New records of free-living microturbellarians from the Chillón River, Peru

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Abstract

Microturbellarians are all mostly small free-living Platyhelminthes that do not belong to either the Polycladida or Tricladida order. This group includes species of the clades Catenulida and Rhabditophora. Species of microturbellarians are known to live in marine and continental waters such as rivers, where they are diverse and abundant. However, there are few records of microturbellarians in most of the rivers of the Pacific slope of Peru. Here, we report eight species of microturbellarians from the Chillón River, in the central region of Peru. Of the total species recorded, five (Gieysztoria cuspidata, G. bellis, Myostenostomum vanderlandi, Stenostomum tuberculatum and S. saliens) represent the first reports on the Pacific slope of the Neotropical region in Peru, thereby increasing the diversity of microturbellarians in this country.

Introduction

Catenulida and Rhabditophora are the clades of Platyhelminthes containing species of microturbellarians that are generally short in length [1,2]. The genital systems of some species of these hermaphroditic flatworms are important for species identification, with the male copulatory apparatus containing sclerotic stylets, spines and/or cirrus [3]. These organisms live in different environments such as marine, brackish and freshwater (rivers, lagoons and temporary pools) habitats [3–6]. They have also been found in moist environments (e.g. moss, vascular plants, wood snags, detritus, gravel, or sand) [7].

Most records of freshwater microturbellarians in the Neotropical region are from Brazil (the states of São Paulo, Rio Grande do Sul and Paraná) and Argentina (the provinces of Santa Fé, Buenos Aires and Entre Ríos) [2,8–26]. In Peru, there have been sporadic approaches to study freshwater microturbellarians. To date, 31 species belonging to Catenulida, Macrostromophora and Rhabdocoela have been reported living in the Amazon floodplain (Loreto and Ucayali regions), altitudinal lagoons (Junín and Puno regions), brackish ponds of different sizes and depths (Lima region), and a few rivers from the Pacific slope (Lima region) (Figure 1) [5,22,24,27–30]. In addition, microturbellarians in Peru are scarcely taken into account when assessing freshwater invertebrate fauna [5]. This lack of reports is due to the need to study these organisms alive whenever possible in order to identify diagnostic structures to help specific identification [3,31], and most importantly because of the lack of specialists in the field.

With the aim of increasing the knowledge of the microturbellarian fauna, we surveyed a hydrographic basin in the center of Peru, the Chillón River. Photographs and schematic representations of the species reported are shown to facilitate recognition in subsequent assessments in other hydrographic basins.

Material and methods

The study area was the Chillón River, between the provinces of Lima and Canta, in the central region of Peru. This river flows from east to west for about 126 km (11°22’–11°56’S; 76°26’W–77°08’W), originating in the Viudada mountain range (~4500 meters above sea level [m.a.s.l]) and flowing towards the Pacific Ocean [32] (Figure 1). Samples from the riverbanks at an altitude of ~500 m.a.s.l were taken almost monthly from February 2013 to November 2017. The sites sampled were characterized by muddy substrates and stones, with herbaceous vegetation and algae. Water temperature and pH of each locality were measured.

Microturbellarians were collected from aquatic herbaceous vegetation and algae using a net with a mesh size of 80 μm, and the samples were carried alive to the laboratory. All specimens were measured and described based on external and internal characteristics. Some microturbellarians were relaxed using MgCl2 7% and examined under light microscopy. When sclerotic structures (e.g. copulatory stylets) were observed, whole-mounted preparations were performed using polyvinyl-lactophenol. Stylets and soft body tissues were measured in micrometers (μm).
using the ImageJ software [33]. The material studied was deposited in the Departamento de Protozoología, Helminthología e Invertebrados Afines, Museo de Historia Natural (MUSM), Peru. Some specimens deteriorated during the observations were not deposited.

Abbreviations used in the figures
ab: anterior brain lobe; ap: adhesive papilla; cp: ciliated pit; e: eyes; eg: egg; ep: excretophores; fg: female gonopore; gd: girdle; ger: germovitellarium; i: intestine; m: mouth; mgu: muscular gut; np: nephridiopore; o: ovary;

Figure 1. Localities sampled where species of microturbellarians are found in Peru. A) Species included in this study, B) species from du Bois-Reymond Marcus [28], C) species from Reyes & Brusa [5], D) species from Reyes et al. [30], E) species from Noreña et al. [29, 34] and Damborenea et al. [22], F) species from Damborenea et al. [24], and G) species from Beauchamp [27].
**Results and remarks**

**Systematics**

Catenuilida Meixner, 1924

Stenostomidae Vej dovsky, 1880

Stenostomum grande Child, 1902 (*Figure 2*)

**Material studied**

Observations of live individuals, three of which were studied in squashed preparations.

**Locality sampled**

Chillón River (11°44′1.8″S; 76°58′19.56″W), near Trapiche in Canta Province, Lima region (2015/11/20) (temperature: 23.4 °C; pH: 8.2).

**Other localities in Peru**

Pacaya Samiría National Reserve in the Loreto region [34].

**Distribution**

United States (US) [35–37], Finland [38], Poland [39], Russia [40, and references therein], Suriname [41], Brazil [13,26], Peru [34] and Argentina [21].

**Description and remarks**

The color of the body is whitish, being darker in the intestinal region (*Figure 2A*). The body size of worms formed by two or three zooids ranges between 797 and 2404 μm long and 108–324 μm wide. The anterior and posterior brain lobes, light-refracting bodies, and pharyngeal glands, are characteristic of the species [21] (*Figure 2B–E*). The body length of specimens from Peru is within the species range (730–2000 μm) [12,13,21,26,36,37].

Stenostomum saliens Kepner & Carter, 1931 (*Figure 3A*)

**Material studied**

A single individual, studied in a squashed preparation.

**Locality sampled**

Chillón River (11°44′4.38″S; 76°58′20.52″W), near Trapiche in Canta Province in the Lima region (2015/10/31) (temperature: 22.3 °C; pH: 7.2).

**Distribution**

US [36,37], Poland [42], Suriname [41], Brazil [13,26] and Argentina [21].

**Description and remarks**

Live animal is yellow-whitish and fusiform. The body is 246.7 μm long and 45.7 μm wide; no zooids were observed. The anterior region has two small ciliated lateral pits, with six pairs of anterior brain lobes associated with these. Light-refracting bodies absent. The intestine has scarce excretophores with no particular arrangement, and the posterior region of the body is free of intestine. These characteristics are in accordance with the diagnosis of the species [36]. The single-zooid specimen from Peru is similar in length to those found in Argentina (150–620 μm) [21], but smaller than those in Poland (600–800 μm) [42], USA (620 μm) [36] and Brazil (400–600 μm) [13,26]. Interestingly, Kepner & Carter [36] did not mention the presence of excretophores in the intestinal region, although Larsson & Willems [43] reported that excretophores in *Stenostomum saliens* from Sweden are absent. Specimens from Peru present scarce excretophores, suggesting that these structures are a variable feature of the species. In addition, *S. saliens* has not previously been recorded in Peru, thereby expanding the distribution range of the species to the occidental coast of the Neotropical region.

Stenostomum tuberculatum Nuttycombe & Waters, 1938 (*Figure 3B*)

**Material studied**

Two individuals, studied in squashed preparations.

**Locality sampled**

Chillón River, Lima region (11°44′1.8″S; 76°58′19.56″W) (2013/04/18, 2013/10/26) (temperature: 23.6 °C; pH: 8.1).

**Distribution**

US [37], Suriname [41], Brazil [13,26], Argentina [21], Finland [44], Poland [42] and Germany [45].

**Description and remarks**

Single-zooid animals are whitish. The body is 475 μm long and 66 μm wide, is covered by long semi-rigid sensory cilia, which are more abundant at the anterior and posterior end of the body. The anterior tip of the body has a notorious tubercle. The intestine is lobulated, and the posterior body region is free of intestine. In comparison with single-zooid specimens found in other regions, animals
from Peru are larger than those recorded by Nuttycombe & Waters [37] (200 μm) and Marcus [13] (200–500 μm). However, specimens of four zooids are larger than Peruvian worms [13,37], and those of two zooids are similar in length [42,44] or longer [21]. The finding of S. tuberculosum, which represents the first report in Peru, expands its distribution to the occidental border of the Neotropical region.

**Myostenostomum Luther, 1960**

**Myostenostomum vanderlandi** Rogozin, 1992

(Figure 3C)

**Material studied**

Two individuals, one animal studied in squashed preparations.
Locality sampled
Chillón River, Lima region (11°44′1.8″S; 76°58′19.56″W) (2013/03/05) (temperature: 24.5 °C; pH: 8.4).

Distribution
Suriname [41], Russia [46] and Brazil [26].

Description and remarks
Single-zooid animals are whitish in color. The body is 1103 μm long and 182 μm wide. The anterior brain lobe has 7 paired metameric ganglia. The posterior brain lobe has a triangular shape. The blind-sac intestine has a muscular belt, 78.6 μm long and 155.2 μm wide at its anterior portion. Excretophores are randomly arranged along the intestine. These features agree with the specimens of M. vanderlandi from Suriname [41], Brazil [26] and Russia [46]. In addition, the body length of the individuals from Peru is within the range of the species (500–1300 μm) [26,41,46]. This is the first record of M. vanderlandi in Peru.

Rhabdocoela Ehrenberg, 1831

Dalytyphloplanida Willems, Wallberg, Jondelius, Littlewood, Backeljau, Schockaert & Artois, 2006

Dalyelliidae Graff, 1905

Gieysztoria Ruebush & Hayes, 1939

Gieysztoria cuspidata (Schmidt, 1861) Ruebush & Hayes, 1939 (Figure 4)

Material studied
Nine individuals observed alive, five of which were studied as whole-mounted specimens (MUSM-INV 4790–4794).
Locality sampled
Chillón River (11°43′30.9″S, 76°57′54.1″W; 11°44′1.8″S, 76°58′19.56″W; 11°43′53.64″S, 76°58′16.38″W) in temporary ponds, near the river, in Trapiche, Canta Province, Lima region (2015/6/8; 2015/7/23) (temperature: 24.1–25 °C; pH: 8.4–7.9).

Distribution
Broadly distributed in the Palearctic, Afrotropical and Nearctic regions ([47], and references therein).

Description and remarks
Live mature animals are 900–1600 μm long. The body has dark-brownish anastomosed dots (Figures 4A–B) not mentioned in specimens studied in other regions [48,49]. In the male reproductive system, the globular seminal vesicle shows the same size as the prostatic vesicle. The penis stylet has five claw-like spines similar in length, with digitiform projections at the proximal region, without a real girdle. Each spine has a skirt-like projection for muscular insertion (Figures 4C–D). The tips of the spines are slightly curved outward. Spines are 20.5–32.1 μm long (48.5–56.7 μm long including the proximal digitiform projections). The egg is 252.7–164.8 μm long and 108.1–153 μm wide (Figure 4A–B). Two tubular brown-greenish vitellaria branches extend from the ventral posterior zone to the anterior dorsal zone, reaching the pharynx level (Figure 4A). This is the first record of Gieysztoria cuspidata in the Neotropical region.

Gieysztoria bellis (Marcus, 1946) Luther, 1955
(Figure 5)

Material studied
Five individuals observed alive, studied in whole-mounted preparations (MUSM-INV 4795–4799).

Locality sampled
Chillón River (11°44′1.8″S, 76°58′19.56″W; 11°44′4.38″S, 76°58′20.52″W; 11°43′57″S, 76°58′17.58″W) in temporary ponds, near Trapiche, Canta Province, Lima region (2015/10/31) (temperature: 25.4 °C; pH: 9.16).

Distribution
Tietê River, city of São Paulo, Brazil [14].

Description and remarks
Live mature animals are 300–700 μm long and 67–162.2 μm wide. Reddish-brown pigmentation present throughout the body (Figure 5A). In the male reproductive system, the thick-walled seminal vesicle is 28.5–36.1 μm long and 31.2–37.1 μm wide and is completely filled with spermatozoa (Figure 5C). The seminal vesicle is partly separated from the prostatic vesicle by a septum (Figure 5C). The “U”-shaped prostatic vesicle is 20.6–21.8 μm long and 26.7–27.3 μm wide, with granular prostatic glands (Figure 5C). The 37–45.1 μm-long crown-like penis stylet has a smooth girdle, 2.7–8 μm high. In some styles, the girdle has two weak distal rings (Figures 5B, E). The girdle has 20–21
fang-like spines of similar size and shape. Spines are 31.2–38.9 µm long and 2.5–3.7 µm wide, directed slightly outwards (Figure 5E, F). The egg is 60.9 µm long and 37.3 µm wide. A pair of dark green vitellaria with finger-like projections run from the posterior end of the pharynx to the end of the intestine (Figure 5A).

The body length and the number of spines of the penis stylet of individuals from Peru are within the species range, with some differences regarding specimens from Brazil. Peruvian individuals have a shorter and smooth non-fenestrated girdle, not reported by Marcus [14]. In addition, Marcus [14] described ~12 bridges between the girdle and the 20–21 spines on the stylet, and the spines were not projections of the bridges. In this sense, individuals from Peru have no bridges between the girdle and the 20–21 spines, but two weak rings were present in some individuals. Also, individuals from Peru have larger fang-like spines, while Marcus [14] reported shorter and slender spines with cone-like distal tips. The girdle, could be a variable structure within species of Gieysztoria since observations in other species suggest a remarkable variation in the height of the girdle during maturity [50]. Weak distal rings observed in Peruvian individuals could suggest that the girdle presents intraspecific differences. Morphological variations of the penis stylet within species populations are usual within Gieysztoria [47,49], but the number and size of spines are usually stable [50]. This is the first record of G. bellis in Peru and the second for the Neotropical region.
Prorhynchida Laumer and Giribet, 2014

Prorhynchidae Hallez, 1894

Prorhynchus Schultze, 1851

Prorhynchus stagnalis Schultze, 1851 (Figure 6A-B)

**Material studied**
A single individual observed alive, studied on a whole-mounted preparation.

**Locality sampled**
Chillón River (11°43'30.9"S, 76°57'54.1"W) in temporary pond, near the river in Trapiche, Canta Province, Lima region (2015/7/23) (temperature: 26 °C; pH: 8.3).

**Other localities in Peru**
Loreto Region [34].

**Distribution**
 Widely distributed [51].

**Description and remarks**
Live mature individual ~3200 µm long and slender (Figure 6A). The hollow spine of the male reproductive system is ~77.2 µm long, and concentric bars are ~33 µm long. The female reproductive system is at the middle of the body and is composed of a germovitellarium. The

![Figure 6. Prorhynchus stagnalis Schultze, 1851. A, Dorsal view of the habitus; B, Detail of the anterior end of the body in sequence of movements (no scale bar available). Geocentrophora applanata (Kennel, 1888). C, Dorsal view of the habitus, sequence of movement; D, Detail of the anterior region of the body; E, Schematic representation of the habitus.](image-url)
hollow spine of the penis stylet and the concentric bars of the individual studied from Peru are within the species range (50–123 μm and ~51 μm, respectively) [6,11,26,52,53]. These findings extend the distribution of this species to the central coast of Peru.

**Geocentrophora de Man, 1876**

**Geocentrophora applanata** (Kennel, 1888) *(Figure 6C–E)*

**Material studied**
A single individual observed alive, studied on a whole-mounted preparation.

**Locality sampled**
Chillón River (11°43’30.9”S, 76°57’54.1”W) in temporary pond, near the river in Trapiche, Cantala Province, Lima region (2015/7/23) (temperature: 22.1 °C; pH: 8.2).

**Other localities in Peru**
Loreto Region [34].

**Distribution**
Widely distributed [51].

**Description and remarks**
Live mature, whitish-colored individual, ~2230 μm long and flattened. The anterior end is flabelliform, while the posterior end is rounded. Two brownish eyes at both sides of the pharynx. Features observed in the single individual from Peru agree with descriptions of *Geocentrophora applanata* [6,11,31,53]. This is the first record in Lima, on the central coast of Peru.

**Discussion**
Peru presents great invertebrate diversity [54–57], but microscopic aquatic invertebrates such as microturbellarians are not usually considered in assessments and inventories of biodiversity [5,30]. In the last two decades, sporadic investigations on microturbellarians were mainly performed in the rainforest region of this country [5,22,24,29,30,34]. These investigations demonstrated the potential high diversity of microturbellarians with the description of six new species [22,24,29]. However, a vast territory in which these organisms are very likely to occur remains unexplored. In this sense, the central coastal region of Peru has rivers, such as the Chillón River, which has the following features: it is a few km long, with altitudinal gradient and with notorious seasonal water level fluctuations [32]. These features create heterogeneous environments in which microturbellarians can be expected to inhabit. Nevertheless, there are only three records of microturbellarians (*Macrostomum quitinium* Beklemishev, 1951; *M. tuba* Graff, 1882 and *Yagua luteri* du Bois-Reymond Marcus, 1958) living in these rivers [5,28].

*Stenostomum* is a rich and widely distributed genus [3,24], but species identification can be difficult to discern. Diagnostic features need to be examined in live individuals [43]. The species of *Stenostomum* reported here show most of the diagnostic features mentioned in their original descriptions and posterior reviews [14,21,36,37]. Individuals of *Myostenostomum* from Peru present a muscular belt, in addition to features on the anterior and posterior brain lobes that correspond to descriptions for *M. vanderlandi* [26,46,58].

The species of *Gieysztoria* from Peru are only represented by the *Inaequales* group, that is, species with spines of different shapes and stylet sizes (*G. complicata* (Fuhrmann, 1914), *G. chiqchi* Damborenea, Brusa & Noreña, 2005, *G. kasasapa* Damborenea, Brusa & Noreña, 2005, *G. sasa* Damborenea, Brusa & Noreña, 2005) [22]. Thus, the two new records (*G. cuspidata* and *G. bellis*) represent the first record of an *Aequales* group species (specimens with spines of similar shape and stylet sizes) in Peru. In addition, evidence suggests that the morphological complexity of the penis stylet in the species of *Gieysztoria* is greater in South America [17,20,22,59]. However, consolidated information on species diversity, distribution and dispersal patterns makes it difficult to observe clearer traits in the penis stylet configuration among biogeographical regions [60]. The finding of *G. cuspidata* in the Neotropical region provides greater support to the hypothesis that certain groups of species (e.g. *cuspidata-isoldeae*) could represent an ancient lineage, with *G. cuspidata* already well established in the Mesozoic [60].

The species of *Prorhynchidae* found in Peru are well-known and are found worldwide [6,11,34,51–53]. Unfortunately, few individuals of *P. stagnalis* were collected. These animals are known to be fragile when studied alive and are also sensitive to temperature variations [61] thereby preventing further information gathering.

Microturbellarians play important roles in continental aquatic environments. For instance, experiments have demonstrated that species of *Stenostomum* prey on cladocerans and rotifers and can affect population densities and structured microinvertebrate communities [62,63]. Similarly, species of *Gieysztoria* prey on microinvertebrates as well as individuals of the same species [64]. Therefore, understanding the population dynamics of microturbellarian species in freshwater ecosystems is necessary to obtain better understanding of the trophic chains in Peruvian microinvertebrates.

In conclusion, the diversity of microturbellarians in Peru is far from being properly cataloged, with only 31 species having been recorded to date. More data is
needed to provide a better panorama of the freshwater invertebrate communities and to obtain clearer evidence to assess species richness in continental waters, such as those in rivers of the Pacific slope in Peru. The results of the present study increase the number of microturbellarian species in Peru to 36 species, five of which were recorded for the first time. Likewise, one species (G. cuspidata) is recorded for the first time in the Neotropical region, providing evidence to establish a biogeographical hypothesis of distribution from geological eras.

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