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Cryptic diversity of the semi-terrestrial amphipod *Platorchestia japonica* (Tattersall, 1922) (Amphipoda: Talitrida: Talitridae) in Japan and Taiwan, with description of a new species

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Abstract

The talitrid amphipod *Platorchestia* Bousfield, 1982 is common in sandy beaches, estuarine marshes, shores of lakes and rivers. They are detritivores, and being prey for birds and other animals, they play an important role in the food chain. In the present study, we identified a new *Platorchestia* from Guan-du, Taiwan, based on morphological (light microscopy and scanning electron microscopy) and molecular approach (sequence divergence in the mitochondria DNA, COI) and described herein. *Platorchestia paludosus* **sp. nov.** from the Guan-du was morphologically different from all reported *Platorchestia* species, but it was very close to *P. japonica* (Tattersall, 1922). The distribution pattern of setae in the lateral margin of the telson and sharpness of ramus tip of uropod 3, however, exhibit diagnostic differences between the two species. From sequence divergence in COI, *Platorchestia paludosus* differed from *P. japonica* 13.3% in average, values that are comparable to inter-specific differences in other amphipod taxa.

Key words: Marsh, Amphipoda, Talitridae, *Platorchestia*, Beach fleas, Taiwan

Introduction

Talitrid amphipods inhabit a wide range of habitats including forests, marshes and coastal environments (Bousfield 1982). Bousfield (1984) subdivided the Talitridae into four systematic-ecological groups, (1) palustral talitrids (marsh-hoppers), (2) beach fleas (beach-hoppers), (3) sand hoppers and (4) land hoppers. Beach fleas do not modify their substrate but occupy semi-terrestrial to terrestrial habitats in supralittoral and coastal forests. The beach fleas are represented by the genus *Platorchestia*, which includes seventeen species (Serejo & Lowry 2008 and the new species described here). In the N.W. Pacific, *P. japonica* (Tattersall, 1922) was reported to be widely distributed in the W. Pacific region including China, Japan and Taiwan (Tattersall 1922; Morino & Dai 1990; Morino 1999; Miyamoto & Morino 2004). *Platorchestia japonica* inhabits leaf litter found at the waterfront of rivers, lakes and paddy fields (Tattersall 1922; Iwasa 1965; Morino & Dai 1990; Morino 1999; Hou & Li 2003; Miyamoto & Morino 2004; Hou & Li 2005), playing important ecological roles in the detritus food chains (Friend & Richardson 1986; Richardson & Morton 1986; Morritt & Spicer 1998; Graça *et al*. 2000).

Platorchestia japonica was first described from Lake Biwa, Japan by Tattersall (1922), and descriptions of *P. japonica* from previous studies mainly relied on light microscopy, which can only reflect limited morphological details (see Miyamoto & Morino 2004). Recent advances in using Scanning Electron Microscopy (SEM) can reflect more sophisticated morphological details (see Lowry & Springthorpe 2009), especially the types of setae (also see Miyamoto & Morino 2004 for the importance of setae in *Platorchestia* taxonomy based on light microscopy), allowing identification of individual species from species complexes (Lowry & Springthorpe 2009; Zimmer *et al*. 2009). Recent SEM studies have started in the family Dogielinotidae, reporting seven groups of setae which can be used for taxonomic studies (Zimmer *et al*. 2009).

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TABLE 1. Classification of different types of setae of *Platorchestia*, modified from Zimmer *et al.* (2009).

Setae classification under SEM	Figure	Description
Simple setae: Shaft long and smooth, without any setules, tips can have or have no pores.	Type I (Fig 7–a)	Length 30~40μm,tip sharp without pores.
	Type II (Fig 7–b)	Length 200~400μm,tapering gradually towards the apex, without terminal pore.
	Type III (Fig 7–c)	Length 40~50μm, shaft width uniform, tip rounded with terminal pore.
	Type IV (Fig 7–d)	Length 80~90μm,apex lamellae, apex length about 1/2–1/5 shaft length, terminal pore.
	Type V (Fig 7–e)	Length 20~25μm, shaft width uniform, tip flattened.
	Type VI (Fig 7–f)	Length 30~40μm, base wide, apex lamellae, apex length 1/3 shaft length, terminal pore.
Cuspidate setae: Shaft robust, length width ratio < 8, tip with or without terminal pore.	Type I (Fig 7–g)	Length 10~20μm, base width about 10μm, smooth, apex sharp.
	Type II (Fig 7–h)	Length 70~250μm, base width about 10μm, smooth, apex sharp.
	Type III (Fig 7–i)	Length 30~100μm, apex sharp with additional accessory lamaella seta.
Serrate setae: Shaft with setules from mid region to tip, base naked, setules slender or robust.	Type I (Fig 7–j)	Length 50~60μm, shaft robust, single row of robust setules, apex sharp.
	Type II (Fig 7–k)	Length 60~90μm, shaft long and slender, with single row of slender setules starting from the mid region of shaft to tip.
	Type III (Fig 7–l)	Length 60~90μm, shaft long and slender, with double row of slender setules starting from the mid region of shaft to tip.
	Type IV (Fig 7–m)	Length 25~30μm, similar to type I but with sharp and short setules.
Serrulate setae: Shaft with setules from mid region to tip, base naked, setules wide and flattened.	Type I (Fig 7–n)	Length 70~80μm, shaft long and slender, double row of flattened setules.
Plumose setae Shaft long and slender, with double rowed, long and fine setules, starting from base to tip	type I (Fig 7–o)	Length 200~300μm.
Pappose setae Shaft with very dense, long and slender setules, without terminal pore.	Type I (Fig 7–p)	Length 60~80μm，dense short setules on one side of shaft，the base region of the opposite side of shaft with a group of long setules.
	Type II (Fig 7–q)	Length 150~200μm，shaft long and slender, with dense and randomly distributed setules.
Papposerrate setae Shaft long and slender with flattened shape setules randomly distributed on shaft	Type I (Fig 7–r)	Length 50~100μm，setules long and flattened 。
	Type II (Fig 7–s)	Length 20~30μm, shaft robust, setules wide, flattened and leaf-shaped.
Setule Short and slender, flattened or leaf-shaped, on surface of setae or cuticle surface	Type I (Fig 7–t)	Length15~20μm，flattened, serrated on both sides。
	Type II (Fig 7–u)	Length 20μm，flattened, tapering apex.

From our recent studies on morphological and molecular variation of *P. japonica* collected from Lake Biwa in Japan and Guan-du in Taiwan, we revealed *P. japonica* populations from Japan and Taiwan exhibit significant distinct genetic variations, suggesting *P. japonica* contains a cryptic species complex. The objectives of this study is to use SEM and molecular phylogenetic analysis to study the morphological variation and sequence divergence of the mitochondria gene in Cytochrome c oxidase subunit I (COI) mitochondria DNA in the *P. japonica* collected from the Lake Biwa, Japan and from Guan-du, Taipei, Taiwan, to investigate the potential cryptic diversity of *Platorchestia* in the N.W. Pacific region. Our studies showed that the population from Taiwan is different from that collected from the type locality, in terms of both morphology and genetic distance. We regard the Taiwan population as a new species and described herein. Taxonomic terminology in the present study follows Morino (1999) and setal SEM terminology used in the present were based on *P. japonica* from Lake Biwa, Japan and modified from Zimmer *et al*. (2009) (Fig. 1; Tab. 1). Specimens studied were deposited in the collections in National Museum of Natural Science (NMNS), Taichung, Taiwan, Biodiversity Research Museum, Academia Sinica (ASIZCR) and Australian Museum (AM).

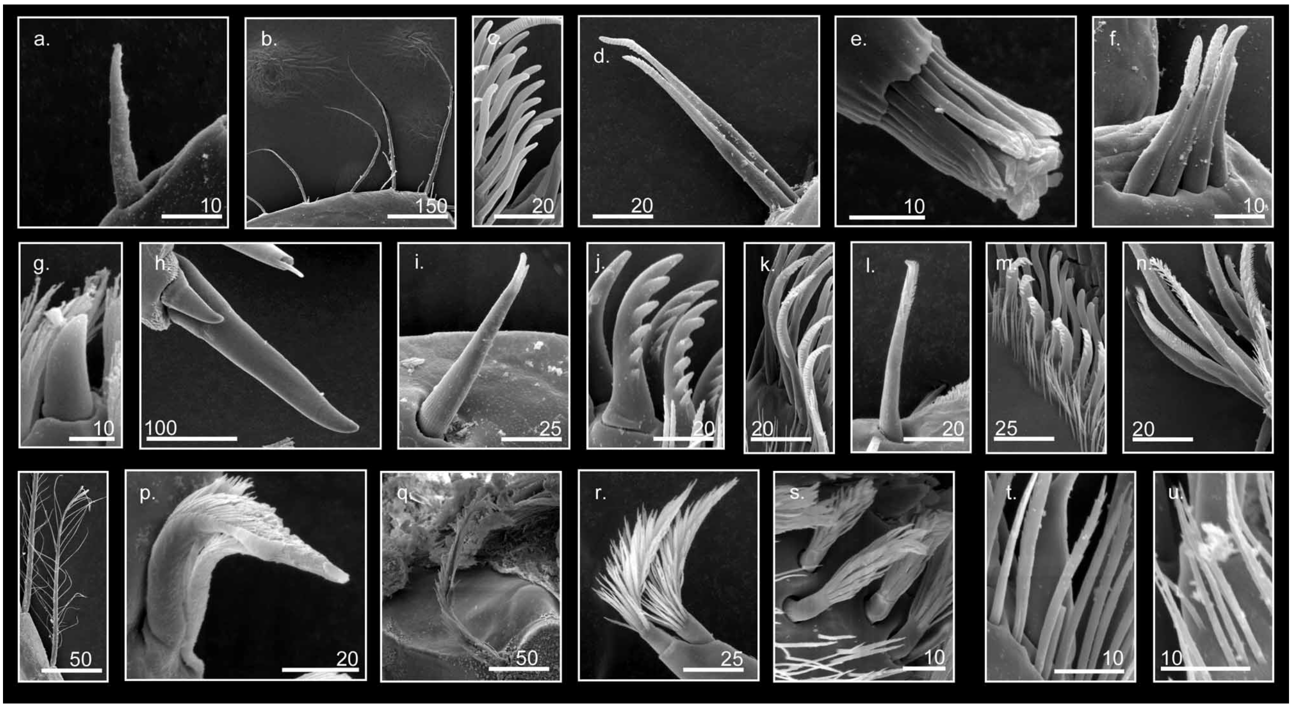

FIGURE 1. *Platorchestia paludosus* **sp. nov.** Types of setae revealed from SEM. a–f. Simple setae. g–i, Cuspidate setae, j–m Serrate setae, n. Serrulate setae, o. Plumose setae, p–q. Pappose setae, r–s Papposerrate setae, t–u. Setule.

Material and methods

Sample collection and morphology observation. Specimens in Taiwan were collected from salt-marsh in Guan-du, during Sept. 2007 to Oct. 2008, and preserved in 70% ethanol upon collection. Four *Platorchestia japonica* (Tattersall) specimens were collected from Lake Biwa, Japan (Type locality) for comparative study. Specimens were dissected under stereo-microscopes, appendages and mouthparts were dehydrated in grading Ethanol (75%, 95% and 100%, each with 30 minutes) and dried using a Critical Point Dryer (CPD) (Pelco CPD2) and subsequently observed using Scanning Electron Microscope (FEI Quanta 200, USA).

Molecular analysis. Total genomic DNA was extracted from soft tissue of amphipods, using the commercial QIAamp Tissue Kit (QIAGEN, Hilden, Germany). For amplifying mitochondrial COI sequences with a polymerase chain reaction (PCR), the universal primers LCO1490 and HCO2198 (Folmer *et al.* 1994) was used: 5’-GGTCAACAAATCATAAAGATATTGG-3’ and 5’-TAAACTTCAGGGTGACCAAAAAATCA-3’. The PCR conditions for COI were as follows: 2 min at 95°C for initial denaturing, then 35 cycles of 1 min at 95°C, 1 min. at 50°C, 1 min. at 72°C with a final extension for 5 min. at 72°C. Sequences were generated using the same sets of primers and determined on an Applied Biosystems (ABI) 3100 automated sequencer using the ABI Big-dye Ready-Reaction mix kit, following the standard cycle sequencing protocol and were aligned with the aid of MEGA ver. 4 (Tamura *et al*. 2007), after verification with the complementary strand. Sequences were deposited in the GenBank (NCBI).

Neighbor joining (NJ) tree were generated by MEGA ver. 4.0 (Tamura *et al*. 2007), the Bayesian inference (BI) tree by the program MrBayes ver. 3.12 (Ronquist & Huelsenbeck 2003), and the maximum likelihood (ML) tree using RAxML (Stamatakis *et al*. 2005) through on line server Cyberinfrastructure for Phylogenetic Research (CIPRES; http://www.phylo.org; conducted on 7 May, 2010). Substitution models TIM1+I+G model, which were used to specify a priori parameter in BI analysis, based on the corrected Akaike information criterion implemented in jModelTest ver. 0.1.1. Two independent Markov-chain-Monte-Carlo (MCMC) searches with random starting points were conducted in BI for each dataset until the divergence between the two runs became small and stationary. Trees were sampled every 1,000 cycles in 20,000,000 generations with the burn-in value set to the first-quarter of the sampling trees, in which consensus was reached for the two parallel runs.

Results

Systematics

Infaorder Talitrida Rafinesque, 1815, emended Serejo 2004

Superfamily Talitroidea Bulycheva, 1957, emended Serejo 2004

Family Talitridae Raphinesque, 1815, emended Serejo 2004

Genus *Platorchestia* Bousfield, 1982

Platorchestia paludosus sp. nov.

(Figures 2–9)

Platorchestia japonica. —Miyamoto & Morino 2004 : 83–88 (non *P. japonica*).

Materials examined. Holotype (NMNS—6519-001) 1 male, Guan-du, Taipei County, Taiwan, Aug 2008, Coll. Y-T. Cheng. Paratypes (NMNS—6519-002–06519-009) 4 males and 4 females, data same as holotype. Paratypes (AM—P.84807) 2 males and 2 females, data same as holotype. Paratypes (ASIZCR—000230) 2 males and 2 females, data same as holotype.

Comparative material. *Platorchestia japonica* (Tattersall, 1922). Lake Biwa, Japan. 2 males 2 females.

Description. Male. Body smooth, without protuberance. Eyes sub-rounded, larger than 1/3 of head height. Antenna 1 (Figs 3–a, b, c and d) short with 6–7 articles (3 peduncle articles, 3–4 flagellated articles), slightly longer than article 4 of antenna 2, peduncle articles 1–3 equal in length, dorsal side convex, ventral side flattened, each peduncle article with simple setae, article 4 slightly curved, each flagellated article with 2 groups of simple setae type IV (Fig. 1–d) on the inner and outer side of the dorsal surface.

Antenna 2 (Figs 3–e, f, and g) up to 1/3 of the body length, outer margin of ventral side of peduncle article 3 with 3–5 simple setae type IV (Fig. 1–d), a medial furrow extend from distal 1/3 of peduncle article 4 to entire peduncle article 5, length of article 5 equals to combined length of article 3 and 4, 11–14 flagellated articles each with medial furrow, flagellum subequal to peduncle 3–5 combined in length; tip of antenna 2 with simple setae type V (Fig. 1–e).

Upper lip entire, apical margin with fine setule.

Incisor of mandible (Figs 3–i, j) 5 to 6 dentate, left lacinia mobilis 5–dentate, right lacinia mobilis bi-fid, with serrated cutting edges; molar finely serrated, right molar convex, left molar concave; 3–4 pappose setae type I (Fig. 1–p) located in the region between the lacinia mobilis and molar, 1 pappose seta type II (Fig. 1–q) on the dorsal side of molar.

Lower lip bi-lobed, lobes wide, with fine setule on the inner margin.

Inner plate of maxilla 1 (Figs 3–l, m) narrow, with 2 apical papposerrate setae type I (Fig. 1–r); outer plate with 9 serrate setae type I (Fig. 1–j), with a row of 13–19 setule type I (Fig. 1–t) on ventral base of inner 4 serrate setae; palp very small, 2 articulate.

Inner plate of maxilla 2 (Fig. 3–n, o, p, q, r and s) smaller than outer plate, distal margin with 17–19 simple setae type III (Fig. 1–c), a row of 8–10 serrate setae type II (Fig. 1–k) on ventral and a row of 6–11 serrate setae type IV (Fig. 1–m) on dorsal, inner margin with 2 papposerrate setae type I (Fig. 1–r) , inner one longer; distal margin of outer plate with 15–20 simple setae type III (Fig. 1–c), a row of 9–12 serrate setae type II (Fig. 1–k) on ventral surface, a row of 3 serrate setae type IV (Fig. 1–m) and 2 serrulate setae type I (Fig. 1–n) on dorsal surface.

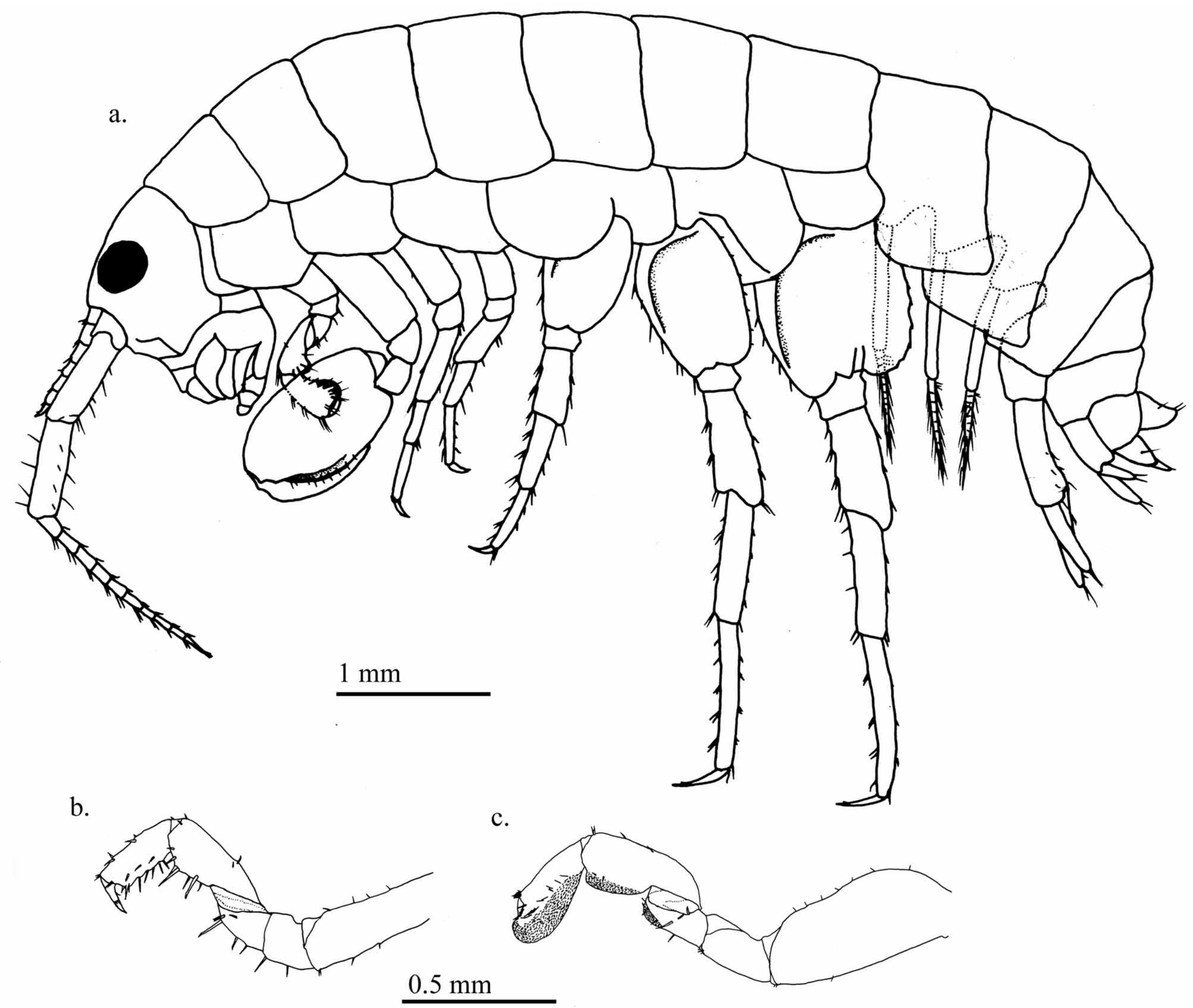

FIGURE. **2**. *Platorchestia paludosus* **sp. nov.** a. external view, male. b. female gnathopod 1. c. female gnathopod 2.

Distal end of maxilliped inner plate (Figs 4–b, e) with 3 cuspidate setae type I (Fig. 1–g), a row of 3–4 papposerrate setae type II (Fig. 1–s) behind the cuspidate setae, dorsal surface (Fig. 4–b) with a row of papposerrate setae type II (Fig. 1–s) along inner margin, with fine setae situating behind the papposerrate setae; ventral surface (Fig. 4–e) with several papposerrate setae type II (Fig. 1–s); distal end of outer plate (Figs 4–n, o) with a row of 4–5 papposerrate setae type II (Fig. 1–s) along margin, along inner margin to ventral surface of distal corner with 2 rows of simple setae type VI (Fig. 1–f), ventral surface of medial inner margin (Fig. 4–q) with a row of 3–5 simple setae type VI (Fig. 1–f); palp article 1 wider than high, with a row of 3–4 simple setae type VI (Fig. 1–f) on ventral surface of distal inner corner (Fig. 4–a, m), distal outer margin (Figs 4–a, c, j and l) with 1–2 cuspidate setae type III (Fig. 1–i) and 2–3 simple setae type VI (Fig. 1–f); distal inner corner of ventral surface of palp article 2 (Figs 4–a, and h) with a row of 3–4 simple setae type VI (Fig. 1–f), along the inner margin (Figs 4–a, and k) with 2 rows of simple setae VI (Fig. 1–f), distal outer margin (Figs 4–a, c, g and i) with 1–2 cuspidate setae type III (Figs 1–i) and 2–3 simple setae type VI (Fig. 1–f), article 3 rounded apically, dorsal surface (Figs 4–c, and f) with a row of 4–5 serrate setae type III (Fig. 1–l) and 2–3 simple setae type VI (Fig. 1–f) along the base of palp article 4, ventral surface with (Figs 4–a, d) several simple setae type VI (Fig. 1–f), outer distal corner (Fig. 4–a, c, d and f) with a group of 2–3 simple setae type IV (Fig. 1–d); article 4 small with several simple setae type III (Fig. 1–c) and simple setae type VI (Fig. 1–f) apically.

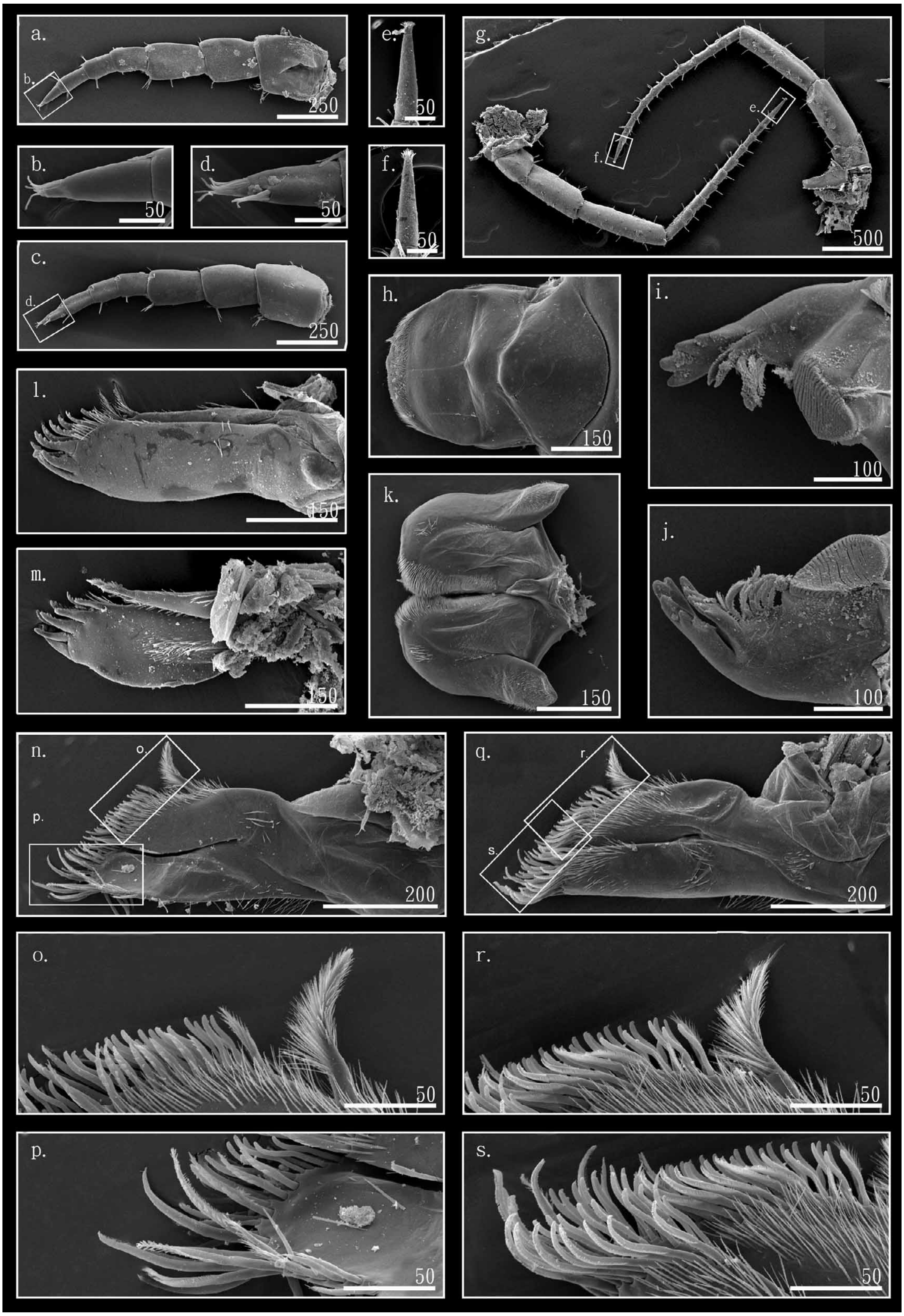

FIGURE 3. *Platorchestia paludosus* **sp. nov.** male. R At 1, a) ventral view, b) tip, ventral view. L At 1, c) dorsal view, d) tip, dorsal view. R At 2, e) tip, inner lateral view. f) tip, outer lateral view. g) R At 2 (L one), L At 2 (R one). h) Ul, dorsal view. i) R Md, dorsal inner lateral view. j) L Md, dorsal inner lateral view. k) Ll, ventral view . l) R Mx 1, ventral view. m) L Mx 1, dorsal view. R Mx 2, n) ventral view, o) inner plate margin, ventral view, p) outer plate margin, ventral view. L Mx 2, q) dorsal view, rs) inner plate margin, dorsal view, s) outer plate margin, dorsal view. R: right; L: left; At: antenna; Ul: upper lip; Md; mandible; Mx: maxilla; Ll: lower lip; Mp: maxilliped; Pa: palp; Gp: gnathopod; Pp: perepod; Plp: pleopod; Up: uropod; Tl: telson.

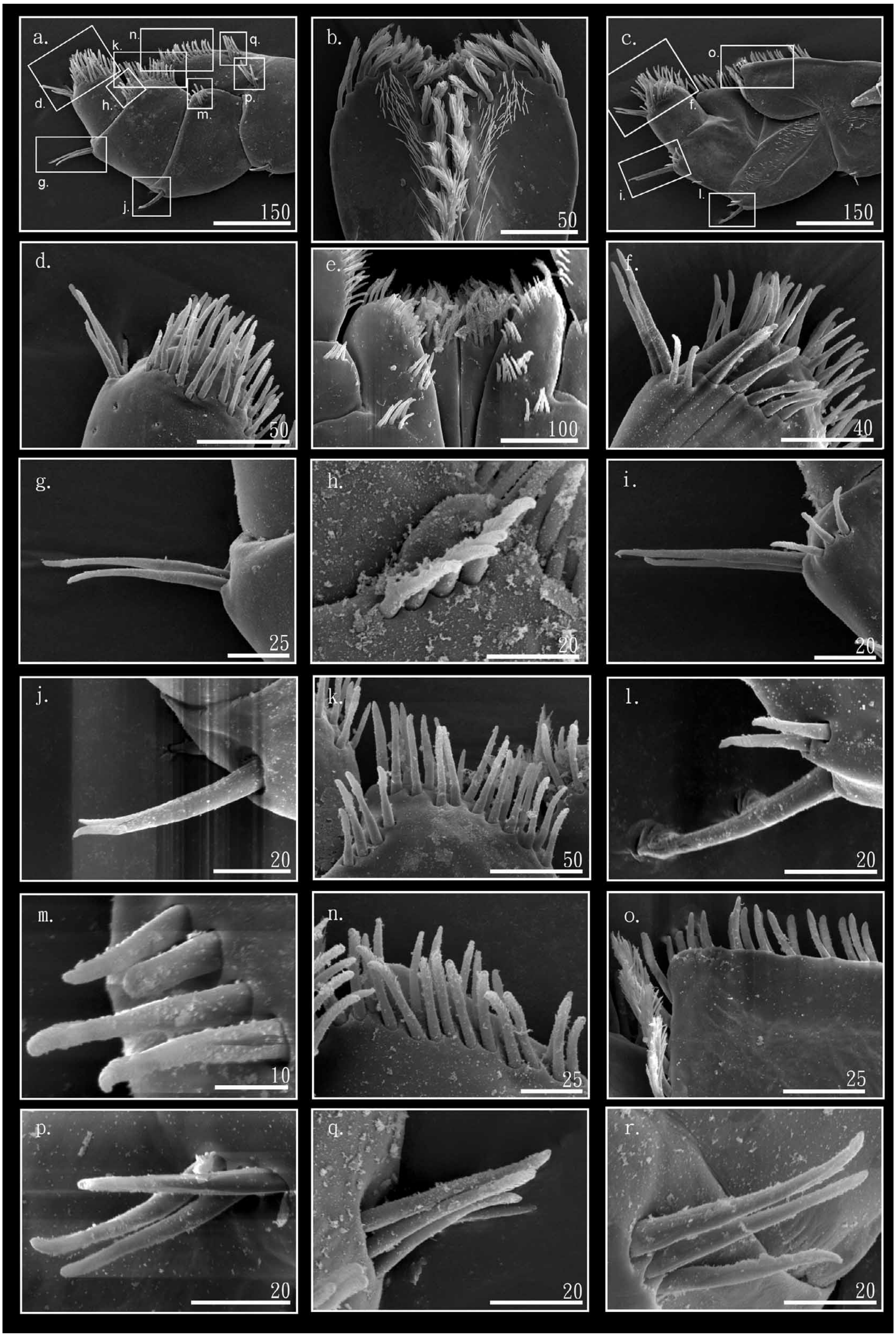

FIGURE 4. *Platorchestia paludosus* **sp. nov.** male. Mp, a) R Pa and outer plate, ventral view, b) inner plate, dorsal view, c) L Pa and outer plate, dorsal view, d) Pa article 3, ventral view, e) outer and inner plate, ventral view, f) Pa article 3, dorsal view, g) Pa article 2, spines on ventral outer lateral surface, j) Pa article 2, inner distal corner, spines on ventral surface, i) Pa article 2, spines on distal outer lateral surface, j) Pa article 1, spines on ventral outer lateral surface, k) Pa article 2, spines on inner margin, l) Pa article 1, spines on distal outer lateral surface, m) Pa article 1, inner distal corner, spines on ventral surface, n) outer plate, ventral view, o) outer plate, dorsal view, p) outer plate, spines on ventral medial surface, q) outer plate, spines on medial inner lateral margin, r) inner plate, spines on ventral medial surface. For abbreviations, see legend in Fig. 3.

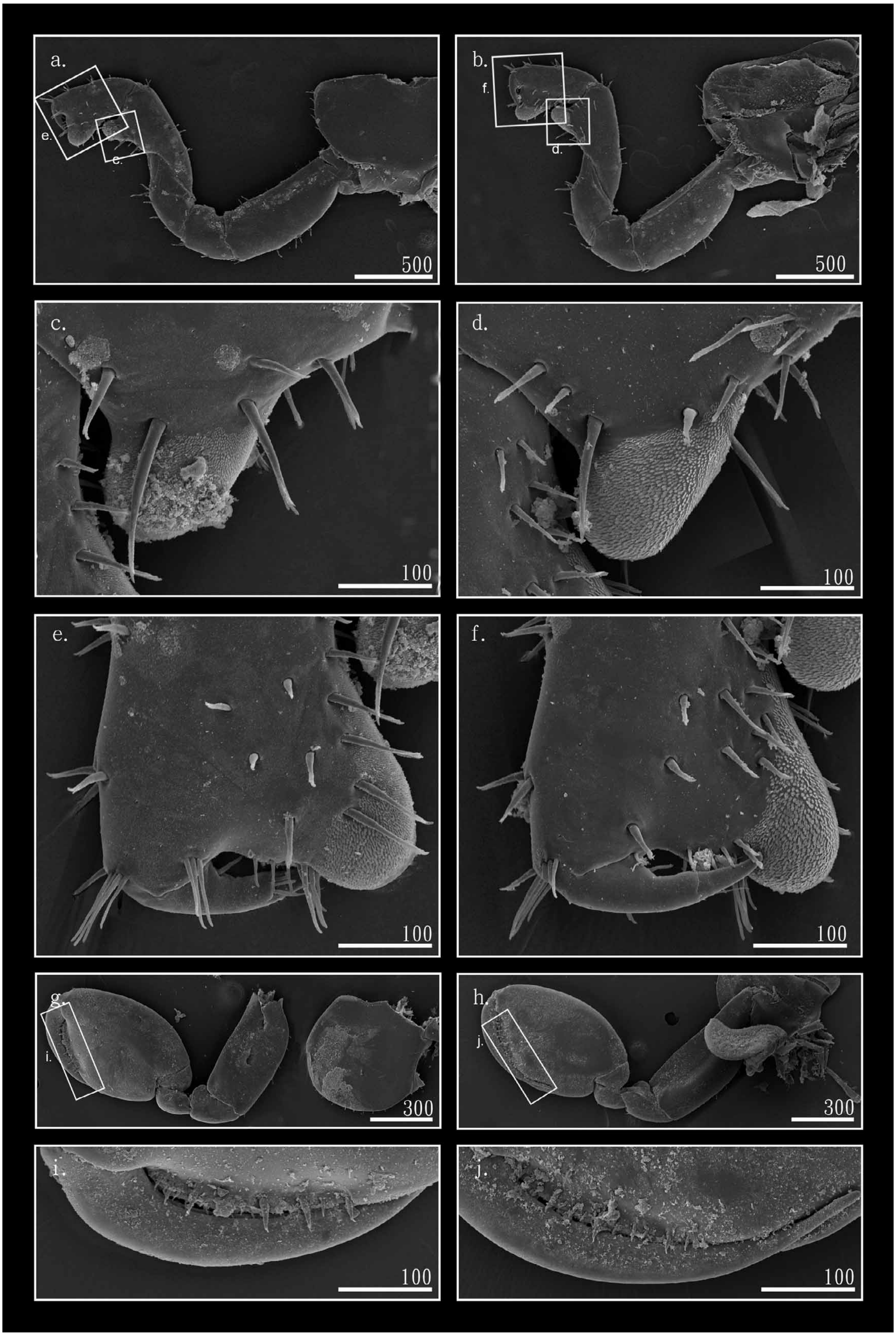

FIGURE 5. *Platorchestia paludal* **sp. nov.** male. a) L Gp 1 outer lateral view. b) R Gp 1 inner lateral view. c) L Gp 1 carpus posterodistal angle, outer lateral view. d) R Gp 1 carpus posterodistal angle, inner lateral view. e) L Gp 1 palm, outer lateral view. f) R Gp 1 palm, inner lateral view. g) L Gp 2 outer lateral view. h) R Gp 2 inner lateral view. i) L Gp 2 palm, outer lateral view. j) R Gp 2 palm, inner lateral view. For abbreviations, see legend in Fig. 3.

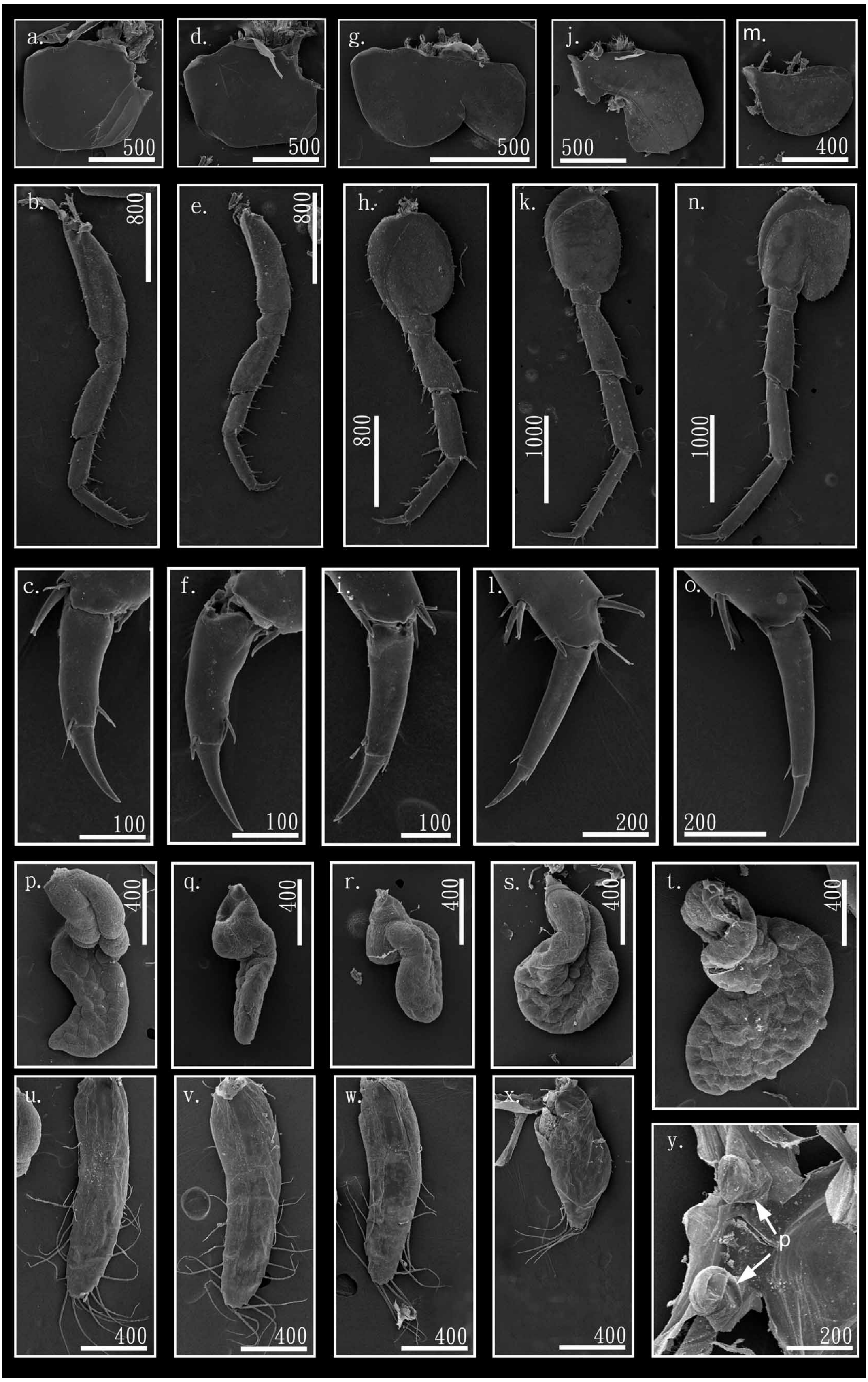

FIGURE 6. *Platorchestia paludosus* **sp. nov.** male. Pp 3, a) coxae, b) basis-dactylus, c) dactylus. Pp 4, d) coxae, e) basis-dactylus, f) dactylus. Pp 5, g) coxae, h) basis-dactylus, i) dactylus. Pp 6, j) coxae, k) basis-dactylus, l) dactylus. Pp 7, m) coxae, n) basis-dactylus, o) dactylus. Coxal gills, p) of Gp 2, q) of Pp 3, r) of Pp 4, s) of Pp 5, t) Pp 6. Brood plates, u) of Gp 2, v) of Pp 3, w) of Pp 4, x) of Pp 5. y) Pair of penial papillae. For abbreviations, see legend in Fig. 3.

FIGURE 7. *Platorchestia paludosus* **sp. nov.** male. a) R Plp 1, posterior view, b) L Plp 1, anterior view, c) R Plp 2, posterior view, d) L Plp 2, anterior view, e) R Plp 3, posterior view, f) L Plp 3, anterior view, g) L Up 1, outer lateral view, h) R Up 1, inner lateral view, i) L Up 2, outer lateral view, j) R Up 2, inner lateral view, k) L Up 3, outer lateral view, l) R Up 3, inner lateral view, m) Tl, dorsal view. For abbreviations, see legend in Fig. 3.

Gnathopod 1 (Figs 5–a, and b) coxa with straight or convex anterior margin, ventral margin convex with a few setae; basis slightly expanded posterodistally, subequal with merus and carpus when combined in length; ischium shortest, anteroproximal margin with a notch; merus sub-triangular, rounded posteriorly; carpus (Figs 5–c, and d) with scabrous tumescent protuberance posterodistally, outer lateral surface (Figs 5–c) with a row of 2–3 serrate setae type III (Fig. 1–l) and 2–3 cuspidate setae type III (Fig. 1–o) at base of tumescent protuberance, inner lateral surface (Fig. 5–d) with 7–10 serrate setae type III (Fig. 1–l) without cuspidate setae; propodus (Figs 5–e, f) with scabrous tumescent protuberance posterodistally, outer lateral surface with a row of 4–6 serrate setae type III (Fig. 1–l) along posteroproximal margin to medial base of tumescent protuberance, outer lateral surface with a group of 4–5 simple setae type IV (Fig. 1–d) on protuberance base distal end, dactylus base with a group of 2–3 simple setae type IV (Fig. 1–d), anterodistal corner with a group of 2–3 simple setae type IV (Fig. 1–d); length of dactylus subequal to palm, and not reach posterodistal angle of propodus.

Gnathopod 2 (Figs 5–g, h) coxa as wide as deep with posterior process, ventral margin convex, basis anterior margin straight, posterior margin convex and proximal end necked; ischium larger than merus, with a notch on proximal end of anterior margin; length of merus subequal to carpus, with convex posterior margin; carpus with convex anterior margin; propodus oval, palm margin curved, without notch, both lateral surface with a row of cuspidate setae type III (Fig. 1–i); dactylus curved, as long as palm.

Pereopods 3–7 cuspidactylate. Pereopod 3 coxa (Fig. 6–a) subquadrate with posterior process, ventral margin slightly convex; basis longest, subequal to combined length of merus and carpus; ischium shortest, anteroproximal margin notched; merus longer than carpus, anterior margin slightly convex, posterior margin straight; carpus anterior and posterior margins parallel; propodus slender, longer than carpus, slightly shorter than merus; dactylus (Fig. 6–c) subequal to ischium in length, posterior nail base with 1 simple setae type I (Fig. 1–a).

Pereopod 4 (Figs 6–d, and e) similar to pereopod 3, shorter, coxa (Fig. 6–d) subquadrate, with posterior process, ventral margin straight; basis longest, subequal to combined length of merus and carpus, slightly convex; ischium shortest, anteroproximal margin notched; merus longer than carpus, anterior margin slightly convex, posterior margin straight; carpus shorter than that of pereopod 3, both margins parallel; propodus slender, subequal to merus, dactylus (Fig. 6–f) pinched, posterior nail base with 1 simple seta type I (Fig. 1–a).

Pereopod 5 coxa (Fig. 6–g) bilobed, anterior lobe larger than posterior lobe; basis oval; ischium shortest, posteroproximal margin notched; merus subequal to carpus in length, anterior margin straight, posterior margin slightly convex; carpus both margins parallel; propodus slender; dactylus (Fig. 6–i) with 1 simple seta type I (Fig. 1–a) on anterior nail base.

Pereopod 6 coxa (Fig. 6–j) bilobed, anterior lobe very small, anterior margin of posterior lobe nearly vertical, anterodistal corner strongly curved; basis oval; ischium shortest, posteroproximal margin notched; merus subequal to carpus in length, anterior margin straight, posterior margin slightly convex; carpus both margins parallel; propodus slender, longest; dactylus (Fig. 6–l) with 1 simple seta type I (Fig. 1–a) on anterior nail base.

Pereopod 7 coxa (Fig. 6–m) rounded posterodistally; basis oval, posterodistal lobe broader than pereopod 5–6; ischium shortest, posteroproximal margin notched; merus subequal to carpus, anterior margin straight, posterior margin slightly convex; carpus anterior and posterior margins parallel; propodus slender, longest; dactylus (Fig. 6–o) with 1 simple seta type I (Fig. 1–a) on anterior nail base.

Pleopods (Figs 7–a, b, c, d, e and f) biramous, peduncle and ramus subequal in length, pleopod 3 peduncle shortest, ramus with 7–10 articulated segments.

Peduncle of uropod 1 (Figs 7–g, h) slightly longer than ramus, with 1 row of 4–5 cuspidate setae type III (Fig. 1–i) along each dorsal lateral margin; outer ramus with 3–5 cuspidate setae type II (Fig. 1–h) on distal end, without seta on medial region; inner ramus with 3–5 cuspidate setae type II (Fig. 1–h) on distal end, a row of 3–4 cuspidate setae type III (Fig. 1–i) along dorsal margin, without 1–2 cuspidate setae type III (Fig. 1–i) along outer lateral region.

Peduncle of uropod 2 (Figs 7–i, j) subequal to ramus in length, with 3 cuspidate setae type III (Fig. 1–i) on dorsal region; outer ramus with 3 cuspidate setae type II (Fig. 1–h) on distal end, with 1 cuspidate seta type III (Fig. 1–i) on medial region; inner ramus with 5 cuspidate setae type II (Fig. 1–h) on distal end, 2–3 cuspidate setae type III (Fig. 1–i) on dorsal margin, with 1–2 cuspidate setae type III (Fig. 1–i) on outer lateral region.

Uropod 3 (Figs 7–k, l), 1– 2 cuspidate setae type III (Fig. 1–i) on peduncle medial region, ramus with a group of 3 simple setae type I (Fig. 1–a) on distal end, 0–1 simple setae type I (Fig. 1–a) on medial region.

Telson (Figs 7–m) bilobed, longer than wide, with a group of 3–4 cuspidate setae type III (Fig. 1–i) on distal end, 1–2 cuspidate setae type III (Fig. 1–i) as a group on dorsal lateral margin.

Female. Antenna 1 short, slightly beyond article 4 of antenna 2, with 6–7 articles (3 peduncle articles, 3–4 flagellated articles), peduncle articles 1–3 equal in length, article 4 slightly curved, each flagellated article with two groups of simple setae on the inner and outer side of dorsal surface.

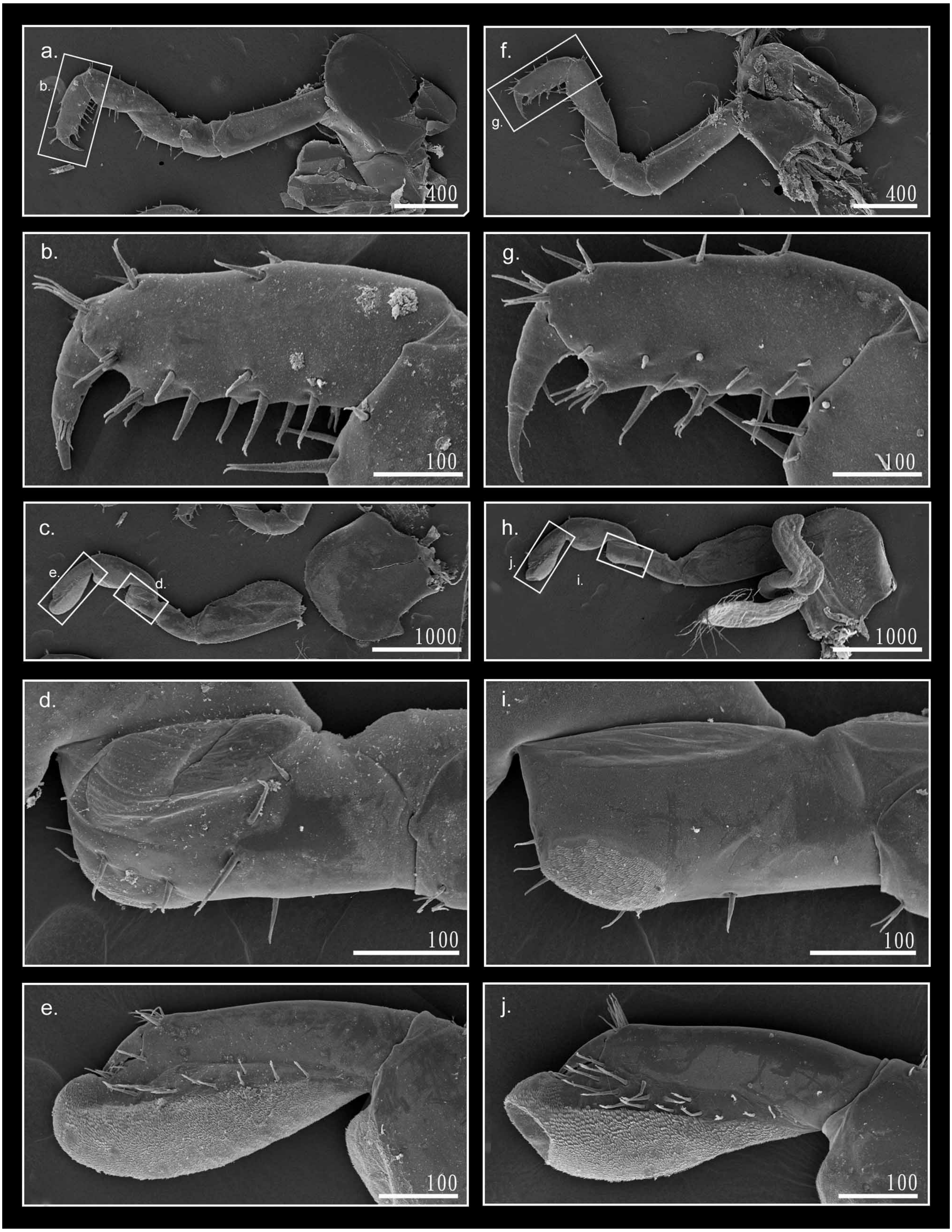

FIGURE 8. *Platorchestia paludosus* **sp. nov.** female. L Gp 1, a) outer lateral view, b) propodus, outer lateral view. L Gp 2, c) outer lateral view, d) merus, outer lateral view, e) propodus, outer lateral view. R Gp 1, f) inner lateral view, g) propodus, inner lateral view. R Gp 2, h) inner lateral view, i) merus, inner lateral view, j) propodus, inner lateral view. For abbreviations, see legend in Fig. 3.

FIGURE 9. Neighbour joining trees, showing bootstrap values NJ/BI/ML showing sequence divergence in *Platorchestia paludosus* **sp. nov.**, *P. japonica* from Lake Biwa, *P. japonica* from China (from Genbank), *P. platensis* from Canada (from GenBank) and outgroup species including *Orchestia cavimana* (from GenBank; see Browne *et al.*) and *Parorchestia* sp. from Japan (for details of GenBank no., see Table 3).

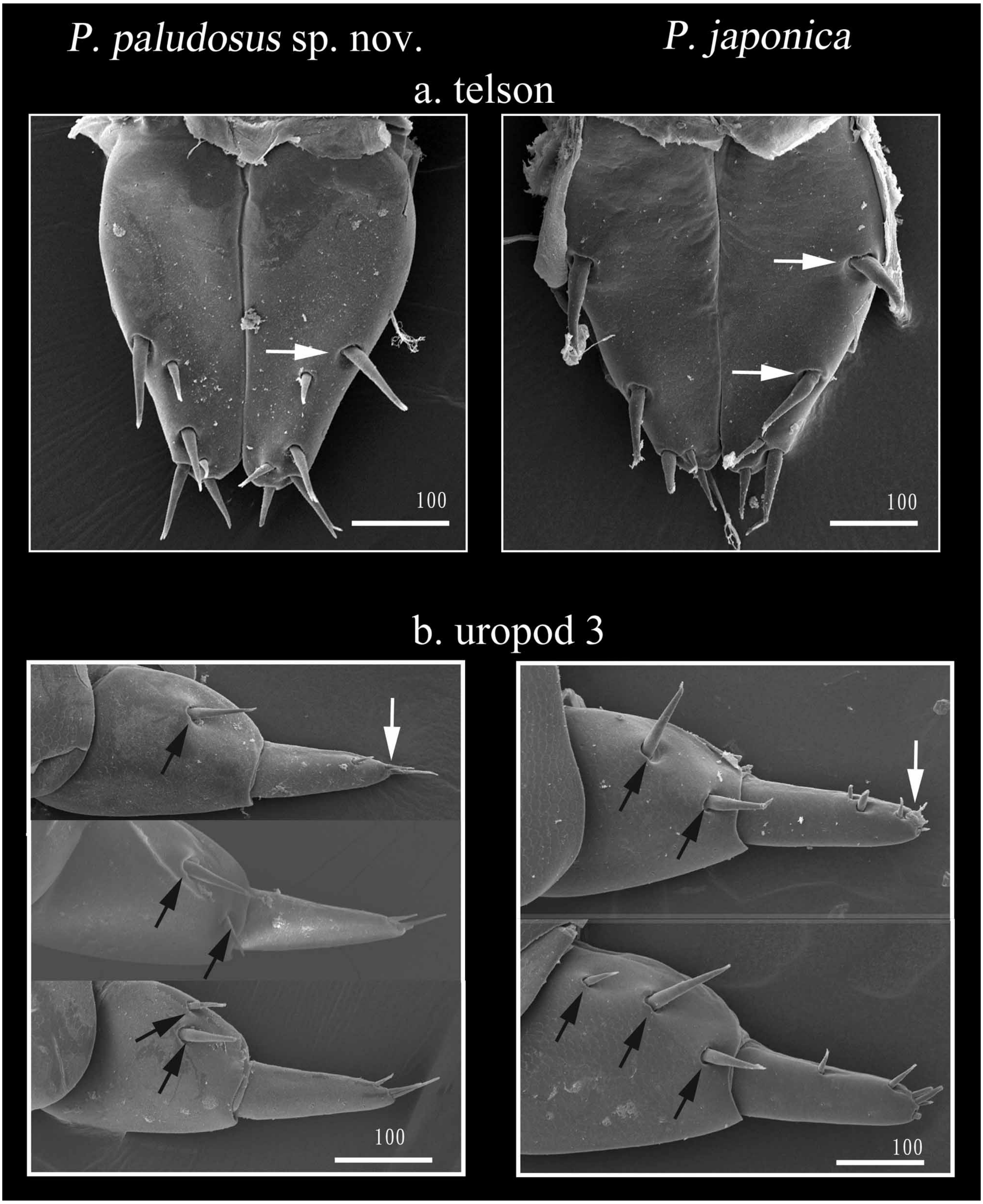

FIGURE 10. *Platorchestia paludosus* **sp. nov.** and *P. japonica*. a) telson, showing the interspecific variation in the number of setal groups at the lateral margin (indicated by white arrows). P. paludosus has one group of setae, whilst *P. japonica* has two groups of setae (indicated by white arrows). b) uropod, showing the interspecific differences in the shape of the ramus (indicated by white arrows). Note the intra-specific variation in number of setae in the peduncle (indicated by black arrows, for details, see discussion).

Antenna 2 up to 1/3 of body length. Outer margin of ventral side of article 3 with 3–5 simple setae, a medial furrow extending from distal 1/3 of article 4 to the entire article 5, length of article 5 equals to total of article 3 and 4, 12–14 flagellated articles, each with medial furrow. Tip of antenna 2 with a group of simple setae.

Coxa of gnathopod 1 (Figs 8–a, f) anterior margin straight or convex, ventral margin convex; basis straight; carpus without tumescent protuberance, posterior margin with 2 long and 4–6 short cuspidate setae type III (Fig. 1–i); propodus (Figs 8–b, g) without tumescent protuberance, inner lateral surface medial region with 4–5 serrate setae type III (Fig. 1–l), outer lateral surface medial region with a row of 2–3 cuspidate setae type III (Fig. 1–i) along margin, posterior margin with 2 rows of cuspidate setae type III (Fig. 1–i), outer lateral surface with a group of 3–4 simple setae type IV (Fig. 1–d) on dactylus base, 3–4 simple setae type IV (Fig. 1–d) on posterodistal corner, anterodistal corner with 3–6 simple setae type IV (Fig. 1–d); dactylus longer than width of propodus.

Gnathopod 2 (Figs 8–c, h) coxa as wide as deep with posterior process, convex ventral margin, basis expanded anteroproximally, posterior margin straight; ischium subequal to merus in length; merus (Figs 8–d, i) with small scabrous region posterodistally; carpus with scabrous tumescent protuberance posterodistally; propodus (Figs 8–e, j) with posterodistal tumescent protuberance elongated distally, scabrous region from posteroproximal end to posterodistal end, outer lateral surface with 10–14 serrate setae type III (Fig. 1–l) along scabrous region, inner lateral surface with 16–19 serrate setae type III (Fig. 1–l) along scabrous region, palm margin short, and outer lateral palm margin with a row of 4–5 simple setae type I (Fig. 1–a), anterodistal corner with 6–8 simple setae type I (Fig. 1–a); dactylus curved as long as palm.

Habitat. This salt marsh in Guan-du, Taiwan is dominated by *Phragmites communis* (L.) Trin. and *Brachiaria mutica* (Forsk) Stapf., with irregular flooding after raining. *Platorchestia paludosus* **sp. nov.** was found under leaf-litter of *Brachiaria mutica* (Forsk) on the water front of estuarine marshes.

Etymology. The word 'paludosus' indicates that the species is common in marshy habitats.

Molecular analysis. We got 618 b.p. aligned sequence of COI (GC ratio: 0.359), from 43 samples of *P. paludosus* **sp. nov.** and four samples of *P. japonica*. The number of total polymorphic sites was 102 within which 75 were parsimony informative sites (PIS), defining 13 haplotypes. There were 9 (3 PIS) and 50 (11 PIS) polymorphic sites for *P. paludosus* and *P. japonica* with 10 and 3 haplotypes respectively. The haplotype diversity (*h*) and nucleotide diversity (π) of *P. paludosus* samples were 0.743 ± 0.047 and 0.00183 ±0.00024. Those of *P. japonica* samples were 0.833 ±0.222 and 0.04396 ± 0.01253 (total: 0.784 ± 0.043 and 0.02088 ± 0.00826). Neighbor joining tree of *P. paludosus*, *P. japonica* and *P. platensis* (Radulovici *et al.* 2009, NCBI, FJ581856–FJ581858) formed three distinct clades, with bootstrap value > 99 suggesting the clades were well separated (Fig. 9), and topology of BI and ML trees were essentially consistent with NJ. Average sequence divergence (Tab. 2) of *P. paludosus* from *P. japonica* was 13.3% (11.9–15.3%) from *P. japonica*, which was comparable to the interspecific difference of other *Platorchestia* species including *P. platensis* (Radulovici *et al.* 2009, NCBI, FJ581856–FJ581858; 14.5 and 17.2%).

TABLE 2. Pairwise K2P difference (%) in mtDNA (COI) gene.

	Platorchestia **sp. nov.**	*P. japonica* Japan	*P. japonica* China	*P. platensis*	*Orchestia cavimana*	*Parorchestia* sp.
Platorchestia **sp. nov.**	0.2					
P. japonica (Japan)	13.3	4.8				
P. japonica (China)	10.4	14.3	_			
P. platensis	14.5	17.2	16.1	0.01		
Orchestia cavimana	19.1	21.1	19.9	23.2	_	
Parorchestia sp.	22.0	24.0	23.4	22.6	26.0	_

Discussion. In the present study, *Platorchestia paludosus* **sp. nov.** is considered as a new species based on morphological and molecular evidence. The morphology is very close to *P. japonica sensu stricto* (Tattersall 1922). Miyamoto & Morino (2004) considered their material collected from North Taiwan as *P. japonica* and Miyamoto & Morino (2004) noted their Taiwanese specimens differed from *P. japonica* collected from the Lake Biwa, Japan in the degree of sexual dimorphisms in peduncle articles 4 and 5 of antenna 2 and carpus of pereopod 7, shape of coxal plate of pereopod 6 and anterodistal corner of the posterior, morphology of marginal spines in peduncles of pleopods 2 and 3 and the spine patterns in pereopod 6 and 7. In the present study, we found that *P. japonica* identified by Miyamoto & Morino (2004) in Taiwan was *P. paludosus* identified in the present study. We used scanning electron microscopy and additionally noted consistent morphological differences between *P. paludosus* (10 individuals) and *P. japonica* (4 individuals) collected from the Lake Biwa, in the distribution pattern of setae on the lateral margin of telson, and in the shape of the ramus tip of uropod 3. *Platorchestia paludosus* has one group of cuspidate setae type III on the lateral margin of telson, whilst *P. japonica* contains two groups of cuspidate setae type III on the lateral margin of telson (Fig. 10a). *P. paludosus* has a sharper ramus of uropod 3, whilst the tip of ramus in uropod 3 of *P. japonica* is blunt (Fig. 10b). However, the number of setae on peduncle of uropod 3 varied from 1–3 in both *P. paludosus* and *P. japonica*, and thus, this cannot be considered as a taxonomic important character (Fig. 10b).

Miyamoto & Morino (2004) divided the genus *Platorchestia* into three groups based on the different gradient of sexual dimorphism character of antenna 2 and pereopod 6 and 7. The "group 3" defined by Miyamoto & Morino (2004) included species "displaying no sexual dimorphism" and having "antenna 2 and pereopod 6 and 7 barely (or slightly) sexual dimorphic". Currently six species are placed within this group, including *P. japonica* (Tattersall 1922), *P. humicola*, *P. kaalensis*, *P. lanipo* and *P. pickeringi*, and the *P. paludosus* in the present study also belong to this group. *Platorchestia paludosus* can be differentiated from *P. pickeringi*, *P. kaalensis* and *P. lanipo* by having normal maxilliped palps (*vs*. slender maxilliped palps) and differs from *P. humicola* by possessing longer pleopod ramus: 0.8–0.9 times as long as peduncle (v.s. 0.5–0.6 times in *P. humicola*). Results from DNA barcoding further supports *P. paludosus* as different from *P. japonica* and is therefore a new species. Average sequence divergence of *P. paludosus* from *P. japonica* was 13.3% (11.9–15.3%), which is comparable to the interspecific difference *P. platensis* (NCBI, FJ581856–FJ581858; 14.5 and 17.2%). *Platorchestia platensis* differs from *P. japonica* in the morphology of palm of mature male gnathopod 2 and degree of sexual dimorphism in antenna 2 and pereopod 6 and 7 (Miyamoto & Morino 2004). Our phylogenetic analysis also included a COI sequence (from GenBank) of *P. japonica* collected from Hebei, China (NCBI, EF570353; Hou, Fu *et al.* 2007). Sequence divergence of *P. japonica* from China and specimens from Lake Biwa, Japan reached about 14.3% and *P. japonica* from China is closer to *P. paludosus*, in having a sequence divergence of 10.4 %. Miyamoto & Morino (2004) claimed their *P. japonica* collected from Taiwan had slight morphological differences (in the sharpness of coxal plate of pereopod 6 and number of margin spine in uropods 1–3) from *P. japonica* from China in Morino & Dai (1990). Morphological and molecular data suggests that *P. japonica* from the mainland China could be another cryptic species and awaits further morphological and molecular analysis.

Iwasa (1965) recorded *Orchestia platensis* Krøyer [= *Platorchestia platensis* (Krøyer), Bousfield 1982] from Tanshui, N. Taiwan but there were no detailed text or figure illustrations for this species in Iwasa (1965). It is therefore difficult to check the validity of the record from Iwasa (1965). However, Miyamoto & Morino (2004) described their collections in Taiwan from 1976–1981 but they have not reported any *P. platensis* (Krøyer), and their records in N. Taiwan were '*P. japonica*'.

TABLE 3. GenBank accession no. for *Platorchestia paludosus* **sp. nov.**, *P. japonica* & *Parorchestia* sp. used in the present study. The sampling locality was indicated in the bracket. Gender, m = male, f = female.

Species name	Gender	Id. no.	GenBank no.
P. paludosus **sp. nov.**	m	Apj 1	HQ010294
(Taipei, Taiwan.)	f	Apj 2	HQ010295
	f	Apj 3	HQ010296
	m	Apj 5	HQ010297
	m	Apj 6	HQ010298
	m	Apj 7	HQ010299
	m	Apj 8	HQ010300
	f	Apj 9	HQ010301
	m	Apj 10	HQ010302
	*	Apj 12	HQ010303
	*	Apj 13	HQ010304
	*	Apj 14	HQ010305

continued next page

TABLE 3. (continued)

Species name	Gender	Id. no.	GenBank no.
	f	Apj 15	HQ010306
	*	Apj 16	HQ010307
	*	Apj 17	HQ010308
	*	Apj 18	HQ010309
	*	Apj 19	HQ010310
	*	Apj 21	HQ010311
	m	Apj gp 2	HQ010312
	m	Apj gp 4	HQ010313
	m	Apj gp 5	HQ010314
	m	Apj gp 6	HQ010315
	m	Apj gp 7	HQ010316
	m	Apj gp 8	HQ010317
	m	Apj gp 9	HQ010318
	m	Apj gp 10	HQ010319
	m	Apj gp 11	HQ010320
	m	Apj gp 12	HQ010321
	m	Apj gp 13	HQ010322
	f	Apj gp 14	HQ010323
	m	Apj gp 15	HQ010324
	m	Apj gp 16	HQ010325
	m	Apj gp 17	HQ010326
	m	Apj gp 18	HQ010327
	m	Apj gp 19	HQ010328
	m	Apj gp 20	HQ010329
	m	Apj gp 21	HQ010330
	m	Apj gp 23	HQ010331
	m	Apj gp 24	HQ010332
	m	Apj gp 26	HQ010333
	m	Apj gp 27	HQ010334
	m	Apj gp 28	HQ010335
	m	Apj gp 29	HQ010336
P. japonica	m	H143-3-1	HQ010293
(Lake Biwa, Japan.)	f	H143-3-2	HQ010337
	f	H143-3-3	HQ010338
	m	H143-3-4	HQ010339
Parorchestia sp. (Lake Biwa, Japan.)	f	H100-4-1	HQ010292

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