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# A new species of *Gieysztoria* (Platyhelminthes, Rhabdocoela) from Argentina and a kinship analysis of South American species of the genus

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This study describes a new species of *Gieysztoria* found in lentic environments in Argentina. *Gieysztoria falx* sp. n. appears to be closely related to *G. therapaina* and *G. intricata*, and is differentiated principally by the presence of two strongly developed pincer-shaped spines of the cuticular stylet. A preliminary analysis of the phylogeny among the South American species of this genus is undertaken, and the monophyly verified. This study constitutes the first approach to interpreting these relationships.

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## Introduction

The genus *Gieysztoria* Ruebush & Hayes, 1939 (Dalyelliidae, Rhabdocoela) includes approximately 70 species distributed throughout both the Northern and Southern Hemispheres. Despite the wide distribution of this genus, few species can be considered cosmopolitan (e.g. *Gieysztoria ornata* (Hofsten, 1907) and *G. rubra* (Fuhrmann, 1894)) according to the criteria established nearly fifty years ago by Luther (1955) in his comprehensive monographic compilation 'Die Dalyelliiden' and confirmed by species descriptions in more recent years (Young 1977; Noreña-Janssen 1995; Therriault & Kolasa 1999; Hartenstein & Dwine 2000; Hochberg & Cannon 2001).

Within the Dalyelliidae, one of the diagnostic characteristics that differentiates genera, as well as species, is the shape and structure of the stylet located on the extreme end of the male copulatory apparatus. Within the Dalyelliidae, *Gieysztoria* possesses the most complex stylet. Based on the complexity and symmetry of the stylet spines, species can be assigned to the Aequales or Inaequales groups and further separated into Fenestratae or Radiatae subgroups (Luther 1955). The new species described in this study belongs to the group of Inaequales, subgroup Fenestratae.

The species with the most complex stylets are generally found in South America (Marcus 1946; Noreña-Janssen 1995). This information, together with the discovery of the new species *G. falx* was used as the basis for a preliminary kinship analysis of the South American species of this genus and the others that are considered Inaequales following Luther's criteria. Thirty-eight morphological characters were analysed in this study; 21 of which were directly related to the stylet, while the remaining 17 described general anatomical features.

## Materials and methods

The specimens studied were extracted from floating vegetation (*Pistia stratiotes* L., *Azolla filiculoides* Lam. and species of Lemnaceae) found in artificial lentic environments located in Buenos Aires province between September 1998 and March 2000. Water temperature fluctuated between 7 °C in winter and 28 °C in summer. The pH was relatively constant, with values around 7. Conductivity increased gradually from 600 µS in September 1998, exceeding 1000–1200 µS in March 2000. A decline in water levels was observed as conductivity increased.

The collected material was transported live to the laboratory and processed under stereomicroscopy. Specimens were

fixed in Bouin, embedded in Paraplast, and cut into a series of 4 µm thick sagittal sections. Histological sections were stained with AZAN. Stylet morphology was determined from observation of *in toto* preparations mounted on polyvinyl-lactophenol, and stylets extracted from selected specimens. The stylets were placed in a solution of sodium hydrochloride to remove the soft parts, washed in distilled water, mounted and left to air dry. They were coated with gold and observed under a Scanning Electronic Microscope (JEOL 100). Figures and photographs were scanned and edited using Adobe

Photoshop. The material was stored in the Helminthological Collection of the Museo de La Plata, Argentina (CHMLP).

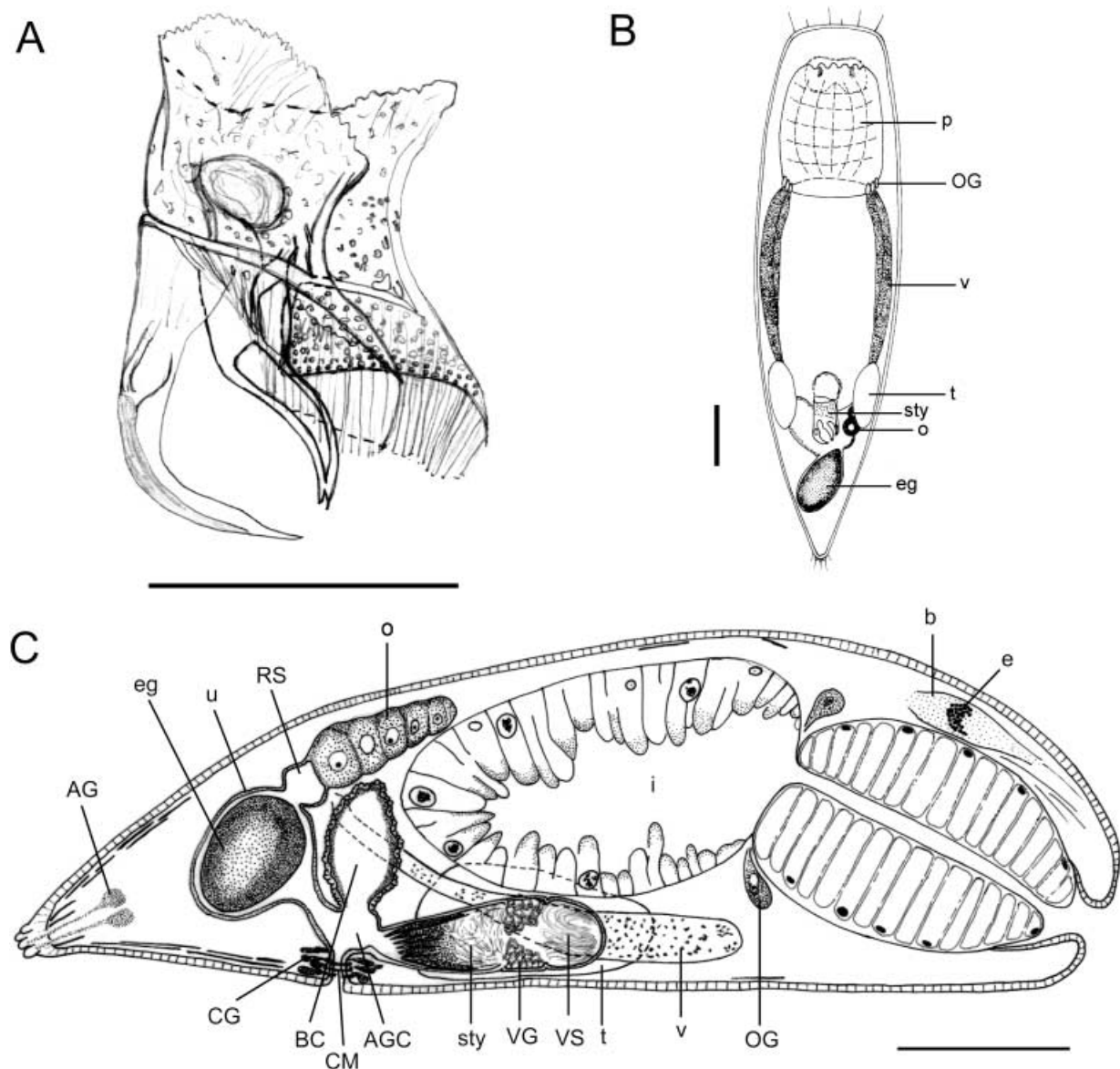
## Results

### Species description

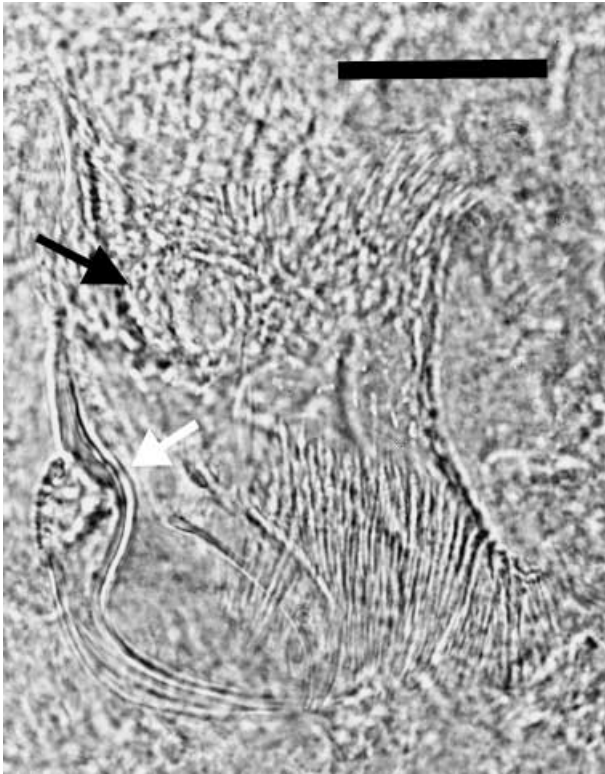
Genus *Gieysztorina* Ruebush & Hayes, 1939

*Gieysztorina falx* sp. n. (Figs 1–3)

*Syntype*. Three sagittally sectioned specimens, four whole mounted specimens, embedded in polyvinyl-lactophenol.



**Fig. 1** A–C. *Gieysztorina falx* sp. n. —A. Compressed stylet (scale bar: 50 µm). —B. Ventral view of body (scale bar: 100 µm). —C. Sagittal reconstruction (scale bar: 100 µm). Abbreviations: AG, adhesive glands; AGC, atrium genitale communis; b, brain; BC, bursa copulatrix; CG, cement glands; CM, circular muscle; e, eye; eg, egg; i, intestine; o, ovary; OG, oesophageal glands; RS, receptaculum seminis; sty, stylet; t, testes; u, uterus; VG, vesicula granulorum; v, vitellaria; VS, vesicula seminalis.



**Fig. 2** Microphotograph of compressed stylet (scale bar: 25  $\mu$ m). Black arrow indicates the window (opening); white arrow indicates the bulb of the largest spine.

ARGENTINA, Buenos Aires, Berisso, 'Los Talas' (34°53' S, 57°50' W) (CHMLP 4928).

**Other material.** One sagittally sectioned specimen and one specimen fixed in Bouin and preserved in 70° alcohol (CHMLP 4929).

**Etymology.** Refers to the large sickle-like spine of the stylet (Greek *falx* = sickle).

**Diagnosis.** Stylet girdle reticulate with a 'window-like' structure opposite to the lateral stylet opening. Two types of spine are present: (1) a pair of large, pincer-like and grooved spines, strongly curved (the external spine curves toward the interior, the internal one in the opposite direction) and (2) numerous fine straight spines.

#### Description

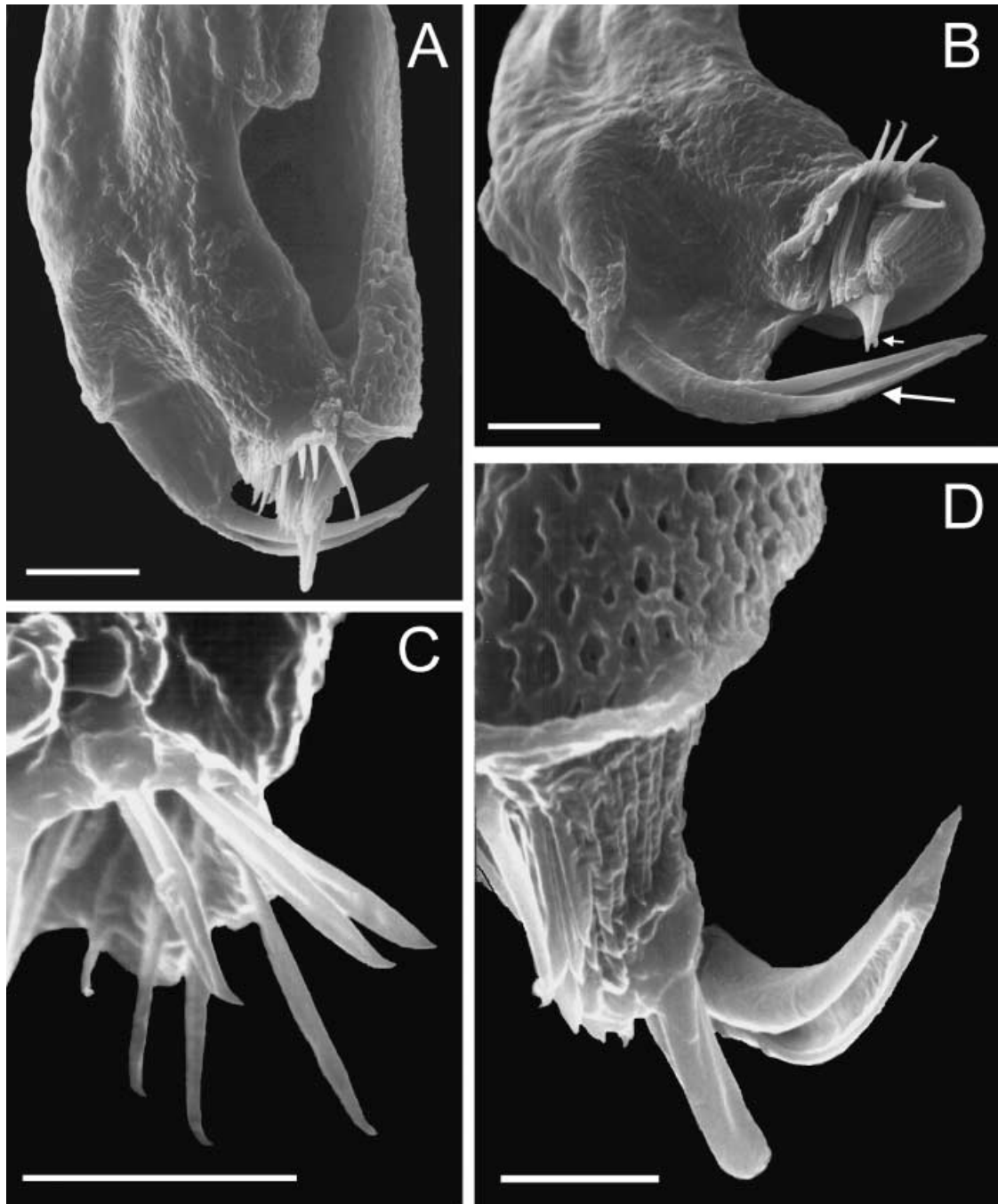
Length of sexually mature individuals ranges from 630 to 670  $\mu$ m, maximum width 190  $\mu$ m. Body spindle-like, rounded anteriorly and pointed posteriorly with small adhesive papillae (Fig. 1B). Colour of body whitish-transparent. Epithelium with polygonal, homogeneous ciliated cells, longer and semirigid cilia in the posterior extreme and anterior parts

of the body. Adenal rhabdites distributed in groups of 3–4. Basement membrane well developed. The body musculature consists of longitudinal inner muscle layers and circular outer muscle layers that are more developed in the posterior half of the body. Adhesive glands are also located in posterior region. Oral pore located antero-ventrally. Pharynx is barrel-like (1/3 the length of the body) with 10 short cylindrical papillae in the anterior end. Oesophageal glands open in the transition area between the pharynx and the intestine. Intestinal epithelium differs among the studied specimens.

Brain dorsal to the pharynx with prolongations extending toward the anterior and posterior end of the body. Two black, bean-like eyes with accumulations of pigment attached to the brain. A single ovary lies dorsal to the intestine in the posterior third of the body. The oviduct dilates to form the receptaculum seminis. The uterus lies between the short ductus communis and the atrium genitale, and may contain a single ovoid egg. A pair of simple smooth vitellaria runs first (from the anterior end of the intestine) latero-ventrally, and ends dorsally. The vitelline ducts merge into a common duct before reaching the ductus communis. The saculiform bursa copulatrix lies between the sexual organs and opens into the atrium communis. Its walls are sinuous and lined with well-developed muscle layers. The cement glands open near the genital pore, between four strong muscle layers (Fig. 1C).

The testes sit near the intestine in the middle of the body, lying ventrally to the vitellaria. The vasa deferentia originate in the testes and extend into the vesicula seminalis. The vesicula seminalis is located proximally in the male organ, and distally are the vesicula granulorum and the ductus ejaculatorius. The stylet is open laterally and is 85–88  $\mu$ m long. Stylet girdle reticulate. The total length of the girdle is greater than the total length of the spines (Fig. 1A). Girdle is fibrous proximally with clear perforations distally (Fig. 3D). In the middle of the girdle, approximately opposite the lateral opening, fibres join together to produce a 'window-like' structure. We can distinguish two types of spines (Fig. 3A,B); group (a) consists of a pair of large grooved spines, strongly curved and pincer-like, while group (b) comprises numerous fine, straight spines.

Spines of group (a) protrude laterally from the area of the girdle located opposite the lateral opening. The most external spine curves toward the interior, and has a conspicuous bulb-like structure proximally near the spine's point of origin (Figs 2 and 3B,D). The second spine is strongly curved in the opposite direction, and forked (Fig. 3B). Both spines are grooved (Fig. 3B,D). Coming together distally, these two spines form a pincer-like complex. These large spines divide the finer spines of group (b) into two subgroups: (1) a single row of spines that protrudes from the distal edge of the girdle; (2) numerous, fine spines on the opposing side of the girdle that appear at various levels along the girdle. The fine spines



**Fig. 3** A–D. SEM of extracted male copulatory organ of *Gieysztoria falx*. —A. Dorsal view of stylet (scale bar: 10 µm). —B. Lateral view of stylet; large arrow shows strongly grooved spine; short arrow shows the extreme forking of other large spine (scale bar: 10 µm). —C. Detail of spines showing extreme curvature (scale bar: 5 µm). —D. Detail of girdle and spines (scale bar: 5 µm).

of group (b) all reach the same level distally creating a brush-like appearance; all have a longitudinal groove and a slightly curved distal end (Fig. 3C).

*Remarks.* The morphology of the stylet places *Gieysztoria falx*

into the Inaequales group (Fenestratae subgroup) (Luther 1955). It is important to note that the most complex stylet within the genus is found in species of the subgroup Fenestratae (e.g. *G. intricata* (Marcus, 1946), *G. therapaina* (Marcus, 1946) and *G. complicata* (Fuhrmann, 1914)).

The stylet of the new species strongly resembles that of *G. therapaina*, although the latter's is generally shorter (33 µm) and has only one large curved spine. Additionally, a 'pedunculate comb' (a group of spines that originates on a peduncle) (Marcus 1946) is present on *G. therapaina* — a characteristic that is not observed on *G. falx*.

The stylet of *G. intricata* resembles that of *G. falx*, but has four, instead of two, groups of clearly differentiated spines. Other differences between these species include the presence of fine hairs on the large spine of *G. intricata* (which are absent in *G. falx*), and the greater overall length of the stylet of *G. intricata* (120 µm).

The stylet of *G. complicata* also displays some similarities with that of *G. falx*, but the large spines of the latter are curved toward the interior and converge distally to form a pincer-like structure. In contrast, the large spines of *G. complicata* are parallel, curve toward the exterior and are proportionally shorter, while the fine spines that form 'the brush' are longer. The stylet of *G. complicata* is generally longer (108 µm) than that of *G. falx*.

Based on these comparisons, we have determined that the diagnostic features of the stylet of *G. falx* are: (1) presence of a bulb on the base of the largest spine (2) forked end of the second largest spine and (3) pincer-like shape of both large spines (Fig. 2).

## Phylogenetic analysis

### Methods

*Gieysztoria falx* and 32 other *Gieysztoria* species were used for the phylogenetic analysis. All the species belonging to the Inaequales group were included as well as the rest of the species previously identified for South America (Table 1, Fig. 4). The following species, all placed within Inaequales by Luther (1955) were not used in the analysis owing to their scarce descriptions which did not allow us to obtain enough characters for the analysis: *Gieysztoria beklemischevi* Luther, 1955, *G. beltrani* (Gieysztor, 1931) *G. capreola* (Steinböck, 1948) *G. koiwi* (Eggers, 1925), *G. (Marcusiella) okugawai* Ruebush & Hayes, 1939, *G. semispinosa* (Fulinski & Szynal, 1927) and *G. triquetra orientalis* Nasonov, 1929. In addition, *G. reggae* Therriault & Kolasa, 1999 and *G. rastafariae* Ther-

**Table 1** All *Gieysztoria* species previously identified for South America and others belonging to the 'Inaequales' group used for the phylogenetic analysis. Their distribution is included.

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|                                                                                                                                       |
|---------------------------------------------------------------------------------------------------------------------------------------|
| <i>Gieysztoria (Marcusiella) thymara</i> (Marcus, 1946) Luther, 1955. Sao Paulo, Brazil.                                              |
| <i>Gieysztoria acaraiia</i> (Marcus, 1946) Luther, 1955. Sao Paulo, Brazil.                                                           |
| <i>Gieysztoria bellis</i> (Marcus, 1946) Luther, 1955. Sao Paulo, Brazil.                                                             |
| <i>Gieysztoria complicata</i> (Fuhrmann, 1914) Luther, 1955. Curitiba, Brazil. High plateau of Bogotá, Colombia. Lake Titicaca, Peru. |
| <i>Gieysztoria coronae</i> Noreña-Janssen, 1995. El Tigre lagoon, Santa Fe and Puerto Blest, Rio Negro, Argentina.                    |
| <i>Gieysztoria cypris</i> (Marcus, 1946) Luther, 1955. Sao Paulo, Brazil.                                                             |
| <i>Gieysztoria dodgei</i> (Graff, 1911) Luther, 1955. United States of America.                                                       |
| <i>Gieysztoria donnae</i> Young, 1977. Kenya.                                                                                         |
| <i>Gieysztoria euchroa</i> (Gieysztor, 1926) Luther, 1955. Germany, Poland, Italy.                                                    |
| <i>Gieysztoria evelinae</i> (Marcus, 1946) Luther, 1955. Sao Paulo, Brazil.                                                           |
| <i>Gieysztoria falx</i> n. sp. Los Talas, Berisso. Buenos Aires, Argentina.                                                           |
| <i>Gieysztoria hymanae</i> (Marcus, 1946) Luther, 1955. Sao Paulo, Brazil.                                                            |
| <i>Gieysztoria infundibuliformis</i> (Fuhrmann, 1894) Luther, 1955. Europe.                                                           |
| <i>Gieysztoria intricata</i> (Marcus, 1946) Luther, 1955. Sao Paulo, Brazil.                                                          |
| <i>Gieysztoria joannae</i> Young, 1977. Kenya.                                                                                        |
| <i>Gieysztoria kolasai</i> Young, 1977. Kenya.                                                                                        |
| <i>Gieysztoria ornata</i> (Hofsten, 1907) Luther, 1955. Europe. Sao Paulo, Brazil.                                                    |
| <i>Gieysztoria pavimentata</i> (Beklemishev, 1926) Luther, 1955. Scandinavia, Russia, Yugoslavia.                                     |
| <i>Gieysztoria pseudodiadema</i> Noreña-Janssen, 1995. El Tigre lagoon, Santa Fe, Argentina.                                          |
| <i>Gieysztoria quadrata</i> Noreña-Janssen, 1995. El Tigre lagoon, Santa Fe, Argentina.                                               |
| <i>Gieysztoria quadridensoides</i> (Fuhrmann 1914) Luther, 1955. Fault of Tequendama, Colombia.                                       |
| <i>Gieysztoria saganae</i> Young, 1977. Kenya.                                                                                        |
| <i>Gieysztoria santafeensis</i> Noreña-Janssen, 1995. El Tigre lagoon, Santa Fe, Argentina.                                           |
| <i>Gieysztoria therapaina</i> (Marcus, 1946) Luther, 1955. Sao Paulo, Brazil.                                                         |
| <i>Gieysztoria thienemanni</i> (Reisinger, 1933) Luther, 1955. Java.                                                                  |
| <i>Gieysztoria tigrensis</i> Noreña-Janssen, 1995. El Tigre lagoon, Santa Fe, Argentina.                                              |
| <i>Gieysztoria tridesma</i> (Marcus, 1946) Luther, 1955. Sao Paulo, Brazil.                                                           |
| <i>Gieysztoria triangulata</i> (Robeson, 1931) Luther, 1955. United States of America.                                                |
| <i>Gieysztoria triquetra</i> (Fuhrmann, 1894) Luther, 1955. Germany, Switzerland, Italy, Yugoslavia.                                  |
| <i>Gieysztoria trisolena</i> (Marcus, 1946) Luther, 1955. Sao Paulo, Brazil.                                                          |
| <i>Gieysztoria uncia</i> (Marcus, 1946) Luther, 1955. Sao Paulo, Brazil.                                                              |
| <i>Gieysztoria variata</i> Noreña-Janssen, 1995. El Tigre lagoon, Santa Fe, Argentina.                                                |
| <i>Gieysztoria virgulifera</i> (Plotnikov, 1906) Luther, 1955. Europe.                                                                |

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**Table 2** Characters and character states.

|    |                                                                                                                                                                                                                                                                            |
|----|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1  | Pharynx size. Small, smaller than 1/4 of the body length (0); large, between 1/4 and 1/3 of the total length (1).                                                                                                                                                          |
| 2  | Pharyngeal papillae. Absent (0); present (1).                                                                                                                                                                                                                              |
| 3  | Number of pharyngeal papillae: 4–6 (0); 8–12 (1); 14–20 (2).                                                                                                                                                                                                               |
| 4  | Intestinal diverticles. Absent (0); present (1).                                                                                                                                                                                                                           |
| 5  | Vitellaria. Smooth, cylindrical (0); feathery, with finger-like prolongations that can imbricate centrally (1).                                                                                                                                                            |
| 6  | Receptaculum seminis. A dilatation of the oviduct (0); pedunculate (1); with basal constriction (2).                                                                                                                                                                       |
| 7  | Shape of the bursa copulatrix. Sac-like, irregular, with undulated wall (0); claviform, bursa-stalk originated in the atrium genitale (1).                                                                                                                                 |
| 8  | Musculature of the bursa copulatrix. Partially covered with muscles (0); totally covered with muscles (1).                                                                                                                                                                 |
| 9  | Sperm-box ('Spermatodose'). Absent (0); present (1). Luther (1955) described the 'Spermatodose' as an internal layer present in some bursas. The function of this structure is to protect the bursa during the copulation. A chitinous nature of the sperm-box is assumed. |
| 10 | Number of eggs. One (0); more than one (1).                                                                                                                                                                                                                                |
| 11 | Egg-shape. Ovoid, with a tapered end (0); ellipsoid or triangular (squash preparations) (1); 'lemon-like', one end with a small projection (2); oval (3).                                                                                                                  |
| 12 | Origin of the vasa deferentia. Medial area of the testes (0); at one end of the testes (1).                                                                                                                                                                                |
| 13 | Entrance of the vasa deferentia into the copulatory organ. Between the vesicula seminalis and vesicula granulorum (0); directly into the vesicula seminalis (1).                                                                                                           |
| 14 | Mode of entrance of the vasa deferentia into the copulatory organ. Together (0); separate (1).                                                                                                                                                                             |
| 15 | Transversal duct. Absent (0); present (1).                                                                                                                                                                                                                                 |
| 16 | Storage of the eggs. In the uterus (0); in the parenchyma (1); both (2).                                                                                                                                                                                                   |
| 17 | Proximal end of the stylet. Two branches (0); girdle (1).                                                                                                                                                                                                                  |
| 18 | Texture of the proximal end of the stylet. Smooth (0); fibrous (1); reticulate (2).                                                                                                                                                                                        |
| 19 | Ratio between the height of the proximal end of the stylet and the length of the spines. Minor or equal to the length of the spines (0); greater than the length of the spines (1).                                                                                        |
| 20 | Discontinuity in the girdle. Absent (0); present (1).                                                                                                                                                                                                                      |
| 21 | Position of the girdle discontinuity. Lateral (0); dorsal (1); ventral (2).                                                                                                                                                                                                |
| 22 | Girdle with two rings united by bridges. Absent (0); present (1).                                                                                                                                                                                                          |
| 23 | Girdle with a window. Absent (0); present (1).                                                                                                                                                                                                                             |
| 24 | Number of spines. Fewer than 30 (0); more than 31 (1).                                                                                                                                                                                                                     |
| 25 | Kind of spines. Equal or subequal to each other (0); different from each other (1).                                                                                                                                                                                        |
| 26 | Disposition of the spines. Symmetrical (0); asymmetrical (1).                                                                                                                                                                                                              |
| 27 | Length of the spines. Equal length (0); different length (1).                                                                                                                                                                                                              |
| 28 | Oblong spines (at least one of them). Absent (0); present (1).                                                                                                                                                                                                             |
| 29 | Total length of the stylet. Minor than 60 µm (0); between 60 and 100 µm (1); greater than 100 µm (2).                                                                                                                                                                      |
| 30 | Robust spines. Absent (0); present (1).                                                                                                                                                                                                                                    |
| 31 | Number of robust spines. Fewer than four (0); more than four (1).                                                                                                                                                                                                          |
| 32 | One or two spines much more stronger than the rest. Absent (0); present (1).                                                                                                                                                                                               |
| 33 | Present of a medial appendix in the stylet. Absent (0); present (1).                                                                                                                                                                                                       |
| 34 | Spines originating at the same level. Absent (0); present (1).                                                                                                                                                                                                             |
| 35 | Ratio length/base of the spines. Conic section (conical) (0); tubular (1); both (2).                                                                                                                                                                                       |
| 36 | Cuticular hairs on some spines. Absent (0); present (1).                                                                                                                                                                                                                   |
| 37 | Bifid spine. Absent (0); present (1).                                                                                                                                                                                                                                      |
| 38 | Zoochlorellae. Absent (0); present (1).                                                                                                                                                                                                                                    |

riault & Kolasa, 1999 were not considered in the analysis, since descriptions are based only on the characters of the stylets. Representatives of other freshwater genera of the Dalyelliidae family were selected as outgroup, those described for South America as well as other species from other geographical regions: *Microdalyellia sawayai* Marcus, 1946, *M. fairchildi* (Graff, 1911), *Dalyellia viridis* (Shaw, 1791) *D. obscura* Noreña-Janssen, 1995, *D. tatriva* Gieysztor, 1934, *D. cetica* Reisinger, 1924 and *D. styriaca* Reisinger, 1924.

Characters and character states selected for analysis (Table 2) were obtained from direct observation of collected material, and from an exhaustive review of the bibliographic literature in those instances where material was lacking (Reisinger 1924; Marcus 1946; Luther 1955; Young & Young 1976; Noreña-Janssen 1995; Therriault & Kolasa 1999). The characters were coded according to the format required for

NONA 2.0 (Goloboff 1993). A heuristic search with random addition sequences and swapping by tree-bisection reconnection was applied (option mult\*N) to find the most parsimonious cladogram. Unknown character states or states that had not been previously described were coded using a question mark ('?'), while nonapplicable character state codes were followed by a dash ('-') (Table 3). All characters were treated as unordered and with equal weights. WINCLADA 1.00.08 (Nixon 2002) was employed for optimization of characters (fast optimization).

## Results and discussion

Four equally parsimonious trees were obtained, with 167 steps, consistency index (CI) of 0.25, retention index (RI) of 0.59, starting from the matrix (Table 3); characters 4 and 36 were excluded because they are not informative and charac-

Table 3 Data matrix used in parsimony analysis.

| Taxa/character number       | 12345 | 1<br>67890 | 12345 | 2<br>67890 | 12345 | 3<br>67890 | 12345 | 678 |
|-----------------------------|-------|------------|-------|------------|-------|------------|-------|-----|
| <i>G. acariaia</i>          | 11100 | 01000      | 21100 | 01200      | –0001 | 01001      | 00110 | 001 |
| <i>G. bellis</i>            | 00–01 | 21000      | 11010 | 01001      | 11000 | 00001      | 10011 | 000 |
| <i>G. complicata</i>        | 1??00 | 01000      | 31??0 | 01211      | 10011 | 11111      | 01000 | 000 |
| <i>G. coronae</i>           | 0??00 | 10100      | 0???0 | 01000      | –0000 | 00000      | –0010 | 000 |
| <i>G. cypris</i>            | 10–0? | 01000      | ?1110 | 01201      | 20001 | 01121      | 10002 | 000 |
| <i>G. dodgei</i>            | ?1100 | 00100      | 3?100 | 0120?      | ?0001 | 011?1      | 01011 | 001 |
| <i>G. donnae</i>            | 0??01 | 21000      | ?1110 | 0100?      | ?0001 | 01101      | 01002 | 000 |
| <i>G. euchroa</i>           | 01100 | 01001      | 2???0 | 01101      | ?0101 | 11011      | 01002 | 011 |
| <i>G. evelinae</i>          | 00–01 | 11000      | 11100 | 01101      | 11001 | 01110      | –0002 | 000 |
| <i>G. falx</i>              | 11100 | 00100      | 0???0 | 01211      | 00111 | 11111      | 01002 | 010 |
| <i>G. hymanae</i>           | 10–01 | 21010      | 30110 | 01101      | 11001 | 00110      | –0012 | 000 |
| <i>G. infundibuliformis</i> | ???00 | 01000      | 3???0 | 0120?      | ?0101 | 01010      | –1110 | 001 |
| <i>G. joannae</i>           | 0??00 | 21000      | ?1110 | 0100?      | ?1001 | 01101      | 00000 | 000 |
| <i>G. intricata</i>         | 1??00 | 2?100      | 01110 | 01211      | 10111 | 11121      | 01000 | 100 |
| <i>G. kolasai</i>           | 1??00 | 01000      | ?1110 | 0100?      | ?0001 | 11111      | 01002 | 000 |
| <i>G. pavimentata</i>       | 01100 | 01110      | 3?100 | 0120?      | ?0101 | 10110      | –1002 | 001 |
| <i>G. ornata</i>            | 00–01 | 11000      | 01010 | 0100?      | ?1000 | 00000      | –0010 | 001 |
| <i>G. pseudodiadema</i>     | 0??00 | 01000      | ?1110 | 01001      | ?0000 | 11101      | 10010 | 000 |
| <i>G. quadrata</i>          | 11?00 | 11000      | 30100 | 01001      | ?1000 | 01011      | 00010 | 000 |
| <i>G. quadridensoides</i>   | 0???? | ?????0     | ????0 | 0100?      | ?0000 | 00111      | 00010 | 000 |
| <i>G. saganae</i>           | 0??00 | 01000      | ?0110 | 0100?      | ?0001 | 11111      | 01000 | 000 |
| <i>G. santafeensis</i>      | 11?00 | 10110      | 11010 | 01001      | ?0001 | 01020      | –0012 | 000 |
| <i>G. therapaina</i>        | 11100 | 01000      | 01110 | 01101      | 10111 | 11121      | 01002 | 000 |
| <i>G. thienemanni</i>       | 11200 | 00110      | 31??0 | 0?00?      | ???01 | 11111      | 01002 | 001 |
| <i>G. thymara</i>           | 01110 | 01000      | 31010 | 01201      | 00011 | 11111      | 00002 | 001 |
| <i>G. tigrensis</i>         | 11100 | 10110      | 3?210 | 01100      | –0000 | 01010      | –0012 | 000 |
| <i>G. triangulata</i>       | 0??01 | 01?00      | 1???0 | 01001      | ?1001 | 011?0      | –0002 | 000 |
| <i>G. tridesma</i>          | 10–01 | 21000      | 01110 | 01111      | 11000 | 01001      | 10000 | 000 |
| <i>G. triquetra</i>         | 0??01 | 20100      | 31100 | 0110?      | ?0001 | 01120      | –0012 | 001 |
| <i>G. trisolena</i>         | 11201 | 20100      | 31100 | 01101      | 11001 | 00010      | –0011 | 000 |
| <i>G. uncia</i>             | 11100 | 21000      | 31110 | 01101      | 10011 | 11101      | 00000 | 000 |
| <i>G. variata</i>           | 11000 | 10110      | 31100 | 01101      | ?0011 | 01010      | –0002 | 000 |
| <i>G. virgulifera</i>       | 11100 | 01010      | 31010 | 0120?      | ?0111 | 11101      | 01000 | 000 |
| <i>M. fairchildi</i>        | 1??00 | 00100      | 300?0 | 0000–      | –––11 | 01110      | –0101 | 000 |
| <i>M. sawayai</i>           | 11201 | 01001      | 31110 | 2010–      | –––01 | 01110      | –0100 | 000 |
| <i>D. obscura</i>           | ????? | 10100      | 1?0?1 | 1010–      | –––00 | 00121      | 00010 | 00? |
| <i>D. viridis</i>           | 01101 | 10?01      | 31011 | 1011–      | –––00 | 01020      | –0000 | 001 |
| <i>D. tatrica</i>           | 01?01 | 10?01      | 31111 | 1000–      | –––10 | 01020      | –0100 | 001 |
| <i>D. styriaca</i>          | 01201 | 10?01      | 31101 | 1000–      | –––10 | 01020      | –0100 | 001 |
| <i>D. cetica</i>            | 01201 | 10?01      | 31101 | 1000–      | –––11 | 11020      | –0100 | 001 |

ters 1 and 28 because they have a high homoplasy. Figure 5 shows the strict consensus of the four trees.

The analysis carried out proves the monophyly of the species studied (Fig. 5), their synapomorphy being the presence of a girdle in the proximal end of the stylet (character 17). *Microdalyellia fairchildi* is the sister species of the ingroup.

The analysis shows that the species allocated to either Aequales and Inaequales following Luther's (1955) criteria do not in fact form natural groups. *Gieysztoria tridesma*, *G. coronae*, *G. ornata* and *G. bellis* on the one hand and *G. pseudodiadema*, *G. quadrata* and *G. quadridensoides* on the other, both considered as Aequales, are interspersed among

the species considered as Inaequales. Between these two groups of Aequales, *G. uncia* and *G. joannae* are found. *Gieysztoria uncia* was hitherto considered to belong to Aequales (species with similar types of spines); however, based on the results of our analysis which clearly reveal heterogeneity of spine structure — 4 large spines (18 µm in length) and numerous small spines (from 7 to 12 µm in length) — it should be assigned to Inaequales. *Gieysztoria joannae* is assigned to Inaequales owing to the presence of spines differing from one another in the stylet, in spite of their being symmetrically disposed.

Results from the analysis demonstrated that *G. falx* is closely

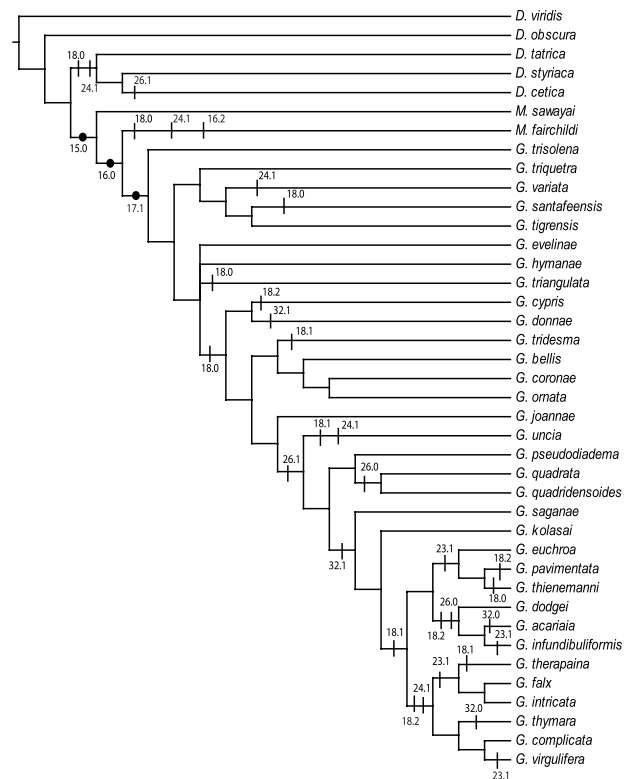


**Fig. 4** Map. 1, Sao Paulo, Brazil; 2, Curitiba, Brazil; 3, 'El Tigre' lagoon, Santa Fe, Argentina; 4, Los Talas, Berisso, Argentina; 5, Puerto Blest, Argentina; 6, Lake Titicaca, Peru; 7, High plateau of Bogotá, Colombia.

related to *G. intricata* (sister species) and more distantly related to *G. therapaina*. Even though *G. falx* presents a stylet comparable to that of *G. complicata*, the latter is more closely related to *G. virgulifera* and *G. thymara* than to the new species.

*Gieysztoria falx* and the other species with the presence of a window in the girdle (character 23) and/or reticulate proximal end of the stylet (character 18) form a group (the exception being *G. cypris*, in spite of its having a reticulate girdle). It is within this group that the species with unequal spines are found (character 25). There are two subgroups. The first comprises *G. therapaina*, *G. falx*, *G. intricata*, *G. thymara*, *G. complicata* and *G. virgulifera*, all with a large number of spines (character 24) arranged asymmetrically (character 26) and excepting *G. virgulifera*, all of them South American. The second, with fewer spines, comprises *G. eubroa*, *G. pavimentata* and *G. thienemanni* (with asymmetrical spines) and *G. dodgei*, *G. acariaia* and *G. infundibuliformis* (with symmetrical spines); only *G. acariaia* is South American.

Luther (1955) assigned the specimens described by Marcus (1946) as *G. ornata* to *G. rubra* based on the shape of their vitellaria. From morphological characters and an exhaustive



**Fig. 5** Strict consensus of four equal parsimonious trees. Circles represent synapomorphic characters, lines represent some relevant characters and their states.

review of the descriptions offered by Marcus (1946) and Luther (1955), we consider that the Brazilian specimens belong to the species *G. ornata* and not *G. rubra*, due to the shape and structure of the spines that make up the stylet. The shape of the vitellaria is a character that varies within the same species, depending on the state of development; therefore it ought not to be the only criterion on which to base a change of species. Reconsidering the species described by Marcus (1946) for Brazil as *G. ornata* extends the distribution range of this species, hitherto considered limited to the Holarctic region, to the Neotropical region.

To reach a definitive resolution of the phylogenetic relationships of *Gieysztoria* species requires more morphological information than we currently have, since some characters appear to be contradictory. There is, in fact, a general lack of information about turbellarian fauna in South America. The preliminary results presented here have, however, established the monophyly of *Gieysztoria*, as well as the importance of the stylet morphology in establishing the relationship among the species. This study constitutes the first stage of our study of the relationships among the species of the genus.

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