



The “Falcatae”, a new Gondwanan species group of *Gieysztoria* (Platyhelminthes: Dalyelliidae), with the description of five new species

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ABSTRACT

Gieysztoria Ruebush and Hayes, 1939, the most species-rich genus of limnic rhabdoceols (Platyhelminthes: Dalyelliidae), is subdivided into several groups based on stylet morphology. Most taxa are known from the Palearctic and the Neotropics. Very little is known about limnic, free-living flatworms in other tropical and subtropical regions. In this contribution five new species of *Gieysztoria* are described from India, Australia and South Africa, all former Gondwanan fragments. *Gieysztoria garudae* n. sp., *G. ashokae* n. sp. and *G. ramayana* n. sp. were collected in Goa (India), *G. stokesi* n. sp. in the Northern Territory (Australia) and *G. zuluensis* n. sp. in KwaZulu-Natal (South Africa). All these new species share a typical, very complex stylet morphology, which deviates strongly from that of the groups into which the bulk of the other species within this genus are classified. Therefore a new subgroup, the “Falcatae”, is erected. In addition, four known species could also be included in this subgroup. Possible homologies, common ancestry and biogeographic distribution patterns are discussed.

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1. Introduction

In the framework of the Freshwater Animal Diversity Assessment project (FADA; see Balian et al., 2008), a census was made of all freshwater turbellarians, omitting all doubtful species and *species inquirendae* (Schockaert et al., 2008). This census revealed that, with 84 species known, *Gieysztoria* Ruebush and Hayes, 1939 is the most species-rich genus within Dalyelliidae Graff, 1908, one of the major families of free-living flatworms and an important component of the freshwater meiofauna (own unpublished data). Eighty species of *Gieysztoria* are limnic, a few of which also can occur in brackish water environments (including the Caspian Sea and Lake Aral). Only four species exclusively occur in brackish or marine waters (*G. expeditoides* Luther, 1955, *G. maritima* Luther, 1955, *G. reggae* Therriault and Kolasa, 1999 and *G. subsalsa* Luther, 1955; see Luther, 1955; Therriault and Kolasa, 1999; Ax, 2008). Of these 80 limnic species, most are known from the Palearctic (37 species; e.g. Luther, 1955; Kolasa, 1981; Wang and Wu, 2005; Wang and Deng, 2006; Van Steenkiste et al., 2011a) and the Neotropics (28 species; e.g. Marcus, 1946; Luther, 1955; Noreña-Janssen, 1995; Brusa et al., 2003, 2008; Noreña et al., 2004; Damborenea et al., 2005, 2007). From the Nearctic only nine species are recorded with certainty

(for an overview see Van Steenkiste et al., 2011b). The Afrotropical, Oriental, Australian, Antarctic and Pacific regions have practically not been sampled (see also Schockaert et al., 2008). Most of this poorly sampled land surface, except for the Pacific and parts of the Oriental, was once part of Gondwana, the vast continental area in the southern hemisphere resulting from the breakup of Pangaea during the Mesozoic.

Ten species have been recorded from the Afrotropical region. Most species of *Gieysztoria* described from subsaharan Africa were collected in Kenya and Tanzania, East Africa [eight species: *G. donnae* Young, 1977, *G. expedita* (Hofsten, 1907) Ruebush and Hayes, 1939, *G. joannae* Young, 1977, *G. kolasai* Young, 1977, *G. papii* Young, 1977, *G. rubra* (Fuhrmann, 1894) Ruebush and Hayes, 1939, *G. saganae* Young, 1977; see Young, 1976, 1977]. Isolated records also exist for Nigeria, West Africa [one species: *G. cuspidata* (Schmidt, 1861) Ruebush and Hayes, 1939; see Mead and Kolasa, 1984] and Botswana, southern Africa (two species; *G. isoldeae* Artois et al., 2004 and *G. faubeli* Artois et al., 2004; see Artois et al., 2004).

For other zoogeographical regions, only sporadic records are known. Three species are recorded from Australia, more specifically from the Darwin area, Northern Territory (one species: *Gieysztoria billabongensis* Jondelius, 1997; see Jondelius, 1997) and from the Brisbane area, Queensland (two species: *G. superba* Hartenstein and Dwine, 2000 and *G. queenslandica* Hochberg and Cannon, 2001; see Hartenstein and Dwine, 2000; Hochberg and Cannon, 2001). In addition, one species is described from Java,

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Indonesia [*G. thienemanni* (Reisinger, 1933) Ruebush and Hayes, 1939; see Reisinger, 1933], but the geological history of this region in relation to the breakup of Gondwana is very complicated (for a review see van Tol and Gassmann, 2007). Specimens clearly belonging to the genus *Gieysztoria* have also been collected in Papua New Guinea by Jurek Kolasa, but these were never formally described (Therriault and Kolasa, 1999; J. Kolasa, pers. comm. 2009). For India, Madagascar and Antarctica, no records exist.

These provisional distribution patterns are difficult to interpret in terms of vicariance or dispersal, the two hypotheses often competing in historical biogeographical studies. Although *Gieysztoria* has a worldwide distribution, some species “groups” that seem closely related based on stylet morphology, are clearly confined to a particular zoogeographical region [e.g. the *therapaina-intricata-complicata-kasasapa-namuncurai-matilde-falx* group and the *sasa-cypris* group from the Neotropics (see general discussion) or the *pavimentata-virgulifera-beltrani* group and *dodgei-infundibuliformis-koivi* group from the Holarctic (see Van Steenkiste et al., 2011b)]. For these groups, the breakup of Pangaea and Gondwana could have been an important mechanism explaining their evolutionary and biogeographical history. In contrast, several species “groups” have a disjunct and possibly worldwide distribution [e.g. the *rubra-ornata-maritima-expedita-expeditoides* group (see Van Steenkiste et al., 2011b) and the *cuspidata-isoldeae* group], suggesting the taxon *Gieysztoria* was already present and well established in the early Mesozoic.

However, many other invertebrate taxa combine an ancient vicariance pattern with relatively recent dispersal events (Sanmartín and Ronquist, 2004). It is assumed that long-distance dispersal through different vectors (wind, rain, animals, humans) is very important in many freshwater invertebrates with resistant propagules (Artois et al., 2011; Bilton et al., 2001; Bohonak and Jenkins, 2003; Havel and Shurin, 2004; Panov et al., 2004; Green and Figuerola, 2005; Vanschoenwinkel et al., 2008; Schabetsberger et al., 2009); however, limnic rhabdocoels (and other freshwater “Turbellaria”), nevertheless an important component of the limnic meiofauna, have seldom been included in ecological and biogeographical studies (see e.g. Schockaert et al., 2008; Tranchida et al., 2009). Although their survival strategies to overcome unfavourable conditions (dormant eggs, cysts) are similar to other long-distance dispersers (e.g. rotifers, nematodes, cladocerans, copepods), very little is known about their dispersal capacities. Their worldwide distribution and occurrence in very isolated freshwater habitats on oceanic islands such as Kerguelen (*Castrada trispina* Willems et al., 2005; see Willems et al., 2005) and Hawai’i (undescribed species of Typhloplanidae Graff, 1905 and *Gieysztoria*; own unpublished data) suggests dispersion could have played an important role for at least some limnic rhabdocoels and *Gieysztoria* in particular.

In this contribution, five new species of *Gieysztoria* are described, collected during several sampling campaigns in India, Australia and South Africa. All species share a spectacular and complex stylet morphology. Some English terminology for morphological structures is, for clarity, accompanied (in parenthesis) by the German terms as used in Luther’s (1955) standard work on Dalyelliidae.

2. Materials and methods

All animals were collected in various wetland habitats by members of the research group Zoology: Biodiversity and Toxicology during several sampling campaigns in November–December 2008 (Goa, India: NVS and BT), November–December 2009 (Kwazulu-Natal, South Africa: BT and WW) and November 2010 (Northern Territory, Australia: BT and WW).

Specimens were collected by the oxygen depletion method (see Schockaert, 1996), studied with a compound microscope and drawn alive. Of most species one or more specimens were

whole-mounted with lactophenol. Specimens intended for sectioning were fixed in hot (50°C) Bouin’s solution, embedded in paraffin, serially sectioned (4 µm sections), and stained with Heidenhain’s iron haematoxylin, using erythrosin as counterstain.

The positions of the gonopore and internal organs, and the measurements of the pharynx are expressed in percentages of the total body length (distance from the anterior tip of the body).

Holotypes of *G. garudae* n. sp., *G. ashokae* n. sp., *G. ramayana* n. sp. and *G. zuluensis* n. sp. and the paratype of *G. stokesi* n. sp. are deposited in the collections of the Swedish Museum of Natural History (Stockholm, Sweden). The holotype of *G. stokesi* n. sp. is deposited in the Museum and Art Gallery of the Northern Territory (Darwin, Australia). All other material (paratypes), is deposited in the collections of the research group Zoology: Biodiversity and Toxicology of Hasselt University, Belgium.

The description of the new species is the responsibility of three of the authors in different combinations (NVS, EVM, TA). The correct authorship for every newly described taxon is mentioned after the new name in the respective taxon heading. The other two authors (BT, WW) helped during the collection of specimens.

Abbreviations used in the text

SMNH: Swedish Museum of Natural History (Stockholm, Sweden)
 HU: Hasselt University (Hasselt, Belgium)
 NTM: Museum and Art Gallery of the Northern Territory (Darwin, Australia)

Abbreviations used in the figures

ab: abductor muscles; b: bursa; cag: caudal glands (“Klebdrüsen”); cc: stylet cross-connection; ceg: cement glands (“Kittdrüsen”); cga: common genital atrium; co: copulatory organ; de: ejaculatory duct; e: egg; ey: eye; fd: female duct; fg1–2: eosinophilic female glands; gg1: eosinophilic prostate glands; gg2: basophilic prostate glands; gp: gonopore; i: intestine; ma: male atrium; mo: mouth; od: oviduct; ov: ovary; p1–2: stylet plate/part; pc: pseudocuticula; ph: pharynx; pi: pigment; pv: prostate vesicle; rs: seminal receptacle; sk: stylet stalk; sph: sphincter; st: stylet; s1–8: stylet spines; t: testis; u: uterus; ub: uterine bend (“Uterusbucht”); vd: vitelloduct; vi: vitellarium; vs: seminal vesicle.

3. Taxonomy

RHABDOCOELA
 DALYTYPHLOPLANIDA WILLEMS ET AL., 2006
 DALYELLIIDAE GRAFF, 1908

3.1. *Gieysztoria garudae* Van Steenkiste, Van Mulken and Artois n. sp.

(Figs. 1 and 2)

3.1.1. Localities

- 1) Carambolim lake, Corlim, Tiswadi, Goa, India (15°29’33”N, 73°55’50”E). Marshy lake with different kinds of aquatic vegetation (e.g. water lilies, grasses) (23/11/2008): Type locality.
- 2) Mayem lake, Bicholim, Goa, India (15°34’33”N, 73°56’32”E). Rich submersed aquatic vegetation in an eastern inlet of the lake (01/12/2008; 15/12/2008).

3.1.2. Material

Observations on several live animals. Nine whole mounts, one of which designated as the holotype (SMNH, no. 8225), all other

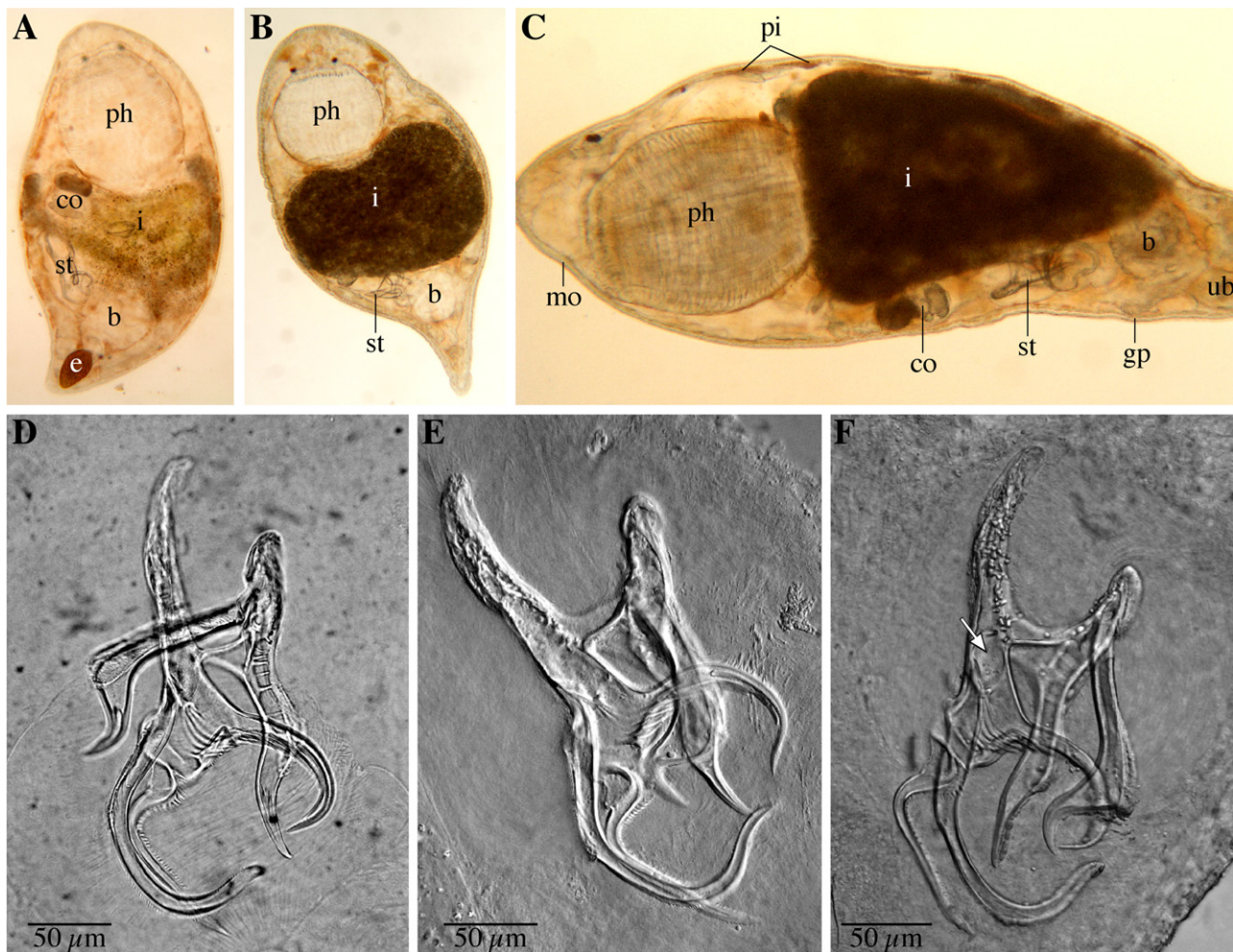


Fig. 1. *Gieysztorina garudae* n. sp. (A and B) Squash preparations of live animals, dorsal view. (C) Squash preparation of a live animal, lateral view. (D–F) Stylets (paratypes).

material automatically becoming paratypes (ICZN Art. 72.4.5; ICZN, 1999): eight whole mounts (HU, nos. 495–502) and five serially sectioned specimens (HU nos. 503–507).

3.1.3. Etymology

Named after Garuda, an eagle-like being that Vishnu, one of the important deities in Hinduism, rides as his mount.

3.1.4. Diagnosis

Species of *Gieysztorina* with dark, giraffe-like spots over the entire body. Stylet very complex, $\pm 330 \mu\text{m}$ long (longest length measured axially) and consisting of two proximal stalks of different sizes, interconnected by a broad cross-connection. Shortest stalk continuing in two large, robust spines, longest stalk distally broadening to form a group of long, serrulate spines resembling tongue-and-groove pliers. Copulatory bursa lined with strongly developed pseudocuticula.

3.1.5. Description

Animals up to 1.7 mm long. Live animals have a reddish-brown parenchymatous pigmentation (Fig. 1A–C) and display giraffe-like dark brown spots caused by dorsal, subepidermal pigment accumulations (pi) (Figs. 1C and 2C). These spots were only visible when observing the animals under reflective light with a dissecting microscope. Habitus typical for species of Dalyelliidae with front end bluntly rounded and caudal end pointed, having a tail-like shape (Figs. 1A,B and 2B,C).

Epidermis about $7 \mu\text{m}$ thick, consisting of polygonal cells, $6 \mu\text{m}$ -long cilia and a relatively thick basement membrane. Adrenal rhabdite glands are subepidermally spread throughout the parenchyma producing lanceolate, $6\text{--}8 \mu\text{m}$ -long rhabdites, which are abundantly present throughout the epidermis, often grouped in numbers of three to five. The darkly stained apical side of the epidermal cells is probably caused by the presence of ultrarhabdites (see Bedini and Papi, 1970). Body wall provided with outer circular muscles and inner longitudinal muscles. The latter are especially well-developed at the dorsal side of the body. In addition to caudal rhabdite glands producing rhabdites similar to those present throughout the epidermis, caudal adhesive glands (cag) (“Klebrüsen”) discharge a rod-shaped secretion through the terminal adhesive papillae of the tail-like caudal end (Fig. 2B).

Subterminal, ventrally located mouth (mo) at the anterior body end, surrounded by a sphincter (Fig. 1C). Typical prepharyngeal cavity and very large pharynx doliiformis (ph) (for a detailed description see Luther, 1955), which is about 1/3 of the entire body length long. Numerous conspicuously large papillae line the anterior edge of the pharynx. Intestine greenish, most likely because of the presence of zoochlorellae.

Testes (t) ventro-laterally in caudal body half, extending behind and often at both sides of the intestine (Fig. 2B). The ovary (ov) lies on the right hand side, posterior to the intestine. Smooth to slightly papillose vitellaria (vi) extend dorso-laterally from the pharynx to the end of the intestine, where they join to form a long and slender common vitelloduct (vd) entering the female duct. The

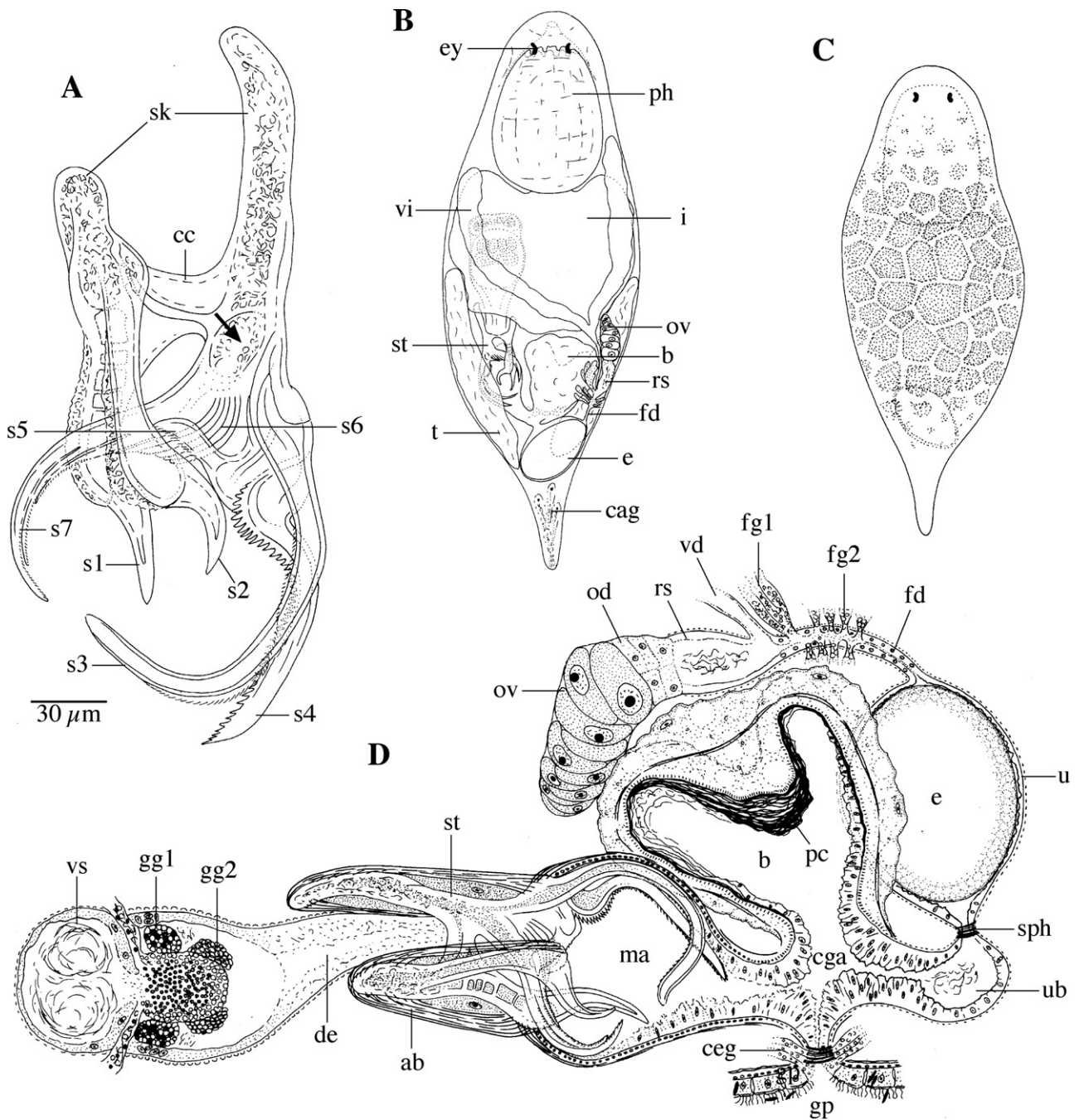


Fig. 2. *Gieysztoria garudae* n. sp. (A) Stylet (from the holotype). (B) Habitus from a live animal based on a squash preparation. (C) Habitus from a live animal under reflective light. (D) Reconstruction of the genital system from the left hand side.

medio-ventral gonopore (gp) is at 3/4 of the body length and can be closed by a strong sphincter. At this point, eosinophilic cement glands (ceg) (“Kittdrüsen”) also surround and enter the gonopore (Fig. 2D).

The large, elongated male copulatory organ (co) is situated ventrally at the left hand side of the body (Fig. 2B). Proximally, it consists of a large seminal vesicle (vs), usually completely filled with sperm. The seminal vesicle is surrounded by circular muscles and partly separated from the prostate vesicle by a septum. Granular eosinophilic prostate glands (gg1) enter the prostate vesicle proximally and form a globular mass proximally surrounded by several lobular, glandular partitions. Distally, another, more basophilic type of prostate secretion (gg2) is present around this globular mass. The seminal vesicle and the prostate glands

continue to the male genital atrium (ma) through the ejaculatory duct (de) and a short penial papilla surrounded by a large stylet (st). The wall (most probably the basement membrane) of this atrium is thickened and provided with a low epithelium and inner circular and very well-developed outer longitudinal muscle layer. The entire copulatory organ is surrounded by circular muscles.

The large stylet is very complex (Figs. 1D–F and 2A). Its longest axial length varies from 240 to 360 μm (\bar{x} = 330 μm; n = 7). Proximally it consists of two robust fibrous stalks (sk), one conspicuously longer than the other and each bearing different kinds of spines. Both stalks flank the distal part of the copulatory bulb and are linked by a broad cross-connection (cc). The shortest stalk almost immediately splits into two broad axes, one of which is supported by inner cross-connections and terminates

in a slightly curved spine (s1). The other axis initially continues straight to make a perpendicular angle distally, ending in a large falcate spine (s2). Stalk and spines together measure 155–190 μm (\bar{x} = 180 μm ; n = 7). The largest stalk is slightly more slender and distally differentiates into a very complex group of serrate spines and plates, its overall appearance being somewhat reminiscent of tongue-and-groove pliers. At the base of this group of spines and plates, a window-like opening (“Fenestra”) is present (arrow in Figs. 1F and 2A). The outermost spine (s3) is falcate with a dentate edge and in some specimens somewhat bluntly ending. Its length is about 155–190 μm (\bar{x} = 180 μm ; n = 7). It is followed by another relatively long and slender spine (s4) with a broadened, S-shaped base provided with a serrate inner edge becoming serrulate towards the distal tip of the spine. From base to tip it measures around 105–145 μm (\bar{x} = 130 μm ; n = 7). A smaller, saw-like spine (s5) originates from the same base, but points to the other direction. Several plate-like spines (s6), partially overlapping each other and all provided with a serrulate edge make up the central part of the stylet. These become gradually longer towards the opposite stylet edge. The three outermost plates (s7), which are difficult to discern because they partly overlap, are very slender and conspicuously longer, thus forming the other falcate stylet edge.

Ventro-rostrally, the male genital atrium (ma) enters the common genital atrium (cga) at the left hand side. The common genital atrium is lined with a high, cellular epithelium and surrounded by inner circular and outer longitudinal muscles (Fig. 2D). Medio-rostrally it connects to a large blind sac, the copulatory bursa (b). The distal part of this bursa is somewhat continuous with the common genital atrium and initially has a high, nucleated and cellular epithelium provided with strongly developed inner circular and outer longitudinal muscles. More proximally, the basement membrane of the epithelium soon becomes a very thick and darkly stained pseudocuticula (pc). This pseudocuticula almost completely lines the proximal parts of the bursa. Medio-proximally, a concave lobe divides the bursa into several lobular parts. These proximal parts are also provided with inner circular muscles and outer longitudinal muscles. The latter are continuous with numerous fixators running to the common genital atrium and the body wall. The entire copulatory bursa is embedded in a mantle of bulgy sarcoplasmic material. Dorso-caudally, the common genital atrium forms the uterine bend (ub) (“Uterusbucht”).

The oviduct (od) is short and made up of only a couple of large cells (Fig. 2D). Distally it enters a widened, somewhat globular portion of the female duct. This part is lined with a syncytial epithelium, surrounded by circular muscles and contains sperm, thus functioning as the seminal receptacle (rs). Just distal of the seminal receptacle, the female duct narrows and receives the vitellogoduct (vd) and a bundle of more darkly stained eosinophilic glands (fg1). The female duct (fd) continues as a slender canal in which many eosinophilic glands empty (fg2). This glandular part is surrounded by weak, circular muscles and lined with a cellular epithelium. Distally, the female duct widens again and becomes a large, single egg-containing uterus (u), which connects caudally to the uterine bend (ub) of the common genital atrium through a well-developed sphincter (sph). Both the common genital atrium and the uterus are lined with a nucleated, cellular epithelium and surrounded by inner circular muscles and well-developed outer longitudinal muscles. Eggs (e) are oval (Figs. 1A and 2B–D) and average 165 μm in length and 120 μm in width.

3.1.6. Remarks

The overall morphology of *G. garudae* n. sp. is very reminiscent of that of *G. thienemanni*, a species from western Java, Indonesia (originally described as *Dalyellia thienemanni* by Reisinger, 1933), although many details in the description of Reisinger (1933) are lacking. Especially the construction of the stylet and the copulatory

bursa, the latter also with a thick pseudocuticula, are strikingly similar. However, *G. thienemanni* is somewhat smaller (up to 1 mm), lacks the dark brown spots, and has a larger pharynx/body length ratio (up to 1/2) and a seminal receptacle clearly separated from the oviduct (see Reisinger, 1933; Luther, 1955). Unfortunately, no whole mounts are available of this species, making a thorough comparison of the stylet morphology impossible. Although the somewhat confusing sketches of Reisinger (1933) also suggest the presence of two proximal stalks, they are not drawn in detail. A large falcate spine (s2) as in *G. garudae* n. sp. is also not shown. The spine on this position (spine d in Reisinger’s drawings) seems much more straight or slightly curved. The other stylet spines of *G. garudae* roughly correspond with the following spines described by Reisinger (1933): s1 with c, s3 with b, s4 with a, s7 with e. The saw-like spine (s5) and the smaller, centrally located, serrulate plates (s6) are lacking in *G. thienemanni*. Another noteworthy feature is the shape and construction of the copulatory bursa. Although some congeneric species also display a thickened and darkly stained basement membrane in parts of the bursa (e.g. *G. evelinae* Marcus, 1946, *G. choctaw* Van Steenkiste et al., 2011b), *G. hymanae* Marcus, 1946 and *G. thienemanni* are provisionally the only species within *Gieysztoria* in which a genuine bursal pseudocuticula as in *G. garudae* n. sp. is present. Within Dalyelliidae, a bursal pseudocuticula is well-known for *Dalyellia styriaca* Reisinger, 1924 and *D. cetica* Reisinger, 1924 (see Reisinger, 1924). Both these species, in addition to the above-mentioned species of *Gieysztoria*, also have a very complicated stylet construction with many powerful spines. Although the function of the copulatory bursa is not fully understood, it is possible that this pseudocuticula and the thickened basement membrane, the latter albeit to a lesser extent, have a protective purpose during copulation. Moreover, it could be hypothesised that the conspicuous concave bursal lobe in *G. garudae* n. sp. creates a key-and-lock mechanism during copulation.

Based on the above-mentioned differences, we therefore provisionally prefer to describe the Indian specimens as a new species, until more material of *G. thienemanni* is available for study. It seems, however, that *G. garudae* from Goa (India) and *G. thienemanni* from Java (Indonesia) share a common ancestor and probably many other closely related species are present in the very undersampled Indo-Malayan region.

3.2. *Gieysztoria ashokae* Van Steenkiste, Van Mulken and Artois n. sp.

(Fig. 3)

3.2.1. Localities

- 1) Mayem lake, Bicholim, Goa, India (15°34'33"N, 73°56'32"E). Rich submersed aquatic vegetation in an eastern inlet of the lake (01/12/2008; 15/12/2008): Type locality.
- 2) Parcem Wetlands, Parcem, Pernem, Goa, India (15°39'49"N, 73°46'33"E). Aquatic vegetation in river between paddy fields (06/12/2008).
- 3) Carambolim lake, Corlim, Tiswadi, Goa, India (15°29'33"N, 73°55'50"E). Marshy lake with different kinds of aquatic vegetation (e.g. water lilies, grasses) (15/12/2008).

3.2.2. Material

Observations on several live animals. Nine whole mounts, one of which designated as the holotype (SMNH, no. 8226). The eight other whole mounts automatically becoming paratypes (ICZN Art. 72.4.5; ICZN, 1999) (HU, nos. 508–515).

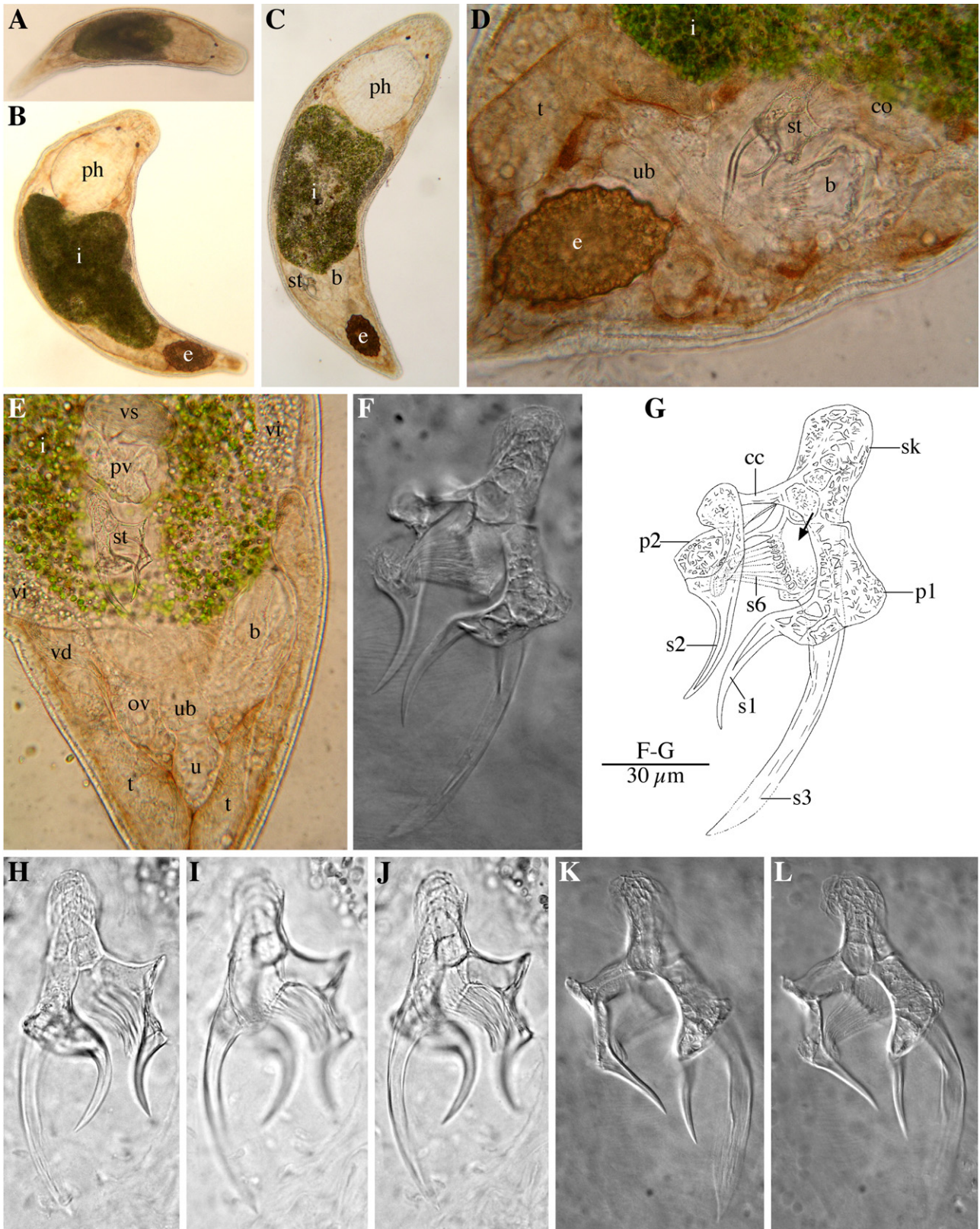


Fig. 3. *Giaysztoria ashokae* n. sp. (A–C) Squash preparations of live animals, dorsal view. (D) Detail of the genital system in a live animal, lateral view. (E) Detail of the genital system in a live animal, dorsal view. (F–G) Stilet (from the holotype). (H–L) Stylets (paratypes).

3.2.3. Etymology

The species epithet refers to Ashoka the Great (ca. 304–232 BC), the iconic Mauryan emperor and the first ruler to reign over nearly the entire Indian subcontinent. The Lion Capital of Ashoka from Sarnath is the national emblem of India and the wheel “Ashoka Chakra” from its base appears on the national flag of India.

3.2.4. Diagnosis

Brownish-red species of *Gieysztoria* with intestinal zoochlorellae. Stylet very complex, $\pm 135 \mu\text{m}$ long (longest length measured axially) with main axis formed by a proximal, fenestrated stalk and a long, slightly curved spine; the latter spine originates on a large, window-like base carrying a group of around eight, distally curved, plate-like spines; window-like base continuous with a triangular plate with a large falcate spine; main axis connected to a parallel, smaller stylet portion distally ending in another falcate spine.

3.2.5. Description

Animals up to 0.9 mm long, with a vivid, reddish-brown parenchymatous pigmentation (Fig. 3A–E). Habitus typical for species of Dalyelliidae. Lanceolate rhabdites present throughout the epidermis.

Pharynx doliiformis (ph) about 1/5–1/4 of the entire body length long (Fig. 3B and C). Numerous large papillae line the anterior edge of the pharynx. Intestine bright green because of the presence of zoochlorellae (Fig. 3A–E).

Caudal testes (t) laterally extending from the tail-like body end up to the posterior portion of the intestine (Fig. 3D and E). Sperm-filled vasa deferentia (vd) originate at the rostral end of the testes and run anteriorly to the seminal vesicle of the male copulatory organ (co). Smooth vitellaria (vi) run dorsolaterally from the anterior to the posterior end of the intestine, where they join and enter the female system (Fig. 3C and E). Medio-ventral gonopore is at 4/5 of the body length and surrounded by cement glands (“Kittdrüsen”).

The large male copulatory organ (co) is situated ventrally of the intestine and consists of a proximal seminal vesicle (vs), a prostate vesicle (pv) filled with prostate secretion and a distal stylet (st) (Fig. 3E).

The conspicuous stylet has a very complex 3D-structure (Fig. 3F–L). The main axis is formed by a proximal, robust stalk (sk) that continues distally in a long, slender spine (s3). The stalk is broad and fibrous and has several window-like openings (“Fenestrae”). The long spine (s3) initiates from a large window-like structure (arrow in Fig. 3G) and measures 80–90 μm ($\bar{x} = 82 \mu\text{m}$; $n = 8$). On the inner edge of this window, around eight slender, plate-like spines (s6) originate. The distal edges of these plates seem serrulate and their distal tips are somewhat spoon-shaped and perpendicular bent. On one of its sides, the stalk continues in a triangular fibrous plate (p1) with a number of smaller “Fenestrae” and a somewhat protruding outer ridge for the insertion of abductor muscles (see Luther, 1955). Distally, this plate ends in a robust, falcate spine (s1) of about 30–40 μm ($\bar{x} = 34 \mu\text{m}$; $n = 8$). On its other side, the main stalk (sk) is connected to an irregular fibrous stylet part (p2) by a broad cross-connection (cc). The latter partly caps the group of plate-like, serrulate spines (s6) and is also continuous with the triangular fibrous plate (p1) on the main axis. Proximally, this irregular fibrous part (p2) is ear-shaped and protrudes slightly proximally to form a very small stalk. Distally, it continues in a falcate spine (s2) of 35–40 μm ($\bar{x} = 37 \mu\text{m}$; $n = 8$), i.e. about the same size as the falcate spine (s1) on the triangular plate of the main axis. The longest axial length of the entire stylet varies from 130 to 140 μm ($\bar{x} = 135 \mu\text{m}$; $n = 8$).

Adjacent and somewhat caudal to the male copulatory organ, a large sack-shaped copulatory bursa (b) is present. In some specimens, a wrinkled, darker-stained inner lining was visible, most

probably caused by a thickening of the basement membrane or a pseudocuticula (Fig. 3D and E).

Only parts of the female system were visible in live specimens (Fig. 3D and E). The ovary (ov) was often observed on the left hand side of the body, posterior to the male genital system. The caudal uterus (u), when empty, has a conspicuous, bulgy inner lining (Fig. 3E). When containing a single egg, this bulgy uterus lining seems to imprint the egg-shell, giving it a sinuate outline (Fig. 3B–D). Rostrally, the uterus enters the uterine bend (ub) (“Uterusbucht”) of the common genital atrium (Fig. 3D and E).

3.2.6. Remarks

G. ashokae n. sp. is very reminiscent of, and probably also very closely related to the sympatric *G. garudae* n. sp. and its Indonesian relative *G. thienemanni* (see discussion *G. garudae* n. sp.). It shares their basic stylet morphology (see general discussion on the Falcatae group), but lacks a second, well-developed proximal stalk, elongated spines in the group of central, serrulate plates (s7 in *G. garudae* n. sp.) and additional falcate or saw-like spines (s4 and s5 in *G. garudae* n. sp.). In addition, *G. ashokae* n. sp. is somewhat smaller than *G. garudae* n. sp. and giraffe-like pigment spots are completely lacking. *G. ashokae* n. sp., *G. thienemanni* and most likely also *G. garudae* n. sp. all have intestinal zoochlorellae. Many other species of *Gieysztoria* share this feature; moreover the presence of symbiotic algae in the intestine occurs in all other groups and subgroups of this genus (Aequales, Fenestratae, Radiatae, Aberrantes) (e.g. see Luther, 1955; Brusa et al., 2003). Unfortunately, very little is known about this symbiotic relationship.

The abundant occurrence of *G. ashokae* n. sp. and *G. garudae* n. sp. and their coexistence in the same geographical range and habitats, evoke many questions on niche differentiation and sympatric speciation of the Falcatae in general (see general discussion) and these two species in particular, on the Indian subcontinent.

3.3. *Gieysztoria ramayana* Van Steenkiste, Van Mulken and Artois n. sp.

(Fig. 4)

3.3.1. Locality

Wetlands of the Baga river, Baga, Bardez, Goa, India (15°33'29"N, 73°45'34"E). Aquatic vegetation (e.g. sedges, water lilies, filamentous algae) in marshy field (20/11/2008): Type locality.

3.3.2. Material

Observations on some live animals. Two whole mounts, one of which designated as the holotype (SMNH, no. 8227). The other is an immature specimen, and therefore considered unsuitable as paratype.

3.3.3. Etymology

The species epithet refers to the Ramayana (composed ca. 300 BC), one of the two great Sanskrit epics of India (the other being the Mahabharata), which has had a massive influence on Indian life and culture.

3.3.4. Diagnosis

Large, brownish-red species of *Gieysztoria*. Stylet, $\pm 85 \mu\text{m}$ long and consisting of two proximal fibrous stalks of different sizes interconnected by a cross-connection, two large spines continuous with the smaller stalk, a funnel-shaped structure on the cross-connection and a fenestrated, elongated plate continuous with the larger stalk and provided with numerous smaller spines.



Fig. 4. *Gieysztoria ramayana* n. sp. (A–C) Stylets (from the holotype).

3.3.5. Description

Animals reddish-brown and up to 2 mm long. Habitus and pharynx typical for species of Dalyelliidae (see Luther, 1955). Vitellaria smooth. Other than the stylet, very few details of the internal morphology are known of this species.

Stylet about 85 μm long and consisting of two, fibrous stalks (sk), one slightly longer than the other, both distally continuing in a number of heteromorphic spines and linked by a broad cross-connection (cc) (Fig. 4A–D). The shortest stalk splits into two large spines of about 50 μm long; one runs nearly straight to end in a slightly curved tip (s8), while the other basically consists of a straight axis that carries a large falcate spine distally (s2). The latter axis is provided with a fibrous edge for the attachment of abductor muscles. The cross-connection is continuous with a very broad, funnel-shaped structure ($\pm 25 \mu\text{m}$ long), distally ending in a short spine (s1). Distally, the larger stalk forms an elongated plate-like structure, which appears to be fenestrated (arrow in Fig. 4A). The inner edge of this structure is provided with a large number of spines and plates (s6). The most proximal spine of this group is clearly slender and straight, while the most distal spines are broadened and plate-like. Serrulate edges could be observed when carefully fine focussing. Distally, the whole plate-like structure ends in a small spine (s3) which is provided with a broad ridge, probably also for the insertion of abductor muscles.

Observations on the female system and the copulatory bursa are lacking.

3.3.6. Remarks

Unfortunately, only the hard parts of the male copulatory organ are known in this species; however, since stylet morphology is of key importance in the identification and description of Dalyelliidae, this allows us to establish this specimen as a new species of *Gieysztoria*.

The general construction is somewhat reminiscent of *G. kola-sai*. The latter species from Kenya, East Africa, also has two short, proximal stalks, a broad cross-connection (described as a girdle by Young, 1977), two large spines continuous with one of the stalks and a large number of spines originating on the girdle and a robust spine continuous with the other stalk; however, the new species from India clearly differs from its East African congener by the broad, funnel-shaped structure on the cross-connection and the less “girdle-like” appearance of the proximal stylet parts (two stalks interconnected by a cross-connection).

The stylet of *G. ramayana* n. sp. also displays some remarkable similarities with that of *G. stokesi* n. sp., the newly described species from the Northern Territory, Australia and also with that of *G. okugawai* (Ruebush and Hayes, 1939) Marcus, 1946 from Japan (see description and discussion of *G. stokesi* n. sp. below). Both above-mentioned species have two proximal stalks, a cross-connection with a distal, funnel-shaped spine and a fenestrated plate-like

part provided with a group of smaller spines or plates; however, in both species the central, funnel-shaped spine on the cross-connection (s1) and the large spine originating on the fenestrated plate-like part (s3) are conspicuously longer and more slender than in *G. ramayana* n. sp. Other differences include the more prominent saw-like outline of the grouped spines (s6) and the presence of only one falcate spine (s2) continuous with the smaller stalk in *G. stokesi* n. sp.

One of the stylet spines (s8) in *G. ramayana* n. sp. is lacking in other closely related species described and mentioned in this paper (see general discussion). Possibly it is homologous with the straight spine in *G. okugawai*.

Although it should be clear from the discussion above that *G. ramayana* n. sp. is clearly recognisable from all other species of *Gieysztoria*, more material is needed to establish its relation to other putatively closely related species.

3.4. *Gieysztoria stokesi* Van Steenkiste and Artois n. sp.

(Fig. 5A–D).

3.4.1. Locality

Fogg Dam Conservation Reserve, Middle Point, Litchfield Municipality, Northern Territory, Australia ($12^{\circ}34'13''\text{S}$, $131^{\circ}18'10''\text{E}$; $12^{\circ}34'10''\text{S}$, $131^{\circ}18'16''\text{E}$). *Pistia stratiotes* and other aquatic vegetation in swampy area at the edge of a paperbark forest; aquatic vegetation (*Myriophyllum*-like) in shaded forest pool (14/11/2010): Type locality.

3.4.2. Material

Observations on live animals. Two whole mounts, one of which designated as the holotype (NTM, no. D1396), the other one paratype (SMNH, no. 8228).

3.4.3. Etymology

Species name in honour of John Lort Stokes, Lieutenant on the HMS Beagle under Commander J. C. Wickham during a voyage (1837–1843) to survey large parts of the coast of Australia. Previously, J. L. Stokes had served onboard with Charles Darwin. He was the first to sight the natural harbour of Port Darwin (1839) and completed the survey as Commander of the Beagle when J. C. Wickham fell ill.

3.4.4. Diagnosis

Brownish-red species of *Gieysztoria*. Complex stylet, $\pm 115 \mu\text{m}$ long and consisting of two proximal fibrous stalks of different sizes interconnected by a cross-connection, three large heteromorphic spines with an open base and a broad plate-like structure carrying nine falcate, dentate plates.

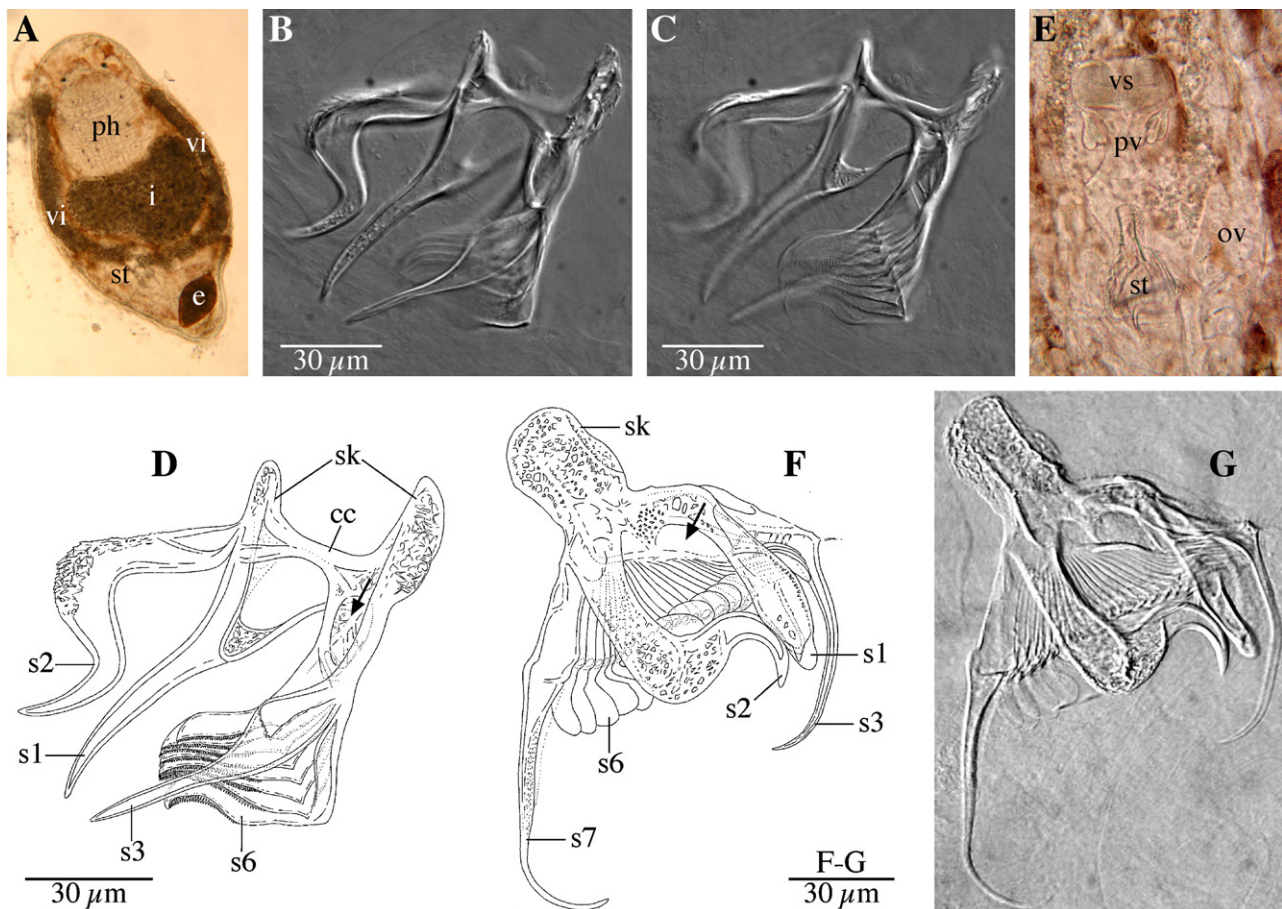


Fig. 5. *Gieysztoria stokesi* n. sp. (A) Squash preparation of a live animal, dorsal view. (B–D) Stylet (from the holotype). *Gieysztoria zuluensis* n. sp. (E) Detail of the copulatory organ and the ovary in a live animal. (F–G) Stylet (from the holotype).

3.4.5. Description

Animals up to 1.1 mm long. Live animals with a vivid reddish-brown parenchymatous pigmentation (Fig. 5A). Habitus typical for species of Dalyelliidae with front end bluntly rounded and caudal end pointed, having a tail-like shape.

Large pharynx doliiformis (ph) (see Luther, 1955), which is about 1/3 of the entire body length.

Position of testes unknown. The ovary lies at the right hand side of the body. Smooth to slightly bulgy vitellaria (vi) extend from the anterior of the pharynx to the end of the intestine, where they join. Cement glands (“Kittdrüsen”) surround the gonopore.

Male copulatory organ at the left hand side of the body and consisting of a proximal seminal vesicle, median prostate vesicle, the ejaculatory duct and a distal stylet (st).

Stylet very complex (Fig. 5B–D). Proximally it consists of two, fibrous stalks (sk), one more robust and longer than the other, both flanking the distal part of the copulatory bulb and linked by a broad cross-connection (cc). The shortest stalk initially continues in a straight axis to make a perpendicular angle distally, ending in a large falcate spine (s2). At this turning point, considerable surface unevenness provides attachment for well-developed abductor muscles. Axis and falcate spine measure about 115 μm axially. Both stalks and their cross-connection form the base of a median, very large and slightly curved spine (s1) about 85 μm long. This spine has a broad, funnel-shaped base, which is partly open and has a fibrous, slightly protruding edge, probably also for the insertion of abductor muscles. The largest stalk initially extends into a fibrous part provided with an oval window-like opening (arrow in Fig. 5D). The outer edge of this fibrous part is carrying a slender, relatively

straight, 75 μm -long spine (s3) with a broad, funnel-shaped base and proximal opening. Additionally, the fenestrated part fans out in a broad piece bearing nine very conspicuous falcate plates (s6) with a dentate inner edge, giving them a saw-like appearance. From the tip of the longest stalk to the tip of the central spine, the stylet measures around 115 μm .

Unfortunately no details of the female system and the copulatory bursa have been observed. Uterus with a single oval to rhomboid egg (Fig. 5A) of about 180 μm long.

3.4.6. Remarks

The stylet of *G. stokesi* n. sp. is almost completely identical to the stylet of *G. okugawai*, a poorly described species from Awaji, Japan. Originally described as *Dalyellia gracilis* Okugawa, 1930 (see Okugawa, 1930), this species was later renamed *Microdalyellia okugawai* by Ruebush and Hayes (1939), because the former name was already preoccupied by *D. gracilis* Fulinski and Szynal, 1927. Eventually, Marcus (1946) and Luther (1955) disputed the homology of the proximal stylet stalks with those of *Microdalyellia* Gieysztor, 1938 and *M. okugawai* was placed within *Gieysztoria*.

The stylet of *G. stokesi* n. sp. has about the same size ($\pm 100 \mu\text{m}$ long in *G. okugawai*) and construction as that of *G. okugawai*; however, it clearly differs from its Japanese congener in lacking the large straight spine, which in *G. okugawai* originates at the proximal stalk that carries the straight axis with the large falcate spine (s2). The broad-based, central spine (s1) on the cross-connection is homologous with spine “m” in the stylet sketch of Okugawa (1930), but the latter is considerably shorter than the other large spines of the stylet. The bundle of spines in *G. okugawai* corresponding

with the saw-like plates (s6) of *G. stokesi* n. sp. is described as a “lantern-shaped” terminal branch composed of about ten spines (“l” in Okugawa, 1930). A window-like opening at the base of this bundle and spine is not mentioned by Okugawa (1930). The large spine accompanying the bundle of plates (s3 in *G. stokesi* n. sp.) is relatively longer and more curved in *G. okugawai*. A final minor difference between *G. stokesi* n. sp. and *G. okugawai*, which can be drawn from the very incomplete description of the Japanese species, includes the smaller pharynx size/body length ratio.

Although closely resembling *G. okugawai*, we consider the Australian specimens as belonging to another species, mainly because they clearly lack the larger, straight stylet spine of the Japanese species. The smaller above-mentioned differences (relative length and curvature of the spines, pharynx/body length ratio) are noteworthy in respect to this taxonomical decision. Hopefully, a more thorough study of new specimens of *G. okugawai* in the future will be able to clarify the relationship between these two putatively closely related species.

3.5. *Gieysztoria zuluensis* Van Steenkiste and Artois n. sp.

(Fig. 5 E–G)

3.5.1. Locality

Monzi, Mtubatuba, Umkhanyakude, KwaZulu-Natal, South Africa (28°27'57"S, 32°17'36"E). Aquatic vegetation from drainage channel in a sugarcane plantation entering the Umfolozi River (30/11/2009; 01/12/2009).

3.5.2. Material

Observations on live animals. One whole mount, designated as the holotype (SMNH, no. 8229).

3.5.3. Etymology

Named after the Zulu people, the major ethnic group in South Africa and Kwazulu-Natal in particular, the province of South Africa where this species was found.

3.5.4. Diagnosis

Reddish-brown, irregularly spotted species of *Gieysztoria*. Stylet very complex, $\pm 140 \mu\text{m}$ long with a proximal, fibrous stalk carrying a large falcate spine, a blunt axis with a small distal spine and a very long and slender slightly curved spine on the one hand, and a very broad plate consisting of around 25–30 spoon-shaped plates edged by a long, slender spine on the other hand.

3.5.5. Description

Animal about 1 mm long, with irregular brown spots and a vivid, reddish-brown parenchymatous pigmentation (Fig. 5E). Habitus very elongate, which is somewhat atypical for species of Dalyeliidae.

Pharynx doliiformis about 1/4 of the entire body length long. Intestine green, most likely because of the presence of zoochlorellae.

Testes caudal, with sperm-filled vasa deferentia originating at the rostral end and running anteriorly to the seminal vesicle of the male copulatory organ. Vitellaria smooth and extending from the transition pharynx-intestine to the female system in the caudal body half.

Large male copulatory organ with proximal seminal vesicle (vs), a prostate vesicle (pv) filled with a granular prostate secretion and a distal stylet (st) (Fig. 5E).

The elaborate stylet consists of a very large number of heteromorphic spines (Fig. 5F–G). At its maximum, it is $140 \mu\text{m}$ long. Proximally, a robust, fibrous stalk (sk) connects to the copulatory

bulb. At one side this stalk splits into two fibrous axes: one is relatively straight and carries a $45 \mu\text{m}$ -long, robust, falcate spine very reminiscent of a raptor claw (s2), while the other one almost immediately splits into a very long and slender, slightly curved spine (s3) measuring $95 \mu\text{m}$, and a bluntly ending axis with a small, spinous protrusion (s1). On the other side, the proximal stalk fans out in a very broad plate composed of a very large number (25–30) of elongate spoon-shaped plates (s6). Laterally, these plates are flanked by a very long, slender spine, which is clearly a modified plate-like spine (s7). It measures $120 \mu\text{m}$ (axially) from its base and initially runs straight to end in a falcate tip.

The bursa was not observed. Only the ovary (ov) was clearly visible at the right hand side of the copulatory organ (Fig. 5E). The uterus contains one single egg with a sinuate outline.

3.5.6. Remarks

Although only the stylet morphology of *G. zuluensis* is well-known, this species is clearly closely related to other species of the Falcatae group (see general discussion). On first sight, the stylet seems very different from all previously known stylets of this group, but closer study reveals the overall construction of the stylet to be quite reminiscent of *G. garudae* n. sp. and consequently also of *G. thienemanni* (see discussion of *G. garudae* n. sp.). The most important similarities, which are most probably homologous structures, include the large falcate spine (s2) originating on a broad straight axis, the relatively long, curved spine (s3) laterally from the series of plates (s6), and the modified, elongated spine on the outer edge of the plates (s7). The stylet of the South African species differs from its Asian congeners by the presence of only one stalk, the number and form of the central plates, and the overall morphology of the other spines that constitute the stylet.

Other African species of *Gieysztoria* belonging to the Falcatae (i.e. *G. kolasai* and *G. saganae*; see general discussion) display a very different stylet morphology, making the morphological resemblance with *G. garudae* n. sp. interesting from a biogeographical point of view. A common ancestor with the species from India and to a lesser extent Indonesia could indicate a Gondwanan origin; however, dispersion cannot be excluded (see introduction and general discussion). Broader sampling in subsaharan Africa, Madagascar and the Indian subcontinent and a thorough (molecular) phylogenetic study could provide answers to these questions.

4. General discussion

4.1. Morphology

The species described here all share a similar stylet morphology, very aberrant from the basic *Gieysztoria* type stylet with a proximal girdle and distal spines (Luther, 1955). Based on stylet morphology, species within this genus are traditionally classified into two groups: Aequales (having stylets with spines of similar shapes and sizes) and Inaequales (having stylets with spines of different shapes and sizes). The Inaequales were further subdivided into three subgroups: Fenestratae (with one or more openings in the girdle), Radiatae (stylet radially symmetrical without openings in the girdle) and Aberrantes (with an aberrant stylet morphology) (see Luther, 1955 and, more recently, Damborenea et al., 2005). However, the uncertain position of some species and doubtful features diagnosing these (sub)groups, are problematic. For instance, several indications suggest that the openings in the girdle, the feature diagnosing the Fenestratae, probably have limited value as a phylogenetic feature: (1) the stylets of the closely related *G. virgulifera* (Plotnikow, 1906) Ruebush and Hayes, 1939, *G. pavimentata* (Beklemischew, 1926) Ruebush and Hayes, 1939, *G. beltrani* (Gieysztor, 1931) Ruebush and Hayes, 1939 and to a lesser extent

G. atalaya Brusa et al., 2008, are considered to be typical for the Fenestratae (Luther, 1955; Brusa et al., 2008), but clearly differ from other species within the same subgroup. Their stylet girdle has one large, round to oval opening that most likely contains the nucleus and nucleolus of a big cell forming the stylet (Luther, 1955). *G. intricata* Marcus, 1946, *G. therapaina* Marcus, 1946, *G. falx* Brusa et al., 2003 and *G. matilde* Brusa et al., 2008 display a similar opening in their girdle, but are undoubtedly more closely related to *G. complicata* (Fuhrmann, 1914) Ruebush and Hayes, 1939, *G. kasasapa* Damborenea et al., 2005 and *G. namuncurai* Damborenea et al., 2007, which have no openings in their girdle (see below); (2) in contrast to the above-mentioned species with a single fenestra, the girdle openings of other species of Fenestratae are clearly different in nature, i.e. larger spaces in between the distal girdle fibres in *G. triquetra* (Fuhrmann, 1894) Ruebush and Hayes, 1939 and a proximal opening of the sheath surrounding the central tube in *G. dodgei* (Graff, 1911) Ruebush and Hayes, 1939 and *G. infundibuliformis* (Fuhrmann, 1894) Ruebush and Hayes, 1939 (Luther, 1955; Van Steenkiste et al., 2011b). Especially *G. infundibuliformis* and *G. dodgei* are more closely related to *G. koiwi* (Eggers, 1925) Luther, 1955, a species also lacking fenestrae, than to the other species of Fenestratae; (3) the stylet of *G. euchroa* (Gieysztor, 1926) Luther, 1955 is clearly more reminiscent of the stylets of the new species group proposed in this study, than to the other representatives of Fenestratae (see below); (4) the problematic position of *G. choctaw* combining features of both the Fenestratae (a round opening in the girdle) and the Radiatae (a radially symmetrical stylet) (Van Steenkiste et al., 2011b). It should be clear from the above-mentioned examples that the taxonomical value of the feature diagnosing the Fenestratae is questionable. In addition, a more recent (albeit preliminary) phylogenetic analysis based on morphological data, with a majority of the characters directly relating to the stylet, suggests that the (sub)groups within *Gieysztoria* are not monophyletic (Brusa et al., 2003).

The Aberrantes comprise an even more varied amalgam of species with divergent stylet morphologies not fitting the diagnoses of the other groups and subgroups. About nine species can be considered belonging to this group: *G. acariaia* Marcus, 1946, *G. cypris* Marcus, 1946, *G. thymara* Marcus, 1946, *G. sasa* Damborenea et al., 2005 and *G. namuncurai* from the Neotropics; *G. kolasai* and *G. saganæ* from the Afrotropics; *G. thienemanni* from Indonesia; and *G. okugawai* from Japan. Nearly all are southern hemisphere species except for *G. okugawai*.

The discovery of new species in India, Australia and South Africa, described in this paper, brings new insights into the stylet morphology of this group. In contrast to the other species of Aberrantes, *G. garudae* n. sp., *G. ashokae* n. sp., *G. ramayana* n. sp., *G. stokesi* n. sp., *G. zuluensis* n. sp., *G. thienemanni*, *G. okugawai*, *G. kolasai* and to a lesser extent also *G. saganæ* all share a number of stylet features, which justify the erection of a new subgroup, the “Falcatae” within the Inaequales.

The most conspicuous character of this new subgroup is the presence of a large and robust falcate stylet spine (s2), which is often very reminiscent of a raptor claw. In *G. ramayana* n. sp., *G. kolasai* and *G. saganæ*, this spine is not as strongly developed as in the other species. The girdle is modified into one (*G. ashokae* n. sp. and *G. zuluensis* n. sp.) or two (*G. garudae* n. sp., *G. ramayana* n. sp., *G. stokesi* n. sp., *G. thienemanni*, *G. okugawai*, *G. kolasai* and *G. saganæ*) fibrous stalks (sk). The proximal part of the stylet of *G. thienemanni* is inadequately described. If two stalks are present, they are interconnected by a broad cross-connection (cc).

The second shared feature in this stylet subtype is the presence of a grouped, variable number of nearly always plate-like spines. In *G. garudae* n. sp., *G. stokesi* n. sp. and to a lesser extent also *G. ramayana* n. sp., these plates are saw-like with a serrulate ridge, while in *G. ashokae* n. sp. and *G. zuluensis* n. sp. a more or less

spoon-shaped design occurs. For *G. thienemanni*, *G. okugawai* and *G. kolasai*, no detailed information on the morphology of this group of spiny plates is given; however, Reisinger's (1933) sketches also suggest the presence of saw-like plates in *G. thienemanni*. *G. saganæ* differs from all other Falcatae in the fact that it has genuine spines, not plates, making its position within the Falcatae somewhat doubtful. Outside the Falcatae, plate-like stylet spines with serrate or dentate edges are only known in *G. euchroa*, a species recorded from central and southern Europe. Although the overall stylet morphology of this enigmatic and rarely encountered species is very aberrant from the known stylet types and subtypes within *Gieysztoria*, it has been placed within Fenestratae because of the presence of a window-like opening in the basis of the stylet (see Luther, 1955). Despite the presence of plate-like spines, this species cannot be included within the Falcatae because it lacks a large falcate spine.

Besides the group of plate-like spines (s6) and the large falcate spine (s2), most of the other spines of the various stylets within the Falcatae can be considered homologous with varying degrees of certainty. In nearly all stylets, the grouped, plate-like spines are accompanied by a mostly slender and curved spine (s3; b in *G. thienemanni*, see Reisinger, 1933) also originating at the base of the structure supporting this group of plate-like spines. Only in *G. ramayana* n. sp. and *G. kolasai*, is this spine short, while in *G. saganæ* it is completely lacking (see Young, 1977). In *G. garudae* n. sp., *G. ramayana* n. sp., *G. stokesi* n. sp. and *G. okugawai*, a large spine (s1) is present on the cross-connection and can almost without any doubt be considered a homologous feature. In *G. garudae* n. sp., this spine partly originates on the cross-connection, but it has somewhat shifted towards the large falcate spine (s2), while in *G. ashokae* n. sp. and *G. zuluensis* n. sp., it is possibly homologous with the large spine continuous with the triangular plate (p1) and the small spinuous protrusion on the bluntly ending axis respectively. As already mentioned in the discussion of *G. ramayana* n. sp., s8 and the possible homologous straight spine in *G. okugawai* are provisionally unique within the Falcatae. Other separately named spines (s4–7) in *G. garudae* n. sp. and *G. zuluensis* n. sp. are all modifications of the plate-like spines.

A number of Neotropical species also display a conspicuously large, curved or falcate spine, often in combination with other larger spines. All these species, i.e. *G. complicata*, *G. intricata*, *G. therapaina*, *G. falx*, *G. kasasapa*, *G. namuncurai* and *G. matilde*, also have a comparable stylet morphology with a fibrous girdle (with or without a window-like opening) and numerous rows of distal spines sometimes accompanied by an additional spine group. The above-mentioned species are partly interspersed between the Fenestratae and the Aberrantes, and partly have not been assigned to any subgroup within Inaequales (see Damborenea et al., 2005, 2007). Because they lack the plate-like spines typical for the Falcatae, it could be argued that a new subgroup would also be appropriate for the classification of the above-mentioned Neotropical species. However, it is not within the scope of this study to formally erect a second new subgroup within the Inaequales. This hypothesis and an overall revision of the genus *Gieysztoria* as a whole, should be backed up by a thorough morphological and preferably molecular phylogenetic study of all species in general and the southern hemisphere representatives in particular.

4.2. Biogeography

In general, biogeographical patterns for limnic free-living flatworms are poorly understood, mainly because a large fraction of their diversity is still undescribed and because the phylogenetic relationships between the species are unknown. As is the case for many other micro-organisms, known species richness and distribution data of microturbellaria reflect scientific activity from the past rather than real distribution patterns derived from geological

(vicariance) or ecological (dispersion) conditions (Foissner, 2006; Schockaert et al., 2008; Artois et al., 2011).

Although a large number of species of *Gieysztoria* have been described in the Palearctic, Nearctic and Neotropics, the recent discovery of several new species in Spain (Van Steenkiste et al., 2011a), Alabama (Van Steenkiste et al., 2011b), Peru (Damborenea et al., 2005), Argentina (Brusa et al., 2003, 2008; Damborenea et al., 2007) and Brazil (own unpublished data) suggests that the actual diversity in most of these regions is probably much higher. It is clear that *Gieysztoria* has a worldwide distribution, but our knowledge of other regions in the southern hemisphere is only based on a handful of records (see introduction for an overview and references). This greatly hampers meaningful biogeographical conclusions; however, nearly all species of Falcatae including the new ones described in this paper seem to have a Gondwanan distribution, since this group does not occur in “relatively” well-sampled western Laurasia. Only *G. okugawai* from Japan and *G. thienemanni* from Indonesia do not completely fit this vicariance distribution pattern. If the breakup of Gondwana was indeed the main mechanism driving the evolution of this new subgroup of *Gieysztoria*, dispersal through wind, birds or other vectors could possibly explain actual aberrant distributions. Denser sampling on the different Gondwanan fragments and lesser-known Laurasian regions and a thorough phylogenetic study of the genus *Gieysztoria* are indispensable to shed some light on the evolutionary history of this important genus of limnic meiofauna.

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