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New Species of *Gieysztoria* (Platyhelminthes, Rhabdocoela) from Peruvian Amazon Floodplain with Description of their Stylet Ultrastructure

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ABSTRACT—The free-living Platyhelminthes of the Amazon basin are poorly known. Presently only four turbellarian species have been mentioned from the Amazon river, a fact that confirms the lack of information on this kind of faunas in this huge basin. Three new species of *Gieysztoria* from Amazonian floodplain in Peru are described herein: *G. chiqchi* n. sp., *G. kasasapa* n. sp. and *G. sasa* n. sp. The samples were taken in the Pacaya-Samiria National Reserve (Peru) during September 2002. Besides the usual description of the stylet based on whole mounted specimens, we provide a complementary description using SEM, which allowed differentiation of the new species within the currently yet imperfect picture of the Amazonian turbellarian fauna. Although further research is desired, current findings are suggestive of high diversity of free-living Turbellaria in the surveyed region.

Key words: Turbellaria, *Gieysztoria*, Peru, new species, Amazon basin

INTRODUCTION

Most of the well-known Platyhelminthes from the Amazon basin belong to the parasitic classes (Neodermata): Cestoda, Trematoda and Monogenea (e.g. Thatcher, 1993; Carvalho *et al.*, 2001; Marques *et al.*, 2003; Belmont-Jegu *et al.*, 2004; Rego, 2004) whereas the free-living Platyhelminthes are almost unknown. Presently only four species have been mentioned from the Amazon river (floodplain and tributaries): *Xenostenostomum microstomoides* Reisinger, 1976, *Dugesia fissipara* (Kennel, 1888) Ball, 1974 (Marcus, 1948) captured near the Amazon estuary, and more recently, *Sergia* sp. and *Mesostoma* sp. from the Peruvian Amazon (Noreña, *et al.*, in press). Therefore, data or information concerning this group are still very inadequate for over 350,000 km² (during the wet season) of Amazonian flooded area (Amazon river and tributaries).

In this study we describe three new species of the genus *Gieysztoria* Ruebush & Hayes, 1939 from the Peruvian Amazon floodplain. The samples were taken during an expedition to the Pacaya-Samiria National Reserve (Department of Loreto, Peru) under the aegis of the project “Aquatic

Faunal Survey of Peruvian Amazon floodplain” during September 2002.

The genus *Gieysztoria* is one of the richest in the Dalyelliidae family. Currently, about 70 species of this genus have been reported from all the continents. In spite of the few localities studied in South America, 21 species have been cited from Brazil, Argentina, Colombia and Peru (Fuhrmann, 1914; Beauchamp, 1939; Marcus, 1946; Noreña-Janssen, 1995; Brusa *et al.*, 2003; Noreña *et al.*, 2003; Gamo and Leal-Zanchet, 2004). It is a genus with a homogeneous anatomy in which the specific identification is based mainly on the morphology of the male copulatory stylet. This structure is traditionally studied from whole mounted specimens. In this research we complemented the usual description of the stylet based on whole mounted specimens with Scanning Electron Microscope observations.

The three new species of the genus *Gieysztoria* described in this paper can be added to the list of micro-turbellarians described by Noreña *et al.* (in press) from the Pacaya-Samiria National Reserve in the Peruvian Amazon. Of course, this study renders only an imperfect picture of the Amazonian turbellarian fauna. Further sampling and research are needed, but the discovery of these species allows us to infer that this area has a high specific diversity within *Gieysztoria*.

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MATERIAL AND METHODS

The material studied was collected in September 2002 during an expedition to the Pacaya-Samiria National Reserve, in the department of Loreto, Peru (Fig. 1). This reserve is the largest in Peru, the second in the Amazon basin and the fourth in South America. As a protected area, the Pacaya-Samiria Reserve retains relatively intact forest and aquatic ecosystems. The diversity of aquatic invertebrates in this region is likely to be very high.

The localities spread over an area crossed by many connecting channels, called "caños" (secondary beds), and lagoons of different size called "cochas" (abandoned beds), all under the fluvial influence of the Pacaya Samiria river (Fig. 1), a tributary of the Ucayali river. The collections were made in the littoral zone, to a depth of 50–100 cm, using plankton-nets (60 µm of mesh). The nets were dragged several times through the floating and rooted aquatic vegetation (*e.g.*, *Pistia* sp., *Salvinia* sp., *Eichornia* sp., *Paspalum* sp., *Scirpus* sp.).

Specimens were studied *in vivo* under a stereomicroscope and drawings of dorsal view were done (freehand drawings). Some specimens were fixed in polyvinyl-lactophenol (whole-mounts), and others were fixed in Bouin (for subsequent histological study). Histological sections (4 µm thick) were stained with Haematoxylin-Eosin. Stylet morphology was studied with optical microscope from *in toto* preparations mounted in polyvinyl-lactophenol, and also using Scanning Electron Microscope (SEM). For SEM study the

stylet was dissected, treated in sodium hypochlorite, washed in distilled water, mounted, left to air-dry, and coated with gold. Photographs of the stylet mounted in polyvinyl-lactophenol were taken in a Nikon microscope with interferential contrast. Figures and photographs were scanned and edited using Adobe Photoshop.

The species described here we found between the roots of both floating and rooted vegetation (*Eichornia* sp., *Paspalum* sp., *Pistia* sp., *Salvinia* sp., *Nimphaea* sp.). Other Turbellaria species (*Stenostomum* spp., *Macrostomum* sp., *Prorhynchus* sp., *Gieysztor* spp. and triclads), and other invertebrates (Hydroidea, Oligochaeta, Acari, Ostracoda, Amphipoda, Copepoda, Chironomida, other insects, Rotifera, Nematoda and Gastropoda) were found.

Type and voucher specimens were deposited in the Helminthological Collection of the Museo de La Plata, Argentina (CHMLP).

RESULTS

Family Dalyelliidae von Graff, 1905

Genus *Gieysztor* Ruebush & Hayes, 1939

Gieysztor *chiqchi* n. sp.

(Figs. 2, 3)

Type locality: Pacaya-Samiria National Reserve, Loreto, Peru. "Cocha" Yarina: lagoon connected with the "caño" Yarina (5°24'42"S, 74°30'23"W). Date: 17.09.02

Holotype: one whole mounted specimen embedded in polyvinyl-lactophenol, (CHMLP 5436).

Paratypes: two whole mounted specimen embedded in polyvinyl-lactophenol, (CHMLP 5437); four sagittally sectioned specimens (CHMLP 5438).

Other material: six whole-mounted specimens embedded in polyvinyl-lactophenol (CHMLP 5439 and 5440). One stylet mounted for SEM (CHMLP 5449).

Etymology: the species name, *chiqchi* means freckles in Quechua, making reference to its *in vivo* aspect.

Description: Length of fixed sexually mature individuals 800–1100 µm. Oval body with posterior region widened and posterior end pointed (Fig. 2). *In vivo* specimens whitish with dark conspicuous spots. Epithelium with polygonal, homogeneous ciliated cells. Body musculature consisting of longitudinal inner muscle layers and circular outer muscle layers, more developed in the posterior half of the body. Adhesive glands located in posterior region.

Oral pore located antero-ventrally. Long (1/3–1/4 of the body length) barrel-like pharynx. Small oesophageal glands opening in transition area between pharynx and intestine. Intestinal epithelium long, cells vacuolated. Posterior region of body intestine-free.

Brain dorsal to pharynx, associated with two black, bean-like eyes formed by pigment.

Single ovary dorsal and posterior to intestine, placed in posterior third of body. Very large seminal receptacle, bag-shaped, joined to oviduct by thin duct. Uterus lying between short ductus communis and female duct; may contain single ovoid egg. Pair of simple feathery vitellaria running first (from posterior end of pharynx) latero-ventrally, and ending dorsally. Sacculiform *bursa copulatrix* opening into *atrium communis*; walls sinuous and lined with muscle layers. Api-

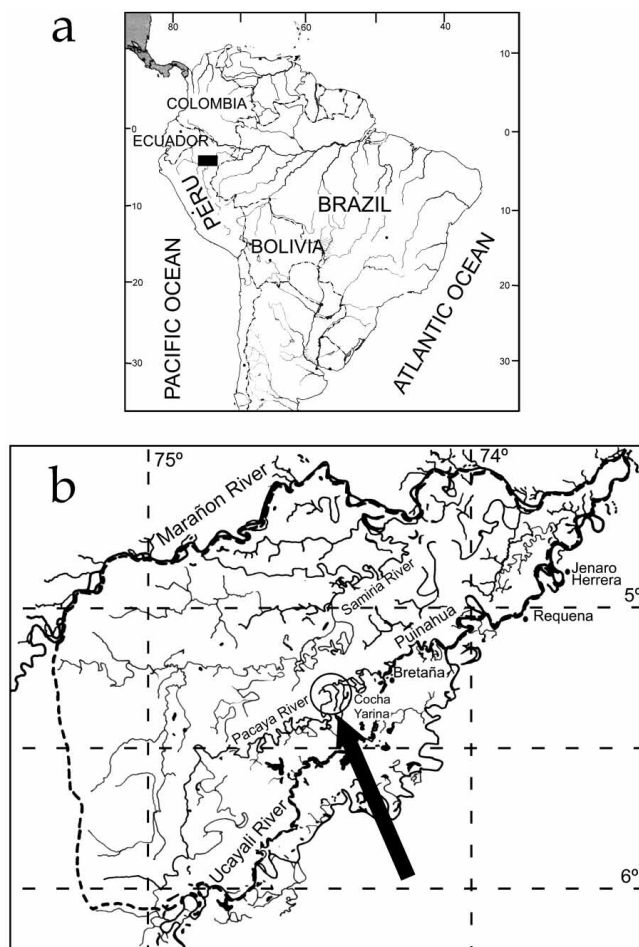


Fig. 1. a. North region of South America with the location of Pacaya Samiria National Reserve, Loreto, Peru; b. Detail of the area. The circle and the arrow indicate the location of the sampling place.

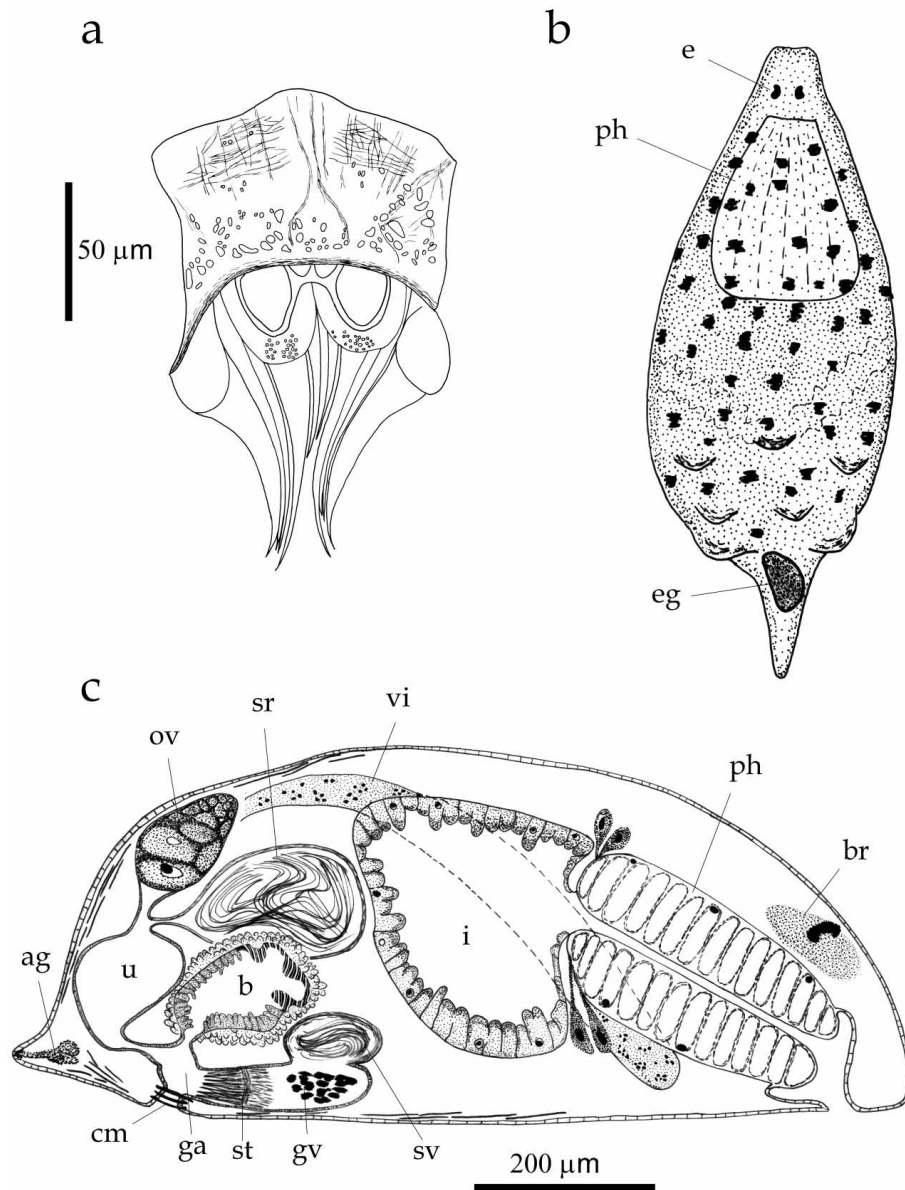


Fig. 2. *Gieysztoria chiqchi* n. sp. **a.** Compressed stylet. **b.** Dorsal view of live specimen (freehand drawing). e, eye; eg, egg; ph, pharynx. **c.** Sagittal reconstruction. ag, adhesive glands; b, bursa copulatrix; br, brain; cm, circular muscle; ga, genital atrium; gv, granular vesicle; i, intestine; ov, ovary; sr, seminal receptacle; st, stylet; sv, seminal vesicle; u, uterus; vi, vitellaria.

cal wall of *bursa* thicker, strongly muscular and more sinuous than rest of wall. Parenchymal cells surrounding the bursa copulatrix. Cement glands opening near genital pore. Strong sphincter surrounding gonopore.

Testes in posterior region of body, lying ventrally to vitellaria. Sperm ducts originating in testes and reaching into seminal vesicle. Seminal vesicle located proximally in male organ; granular vesicle distal. Both vesicles with muscular walls. Granular vesicle followed by ejaculatory duct.

Stylet hyaline, measuring 170 µm long (Figs. 2, 3). Girdle fibrous, reticulate and open on one side. Ten hollow spines of different length distributed symmetrically from girdle. A pair of small spines – 75 µm long and with rounded base – is placed centrally; at each side of these is inserted

another spine 100 µm long, with rounded base, wider and more rugose than rest of spines. External to the latter, towards edges of stylet, two spines 100 µm long, with the same characteristics but with smaller rectangular bases. Outside these and on each side of open edge of stylet is spine 100 µm long with triangular bases. All spines, except central ones, slightly curved outwards.

Discussion: The presence of spines of different size in the cuticular stylet and their symmetrical disposition is a characteristic that *G. chiqchi* n. sp. shares with several species of this genus. *Gieysztoria rastafariae* Theriault & Kolasa 1999 seems to bear some general similarity to *G. chiqchi* n. sp. but the stylet of the *G. rastafariae* are 100 µm in length and

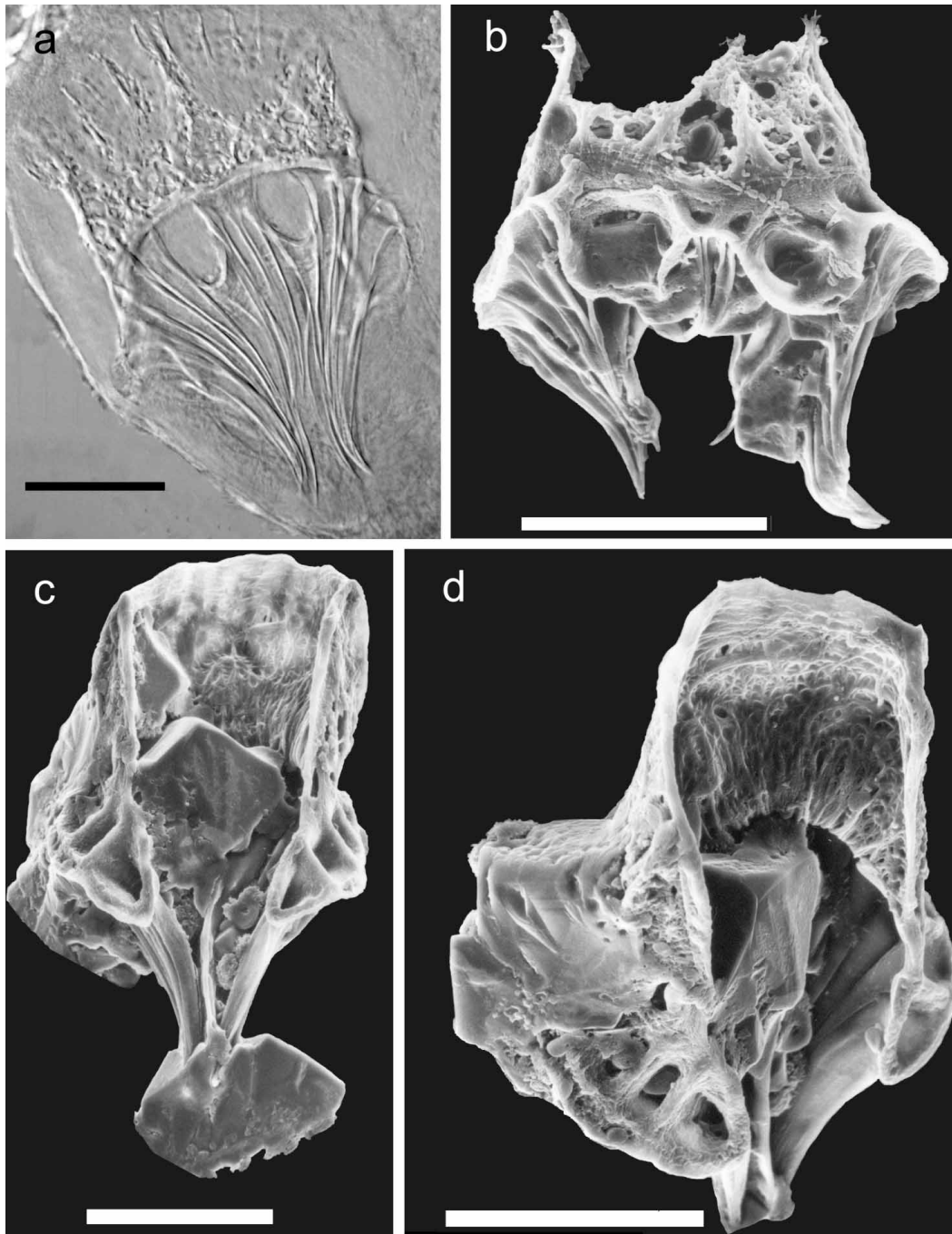


Fig. 3. *Gieysztoria chiqchi* n. sp. **a.** Microphotograph of squashed stylet. **b–d.** SEM of extracted stylet. **b.** General view showing the two central spines and their bases, the bases of the big lateral spines and lateral spines. **c.** Open side of the stylet and the rectangular bases lateral spines and triangular bases of the external lateral spines. **d.** Spines bases detail and girdle. Scale bar 50 µm.

in *G. chiqchi* n. sp. 170 µm. The girdle of *G. rastafariæ* stylet has 2–4 fenestrae, and the outward spines decrease in size in *G. rastafariæ* but not in *G. chiqchi* n. sp.

In South America, species with similar stylets are *Gieysztoria hymanæ* (Marcus, 1946) Luther, 1955 and *Gieysztoria evelinæ* (Marcus, 1946) Luther, 1955. In these species, thick and thin spines alternate, as observed in the new species, but the arrangement, number and size of the spines are different. On the other hand, the girdle in these

species has two rings joined by bridges, whereas in *G. chiqchi* n. sp. it is totally fibrous, with no bridges.

Gieysztoria kasasapa n. sp.
(Figs. 4, 5a and 6)

Type locality: Pacaya-Samiria National Reserve, Loreto, Peru. Pacaya river (5°16'28"S, 74°29'55"W). Date: 16.09.02.

Holotype: one whole-mounted specimen embedded in poly-vinyl-lactophenol. (CHMLP 5431).

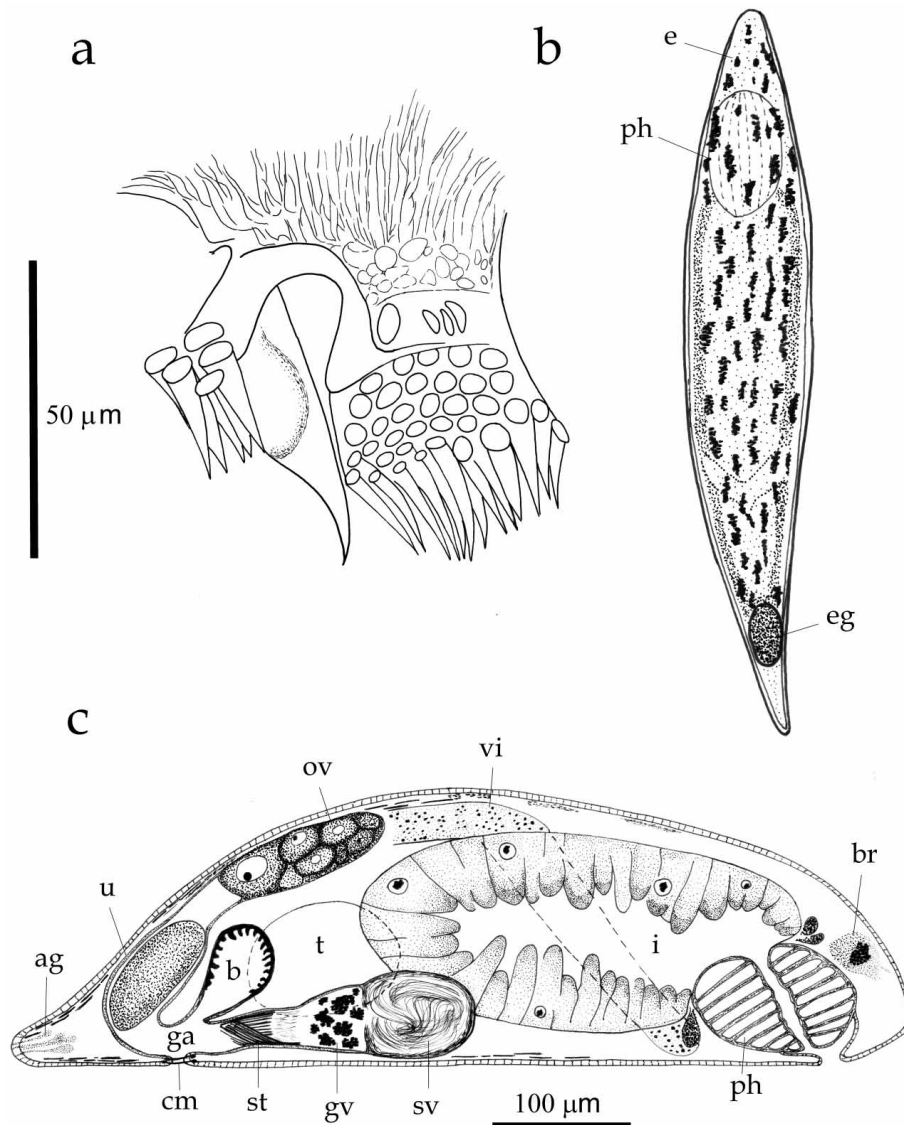


Fig. 4. *Gieysztor kasasapa* n. sp. **a.** Compressed stylet. **b.** Dorsal view of live specimen (freehand drawing). e, eye; eg, egg; ph, pharynx. **c.** Sagittal reconstruction. ag, adhesive glands; b, bursa copulatrix; br, brain; cm, circular muscle; ga, genital atrium; gv, granular vesicle; i, intestine; ov, ovary; st, stylet; sv, seminal vesicle; t, testicle; u, uterus; vi, vitellaria.

Paratypes: two whole-mounted specimens embedded in polyvinyl-lactophenol (CHMLP 5432) and two sagittally sectioned specimens (CHMLP 5433). One whole-mounted specimen embedded in polyvinyl-lactophenol (CHMLP 5434).

Other material: one whole-mounted specimen embedded in polyvinyl-lactophenol from Cocha Yarina, Pacaya-Samiria National Reserve, Loreto, Peru. Date: 17.09.02 (CHMLP 5435). One stylet, mounted for SEM from type locality (CHMLP 5450).

Etymology: The species name, *kasasapa*, means thorny in Quechua.

Description: Length of sexually mature specimens 550–750 µm. Body oval, elongate and pointed posteriorly (Fig. 4). Body colour amber, with abundant dark subepidermic pig-

mentation arranged in stretched spots. Polygonal epithelial cells with homogeneous cilia. The body musculature consisting of longitudinal inner muscle layers and circular outer muscle layers, more developed in posterior half of body. Adhesive glands located in posterior region.

Oral pore located antero-ventrally. Pharynx barrel-like, small and occupying about the 1/6 of body length. Oesophageal glands opening in transition area between pharynx and intestine. Intestinal epithelium very long; cells vacuolated. Posterior region of body intestine-free.

Brain dorsal to pharynx, with prolongations extending toward anterior and posterior ends of body. Two black, bean-like eyes - with accumulations of pigment - attached to brain.

Single ovary lying dorsal to intestine in posterior third of body. Uterus lying between short ductus communis and

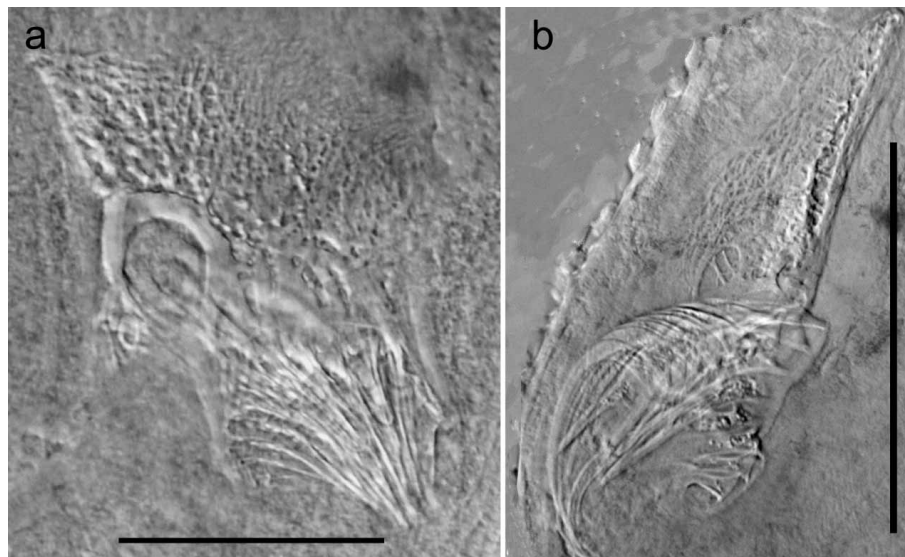


Fig. 5. Microphotographs of squashed stylets. **a.** *Gieysztoria kasasapa* n. sp. **b.** *Gieysztoria sasa* n. sp. Scale bar: 50 μ m.

genital atrium; may contain single ovoid egg. Pair of simple smooth vitellaria running latero-ventrally from anterior end of the intestine, ending dorsally. Sacculiform bursa copulatrix with sinuous walls lined with well developed muscle layers, opening into atrium communis. Gonopore surrounded by sphincter, numerous cement glands opening in its proximity.

Testes near intestine in middle of body, ventral to vitellaria. Sperm ducts originating in testes and reaching into seminal vesicle. Seminal vesicle located proximally in male organ, granular vesicle and ejaculatory duct distal. Walls of both vesicles strongly muscular and are separated by septum leaving central light for passage of spermatozooids. Content of granular vesicle is arranged in cumuli.

Stylet 75–85 μ m in total length (Figs. 4, 5a and 6). Girdle formed by fiber reticule. Reticule lax in proximal stylet region; more compact in distal region. Small perforations among the cuticularized area. Girdle openings towards dorsal face. Thorny region of stylet with two groups of robust spines separated by strongly sclerotized arc, distinguishable under optic microscope and SEM. First group formed by crown of at least four rows of convergent spines, each one with between six and ten straight and hollow spines. These spines originating at different heights; proximal spines longer (27 μ m) than distal ones (16 μ m), thus all reaching same level. On external edge of this group, there is a spine larger than rest. Second group formed by five straight and hollow spines between 18 and 24 μ m long, and a large hollow spine, 44 μ m long, with large base for muscular insertion, running towards first group of spines but distal end curved outwards. Total length of thorny region reaching 55 μ m.

Discussion: Because of the stylet characteristics, *G. kasasapa* n. sp. belongs to the Inaequales group according to Luther (1955). However few species in this group have a

spine disposition comparable to that observed in the new species. *G. complicata* (Fuhrmann, 1914) Luther, 1955 has a similar stylet to that appearing in the new species. In this species we can distinguish a group of numerous spines in the shape of a crown, and another group formed by two strong spines and a series of spines organized in rows. Even though the arrangement is similar to that observed in *G. kasasapa* n. sp., in *G. complicata* the number of rows that forms the crown - and the number of spines in each row - are greater than in the new species. In the second group of spines, *G. complicata* presents two strong spines instead of the only one observed in the new species, and the arrangement and number of the small spines accompanying the latter is different. Both *G. kasasapa* n. sp. and *G. complicata* lack the window in the girdle, though an inconspicuous window was observed in one of the stylets of the new species studied.

Another species with a stylet similar to that of *G. kasasapa* n. sp. is *G. intricata* (Marcus, 1946) Luther, 1955, although the stylet in the latter is more complex. The presence of a strong spine with cuticular hairs, the reduction of the size of the spines in the crown towards the proximal region of the stylet, and the presence of the strong window in the girdle, differentiates it clearly from *G. kasasapa* n. sp.

In the specimens studied here, neither the communication of the vitellaria with the ductus communis nor with the seminal receptacle were observed.

Gieysztoria sasa n. sp.
(Figs. 5b, 7 and 8)

Type locality: Pacaya-Samiria National Reserve, Loreto, Peru. "Cocha" Yarina: lagoon connected with the "caño" Yarina (5°24'42"S, 74°30'23"W). Date: 17.09.02.

Holotype: one whole-mounted specimen embedded in poly-vinyl-lactophenol (CHMLP 5426).

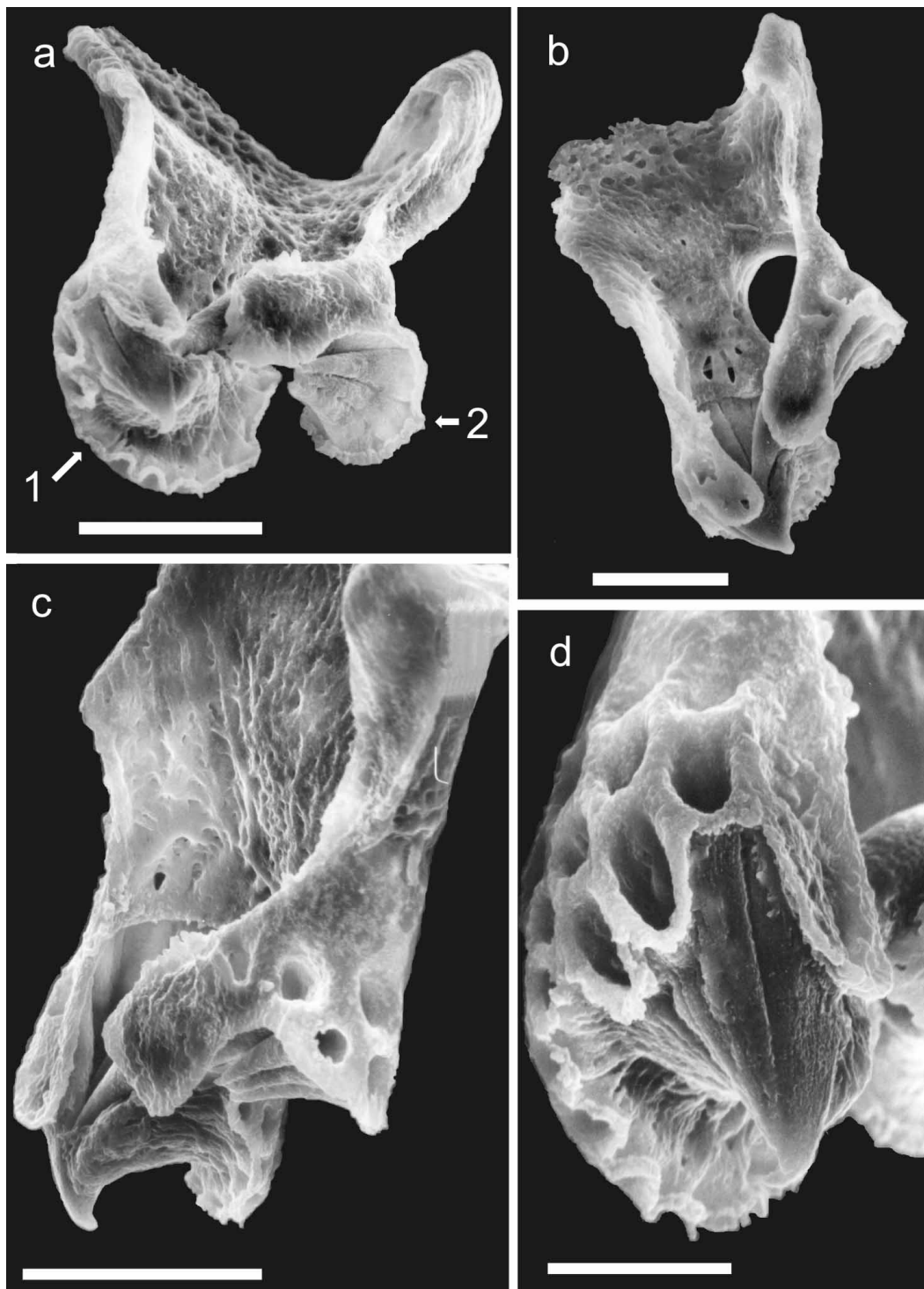


Fig. 6. *Gieysztoria kasasapa* n. sp. SEM of extracted stylet. **a.** General view of the dorsal open side of the stylet, with the two groups of spines (1 and 2). **b.** View of the large spine and its base for muscular insertion, behind the sclerotized arc. **c.** Lateral view of the stylet showing the spine's bases of the second group and the girdle's reticule. **d.** Detail of the first group of spines and their bases. Scale bar 50 μ m.

Paratypes: one sagittally sectioned specimen from the type locality (CHMLP 5427); one sagittally sectioned specimen from a pool located in the semi-dry connection channel between the Cocha Zapote (05°20'16"S, 74°9'40"W) and the secondary arm of the Ucayali river, the "caño" Yarina. Date: 20.09.02 (CHMLP 5428).

Other material: one sagittally sectioned specimen (CHMLP 5429); three sagittally sectioned specimens (CHMLP 5430). One stylet, mounted for SEM (CHMLP 5451).

Etymology: The species name *sasa* in Quechua means difficult.

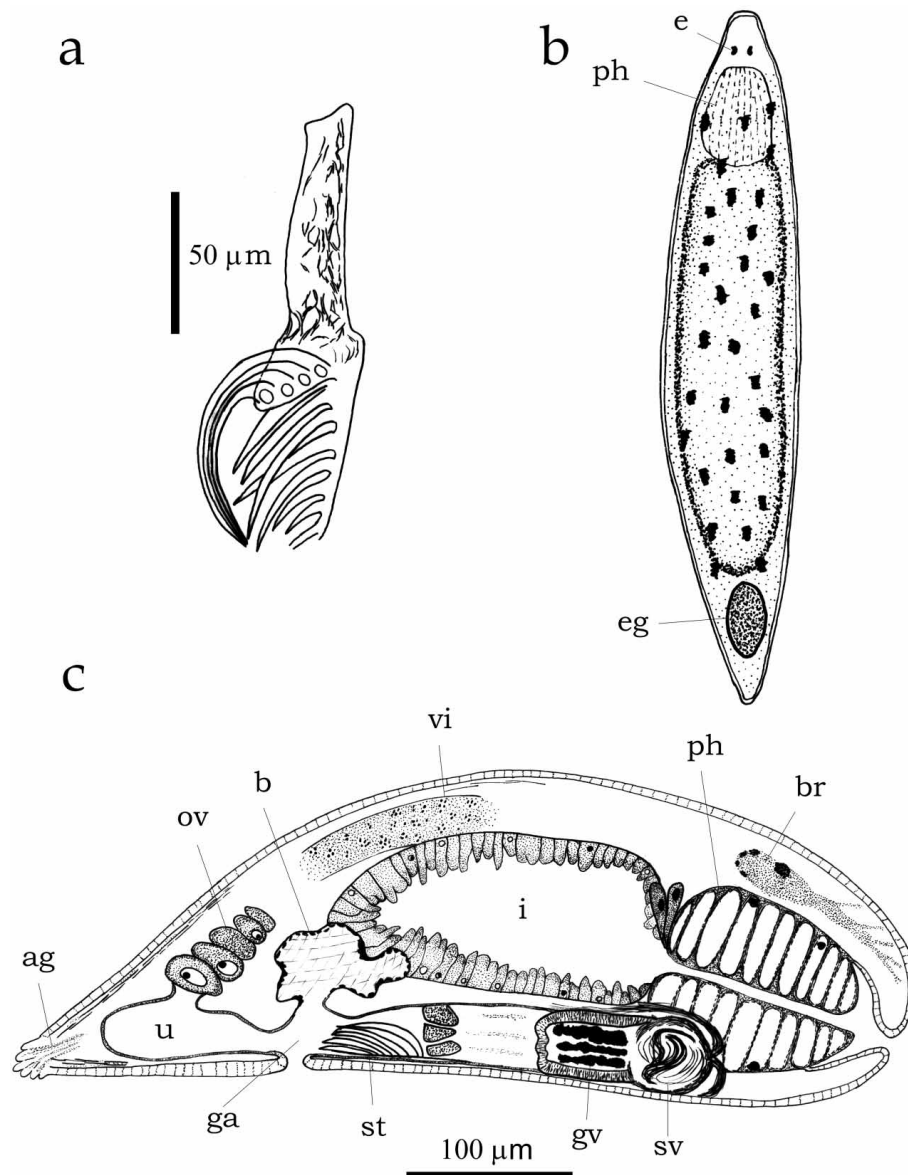


Fig. 7. *Gieysztoria sasa* n. sp. **a.** Compressed stylet. **b.** Dorsal view of live specimen (freehand drawing). e, eye; eg, egg; ph, pharynx. **c.** Sagittal reconstruction. ag, adhesive glands; b, bursa copulatrix; br, brain; ga, genital atrium; gv, granular vesicle; i, intestine; ov, ovary; st, stylet; sv, seminal vesicle; u, uterus; vi, vitellaria.

Description: Sexually mature and fixed specimens measuring between 500–600 µm. Body oval elongated and with posterior end tapering (Fig. 7). Live specimens with elongate amber spots, owing to sub-epidermic pigment. Epithelial cells polygonal and ciliated. Body musculature consisting of inner longitudinal and external circular muscular layers; both layers better developed in posterior half of body. Numerous adhesive glands situated in posterior region.

Mouth ventral and anterior. Pharynx barrel-like occupying a third of body length. Oesophageal glands in the transition between pharynx and intestine. Intestine short; posterior region of body intestine-free; intestinal epithelium formed by very high and vacuolated cells.

Brain dorsal to pharynx, with prolongations towards

anterior and posterior region of body. Two small bean-like black eyes - formed by pigment cumuli - associated to brain.

Single ovary dorsal to intestine, located in posterior third of body. Uterus placed between short ductus communis and female duct; may contain single egg. Pair of simple smooth vitellaria originating at same level at anterior end of intestine, and reaching from lateral region to dorsal region of body. Bursa copulatrix sacculiform, with sinuous wall, strong musculature, and opening into common genital atrium.

Testes near intestine in the middle of body, ventral to vitellaria. Sperm duct originating in testes and extending to seminal vesicle. Seminal vesicle ventral and anterior, located at same level as pharynx. Granular vesicle with thick

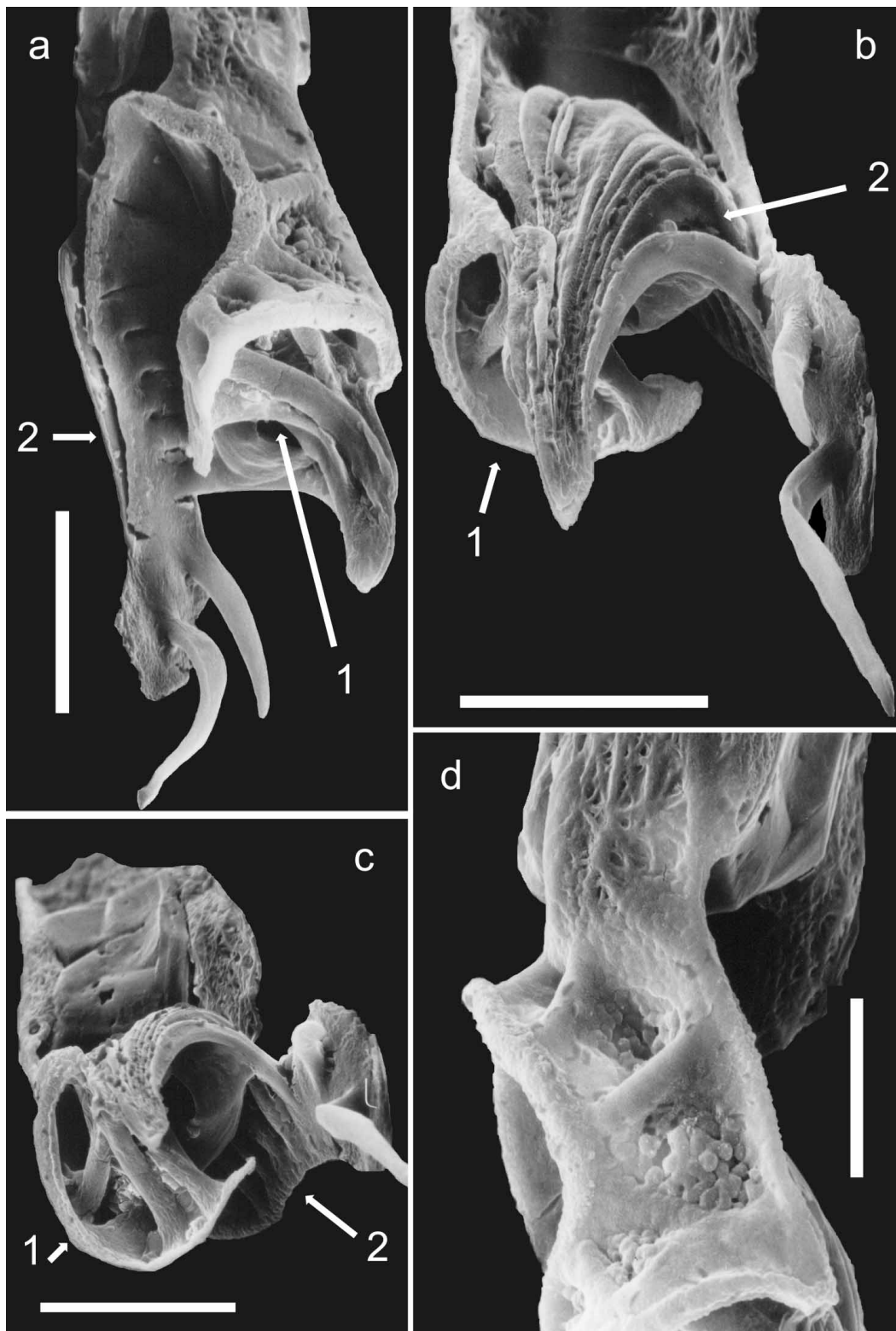


Fig. 8. *Gieysztoria sasa* n. sp. SEM of extracted stylet. **a.** Distal end of the stylet with the two groups of spines (1 and 2). **b** and **c.** Posterior and lateral view of the stylet respectively showing the convergency of both groups of spines (1 and 2), and the girdle opening. **d.** Detail of the proximal spine's bases of the group 2 and the girdle's texture. Scale bar 50 μ m.

wall, very long, and containing abundant granular secretion.

Stylet 160 μ m in total length (Figs. 5b, 7 and 8). Girdle 90 μ m in length, formed by reticule of irregular fibers. One

of its proximal ends longer. Two groups of spines originating from distal region of girdle. One (1) formed by four large, hollow spines; spines circular in section, curved and

arranged in a single row, measuring 70–100 µm; spines rising from broad surface, perpendicular to major axis of stylet and joined by one of their ends with longer edge of girdle; spines curving towards opposite edge of stylet. Second group (2) formed by nine or ten oval-sectioned spines; the two proximal spines 50–60 µm long and with broad bases; the remaining seven or eight spines with narrower bases, becoming shorter towards the distal end of the stylet, reaching 65–25 µm; spines in second group starting from longitudinal surface parallel to major axis of stylet, and related to the shorter edge of the girdle; these spines are perpendicular to major axis of stylet, running towards opposing edge, their ends converging with ends of spines of first group.

Discussion: The species with a stylet more similar to *G. sasa* n. sp. is *G. cypris* (Marcus, 1946) Luther, 1955, which was considered by Luther (1955) as belonging to the group Inaequales, subgroup Aberrantes. In the girdle of the stylet of *G. cypris*, the dorsal side is higher than the ventral one. From each distal side of the girdle there is a triangular base, each one with eight spines, the ones on the right side curving outwards and those on the left side curving outwards proximally and inwards distally. The girdle in *G. sasa* n. sp. is only slightly higher at one of its proximal ends. On each side of the distal region of the girdle arise spines, but their arrangement (on one side parallel to the major axis of the stylet and on the other perpendicular to it) and number (9–10 on one side and 4 on the other) is different to that observed in *G. cypris*. Even though Marcus (1946) did not mention the length of the stylet in *G. cypris*, it can be inferred from the original figure of the species that it was 125 µm long, *i.e.*, shorter than that of *G. sasa* n. sp.

Other characteristics that *G. sasa* n. sp. shares with *G. cypris* are (1) the position of the seminal vesicle anterior and ventral to the pharynx; (2) the great length of the granular vesicle, although in the new species, its walls are thinner and they present more secretion than in *G. cypris*; and (3) the scarce length of the intestine which leaves the posterior portion of the body free of it, even though in *G. sasa* n. sp. the intestine is longer than the one describe for *G. cypris*.

In the specimens studied here neither the seminal receptacle nor the viteloducts could be observed.

Viewpoints on the male copulatory stylet

The cuticular stylet of *Gieysztoria* is a structure of special interest from comparative anatomical as well as taxonomical viewpoints. The anatomy of this structure is the same within the genus: a girdle and spines, but it shows a great inter-specific variability in the consistency and morphology of the girdle, and in the shape, number, and disposition of the spines (Marcus, 1946; Luther, 1955; Brusa *et al.*, 2003).

The girdle can be continuous [*Gieysztoria thymara* (Marcus, 1946) Luther, 1955, *G. acaraiia* (Marcus, 1946) Luther, 1955] or discontinuous [*G. evelinae* (Marcus, 1946) Luther, 1955, *G. hymanae* (Marcus, 1946) Luther, 1955],

with windows or holes and ridges [*G. intricata*, *G. therapaina* (Marcus, 1946) Luther, 1955] or decrease to form a structure like a handle [*G. cypris* (Marcus, 1946) Luther, 1955, *G. sasa* n. sp.], and sometimes the girdle is lacking (*G. isoldeae* Artois, Willems, De Roeck, Jocqué & Brendonck, 2004).

Among the spines we find three different types. Type a): fine spines, generally hollow or provided with a narrow longitudinal channel, with a rounded or rectangular base (as those described for *Gieysztoria chiqchi* n. sp.). This type of spines is placed generally in a ring that forms an inverted crown with great mobility due to the action of multiple muscles. The length of the type a) spines is the same (*G. bellis* (Marcus, 1946) Luther, 1955, *G. coronae* Noreña-Janssen, 1995) or different (*G. complicata*, *G. intricata*). The type b) are stronger spines than those of type a), they are located at the ends of the stylet or in the central part (like in *G. chiqchi* n. sp., *G. kasasapa* n. sp., and *G. acaraiia*). Both types can be arched (concave or convex), straight or lightly curved at the distal end. Occasionally, a third type of spines (type c) is present. These are extremely developed and form a hook or pliers located laterally to the crown of spines [*e.g.* *G. falx* Brusa, Damborenea & Noreña, 2003, *G. euchroa* (Gieysztor, 1926) Luther, 1955, *G. donnae* Young, 1977].

We can find this complex stylet variety throughout the genus *Gieysztoria*, but it is extremely manifest in the southern hemisphere species, *e.g.* in the species described by Marcus (1946) for Brazil, by Hartenstein and Dwine (2000) and Hochberg and Cannon (2001) for Australia, by Brusa *et al.* (2003) for Argentina and more recently by Artois *et al.* (2004) for Botswana.

The origin of the formation of the stylet has not always been clear. At first it was supposed to be a cuticle secretion in an external wall fold of the proximal region of the male channel (Karling, 1943). Some authors considered that the stylet is a sclerotic structure formed by differentiations of the basement membrane ("pseudocuticles" after Karling, 1985). Studies under electron microscope showed that some stylets are intracellular differentiations (Rieger *et al.*, 1991). Not only because of their origin but also because of their complexity, these structures allow the establishment of relationships among the different taxa (Ehlers, 1985, Brusa *et al.*, 2003).

In general, and with regard to the genus *Gieysztoria*, Luther (1955) distinguished two large groups based on the stylet: a group including the species with symmetrical stylets (Aequales) and another group with asymmetric or very irregular stylets (Inaequales). The first group is broadly represented in the northern hemisphere, while the second group is distributed mainly in the southern hemisphere (Noreña *et al.*, 2003).

A further analysis of the morphological features of the stylet and a cladistic study are necessary to answer several questions about the genus *Gieysztoria*, *e.g.* why there are such differences between the geographic distributions of Aequales and Inaequales stylet morphologies; whether the

current genus *Gieysztoria* is monophyletic or not; which of the two stylet morphologies is basal, among others. Turbellaria from many regions of the south hemisphere (including South America) are still very poorly known and the study of the fauna of these areas might help to provide the answers.

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