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Gieysztoria reta n. sp. CLSM images of whole-mounts labeled with rhodamine-phalloidin showing the muscle arrangement.

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A new species of *Gieysztoria* Ruebush & Hayes, 1939 (Platyhelminthes, Rhabdocoela, Dalyelliidae) from Argentina with comments on geographical distribution of the genus in the Neotropical region

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Adami M. L. & Damborenea C. 2024. — A new species of *Gieysztoria* Ruebush & Hayes, 1939 (Platyhelminthes, Rhabdocoela, Dalyelliidae) from Argentina with comments on geographical distribution of the genus in the Neotropical region. *Zoosystema* 46 (28): 723-732. https://doi.org/10.5252/zoosystema2024v46a28. http://zoosystema.com/46/28

ABSTRACT
A new *Gieysztoria* Ruebush & Hayes, 1939 species from limnic environments in Argentina is described. The arrangement of its genital muscle system is described using rhodamine-labeled phalloidin and confocal laser scanning microscopy (CLSM), a novel methodological approach for this genus. *Gieysztoria reta* n. sp. differs from its congeners in terms of stylet shape and length. The complex stylet is 50-75 µm long, with a fenestrate fibrous girdle. Distally it has four sets of spines: the largest, robust, ventral fang-shaped spine slightly curved toward the center and proximately grooved; a shorter ventral fang-shaped spine; a dorsal group of seven to eight fine spines arranged like bristles; and a group of 12 to 13 dorsal hollow, broad-based spines of different lengths. These are arranged in three rows, forming a fan shape. The major advantage of using rhodamine-labeled phalloidin is that we have been able to visualize the 3D structure of the genital system and the relationship between different sets of muscle fibers, both in the female and male genital organs. Finally, the Neotropical biogeographic distribution of the genus is provided and its relation to stylet morphology is discussed.

KEY WORDS
Microturbellarian, freshwater flatworm, phalloidin, Buenos Aires, confocal laser scanning microscopy, new species.

RÉSUMÉ
Une nouvelle espèce de Gieysztoria *Ruebush & Hayes, 1939 (Platyhelminthes, Rhabdocoela, Dalyelliidae) d'Argentine, avec une discussion sur la répartition du genre en région néotropicale.*
Une nouvelle espèce de *Gieysztoria* Ruebush & Hayes, 1939 provenant des environnements limniques en Argentine est décrite. La disposition de son système musculaire génital est décrite en utilisant de la phalloïdine marquée à la rhodamine et la microscopie confocale à balayage laser (CLSM), une approche méthodologique nouvelle pour l'étude de ce genre. *Gieysztoria reta* n. sp. diffère de ses congénères dans sa morphologie et la longueur du stylet. Le complexe stylet a une longueur de 50 à 75 µm, avec une gaine fibreuse fenestrée. Quatre groupes d'épines sont situés distalement : l'épine la plus longue, robuste, ventrale et en forme de croc légèrement incurvée vers le centre, cannelée dans la partie proximale ; une épine plus courte, ventrale et en forme de croc ; un groupe dorsal de sept à huit épines fines, disposées comme des soies ; et un groupe de douze à treize épines creuses, larges à la base et de différentes longueurs. Celles-ci sont disposées en trois lignes suspendues en forme d'éventail. Le principal avantage de l'utilisation du phalloïdine marquée à la rhodamine est d'avoir pu visualiser la structure tridimensionnelle du système génital et la relation entre les différents ensembles de fibres musculaires, aussi bien dans les dans les organes génitaux femelles que mâles. La distribution biogéographique du genre dans la région néotropicale est donnée et sa relation avec la morphologie du stylet est discutée.

MOTS CLÉS
Microturbellaire, ver plat d'eau douce, phalloïdine, Buenos Aires, microscopie confocale à balayage laser, espèce nouvelle.

INTRODUCTION

Dalyelliidae Graff, 1908 is one of the main families of free-living and symbiotic freshwater flatworms. Within this family, *Gieysztoria* Ruebush & Hayes, 1939 is the most species-rich genus, with almost 100 free-living species distributed worldwide (Tyler *et al.* 2006-2024). About 37 species are found in the Palearctic region (Van Steenkiste *et al.* 2011, 2012) and 33 in the Neotropical region (Marcus 1946; Noreña-Janssen 1995; Therriault & Kolasa 1999; Noreña *et al.* 2003; Brusa *et al.* 2003, 2008; Damborenea *et al.* 2005, 2007; Reyes *et al.* 2021, 2022), with fewer occurrences in other regions of the world: at least nine *Gieysztoria* species were reported in the Nearctic region (see table in Van Steenkiste *et al.* 2011). Both in the Afrotropical region (Young 1977; Artois *et al.* 2004) and in the Oriental region ten species have been reported (Wang & Wu 2005a, b; Wang & Deng 2006; Van Steenkiste *et al.* 2012; Lai *et al.* 2013; Lu *et al.* 2013; Xia *et al.* 2014; Zhang *et al.* 2014), while three species have been found in the Australian region (Young 1977; Jondelius 1997). Within the Neotropical region, ten *Gieysztoria* species have been described in Argentina (*G. atalaya* Brusa, Damborenea & Noreña, 2008, *G. coronae* Noreña-Janssen 1995, *G. falx* Brusa, Damborenea & Noreña, 2003, *G. matilde* Brusa, Damborenea & Noreña, 2008, *G. namuncurai* Damborenea, Brusa & Noreña, 2007, *G. pseudodiadema* Noreña-Janssen 1995, *G. quadrata* Noreña-Janssen 1995, *G. santafensis* Noreña-Janssen 1995, *G. tigrensis* Noreña-Janssen 1995, and *G. variata* Noreña-Janssen 1995). The Brazilian congeners *G. evelinae* (Marcus 1946) and *G. hymanae* (Marcus 1946) have also been reported in Argentina (Noreña-Janssen 1995; Brusa *et al.* 2003, 2008; Damborenea *et al.* 2007).

The stylet is the most important structure for identification of *Gieysztoria* species. A typical stylet has a proximal fibrous girdle (with one or more openings) and distal spines (with different sizes, shapes and arrangements). Based mainly on stylet shape and arrangement, *Gieysztoria* is divided into two groups: Aequales and Inaequales (Luther 1955). These groups of species have been found to predominate in different zoogeographic regions of the world, while few species seem to have worldwide distribution (Brusa *et al.* 2003; Van Steenkiste *et al.* 2012). However, the phylogenetic significance of these morphologies has not yet been resolved.

This contribution describes a new species of the genus *Gieysztoria* from Buenos Aires, Argentina (Neotropical region), including a description of the musculature associated with the sexual organs and body-muscle arrangement, using phalloidin as marker.

MATERIAL AND METHODS

Sampling and morphological study

Specimens were collected on February 2, 2019, from a lentic pond to a depth of 50 cm, using a 125 µm plankton net (Fig. 1A, B). The net was dragged several times through the floating sections and roots of the predominant aquatic vegetation, *Schoenoplectus americanus* (Pers.) Volkart ex Schinz & R. Keller. The sample was transported to the laboratory and observed alive under a dissecting microscope. *Gieysztoria* specimens were separated and photographed *in vivo* in whole squash mounts under a compound microscope. Whole mounts in polyvinyl-lactophenol were prepared to study stylet morphology.

In this study we use a rhodamine-phalloidin histological method to visualize the three-dimensional muscle structure of the organs, following Adami *et al.* (2012, 2017, 2018). This technique provides more information about 3D arrangement than other classic methods and allows a more precise morphological reconstruction.

To analyze muscle arrangement, specimens were fixed in 4% formaldehyde-phosphate buffered saline (PBS) for 12h, washed in 0.05% PBS-Tween (PBS-T) and permeabilized in 1% Triton X-100 for 24h at 4°C. They were incubated overnight at 4°C in rhodamine-phalloidin solution (1/1000 Sigma-Aldrich; Adami *et al.* 2012), washed in 0.05% PBS-Tween (PBS-T) three times and finally, mounted in Vectashield mounting medium. The resulting material was observed with a Leica SP5 confocal laser scanning microscope, using an excitation wavelength at 543 nm and emission at 560-600 nm. The step size of the image stack was 3 µm. The images were analyzed using Leica LASX Image Examiner software. Serial images obtained with confocal laser scanning microscope of the specimens stained with phalloidin were deposited in a public repository of Universidad Nacional de La Plata (Supplementary material 1, https://doi.org/10.35537/10915/149823).

Voucher specimens of the examined material were deposited in the Invertebrate Collection of Museo de La Plata, Argentina (MLP-He).

Abbreviations

Institution

MLP-He	Invertebrate Collection of Museo de La Plata, La Plata.

Morphological terms used in the text and/or figures

ag	accessory glands;
b1	copulatory bursa cavity 1;
b2	copulatory bursa cavity 2;
c	cilia;
cb	copulatory bursa;
cga	common genital atrium;
cm	circular muscle fibers of the body wall;
cma	circular muscle fibers of common genital atrium;
cmb1	circular muscles of the bursa cavity 1;
cmb2	circular muscles of bursa cavity 2;
dm	diagonal muscle fibers of the body wall;
e	egg;
ed	ejaculatory duct;
ep	ciliated epidermis;
ey	eye;
f	fenestra;
i	intestine;
ic	insertion cavity of s1 spine;
lma	longitudinal muscles fibers of common genital atrium;
lmb2	longitudinal muscle of bursa cavity 2;
ma	male genital atrium;
ov	ovary;
ph	pharynx;
pp	prepharyngeal cavity;

pv	prostate vesicle;
s	septum;
scm	muscle fibers of gonopore sphincter;
sfb1	strong muscle fiber of bursa cavity 1;
st	stylet;
sv	seminal vesicle;
svm	muscular wall of seminal vesicle;
s1	the largest spine;
s2	large spine;
s3	group of fine short spines;
s4	group of shorter hollow spines;
t	testes;
ub	uterus bend;
vd	vasa deferentia;
vi	vitellaria.

SYSTEMATIC

Order RHABDOCOELA Graff, 1904
Family DALYELLIIDAE Graff, 1908

Genus *Gieysztoria* Ruebush & Hayes, 1939

TYPE SPECIES. — *Gieysztoria expedita* (Hofsten, 1907) Ruebush & Hayes, 1939, by original designation.

Gieysztoria reta n. sp.
(Figs 1-4)

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TYPE LOCALITY. — Argentina, Buenos Aires Province, Reta.

TYPE MATERIAL. — **Holotype**. **Argentina** • Reta, Buenos Aires Province, natural wetland close to a dune field; 38°54'06"S, 60°21'4"W; 16 m a.s.l.; 2.II.2019; Adami M. L. leg.; 125 µm plankton net, whole mount in polyvinyl-lactophenol; MLP-He, 8020. **Paratypes**. **Argentina** • 8 whole mounts in polyvinyl-lactophenol; same data as for holotype; Adami M. L. leg.; MLP-He, 8021.

OTHER MATERIAL. — **Argentina** • 2 specimens preserved in 100% ethanol; same data as for holotype; MLP-He, 8022.

ETYMOLOGY. — Name refers to the geographic area where the species lives.

DIAGNOSIS. — A small, complex stylet, 50-75 µm long, with a proximal fenestrate fibrous girdle and distally with four sets of spines: 1) the largest, robust, ventral fang-shaped spine slightly curved toward the center and proximately grooved; 2) a shorter ventral fang-shaped spine; 3) a dorsal group of seven to eight fine spines arranged as bristles; and 4) a group of 12 to 13 dorsal hollow, broad-based spines of different lengths, arranged in three rows, forming a fan shape. Copulatory bursa with two cavities separated by incomplete septum. Uterus with a conspicuous bend.

DESCRIPTION

Living adult specimens up to 500 µm long and 260 µm wide (Fig. 2A); mature fixed adult specimens approximately 336 µm to 600 µm (n = 11) long, and 175 µm to 234 µm (n = 11) wide (Fig. 2B). Body oval, rounded at the anterior end and tapering towards the posterior end. Overall colour light brown with two pigmented black eyes (ey). Pharynx doliformis (ph): 135 µm to 150 µm long and 112 µm to 150 µm wide (n = 10), with prepharyngeal cavity (pp) (Fig. 2A, B).

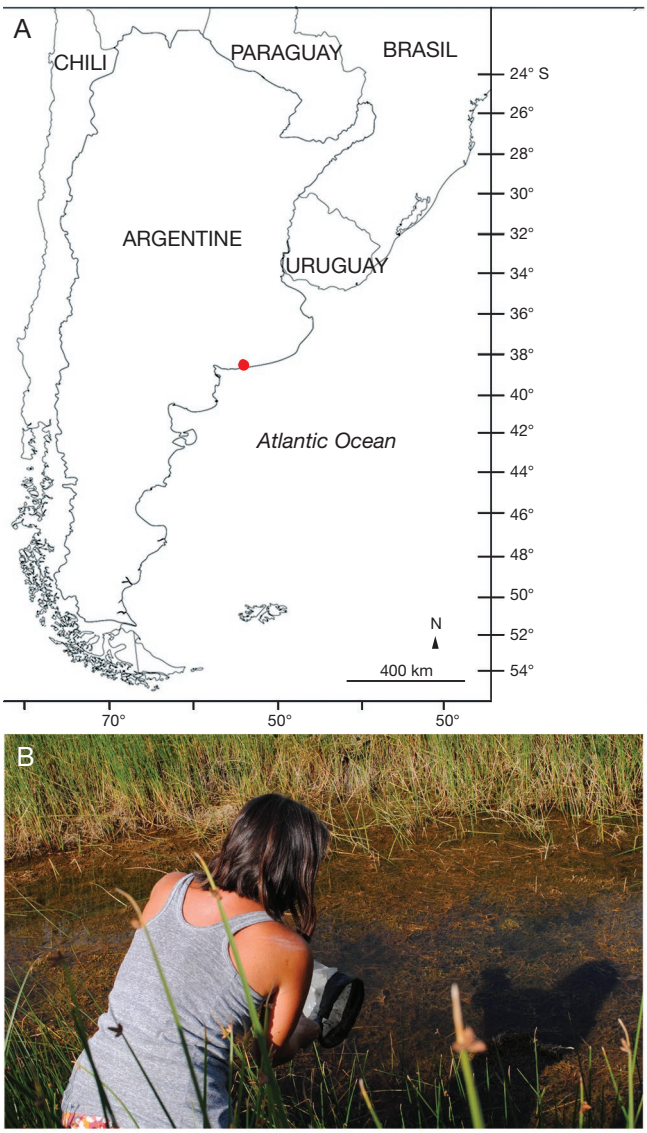

FIG. 1. — Study area: **A**, geographical location (● Reta locality); **B**, sampling site.

Body wall musculature with an outer layer of circular muscles fibers (cm) and diagonal muscles fibers (dm). Inner longitudinal muscle fibers not observed in the stacks. Cellular epidermis about 5 µm, with cilia (c) of approximately 6 µm (Fig. 3A, B).

Male reproductive system, testes (t) ventro-lateral, paired, 75 µm long in caudal half of the body. Testes and copulatory organ connected by vasa deferentia (vd) (Figs 3C; 4A). Copulatory organ on the middle axis in three individuals, and on the right side of the body in other specimens. Seminal vesicle (sv) oval, 57 µm long and 36 µm wide, with muscular wall (svm), posteriorly connected with prostatic vesicle (pv) (Figs 2B, C; 3C, D). Prostatic vesicle 29 µm long and 18 µm wide, with at least four accessory glands (ag) arranged as elongated sacs reaching the stylet girdle (Figs 2C; 3D). Prostatic vesicle anterior to ejaculatory duct (ed) which runs inside the sclerotic stylet (Fig. 3F).

TABLE 1. — Main stylet features of *Gieysztoria reta* n. sp. and others similar species.

Species of *Gieysztoria*	Length (µm)	Number of single spines	Number of groups of spines	Fenestra in the girdle	References
G. reta n. sp.	50-75	2	1	yes	This study
G. hermes Reyes & Brusa, 2021	34.2	3	2	yes	Reyes *et al.* 2021
G. matilde Brusa, Damborenea & Noreña, 2008	54-65	1	2	yes	Brusa *et al.* 2008
G. namuncurai Damborenea, Brusa & Noreña, 2007	100	2	3	no	Damborenea *et al.* 2007
G. kasasapa Damborenea, Brusa & Noreña, 2005	75-85	1	2	no	Damborenea *et al.* 2005
G. therapaina (Marcus, 1946)	33	1	3	yes	Marcus 1946
G. complicata (Fuhrmann, 1914)	100	2	2	no	Marcus 1946
G. intricata (Marcus, 1946)	120	1	3	yes	Marcus 1946

Stylet (st) about 50-75 µm (n = 11) long, and 40-69 µm (n = 11) wide. Proximal portion: fibrous girdle 40-60 µm (n = 4) wide and 17-21 µm (n = 4) long, with dorsal discontinuity about 18 µm wide, and circular fenestra (f) traversed by few fibers (Fig. 2D). Distal portion with four different sets of spines of variable shape and size. Two large, single ventral spines (s1 and s2). S1, on the ventral side of stylet, the largest, robust fang-shaped spine, 27-38 µm (n = 5) long, slightly curved toward male genital atrium. Proximally fibrous and grooved, and distally inserted in a cavity (ic) connected with male atrium (Figs 2D; 3E; 4A, B). S2 shorter than s1, also ventral, 20 µm long, fang-shaped, similar to s1 (Figs 2D; 4B). Opposite to s1 and s2, there is a dorsal group of seven or eight fine spines arranged like the bristles of a paintbrush (s3) (Figs 2D; 4B). Also dorsal, but on the opposite side, group of 12 or 13 hollow spines with broad bases arranged in three rows, forming a fan shape (s4) with variable length: shorter at both ends, and longer in the center of the fan, 13 and 26 µm long, respectively (Figs 2D; 3C, D; 4B). Distal part of stylet anterior to male genital atrium (ma), which enters the common genital atrium (cga) (Figs 3C-E; 4A).

Female reproductive system, ovary single (ov), at least 60 µm long, posterior and dorsal to the intestine, located on the left side (Fig. 3B, C). Vitellaria (vi) extended dorsally in the center region of the body (Figs 2A, B; 3B). Vitelloduct, female duct and seminal receptacle not observed. Single yellowish shelled egg (e) about 160 µm long (n = 8) carried in uterus (Figs 2A, B; 3B, C). Uterine bend (ub) caudal to uterus. Large, globular, conspicuous, 50 µm long and 30 µm wide, associated by means of muscle fibers with copulatory bursa (cb) (Fig. 3B), opening into common genital atrium (cga) (Fig. 3D, E). Common genital atrium surrounded by circular muscle fibers (cma) and to a lesser extent by longitudinal muscle fibers (lma) continuous with muscle fibers of male genital atrium (Figs 3C, D; 4A).

Copulatory bursa 57 µm long and 34 µm wide (n = 2), dorsal to common genital atrium, opening into it through a ductus. Bursa with two cavities (b1 and b2) separated by an incomplete septum (s). Content observed inside both cavities (Figs 2B; 3C, D). Proximal cavity (b1) with muscular wall with strong circular fibers (cmb1) (Figs 3B; 4A). Single thick muscular fiber extending from b1 wall (sfb1) to anterior end (Figs 3C; 4A). Distal cavity (b2) with strong outer circular muscle fibers (cmb2) and fine inner longitudinal muscle fibers (lmb2) connecting with ductus communis (Figs 3C, D; 4A).

Common gonopore (gp) ventral, with four circular muscle fibers forming a sphincter (scm) (Figs 3E, F; 4A).

REMARKS

Regarding stylet morphology, *Gieysztoria reta* n. sp. has spines of different shapes and sizes and a circular opening (fenestra) in the girdle, so it belongs to the Inaqueales Group, subgroup Fenestratae (Luther 1955).

Considering stylet morphology, *G. reta* n. sp. resembles its Brazilian congeners *G. hermes* Reyes & Brusa, 2021, *G. therapaina* Marcus, 1946, *G. complicata* (Fuhrmann, 1914), and *G. intricata* Marcus, 1946; and Argentinian congener *G. matilde* Brusa, Damborenea & Noreña, 2008, *G. namuncurai* Damborenea, Brusa & Noreña, 2007, and Peruvian congener *G. kasasapa* Damborenea, Brusa & Noreña, 2005 (Table 1). The most similar stylet to that of *G. reta* n. sp. is that of *G. hermes.* However, the stylet is larger in *Giesystoria reta* n. sp. than in *G. hermes* (50-75 µm and 34.2 µm, respectively). *Gieystoria reta* n. sp. has four sets of spines (two single strong ventral spines, and two groups of dorsal spines), while *G. hermes* has three single ventral spines (the new species has one less ventral spine) (Reyes *et al.* 2021: fig. 12C, blue). In *G. reta* n. sp., the two ventral spines seem not to be associated with the dorsal groups of spines, although Reyes *et al.* (2021) reported that in *G. hermes*, the two lateral ventral spines are associated with the dorsal groups of spines. Spine s1 in the new species is larger than the largest fang-shaped spine in *G. hermes* (Reyes *et al.* 2021: fig. 12C, red).

Like *G. hermes*, *G. reta* n. sp. has two groups of dorsal spines: a group of fine spines on one side, and a group of thicker hollow spines arranged in three rows on the other side. However, in *G. reta* n. sp., these three rows of spines are arranged forming a fan shape, while in *G. hermes*, this arrangement is not observed, and the number of rows of spines is not mentioned, though more than three rows are shown in the drawing of the holotype (Reyes *et al.* 2021: fig. 12C). In *G. hermes*, the dorsal group of fine spines is arranged forming a comb-like structure in the stylet, whereas *G. reta* n. sp. has a single row of spines. Regarding the copulatory bursa, *G. hermes* has a single cavity with a thick wall, while *G. reta* n. sp. has two cavities with circular and longitudinal muscle fibers separated by an incomplete septum.

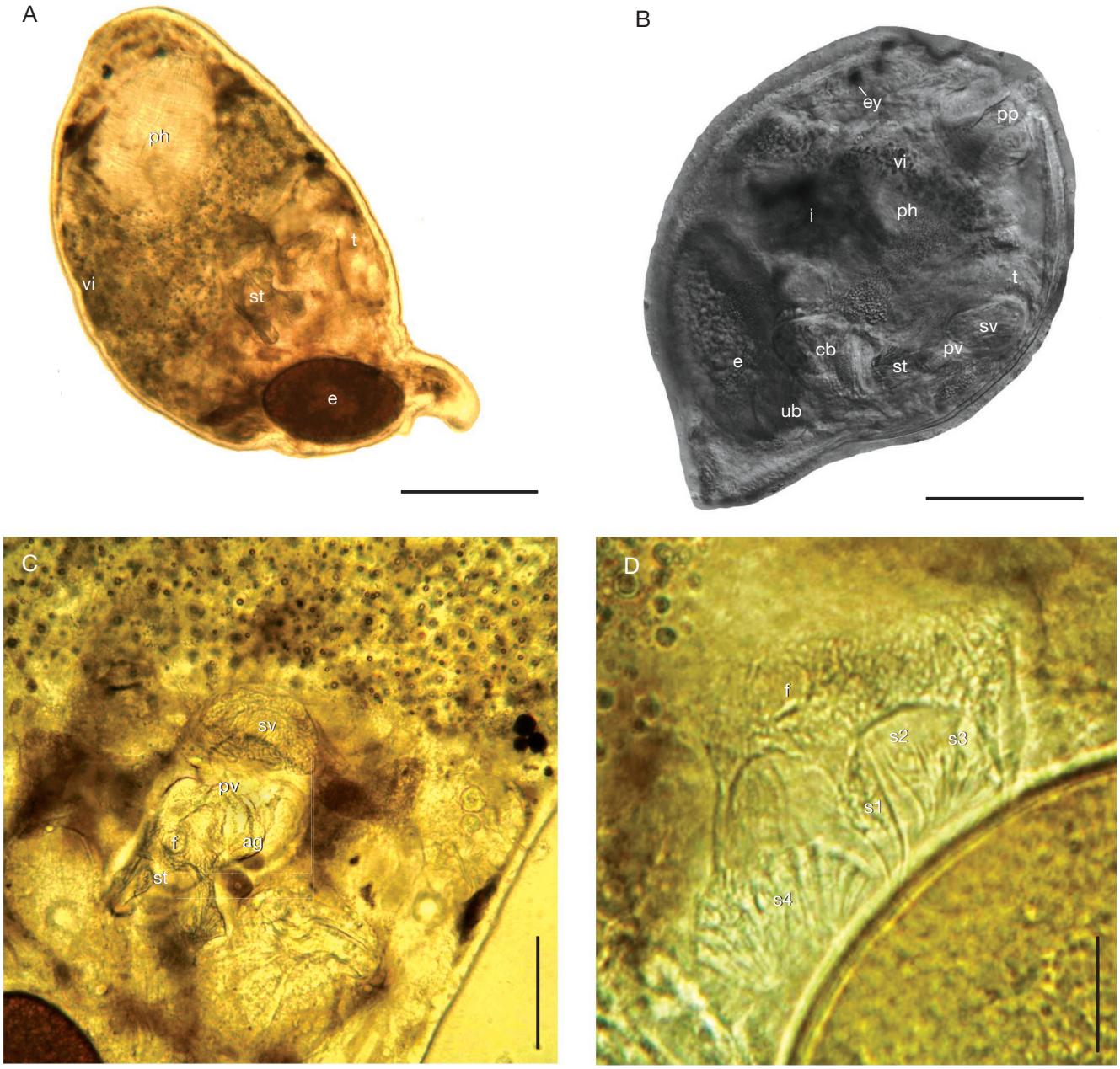

Fig. 2. — *Gieysztoria reta* n. sp.: **A**, **C**, **D**, specimens viewed under compound microscope: **A**, living specimen whole mount; **B**, fixed specimen viewed under a phase contrast light microscope; **C**, detail of seminal vesicle, prostate vesicle and additional glands; **D**, detail of stylet. Abbreviations: **ag**, accessory glands; **cb**, copulatory bursa; **e**, egg; **ey**, eye; **f**, fenestra; **i**, intestine; **ph**, pharynx; **pp**, prepharyngeal cavity; **pv**, prostate vesicle; **st**, stylet; **sv**, seminal vesicle; **s1**, largest spine; **s2**, large spine; **s3**, group of fine short spines; **s4**, group of shorter hollow spines; **t**, testes; **ub**, uterus bend; **vi**, vitellaria. Scale bars: A, B, 100 µm; C, 50 µm; D, 20 µm.

G. matilde and *G. reta* n. sp. have similar stylet lengths (54-65 µm and 50-75 µm, respectively), but *G. reta* n. sp. has two ventral large spines, while *G. matilde* has a single large hollow blade-like spine. Like *G. reta* n. sp., *G. matilde* has two major groups of thin spines, one group with thicker spines than the other, though the original description does not specify their sizes. *Gieystoria namuncurai* and the new species share several stylet traits, but clear differences distinguish *G. namumcurai* from *G. reta* n. sp.: the lack of a circular opening (fenestra) in the girdle, a total stylet length of 100 µm, and the presence of several rows of spines arranged in two groups, while in the new species there is only one group. *Gieystoria kasasapa* also shows differences from *G. reta* n. sp.: the stylet is larger in *G. kasasapa* than in *G. reta* n. sp. (75-85 µm and 50-75 µm, respectively). The Peruvian species has two groups of spines, both with less spines than the new species, and a single large hollow spine. Another species similar to *G. reta* n. sp. is *G. therapaina*, which has a stylet 33 µm long, almost half the

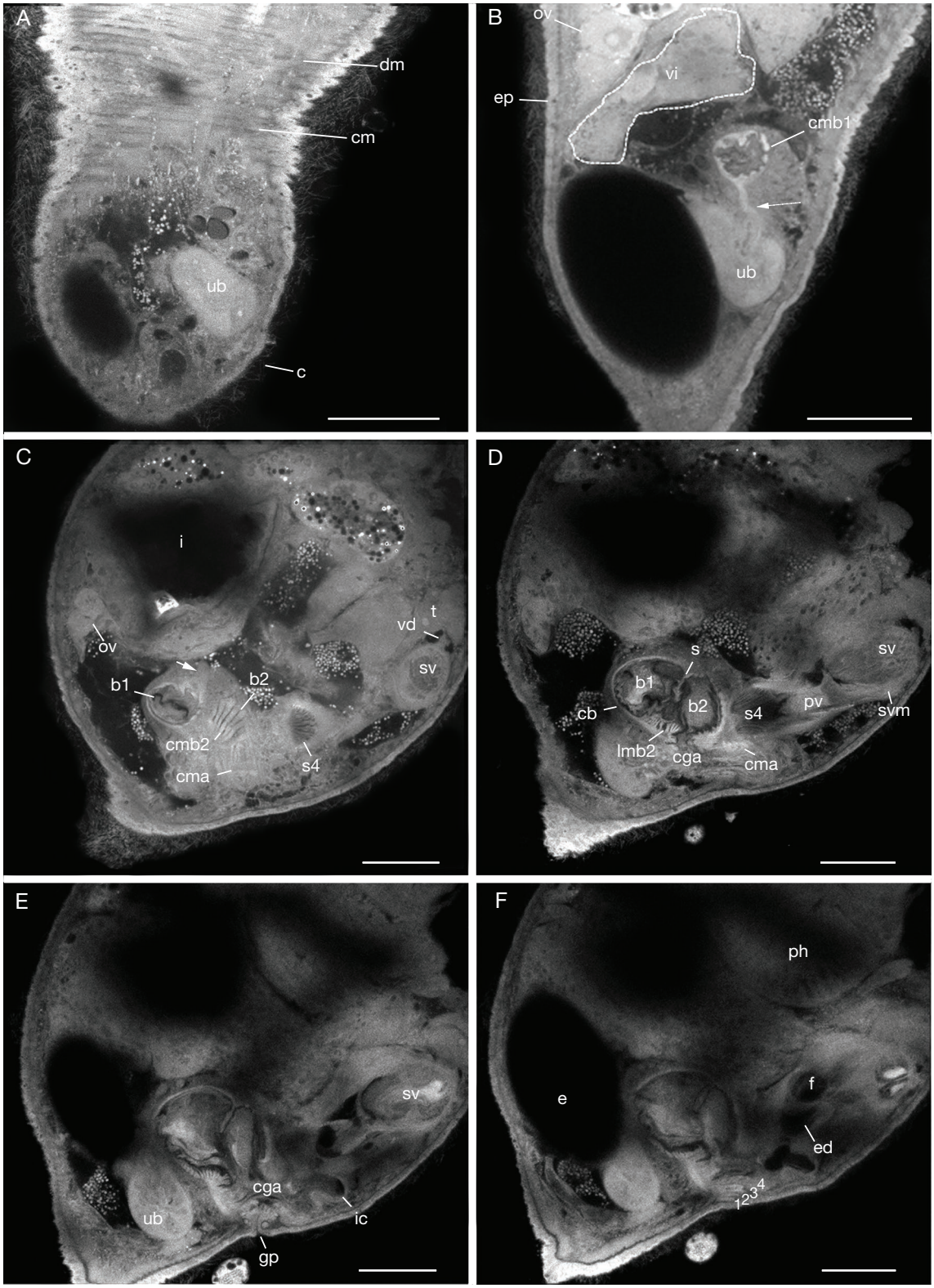

Fig. 3. — *Gieysztoria reta* n. sp. CLSM images of whole-mounts labeled with rhodamine-phalloidin showing the muscle arrangement: **A**, **B**, caudal body section from dorsal to ventral plane; **C**-**F**, caudal body section viewed from the right side toward the left side to sagittal plane. Images **A**, **B** and **C**-**F** correspond to two different specimens. Numbers **1**-**4** indicate circular fibers of gonopore sphincter; **dashed lines outline** the vitellaria area. **Continuous arrow** indicates strong muscle fiber of bursa cavity. **Dashed arrow** indicates muscle fiber that associates the uterus bend with the copulatory bursa. Abbreviations: **b1**, copulatory bursa cavity 1; **b2**, copulatory bursa cavity 2; **c**, cilia; **cb**, copulatory bursa; **cga**, common genital atrium; **cm**, circular muscle fibers of the body wall; **cma**, circular muscle fibers of common genital atrium; **cmb1**, circular muscles of the bursa cavity 1; **cmb2**, circular muscles of bursa cavity 2; **dm**, diagonal muscle fibers of the body wall; **e**, egg; **ed**, ejaculatory duct; **ep**, epidermis; **gp**, gonopore; **i**, intestine; **ic**, insertion cavity of s1 spine; **ov**, ovary; **ph**, pharynx; **pv**, prostate vesicle; **s**, septum; **sv**, seminal vesicle; **svm**, muscular wall of seminal vesicle; **s4**, group of dorsal hollow spines; **t**, testes; **ub**, uterus bend; **vd**, vasa deferentia; **vi**, vitellaria. Scale bars: A, B, 50 µm; C, D, E, F 40 µm.

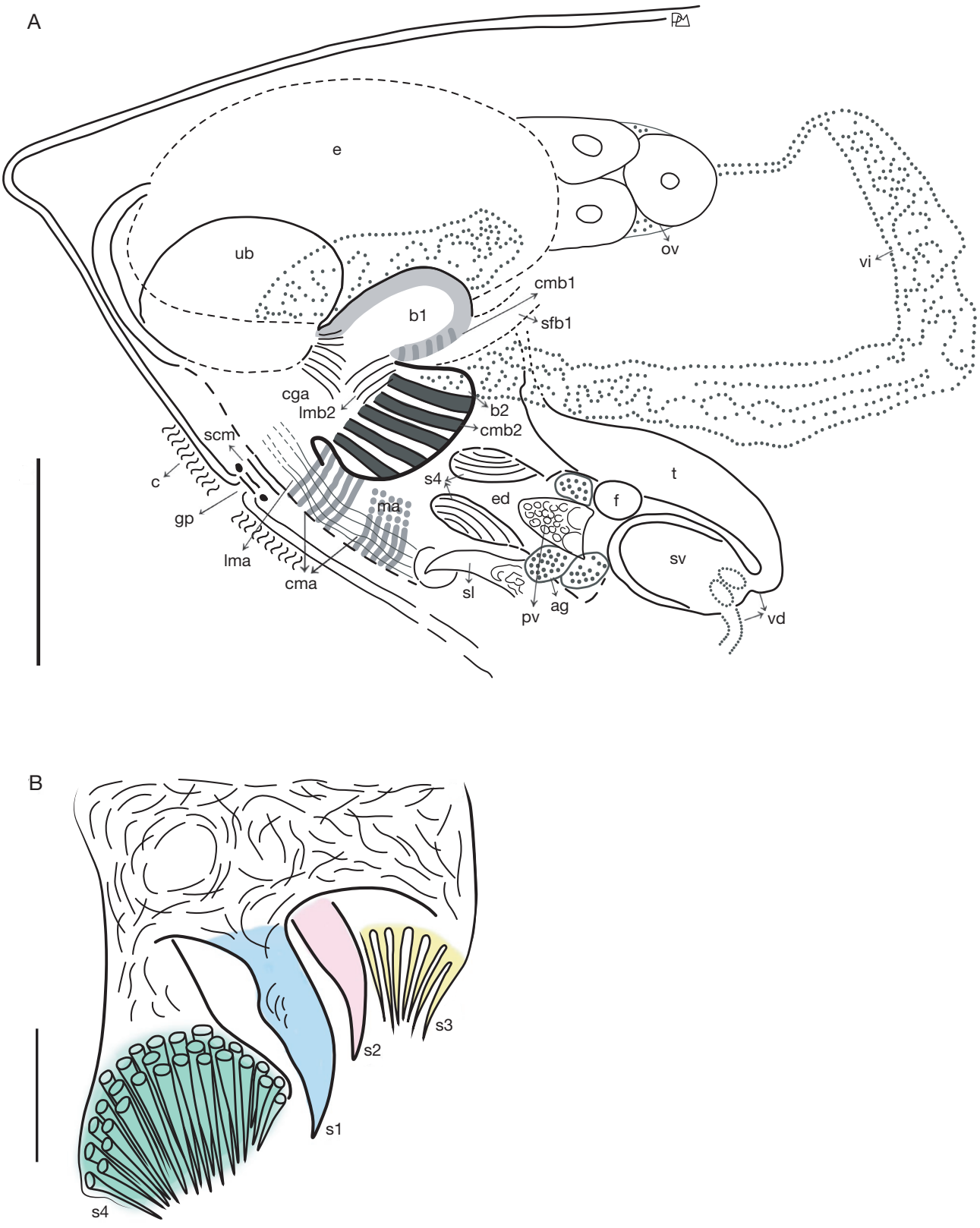

FIG. 4. — Graphic reconstruction of the genital system of *Gieysztoria reta* n. sp.: **A**, genital reconstruction using confocal microscopy images; **B**, penis stylet from the holotype, blue (**s1**), pink (**s2**), yellow (**s3**) and green (**s4**). Abbreviations: **ag**, accessory glands; **b1**, copulatory bursa cavity 1; **b2**, copulatory bursa cavity 2; **c**, cilia; **cga**, common genital atrium; **cma**, circular muscle fibers of common genital atrium; **cmb1**, circular muscles of the bursa cavity 1; **cmb2**, circular muscles of bursa cavity 2; **e**, egg; **ed**, ejaculatory duct; **f**, fenestra; **gp**, gonopore; **ma**, male genital atrium; **lma**, longitudinal muscles fibers of common genital atrium; **lmb2**, longitudinal muscle of bursa cavity 2; **ov**, ovary; **pv**, prostate vesicle; **sfb1**, strong muscle fiber of bursa cavity 1; **scm**, muscle fibers of gonopore sphincter; **sv**, seminal vesicle; **s1**, largest spine; **s2**, large spine; **s3**, group of fine short spines; **s4**, group of hollow spines; **t**, testes; **ub**, uterus bend; **vd**, vasa deferentia; **vi**, vitellaria. Scale bars: A, 50 µm; B, 20 µm.

TABLE 2. — A detailed list of *Gieysztoria* Ruebush & Hayes, 1939 species reported in the Neotropical Region, showing group designed on the stylet morphology, country where the species was originally described and geographical distribution. Information sources: [1] Original description of species; [2] Luther 1955; [3] Brusa *et al.* 2003; [4] Brusa *et al.* 2008; [5] Reyes *et al.* 2021; [6] Reyes *et al.* 2022; [7] Xia *et al.* 2014.

***Gieysztoria* species**	**Group and Subgroup after stylet morphology**	**Original description**	**Geographic distribution**
G. acariaia (Marcus, 1946)	Inaequales (Aberrantes)[2]	Brazil	Brazil
G. atalaya Brusa, Damborenea & Noreña, 2008	Inaequales (Fenestratae)[1]	Argentina	Argentina
G. bellis (Marcus, 1946)	Aequales[2,6]	Brazil	Brazil and Peru
G. complicata (Fuhrmann, 1914)	Inaequales (Aberrantes)[2,5]	Colombia	Colombia, Brazil and Peru
G. coronae Noreña-Janssen, 1995	Aequales[3]	Argentina	Argentina
G. cuspidata (Schmidt, 1861)	Aequales[2,6]	Greece?	Cosmopolitan
G. cypris (Marcus, 1946)	Inaequales (Aberrantes)[2]	Brazil	Brazil
G. chiqchi Damborenea, Brusa & Noreña, 2005	Inaequales (without subgroup assigned)[1]	Peru	Peru
G. duopunctata Reyes & Brusa, 2021	Inaequales (Radiatae)[1]	Brazil	Brazil
G. evelinae (Marcus, 1946)	Inaequales (Radiatae)[2,4]	Brazil	Brazil and Argentina
G. falx Brusa, Damborenea & Noreña, 2003	Inaequales (Fenestratae)[1,5]	Argentina	Argentina and Brazil
G. hermes Reyes & Brusa, 2021	Inaequales (Aberrantes)[1]	Brazil	Brazil
G. hymanae (Marcus, 1946)	Inaequales (Radiatae)[2,4]	Brazil	Brazil and Argentina
G. intricata (Marcus, 1946)	Inaequales (Fenestratae)[2]	Brazil	Brazil
G. kasasapa Damborenea, Brusa & Noreña, 2005	Inaequales (Aberrantes)[5]	Peru	Peru
G. matilde Brusa, Damborenea & Noreña, 2008	Inaequales (Aberrantes)[5]	Argentina	Argentina
G. namuncurai Damborenea, Brusa & Noreña, 2007	Inaequales (Aberrantes)[5]	Argentina	Argentina
G. ornata (Hofsten.1907)	Aequales[2]	Switzerland	Cosmopolitan
G. pseudodiadema Noreña-Janssen, 1995	Aequales[7]	Argentina	Argentina
G. quadrata Noreña-Janssen, 1995	Aequales[7]	Argentina	Argentina
G. quadridensoides (Fuhrmann, 1914)	Aequales[2]	Colombia	Colombia
G. reggae (Therriault & Kolasa, 1999)	Inaequales (Radiatae)[5]	Jamaica	Jamaica
G. reta n. sp.	Inaequales (Fenestratae)[1]	Argentina	Argentina
G. rubra (Fuhrmann, 1894)	Aequales[2]	Switzerland	United Kingdom, Switzerland, Denmark and Brazil
G. santafeensis Noreña-Janssen, 1995	Aequales[3]	Argentina	Argentina
G. sasa Damborenea, Brusa & Noreña, 2005	Inaequales (Aberrantes)[1]	Peru	Peru
G. therapaina (Marcus, 1946)	Inaequales (Fenestratae)[2]	Brazil	Brazil
G. thymara (Marcus, 1946)	Inaequales (Aberrantes)[2]	Brazil	Brazil
G. tridesma (Marcus, 1946)	Inaequales (Radiatae)[2]	Brazil	Brazil
G. tigrensis Noreña-Janssen, 1995	Aequales[7]	Argentina	Argentina
G. triquetra (Fuhrmann, 1894)	Inaequales (Fenestratae)[2]	Switzerland	Brazil and Europe
G. trisolena (Marcus, 1946)	Inaequales (Radiatae)[2]	Brazil	Brazil
G. uncia (Marcus, 1946)	Aequales[2]	Brazil	Brazil
G. variata Noreña-Janssen, 1995	Aequales[7]	Argentina	Argentina

length of the stylet of the new species (50-75 µm). Distally to the girdle, *G. therapaina* has four groups of spines (Marcus 1946): three groups with several spines of different sizes and thicknesses, while the fourth group consists of a single large spine 20 µm long, almost ⅔ of the total stylet size. *Giestoria reta* n. sp. has two large ventral spines, and the largest spine is about half the length of the stylet.

Another species with similar stylet morphology to the new species is *G. complicata*, which has three groups of spines: one group with two strong parallel outward-curving spines (spines a), the second group with numerous spines in shape of a crown (spines b), and the third group with numerous spines arranged like a 'brush' (spines c), longer and broader than spines b (Marcus 1946). However, in contrast to the new species, *G. complicata* does not have a fenestrated girdle, and the stylet is longer (100 µm).

The stylet in *G. reta* n. sp. is also similar to the one in *G. intricata*, which has different sets of spines: single and clustered shorter spines, and a fenestrated girdle. However, it can be clearly distinguished from the new species because its stylet is longer (120 µm) and has a single strong spine with cuticular hairs.

DISCUSSION

TAXONOMY AND BIOGEOGRAPHIC NOTES

Gieysztoria species were divided into two groups, Aequales and Inaequales, based on stylet morphology (Luther 1955; Brusa *et al.* 2003; Van Steenkiste *et al.* 2012; Xia *et al.* 2014). Aequales have symmetrical stylets, and are well represented in the Holarctic realm, while Inaequales have complex, irregular, asymmetric stylets, and highest richness in the Neotropic (Noreña *et al.* 2003; Brusa *et al.* 2003; Van Steenkiste *et al.* 2012; Reyes *et al.* 2021). Inaequales is divided into 4 subgroups: Fenestratae, Radiatae, Aberrantes and Falcatae (Luther 1955; Damborenea *et al.* 2005; Van Steenkiste *et al.* 2012). However, despite the complex stylet morphologies found throughout the genus *Gieysztoria*, mainly in the southern hemisphere, the position of some species is uncertain because they do not fit the diagnoses of the subgroups (Noreña *et al.* 2003; Van Steenkiste *et al.* 2012). For instance, some species display intermediate features between Aberrantes and Fenestratae, and have not been assigned to either subgroup (Damborenea *et al.* 2005, 2007). In this regard, girdle openings may have limited taxonomical value (Van Steenkiste *et al.* 2012).

According to stylet morphology, *G. reta* n. sp. was compared to species from the subgroup Fenestratae (*G. therapaina*, *G. intricata*, and *G. matilde*) and the subgroup Aberrantes (Inaequales) (*G. hermes*, *G. complicata*, *G. namumcurai* and *G. kasasapa*). All these species have fibrous girdles and numerous rows of distal spines that may be accompanied by another group of spines.

Gieystoria is a well-known genus in both the Paleartic and in the Neotropic, and to a lesser extent in the Neartic and Afrotropical regions. Other zoogeographical regions are poorly explored (Noreña *et al.* 2003; Van Steenkiste *et al.* 2012). Records show 33 species distributed in the Neotropical region, of which only four, *G. cuspidata* (Schmidt, 1861), *G. ornata* (Hofsten, 1907), *G. rubra* (Fuhrmann, 1894) and *G. triquetra* (Fuhrmann, 1894), were not originally described in the region (Table 2). *Gieystoria cuspidata*, *G. ornata* and *G. rubra* are cosmopolitan species, while *G. triquetra* is restricted to the Paleartic and Neotropical regions (Noreña *et al.* 2003; Braccini *et al.* 2016; Tyler *et al.* 2006-2024). This suggests a high level of endemism in the Neotropical region. However, most of the species have been reported only once, when they were originally described, with some exceptions such as *G. bellis*, *G. falx*, *G. hymanae*, *G. complicata* and *G. evelinae* (Beauchamp 1939; Marcus 1946; Brusa *et al.* 2008; Reyes *et al.* 2021, 2022; Tyler *et al.* 2006-2024) having an extensive distribution range in the Neotropical region (Table 2). Even so, the interpretation of the results in a broader biogeographical context is preliminary, due to the fragmentary knowledge of the genus in the region. Studies by Noreña *et al.* (2003) and Van Steenkiste *et al.* (2012) suggest that the breakup of Pangaea and Gondwana could have been an important mechanism influencing the evolutionary and biogeographical history of *Gieysztoria* species. These studies report that stylet shape is more complex in *Gieysztoria* species from the Gondwanian region than in those from the Laurasian regions, suggesting that the significant speciation events occurred after the separation of Gondwana and Laurasia which drove the evolution of new subgroups. Thus, *Gieysztoria* species can be assigned to two groups (Aequales and Inaequales) and four subgroups with different stylet traits (Luther 1955; Brusa *et al.* 2003; Noreña *et al.* 2003; Van Steenkiste *et al.* 2012, see Table 2). Interestingly, two thirds of all species recorded in the Neotropical region have complex stylet morphology and are into the Inaequales group (see references in Table 2).

Finally, in the hope of improving knowledge on the process that has shaped the biogeographic pattern of *Gieystoria* and other freshwater invertebrates, it would be necessary to consider regional and local geological history, as well as the environmental history of the hydrological system where they live (Adami & Damborenea 2021).

CONCLUSIONS

This study contributes to increasing the taxonomic knowledge on the genus *Gieysztoria*, and discusses its geographical distribution in South America. Considering the diagnostic characteristics of *G. reta* n. sp., it can be assigned to the Inaequales group, like most other Neotropical *Gieysztoria* species. The use of phalloidin-rhodamine and laser confocal microscope enabled observation of the details of several muscle groups associated to the genital organs.

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY MATERIAL 1. — Stack 1 and Stack 2 with images of two of *Gieysztoria reta* n. sp. specimens labeled with phalloidin-rhodamine. Images were taken with CLSM. Some of the serial images were used to show relevant structures in Figure 3. https://doi.org/10.35537/10915/149823