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# Species of *Macrostomum* (Macrostomorpha: Macrostomidae) from the coastal region of Lima, Peru, with comments on *M. rostratum* Papi, 1951

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## Abstract

Species of *Macrostomum* are present in almost all regions of the world. These flatworms occur in different kinds of environments, from freshwater to marine ecosystems. Although Peru is considered one of the megadiverse countries in South America, little is known about freshwater microturbellarians in this country. Here we report the presence of *M. rostratum* Papi, 1951 for the first time in Peru, in a coastal brackish water wetland, and that of *M. quiritium* Beklemischev, 1951 and *M. tuba* Graff, 1882 in an inland portion of a river near the city of Lima. All the species were studied alive. Comments on the habitat and associated fauna are given. Detailed information on the copulatory system is provided for *M. rostratum*; the male system has a bilobed seminal vesicle with thick walls and full of spindle-shaped sperm cells, and a stylet in the form of a solid curved inverted cone with a subterminal opening located on the concave side of the stylet. These findings led us to suggest that *M. rostratum* may exhibit a hypodermic mating syndrome. The three species of *Macrostomum* reported here are widespread and, although the diagnostic characters of the species are present, some morphological differences in the copulatory stylet suggest they are cryptic species.

**Key words:** Platyhelminthes, Chillón River, Humedales de Ventanilla, wetlands, taxonomy, turbellarians

## Introduction

The genus *Macrostomum* Schmidt, 1848 consists of a group of microturbellarians, which live in a variety of freshwater, brackish, and marine environments (Ax 2008; Ferguson 1938). The species of the genus are spatula-shaped with rounded anterior end and widened posterior end. The reproductive organs are in the middle and posterior parts of the body. The female reproductive system is composed of two ovaries and a female atrium, whereas the male system is composed of two testes, vas deferens, seminal vesicles, prostatic visicle and a copulatory stylet. The morphology of the stylet is the most important feature used in the morphological identification of the species of the genus (Ferguson 1954; Rieger 1977; Schärer *et al.* 2011). So far, approximately 170 valid species have been recognized in the genus *Macrostomum* (Tyler *et al*. 2006–2016, http://macrostomorpha.info/) worldwide. However, species identification is sometimes difficult because some species do not have type specimens and the stylet has intraspecific morphological plasticity (Rieger 1977).

Although Peru is considered one of the megadiverse countries in South America (Chang & Ortega 1995; Rodríguez & Young 2000; Zamora 1996), little is known about freshwater microturbellarians in this country (Noreña *et al*. 2004). Nineteen species of *Macrostomum* have been reported for South America in freshwater, brackish and marine environments (Adami *et al*. 2012, 2016, Braccini *et al*. 2016; Brusa 2006), and only two species (*M. johni* Young, 1972 and *M. tuba* Graff, 1882) have been recorded for Peru (Noreña *et al.* 2006).

In this paper, we report the presence of *M. rostratum* Papi, 1951 and *M. quiritium* Beklemischev, 1951 for the first time, and the presence of *M. tuba* Graff, 1882 for the second time in Peru. *M. rostratum* was found in a lentic

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wetland, and *M. quiritium* and *M. tuba* in a river near the city of Lima. We also add new data on the morphology of *M. rostratum*, providing detailed information on the stylet and spermatozoa.

## Materials and methods

Samples were taken in two localities of Lima Region: the regional Conservation area Humedales de Ventanilla and the Chillón River, near Trapiche.

The regional Conservation area Humedales de Ventanilla (11°51'23"–11°52'42"S and 77°7'43"–77°9'32"W) is located in the central western region of Ventanilla district. Samples were taken monthly from August to December 2015 (Table 1; Figure 1) from the littoral benthos and marsh vegetation in lagoons of Humedales de Ventanilla. The samples from the benthos were taken with corer tubes of 5 cm in diameter in a transect of one meter just below the water level. The first two centimeters of sediment were carried to the laboratory to observe the flatworms alive. The samples from the vegetation were taken with 65-µm mesh sieves and carried alive to the laboratory.

The Chillón River is located in the central region of Peru, in Canta and Lima Provinces (Table 1; Figure 1). The river flows from east to west for ~126 km between 11°22'S and 11°56'S and 76°26'W and 77°08'W (MINAM 2010). Samples were collected monthly from June to November 2015 from aquatic herbaceous vegetation with 80-µm mesh sieves and carried alive to the laboratory.

Once in the laboratory, the flatworms were extracted by the oxygen depletion method (Schockaert 1996) and observed *in vivo* using the squash method. Other specimens were mounted in polyvinyl–lactophenol to observe the copulatory stylet. The stylets were measured following Schockaert's (2014) recommendations (Figure 3D). The specimens studied were deposited at the Departamento de Protozoología, Helmintología e Invertebrados Afines at Museo de Historia Natural of the Universidad Nacional Mayor de San Marcos (MUSM), Peru.

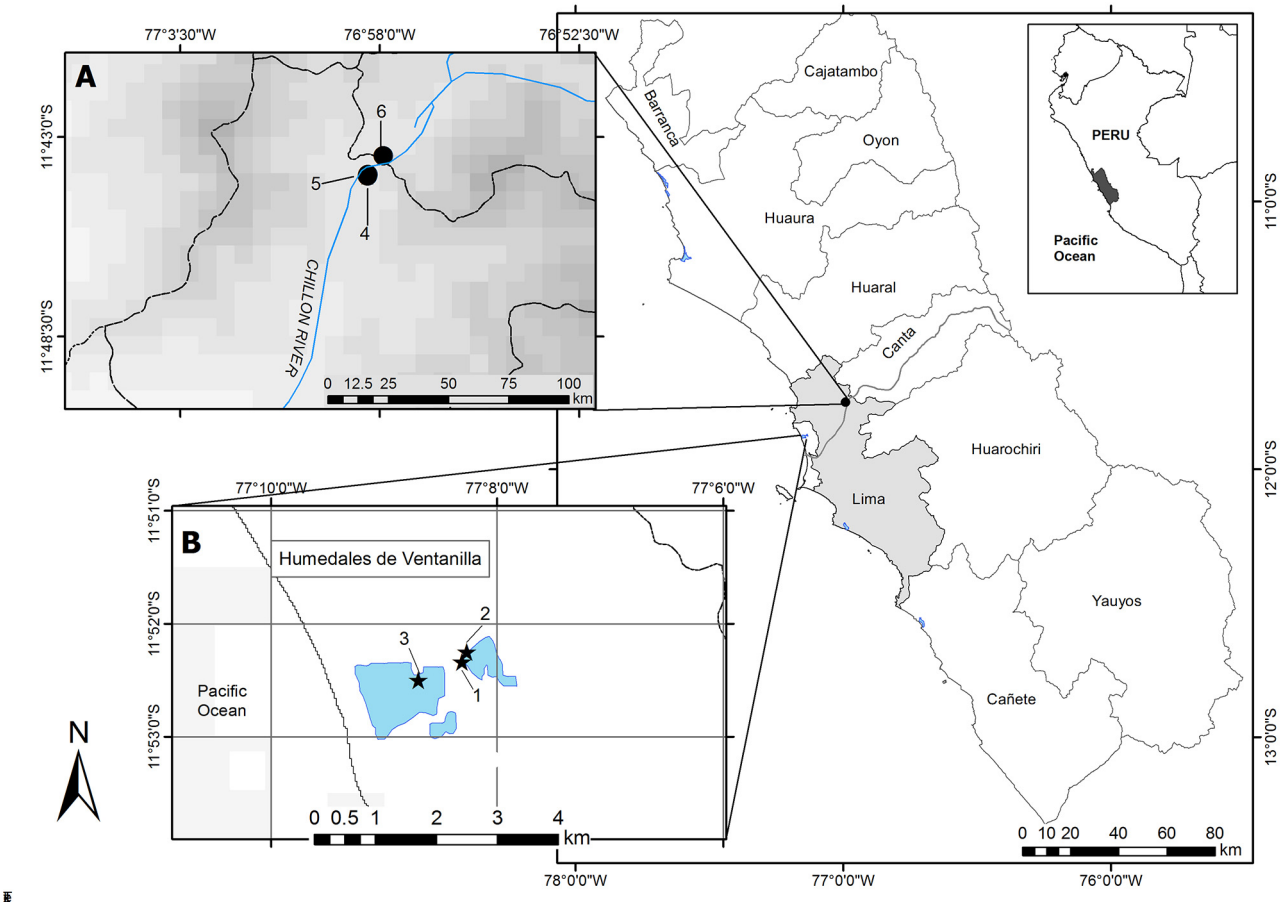


**FIGURE 1.** Sample sites where species of *Macrostomum* were found. A, *M. quiritium* and *M. tuba* in Chillón River (sites 4, 5 and 6). B, *M. rostratum* in Humedales de Ventanilla (sites 1, 2 and 3).

The conductivity, pH and water temperature were recorded at 15 cm depth. The type of substrate and accompanying fauna were also recorded (Table 2).

**Abbreviations used in the figures. a**: proximal region of the penis stylet; **b**: body; **c**: cilia; **cg**: cement glands; **eg**: egg; **e**: eyes; **fa**: female antrum; **fg**: female gonopore; **fl**: feeler; **fvs**: false vesicula seminalis; **i**: intestine; **h**: total stylet height; **L**: total stylet length; **m**: moth; **o**: ovary; **p**: pharynx; **pg**: pharyngeal glands; **ps**: penis stylet; **pso**: penis stylet opening; **rc**: rigid cilia; **sf**: shaft; **t**: testes; **vg**: prostatic vesicle; **vs**: vesicula seminalis.

**TABLE 1.** Sample sites in Humedales de Ventanilla (HV) and Chillón river (CR).

| Sites | Latitude | Longitude | Characteristics of the sample sites |
|---|---|---|---|
| HV-1 | 11°52'20.2"S | 77°08'18.9"W | Littoral zone with *Salicornia* sp. and *Chara* sp. |
| HV-2 | 11°52'15"S | 77°08'16.4"W | Muddy litoral with *Cypers* sp. and *Arundo* sp. |
| HV-3 | 11°52'30.8"S | 77°08'42.5"W | Littoral zone with *Chara* sp, *Typha domingensis* and *Cyperus* sp. |
| CR-4 | 11°44'1.8"S | 76°58'19.56"W | Temporary ponds formed by irrigation channels filtration. Presence of shrub and herbaceous vegetation. |
| CR-5 | 11°44'4.38"S | 76°58'20.52"W | Main river channel. Presence of herbaceous vegetation. |
| CR-6 | 11°43'30.9"S | 76°57'54.1"W | Temporary pond formed by main river channel filtration. Presence of shrub and herbaceous vegetation. |

## Results

### Family Macrostomidae

### Genus *Macrostomum* Schmidt, 1848

### *Macrostomum rostratum* Papi, 1951

Synonyms: *Macrostomum viride rostrata* Papi, 1951
*Macrostomum phytophilum* Beklemischev, 1951
*Macrostomum rostratus* Ferguson, 1954

**Localities.** The specimens were found in the sampling sites coded as HV-1 (11°52’20.2’’S; 77°08’18.9’’W), HV-2 (11°52'15’’S; 77°08’16.4’’W) and HV-3 (11°52’30.8’’S; 77°08’42.5’’W) (Figure 1, Table 1) in Humedales de Ventanilla in Lima.

**Other localities in the world.** The species has been found in several countries around the world: Russia, Finland (Luther 1960), Italy (Papi 1951), United Kingdom, Ireland (Young 2001), Spain (Farías *et al.* 1995; Noreña *et al*. 2007), The Netherlands (Tulp 1974), Kenya (Young 1976) and Brazil (Braccini & Leal-Zanchet 2013).

**Studied material**. Twenty-five specimens observed *in vivo* (squash preparations) and 28 (MUSM 3320–3346) specimens fixed in polyvinyl-lactophenol.

**Description**. Body usually whitish that turns to yellow–mustard (Figure 2A) when the intestinal contents (Figures 2A; 3C) reflect the light (algae, rotifers, ostracods and sometimes larvae of dipterans). The anterior part of the body is truncated, and widens to reach its maximum width at about 2/3 of the body length. The posterior end is spatula–shaped with a large number of adhesive papillae on its edge. The length of the body varies from 1.5 to 2 mm. The epidermis has locomotor cilia and longer sensory cilia throughout the body. Abundant rhabdites grouped in packs of seven to twelve rhabdites each and distributed mainly on the dorsal surface of the body. Cerebral ganglia paired and linked by a weak cerebral commissure. The ciliated mouth is located just behind the eyes. The pharynx simplex possesses a large quantity of thin pear-shaped glands (Figures 2A–B; 3A).

Male reproductive system: Oval, thin-walled testes latero-ventral in the mid-part of the body, flanking the intestine (Figures 2C; 3A). The seminal vesicle is bilobed and globular in shape, with thick walls and full of spermatozoa. When swimming, the anterior region of the seminal vesicle (anterior lobe) is positioned close to the left side and to the ventral surface of the body, while the posterior region (posterior lobe) is located in the dorsal

position, slightly posterior to the anterior lobe and on the central body axis. Both lobes are separated by a constriction. The seminal vesicle continues at its distal end with the smaller prostatic vesicle, which immediately joins the proximal end of the stylet (Figure 2A, E). Digitiform prostate glands fill the prostate vesicle and the proximal region of the stylet. The stylet is a conical tube that is housed in a muscular tunica. Proximally, the stylet

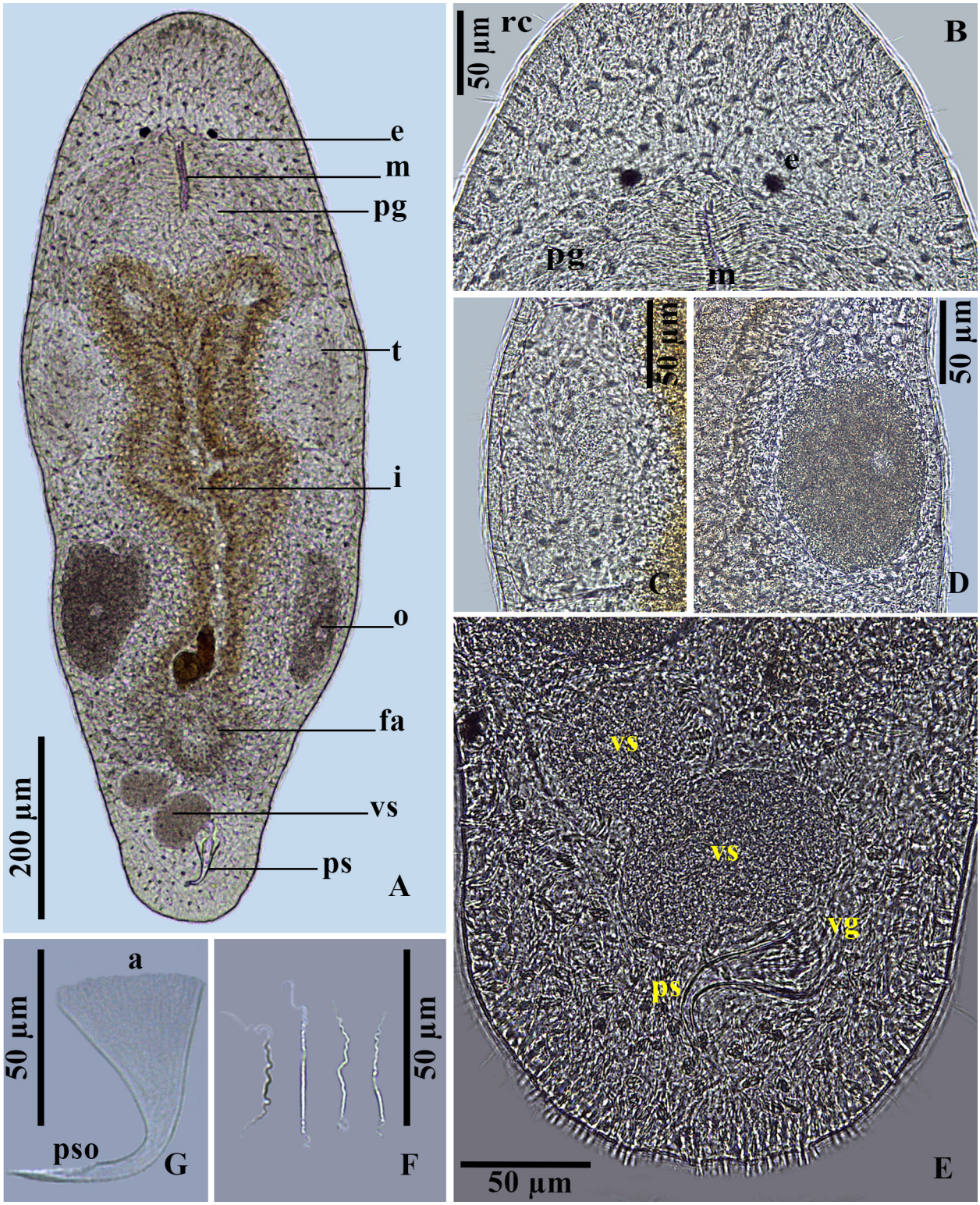


**FIGURE 2.** Photographs of a live specimen of *Macrostomum rostratum* Papi, 1951. A, Dorsal view of a mature specimen; B, Detail of the anterior body region; C, Detail of the testes; D, Detail of the ovary; E, Posterior body region with details of the copulatory organs; F, Detail of four spermatozoa in different focal planes; G, Penis stylet.

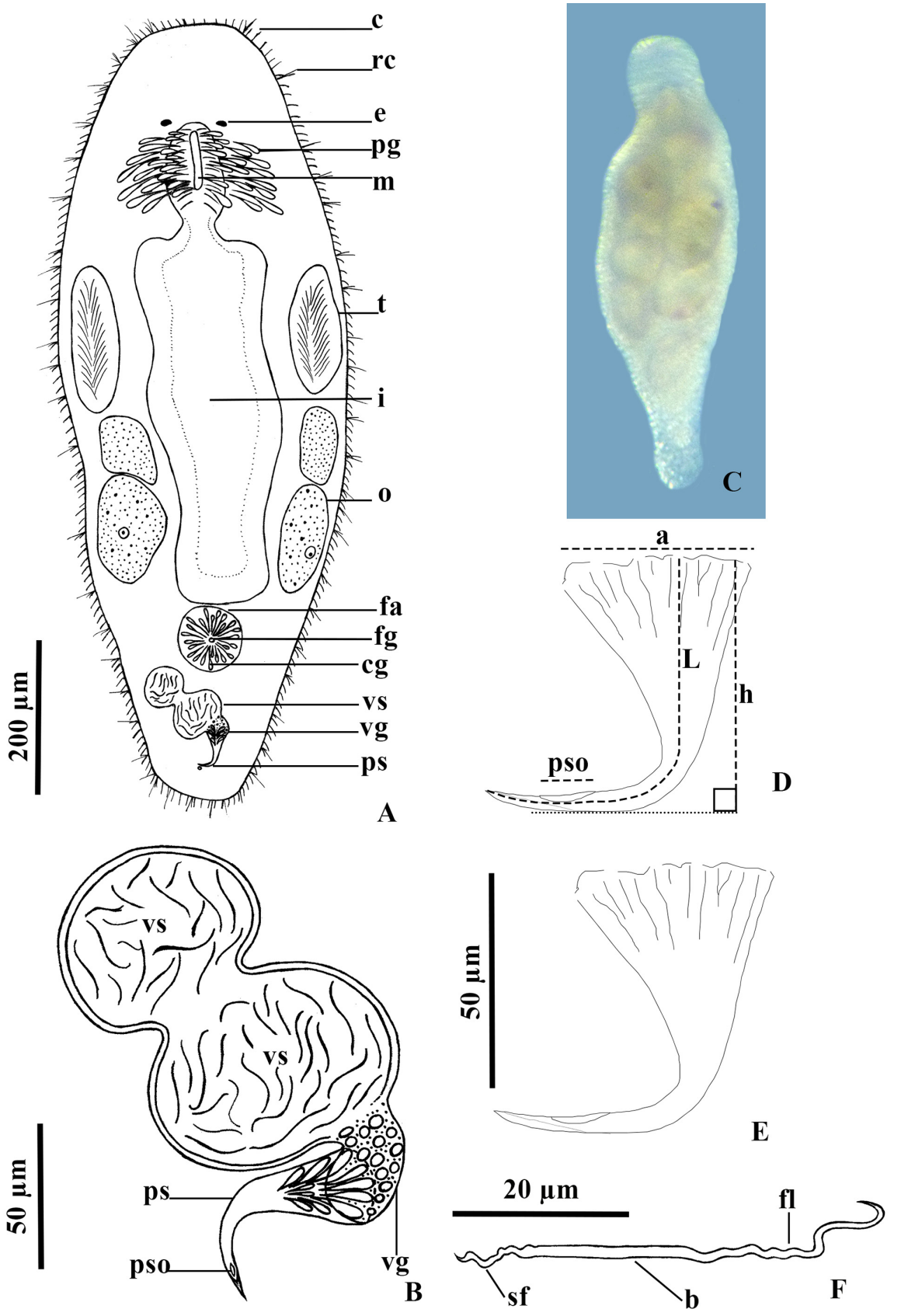


**FIGURE 3.** *Macrostomum rostratum* Papi, 1951. A, Schematic reconstruction of the body; B, Detail of the male copulatory system; C, Specimen of *M. rostratum* with mainly microcrustaceans in the intestine; D–E, Schematic representation of the stylet with measurements taken; F, Detail of a spermatozoon.

has a slightly crenellated base, which is 38.4 µm in length (26.8–49.8 µm; *n* = 27; *sd* = 6.8), whereas distally, its shaft is curved at an angle of ± 90° (with the curvature attenuated). The subterminal opening of the stylet is 9.6 µm long (7.4–12.7 µm; *n* = 22; *sd* = 1.5) and located on the concave side of the stylet (Figures 2G; 3D, E). The total length of the stylet (*sensu* Schockaert 2014) is 82.4 µm (70.4–92.5 µm; *n* = 18; *sd* = 6.6), whereas the total height of the stylet is 49.2 µm (37.3–59.6 µm; *n* = 26; *sd* = 5.7) (Figures 2G; 3D, E).

Spermatozoa consist of body and shaft (Figures 2F; 3F) and their total length is 31.9 µm (24.2–44.4 µm, *n* = 14; *sd* = 5.1).

Female reproductive system: Typical of the genus. The ovaries are located behind the middle of the body, and have brownish–yellow coloration. The female atrium is located posterior to the intestine, ventrally, and is surrounded by shell glands. The female atrium usually contains eggs in formation (Figures 2A, D; 3A).

**Remarks.** Based on the morphological characteristics observed, the individuals collected belong to the species with a hook-shaped stylet. The species of this group are characterized by a stylet with a broad base, a funnel-shaped shaft, and a pointed distal portion which is curved into a hook at an angle of ± 90°. The opening of the ejaculatory duct lies at the level of the stylet curvature, in the concave or convex portion (Figures 2G; 3E). In most species of this group (*M. appendiculatum* Fabricius, 1826, *M. balticum* Luther, 1947, *M. distinguendum* Papi, 1951, *M. hystricinum* Beklemischev, 1951, *M. hystrix* Ørsted, 1843, *M. pusillum* Ax, 1951, *M. romanicum* Mack-Fira, 1968, and *M. sileciacum* Kolasa, 1973), the general shape of the stylet is very similar, with the distal opening on the convex side of the curvature. However, in *M. rostratum*, the stylet opening occurs on the concave side.

Based principally on the copulatory apparatus, we here report *M. rostratum* Papi, 1951 for the first time in Peru. This species has been described for Europe and inhabits both brackish and freshwater environments (Braccini & Leal-Zanchet 2013; Noreña *et al*. 2007; Papi 1951, 1959; Young 1976). The morphological characteristics of the specimens found in Humedales de Ventanilla are similar to those described by Papi (1951, 1959) and Luther (1960); however, there are some differences in the copulatory apparatus. The stylets of the specimens from Humedales de Ventanilla have the shape of a solid inverted cone, whereas those reported by Papi (1951, 1959) are slightly spiral- or "S"-shaped as is also the case in *M. hystricinum*. However, in the latter species, the distal opening is on the convex side of the stylet. In the specimens found in Humedales de Ventanilla, the distal opening is clearly seen as described by Papi (1959), while in the specimens reported by Papi (1951), this opening is not clearly stated.

The size of the stylets of the specimens found in Humedales de Ventanilla differs slightly from those recorded so far. In Europe, lengths of 67 µm (Papi 1959), 52–60 µm (Luther 1960), 63 µm (Tulp 1974), 53 µm (Young 1976) and 36–77 µm (Heitkamp 1979) have been recorded, whereas the height of the stylet of the specimens studied here are similar to those found by the authors mentioned above. We thus infer that the stylet dimensions of this species are variable, as in other species of the genus. The morphology of the spermatozoa and stylet of *M. rostratum* suggests hypodermic insemination (Schärer *et al*. 2011). However, new observations are needed to confirm this hypothesis.

In some occasions, *M. rostratum* was found with its intestinal content full of cladocerans (Figure 3C). Moreover, this species was associated with vegetation such as *Cyperus* sp., *Arundo* sp., *Salicornia* sp., *Chara* sp. and *Typha domingensis* Pers.; the water temperature was 20.88 °C on average, the pH was 9.16 and the conductivity was 20 mS/cm (Table 2).

### *Macrostomum quiritium* Beklemischev, 1951

**Localities**. The specimens were found in the sampling sites coded as CR-4 (11°44'1.8''S; 76°58'19.56''W), CR-5 (11°44'4.38''S; 76°58'20.52''W) and CR-6 (11°43'30.9''S; 76°57'54.1''W)(Figure 1, Table 1) in the Chillón River in Lima.

**Other localities in the world.** The species has a worldwide distribution; it occurs in Russia, Poland, Switzerland (Schärer *et al*. 2011) and Argentina (Río de la Plata River and Paraná River) (Adami *et al*. 2016).

**Studied material.** Ten specimens observed alive (squash method) and 20 specimens (MUSM 3414–3433) fixed in polyvinyl-lactophenol.

**Description**. Adult specimens are whitish in reflected light. The body is 0.9–1.9 mm long and 0.3–0.4 mm wide. The whole body is covered by cilia. Large amounts of homogeneously distributed rhabdites are observed on the surface of the body. The anterior region of the body has a rounded shape, while the posterior region has the

shape of a rounded spatula. The mouth is just behind the kidney–shaped eyes, with many cilia in its outline. The simplex pharynx has sac-shaped pharyngeal glands that are arranged beside and behind the pharynx. The intestine occupies 3/4 of the total length of the body and extends almost to the posterior region of the body.

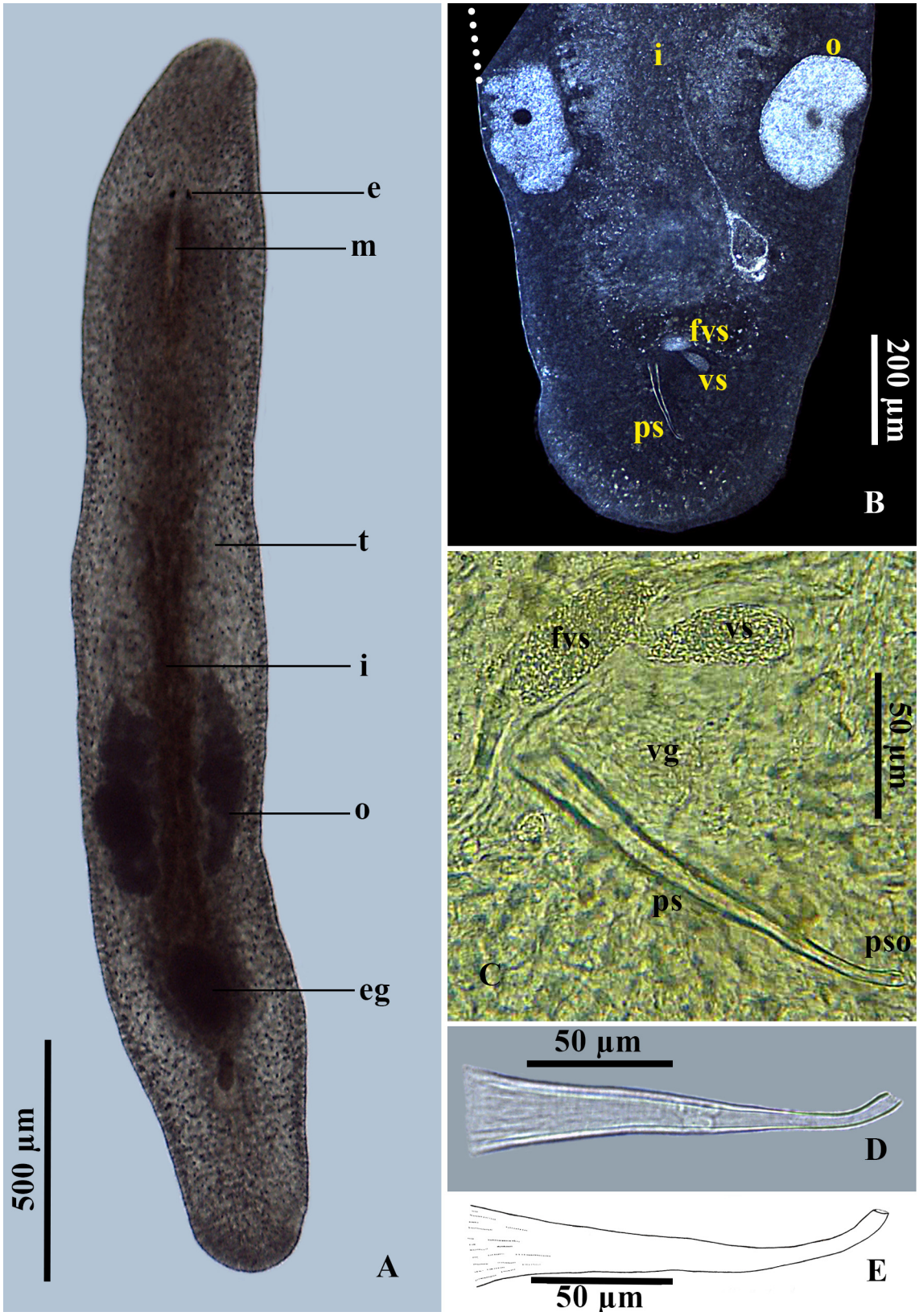


**FIGURE 4.** Photographs of a live specimen of *Macrostomum quiritium* Beklemischev, 1951. A, Dorsal view of a mature specimen; B–C, Posterior body region with details of the copulatory organs observed in dark and normal field; D–E, Photograph and schematic reconstruction of the penis stylet.

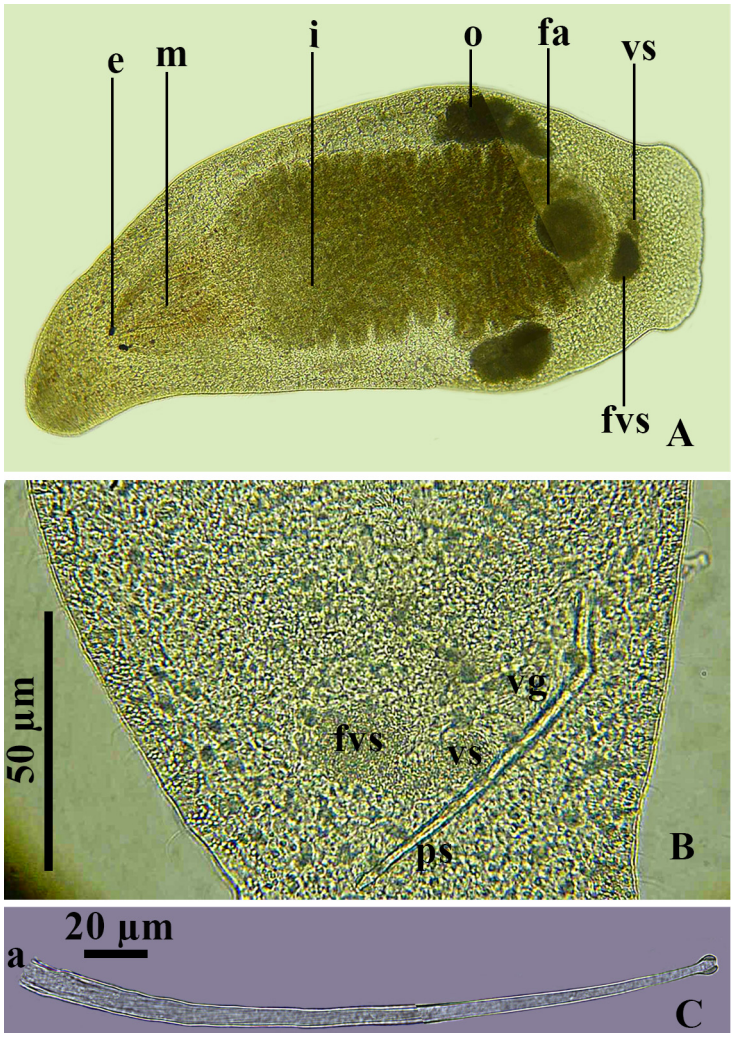


**FIGURE 5.** *Macrostomum tuba* Graff, 1882. A, Dorsal view of a mature specimen; B, Posterior body region with details of the copulatory organs; C, Detail of the penis stylet.

Male reproductive system: Two oval–shaped testes arranged at the sides of the body, behind the beginning of the intestine and anterior to the ovaries (Figure 4A). The copulatory apparatus is located posterior to the intestine, and has a false thin–walled seminal vesicle, which stores the spermatozoa. It is continued through a thin intervesicular duct with the seminal vesicle, which has thick muscular walls and is smaller than the false seminal vesicle (Figure 4B–C). The prostatic vesicle is larger than the seminal vesicle and communicates with it through its proximal end. Within the prostatic vesicle, granulations are observed. These are grouped in small and long sacs that distally enter the stylet. The stylet is tubular with a slight curvature. The length of the stylet is 144.9 µm (99.6–181.9 µm; $n$ = 20; $sd$ = 21). The proximal base of the stylet is 25.7 µm wide (15.1–34 µm; $n$ = 20; $sd$ = 5.6) and narrows towards the distal opening, which is 5.4 µm wide (3.9–6.5 µm; $n$ = 20; $sd$ = 0.7) (Figure 4C–E). The distal tip of the stylet presents a slight curvature, without lobulation or expansion, but the walls of the stylet in the distal region terminate in a very slight slope (Figure 4D–E). The stylet protrudes through the male gonopore, which is covered with cilia and is located in the posterior part of the body, behind the female gonopore.

Female reproductive system: The ovaries are approximately located at the mid-part of the body and are arranged lateroventrally to the intestine. The female genital atrium is located anterior to the false seminal vesicle of the male reproductive system and ventral to the intestine. The atrium is lined with abundant cells corresponding to the cellular valve (Ladurner *et al*. 2005) and in some cases, spermatozoa were observed anchored to this epithelium. The atrium communicates immediately with the female gonopore. In some adult specimens, egg formation was observed in the atrium (Figure 4A).

**Remarks.** The specimens described here represent the first record of *M. quiritium* for Peru, and the second for

the Neotropical Region. *M. quiritium* has a tubular- or "J"-shaped penis stylet (Beklemishev 1951; Adami *et al*. 2016), which resembles 16 species of *Macrostomum* (*M. amurense* Beklemischev, 1950, *M. axi* Papi, 1959, *M. balticum* Luther, 1947, *M. bulbostylum* Kepner & Stiff, 1932, *M. christinae* Young, 1976, *M. coxi* Young, 1976, *M. curvituba* Luther, 1947, *M. dongyuanensis* Wang & Sun, 2015, *M. georgeense* Young, 1976, *M. longistylifernum* Ax, 1956, *M. lignano* Ladurner *et al*., 2005, *M. magnacurvituba* Ax, 1994, *M. parmum* Ball, 1977, *M. sinensis* Wang, 2005, *M*. spec 1 Ax, 2008, and *M*. spec 2 Ax, 2008). Among them, five species, namely *M. axi*, *M. balticum*, *M. sinensis*, *M*. spec 1 and *M*. spec 2, have the distal part of the stylet ended in a sharp point. In contrast, *M. quiritium* has the stylet with its distal region terminating in a very slight slope. The stylet of *M. bulbostylum*, *M. christinae*, *M. coxi*, *M. curvituba*, *M. longistylifernum*, *M. lignano*, and *M. magnacurvituba* has notably thickened walls at its distal end, which is not observed in the distal part of the stylet of *M. quiritium* found at the Chillón River. As for *M. amurense*, *M. georgeense* and *M. parmum*, the penis stylet is clearly bent; in contrast, in *M. quiritium*, the distal part of the penis stylet is slightly bent (Young 1976, 2001; Sun *et al*. 2015).

The internal morphology, i.e., the penis stylet, of *M. dongyuanensis* is very similar to that of *M. quiritium*; however, *M. dongyuanensis* has a "U"-shaped prostate vesicle (Sun *et al*. 2015), while *M. quiritium* has a sacciform prostate vesicle (Beklemishev 1951; Adami *et al*. 2016). Moreover, the shape of the stylet and the fact that the female atrium is lined with a cellular valve suggest reciprocal insemination (Schärer *et al*. 2011).

*M. quiritium* was found associated with algae such as *Spirogyra* sp., *Hydrodictyon* sp., *Enteromorpha* sp. and *Cladophora* sp. The average water temperature was 24.3 °C, the pH was 8.34 and the conductivity was 0.85 mS/cm (Table 2).

**TABLE 2.** Average values of the physicochemical parameters and characteristics of the sample sites in Humedales de Ventanilla (HV) and Chillón river (CR).

| Sites | Conductivity (mS/cm) | pH | Temperature (C°) | Type of bottom | Type of vegetation | Other invertebrates in the sites |
|---|---|---|---|---|---|---|
| HV-1 | 20.0 | 9.03 | 20.80 | muddy | *Cyperus* sp., *Arundo* sp. | Coleoptera, Poecilidae, Anisopetara, Diptera. |
| HV-2 | 20.0 | 9.95 | 21.85 | muddy | *Salicornia* sp., *Chara* sp. | Rotifera, Coleoptera. |
| HV-3 | 20.0 | 8.50 | 20.0 | muddy | *Chara* sp., *Typha domingensis, Cyperus* sp. | Neuroptera, Anisoptera, Amphipoda, Ostracoda. |
| CR-4 | 0.85 | 8.43 | 24.03 | Floating vegetation and algae | *Tessaria* sp., *Plantago major, Ludwigia peploides, Spirogira* sp., *Hydrodictyon* sp., *Enteromorpha* sp., *Cladophora* sp. | Colembola, Gastropoda, Aranea, Diptera larvae, Odonata larvae, Nemertea, Rotifera, Oligochaeta |
| CR-5 | 0.83 | 8.49 | 25.30 | Floating vegetation, algae and muddy | *Nasturtium* sp., *Plantago major, Ludwigia peploides, Hydrodictyon* sp., *Enteromorpha* sp., *Cladophora* sp. | Hemiptera, Neuroptera, Colembola, Gastropoda, Oligochaeta, Diptera larvae, Odonata larvae, Acari, Rotifera. |
| CR-6 | 0.89 | 8.11 | 23.67 | Floating vegetation, algae and muddy | *Lemna* sp., *Cyperus* sp., *Miriophylum acuaticum, Hydrocotile* sp., *Nasturtium* sp., *Tessaria* sp., A*rundo donax, Plantago major.* | Coleoptera, Odonata larvae, Diptera larvae, Nemertea, Hirudinea, Hydroidea, Oligochaeta, Acari, Amphipoda, Rotifera. |

### ***Macrostomum tuba* Graff, 1882**

Synonyms: *Macrostomum bulbostylum* Kepner & Stiff, 1932
*Macrostomum gigas* Okugawa, 1930
*Macrostomum tuba bulbostylum* Ferguson & Jones, 1940
*Macrostomum tuba gigas* Okugawa, 1930
*Macrostomum tubum* Hyman, 1936

**Localities.** The specimens were found in the sampling sites coded as CR-4 (11°44'1.8''S; 76°58'19.56''W), CR-5 (11°44'4.38''S; 76°58'20.52''W) and CR-6 (11°43'30.9''S; 76°57'54.1''W) (Figure 1, Table 1) in the Chillón River in Lima.

**Other localities in Peru**. The species has been previously found at the Pacaya-Samiria National Reserve in Loreto region: "cocha" Zapote and the "caño" Yarina (5°20'16''S; 74°29'40''W) (Noreña *et al*. 2006).

**Other localities in the world**. The species has a worldwide cosmopolitan distribution (Adami *et al*. 2016; Ferguson 1940; Gamo & Leal-Zanchet 2004; Hyman 1955; Marcus 1946; Noreña *et al*. 2003; Papi 1951; Wang *et al*. 2004; Young 1976).

**Studied material**. Eight specimens observed alive (squash method) and eight (MUSM 3406–3413) specimens fixed in polyvinyl–lactophenol.

**Description**. Adult specimens exhibit whitish–orange body coloration when observed in reflected light. The body is 2.2–2.7 mm long and 0.8–1.2 mm wide. The anterior region is rounded in shape, whereas the posterior region is spatula–shaped when the specimen is attached to the substrate (Figure 5A).

Male reproductive system: Two ellipsoid–shaped testes at the sides of the body, approximately at the beginning of the intestine. A thin-walled false seminal vesicle stores sperm and continues into a thick-walled, muscular seminal vesicle through a thin and short intervesicular duct. The seminal vesicle is about a quarter of the size of the false seminal vesicle. Distally, the seminal vesicle connects to the prostate vesicle. Within the prostate vesicle, granulations are observed. These are grouped in small and long sacs that enter the stylet proximally (Figure 5B). The stylet is a slightly-curved, long and slender tube (Figure 5C). The stylet is 206.9 µm long (162.5–291.5 µm; $n$ = 8; $sd$ = 46.7), and progressively narrows from the base, which is 10.2 µm wide (7.8–15.3 µm; $n$ = 6; $sd$ = 2.6), to the distal opening, which is 2.8 µm wide (2.2–5 µm; $n$ = 8; $sd$ = 0.9). The stylet wall is thickened in its distal portion, which is typical for this species (Figure 5C).

The female reproductive system is typical for the genus. The ovaries are located in the third part of body, arranged lateroventrally to the intestine (Figure 5A). The female genital atrium is anterior to the false seminal vesicle. In some adult specimens, egg formation was observed in the atrium.

**Remarks.** The most remarkable feature of *M. tuba* is the penis stylet, although the diameter of the base, size of shaft and diameter of the distal opening vary according to the region and habitat (Hyman 1936; Ferguson 1939, 1940; Papi 1951; Young 1976). The shape of the distal region of the stylet, with thickened walls, remains constant (Wang *et al*. 2004) (Figure 5C), making easily the recognition of the species. Also, the stylet and body length in *M. tuba* are variable; smaller body lengths usually have a straight and not slightly curved stylet, as observed in larger specimens (greater than 3 mm) (Young 1976; Ferguson 1939, 1940; Hyman 1936; Bois-Reymond Marcus 1957). These characteristics were observed in the specimens found in the Chillón River.

*M. tuba* was associated with the same vegetation and physicochemical parameters as *M. quiritium* (Table 2).

## Discussion

In Peru, microturbellarians have been scarcely studied. Only two species of the genus *Macrostomum* have been recorded for the eastern region of Peru, although this region has a high diversity of fauna and flora (Noreña *et al*. 2006). In addition, the coastal region has only one record of microturbellarians (i.e., *Yagua luteri* Bois-Reymond Marcus 1958).

The three species of *Macrostomum* studied use algae as a source of food and refuge. In the Chillón River, *M. quiritium* and *M. tuba* were observed mainly associated with the algae *Hydrodictyon* sp., *Enteromorpha* sp. and *Cladophora* sp., which are substrate for potential food items (rotifers, protozoa, unicellular algae, cladocerans and insect larvae) (Ferguson 1938). The Chillón River presents basic waters and high conductivity (Table 2), which are not ideal for microturbellarians (Noreña *et al*. 2004). However, most specimens of *Macrostomum* are widely distributed and tolerant to ecological changes (Noreña *et al*. 2006). In addition, *M. quiritium* and *M. tuba* can withstand temperature variations (14–35 °C) (Gamo 1987; Young 1976) and coexist in apparent harmony without large fluctuations in the population (Ferguson 1938). Thus, the Chillón River would represent an adequate habitat for their existence.

As mentioned above, *M. rostratum* has been recorded for Europe and South America in brackish and freshwater environments (Braccini & Leal-Zanchet 2013; Farías *et al*. 1995; Noreña *et al*. 2003, 2007). Therefore,

it is possible that the environmental characteristics of the Humedales de Ventanilla resemble those in which *M. rostratum* has been found. In Humedales de Ventanilla, *M. rostratum* was principally found associated with the green algae *Chara* sp. This affinity is probably due to the fact that the apical branches of the algae are a net which provides protection against fish and odonate larvae for rotifers, cladocerans, bacteria, protozoa and unicellular algae, which serve as food for *M. rostratum* since this microturbellarian was found principally in the apical region of *Chara* sp. (Figure 3C).

The species of *Macrostomum* reported here are widespread and probably cryptic species. This hypothesis has been shown for other groups of microturbellarians (*e.g.* Casu & Curini-Galleti 2006; Rundell & Leander 2014) Even though the diagnostic characters of the species are present, evidence indicates this assumption. The stylet of the Peruvian specimens of *M. rostratum* is a solid inverted cone and specimens from Italy have stylets with a slight spiral shape (Papi 1951). Also, the size of the stylets is slightly different from those of species from Europe and Africa (Young 1976). In *M. quiritium*, no morphological differences are present, probably because this is a Neotropical species which has been reported in artificial ponds of Europe containing aquatic vegetation from tropical regions of the world. However, the origin of the species remains unknown (Adami *et al*. 2016). The body length of specimens of *M. tuba* from Peru is in the species range, but small specimens have been reported and this fact affects the length of the stylet. The stylets are larger than those described by Young (1976) for specimens from Africa, but shorter than those reported by Kepner and Stiff (1932) (Hyman 1936) for specimens from North America.

In summary, more morphological and molecular analyses are needed to understand the possible cryptic *Macrostomum* species, especially in Peruvian populations. Furthermore, additional sampling campaigns will be necessary to discern the role they occupy in trophic chains and assemblages with other species of both turbellarians and other aquatic invertebrate fauna.

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