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Authors: Artois, Tom, Willems, Wim, De Roeck, Els, Jocqué, Merlijn, and Brendonck, Luc

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Freshwater Rhabdoceola (Platyhelminthes) from Ephemeral Rock Pools from Botswana, with the Description of Four New Species and One New Genus

Tom Artois^{1*}, Wim Willems¹, Els De Roeck², Merlijn Jocqué² and Luc Brendonck²

¹*Limburgs Universitair Centrum, Centre for Environmental Sciences, Research Group Biodiversity, Phylogeny and Population Studies, Dept. SBG, Universitaire Campus Gebouw D, B-3590 Diepenbeek, Belgium.*

²*Catholic University of Leuven, Laboratory of Aquatic Ecology, Charles de Bériotstraat 32, B-3000 Leuven, Belgium*

ABSTRACT—Four new species of freshwater rhabdoceol flatworms from ephemeral rock pools in south-eastern Botswana are described and discussed. Two of them, *Syringoplana kolasai* n. gen. n. sp. and *Mesostoma thamagai* n. sp. belong to the Typhloplanidae Graff, 1905. The unique construction of the excretory system is the main characteristic of *S. kolasai*. *M. thamagai* can be separated from other *Mesostoma* Ehrenberg, 1837 species by the presence of a bundle of eosinophilic glands at the transition from oviduct to seminal receptacle. The other two taxa, *Gieysztoria isoldeae* n. sp. and *G. faubeli* n. sp. belong to the Dalyelliidae Graff, 1905. *G. isoldeae* is characterised by the presence of four separate hollow spines in the male atrium, which are connected to two accessory glandular organs. *G. faubeli* can be separated from other *Gieysztoria* Ruebush and Hayes, 1939 species by the detailed construction of the stylet. Apart from these two species the occurrence of an unidentified *Microdalyellia* Gieysztor, 1938 species is mentioned.

Key words: Rhabdoceola, *Syringoplana*, *Mesostoma*, *Gieysztoria*, new taxa

INTRODUCTION

Although free-living flatworms (“Turbellaria”) are of major importance in freshwater and marine ecosystems, our knowledge of this group is very scant. This is especially true for tropical and subtropical areas, such as South America, Australia and Africa. Knowledge of the African freshwater Turbellaria mostly comes from older literature. A comprehensive list of the African species of freshwater Turbellaria and of the literature concerning them is given by Young (1976). This author recognises 83 valid species. Since then not much work on African freshwater Turbellaria has been done (De Vries, 1988; Kolasa, 1976; Kolasa and Mead, 1981; Young, 1977).

In this contribution we describe four new rhabdoceol species from ephemeral rock pools in southeastern Botswana. These pools were sampled within the framework of an intercontinental comparative study on the faunal community structures of ephemeral rock pools in subtropical

areas. Two of the species described here, *Mesostoma thamagai* n. sp. and *Syringoplana kolasai* n. gen. n. sp., belong to the large and widespread rhabdoceol taxon Typhloplanidae Graff, 1905. The other two, *Gieysztoria isoldeae* n. sp. and *G. faubeli* n. sp. fit into the Dalyelliidae Graff, 1905, another species-rich and widespread taxon of rhabdoceol flatworms. A third representative of the Dalyelliidae, a species of *Microdalyellia* Gieysztor, 1938, was also collected. However, the only whole mounted specimen of this species is in such bad condition that it does not allow further identification.

MATERIAL AND METHODS

All turbellarians were hatched from dry sediment samples containing the resting propagules collected from ephemeral rock pools on a granite escarpment in southeastern Botswana. The samples were taken with a spoon and a little brush during the dry phase of the pools. Sediment was transported to Belgium in plastic bags. To obtain hatchlings, about 200 g of sediment was incubated in distilled water, under 24-hour light conditions and at a temperature of 25°C. Usually the first turbellarians were visible after one or two days. They were fed with living *Daphnia* and fairy shrimps (freshwater Anostraca).

* Corresponding author: Tel. +32-11-268383;
FAX. +32-11-268301.
E-mail: tom.artois@luc.ac.be

Specimens of most species could be studied alive. If hard parts were present, whole mounts were prepared using lactophenol. Specimens intended for sectioning were fixed using hot (50°C) Bouin's fixative. They were embedded in paraffin and serially sectioned (5 µm), then stained with Heidenhain's haematoxylin, using erythrosine as counterstain.

Hard parts were measured axially. Drawings without a scale are freehand.

Type material will be deposited in the collections of the research group Biodiversity, Phylogeny and Population Studies of the Limburgs Universitair Centrum (LUC), Diepenbeek, Belgium.

TAXONOMIC ACCOUNT

TYPHLOPLANIDAE GRAFF, 1905

Syringoplana kolasai n. gen. n. sp.

(Fig. 1)

Locality. Botswana, Thamaga (24°41'50"S, 25°31'00"E), ephemeral rock pools on isolated granite domes (type locality). Sediment collected 22/12/1998, inundated in the laboratory 4/10/2002.

Material. Observations on a live animal. Several sectioned specimens, a sagittally-sectioned one designated holotype (LUC nr. 232), the others paratypes (LUC nr. 233–238).

Etymology. The genus is named after Syrinx, one of the Naiads (water nymphs) of Greek mythology. Species epithet in honour of Dr. Jurek Kolasa (Hamilton, Canada).

Description. The animals are relatively small, oval-shaped, with a blunt rostral and a pointy caudal end ("tail"). Animals coloured dark brownish green, owing to the presence of a subepidermal pigment, which is less dense at the ventral side. Eyes absent.

Epidermis cellular, ciliated all over the body surface. Dermal rhabdites absent. Anteriorly there are some large rhabdite glands that produce large, adenal rhabdites.

The excretory system consists of two protonephridial ducts, situated at both sides of the body. They end in a common excretory pore, which is just caudal from the gonopore and lies in a ventro-caudal pit. Several large, coarse-grained basophilic glands surround this pit. The excretory pore can be closed by a sphincter.

The mouth is at 1/3 of the body length and is surrounded by a weak sphincter. The prepharyngeal cavity is relatively small and lined with a very low epithelium without nuclei. It is surrounded by a weak internal circular and a strong external longitudinal muscle layer. The globular pharynx is very large and oriented vertically. Its distal border is lined with a very low, membranous, anucleated epithelium, with very short cilia. The pharynx lumen is also lined with a very low, anucleated epithelium that has no cilia. The pharynx musculature consists of a weak internal circular muscle layer (around the pharynx lumen), a thick internal longitudinal muscle layer consisting of 24–26 muscles, an external circular and an external longitudinal muscle layer underneath the septum of the bulb, and a longitudinal muscle layer just outside the septum, continuous with the longitudi-

nal muscles of the prepharyngeal cavity. Strong radial muscles connect the wall of the pharynx lumen with the septum of the bulb. There are two types of pharyngeal glands, with the eosinophilic fine-grained ones entering the pharynx lumen proximally from the basophilic coarse-grained ones.

The genital system lies just caudally from the pharynx, the gonopore situated ventrally at about 2/3 of the body length, just in between the excretory pore and the mouth. The two large testes are situated at both sides of the body. The single ovary is situated at the level of the gonopore. The vitellaria are long and narrow, extending dorsally from the testes at both sides of the body. The common genital atrium is surrounded by an inner circular and an outer longitudinal muscle layer, and lined with a high, nucleated, lightly sclerotised epithelium.

The copulatory organ enters the genital atrium dorsally. It is a large, ovoid bulb, with a septum enclosing the seminal vesicle, the prostate glands and the cirrus (conjuncta-duplex copulatory organ; terminology of Karling, 1956). Two spirally-running muscle layers surround the bulb. The vasa deferentia join each other just before entering the copulatory bulb at its proximal end. Within the bulb the seminal duct enlarges to form a seminal vesicle, which is surrounded by a weak circular muscle layer. The seminal duct enters the cirrus about midway along the bulb. The cirrus consists of a rather broad tube armed with small, sharp spines and is surrounded by an inner circular and an outer longitudinal muscle layer. The coarse-grained basophilic prostate glands enter the copulatory bulb at the same place as do the vasa deferentia, feeding into the prostate vesicle, which in turn enters the cirrus at the same place as does the seminal vesicle. The internal prostate vesicle is surrounded by a weak circular muscle layer.

The female duct enters the genital atrium caudally, while proximally it ends in the small ovary. Somewhat halfway, it makes a 180° turn and starts running anteriorly. At the turn, a large bundle of fine-grained eosinophilic glands enters the duct. Just before it reaches the ovary it widens to a seminal receptacle, containing eosinophilic glandular secretion and many sperm. The duct is lined with a high, nucleated epithelium and surrounded by an inner circular and an outer longitudinal muscle layer.

The vitellogonoduct could not be seen. The single uterus leaves the common genital atrium anteriorly.

Diagnosis. *Syringoplana* n. gen. Typhloplanidae with pharynx rosulatus in the first 1/3 of body. Dermal rhabdites lacking. Excretory system with two lateral nephridial ducts ending in a single ventral excretory pore, caudal from the gonopore. Excretory pore surrounded by large basophilic glands. Testes paired, lying laterally from the pharynx, ventrally from the vitellaria. Copulatory organ of the duplex-type, caudal to the pharynx. Seminal vesicle single, intracapsular. Prostate vesicle intracapsular. With a cirrus with fine spines. Ovary single. Oviduct enlarged to a seminal receptacle. Large bundle of eosinophilic glands entering the female duct

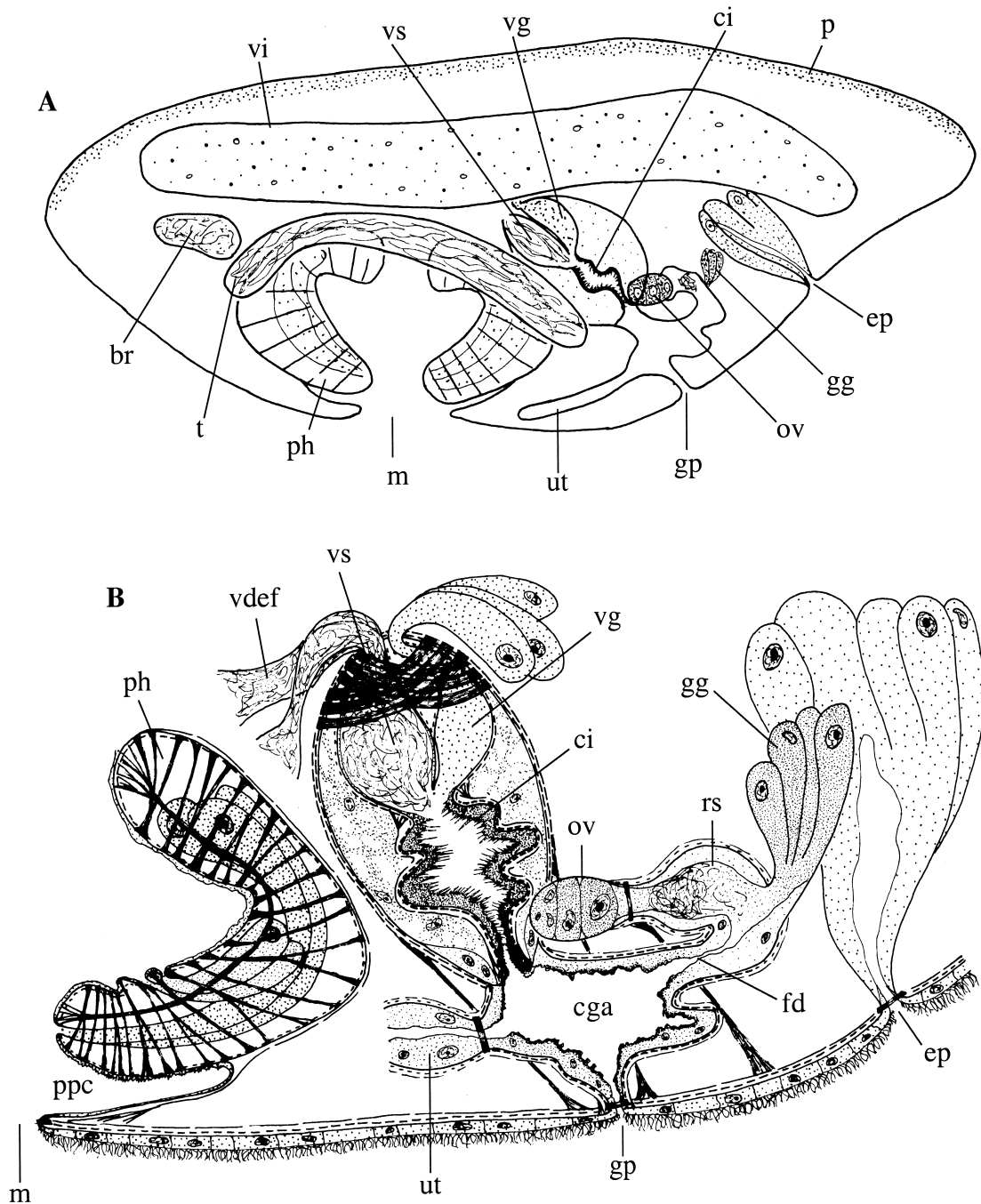


Fig. 1. *Syringoplana kolasai* n. gen. n. sp. (A) Semi-diagrammatic reconstruction of the whole animal from sagittal sections. (B) Reconstruction of the genital system (holotype). br, brain; cga, common genital atrium; ci, cirrus; ep, excretory pore; fd, female duct; gg, glands; gp, gonopore; m, mouth; ov, ovary; p, pigment grains; ph, pharynx; ppc, prepharyngeal cavity; rs, seminal receptacle; t, testis; ut, uterus; vdef, vas deferens; vi, vitellarium; vg, prostate vesicle; vs, seminal vesicle.

distally from the seminal receptacle. Type species: *Syringoplana kolasai* n. sp. Provisionally with the same diagnosis as the genus.

Discussion. *Syringoplana kolasai* differs from all other taxa of Typhloplanidae by the presence of only one ventral excretory pore, separate from the mouth, and lying just caudally from the gonopore. In most of the other species of Typhlo-

planidae, the protonephridial ducts have two separate pores at the body surface (Protoplanellinae Reisinger, 1924, Olisthanellinae Luther, 1904, Ascophorinae Findenegg, 1924, Phaenocorinae Wahl, 1910). Alternatively, the protonephridial ducts join each other and enter the prepharyngeal cavity near the mouth (Mesostominae Luther, 1904, Typhloplaninae Luther, 1904) or in the common genital atrium (Rynchomesostominae Bresslau, 1933).

Not considering the unique construction of the excretory system, the new taxon shows a combination of features characteristic of the Protoplanellinae: a typical globular, ventrally-oriented pharynx rosulatus, testes ventral to the vitellaria and the excretory ducts ending at the body surface (but separately in the Protoplanellinae). The presence of a spiny cirrus makes the copulatory organ resemble that of *Acrochordonoposthia* Reisinger, 1924. The pharynx is, however, in a more central position, in comparison with the anterior position in *Acrochordonoposthia*.

From the above it is clear that the new species cannot be put into any of the existing genera, and therefore must be placed in a genus of its own. The position of this new genus within the Typhloplanidae remains unclear.

***Mesostoma thamagai* n. sp.**

(Fig. 2)

Locality. Botswana, Thamaga (24°41'50"S, 25°31'00"E), ephemeral rock pools on isolated granite domes (type locality). Sediment collected 22/12/1998, inundated in the lab 4/10/2002.

Material. Observations on live animals. Several serially-sectioned animals, a sagittally-sectioned one designated holotype (LUC nr. 239), the others paratypes (LUC nr. 240–247).

Etymology. Named after the type locality.

Description. Elongated, elliptical animals with two eyes. They are 3–4 mm long when reared in the lab. Animals collected in the field were not measured, but appear to be somewhat larger. Adult animals coloured dark brown by subepidermal pigment. Juveniles are somewhat lighter.

Epidermis cellular, consisting of large octagonal cells, ciliated and containing numerous large, rod-shaped rhabdites.

Protonephridia extend at both sides of the body and end separately in the prepharyngeal cavity.

The pharynx is of the rosulatus type and is identical to that of other *Mesostoma* Ehrenberg, 1837 species. It is situated at 1/3 of the body length. The mouth is lined with a degenerating, ruffled epithelium (pseudociliation) and can be closed by a sphincter. The prepharyngeal cavity is lined with a low epithelium with a few nuclei. Most of the nuclei of this epithelium are, however, insunk in a nuclear pouch situated at the junction between the prepharyngeal epithelium and the pharynx bulb. The prepharyngeal cavity is surrounded by longitudinal muscles, which continue around the pharynx bulb. The distal pharynx rim is lined with a low, ciliated epithelium that becomes unciliated towards the pharynx lumen. There are 32 internal longitudinal muscles. There are three types of pharyngeal glands: coarse-grained eosinophilic, coarse-grained basophilic and fine-grained eosinophilic. All three types enter the pharynx lumen near the distal end of the pharynx bulb, the fine-grained eosinophilic ones probably most proximally.

There are two very large testes, extending at both sides of the body. They are connected to each other at three places: caudally, rostrally and at 3/4 of the body length. The very small ovary is situated just behind the pharynx, at the left hand side. The vitellaria are paired and situated ventrally to the testes.

The gonopore lies just behind the mouth, both being situated within a depression of the ventral body wall. The com-

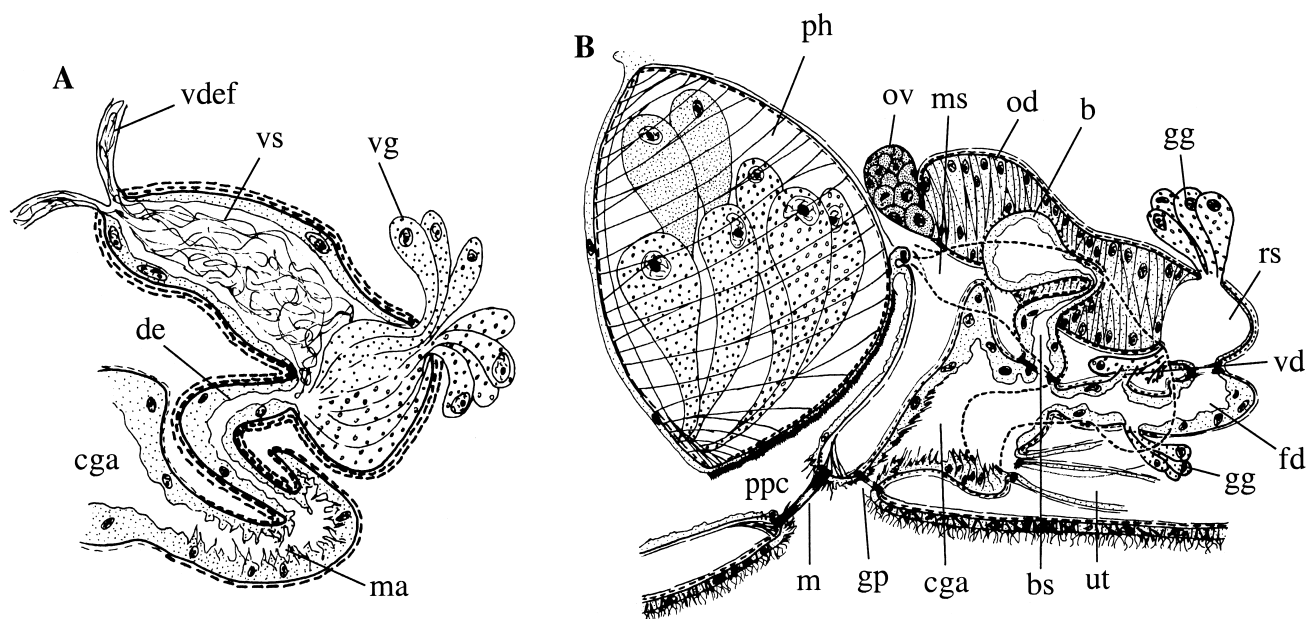


Fig. 2. *Mesostoma thamagai* n. sp. (A) Reconstruction of the male atrial system (holotype). (B) Reconstruction of the genital organs (holotype). b, bursa; bs, bursal stalk; cga, common genital atrium; de, ejaculatory duct; fd, female duct; gg, glands; gp, gonopore; m, mouth; ma, male atrium; ms, male genital system; od, oviduct; ov, ovary; ph, pharynx; ppc, prepharyngeal cavity; rs, seminal receptacle; ut, uterus; vd, vitellocyst; vdef, vas deferens; vg, prostate vesicle; vs, seminal vesicle.

mon genital atrium is very large. It is lined with a high, nucleated epithelium and surrounded by a weak inner circular and an outer longitudinal muscle layer. Towards the gonopore it narrows to a duct that is lined with a pseudociliation and surrounded by a thicker circular muscle layer.

The male atrial system (called "copulatory organ" by Noreña Janssen and Faubel, 1992) consists of a long duct that is surrounded by a pigment similar to that found beneath the epidermis. It is surrounded by two spirally-running muscle layers over its entire length. Proximally the duct is widened to form an elongated seminal vesicle, which is lined with a high, nucleated epithelium. Distally from the seminal vesicle the duct abruptly narrows to form a somewhat coiled, very muscular ejaculatory duct that is lined with a high, nucleated epithelium (the so-called "ductus ejaculatorius exterior" or "penispapilla" of Noreña Janssen and Faubel, 1992). At the transition between the seminal vesicle and the ejaculatory duct eosinophilic and basophilic prostate glands enter the male system through its caudal wall. Both types of glands are coarse-grained. Neither the glands nor the gland necks are surrounded by muscle layers ("false vesicula granulorum" of Noreña Janssen and Faubel, 1992). Distally, the ejaculatory duct merges into the male atrium, which is the most distal part of the male duct and is lined with a pseudociliation. It is surrounded by a strong circular muscle layer.

The oviduct is very long and broad and lined with a very high epithelium. A lumen could not be observed. Distally the oviduct enters a spherical seminal receptacle, which in most specimens contained many sperm. The receptacle is lined with a low epithelium without nuclei, and there is a large sphincter at its distal end. At the transition between female duct and receptacle there is a large bundle of eosinophilic glands. The most distal part of the female duct (the "ductus communis" of Noreña Janssen and Faubel, 1992) is lined with a very high, nucleated epithelium. It enters the common genital atrium dorso-caudally, ventrally from the bursal stalk. It receives the basophilic shell glands ventrally, and the viteloduct dorsally.

The uteri are paired and enter the common genital atrium separately through its caudal wall.

The bursal stalk leaves the common genital atrium dorsally from the female duct. It is lined with a high, nucleated epithelium and is surrounded by a thick circular muscle layer. The bursa proper is spherical, and in many specimens it was inflated and filled with many sperm. It is lined with a high epithelium without nuclei.

Diagnosis. *Mesostoma thamagai* n. sp. *Mesostoma* species with dark brown, subepidermal pigment. Same pigment present around the male genital system. Gonopore very close behind the mouth. Vasa deferentia entering the copulatory bulb separately from the prostate glands. Prostate glands eosinophilic and basophilic, entering the copulatory bulb dorsally. Ejaculatory duct distally very muscular, with a high epithelium. Eosinophilic glands enter the female system at the transition from oviduct to seminal receptacle.

Uteri paired.

Discussion. This species clearly belongs to the Mesostominae as defined by Noreña Janssen and Faubel (1992). The lack of a true prostate vesicle, a spermatid duct and a prepharyngeal dermal pit furthermore mark it as a *Mesostoma* species.

The most recent and comprehensive treatment of this extremely complicated taxon was made by Noreña Janssen and Faubel (1992). These authors recognise 60 species of *Mesostoma*. Twenty eight of them they consider *species dubiae*, one a *species inquirenda*. The remaining 31 species they divide into four morphological groups. Since 1992 only two species of *Mesostoma* have been described: *M. tubiseminalis* Smith, 1998 (designated to the *M. maculatum*-group; see Smith, 1998) and *M. extremiensis* Noreña *et al.*, 1999 (with a typical pincers-like end of the ejaculatory duct; see Noreña *et al.*, 1999). Based on the relative position of the prostate glands (separated from the entrance of the vasa deferentia), the fact that the male duct is directed dorso-caudally (at least the distal part) and the fact that the male system lacks the typical shape of the species of the *M. lingua*-group (dorso-ventrally orientated, globular to pyriform with a relatively short and conical ejaculatory duct or "penispapilla"), *M. thamagai* can be included in the *M. ehrenbergi*-group. Noreña Janssen and Faubel (1992) include five species within this group, of which only *M. ehrenbergi* (Focke, 1836) Örsted, 1844, *M. productum* (Schmidt, 1848) Graff, 1882 and *M. magnum* Kolasa and Schwartz, 1988 have paired uteri that enter the common atrium separately, as is the case in *M. thamagai*. *M. thamagai* most closely resembles *M. magnum*, and they share a number of features that are unique to both: the prostate glands enter the male duct dorsally, the male atrium is tube-shaped, and pigment surrounds the male atrial system. *M. thamagai* differs from *M. magnum* in that the seminal vesicle is much more elongated. A unique feature distinguishing *M. thamagai* from all other species of *Mesostoma* is the presence of a bundle of eosinophilic glands at the transition between oviduct and seminal receptacle. The combination of all these features indicates that *M. thamagai* is indeed a new species.

Whether the four species-groups recognised by Noreña Janssen and Faubel (1992) represent natural, monophyletic groups is doubtful. The subdivision is rather artificial, based on a limited number of morphological features. In addition several features occur in more than one group. For instance, paired uteri are not unique to the *M. ehrenbergi*-group, but also occur in species of other groups (e.g. *M. sibolae* Kolasa, 1976 of the *M. lingua*-group and *M. columbianum* Hyman, 1939 of the *M. maculatum*-group). Moreover, this feature was considered dubious and probably depends on the number of eggs in the uteri (see Kolasa, 1976). A thorough cladistical analysis including a re-evaluation of morphological characters complemented with molecular data is badly needed to reveal the phylogenetic relationships within *Mesostoma*.

Young (1976) mentioned seven valid *Mesostoma* species occurring on the African continent: *M. brincki* Marcus, 1970, *M. ehrenbergi*, *M. evelinae* Marcus, 1955, *M. ewerum* Du Bois-Reymond Marcus, 1951, *M. inversum* Beauchamps, 1954, *M. lacteum* Neppi, 1904 and *M. lingua* (Abildgaard, 1789) Schmidt 1848. Additionally, he mentioned two other South African species as *species dubiae*: *M. antarcticum* Dreyer, 1918 and *M. karrooense* Dreyer, 1914. Of these two, especially *M. karrooense* has often been considered insufficiently described (Ruebush, 1939; Du Bois-Reymond Marcus, 1951; Marcus, 1955), and therefore has not been considered in more recent faunistic or taxonomic studies. In the same year, Kolasa (1976) described *M. africanum* Kolasa, 1976 from south Algeria and gave a list of six “undoubtedly valid” African species: *M. africanum*, *M. brincki*, *M. evelinae*, *M. ewerum*, *M. lingua*, and *M. productum*. His list and that of Young (1976) differ in the fact that Kolasa (1976), at the time of his writing, was not aware of the record of *M. ehrenbergi* by Young and Young (1976) and (probably) considered *M. inversum* a junior synonym of *M. productum* as later also did Noreña Janssen and Faubel (1992), although Kolasa did not mention this explicitly. Moreover, he apparently considered *M. lacteum* to be a *species dubia*. In 1981, Kolasa and Mead described a new species from Nigeria: *M. zariae* Kolasa and Mead, 1981. In their monograph of the Mesostominae, Noreña Janssen and Faubel (1992) erected a new monospecific genus to classify the somewhat aberrant species *M. evelinae*: *Marcomesostoma* Noreña Janssen and Faubel, 1992. Moreover, they considered *M. antarcticum* a junior synonym of *M. mutabile* Böhmig, 1902, probably based on the statement by Dreyer (1918) that there was virtually no difference between the two species except for their geographical distribution and for some minute differences in the female system. From what we could retrieve from literature, this synonymisation seems justified. They also explicitly considered *M. lacteum* a *species dubia*. Following these changes and additions, nine valid *Mesostoma* species are now recorded from Africa: *M. africanum*, *M. brincki*, *M. ehrenbergi*, *M. ewerum*, *M. lingua*, *M. mutabile*, *M. productum*, *M. thamagai* and *M. zariae*. Three of these are cosmopolitan (*M. ehrenbergi*, *M. productum*, *M. lingua*), while *M. mutabile* also occurs in South America. The remaining five are only known from the African continent.

DALYELLIIDAE GRAFF, 1905

Gieysztoria isoldeae n. sp.

(Fig. 3)

Locality. Botswana, Thamaga (24°41'50"S, 25°31'00"E), ephemeral rock pools on isolated granite domes (type locality). Sediment collected 22/12/1998, inundated in the laboratory 4/10/2002.

Material. Observations on live animals. One whole mount, designated holotype (LUC nr. 248). Seven sectioned specimens, designated paratypes (LUC nr. 249–255).

Etymology. Species named after the first author's daughter

Isolde.

Description. Animals \pm 0.8 mm long (measured on sectioned specimens), light brown, with two eyes. The rostral end is rounded, the caudal end tapers off (“tail”).

The cellular epidermis is about 6 μ m thick, with cilia of about 5 μ m long. The epidermis contains numerous rhabdites, which are straight rods, stretching the entire thickness of the epidermis. These rhabdites are distributed evenly over all of the epidermis. Large and conspicuous rhabdite glands are present in the tail area, and end at the body surface near the tail.

The mouth is situated slightly ventrally at the anterior body end. The prepharyngeal cavity is rather short, and lined with a very low epithelium. It is surrounded by relatively strong longitudinal muscles. The pharynx bulb measures about 20–25% of the body length and is situated anteriorly. Morphologically it is the same as in most other dalyelliids (see Luther, 1955). However, distal sensory areas (“Taster”; see Luther, 1955) and ciliated areas in the pharynx lumen (“Brusten”; see Luther, 1955) were not observed. There are 26 internal longitudinal muscles, which alternate with 26 radial muscles on each transverse section.

There are two testes, which extend ventro-laterally at both sides of the gut. Rostrally they start far behind the pharynx; their caudal ends are at the anterior end of the copulatory organ. The single ovary is rather small and ovoid. It is situated dorso-caudally from the gonopore, somewhat on the left hand side. The vitellaria are situated dorsally from the testes. They are slender, and join each other caudally to form a broad single vitellogonaduct that enters the oviduct. The common gonopore is situated ventrally, at \pm 2/3 of the body length. It can be closed by a thick sphincter, and is surrounded by a bundle of large glands, which discharge their content in the gonopore. The common genital atrium is large and globular. It is lined with a very high, nucleated epithelium and surrounded by an inner circular and an outer longitudinal muscle layer. It receives the male system anteriorly, the female system more dorso-laterally.

The male system consists of an elongated, oval copulatory organ. In its most proximal part it contains a single, large seminal vesicle that narrows distally to a seminal duct, which is surrounded by a circular muscle layer (only visible in one of the sectioned specimens). At the place where the seminal vesicle narrows, it is surrounded by prostate secretion (glands), part of which is coarse-grained eosinophilic, the other part is coarse-grained basophilic. More distally the prostate secretion disappears, but empty gland necks conspicuously surround the seminal duct. The nuclei of the prostate glands were not observed. Seminal vesicle and prostate glands are surrounded by a muscular septum, consisting of at least two muscle layers which cross each other. Between seminal vesicle/prostate glands and the septum there is a space filled with loose parenchyma. As such the copulatory organ is clearly of the conjuncta-duplex type (terminology of Karling, 1956). Distally the septum narrows and

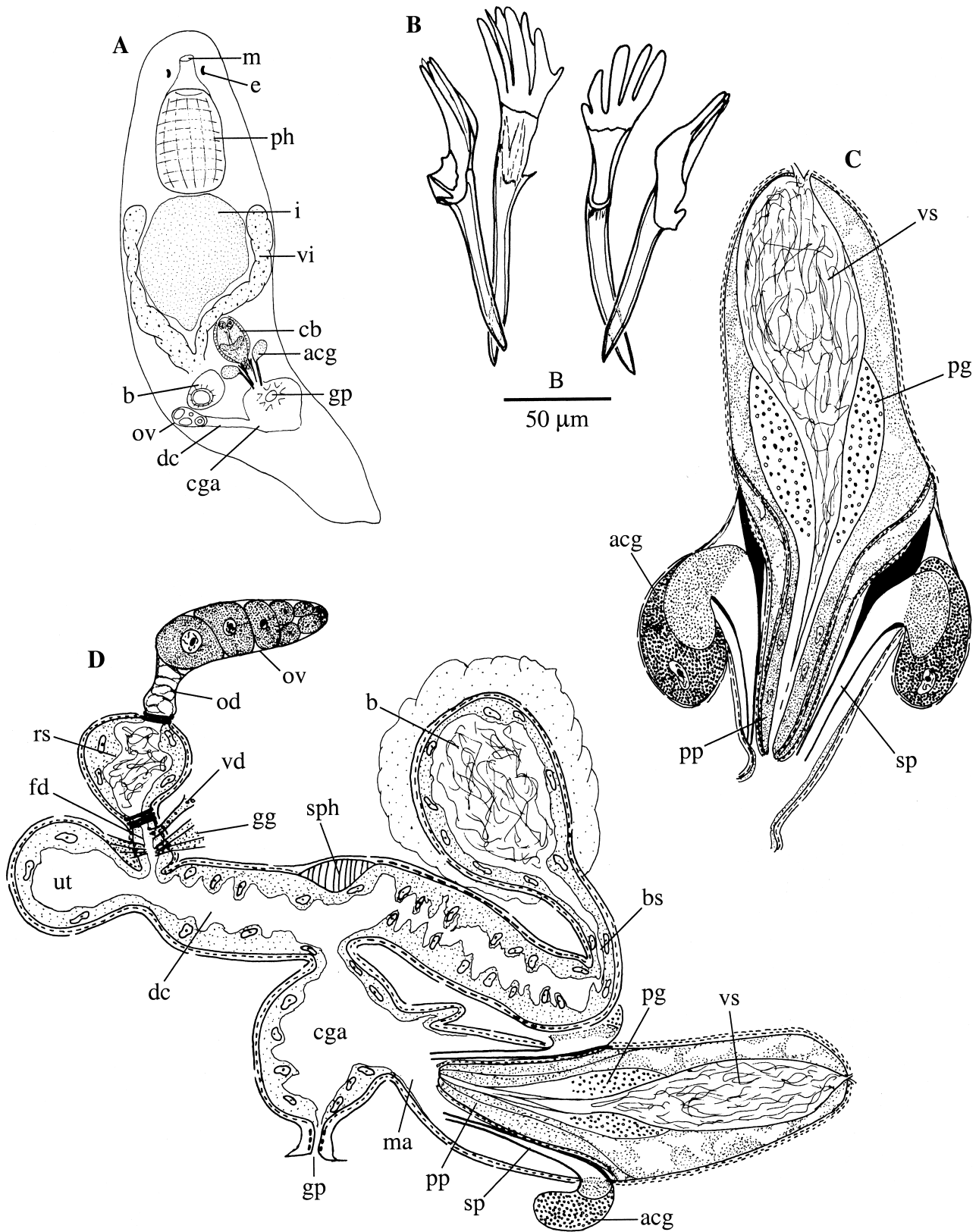


Fig. 3. *Gieysztoria isoldeae* n. sp. (A) Habitus (from a living animal). (B) Spines of the male system (holotype). (C) Reconstruction of the male genital organs (from several paratypes). (D) Reconstruction of the genital system (from several paratypes). acg, accessory glands; b, bursa; bs, bursal stalk; cb, copulatory bulb; cga, common genital atrium; dc, ductus communis; e, eye; fd, female duct; gg, glands; gp, gonopore; i, intestine; m, mouth; ma, male atrium; od, oviduct; ov, ovary; pg, prostate glands; ph, pharynx; pp, penial papilla; rs, seminal receptacle; sph, sphincter; sp, spine; ut, uterus; vd, vitelloduct; vi, vitellarium; vs, seminal vesicle.

splits into two layers, both surrounded by an inner circular and an outer longitudinal muscle layer. In this way a very muscular penial papilla is formed, which is situated centrally in the male atrium. From centre to periphery the penial papilla consists of: the narrow seminal duct, the necks of the prostate glands, parenchyma (distally denser and with many nuclei), a muscular septum, parenchyma and again a muscular septum. The distal opening of this penial papilla can be closed by a sphincter. At the proximal end of the penial papilla (where the septum narrows), four hollow and tubiform spines enter the male atrium, two of them situated ventro-laterally, the other two laterally. The ventrolateral spines (152 and 162 μm) are somewhat larger than the lateral ones (129 and 134 μm). Because of a prolongation of one of the sides, the proximal opening of each spine is large and asymmetrical. These prolonged sides consist of 4–6 digitiform protuberances. They lie next to the septum and support it. The distal part of each spine flanks the penial papilla. A bundle of coarse-grained light basophilic glands enters each lateral spine, and maybe also the ventral ones. These basophilic glands are surrounded by fine-grained eosinophilic ones, which also enter the lateral spines and probably also the ventral ones. These glandular complexes are surrounded by a common muscle layer that is continuous with the muscles forming the septum and surrounding the male atrium. The male atrium itself is lined with a very low epithelium and is surrounded by an inner circular and an outer longitudinal muscle layer.

As it leaves the common genital atrium, the female system immediately splits into the bursal stalk, running anteriorly, and the ductus communis, running caudally. The bifurcation is surrounded by a very thick circular muscle layer, which is extremely thick dorsally, forming an asymmetrical sphincter. Bursal stalk, bursa and ductus communis are lined with the same high, irregular, nucleated epithelium as is the common genital atrium, and are surrounded by an inner circular and an outer longitudinal muscle layer. The bursal stalk is rather broad, but narrows towards the bursa. At the place where it narrows it starts to run caudally. The bursa can have different appearances depending on the state of the animal: very small (underdeveloped; before copulation), or globular or oval filled with many sperm. The fully developed bursa is surrounded by an eosinophilic tissue. The ductus communis is also rather broad, and proximally splits into the uterus and the female duct. The uterus is situated terminally and is globular when no eggs are present. It is lined with the same epithelium and surrounded by the same muscle layers as is the ductus communis. The female duct runs dorsally. It is short and proximally widens to form a seminal receptacle, which often contains sperm. Female duct and seminal receptacle are lined with the same epithelium and surrounded by the same muscle layers as is the ductus communis. At the transition from female duct to seminal receptacle the circular muscle layer thickens to form a sphincter. The vitelloguct and several basophilic and eosinophilic glands (“Schalendrüsen” of Luther, 1955) enter the

female duct just distally from the sphincter. The short oviduct enters the seminal receptacle opposite the female duct. It consists of very flat cells that are piled upon each other. These cells appear to be hollow.

Diagnosis. *Gieysztoria* species with a very well-developed penial papilla. Male system with four separate hollow spines, not connected to each other by a girdle. All spines with proximal digitiform protuberances. Ventrolateral spines larger ($\pm 157 \mu\text{m}$) than the lateral ones ($\pm 132 \mu\text{m}$). A large complex of glands enters the spines.

Discussion. See the discussion of *Gieysztoria faubeli*.

***Gieysztoria faubeli* n. sp.**

(Fig. 4)

Locality. Botswana, Thamaga (24°41'50"S, 25°31'00"E), ephemeral rock pools on isolated granite hills (type locality). Sediment collected 22/12/1998, inundated in the laboratory 4/10/2002.

Material. Observations on live animals. 10 whole mounts, 1 of them designated holotype (LUC nr. 256), the other 9 paratypes (LUC nr. 257–265).

Etymology. Species named in honour of Dr. Anno Faubel (Hamburg).

Description. Small animals, 0.5–0.7 mm long, pale brown, with two eyes. The pharynx is a typical pharynx doliiformis at the rostral end of the body.

The construction of the genital system does not differ from that of the other *Gieysztoria* species. The genital pore is in the caudal 1/3 of the body. Testes were not observed. The ovary is small and is situated at $\pm 2/3$ of the body length. The vitellaria are small and distally join each other in a broad vitelloguct.

The pyriform prostate vesicle contains an internal seminal vesicle at its proximal end and a number of prostate glands at its distal end. Distally it ends in the stylet, which is 69–84 μm long ($m=73 \mu\text{m}$; $n = 8$) and 31–44 μm broad ($m=38 \mu\text{m}$; $n=7$). Proximally the stylet consists of a girdle (terminology of Luther, 1955), which is divided into a proximal part with fine longitudinal and transverse ridges, and a distal rectangular part with only a few longitudinal ridges. Two long S-shaped spines make up the sides of the girdle and protrude into the male atrium. Distally the girdle carries two central slender spines. At each side of these central spines, six broader and stouter spines occur. All spines are hollow (exc. the two large lateral ones?), and their openings are easily visible right up against the rim of the girdle. The longitudinal ridges of the distal part of the girdle are in line with the edges of the spines, and split up and continue as the longitudinal ridges of the proximal part of the girdle. The girdle does not show any holes.

The very muscular bursal stalk ends in the common genital atrium in between the oviduct and the copulatory organ. The uterus never contains more than one egg.

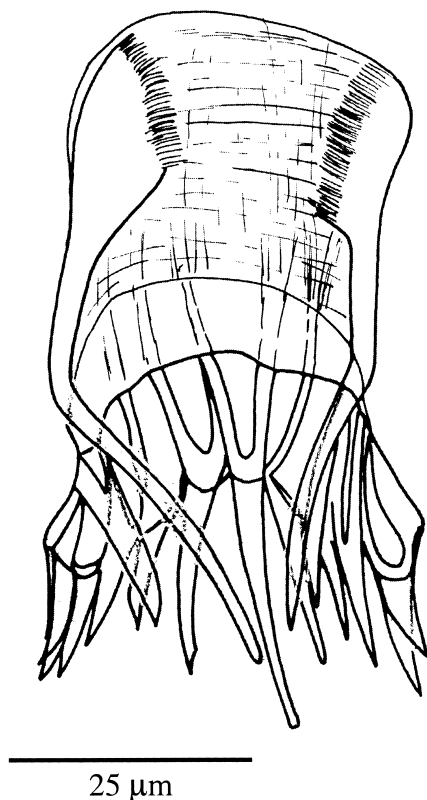


Fig. 4. *Gieysztoria faubeli* n. sp. Stylet (holotype).

Diagnosis. *Gieysztoria* species with a $\pm 73 \mu\text{m}$ long and $38 \mu\text{m}$ broad stylet. Girdle large, with two large spines forming the sides. Apart from these two spines, two long, slender central spines and 12 smaller, stouter spines connected to the distal rim of the girdle. Girdle without holes.

Discussion. *Gieysztoria* is a very species-rich and anatomically rather homogeneous taxon. Large differences between the species can, however, be found in the detailed construction of the stylet. Luther (1955) splits the genus into the "Aequales" with stylets with equal spines, and the "Inaequales", with heteromorph spines on the stylet. Within the Aequales, five species have separate hollow spines in the male system, without a girdle connecting the bases of these spines: *G. cuspidata* (Schmidt, 1861) Ruebush and Hayes, 1939, *G. quadridens* (Böhmig, 1897) Ruebush and Hayes, 1939, *G. inflata* (Okugawa, 1930) Ruebush and Hayes, 1939, *G. eastmanni* (Graff, 1911) Ruebush and Hayes, 1939 and *G. pseudoblodgetti* Luther, 1955. Now also *G. isoldeae* can be added to this list, although its spines are certainly not all of exactly the same length (so not really "Aequales"). *G. quadridens* was described from East Africa (Böhmig, 1897), but the only feature giving some information on its identity is the fact that it has "four simple chitinous spines" in the male system. Apart from *G. cuspidata*, the species mentioned are all badly known and mostly insufficiently described (see Luther, 1955) and we therefore consider them *species inquirendae*. Apart from the lack of a

girdle in the stylet, also the presence of a very well-developed penial papilla is a feature typical of both *G. isoldeae* and *G. cuspidata*. Although it is also present in most of the other *Gieysztoria* species, it is never so well developed there. *G. isoldeae* differs from *G. cuspidata* in the form of the spines, which are more slender than in *G. cuspidata* and have the typical proximal digitiform protuberances. Moreover, the large accessory glands entering the spines are typical of *G. isoldeae*. In *G. cuspidata*, there are small cells at this place (as is the case in most *Gieysztoria* species) that form the spines, but they do not form a glandular organ. The presence of an asymmetrical sphincter at the junction of female duct and bursal stalk also is characteristic of *G. isoldeae*.

It could be argued that the observed similarities between *G. isoldeae* and *G. cuspidata* warrant the erection of a separate genus for these two species. However, here we refrain from doing so because the monophyly of such a group cannot be proven at this moment. The erection of a new taxon and the coining of a new name should fit into a much broader study of the taxonomy of the Dalyelliidae, backed up with a thorough phylogenetic analysis of the group, which is planned by the first author in the near future.

G. faubeli is a more typical representative of the taxon *Gieysztoria*, with the bases of the different spines attached to a proximal girdle. The fact that the spines are of different forms places this species within the Inaequales. Luther (1955) considers the presence of holes in the girdle ("fenestrae") a very important taxonomical feature, dividing the species of Inaequales into the "Fenestratae" (with holes in the girdle) and the "Radiatae" (without holes). *G. faubeli* clearly belongs to the latter. A typical feature of *G. faubeli* is the fact that the two lateral spines have long proximal ends that form both sides of the girdle. This is also the case in *G. kolasai* Young, 1977 and *G. saganuae* Young, 1977, both species from East Africa. In these species, however, one of the lateral spines bears all of the other spines, and the girdle is basically without spines. In *G. faubeli* on the other hand, all spines are attached to the girdle. Other resembling species are *G. pseudodiademata* Noreña Janssen, 1995, *G. santafeensis* Noreña Janssen, 1995, *G. quadrata* Noreña Janssen, 1995 and *G. tigrensis* Noreña Janssen, 1995, all species described from Argentina by Noreña Janssen (1995). The girdle of the stylet of *G. faubeli* is, however, relatively larger, and it is the only species with a stylet with two central spines, flanked by six smaller spines at each side and two large ones forming the sides of the girdle. Regrettably, no whole mounts of the Argentinian species are available, which makes a direct comparison between *G. faubeli* and these species impossible (the same goes for the species described by Young, 1977). The features mentioned above, as exhibited by our specimens, however, indicate that they belong to a different species.

G. isoldeae and *G. faubeli* are, respectively, the ninth and tenth species of *Gieysztoria* to be recorded from Africa. The eight others are [see Young (1977)]: *G. quadridens*, *G. expedita* (Hofsten, 1907) Ruebush and Hayes, 1939, *G.*

rubra (Fuhrmann, 1894) Ruebush and Hayes, 1939, *G. kolasai*, *G. saganae*, *G. donnae* Young, 1977, *G. joannae* Young, 1977 and *G. papii* Young, 1977. *G. isoldeae* and *G. faubeli* are the first to be recorded from the southern part of the continent.

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