



Two new brackish-water species of *Testudinella* (Rotifera: Testudinellidae) from Qi'ao Island in the Pearl River estuary, China, with a key to marine and brackish-water *Testudinella*

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Abstract

Two new species of the rotifer genus *Testudinella* (Rotifera, Monogononta, Testudinellidae), *T. pseudobscura* **sp. nov.** and *T. quadrilobata* **sp. nov.**, are described from the intertidal zone of a brackish-water tidal river and a mangrove swamp on Qi'ao Island in the Pearl River estuary, Guangdong province, southern China. Both species belong to *T. clypeata* (Müller, 1786)—*T. obscura* Althaus, 1957 group on account of possessing a proximal fulcrum opening, a slit-like foot opening and living in thalassic waters.

The trophi of *Testudinella zhujiangensis* Wei, De Smet & Xu, 2010 are redescribed, and a key to the marine and brackish-water *Testudinella* species known to date is presented.

Key words: Rotifera; *Testudinella*; brackish-water; new species; China

Introduction

Marine and brackish-water rotifers are commonly overlooked world-wide, because it is often hard to collect sufficient material, and of the time-consuming and laborious techniques needed to extract and prepare them for study (De Smet 2009). This also holds for China, where rotifer diversity in general is severely underestimated (Zhuge 1998). Data of Chinese brackish-water rotifers, which mainly focused on the Pearl River and Yangtze River estuary, are often outdated or confined to general zooplankton ecology research papers. Gao *et al.* (2008) recorded 28 rotifer species from three cruises during the flood, mean-flow and dry periods of the Pearl River estuary, and more than 100 species were found during zooplankton surveys of the Yangtze River (Han & Hu 1995; Hu *et al.* 2008; Wang *et al.* 1999; Zhang *et al.* 2005). However, almost all these studies were based on samples collected with a 64 µm, or larger, mesh-width zooplankton net, resulting in loss of small-sized species and consequent underestimation of rotifer diversity.

Only few new brackish-water rotifers have been described since the publication of the major taxonomic works on the Chinese rotifer fauna by Wang (1961) and Zhuge (1997). *Synchaeta arcifera* Xu, 1998 was described from the Mu-lan xi river, Fujian (Xu 1998), *Lecane yatseni* Wei & Xu, 2010 and *Testudinella zhujiangensis* Wei, De Smet & Xu, 2010 were described from Qi'ao Island in the Pearl River estuary, Guangdong (Wei & Xu 2010; Wei *et al.* 2010). From the latter region, two new species, *T. pseudobscura* **sp. nov.** and *T. quadrilobata* **sp. nov.** are described in the present study. A key to the marine and brackish-water species of *Testudinella* known to date is presented.

Material and methods

Littoral zooplankton samples of a brackish-water tidal river and a mangrove swamp near the river on Qi'ao Island in the Pearl River estuary, Guangdong province, southern China (Fig. 1) were studied. Qualitative sampling of the intertidal zone of the river was done with a 64 μm mesh width plankton net; quantitative samples of the mangrove swamp were collected with a plastic 2.5 L water sampler. Fixation followed by adding 40% formalin up to a final concentration of 4%. Rotifer specimens were selected and examined with a Nikon E800 microscope. Drawings were made using a camera lucida. The trophi of the specimens were prepared by dissolving the animals in NaOCl for scanning electron microscopy (SEM), following the procedure described in De Smet (1998). SEM was performed with a JEOL JSM-6330F microscope operated at 15KV. Permanent mounts of specimens in glycerine glass slide were prepared according to Jersabek *et al.* (2010).

Number of unci teeth and arched rami scleropili are given from left to right (number left/number right).

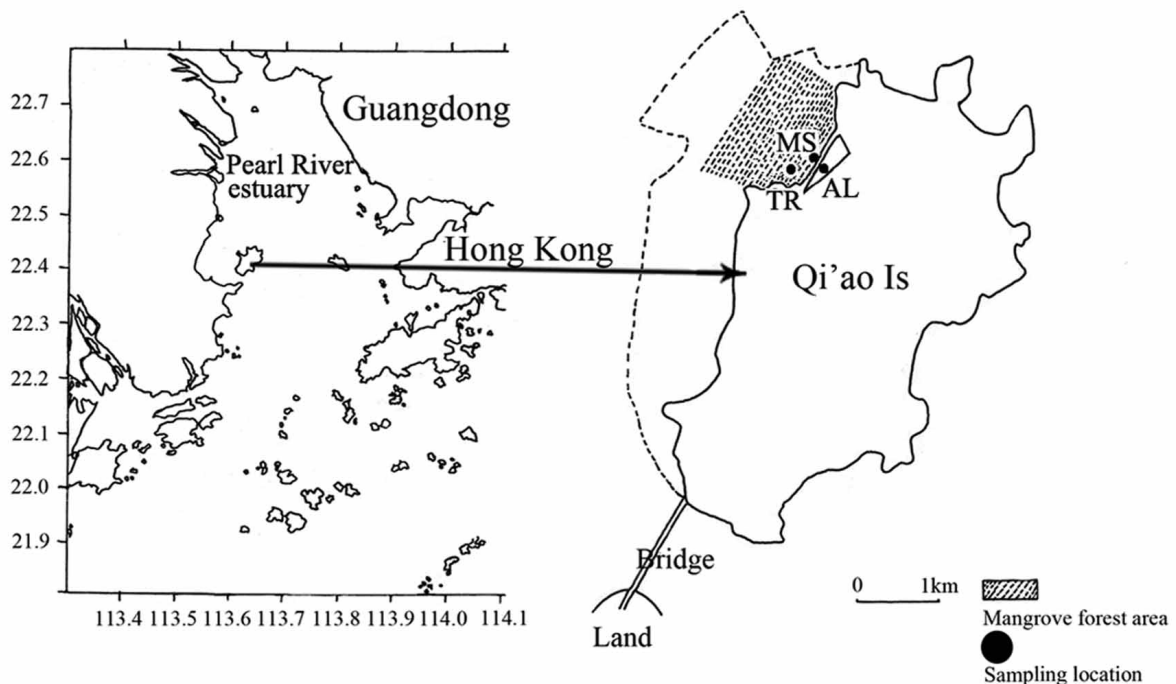


FIGURE 1. Location of the sampling sites on Qi'ao Island in the Pearl River estuary, Guangdong, China. Shadow area stands for mangrove forest; black circles represent the sampling sites, AL: artificial lake site; MS: mangrove swamp site; TR: tidal river site

Systematics

Phylum ROTIFERA Cuvier, 1812

Class EUROTATORIA De Ridder, 1957

Subclass MONOGONONTA Plate, 1889

Order FLOSCULARIACEA Harring, 1913

Family TESTUDINELLIDAE Harring, 1913

Genus *Testudinella* Bory de St. Vincent, 1826

***Testudinella pseudobscura* sp. nov.**

(Figs 2–5, 6A)

Diagnosis. *Testudinella pseudobscura* sp. nov. is characterized by: vase-shaped lorica; dorsal anterior margin with a slightly convex median projection and almost straight or slightly convex lateral parts; ventral anterior margin with a wide V-shaped median sinus and weakly convex lateral edges; longitudinal ventral lorica furrows discontinuous; lateral lorica edges broadly rounded in cross-sectional view; foot opening sub-terminal, inverted U-shaped slit; distal foot pseudosegment moderately long, penultimate one shorter; unci plates with 9–11/10–11 teeth.

Type locality. A tidal river of Qi'ao–Dan'gan Provincial Mangrove Nature Reserve on Qi'ao Island (22° 26' N, 113° 38' E), Zhuhai, Guangdong province, China: depth about 50 cm, distance from shore about 2 m, low tide water, 24 December 2009.

Holotype. A female in a permanent, glycerine glass slide mount deposited in the Biology Museum of Sun Yat-sen University, Guangdong, China (ROT00007).

Paratypes. 4 females from type locality. Two females in Biology Museum of Sun Yat-sen University (ROT00008, ROT00009); one female in the Academy of Natural Sciences of Philadelphia (ANSP 2079); one female in the Royal Belgian Institute of Natural Sciences (RBINS), Brussels, Belgium (No. IG. 312901, RIR 204).

Etymology. The species name *pseudobscura* refers to the shape of the lorica which is similar to that of *T. obscura* Althaus, 1957.

Description of female. The smooth or occasionally very weakly striated lorica (Fig. 2A–D) is vase-shaped, truncate anteriorly. The ratio length/width is on average 1.45 (1.38–1.56). The dorsal anterior margin shows a slightly convex median projection with almost straight (Fig. 2B) or slightly convex lateral parts. The ventral anterior margin shows a shallow wide V-shaped median sinus with two weakly convex lateral edges. The dorsal anterior margin is not or slightly projecting beyond the ventral margin. The posterior margin is smoothly rounded (Fig. 2A) or shows an inconspicuous rounded projection (Fig. 2B). In cross-section the lorica is slightly arched dorsally; the lateral edges are broadly rounded; apparently discontinuous longitudinal ventral furrows delimit a protruding, more or less flat (Fig. 2E) or convex (Fig. 2F) median part. The foot is composed of a long wrinkled proximal part, a short penultimate pseudosegment, and a longer distal pseudosegment ending in a ciliated cup (Fig. 2G). The ratio lorica length/position of antennae relative to dorsal anterior margin averages 2.89 (2.70–3.23) for the dorsal antenna and 2.25 (2.15–2.41) for the lateral antennae. Two red eyespots.

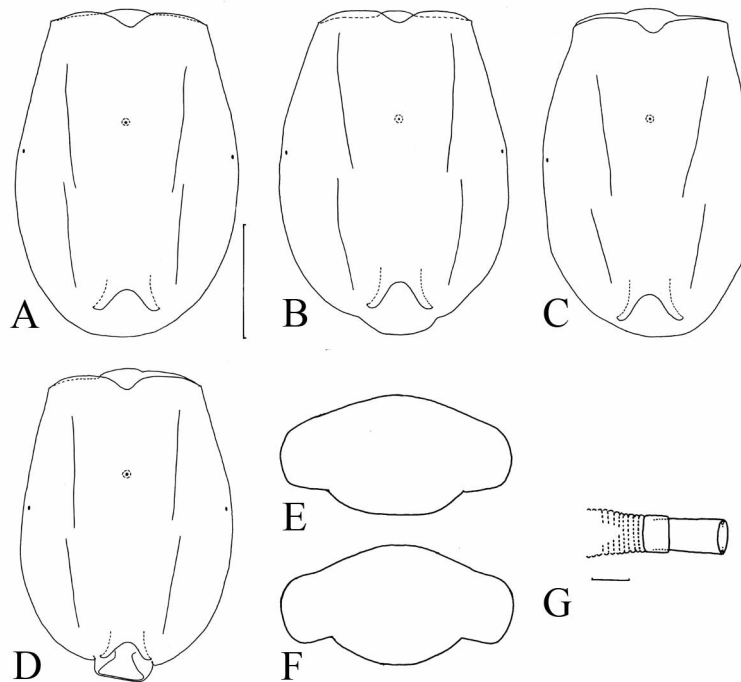


FIGURE 2. *Testudinella pseudobscura* sp. nov. **A–D.** Lorica, ventral view. **E, F.** Lorica, cross-section view. **G.** Foot pseudosegment. Scale bars: A–F: 50 μ m, G: 10 μ m.

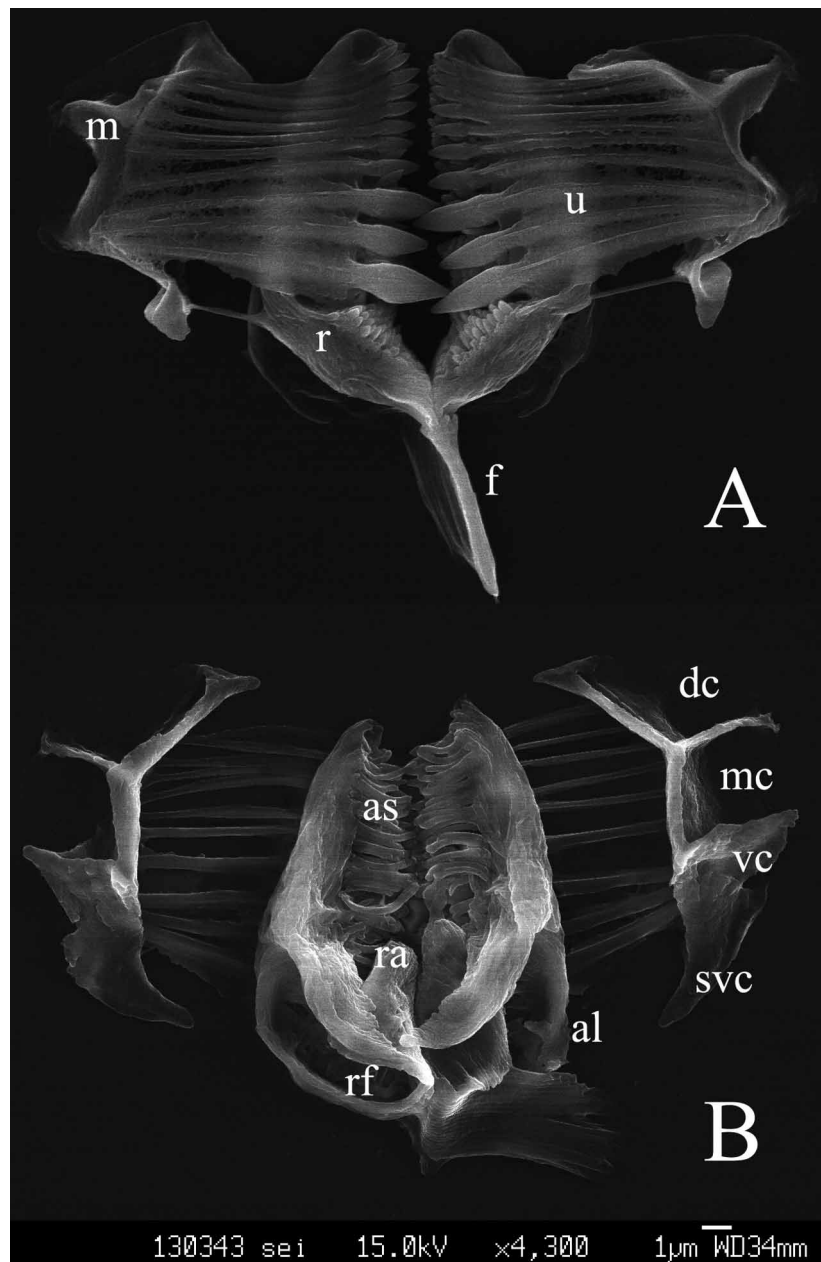


FIGURE 3. *Testudinella pseudobscura* sp. nov., scanning electron microscope photographs of trophi. **A.** Complete set, frontal view. **B.** Complete set, caudal view. al: alula, as: arched rami scleropili, dc: dorsal chamber, f: fulcrum, m: manubrium, mc: median chamber, r: ramus, ra: ramus apophysis, rf: ramus fenestra, svc: sub-ventral chamber, u: uncus, vc: ventral chamber.

Trophi malleoramate (Figs 3, 4). The rami are elongate-triangular with rounded latero-ventral margins. Basal and subbasal chambers forming a single large chamber, open latero-ventrally by a large common fenestra (Fig. 3B: rf). Weakly developed, more or less spiniform, caudally recurved alulae (Fig. 3B: al) situated at the latero-ventral margins of the frontal parts of rami. Asymmetrical median rami apophyses (Fig. 3B: ra) weakly developed. The inner margins of the distal rami sections caudally show 17–22/15–19 arched and webbed rami scleropili (Fig. 3B: as). Frontally, the inner margins of the rami bear several rows of rami scleropili with acute tip (Fig. 5: fs). The basal apophyses (Fig. 4: ba) are moderately developed ridges, composed of a series of basally fused scleropili. The fulcrum is short and plank-shaped, more or less trapezoid in lateral view. It is composed of a double layer of longitudinally oriented and appressed sclerite bodies, the caudal series of which is involved in the formation of the junction with the rami, and an anterior series which border a distinct opening proximally (Fig. 4: fo). The unci plates consist of 9–11/10–11 weakly curved and strongly webbed teeth. Each uncus has 3, occasionally 4, major teeth with moderately offset lanceolate heads of almost similar dimension, the head of the middle teeth is only slightly smaller than the others (Fig. 4). The minor teeth have a weakly offset lanceolate head bearing two minute

lateral knobs at their base; the webbing almost extends up to the base of the heads. The crescent shaped manubria are composed of the superimposed dorsal, median, ventral chamber and weakly developed sub-ventral chamber (Fig. 3B).

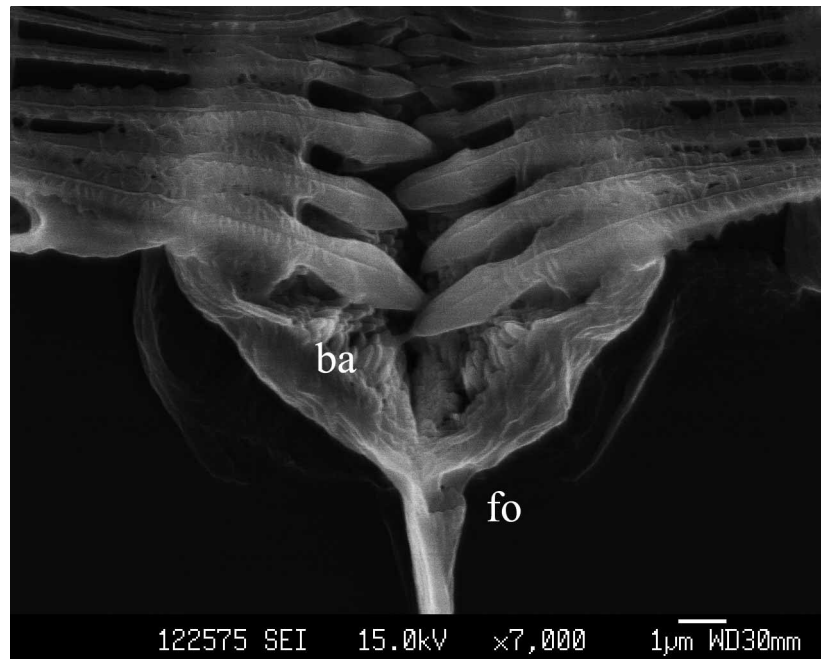


FIGURE 4. *Testudinella pseudobscura* sp. nov., scanning electron microscope photograph of trophi. Detail of major teeth and proximal part of fulcrum, frontal view. ba: basal apophysis, fo: fulcrum opening

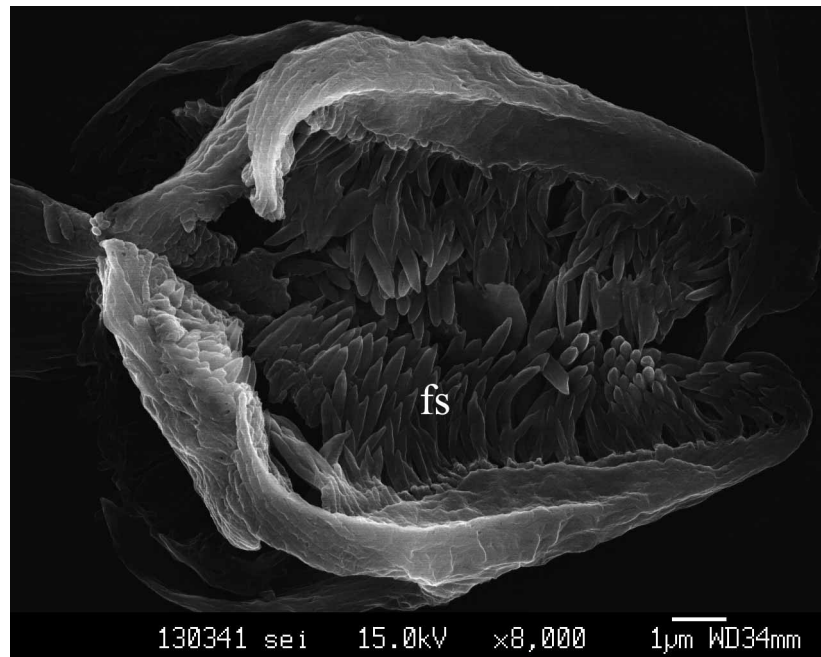


FIGURE 5. *Testudinella pseudobscura* sp. nov., scanning electron microscope photograph of trophi. Detail of rami, frontal view. fs: frontal rami scleropili.

Male and eggs unknown.

Measurements. Lorica length 130–145 μm (mean=140 μm , N=10), lorica width 93–100 μm (mean=96 μm , N=10), anterior aperture width 64–68 μm (mean = 66 μm , N = 10) , penultimate foot pseudosegment 4–6 μm (mean=5 μm , N=10), distal foot pseudosegment 13–15 μm (mean=14, N=10); trophi (N = 5): length \times width 19.1–21.3 \times 23.6–26.1 μm , ramus 11.8–13.6 μm , fulcrum 6.3–8.1 μm , largest major tooth 11.0–12.0 μm , manubrium 10.6–12.5 μm .

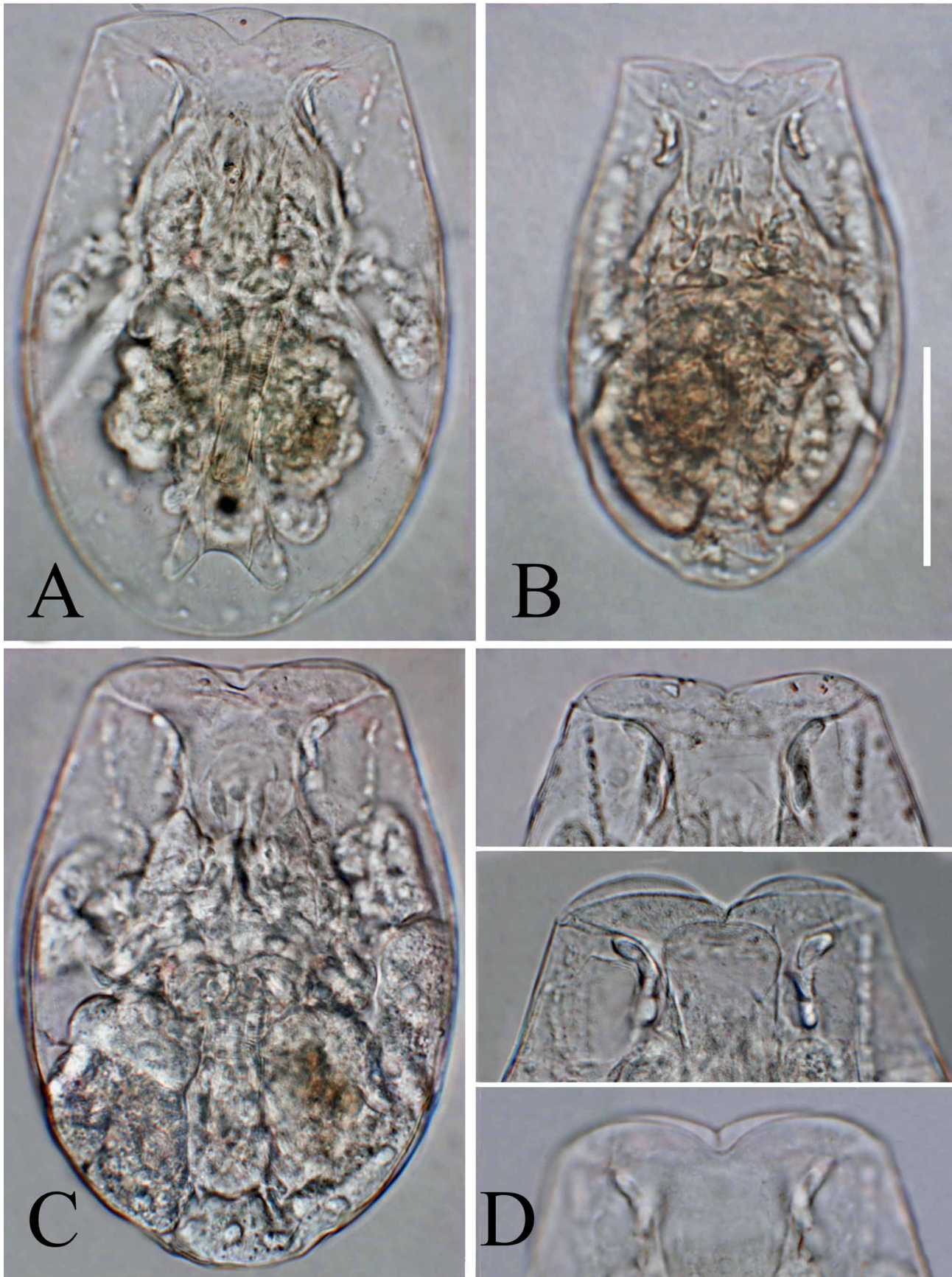


FIGURE 6. Light microscope photograph of three *Testudinella* species recorded from Qi'ao Island, China. **A.** *Testudinella pseudobscura* **sp. nov.** ventral view; **B.** *Testudinella zhujiangensis* ventral view; **C.** *Testudinella quadrilobata* **sp. nov.** dorsal view; **D.** *Testudinella quadrilobata* **sp. nov.** varying shape of anterior aperture. Scale bar: 50 μ m.

Distribution and ecology. The species was collected in the intertidal zone of the brackish-water river on Qi'ao Island of the Pearl River estuary, at depths of about 50 cm, and about 2 m from the shore as tide ebbed. Its occurrence was restricted to 6 and 24 December 2009; water temperature 18.6 and 18.5 °C, salinity 13 and 12 ‰, transparency 29.5 and 16.5 cm.

Comments. The new species can easily be confused with the closely related and morphologically very similar *T. obscura* Althaus, 1957 and *T. zhujiangensis*. The discrimination between *T. pseudobscura* **sp. nov.** and *T. zhujiangensis* indeed, became clear only after studying a large number of specimens by light microscopy and trophi investigation by SEM. In the original description of *T. zhujiangensis* (Wei *et al.* 2010), data on the trophi from both *T. pseudobscura* **sp. nov.** and *T. zhujiangensis* are mixed up, and the SEM photographs of the trophi (i.c., Figs 3, 4) actually concern *T. pseudobscura* **sp. nov.**. Features discriminating both species are as follows: *T. pseudobscura* **sp. nov.** is distinguished by its more robust lorica with relatively wider head aperture (Fig. 6A, B): the ratio lorica length/lorica width averages 1.45 (1.38–1.56, N=10) for *T. pseudobscura* **sp. nov.** and 1.58 (1.49–1.67, N=32) for *T. zhujiangensis*; the ratio lorica width/head aperture width averages 1.46 (1.37–1.52, N=10) for *T. pseudobscura* **sp. nov.** and 1.56 (1.41–1.69, N=32) for *T. zhujiangensis*. In cross-section the body of *T. pseudobscura* **sp. nov.** is arched dorsally, rounded laterally, and ventrally it shows a flat or more or less convex median part, whereas *T. zhujiangensis* shows 4 rounded shallow lobes. Furthermore, the slit-shaped foot opening of *T. pseudobscura* **sp. nov.** is apparently narrower, deeper and placed less distally. Additionally, *T. pseudobscura* **sp. nov.** has a larger body size (130–145 µm, mean 140 µm, N=10) than *T. zhujiangensis* (115–128 µm, mean 123 µm, N=32) (Fig. 7). The ratios lorica length/position of antennae relative to the antero-dorsal margin appear similar for both species: for the dorsal antenna the ratio averages 2.89 (2.70–3.23, N=10) in *T. pseudobscura* **sp. nov.** and 3.02 (2.80–3.24, N=22) in *T. zhujiangensis*, for the lateral antennae these ratios are 2.25 (2.15–2.41, N=10) and 2.26 (2.12–2.33 N=22) respectively. Differences in trophi structure of both species are very little. The alulae (Fig. 3B: al) are short, more or less spiniform and inconspicuous in the new species, whereas large, quadratic and robust in *T. zhujiangensis* (Figs 12A, B, 14: al). The number of unci teeth tends to be smaller in *T. pseudobscura* **sp. nov.** (9–11/10–11, mostly 10–11/10–11, N=15) than in *T. zhujiangensis* (11–13/11–12, mostly 12/12, N=9). Additionally, trophi size tends to be larger in *T. pseudobscura* **sp. nov.** than in *T. zhujiangensis* (Fig. 8).

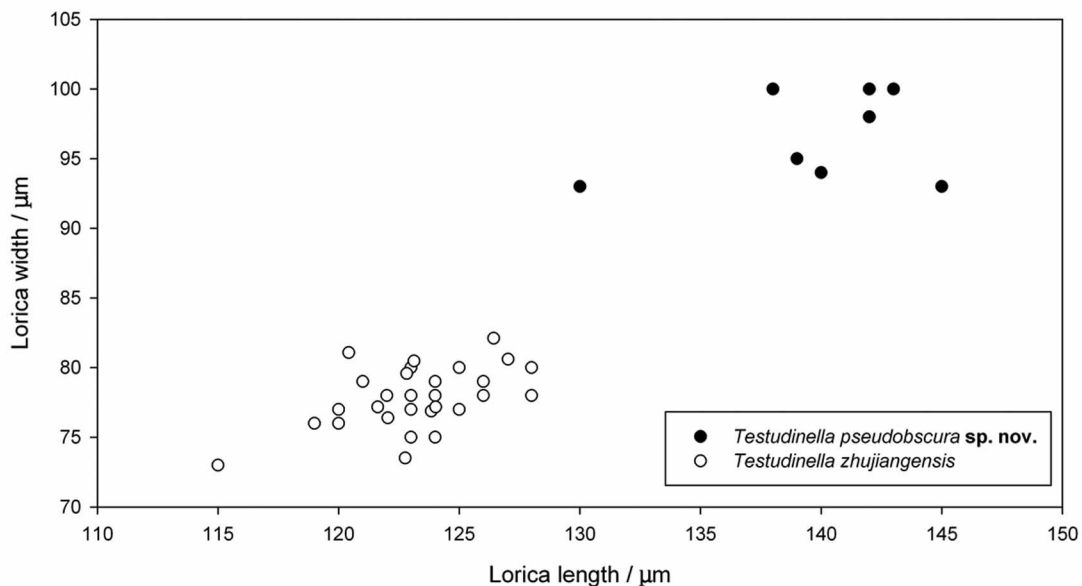


FIGURE 7. Lorica length versus width plot of *Testudinella pseudobscura* **sp. nov.** (N=10) and *T. zhujiangensis* (N=32).

In comparison with the more or less elliptical *T. obscura*, the new species has a distinct vase-shaped appearance. In cross-sectional view, the lateral lorica edges are broadly rounded in *T. pseudobscura* **sp. nov.**, whereas in *T. obscura* they converge to a rather sharp angle. Small differences also appear in the ratio lorica length/position of antennae relative to the antero-dorsal margin: for the dorsal antenna the ratio averages 2.89 (2.70–3.23) in *T. pseudobscura* **sp. nov.** and 2.60 (2.40–2.88) in *T. obscura*, for the lateral antennae these ratios are 2.25 (2.15–2.41) and 2.07 (1.97–2.23) respectively. Main differences in trophi structure concern the number of unci teeth and shape

of the head of the teeth. The teeth formula is 9–11/10–11 in *T. pseudobscura* **sp. nov.** and 10–12/11–12 in *T. obscura*. The heads of the major teeth are weakly offset and lanceolate in the new species, whereas distinctly offset and more or less strongly club-shaped in *T. obscura*. The heads of the minor teeth of *T. pseudobscura* **sp. nov.** are lanceolate as well, whereas cylindrical to elongate-lanceolate, with the webbing not as far extending up to the base as in the latter forming a rake-like structure in *T. obscura*. Additionally, the new species shows less developed sub-ventral manubrium chambers.

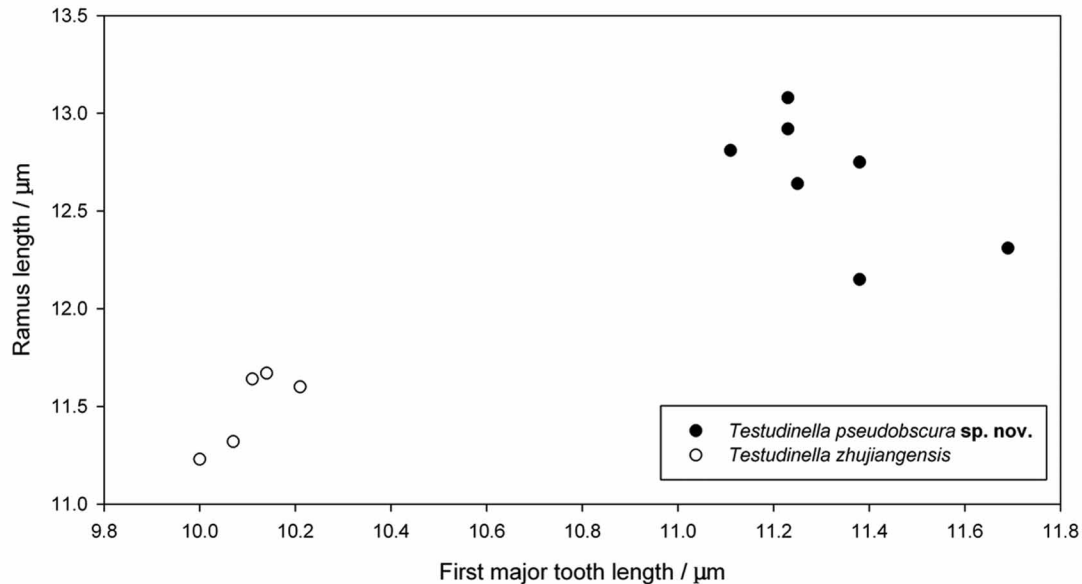


FIGURE 8. First major uncus tooth length versus ramus length plot of *Testudinella pseudobscura* **sp. nov.** (N=7) and *T. zhujiangensis* (N=5).

Testudinella pseudobscura **sp. nov.** resembles *T. bicorniculata* De Smet, 2009 and *T. quadrilobata* **sp. nov.** superficially. The new species is easily differentiated from *T. bicorniculata* by the absence of acute antero-lateral projections and the shape of the anterior margins which are undulate with shallow median sinus in the latter. It is readily distinguished from *T. quadrilobata* **sp. nov.** which shows medially indented anterior margins and a larger number of unci teeth (9–11/10–11 versus 14–15/14–15).

Testudinella quadrilobata **sp. nov.**

(Figs 6C, 9–11)

Diagnosis. *Testudinella quadrilobata* **sp. nov.** is characterized by: vase-shaped lorica; dorsal anterior margin almost straight to weakly undulate with minute median indentation; ventral anterior margin strongly undulate with wide V-shaped median sinus; longitudinal ventral furrows of lorica nearly parallel; foot opening sub-terminal, shallow inverted U-shaped slit; distal foot pseudosegment moderately long; proximal fulcrum opening obvious; unci plates with 14–15/14–15 teeth.

Type locality. A mangrove swamp in Qi'ao–Dan'gan Provincial Mangrove Nature Reserve on Qi'ao Island (22° 26' N, 113° 38' E), Zhuhai, Guangdong province, China: depth about 20 cm, distance from shore about 2 m, 17 January 2009.

Holotype. A female in a permanent, glycerine glass slide mount deposited in the Biology Museum of Sun Yat-sen University, Guangdong, China (ROT00010).

Paratypes. 4 females from type locality. Two females in Biology Museum of Sun Yat-sen University (ROT00011, ROT00012); one female in the Academy of Natural Sciences of Philadelphia (ANSP 2080); one female in the Royal Belgian Institute of Natural Sciences (RBINS), Brussels, Belgium (No. IG. 312901, RIR 205).

Etymology. The name of the species is composed of the prefix *quadri* derived from the Latin *quattuor*, meaning in four parts, and the Latin adjective *lobata*, meaning lobed, and refers to the four-lobed anterior margins of the lorica.

Description of female. The smooth lorica (Figs 6C, D; 9A, B) is vase-shaped, truncate anteriorly. The ratio length/width is on average 1.40 (1.35–1.46). The dorsal anterior margin has two slightly convex (Fig. 9A) or almost straight (Fig. 9B) parts separated by a small median indentation. The ventral margin shows a broader, shallow V-shaped median sinus with two distinct convex lateral parts. The dorsal anterior margin is not or only slightly projecting beyond the ventral margin (Fig. 6C, D). The posterior margin is smoothly rounded (Fig. 9B) or shows an inconspicuous rounded projection (Fig. 9A). Ventrally two almost parallel longitudinal furrows delimit a protruding median part. In cross-sectional view (Fig. 9C, D) the lorica is slightly arched dorsally; the lateral margins are broadly rounded, separated by a shallow sulcus from a weakly protruding median part ventrally; the ventral median part is almost flat (Fig. 9C) or with very shallow sulci laterally from foot channel (Fig. 9D). The foot opening is a shallow inverted U-shaped slit widening distally, situated sub-terminally on the ventral side; an oblique fold may run laterally from the distal edges of the foot opening. The foot is composed of a long wrinkled proximal part, a short penultimate pseudosegment, and a longer distal pseudosegment ending in a ciliated cup (Fig. 9E). The ratio lorica length/position of antennae relative to dorsal anterior margin averages 2.68 (2.50–2.82) for the dorsal antenna and 2.51 (2.34–2.72) for the lateral antennae. Two red eyespots.

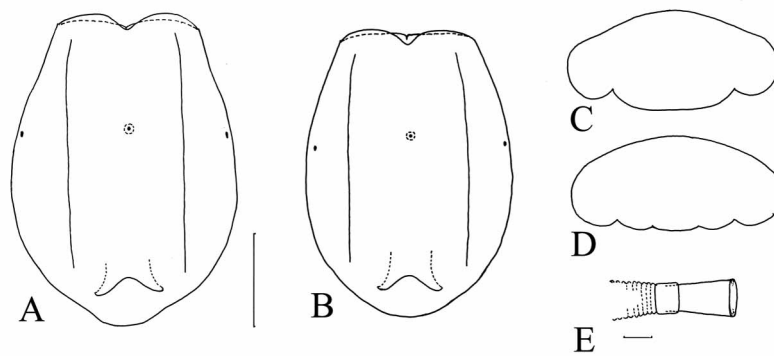


FIGURE 9. *Testudinella quadrilobata* sp. nov. **A, B.** Lorica, ventral view. **C, D.** Lorica, cross-section view. **E.** Foot pseudosegment. Scale bars: A–D: 50 μ m, E: 10 μ m.

Trophi malleoramate (Figs 10, 11). The rami are elongate-triangular with rounded latero-ventral margins, and short blunt alulae (Fig. 10B: al) pointing caudally. Basal and subbasal chambers form a single large chamber, opening latero-ventrally by a large common fenestra (Fig. 10B: rf). Asymmetrical and interlocking median rami apophyses (Fig. 10B: ra) are apparent in caudal view. The inner margins of the distal rami sections bear 33–34/32–33 arched rami scleropili (Fig. 10B: as). The basal apophyses (Fig. 11: ba) are moderately developed ridges, composed of a series of fused scleropili. The fulcrum is short and plank-shaped, more or less trapezoid in lateral view, and composed of a double layer of longitudinally oriented and appressed sclerite bodies. A distinct frontal proximal opening is present (Fig. 11: fo). The unci plates consist of 14–15/14–15 almost straight and webbed teeth with interlocking heads. There are three almost straight major teeth with weakly offset lanceolate head in each uncus; the head of the middle-most teeth is only slightly smaller than the others (Fig. 11). The minor teeth have a sharp, very weakly offset head bearing two minute lateral knobs at their base. The crescent shaped manubria consist of the superimposed dorsal, median and ventral chamber, and very weakly developed sub-ventral chamber (Fig. 10B).

Male and eggs unknown.

Measurements. Lorica length 139–165 μ m (mean=156 μ m, N=10), lorica width 100–119 μ m (mean=111 μ m, N=10), anterior aperture width 66–75 μ m (mean = 70 μ m, N = 10), penultimate foot pseudosegment 4–7 μ m (mean = 5 μ m, N = 10), distal foot pseudosegment 15–19 μ m (mean = 16, N = 10); trophi (N = 5): length \times width 23.1–24.9 \times 28.2–29.4 μ m, ramus 13.7–14.9 μ m, fulcrum 6.7–7.3 μ m, largest major tooth 11.7–12.5 μ m, manubrium 13.7–14.8 μ m.

Comments. *Testudinella quadrilobata* sp. nov. can hardly be confused with any congener. It bears a superficial resemblance with *T. dentata* Myers, 1934, from which it differs by the ventral anterior margin consisting of two convex lobes separated by a broad median sinus, instead of the two more or less straight to concave lobes separated by a rounded median sinus delimited by two acute projections in the latter. The dorsal anterior margin with median sinus appears less convex in *T. quadrilobata* sp. nov. than in *T. dentata*. In cross-sectional view, *T. quadrilobata* sp.

nov. is fairly high, asymmetrical dorsally and ventrally, with distinctly arched dorsal margin and flatter ventral margin, whereas *T. dentata* appears strongly compressed with weakly inflated median part, and symmetrical both dorsally and ventrally. Other *Testudinella* species with a vase-shaped lorica and undulate dorsal and ventral anterior margin showing a median sinus are *T. bicorniculata* and *T. truncata* (Gosse, 1886) which are easily distinguished by the acute antero-lateral lorica projections or angular antero-lateral lorica corners, absent in the new species. As concerns the trophi, *T. quadrilobata* **sp. nov.** shows a higher number of unci teeth (14–15/14–15) and arched rami scleropili (33–34/32–33) than *T. bicorniculata* (10–11/10–11; 11–14/11–16) and *T. truncata* (12/12; 22/25) (De Smet 2005, 2009).

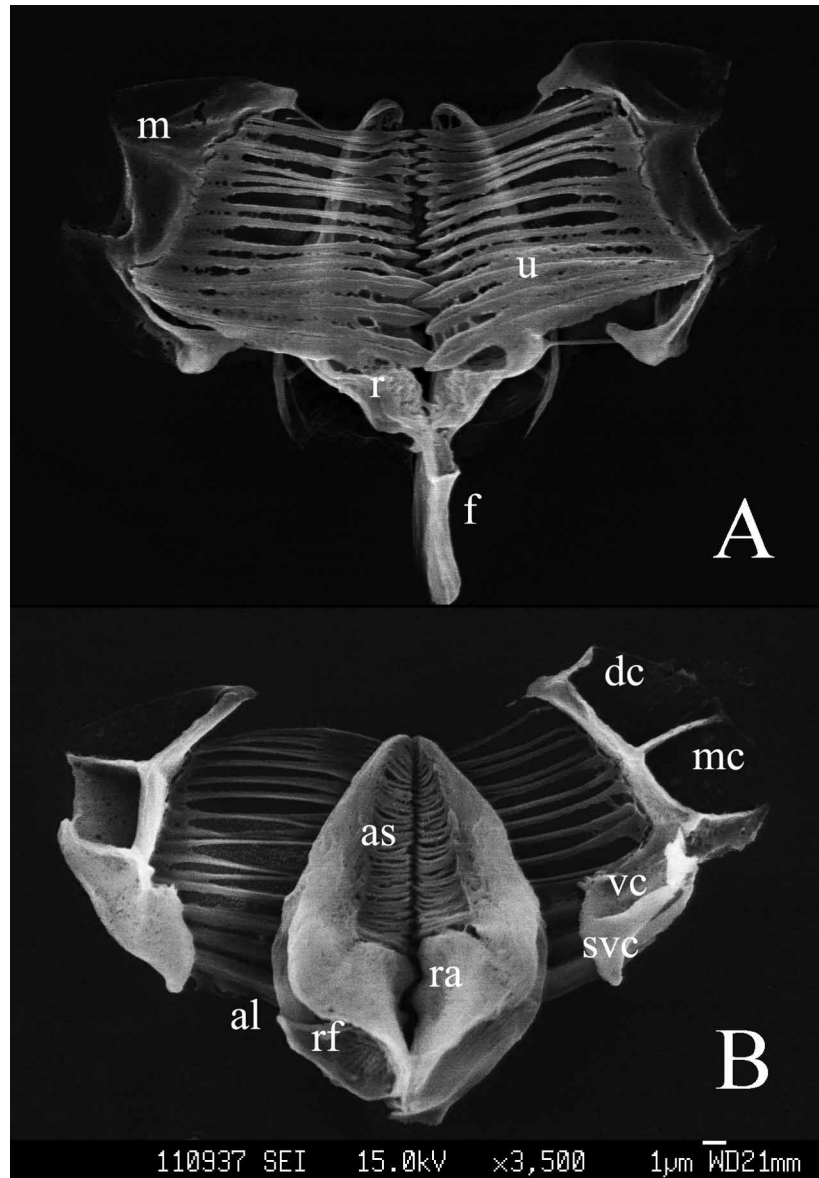


FIGURE 10. *Testudinella quadrilobata* **sp. nov.**, scanning electron microscope photographs of trophi. **A.** Complete set, frontal view. **B.** Complete set (fulcrum lost), caudal view. al: alula, as: arched rami scleropili, dc: dorsal chamber, f: fulcrum, m: manubrium, mc: median chamber, r: ramus, ra: ramus apophysis, rf: ramus fenestra, svc: sub-ventral chamber, u: uncus, vc: ventral chamber.

Testudinella quadrilobata **sp. nov.** belongs to the group of brackish water/marine *Testudinella*-species displaying a slit-like foot opening and proximal fulcrum opening, i.e., *T. bicorniculata*, *T. clypeata* (Müller, 1786), *T. elongata* De Smet, 2009, *T. obscura*, *T. pseudobscura* **sp. nov.** and *T. zhujiangensis* (information on the trophi of the other thalassic species with slit-like foot opening, *T. crassa* (Levander, 1894) **sp. inq.** and *T. dentata*, are unavailable). Of these species, *T. clypeata* and *T. quadrilobata* **sp. nov.** are the only ones sharing a high number of arched

rami scleropili and unci teeth: 28–30/25–26 and 14–16/14–16 versus 33–34/32–33 and 14–15/14–15 respectively (overall range of number of arched rami scleropili and unci teeth from 10–21 and 9–13 respectively in the other thalassic species).

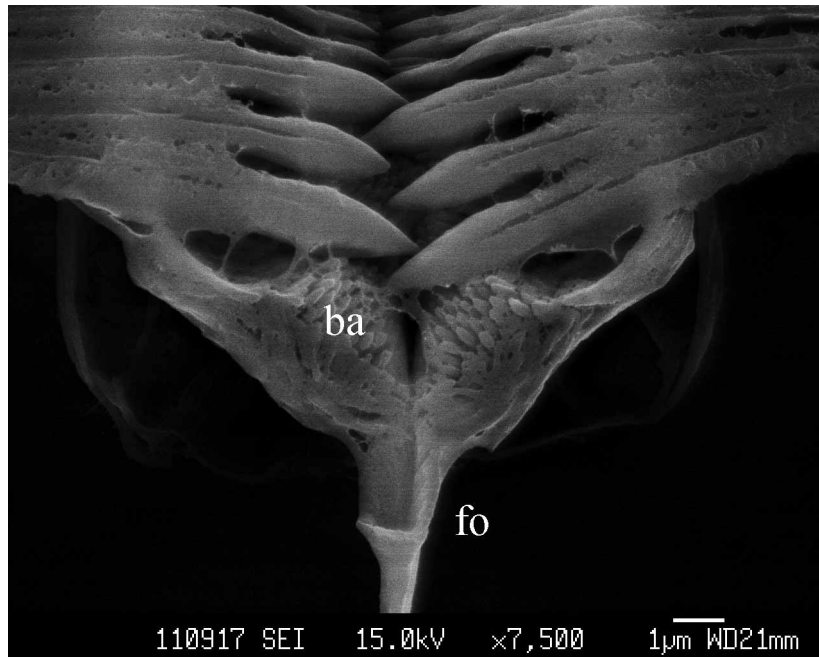


FIGURE 11. *Testudinella quadrilobata* sp. nov., scanning electron microscope photograph of trophi. Detail of major teeth and proximal part of fulcrum, frontal view. ba: basal apophysis, fo: fulcrum opening.

Distribution and ecology. The species was to date only collected in small numbers from a shallow mangrove swamp with varying water depth of 5–30 cm depending of the tide, and distances from the shore of about 2 m, in Qi'ao–Dan'gan Provincial Mangrove Nature Reserve on Qi'ao Island in the Pearl River estuary, on 17 January 2009, 28 February 2009, 27 March 2010, and 26 April 2010. Water temperatures varied from 18–23 °C. Salinity varied from 8–14 ‰. It occasionally co-occurred with *T. zhujiangensis*.

Testudinella zhujiangensis Wei, De Smet & Xu 2010

(Figs 6C, 12–14)

Redescription of trophi. Trophi malleoramate (Figs 12, 13). Frontal latero-ventral margins of rami with a large, quadratic and stout reinforced, caudally recurved alula (Figs 12–14: al). Median rami apophyses (Fig. 12B: ra) weakly developed. Inner margins distal rami sections bearing 14–21/13–21 strongly webbed arched rami scleropili (Figs 12B, 14A: as). Frontal rami scleropili with fairly acute distal tip (Fig. 14B: fs). Basal apophyses moderately developed (Fig. 13: ba). Fulcrum short with a distinct proximal opening frontally (Fig. 13: fo). Unci plates (Fig. 12A: u) consist of 11–13/11–12 weakly curved and strongly webbed teeth. Each uncus has 3, occasionally 4, major teeth with moderately offset lanceolate heads. Minor teeth with lanceolate head bearing two minute lateral knobs at their base; the webbing almost extends up to the base of the heads. Crescent-shaped manubria are composed of a superimposed dorsal, median, ventral and small sub-ventral chamber (Fig. 12B).

Measurements. Trophi (N = 6): length × width 17.4–20.4 × 22.4–24.5 µm, ramus 10.7–12.1 µm, fulcrum 5.2–6.1 µm, largest major tooth 10.0–10.6 µm, manubrium 10.7–12.4 µm.

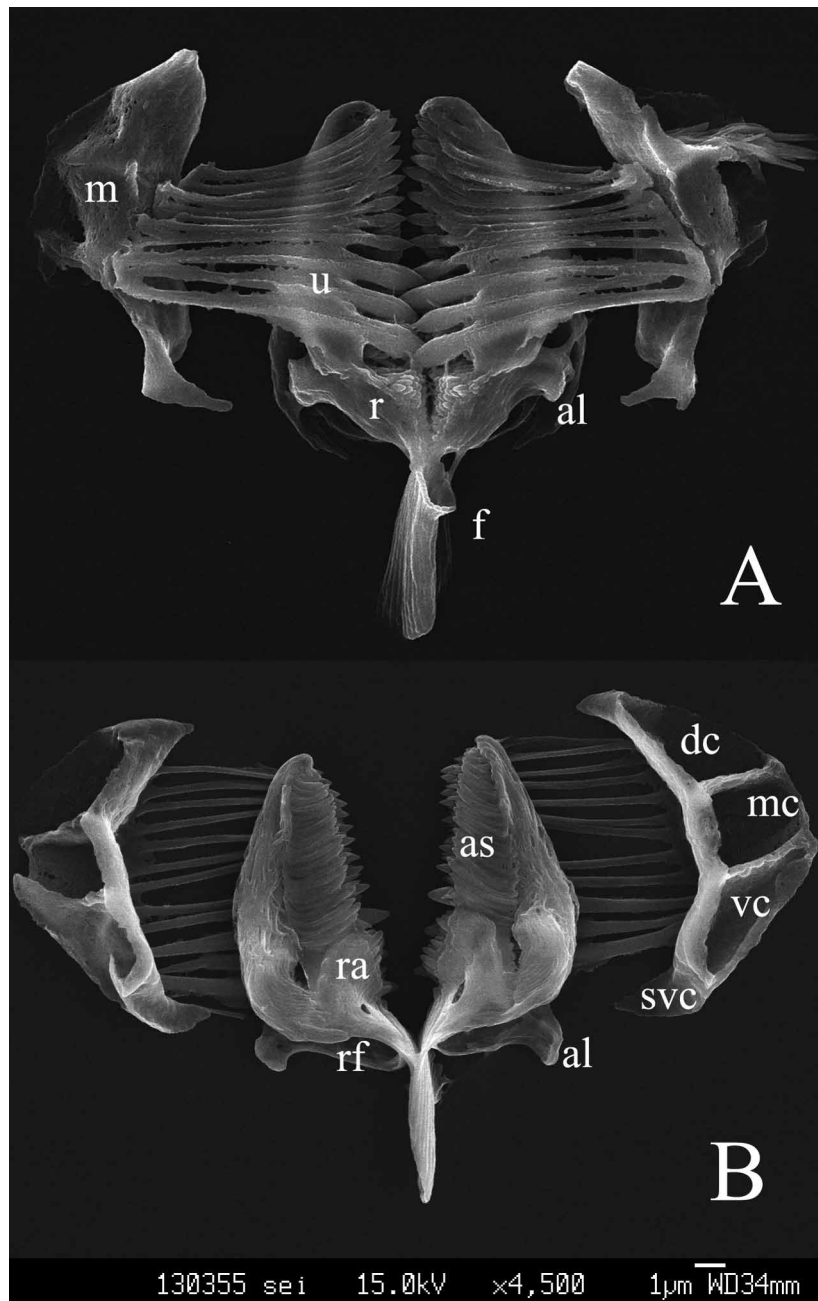


FIGURE 12. *Testudinella zhujiangensis*, scanning electron microscope photographs of trophi. **A.** Complete set, frontal view. **B.** Complete set, caudal view. al: alula, as: arched rami scleropili, dc: dorsal chamber, f: fulcrum, m: manubrium, mc: median chamber, r: ramus, ra: ramus apophysis, rf: ramus fenestra, svc: sub-ventral chamber, u: uncus, vc: ventral chamber.

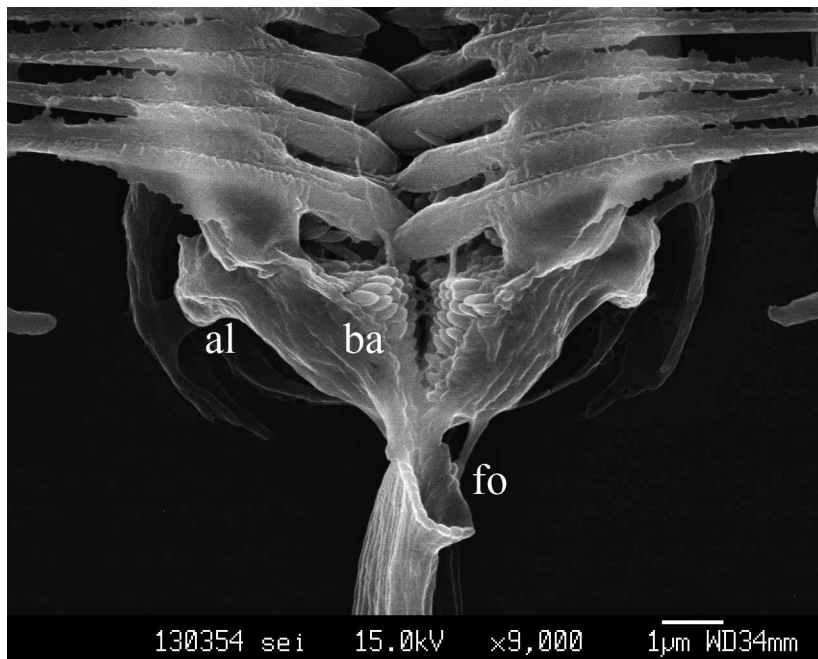


FIGURE 13. *Testudinella zhujiangensis*, scanning electron microscope photograph of trophi. Detail of major teeth and proximal part of fulcrum, frontal view. al: alula, ba: basal apophysis, fo: fulcrum opening

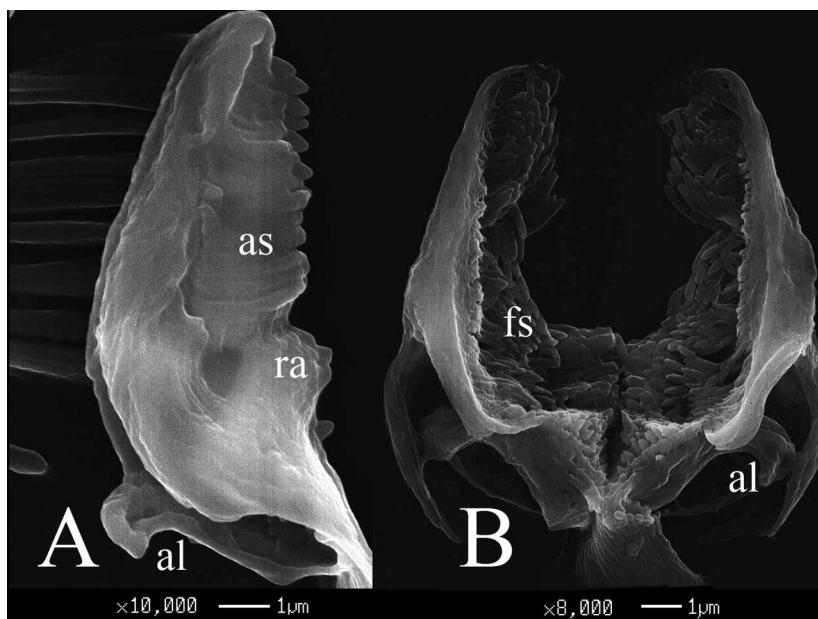


FIGURE 14. *Testudinella zhujiangensis*, scanning electron microscope photograph of trophi. A. Detail of ramus, caudal view. B. Detail of rami, frontal view. al: alula, as: arched rami scleropili, fs: frontal rami scleropili, ra: ramus apophysis

Key to marine and brackish-water species of *Testudinella*

- 1 Foot opening circular or semi-circular 2
- Foot opening a more or less wide, inverted U-shaped slit 3
- 2(1) Foot opening circular, situated near centre or slightly posterior to centre of lorica; lorica almost circular; antero-ventral margin with median sinus; antero-dorsal margin almost straight to weakly convex, or three more or less pronounced shallow lobes. *T. patina* (Hermann, 1783)
- Foot opening semi-lunar, almost terminally; lorica elliptical; antero-dorsal margin convex, antero-ventral margin with broad V-shaped sinus *T. elliptica* (Ehrenberg, 1834)

3(1)	Antero-ventral margin with median sinus bordered by two more or less acute projections	4
-	Antero-ventral margin almost straight or with median sinus not bordered by acute projections	5
4(3)	Antero-dorsal margin convex without median sinus; lorica in cross-section asymmetrical dorsally and ventrally, with arched dorsal margin and smaller ventral margin delimited by rounded lateral margins, not particularly compressed	
-	<i>T. crassa</i> (Levander, 1894) sp. inq.	
-	Antero-dorsal margin with median sinus; lorica in cross section symmetrical both dorsally and ventrally, with rounded lateral margins, distinctly compressed dorso-ventrally	<i>T. dentata</i> Myers, 1934
5(3)	Lorica strongly elongate, ratio length/width > 2.2; antero-ventral margin almost straight, antero-dorsal margin tripartite, with median part and two lateral parts	<i>T. elongata</i> De Smet, 2009
-	Lorica not strongly elongate, ratio length/width < 1.8; antero-ventral margin with distinct median sinus, antero-dorsal margin convex, or with median sinus, or tripartite	6
6(5)	Both antero-dorsal and antero-ventral margin with median sinus or indentation	7
-	Only antero-ventral margin with median sinus or indentation	8
7(6)	Antero-dorsal and antero-ventral margin with broad median sinus; two acute antero-lateral projections	
-	<i>T. bicorniculata</i> De Smet, 2009	
-	Antero-ventral margin with broad median sinus, antero-dorsal margin with small median indentation; antero-lateral projections absent	<i>T. quadrilobata</i> sp. nov.
8(6)	Antero-dorsal margin convex	<i>T. clypeata</i> (Müller, 1786)
-	Antero-dorsal margin tripartite, with median flat or slightly convex part and two lateral parts, whether or not connected by shallow folds	9
9(8)	Lorica more or less elliptical, weakly truncate anteriorly; in cross-section lateral edges converging to a sharp angle; major unci teeth with more or less strongly club-shaped head; minor unci teeth parallel, rake-like	<i>T. obscura</i> Althaus, 1957
-	Lorica vase-shaped, distinctly truncate anteriorly; in cross section lateral edges broadly rounded; major unci teeth lanceolate; minor teeth not parallel, not rake-like	10
10(9)	Lorica in cross-section almost symmetrical both dorsally and ventrally, more or less cruciform with broadly rounded lateral edges and rounded dorsal and ventral median part; rami with stout, quadratic alulae	
-	<i>T. zhujiangensis</i> Wei, De Smet & Xu, 2010	
-	Lorica in cross-section asymmetrical dorsally and ventrally, not cruciform, with arched dorsal margin, lateral edges broadly rounded delimiting a smaller, weakly convex median part ventrally; rami with weakly developed, more or less spiniform alulae	<i>T. pseudobscura</i> sp. nov.

Discussion

Details of the morphology of the lorica and trophi, such as the slit-like foot opening and the proximal fulcrum opening, as well as their brackish-water habitat indicate that *Testudinella pseudobscura* sp. nov. and *T. quadrilobata* sp. nov. belong to the *T. bicorniculata*, *T. clypeata*, *T. elongata*, *T. obscura* and *T. zhujiangensis* complex (De Smet 2009; Wei *et al.* 2010). Trophi structure can further be used to divide the complex into two groups: (1) species with a high number of unci teeth (14 to 16) and arched rami scleropili (25 to 34): *T. clypeata*, *T. quadrilobata* sp. nov.; and (2) species with a low number of unci teeth (9 to 13) and rami scleropili (10 to 21): *T. bicorniculata*, *T. elongata*, *T. obscura*, *T. pseudobscura* sp. nov. and *T. zhujiangensis*. When considering ecological characters, the complex appears strictly thalassic. *Testudinella bicorniculata*, *T. elongata* and *T. obscura* are characteristic elements of marine sub-tidal psammon, *T. clypeata* is a periphytic species inhabiting brackish-waters and the marine littoral, whereas *T. pseudobscura* sp. nov., *T. quadrilobata* sp. nov. and *T. zhujiangensis* were to date only found in littoral plankton of brackish waters, both running or stagnant (De Smet 2009; Wei *et al.* 2010). *Testudinella obscura*, *T. pseudobscura* sp. nov. and *T. zhujiangensis* can be very easily confused, and previous records (e.g. Green 1995) of *T. obscura* from brackish environments may be questioned.

Morphological differences between *Testudinella pseudobscura* sp. nov. and *T. zhujiangensis* are minute, suggesting that the two must be considered sibling species. Although phenotypic plasticity is shown by many rotifers, the coexistence of the latter two elements in the Pearl river estuary and the absence of intermediates with respect to shape and dimensions of the lorica and trophi size, as well as the tendency for a smaller number of unci teeth in *T. pseudobscura* sp. nov. support the validity of the two species. Notwithstanding that these differences in trophi size and number of unci teeth are small, they most probably represent genuine features. Indeed, studies on likewise malteoramate trophi (and other trophi types as well) by SEM of the genera *Filinia* and *Floscularia* showed that trophi elements do not grow after hatching and remain constant during the life of the rotifer (Fontaneto *et al.* 2003; Fontaneto & Melone 2005, 2006), and newborn individuals and adults of *Filinia* have the same number of unci teeth (Sanoamuang 1993a, b). In *Filinia* the number of these unci teeth also usually remains identical when grown at different temperatures, with variations rarely exceeding two teeth (Sanoamuang 1993a). DNA sequence analyses have

shown that many species of rotifer are complexes of sibling species, where morphological differences among distinct taxa are either absent or minute and often overlooked (e.g. Ciroso-Pérez *et al.* 2001; Gómez *et al.* 2002; Gilbert & Walsh 2005; Schröder & Walsh 2007). For example, in the *Brachionus plicatilis* complex, small differences in trophi size (e.g. Ciroso-Pérez *et al.* 2001) or shape of trophi elements (e.g. Fontaneto *et al.* 2007) were found consistent within each taxon and sometimes even the only reliable morphological feature to discriminate among particular cryptic species. According to these observations, it is to be expected that the application of molecular markers to the new *Testudinella* will reveal another complex of cryptic species. The co-occurrence of *T. pseudobscura* **sp. nov.** and *T. zhujiangensis* suggests ecological specialisation and partitioning of resources, that may primarily be influenced by salinity as indicated by studies of *B. plicatilis* (Gómez *et al.* 1995; Ortells *et al.* 2003; Lowe *et al.* 2007). Fontaneto *et al.* (2007) proposed not to use small morphological differences of cryptic species for ecological studies, but to discriminate the largely similar taxa by application of genetic markers. The latter consideration should also hold for *T. pseudobscura* **sp. nov.** and *T. zhujiangensis*, which show few useful discriminating morphological lorica characters and small differences in the trophi.

Marine and brackish-water rotifers from benthic-periphytic habitats have not been well studied and are often neglected in marine meiofauna researches (e.g. Fontaneto *et al.* 2006; De Smet 2009). During our study on the ecology of zooplankton of Qi'ao Island in the brackish Pearl River estuary, carried out at only 3 sampling sites, we now have described *Lecane yatseni* (Wei & Xu 2010) and three new species of *Testudinella*, demonstrating once more that the number of rotifers inhabiting thalassic waters is obviously underestimated.

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