

ORIGINAL ARTICLE

# Two new species of the genus *Gieysztoria* (Rhabdoceola: Dalyelliidae) from China

Hang Zhang<sup>1</sup>, Yi-Kui Li<sup>1</sup>, Cheng-Chen Wu<sup>2</sup>, An-Tai Wang<sup>1\*</sup>

<sup>1</sup> College of Life Science, Shenzhen Key Laboratory of Marine Bioresources and Ecology, Shenzhen 518060, China

<sup>2</sup> Institute of Nuclear Agricultural Sciences, Zhejiang University, Hangzhou 310029, China

\* Corresponding author; E-mail: wang118@szu.edu.cn

**Abstract** Two new turbellarian species, *Gieysztoria shantouensis* Zhang, Li & Wang, **sp. nov.** and *G. huizhouensis* Zhang, Wu & Wang, **sp. nov.** are described, which were collected from ditches in Shantou and Dongjiang River, Huizhou of Guangdong Province, respectively. *G. macrovariata* (Weise, 1942) was firstly recorded in China. All specimens examined were deposited in National Zoological Museum, Institute of Zoology, Chinese Academy of Sciences, China.

**Key word** Platyhelminthes, Rhabdoceola, Dalyelliidae, *Gieysztoria*, new species, China.

## 1 Introduction

Turbellarians, the most primordial triploblastic animals, have few studies about their ecology and zoogeography (Schockaert *et al.*, 2008; Tranchida *et al.*, 2009). The turbellarian genus, *Gieysztoria* Ruebush & Hayes, 1939, belongs to Dalyelliidae, Rhabdoceola, Platyhelminthes. This kind of turbellarian produces resting eggs or vesicles to overcome severe environment, and little is known about the way they spread (Willems *et al.*, 2005). Species of the genus are worldwide distributed and live in freshwater wetlands, as an important group of benthonic animals. So far, 93 species of this genus have been recorded (Tyler *et al.*, 2012; Van Steenkiste *et al.*, 2012; Xia *et al.*, 2014). The sclerotic stylet of *Gieysztoria* is the critical taxonomic character to identify them (Luther, 1955).

Since 2000s, lots of new taxons of freshwater Rhabdoceola, including 3 families, 5 genera and 15 species, have been found in Oriental part of China: 1 species of *Phaenocora*, Typhloplanidae (Wang & Sun, 2011), 1 species of *Gyratrix*, Polycystididae (Wang & Li, 2005), 3 genera and 12 species of Dalyelliidae (Wang, 2004; Wang & Wu, 2005a, b, 2008; Wang & Deng, 2006; Zhang *et al.*, 2011; Lai *et al.*, 2013; Lu *et al.*, 2013; Xia *et al.*, 2014). Taxonomic study about *Gieysztoria* in China was started from 2005 by the team of authors (Wang & Wu, 2005a; Wang & Wu, 2005b; Wang & Deng, 2006; Lai *et al.*, 2013; Lu *et al.*, 2013; Xia *et al.*, 2014). So far, 7 species were recorded in China: *G. shenzhensis*, *G. pulchra*, *G. macrovariata* 9 – *spinosa*, *G. wuyishanensis*, *G. bimaculatas*, *G. guangdongensis*, *G. shiyanensis*.

In this paper, 3 species of *Gieysztoria* are reported, including 2 new species and 1 newly recorded species in China. The materials are collected in Shantou, Shenzhen and Huizhou, Guangdong Province. Taxonomic discussions between these species are provided, in order to provide informations for the study of the diversity of turbellarians in China and ecology of water environment.

## 2 Materials and methods

The animals were collected by the same approaches as Lai *et al.* (2013). Holotypes were selected from mature individuals. The specimens were fixed in F.A.A solution (80 mL 75% ethanol, 15 mL methanol and 5 mL glacial acetic acid) for 6 hours, washed with high purify water for three times (1 minute for each time), then soaked the specimens in high purify water for 12 hours. The materials were stained by H.E method, dehydrated using different concentration ethanol, hyalinized with xylene and sealed with resinene. The sclerotic stylets were isolated by lactophenol (mixture of 1:1 ratio of lactic acid and phenol, and add 3 g polyvinyl alcohol per 100 mL). The alive flatworms were observed with the help of a stereomicroscope Leica MZ 16, and photographed by a Leica DC 300 digital dedicated camera. The specimens and inner construction were observed under a microscope Olympus BX51 and photographed by a DP 72 digital dedicated camera. All specimens were measured by softwares dedicated for the digital microscopes. Figures and photographs were scanned and edited by Adobe Photoshop® version 7.0. The biological patterns were drawn according to the digital photographs.

Abbreviations in the figures: b, brain; bc, bursa copulatrix; cc, stylet cross-connection; cag, caudal glands; eg, egg; ey, eye; ga, genital atrium; i, intestines; ov, ovary; og, oesophageal glands; od, oviduct; ph, pharynx; rs, receptaculum seminalis; sk, stylet stalk; st, stylet; t, testis; u, uterus; vd, vas deferens; vg, vesicula granulorum; vi, vitellaria; vs, vesicula seminalis.

## 3 Taxonomy

Rhabdocoela Meixner, 1925

Dalyelliidae Graff, 1905

*Gieystoria* Ruebush & Hayes, 1939

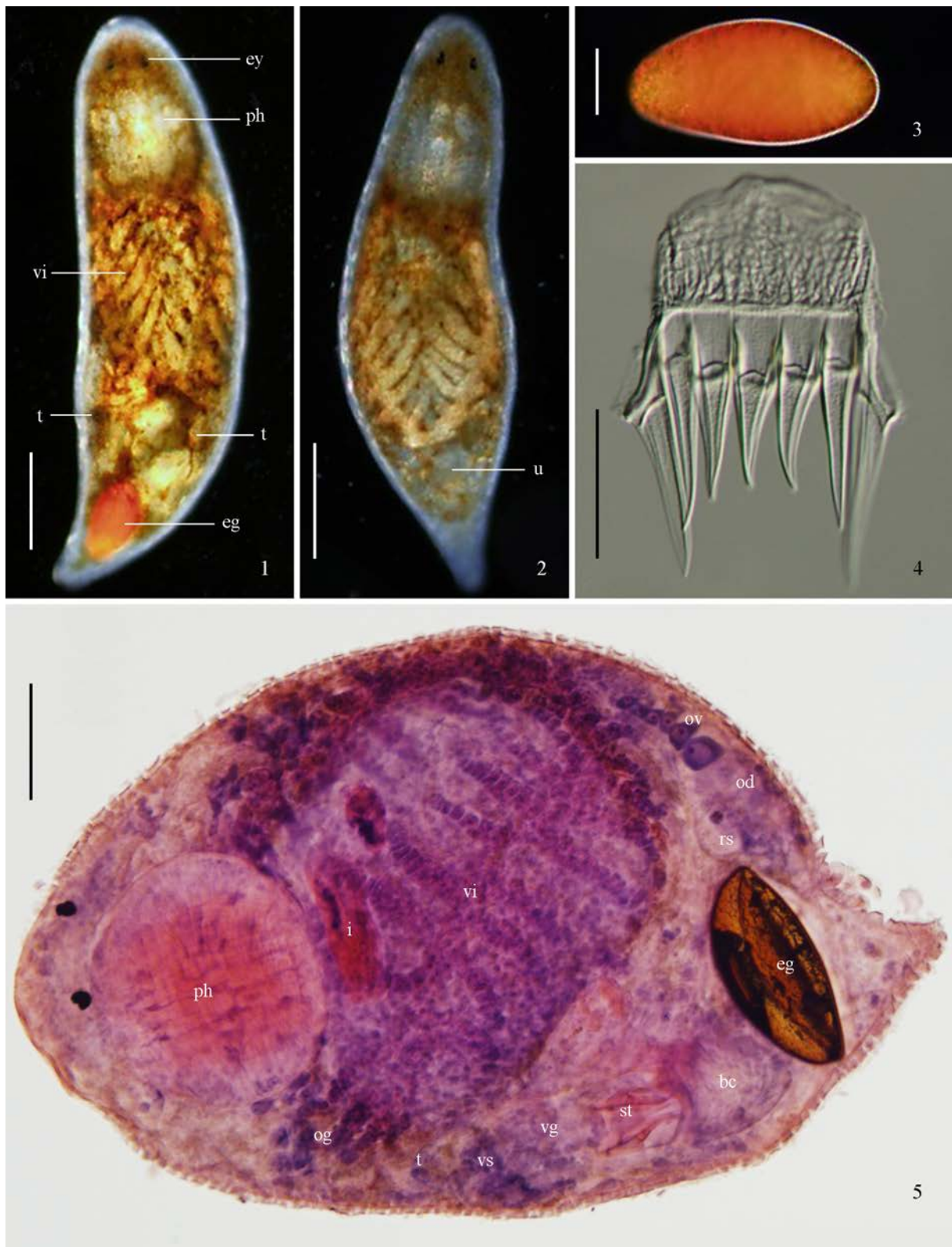
### 3.1 *Gieystoria shantouensis* Zhang, Li & Wang, sp. nov. (Figs 1–8)

Material examined. Holotype PLA-G0060, ditches in Chengtian, Chaonan, Shantou, Guangdong (23°10'57"N, 116°28'18"E) (Fig. 17), 21 January 2013, coll. Yi-Kui Li. saved in Bouin's solution and stained by H.E. method. Paratypes. PLA-G0061–PLA-G0066, same data as holotype (PLA-G0061–PLA-G0063, the whole specimens; PLA-G0064–PLA-G0066, isolated sclerotic stylets). All materials were deposited in National Zoological Museum, Institute of Zoology, Chinese Academy of Sciences (IZCAS), Beijing, China.

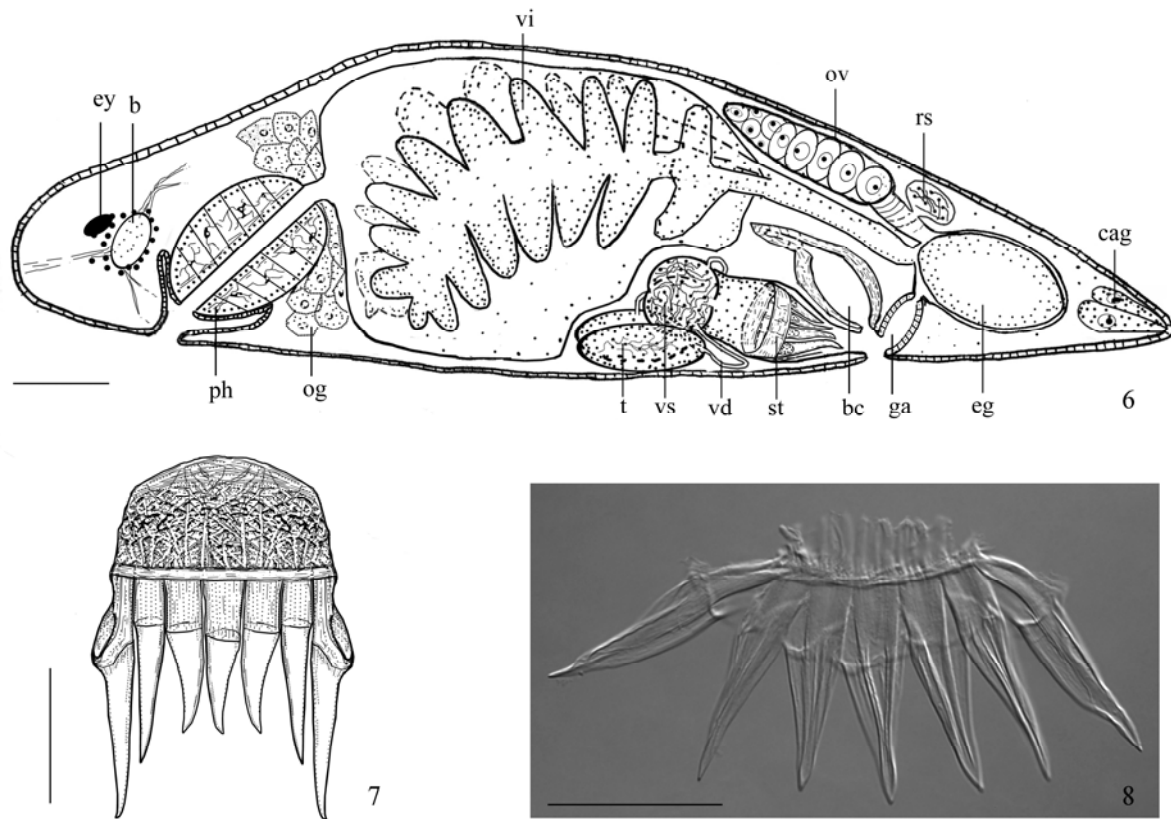
Description. Live individual about 1005–1340 µm long, and middle part of bodies up to 280–350 µm wide. Anterior part of body bluntly rounded, posterior part tapered. Whole body appears spindle-like. Salmon pink pigments distribute under epidermis (Fig. 1). Two reniform eyes locate at back of forebrain near pharynx, space between them about 72 µm. Barrel-shaped pharynx 170 µm long and 160 µm wide. Pocket-like intestinal canal connects to pharynx, with distinct glands in junction (Figs 1–2, 5–6).

Reproductive system. Hermaphrodite, with one gonopore. Female reproductive system consists of an ovary, oviducts, uterus, receptaculum seminis, vitellaria, bursa copulatrix, genital atrium and common gonopore. Strip-shaped ovary lies at back of posterior intestine. Oviducts from rear of ovary lead to uterus near rump. Branches of oviducts lead to spherical receptaculum seminis which contains serried sperms. An orange-yellow elliptic egg, with 190 µm long and 96 µm wide, often appears inside uterus, both ends of it slightly sharp (Fig. 3). Two vitellaria extend dorsolaterally, with 380 µm long. Each vitellarium with numerous finger-shaped branches. Vitelloducts from left and right join together at back of intestines, then lead to uterus, and other side of it connects genital atrium. Finger-shaped bursa copulatrix comprises myofibers, and opens into genital atrium through a pore (Figs 5–6).

Male reproductive system consists of testes, vas deferens, a vesicula seminalis, a vesicula granulorum, prostate tissues and a sclerotic stylet. Paired oval-shaped testes situated laterally at posterior intestine, with a vas deferens at backend of each testis extending to vesicula seminalis. Double-ball-shaped vesicula seminalis contains serried sperms, covered by a muscular layer. Behind it lies a cylindrical-shaped vesicula granulorum, which comprises eosinophilic granules inside, outside lies prostate cells. Sclerotic stylet, with total length 137 µm, connecting to backend of vesicula granulorum, consists of a closed girdle and 7 spines. Girdle formed by sclerotic collagenous fibers, with height 44 µm, diameter 70 µm. Base of each spine connected to a U-shaped muscular layer. Lateral spines appear dagger-shaped, middle ones cynodont-



Figs 1–5. *Gieysztoria shantouensis* Zhang, Li & Wang, **sp. nov.** 1–2. Alive individual. 3. Egg. 4. Stylets. 5. Whole specimen. Scale bars: 1–2=200  $\mu$ m, 3–4=50  $\mu$ m, 5=100  $\mu$ m.



Figs 6–8. *Gieysztoria shantouensis* Zhang, Li & Wang, **sp. nov.** 6. Ventral view. 7. Stylets. 8. Unbaked stylets. Scale bars: 6=100  $\mu\text{m}$ , 7–8=50  $\mu\text{m}$ .

shaped. Spines differ in length, shortening from both sides to middle gradually. Outer spine 94  $\mu\text{m}$  long, and the middle 59  $\mu\text{m}$  long (Figs 4–7). Stylet of immature individual is not fully sclerotized, with some unsclerotized collagenous fibers around girdle (Fig. 8).

**Etymology.** The specific name refers to the type locality, Shantou City, Guangdong Province, China.

**Discussion.** Sclerotic stylet is the critical taxonomic character to identify species of genus *Gieysztoria*. The genus *Gieysztoria* is divided into two groups, Aequales and Inaequales (Luther, 1955). The stylet of Aequales usually contains a proximal girdle and lateral spines which are similar in size and shape. Species of Inaequales have complicated stylets, and are divided into four subgroups: Fenestratae, Radiatae, Aberrantes, Falcatae (Luther, 1955; Damborenea *et al.*, 2005; Van Steenkiste *et al.*, 2012). The proximal girdle of Fenestratae has one or more pores; Radiatae does not have pores, and has actinomorphic stylet; Aberrantes has the stylet irregular (Luther, 1955; Damborenea *et al.*, 2005). The stylet of the Falcatae is a small thick hook-like spine, which is similar to a raptor's claw (Van Steenkiste *et al.*, 2012). By comparing, the new species belongs to *Gieysztoria*, Inaequales, Aberrantes.

Nine species, *G. shantouensis* Zhang, Li & Wang, **sp. nov.**, *G. oligocentra* (Steinböck, 1948), *G. papii* (Young, 1977), *G. pseudodiadema* (Noreña-Janssen, 1995), *G. shenzhensis* (Wang & Wu, 2005b), *G. pulchra* (Wang & Deng, 2006), *G. macrovariata* (Weise, 1942), *G. macrovariata 9-spinosa* (Luther, 1955), *G. octospinosa* (Luther, 1955) are similar because of the number of the terminal spines of stylet. Among them, the stylet of *G. oligocentra*, *G. papii* and *G. pseudodiadema* have 8, 6 and 8 spines, respectively. However, *G. oligocentra* and *G. papii* belongs to Aequales, and *G. pseudodiadema* and the new species belong to Inaequales. The stylet of *G. pseudodiadema* is 60–70  $\mu\text{m}$  long, and the spines are 42  $\mu\text{m}$  long. The shape of stylet between *G. pseudodiadema* and the new species is significantly different. And the length of stylet of the new species is 2 times as long as *G. pseudodiadema* (Luther, 1955; Damborenea *et al.*, 2005). *G. shenzhensis* has 8 spines within its stylet, with 4 thick and 4 flagellous spines, actinomorphic, belongs to Radiatae, a subgroup of Inaequales (Luther, 1955), which is significant different with the new species. *G. pulchra*, *G. macrovariata* and *G. macrovariata 9-spinosa* belong to the same subgroup with the new species (Luther, 1955). They have 13, 10 and 9 spines within their stylets,

respectively, while the new species has 7 spines within its stylet. The stylet of *G. octospinosa* is the most similar to the new species. However, its stylet is 55 µm in length, has 8 sclerotic spines, and spines in the middle are longer than those in the lateral. The stylet of the new species is 137 µm long in total, has 8 spines, and spines in the middle are shorter than those in the lateral.

### 3.2 *Gieysztoria huizhouensis* Zhang, Wu & Wang, sp. nov. (Figs 9–14)

Material examined. Holotype PLA-G0111, tributary of Dongjiang River, Huizhou, Guangdong Province (23°10'57"N, 116°28'18"E), the water temperature 25°C (Fig. 17), 20 July 2014, coll. Hang Zhang. Overall sheeting, H. E. method stained. Paratypes. PLA-G0112–PLA-G0117, the same data as holotype (PLA-G0112–PLA-G0113, mounting specimens; PLA-G0114–PLA-G0117, separated sclerotic mating spines). All the types were deposited in National Zoological Museum, Institute of Zoology, Chinese Academy of Sciences (IZCAS), Beijing, China.

Description. Mature individual about 1000–1100 µm in length, and middle part of body up to 220–280 µm in width. Animal has typical body characteristics of Dalyelliidae. Whole body appears a flat-oval shape, with blunt head, cylindrical-shaped middle part of body, and V-shaped tapering tail epidermis densely covered with ciliums. Dorsally, brown irregular-oval stripes longitudinally distributed from back of its eyes to front area of eggs, with light-colored stripes on pharynx (Fig. 9). Pair of eyes located in anterior end, oval-shaped eyespots formed by many melanin balls. Distance between eyes about 58 µm. Cerebral ganglions distribute under eyes. Barrel-shaped pharynx behind eyes looks 234 µm × 184 µm, accounting for 1/5 of whole body length. Posterior end of pharynx joints a pocket-shaped intestinal canal, no anus present, with distinct pharyngeal glands in junction (Figs 9–11).

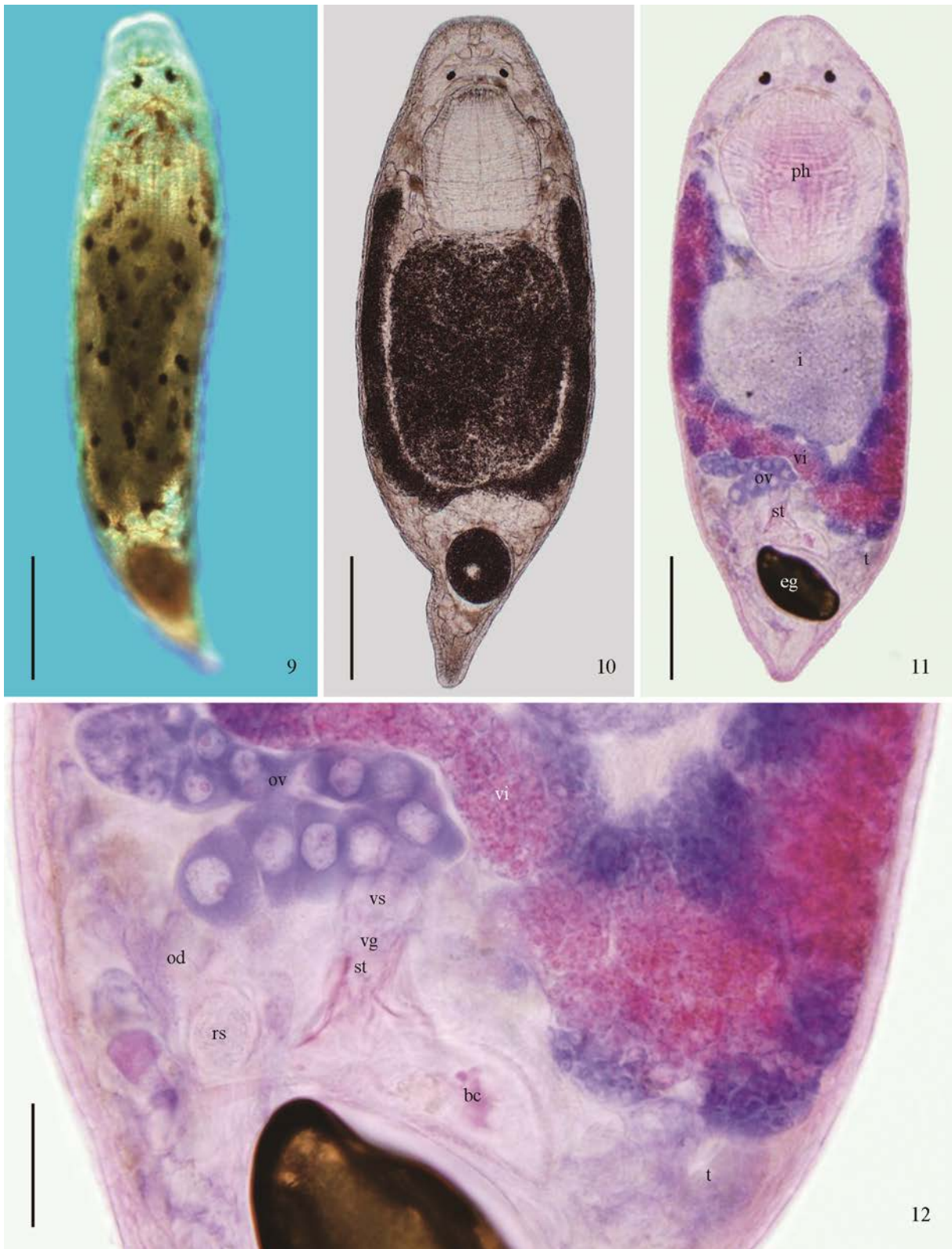
Reproductive system. Hermaphrodite, with a gonopore. Female reproductive system consists of ovary, oviduct, uterus, seminal receptacle, vitellaria, bursa copulatrix, genital atrium and gonopore. Irregular-oval-shaped ovary lies in posterior end of intestine dorsally, which ends to a short oviduct, linking seminal receptacle and uterus. Seminal receptacle filled with spherical-shaped sperms. Within uterus usually exists a oval egg (149 µm × 100 µm) appearing brownish red when mature (Fig. 9). Two rodlike vitellaria, 466 µm in length locate in bilateral sides of intestinal canals. Vitelline ducts from left and right side join and convert together, then extending to uterus. Other end of uterus connected to genital atrium. Utricle-shaped bursa copulatrix, formed by muscular tissues, opens holes from genital atrium (Figs 11–12).

Male reproductive system consists of testes, vas deferens, a vesicula seminalis, a vesicula granulorum, prostate tissue and a sclerotic stylet. Pair of oval-shaped testes lie in ventral side at posterior end of intestinal canal. Vas deferens exists at each end of testis, then flows into vesicula seminalis. Vesicula seminalis filled with sperms and covered with thick circular muscles. Vesicula granulorum located between vas deferens and sclerotic stylet, which comprises eosinophilic granules inside, outside lies prostate cells. (Figs 11–12). Sclerotic stylet appears typical Falcatae characteristics with 88 µm long. Two fiber sclerotic stalks at front end about 15 µm and 28 µm. Base of stylet connected through stylet cross-connection, forming irregular H-shape. Cross-connection (27 µm long, 12 µm wide) stretches backwards along with 2 stalks, which form 6 abnormal spines namely s1, s2, s3, s6, s7 and s8. Outspread parts of cross-connection and short stalk posterior form infundibulate-shaped S1, with 35 µm in length, wide base and tapering distal end. Short stalk posterior stretches out s2 and s8. Length of s2 about 65 µm. Half of part, close to the base, relatively thick, with an oval muscular layer, distal end in canine shape. S8 about 58 µm, saber-shaped, with tip bended slightly. Outspread part of the long stalk posterior forms s3, s6 and s7. Length of s3 about 60 µm, with strip-shaped base 10 µm. Half of near end present a muscular layer. Dagger-shaped far end slightly bended. Inner edge of long stalk extends oval-plate-shaped s6, which stretches out 8 super thin flake-like spines towards the middle part. Each spine looks like a spoon and posterior edge of it has thin serrate spines. Stretching out from inner side of base of long stalk, s7 locates between s1 and s6, about 36 µm (Figs 13–14).

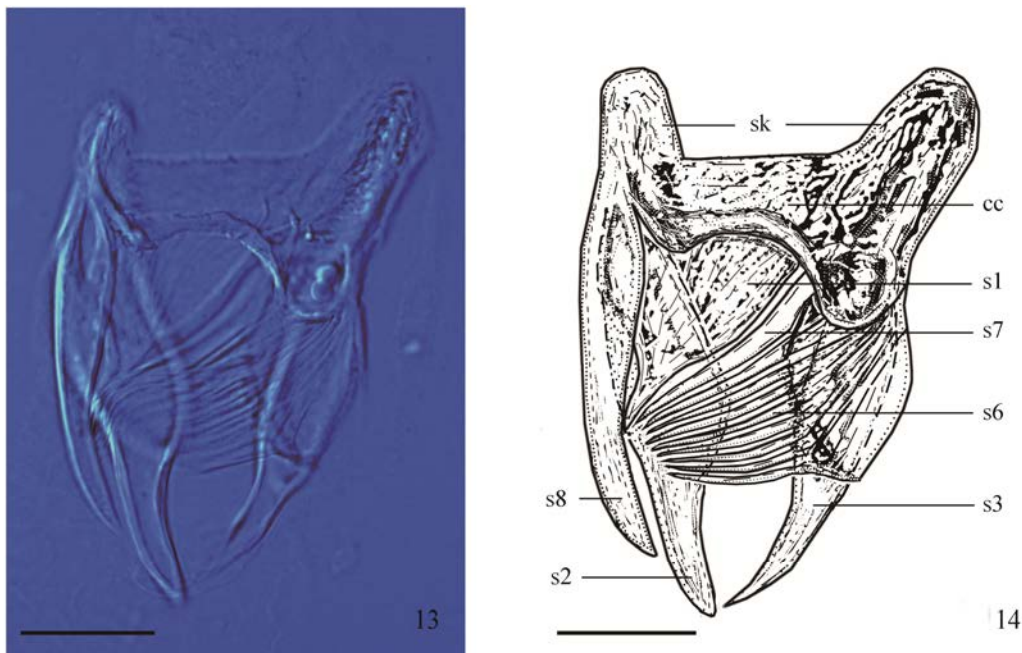
Etymology. The specific name refers to the type locality, Huizhou City, Guangdong Province, China.

Discussion. The new species should belong to *Gieysztoria*, Inaequales, Falcatae. For the subgroup Falcatae, ten species have been recorded: *G. kolasai*, *G. saganai*, *G. ashokae*, *G. zuluensis*, *G. garudae*, *G. thienemanni*, *G. okugawai*, *G. stokesi*, *G. wuyishanensis* and *G. ramayana* (Reisinger, 1933; Ruebush & Hayes, 1939; Young, 1977; Van Steenkiste *et al.*, 2012; Lai *et al.*, 2013). Among these species, *G. kolasai* and *G. saganai* have original sclerotic stylet. *G. ashokae* and *G. zuluensis* have only one stylet stalk. *G. garudae* has stylet with obvious sawtooth on coarse spines of s3 and the surface edges of s4. *G. thienemanni* has s6 in a strip-type shape. *G. okugawai* has 5 coarse spines, s2 fine S-shape, and s6 with 11 small spines. *G. stokesi* and *G. wuyishanensis*, which are very similar to the new species, have discoid spines and hook





Figs 9–12. *Gierystoria huizhouensis* Zhang, Wu & Wang, **sp. nov.** 9–10. Alive individual. 11. Whole specimen. 12. Copulatory apparatus. Scale bars: 9–11 = 200  $\mu$ m, 12 = 100  $\mu$ m.



Figs 13–14. *Gieysztoria huizhouensis* Zhang, Wu & Wang, **sp. nov.**, stilet. Scale bars = 20  $\mu\text{m}$ .

spines. However, *G. stokesi* has *s7* and *s8* absent and *G. wuyishanensis* has *s8* absent. The bending angle of *s2* in both species is significantly hook-like and the discoid spine is smooth.

In *G. huizhouensis* Zhang, Wu & Wang, **sp. nov.**, *s2* is slightly blended and sawtooth serrations lie at its posterior of discoid spine. These are significant different from other species. The sclerotic stilet of *G. ramayana* is the most similar to the new species. Its full length of sclerotic stilet is 85  $\mu\text{m}$ , and the far end has 5 coarse spines, namely *s1*, *s2*, *s3*, *s6* and *s8*. While in *G. huizhouensis* **sp. nov.**, the full length of the stilet is 88  $\mu\text{m}$ , and 6 coarse spines are present, namely *s1*, *s2*, *s3*, *s6*, *s7* and *s8*.

### 3.3 *Gieysztoria macrovariata* (Weise, 1942) New record to China (Figs 15–16)

Material examined. PLA-G0090–PLA-G0092, wetlands of Shiyan Reservoir in Shenzhen, Guangdong Province, China (22°40'58"N, 113°53'42"E) (Fig. 17), 19 June 2013, coll. Yan-Hong Lu, deposited in National Zoological Museum, Institute of Zoology, Chinese Academy of Sciences (IZCAS), Beijing, China.

Diagnosis. The species is similar to *G. shantouensis* Zhang, Li & Wang, **sp. nov.** Sclerotic stilet of *G. macrovariata* is 150  $\mu\text{m}$  in length. Ten sclerotic spines are attached to the distal end. Base of each spine is attached to a U-shaped muscular layer, and lateral spines are dagger-shaped. The spines shorten from both sides to the middle. The lateral one is 97  $\mu\text{m}$  and the middle one 66  $\mu\text{m}$  in length (Figs 15–16).

Discussion. The stilet of type materials of *G. macrovariata* is about 102.5–139.5  $\mu\text{m}$  in length, with 10 sclerotic spines. The longest spine is up to 97  $\mu\text{m}$  and the shortest is 75  $\mu\text{m}$  in length (Weise, 1942). However, the Chinese specimens have little differences with the type. The stilet of the Chinese specimens is 133  $\mu\text{m}$ –150  $\mu\text{m}$  in total length, with 10 sclerotic spines. The longest spine is up to 97  $\mu\text{m}$  and the shortest is 66  $\mu\text{m}$  in length.

## 4 Discussion

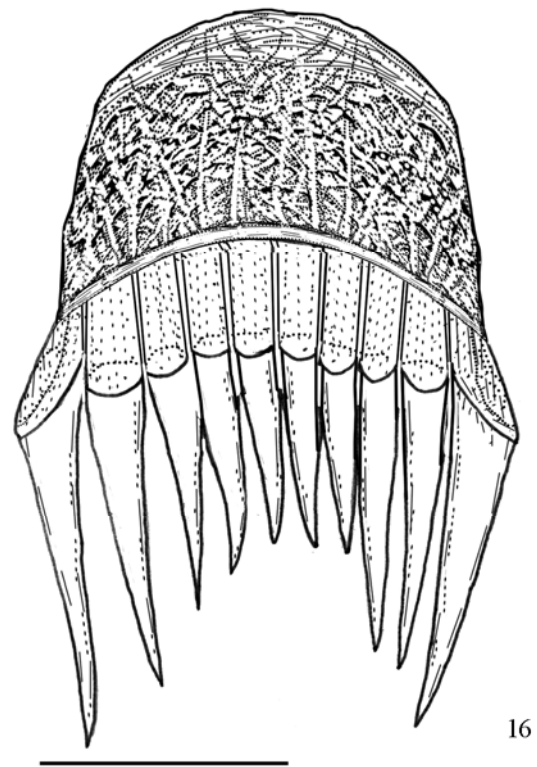
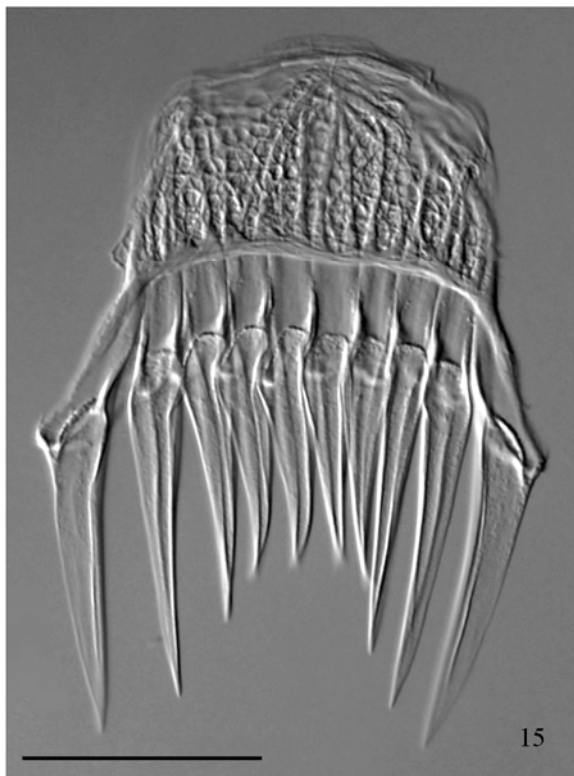
According to the animal geographical fauna division, the Chinese mainland stretches over Palearctic Realm and Oriental Realm. But studies on taxonomy of turbellarian in China are few. At the end of the 1900s, only 21 species of turbellarians were recorded, which were all distributed in Palearctic Realm. The taxonomical study on turbellarian in South

China, which belongs to Oriental Realm, was started since the 2000s (Wang, 2004, 2005; Wang *et al.*, 2004; Wang & Luo, 2004; Wang & Li, 2005; Wang & Wu, 2005a, b, 2008; Peng & Wang, 2006; Wang & Deng, 2006; Peng *et al.*, 2007; Gao *et al.*, 2011; Wang & Sun, 2011; Zhao *et al.*, 2011; Zhang *et al.*, 2011; Lai *et al.*, 2013; Lu *et al.*, 2013; Yu *et al.*, 2013; Ma *et al.*, 2014; Sun & Wang, 2014; Xia *et al.*, 2014). With addition of this paper, 31 species of turbellarians are recorded from South China, and most of them are distributed in freshwater areas.

Turbellarian is a demersal flesh-eater with various feeding habits. For instance, *Gieysztoria huizhouensis* likes preying on anhistozoa and rotifer, freshwater tricladida likes preying on aquatic insects and larvae (mosquitoes and larva), and some other species prey on demersal water fleas (Yu *et al.*, 2013). Meanwhile, turbellarians are high quality baits for tiddlers. Therefore, turbellarian is an important link in the food chain of aquatic ecological environment, and it is necessary to conduct studies relevant to turbellarian.

South China is an evergreen tropical- subtropical region, with high temperature and rainfall, which is densely covered with rivers and streams. It is also rich in plants and species diversity. Based on years of field investigations, the author A-T Wang found that species living in water environment is extremely few, which may due to coastal industrialization, rapid urbanization development and rapid population expansion, shrinking or backfilled natural wetlands, and serious river pollution. In recent 10 years, the turbellarian in South China are only found in original wetlands and water source zones (urban water reservoirs). Some turbellarian species might become extinct before they are found. However, there are few taxonomical studies in China, and other relevant work has not been started yet.

The habitat of the 3 turbellarians reported in this paper are all found in Oriental Realm. The locality for the type specimen of *G. macrovariata* is in Berlin, Germany. It is also found in Romania (Golovitz Lake) (Mack-Fira, 1974) and Spain (Doñana National Park) (Van Steenkiste *et al.*, 2011), which belong to Palaearctic Realm. In this paper, *G. macrovariata* is firstly found in Oriental Realm. The habitat of *G. shantouensis* Zhang, Li & Wang, **sp. nov.** is similar to *G. macrovariata*, which lives near the root of floating water hyacinths in eutrophic water, but the degree of eutrophic water of the new species living in is higher than *G. macrovariata*. The type locality of *G. macrovariata* in China is in the posterior part of Shiyan Reservoir in Shenzhen. This habitat is rich in biodiversity, which form mature food chains. The habitat of



Figs 15–16. *Gieysztoria macrovariata* Weise, 1942, stylets. Scale bars = 50  $\mu$ m.



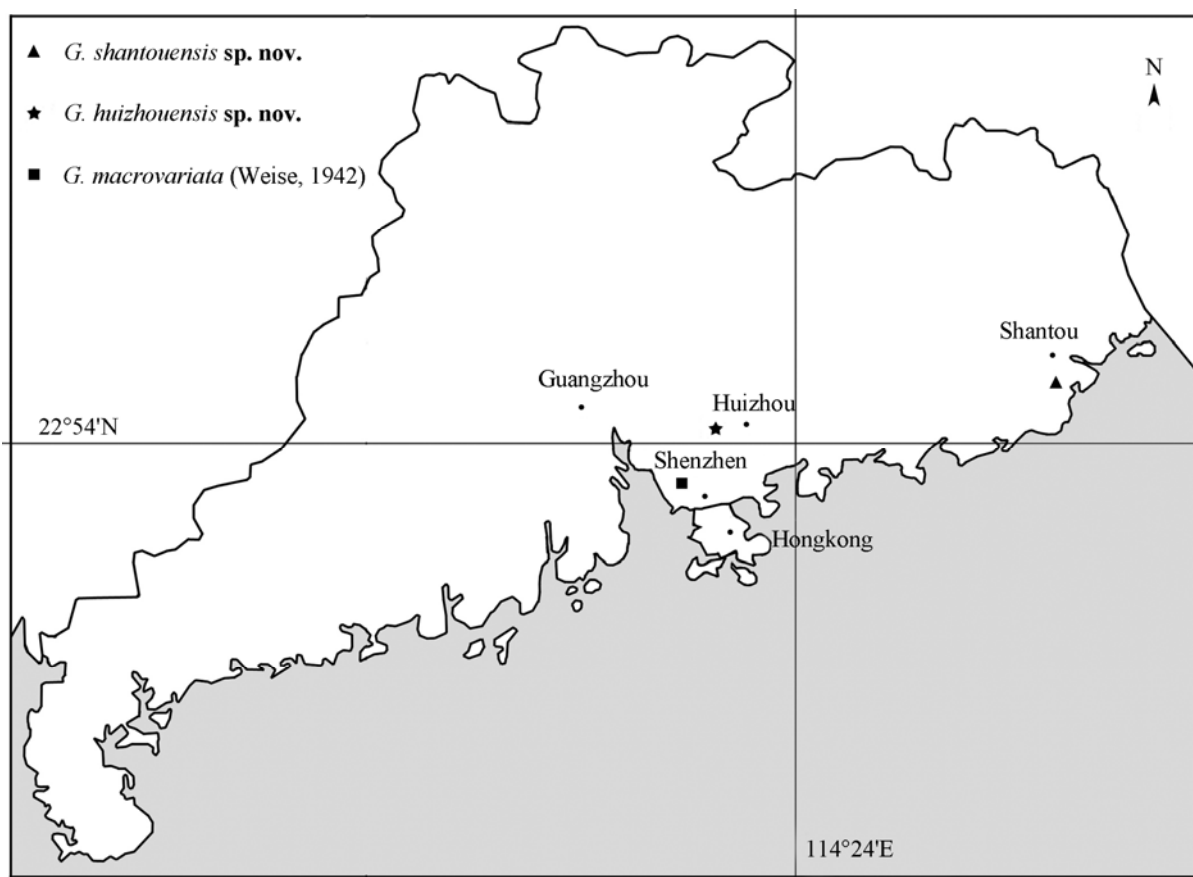


Fig. 17. Distribution of *Gieysztoria* spp.

*Gieysztoria huizhouensis* Zhang, Wu & Wang, **sp. nov.** is located in Dongjiang River (water-supply source for Shenzhen and Hong Kong) and nearby an estuary of river section of Huizhou City. Freshwater environment is protected well. There are various aquatic invertebrates in the collected water sample.

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