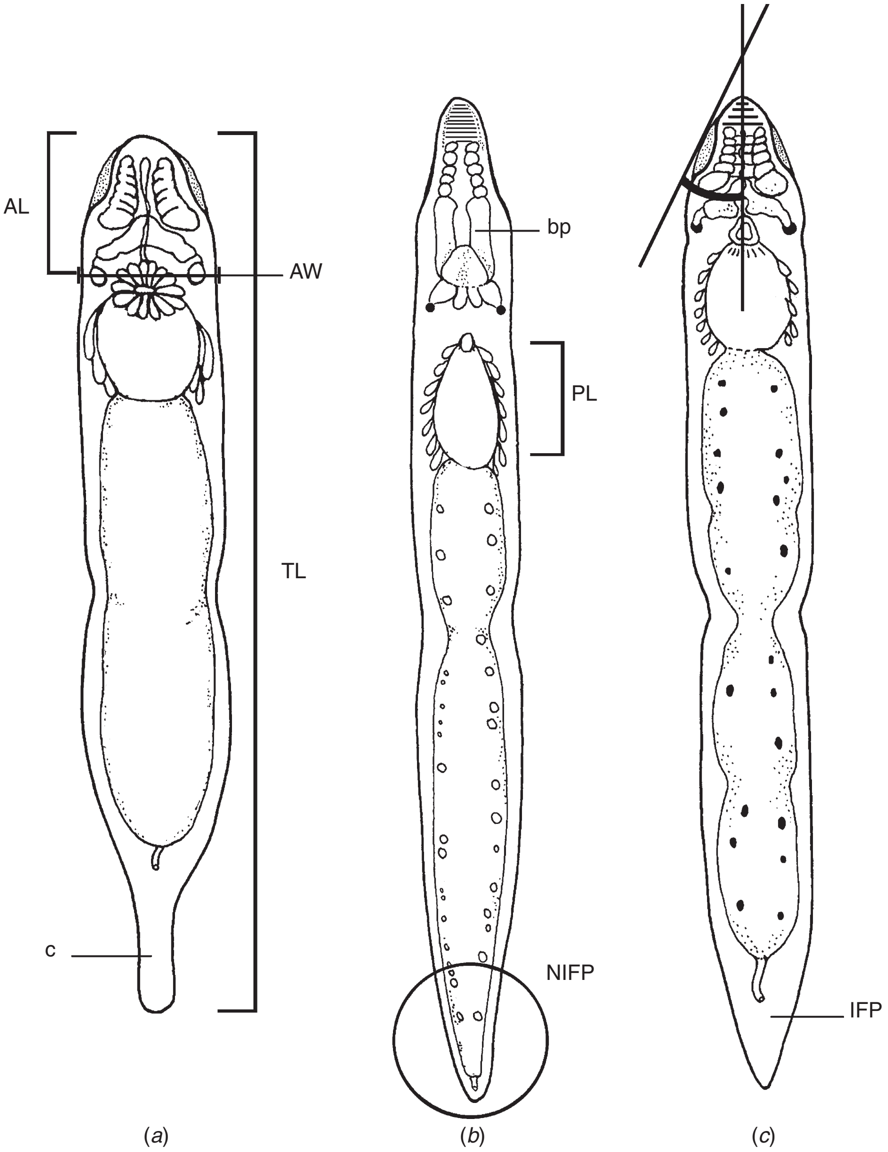

Fig. 2. Drawings showing details of characters used in the descriptions and in the phylogenetic analysis as mentioned in Materials and methods. (*a*) Specimen showing dentate anterior brain lobes, anterior length (AL) from base of the posterior brain lobe to anterior end, anterior width (AW) at the posterior end of the posterior brain lobes, total body length (TL) and presence of a cauda (c). (*b*) Specimen with 'metamerised' anterior brain lobes and the basal portion of the anterior brain lobes (bp) well developed and longer than the posterior brain commissure; posterior end of the animal without an intestine-free portion and the intestine reaching the end (NIFP); pharynx length (PL). (*c*) The intestine not reaching the posterior end of the animal and with an intestine free portion (IFP), ciliated pits angle with respect to axial plane.

Results

Table 3 shows the localities in the study area and the species found in each.

Taxonomy

Family **STENOSTOMIDAE** Vejdovsky, 1880

Genus ***Stenostomum*** Schmidt, 1848

Stylacium Corda, 1838: 71–78.
Weldonia Martin, 1908: 758–763.
Ependytes Picken, 1937: 273–277.
Anokkostenostomum Noreña, Damborenea & Brusa, 2005: 41.

Stenostomum ashanika, sp. nov.

(Fig. 3*a*)

Material examined

Holotype. Peru, Ucayali, Cocha in Breu, near Yurúa River, 9°32′13.5″S-72°45′01.2″W, coll. Damborenea, Noreña, Brusa and Almagro, 21.VII.2008. Whole specimen deposited in UNMSM N° 3013.

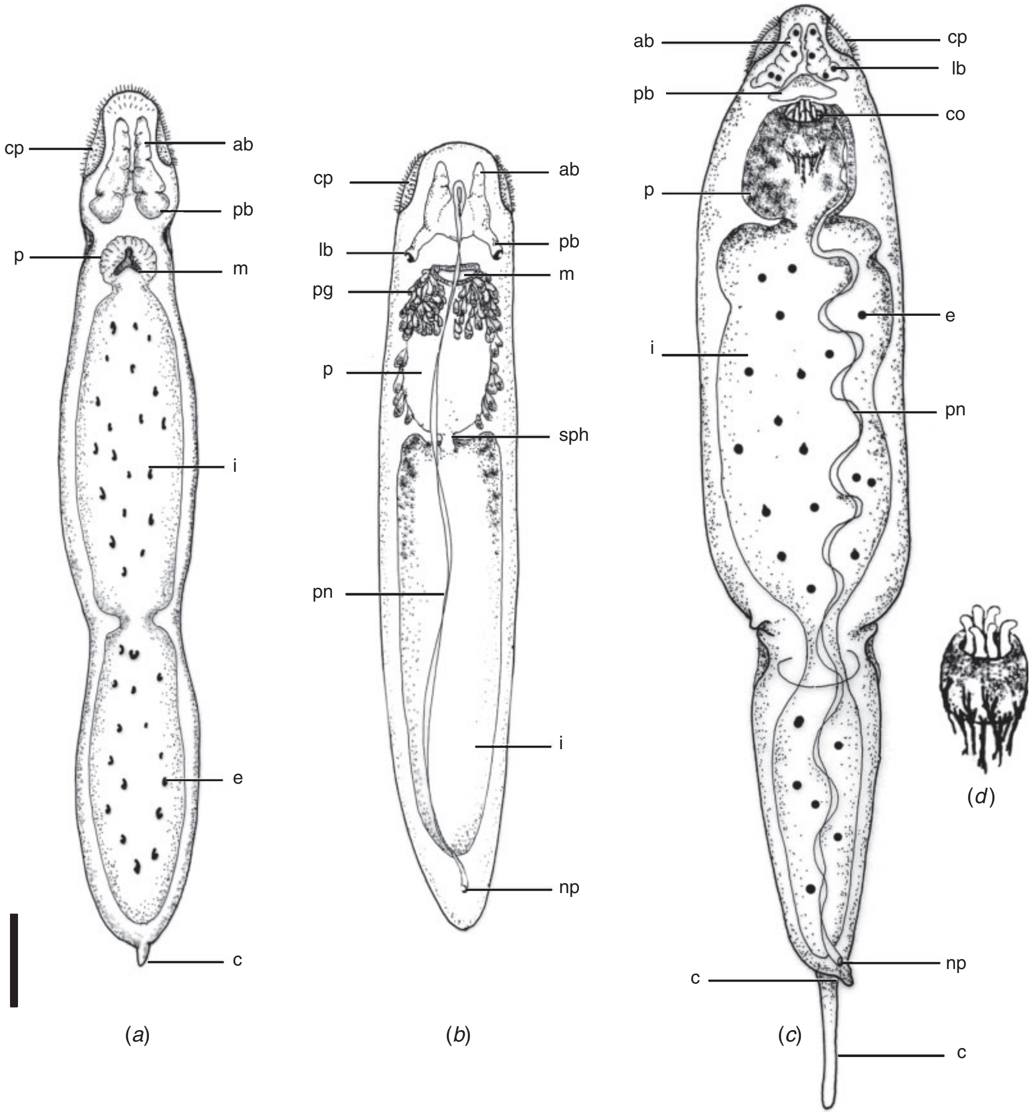

Fig. 3. (*a*) *Stenostomum ashanika*, sp. nov. (*b*) *Stenostomum amphotum*. (*c*) *Stenostomum bicaudatum*. (*d*) Detail of the 'claw organ' of *S. bicaudatum* showing the six tentacles. ab, anterior brain lobe; c, cauda; co, claw organ; cp, ciliated pit; e, excretophore; i, intestine; lb, light-refracting body; m, mouth; np, nephridiopore; p, pharynx; pb, posterior brain lobe; pg, pharyngeal glands; pn, protonephridium; sph, sphincter. Scale bar for A, B and C = 0.1 mm.

Other material examined. Peru, Ucayali, Dulce Gloria, Huacapishtea River and Cocha Lopona; and Breu, Quebrada Antonino (Tables 1, 3). Several specimens were studied alive, captured within the vegetation in stagnant waters.

Diagnosis

Stenostomum ashanika, sp. nov.: species of *Stenostomum* with medium-sized ciliated pits, anterior brain lobes elongated and dentate, a tri-radiate mouth, a small pharynx directed ventrally, rounded posterior end with a small cauda, light-refracting bodies absent.

Description

Specimens are 0.5 mm long. They frequently show chains of two zooids of 0.95 mm. The body is elongated and cylindrical. The anterior end is truncated, approximately one-quarter of the total length and can be extended. It has a pre-pharyngeal constriction. The posterior end is rounded and provided with a small but conspicuous cauda free of intestine. The epidermis is homogeneously ciliated.

The anterior brain lobes are elongated, well developed and slightly dentate. The posterior lobes are globular, well developed and the transverse commissure is not evident. Light-refracting bodies are absent. The ciliated pits are medium-sized and extend along the anterior two-thirds of the length of the anterior brain lobes.

The length of the pharynx is 10–20% of the body length. It is rounded, frequently directed to the ventral side and very mobile. The mouth is characteristically tri-radiate. Pharyngeal glands were not observed. The transition between pharynx and

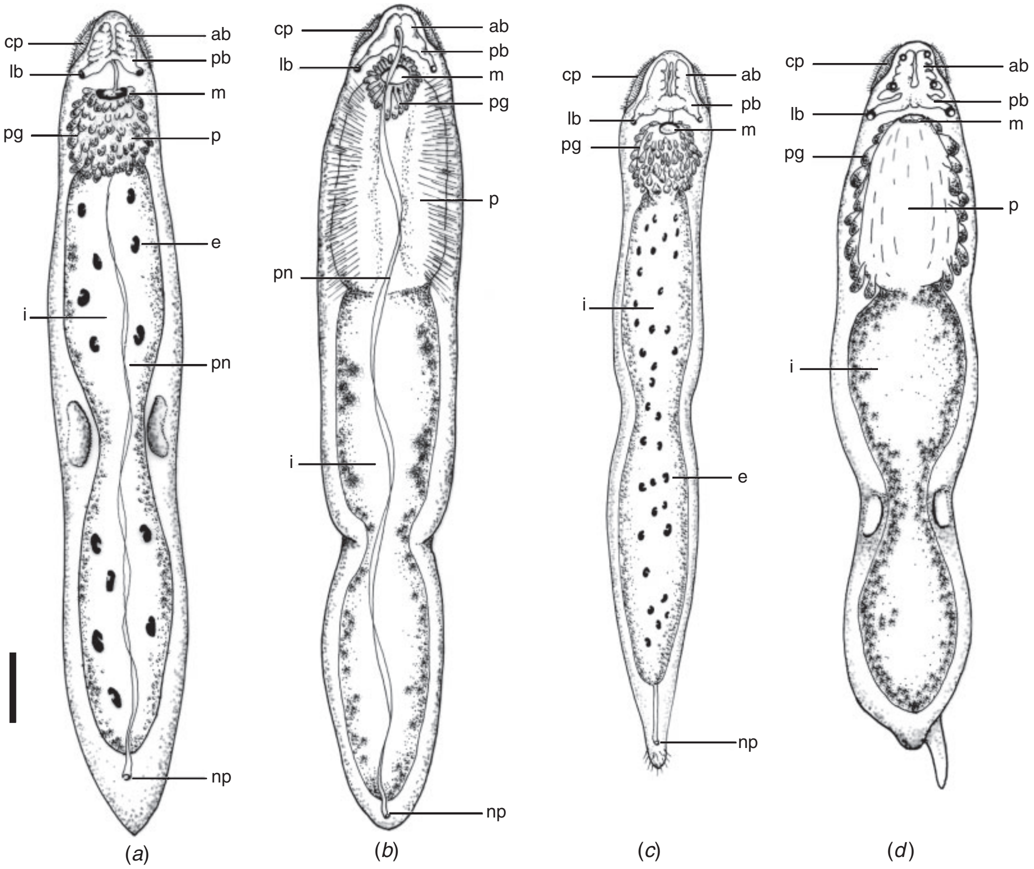

Fig. 4. (*a*) *Stenostomum ciliatum*; (*b*) *S. hemisphericum*; (*c*) *S. leucops*; and (*d*) *S. paraguayense.* Abbreviations as in Fig. 3. Scale bar = 0.1 mm.

intestine is marked by a strong constriction. The intestine, with smooth walls, extends along the whole body length with the exception of the small cauda. Small excretophores are present and distributed homogeneously along the entire intestine region.

Remarks

Stenostomum ashanika, sp. nov. can be compared morphologically with three species of the genus. *Stenostomum saliens* Kepner & Carter, 1931 and *S. ashanika*, sp. nov., share the shape and size of the anterior brain lobes, a conspicuous mouth, the absence of pharyngeal glands and an intestine occupying most of the body. The two differ in the position of the body constriction, which is between the anterior and posterior brain lobes in *S. saliens* and between the posterior brain lobes and the mouth in *S. ashanika*, sp. nov. *Stenostomum saliens* has well delimited ganglia forming the 'metamerised' anterior row, while the new species has slightly dented anterior brain lobes. Both species have a conspicuous mouth, but in *S. saliens* it is not tri-radiate and the pharynx has a special arrangement of muscles, not present in the new species. The ciliate epithelium is heterogeneous in *S. saliens*, while in *S. ashanika*, sp. nov. it is homogeneous.

The new species also resembles *S. ventronephrium* Nuttycombe, 1932 in the shape and relative length of the anterior end, the morphology of the brain, the presence of a pre-pharyngeal constriction and the general shape of the body. However, it is more slender than *S. ventronephrium* and shows a small cauda that is absent in *S. ventronephrium*. The ciliated pits of *S. ventronephrium* extend along the entire length of the anterior brain lobes, while in the new species they are shorter and extend to two-thirds of the anterior brain lobes.

The posterior end of *S. ashanika*, sp. nov. is rounded and with a small cauda. A similar structure has been described in *S. pseudoacetabulum* Nuttycombe & Waters, 1935. However, the latter has an elongate and tapering posterior end, with the free intestine portion curved at its distal end. The morphology of the rear end of the new species is constant in all specimens observed. This rules out the possibility of animals with recent zooid separation.

The shape and position of the mouth, as well as the size and the pharynx of the new species, are unique among the species of the genus. The tri-radiate mouth in the new species is similar to the description of *S. pseudoacetabulum* by Nuttycombe and Waters (1935), who mentioned that the pharynx may be everted. However, Young and Kolasa (1974*b*) mentioned that this feature is variable. *Stenostomum ashanika*, sp. nov. was never seen with an everted pharynx.

Etymology

The name refers to the Ashanika people, one of the ethnic groups inhabiting the region.

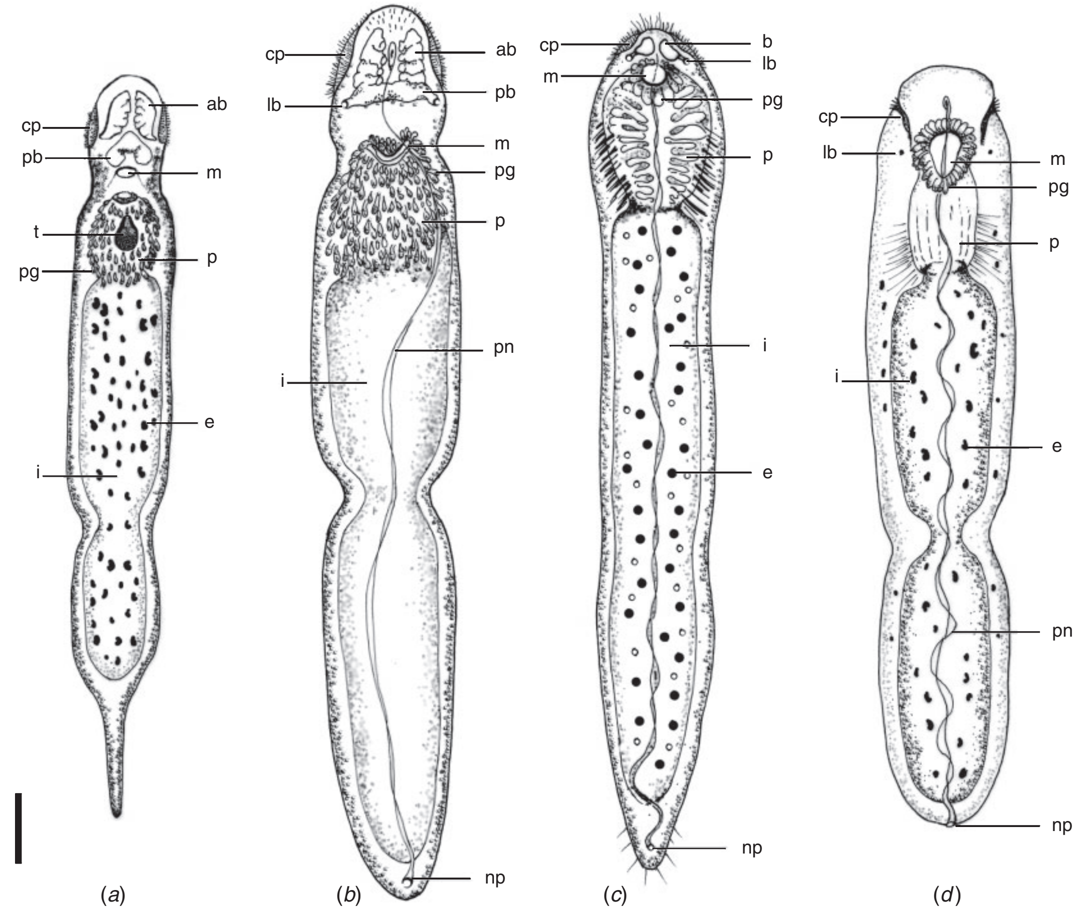

Fig. 5. (*a*) *Stenostomum pseudoacetabulum*; (*b*) *S. tenuicauda*; (*c*) *S. uronephrium*; and (*d*) *S.* aff. *matarazzoi*. Abbreviations as in Fig. 3, and: b, brain; t, testicle. Scale bar = 0.1 mm.

Table 3. Registered species in each sampling site (numbers correspond with sampling sites of Fig. 1 and Table 1)

Sampling sites	1	2	3	4	5	6	7	8	9
S. ashanika, sp. nov.			X				X	X	X
S. amphotum	X					X			
S. bicaudatum		X	X						
S. ciliatum			X						
S. hemisphericum						X			
S. leucops						X			
S. pseudoacetabulum				X					
S. tenuicauda		X							
S. paraguayense				X		X			
S. uronephrium					X				
S. aff. *matarazzoi*		X							

Distribution

Known from the type locality and from other localities of the same region (Table 3).

Biology

Stenostomum ashanika, sp. nov. has been found mainly in stagnant waters, like *cochas* or ponds formed in the riversides, densely covered with aquatic vegetation. Most of the specimens were captured among the roots and leaves of *Paspalum* sp. or *Eichornia* sp.

Stenostomum amphotum Marcus, 1945

(Fig. 3*b*)

Material examined

Peru, Ucayali, Breu, Quebrada Dos y medio and Yurúa River (Table 3). Several specimens were studied alive.

Remarks

The Peruvian specimens agree with the previous descriptions (Marcus 1945*b*; Noreña *et al*. 2005). The pharyngeal sphincter is thinner and more delicate than in the specimens described by Marcus (1945*b*). The protonephridium shows a twist at the base of the anterior brain lobes, differing from the specimens described by Marcus (1945*b*), in which the protonephridium is twisted at the distal end of the lobes. Specimens of one or two zooids were observed, while in previous descriptions the number of zooids was variable (Marcus 1945*b*).

Luther (1960) and subsequently other authors (Gamo and Leal-Zanchet 2004) considered this species a synonym of

S. leucops. However, the shape, type and arrangement of pharyngeal glands, the size of the pharynx, the connection between pharynx and intestine, as well as the shape of the front end of the intestine, are constant features that differentiate both species.

Distribution

This species has been recorded only in South America: Brazil, São Paulo (Marcus 1945*b*) and Argentina, Buenos Aires, Berisso (Noreña *et al*. 2005). The present record is the first for Peru (Table 3).

Stenostomum bicaudatum Kennel, 1889

(Fig. 3*c*, *d*)

Material examined

Peru, Ucayali, Breu, Cocha Aeropuerto and Cocha in Breu (Table 3). Several specimens were studied alive.

Remarks

Specimens studied agree with the description by Nuttycombe and Waters (1938) (North America) and Marcus (1945*b*) (Brazil). Agreeing with statements by Marcus (1945*b*), the chains do not exceed two zooids, of which the second one is always smaller and shows developing ciliate pits. The length of the zooid chains is 1.5 mm without the cauda. They are yellowish and the intestine is darker. The brain is smaller than has been described. The mouth is orbicular and the characteristics of the pharynx – although it is shorter – are as described previously, with longitudinal folds (Marcus 1945*b*) but lacking transverse folds, as those drawn for the specimens from Rio Grande do Sul in Brazil (Gamo and Leal-Zanchet 2004). As mentioned by Nuttycombe and Waters (1938), no pharyngeal glands were observed, although these were mentioned for Brazilian specimens (Marcus 1945*b*; Gamo and Leal-Zanchet 2004). The pharyngeal organ (‘claw organ’) is distinctive, and morphologically similar to previously described ones, and formed by six tentacles (Fig. 3*d*).

Distribution

The species has been recorded from the US, Valdosta, Georgia (Nuttycombe and Waters 1938). In South America: Trinidad (Kennel 1889); Brazil, São Paulo and near Paranaibo (Marcus 1945*b*); Rio Grande do Sul (Gamo and Leal-Zanchet 2004); Argentina, Santa Fe, Paraná River (Noreña-Janssen 1995). Peru: Loreto, Pacaya-Samiria National Reserve (Peruvian Amazon Basin; Noreña *et al*. 2006). The present record (Table 3) is the second from Peru.

Stenostomum ciliatum Kepner & Carter, 1931

(Fig. 4*a*)

Material examined

Peru, Ucayali, Breu, Cocha in Breu (Table 3). Several specimens were studied alive. MLP 6250, one specimen fixed in Bouin’s fixative.

Remarks

The studied specimens are cylindrical with a posterior portion free of intestine, although less elongate and thinner than the specimens described by Kepner and Carter (1931). The anterior end is blunt, with wide and shallow ciliate pits, similar to Marcus’s (1945*b*) description. In the Peruvian specimens the ciliated pits reach the posterior brain lobes, while Kepner and Carter (1931) described them as small. The posterior brain lobes are more rounded, less elongate and more compact than in the North American specimens (Kepner and Carter 1931) and carry light-refracting bodies formed by a vesicle with a single corpuscle in the studied material. Kepner and Carter (1931) stated that they had one to three corpuscles in each vesicle, and made no reference to the pharyngeal glands. Marcus (1945*b*) described some, surrounding the mouth, others lateral and others all around the pharynx. This gland arrangement is found in the Peruvian specimens.

Distribution

The species has been recorded from the US (Kepner and Carter 1931; Kolasa 1991) and in South America from Brazil, São Paulo and Paraná State (Marcus 1945*b*). The present record is the first one from Peru (Table 3).

Stenostomum hemisphericum Nassonov, 1924

(Fig. 4*b*)

Material examined

Peru, Ucayali, Breu, Yurúa River (Table 3). Several specimens were studied alive.

Remarks

Specimens of one to two zooids, cylindrical, 0.62 mm long each. Most of the characters of studied specimens agree with the descriptions by Marcus (1945*a*) and Noreña *et al*. (2005). The anterior brain lobes are compact and short, slightly dentate but not forming rows of ganglia. According to Marcus (1945*a*: fig. 61A) they are long and divided into small ganglia. Enlarged glands are evident around the mouth, as described by Marcus (1945*a*). None of the specimens studied showed the excretophores described by Nassonov (1924) and Marcus (1945*a*).

Distribution

This species has been previously recorded from Europe (Nassonov 1924). In South America it has been recorded from Brazil, São Paulo, in aquaria (Marcus 1945*a*); Surinam, Suriname River (Van der Land 1970). This is the first record from Peru (Table 3).

Stenostomum leucops (Dugès, 1828) O. Schmidt, 1848

(Fig. 4*c*)

Derostoma leucops Dugès, 1828: 141–142, pl. IV, fig. 4, pl. V, fig. 15.
Stenostomum leucops, O. Schmidt, 1848: 59–61, figs 18, 19.
S. sthenum Borkott, 1970: 187, fig. 1.
S. platycaudatum Borkott, 1970: 191, figs 2, 3.
S. plebejum Borkott, 1970: 189, fig. 2.

Material examined

Peru, Ucayali, Breu, Yurúa River (Table 3). Several specimens were studied alive. MLP 6251, three specimens fixed in Bouin's fixative.

Remarks

The collected specimens agree with descriptions of Young and Kolasa (1974*b*) and Noreña *et al*. (2005). The shape of the mouth is a variable character in this species (Noreña *et al*. 2005), and in the Peruvian specimens it is rounded. Pharyngeal glands are on the entire surface of the pharynx (Luther 1960; Borkott 1970). Peruvian specimens, like Brazilian ones (Gamo and Leal-Zanchet 2004) show an elongate posterior end, free of intestine, but not forming a slender cauda as in the European specimens (Luther 1960). Nuttycombe and Waters (1938) and Marcus (1945*b*) considered the description of *Stenostomum leucops* as broad and ambiguous, so its recognition is difficult. Borkott (1970) further split the species into *S. sthenum*, *S. platycaudatum* and *S. plebejum*. Young and Kolasa (1974*b*) identified specimens from Africa as *S. leucops leucops*, due to difficulty differentiating the three species described by Borkott (1970). It was not possible for us to recognise the species described by Borkott (1970).

Distribution

This species is distributed worldwide. In South America it has been recorded from Surinam (Van der Land 1970), Argentina, Santa Fe, Paraná River (Noreña-Janssen 1995) and Brazil, Rio Grande do Sul (Gamo and Leal-Zanchet 2004). This is the first record from Peru (Table 3).

Stenostomum paraguayense (Martin, 1908) Luther, 1908

(Fig. 4*d*)

Weldonia parayguensis Martin, 1908: 758–763, figs 1–5*A*.
Stenostomum paraguayense, Luther, 1908: 300.

Material examined

Peru, Ucayali, Breu, Cocha Segunda and Yurúa River (Table 3). Several specimens were studied alive.

Remarks

The chains of two zooids were 1.2 mm long and 0.35 mm in diameter. The shape of the body, anterior end and cauda coincide with the description by Marcus (1945*b*) and Noreña *et al*. (2005). The anterior brain lobes are markedly dentate in their external face, but not forming ganglia, contrary to Marcus (1945b: fig. 17). The mouth is ovate, transversally elongate. The pharynx is ovoid, with elongate glands, mainly on the lateral part, contrary to the posterior glands mentioned by Marcus (1945*b*). The cells with refracting granules forming a transverse line in the dorsal part of the pharynx (Marcus 1945*b*) were not observed.

Distribution

The species is known only from South America, in Paraguay (Martin 1908), Brazil, São Paulo (Marcus 1945*b*); Surinam (Van der Land 1970), Argentina, Buenos Aires, Berisso (Noreña *et al*. 2005) and Peru, Pacaya-Samiria National Reserve, Loreto, (Noreña *et al*. 2006). The present record is the second for Peru (Table 3).

Stenostomum pseudoacetabulum Nuttycombe & Waters, 1935

(Fig. 5*a*)

Material examined

Peru, Ucayali, Breu, Cocha Segunda (Table 3). Several specimens were studied alive.

Remarks

Specimens studied agree with previous descriptions (Nuttycombe and Waters 1935, 1938; Marcus 1945*b*; Noreña *et al*. 2005). They carry excretophores, as mentioned by Kolasa (1991), but not by Nuttycombe and Waters (1935) and Marcus (1945*b*). The anterior brain lobes are dentate, not forming symmetric rows of decreasing ganglia; the posterior lobes are more globose (Marcus 1945*b*). Some of the studied specimens showed a testicle. Nuttycombe and Waters (1938) stated that about half of the population studied consisted of sexually mature specimens.

Distribution

This species has been recorded in North America (Nuttycombe and Waters 1935, 1938; Kolasa 1991) and Europe (Kolasa 1977; Lanfranchi and Papi 1978). In South America it was recorded in Brazil, São Paulo and Paraná States (Marcus 1945*b*); Surinam (Van der Land 1970); and Argentina, Buenos Aires, Berisso (Noreña *et al*. 2005). This species has not been previously recorded for Peru (Table 3).

Stenostomum tenuicauda Graff, 1911

(Fig. 5*b*)

Material examined

Peru, Ucayali, Breu, Cocha Aeropuerto (Table 3). Several specimens were studied alive.

Remarks

The chains observed did not exceed two zooids. The general body shape, together with the size and arrangement of ciliated pits, allows the identification as *S. tenuicauda* (Marcus 1945*b*; Noreña *et al*. 2005). Differences with the specimens described up to now are in the deep lateral clefts in the anterior brain lobes, which form ganglion like protuberances on the inner lobe face. The pharyngeal glands are also less numerous in the Peruvian specimens, the distal ones being smaller than the proximal ones.

Luther (1960) considered *S. tenuicauda* as a junior synonym of *S. leucops*. However, the body shape, the crescent shape of the mouth, the spherical pharynx and the pharyngeal glands (abundant and covering the entire surface of the pharynx), allow the identification of both species in the samples.

Distribution

This species has been previously recorded from North America (Graff 1911; Nuttycombe 1932; Nuttycombe and Waters 1938); Europe (Beklemichev 1917, 1921; Nassonov 1924, 1926); Brazil, São Paulo (Marcus 1945*b*); Argentina, Buenos Aires, Zapata Stream (Noreña *et al*. 2005). This species had not been previously recorded in Peru (Table 3).

Stenostomum uronephrium Nuttycombe, 1931

(Fig. 5*c*)

Material examined

Peru, Ucayali, Victoria, Quebrada Sabotari (Table 3). Several specimens were studied alive. MLP 6252, two individuals fixed in Bouin's fixative.

Remarks

The features of the studied specimens agree with previous descriptions (Nuttycombe and Waters 1938; Marcus 1945*b*; Noreña *et al*. 2005). The brain of Peruvian individuals is more spherical and compact than those previously described (Marcus 1945*b*), with no clear differentiation into anterior and posterior lobes. The pair of light-refracting bodies are formed by a vesicle and three granules, differing from the four described by Marcus (1945*b*) or the two to four by Nuttycombe and Waters (1938). The posterior end is tapering, with no intestine, and rounded. This feature was described as variable by Marcus (1945*b*), who stated that the length of the cauda reached up to one-quarter of the total body length. Nuttycombe and Waters (1938) mentioned that it could reach up to half the body length. A sphincter separates the pharynx from the intestine, which is less developed than in the specimens described from North America and Brazil. The main nephridial tube near the posterior end of the intestine bends abruptly towards the right, and beyond the intestine end it bends towards the left. The nephridiopore is sub-terminal.

Distribution

This species has been previously recorded from North America; (Nuttycombe 1931; Kolasa 1991); Europe (Kolasa 1977; Lanfranchi and Papi 1978); South America, Brazil, São Paulo State, (Marcus 1945*b*); Argentina, Santa Fe, Paraná River (Noreña-Janssen 1995); Buenos Aires, Berisso (Noreña *et al*. 2005). This is the first record for Peru (Table 3).

Stenostomum aff. ***matarazzoi*** Marcus, 1949

(Fig. 5*d*)

Material examined

Peru, Ucayali, Breu, Cocha Aeropuerto. Several specimens were studied alive.

Remarks

The blunt shape of the anterior end, and the shape and placement of the ciliate pits are similar to the description by Marcus (1949), although the latter are slightly folded in the Peruvian specimens, giving them the appearance of ciliate grooves (Fig. 5*d*). One pair of light-refracting bodies is associated with the posterior brain lobes and is formed by a vesicle with numerous cup-shaped corpuscles (over 20). Their morphology agrees with the description of *S. matarazzoi*. The typical pigmented maculae are evident on the body surface. The mouth is triangular, with a caudal tip, and opens into the sac-shaped pharynx. A constriction dividing the pharynx into two portions, each with a distinct epithelium (Marcus 1949) was not found. Numerous glands surrounding the mouth were observed, but not on the lateral sides of the pharynx. The nephridial channel is evident throughout the body length. The caudal end, contrary to that described for *S. matarazzoi*, is blunt and devoid of cauda, and therefore the nephridiopore is terminal and not sub-terminal. The identification of the collected specimens remains uncertain as information on the brain is not available.

Distribution

Stenostomum matarazzoi has not been found again after its original description: Brazil, São Paulo, near the Pirajussara River, in temporary ponds (Marcus 1949). This taxon has been found in Cocha Aeropuerto, upper Yurúa River, Peru (Table 3).

Phylogeny

Parsimony analysis of the data matrix yielded two most parsimonious trees of length 182 (consistency index 0.29; retention index 0.58) (Fig. 6).

Monophyly of Stenostomidae was supported by three synapomorphies, the absence of a ciliated grove separating the anterior region of the body (13: 1), a lobular brain (14: 1), and the intestine being continuous in the chain (33: 1). The *Rhynchoscolex* species were basal within Stenostomidae, but formed a paraphyletic grade. *Stenostomum* was also paraphyletic with the two species of *Myostenostomum* forming a clade within a majority of the *Stenostomum* species.

The presence of ciliated pits (2: 1) and rhabdoids (9: 1), two non-homoplastic synapomorphies, and the presence of paratomy in adults (41: 1), a homoplastic synapomorphy, supported the large clade of all the *Stenostomum* + *Myostenostomum* species.

The *Stenostomum* + *Myostenostomum* clade has four clear subclades (see Fig. 6). *Stenostomum cryptops*, *S. simplex*, *S. membranosum* and *S. saliens* formed a clade (subclade I) that was sister to the rest of *Stenostomum* + *Myostenostomum.* These four species share plesiomorphic characteristics with the *Rhynchoscolex* species, especially those related to the development of the anterior end and the anterior brain lobes (1: 2, 3: 0 and 15: 2), which represent apomorphies in the remaining *Stenostomum* + *Myostenostomum* clade. The monophyly of subclade I was supported by two homoplastic states; the basal portion of anterior brain lobes being longer than brain commissure (17: 2) and the presence of light-refracting bodies (19: 1). Subclade II was formed by the *Myostenostomum* species, with the non-homoplastic synapomorphy being the presence of a muscular gizzard in the anterior region of the intestine (35: 1), and by the homoplastic synapomorphy of lateral pharyngeal glands (29: 4). Subclade III was formed by 10 *Stenostomum* species and was supported by four homoplastic synapomorphies: ciliated pits between 30° and 40° (3: 2), posterior brain lobes without lateral prolongations (16: 1), more than one pair of light-refracting bodies (20: 1) and the opening of pharyngeal glands at the anterior region of the pharynx (29: 2). Subclade IV members shared the following homoplastic synapomorphies: anterior brain lobes dentate (15: 1), circular mouth (22: 0) and presence of radial pharyngeal muscles (26: 1). The presence of light-refracting bodies (19: 1) and a subterminal nephridiopore (40: 1) are also shared by the majority of species in subclade IV, except *S. heebuktense*.

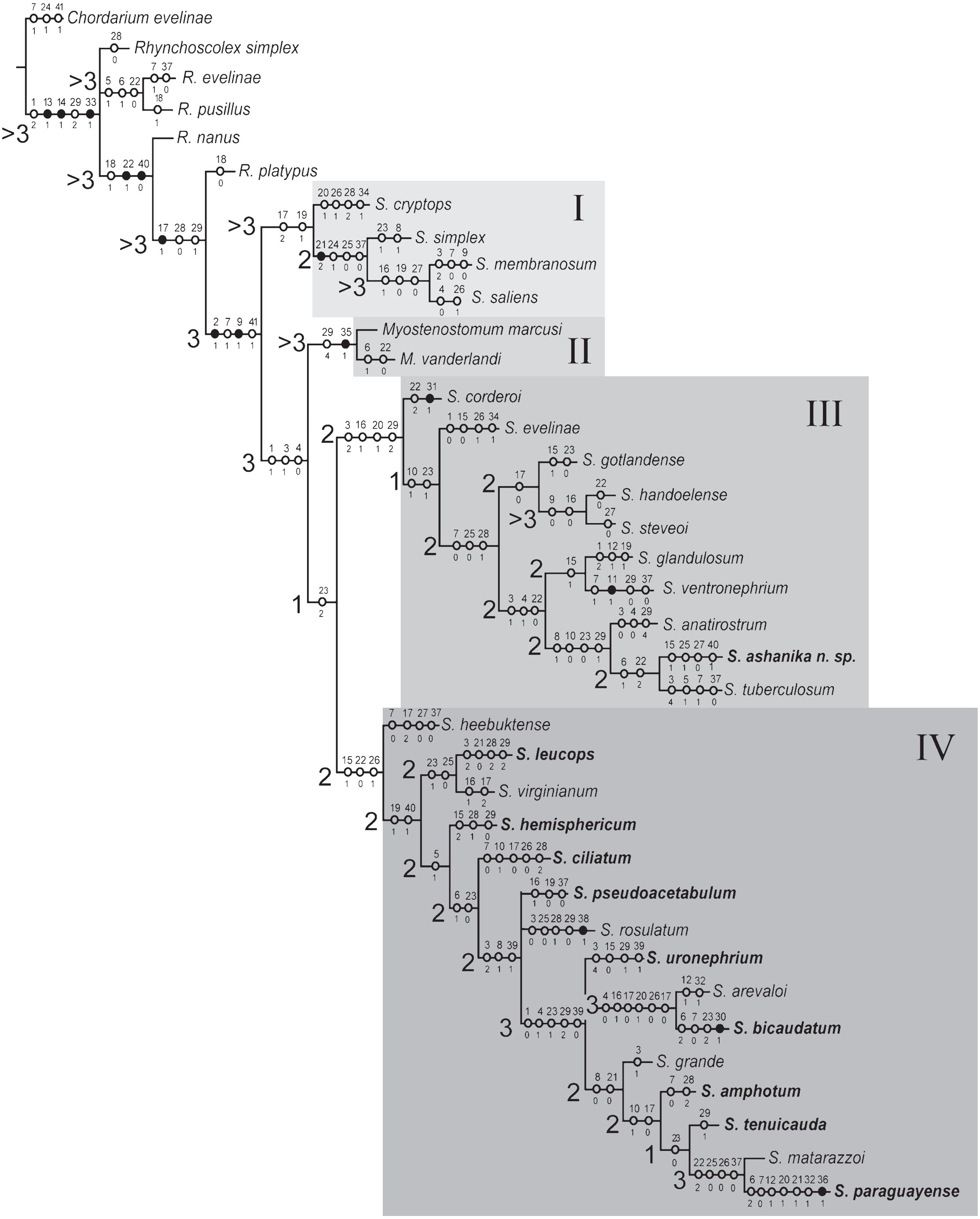

Fig. 6. Strict consensus tree of two equally parsimonious trees (length 182; consistency index 0.29; retention index 0.58) resulting from the cladistic analysis for the species of *Stenostomum* with fast optimisation of characters. Species found in upper Yurúa River are in bold. Character numbers according to Materials and methods, apomorphies in black and homoplasies in white. The numbers on the left of the nodes are the absolute Bremer support values. The four subclades (I, II, III and IV) resulting in the analysis in grey.

Characters related to the orientation of the ciliated pits (3), the type of pharyngeal glands (28), the development of the intestine in the posterior end (5), the type of light-refracting bodies (21) and the presence of excretophores (37) do not support large clade groups (Fig. 6). The consistency index was low (0.29) and is reflected in the high level of homoplastic transformations in Fig. 6.

The neotropical *Stenostomum* species were non-monophyletic. Three Swedish species included in the analysis were part of subclade III and the other of subclade IV and *Myostenostomum* species were within the main clade (*Stenostomum* + *Myostenostomum*).

Homoplastic characters are apomorphies of the new species: anterior brain lobe dentate (15: 1), pharynx with radial musculature (26: 1), absence of pharyngeal glands (27: 0) and nephridiopore subterminal (40: 1).

Discussion

Of the high species richness of Catenulida recorded from the neotropical Region, the majority of the species were studied in localities in Brazil (Marcus 1945*a*, 1945*b*, 1949; Gamo and Leal-Zanchet 2004) and Argentina (Noreña-Janssen 1995; Noreña *et al*. 2005). Before this study only three species were known from Peru, all collected during one sampling event in the Pacaya-Samiria National Park, Loreto (Noreña *et al*. 2006). This study reports 11 stenostomid species from other regions in Peru, i.e. the upper Yurúa River basin, Ucayali. Eight of these species are mentioned for the first time from Peru, and a new species, *Stenostomum ashanika*, sp. nov. is described.

The morphology of the species collected in the Yurúa River is similar to that described previously, although some differences are observed. The structures that show a higher degree of difference to previous descriptions are those associated with the pharyngeal glands, the development of the intestine in the posterior end and the variation in the development of a cauda. These characters show high homoplasy. Luther (1960) stated that the arrangement of the pharyngeal glands could be variable within a species. Other authors considered these structures to be useful for species diagnoses (Nuttycombe and Waters 1938; Marcus 1945*a*, 1945*b*; Noreña *et al*. 2005). These differences could be due to different factors. Observed morphology may be dependent on the way specimens are studied, or on certain environmental characteristics, although no studies have corroborated this yet.

Some very similar species, *S. leucops*, *S. tenuicauda* and *S. amphotum*, were considered synonyms by some authors or were split into additional species (Luther 1960; Marcus 1945*b*; Borkott 1970). They have light-refracting bodies composed of over 10 elements. The types of pharyngeal glands are similar, although differ in arrangement (Marcus 1945*b*). According to our analysis these three species are valid. Specific studies using material from different localities and including molecular data would provide more information to clarify the identity of these species.

Marcus (1945*b*) and Luther (1960) mentioned the morphological variation of the posterior region of the animal, particularly the intestine-free portion and the development of the caudal region. It is worth mentioning that this morphology of the posterior end may vary according to the development of the zooid chains. In the phylogenetic analysis performed here, the characters related to the morphology of the posterior end (characters 5 and 6) are not unambiguous synapomorphies of clades, and show homoplasy.

The morphology of the brain, especially its anterior lobe, proves to be somewhat useful in establishing the phylogenetic relationships among species of *Stenostomum*, and also between them and those of *Rhynchoscolex* included in the analysis. The basal *Stenostomum* species have a well developed anterior brain lobe divided into small ganglia with metameric aspect, while the more derived *Stenostomum* species have a dentate anterior brain lobe without small ganglia. While this phylogenetic analysis is preliminary, it highlights the importance of this feature. Many descriptions of S*tenostomum* species do not include details on the brain.

The presence, number and composition of light-refracting bodies has previously been discussed and used in descriptions (Graff 1913; Nuttycombe and Waters 1938; Marcus 1945*b*). This study shows that the presence of the light-refracting bodies (character 19) is a homoplastic synapomorphy for the members of subclade I (absent in *Stenostomum membranosum* and *S. saliens*), as well as for the majority of subclade IV (absent in *S. heebuktense* and *S. pseudoacetabulum*). However, *Stenostomum glandulosum* from subclade III also has light-refracting bodies. These structures may be useful for specific identification, but their homology needs to be studied in detail. This statement becomes more relevant when considering the two species of subclade I with light-refracting bodies. Thus, *Stenostomum simplex* has light-refracting bodies of special morphology (type 3 after Nuttycombe and Waters 1938), and this character is described in different ways in *S. cryptops* (Nuttycombe and Waters 1938; Marcus 1945*b*). The results obtained in this preliminary analysis show that the different types of light-refracting bodies might not be homologous.

In general, the morphological results obtained in our study and in the molecular analysis of Larsson *et al*. (2008) show a high degree of compatibility. Larsson *et al*. (2008) showed that Retronectidae + Catenulidae is the sister group of Stenostomidae, and *Rhynchoscolex* of *Stenostomum*. We find that *Rhynchoscolex* is a basal paraphyletic grade (Fig. 6). For the present study we used *Chordarium evelinae* (Chordariidae) to root the tree because its morphological features are more comparable than Catenulidae or Retronectidae species with the species studied here. Larsson *et al*. (2008) also found that the genus *Anokkostenostomum* was not a monophyletic group, and considered it a junior synonym of *Stenostomum.* The results of our study based on morphological data also do not recover *Anokkostenostomum* as monophyletic, and the various species fall within *Stenostomum*. Therefore, *Anokkostenostomum* is a junior synonym of *Stenostomum.*

Eight species included in the analysis were formerly *Anokkostenostomum*: *Stenostomum anatirostrum*, *S. corderoi*, *S. evelinae*, *S. membranosum*, *S. pseudoacetabulum*, *S. saliens*, *S. tuberculosum* and *S. ventronephrium.* Five of them belong to the subclade III, together with three Swedish species, *S. glandulosum* and *S. ashanika* sp. nov. The species of this subclade, with the exception of *S. glandulosum*, do not have light-refracting bodies, one diagnostic feature of the genus

Anokkostenostomum (Noreña *et al*. 2005). Six *Stenostomum* species included in Larsson *et al*.'s (2008) analysis were also included in this analysis. Three were found to belong to subclade III (*Stenostomum gotlandense*, *S. handoelense* and *S. steveoi*), whereas the others belong to subclade IV (*Stenostomum arevaloi*, *S. heebuktense* and *S. leucops*). These groups do not coincide with the results of Larsson *et al*. (2008).

Myostenostomum species included in the analysis are closely related to *Stenostomum* species and in fact render *Stenostomum* paraphyletic. *Myostenostomum* show several *Stenostomum* synapomorphies and have a particular development of muscle fibres in the anterior part of the intestine. This result raises new questions about the validity of the genus *Myostenostomum* but this needs to be considered in a broader context of a revision of *Stenostomum.*

'Turbellaria' taxonomy is at a stage in which the boundaries between taxa are not yet fully defined. The simple morphology of Stenostomidae prevents the compilation of a large number of characters for analysis, but this can be remedied with clearly defined characters and states, and the use of histology and ultrastructure. Further taxon sampling and the use of molecular sequence data will allow for more robust hypotheses about the relationships of the group.

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References

Beklemichev, W. (1917). Turbellariés, collectionnés dans les gouvernement de Kalouga, etc. *Annuarie du Musée Zoologique Académie des Sciences* **21**, 347–368.

Beklemichev, W. (1921). Turbellaria. *Faune Petropolitanae Catalogus* **2**, 1–9.

Borkott, H. (1970). Geschlechtliche Organisation, Fortpflanzungsverhalten und Ursachen der sexuellen Vermehrung von *Stenostomum sthenum* nov. spec. (Turbellaria, Catenulida). *Zeitschrift fur Morphologie und Ökologie der Tiere* **67**, 183–262.

Carranza, S., Baguñà, J., and Riutort, M. (1997). Are the Platyhelminthes a monophyletic primitive group? An assessment using 18s rDNA sequences. *Molecular Biology and Evolution* **14**, 485–497.

Corda, A. C. J. (1838). *Stylacium*, ein neues Geschlecht der Schlauchwurmer (Rhabdocoela Ehrenbg.). *Beiträge zur gesammten Natur- und Heilwissenschaft* **4**(1), 71–78.

Dugès, A. (1828). Recherches sur l'organisation et les moeurs des planariees. *Annales des sciences naturelles* **15**, 139–182.

Ehlers, U. (1985). 'Das Phylogenetitische System der Platyhelminthes.' (Gustav Fischer Verlag: Stuttgart, Germany.)

Ehlers, U. (1986). Comments on a phylogenetic system of the Platyhelminthes. *Hydrobiologia* **132**, 1–12. doi:10.1007/BF00046222

Gamo, J., and Leal-Zanchet, A. M. (2004). Freshwater microturbellarians (Platyhelminthes) from Rio Grande do Sul, Brazil. *Revista Brasileira de Zoología* **21**, 897–903. doi:10.1590/S0101-81752004000400026

Goloboff, P., Farris, J., and Nixon, K. (2008). TNT, a free program for phylogenetic analysis. *Cladistics* **24**, 774–786. doi:10.1111/j.1096-0031.2008.00217.x

Graff, L. (1911). Acoela, Rhabdocoela, Alloecoela des Ostens der Vereinigten Staaten von Amerika. *Zeitschrift für Wissenschaftliche Zoologie* **99**, 1–108. t. 1–4.

Graff, L. (1913). Turbellaria. II. Rhabdocoela. Das Tierreich. *Königliche Preussische Akademie der Wissenschaften zu Berlin* **20**, 1–147.

Jondelius, U., Ruiz-Trillo, I., Baguña, J., and Riutort, M. (2002). The Nemertodermatida are basal bilaterians and not members of the Platyhelminthes. *Zoologica Scripta* **31**, 201–215. doi:10.1046/j.1463-6409.2002.00090.x

Karling, T. (1974). On the anatomy and affinities of the Turbellarian orders. In 'Biology of the Turbellaria'. (Eds N. W. Riser and M. P. Morse.) pp. 1–16. (McCraw-Hill Book Company: New York.)

Kennel, J. (1889). Untersuchungen an neuen Turbellarien. *Zoologische Jahrbücher Abteilung für Anatomie der Tiere* **3**, 447–486.

Kepner, W. A., and Carter, J. (1931). Ten well-defined new species of *Stenostomum. Zoologischer Anzeiger* **93**, 108–123.

Kolasa, J. (1977). Turbellaria and Nemertini. Bottom fauna of the heated Konin lakes. *Monografie Fauny Polski* **7**, 27–46.

Kolasa, J. (1991). Flatworms: Turbellaria. In 'Ecology and classification of North American freshwater invertebrates'. (Eds J. M. Thorp and A. P. Covich.) pp. 145–171. (Academic Press: New York.)

Kolasa, J., and Young, J. O. (1974*a*). Studies on the genus *Stenostomum* O. Schmidt (Turbellaria; Catenulida). I. The status of *S. anatirostrum* Marcus 1945 and *S. bryophilum* Luther 1960. *Freshwater Biology* **4**, 145–156.

Kolasa, J., and Young, J. O. (1974*b*). Studies on the genus *Stenostomum* O. Schmidt (Turbellaria; Catenulida). II. A new subspecies and records of two species new to Poland. *Freshwater Biology* **4**, 157–161. doi:10.1111/j.1365-2427.1974.tb00085.x

Lanfranchi, A., and Papi, F. (1978). Turbellaria (excl. Tricladida). In 'Limnofauna Europaea, 2nd edn'. (Ed. J. Illies.) pp. 5–15. (Gustav Fischer Verlag: Amsterdam, Holland/ Swets & Zeitlinger B. V.: Stuttgart, Germany.)

Larsson, K., and Jondelius, U. (2008). Phylogeny of Catenulida and support for Platyhelminthes. *Organisms, Diversity & Evolution* **8**, 378–387. doi:10.1016/j.ode.2008.09.002

Larsson, K., and Willems, W. (2010). Report on freshwater Catenulida (Platyhelminthes) from Sweden with the description of four new species. *Zootaxa* **2396**, 1–18.

Larsson, K., Ahmadzadeh, A., and Jondelius, U. (2008). DNA taxonomy of Swedish Catenulida (Platyhelminthes) and a phylogenetic framework for catenulid classification. *Organisms, Diversity & Evolution* **8**, 399–412. doi:10.1016/j.ode.2008.09.003

Luther, A. (1908). Über *Weldonia parayguensis* CH Martin. *Zoologischer Anzeiger* **33**, 300.

Luther, A. (1960). Die Turbellarien Ostfennoskandiens. *Fauna Fennica* **7**, 1–155.

Marcus, E. (1945*a*). Sobre Catenulida brasileiros. *Boletins da Facultade de Filosofia, Ciencias e Letras Zoologia* **10**, 3–133.

Marcus, E. (1945*b*). Sobre Microturbelarios do Brasil. *Comunicaciones Zoologicas del Museo de Historia Natural de Montevideo* **1**, 1–74. [Est I–XI.]

Marcus, E. (1949). Turbellaria Brasileiros (7). *Boletins da Facultade de Filosofia, Ciencias e Letras Zoologia* **14**, 7–155.

Martin, C. H. (1908). *Weldonia parayguensis*. *Zoologischer Anzeiger* **32**, 758–763.

Nassonov, N. (1924). Les trats généraux de la distribution géographique des Turbellaria rhabdocoelida dans la Russie, etc. *Bulletin de l'Académie des Sciences de Russie* **1924**, 327–352.

Nassonov, N. (1926). Die Turbellarienfauna des Leningrader Gouvernements I. *Bulletin de l'Académie des Sciences de l'Union des Républiques Soviétiques Socialistes* **1926**, 817–836.

Nixon, K. C. (2002). 'WinClada ver. 1.00.08.' (Ithaca: NY.)

Noreña, C., Damborenea, C., and Brusa, F. (2005). A taxonomic revision of South American species of the genus *Stenostomum* O. Schmidt (Platyhelminthes: Catenulida) based on morphological characters. *Zoological Journal of the Linnean Society* **144**, 37–58. doi:10.1111/j.1096-3642.2005.00157.x

Noreña, C., Damborenea, C., Brusa, F., and Escobedo, M. (2006). Free-living Platyhelminthes of the Pacaya-Samiria National Reserve, a Peruvian Amazon floodplain. *Zootaxa* **1313**, 39–55.

Noreña-Janssen, C. (1995). Studies on the taxonomy and ecology of the Turbellaria (Plathelminthes) in the floodplain of the Paraná River (Argentina). II. Taxonomy and ecology of the Turbellaria. *Archiv für Hydrobiologie* **107**(Suppl.), 211–262.

Nuttycombe, J. (1931). Two new species of *Stenostomum* from the southeastern United States. *Zoologischer Anzeiger* **97**, 80–85.

Nuttycombe, J. (1932). Observations on *Stenostomum. Zoologischer Anzeiger* **9**, 123–131.

Nuttycombe, J., and Waters, A. (1935). *Stenostomum pseudoacetabulum* (nov. spec.). *The Biological Bulletin* **68**, 168–171. doi:10.2307/1537260

Nuttycombe, J., and Waters, A. (1938). The American species of the genus *Stenostomum. American Philosophical Society* **79**, 213–301.

Peterson, K. J., and Eernisse, D. J. (2001). Animal phylogeny and the ancestry of bilaterians: inferences from morphology and 18S rDNA gene sequences. *Evolution & Development* **3**, 170–205. doi:10.1046/j.1525-142x.2001.003003170.x

Picken, L. E. R. (1937). A new species of rhabdocoel and its method of feeding. *Journal of the Linnean Society. Zoology* **40**, 273–277.

Rogozin, A. G. (1992). A short revision of *Myostenostomum* (Turbellaria Catenulida). *Zoologicheskij Zhurnal* **71**, 5–11.

Schmidt, E. O. (1848). 'Die rhabdocoelen Strudelwürmer des süssen Wassers.' (Fr. Mauke: Jena.)

Telford, M. J., Lockyer, A. E., Cartwright-Finch, C., and Littlewood, T. J. (2003). Combine large and small subunit ribosomal RNA phylogenies support a basal position of the acelomorph flatworms. *Proceedings of the Royal Society of London* **270**, 1077–1083. doi:10.1098/rspb.2003.2342

Tyler, S., Schilling, S., Hooge, M., and Bush, L. F. (comp.) (2006–2010). Turbellarian taxonomic database. Version 1.6. Available at http://turbellaria.umaine.edu [accessed 1 September 2011]

Van der Land, J. (1970). Kleine dieren uit het zoete water van Suriname verslag van een onderzoek in 1967. *Zoologische Bijdragen* **12**, 1–46.

Young, J. O., and Kolasa, J. (1974*a*). Studies on the genus *Stenostomum* O. Schmidt (Turbellaria; Catenulida). III. A new species from Kenya, East Africa. *Freshwater Biology* **4**, 163–166. doi:10.1111/j.1365-2427.1974.tb00086.x

Young, J. O., and Kolasa, J. (1974*b*). Studies on the genus *Stenostomum* O. Schmidt (Turbellaria; Catenulida). IV. New record of established species from E. Africa, with notes on their anatomy and distribution. *Freshwater Biology* **4**, 167–176. doi:10.1111/j.1365-2427.1974.tb00087.x

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