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A new species of *Manoa* Fittkau (Diptera: Chironomidae), with DNA barcodes from Xianju National Park, Oriental China

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Abstract

The genus *Manoa* and the tribe Pseudochironomini are recorded from the Oriental region for the first time. *Manoa xianjuensis* Qi & Lin sp. n. from Xianju National Park, Zhejiang, China is described and illustrated as adult male and female, the latter associated with the male by standard DNA barcodes. A neighbor joining tree based on available Pseudochironomini DNA barcodes and keys to the adults in *Manoa* are given.

Key words: Culicoidea, Chironominae, taxonomy, COI sequence

Introduction

The genus *Manoa* Fittkau, 1963 was erected by Fittkau (1963) based on *M. obscura* Fittkau, 1963 from Amazonas state, Brazil. Thereafter two additional species were described: *M. tangae* Andersen & Sæther, 1997 from Tanzania and *M. pahayokeensis* Jacobsen, 2002 from North America and the Dominican Republic (Andersen & Sæther 1997; Jacobsen & Perry 2002; Silva *et al.* 2015; R. Jacobsen pers. comm.). *Manoa* belongs to the tribe Pseudochironomini as proposed by Sæther (1977), and has been suspected to be the most plesiomorphic genus within the tribe, dating back to at least mid-Cretaceous (Andersen & Sæther 1997). Originally Pseudochironomini also included the genera *Pseudochironomus* Malloch, 1915, *Riethia* Kieffer, 1917, *Aedokritus* Roback, 1958, *Megacentron* Freeman, 1961, and *Psilochironomus* Sublette, 1966, but the latter genus has been treated as a nomen dubium (see, *e.g.*, Spies & Reiss 1996: 90). A recent addition to the tribe is *Madachironomus* Andersen, 2016 from Madagascar (Andersen 2016). However, the morphology of many members of Pseudochironomini in Sæther's (1977) wide sense is in serious need of revision, the monophyly of this grouping of genera is not firmly established, and is untested due to sampling shortfall in a molecular phylogeny for the Chironomidae (Cranston *et al.* 2012). For this reason Epler *et al.* (2013) considered the validity of a tribe Pseudochironomini as so uncertain that they did not separate it from Chironomini in their work. The adults of *Manoa* are closely related to *Riethia*, but can be separated from the latter genus by the notched floor of the vagina of female of *Manoa* (Andersen & Sæther 1977; Jacobsen & Perry 2002; Trivinho-Strixino *et al.* 2009).

The genus *Pseudochironomus* was incorrectly recorded from Palaearctic China by Wang (2000) based on larvae which later proved to belong to *Rheotanytarsus pentapoda* (Kieffer) (H.Q. Tang pers. comm.). During our recent investigation in the Xianju National Park, China, we collected adults of a *Manoa* species (Figs 1–4) near a small mountain stream in Kuocangshan Nature Reserve. This is thus the first record of the genus *Manoa* and of the tribe Pseudochironomini from the Oriental region. Below we describe and illustrate *M. xianjuensis* sp. n. as adult male and female (associated with the male by DNA barcodes). DNA barcoding using a standard fragment of the

mitochondrial cytochrome *c* subunit 1 gene (COI) has proven a successful method for species-level identification in many animal groups including chironomids (Hebert *et al.* 2003, 2004; Ekrem *et al.* 2007; Lin *et al.* 2015; Montagna *et al.* 2016). However, currently there is no DNA barcode of *Manoa* in the reference library. Consequently we generated the standard DNA barcodes of *M. xianjuensis* **sp. n.** and have uploaded them to the Barcode of Life Data Systems (BOLD, <http://www.boldsystems.org>).

The Xianju National Park is located in the southeastern part of Zhejiang province, China. The park (28°28'14"–28°59'48"N, 120°17'6"–120°55'51"E) is situated at medium to low latitudes of the northern hemisphere, in a region in which a mid-subtropical monsoon climate prevails. The last glacial period had no significant influence on species extinction in the park. Therefore, Xianju National Park provides fully protected habitats and long-term maintenance of the biological diversity. However, the structure and composition of the fauna and flora in the park still remain rather insufficiently known, particularly concerning insects. Previously, only five species of chironomids were recorded from Xianju National Park (Li *et al.* 2013; Lin *et al.* 2012, 2013; Qi *et al.* 2016a, 2016b).

Material and methods

Upon collection the specimens were preserved in 85% ethanol and stored in the dark at 4°C before morphological and molecular analyses. Genomic DNA was extracted from the thorax and head using Qiagen DNA Blood and Tissue Kits at the Department of Natural History, NTNU University Museum, Trondheim, Norway, following the standard protocol. The final elution volume was 100 µl. After DNA extraction, the exoskeleton of each specimen was mounted in Euparal on a microscope slide together with the corresponding wings, legs and antennae, following the procedures outlined by Sæther (1969). Morphological terminology follows Sæther (1980), where applicable. Measurements are given as ranges followed by the mean, when four or more specimens were measured, followed by the number of specimens measured (n) in parentheses.

A 658 bp fragment of the COI region was PCR-amplified using the universal primers LCO1490 and HCO2198 (Folmer *et al.* 1994). DNA amplification was carried out in 25 µl reactions using 2.5 µl 10x Takara ExTaq pcr buffer (CL), 2 µl 2.5 mM dNTP mix, 2 µl 25 mM MgCl₂, 0.2 µl Takara Ex Taq HS, 1 µl 10 µM of each primer, 2 µl template DNA and 14.3 µl ddH₂O. Amplification cycles were performed on a Biorad C1000 Thermal Cycler (Bio-Rad, California, USA) and followed a program with an initial denaturation step of 95°C for 5 min, followed by 34 cycles of 94°C for 30 s, 51°C for 30 s, 72°C for 1 min, and 1 final extension at 72°C for 3 min. PCR products were purified using Illustra ExoProStar 1-Step (GE Healthcare Life Sciences, Buckinghamshire, UK) and shipped to MWG Eurofins (Ebersberg, Germany) for bidirectional sequencing using BigDye 3.1 (Applied Biosystems, Foster City, CA, USA) termination. Sequences were assembled and edited using Sequencher 4.8 (Gene Codes Corp., Ann Arbor, Michigan, USA). Sequence information was uploaded to BOLD SYSTEMS along with an image and collateral information for each voucher specimen. Alignment of the sequences was carried out using the Muscle algorithm (Edgar 2004) on amino acids in MEGA 6 (Tamura *et al.* 2013). The pairwise distances using Kimura 2-Parameter (K2P) model were calculated in MEGA 6. The neighbor joining tree was generated using a K2P substitution model with 1000 bootstrap replications and the “pairwise deletion” option for missing data in MEGA 6.

In addition to our own data, we searched for publicly accessible COI barcodes in BOLD belonging to the genera *Pseudochironomus* and *Riethia* that were longer than 300 base pairs and lacked stop codons. The searches were done on January 3, 2017. Hits were combined with our own data and are available through the public dataset “Pseudochironomini (DS-PSEUDOCH)” in BOLD.

The type specimens of *Manoa xianjuensis* **sp. n.** are deposited in the College of Life Sciences, Nankai University, Tianjin, China (BDN) and at the Zoologische Staatssammlung München, Munich, Germany (ZSM).

Manoa xianjuensis Qi & Lin **sp. n.**

(Figs 1–7)

Type material. Holotype (deposited in BDN): male (BOLD sample ID: XJ17), China, Zhejiang Province, Taizhou,

Xianju, Kuocangshan Nature Reserve, Yukengcun, near stream, 28.63267°N, 120.57606°E, 190 m a.s.l., 24.V.2016, sweep net, X. Qi. Paratypes: 11 males, (BDN no. XJ18–25, XJ33, XJ36, XJ37), 3 females (ZSM no. XJ34, XJ35; BDN no. XJ16), sampling data as for holotype.

Etymology. Named after the type locality. The species epithet is adjectival for the purposes of nomenclature.

Diagnostic characters. The new species conforms to the genus descriptions in Andersen & Sæther (1977) and Jacobsen & Perry (2002). The male of *Manoa xianjuensis* sp. n. can be separated from its congeners by the following combination of characters: frontal tubercles absent; AR 1.13–1.33; wing length 1.43–1.50 mm; wing vein R with 6–8 setae, R₁ with 1–3 setae, squama with 1–3 setae; antepnotum with two lateral setae; LR₁ 0.96–0.98; pars ventralis and digitus present. The female of *Manoa xianjuensis* sp. n. separated from its congeners by the following combination of characters: frontal tubercles absent; wing length 1.58–1.75 mm; R with 10–12 setae, R₁ with 4 setae, R₄₊₅ with 11–12 setae; costal extension with 2–3 non-marginal setae; squama with 2 setae; LR₁ 0.90–0.93.

Description. Adult male (n = 4, unless otherwise stated). Total length 2.67–3.08, 2.91 mm. Wing length 1.43–1.50, 1.49 mm. Total length/wing length 1.80–2.00, 1.92. Wing length/length of profemur 1.84–2.08, 1.97.

Coloration (Figs 1, 2–4). Body pale apricot yellow.

Head (Fig. 5a, b). Antenna with 13 flagellomeres; AR 1.13–1.33, 1.25. Temporal setae 12–16, 14, including 4 inner verticals, 7–12, 9 outer verticals, and 0–2, 1 postorbitals. Clypeus with 5–12, 9 setae. Tentorium 133–138, 135 mm long; 13–20, 17 mm wide. Palpomere lengths (in mm): 28–35, 32; 25–49, 38; 70–78, 75; 83–95, 90; 108–138, 123. Palpomere ratio (5th/3rd) 1.54–1.77, 1.62. Third palpomere with 2–3, 3 lanceolate sensilla clavata.

Wing (Fig. 5c). VR 1.09–1.32, 1.21. Anal lobe reduced. Membrane microtrichia distinctly visible at 100x magnification. Brachiolum with 2–3, 2 setae; R with 6–8, 7 setae; R₁ with 1–3, 2 setae; costal extension 40–60, 50 mm long; with 0–1, 0 non-marginal setae; remaining veins bare. Squama with 1–3, 2 setae.

Thorax (Fig. 6a). Antepnotum with 2 lateral setae. Acrostichals 9–10, 9; dorsocentrals 7–14, 11 in single row; prealars 2–3, 3; supraalar 1. Scutellum with 6 setae.

Legs (Fig. 5d–f). Spur of fore tibia 38–48, 42 mm long; spurs of mid tibia 33–50, 42 and 32–48, 39 mm long; of hind tibia 50–58, 54 and 45–54, 49 mm long. Apical width of fore tibia 38–48, 40 mm; of mid tibia 38–43, 40 mm; of hind tibia 41–50, 46 mm. Lengths (in mm) and proportions of legs as in Table 1.

TABLE 1. Lengths (in mm) and proportions of legs of *Manoa xianjuensis* sp. n., male (n = 4).

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄
p ₁	710–850, 770	680–800, 723	650–780, 695	300–360, 325	240–280, 258	160–200, 180
p ₂	740–850, 795	680–800, 725	330–450, 378	160–200, 175	120–150, 133	90–100, 95
p ₃	810–900, 853	750–810, 775	480–540, 503	230–270, 248	190–200, 195	110–140, 125
	ta ₅	LR	BV	SV	BR	
p ₁	90–100, 98	0.96–0.98, 0.96	2.52–2.59, 2.54	2.11–2.19, 2.15	1.33–2.50, 1.96	
p ₂	60–70, 68	0.48–0.56, 0.52	3.93–4.16, 4.04	3.67–4.42, 4.06	2.00–2.67, 2.25	
p ₃	80–100, 93	0.63–0.67, 0.65	3.17–3.29, 3.23	3.17–3.29, 3.24	1.92–3.33, 2.46	

Hypopygium (Fig. 6b–d). Laterosternite IX with 2–4, 3 setae. Tergite IX with 36–44, 39 setae; anal point absent. Transverse sternapodeme 25–40, 35 mm long, 13–25, 18 mm wide; phallapodeme 85–113, 101 mm long. Gonocoxite 145–163, 152 mm long; with triangular pars ventralis. Superior volsella 63–75, 70 mm long; 7–13, 10 mm wide; dorsal surface with basal microtrichia and 2–3, 2 setae; ventral surface with numerous long microtrichia and 2 apical setae. Digitus 15–30, 19 mm long; with pointed apex. Inferior volsella pediform, 50–58, 53 mm long; with 9–14, 13 long setae. Median volsella vestigial; with 2–3, 2 apical setae. Gonostylus straight; 95–108, 104 mm long; with pointed apex. HR 1.34–1.58, 1.47; HV 2.46–2.86, 2.67.

Adult female (n = 2, unless otherwise stated). Total length 2.92–3.05 mm. Wing length 1.58–1.75 mm. Total length/wing length 1.67–1.94. Wing length/length of fore femur 2.06–2.31.

Coloration. As in male (Figs 3, 4).



FIGURE 1. *Manoa xianjuensis* sp. n., adult male, lateral view.



FIGURE 2. *Manoa xianjuensis* sp. n., adult male, dorsal view.



FIGURE 3. *Manoa xianjuensis* sp. n., adult female, dorsal view.

Head (Fig. 7a, b). Antenna (Fig. 7a) with 6 flagellomeres; AR 0.30–0.32; flagellomere lengths (in mm): 75–88, 65–68, 68–70, 65 (2), 55 (2), 100–110. Temporal setae 15–16, including 3–4 inner verticals, 6–11 outer verticals and 2–5 postorbitals. Clypeus with 10–13 setae. Tentorium 95–135 mm long, 18–19 mm wide. Palpomere lengths (in mm): 33–40, 45–50, 70–78, 88–90, 150 (1). Palpomere ratio ($5^{\text{th}}/3^{\text{rd}}$) 1.92 (1). Third palpomere with 2–3 lanceolate sensilla clavata.

Wing. VR 1.20–1.23. Anal lobe reduced. Membrane microtrichia distinctly visible at 100x magnification. Brachiolum with 2 setae; R with 10–12 setae; R_1 with 4 setae; R_{4+5} with 11–12 setae; costal extension 45–65 mm long, with 2–3 non-marginal setae. Squama with 2 setae.

Thorax (Fig. 7c). Anteprepronotum with 2 lateral setae. Acrostichals 7–9; dorsocentrals 6–11 in single row, prealars 3, supraalar 1. Scutellum with 6 setae.

Legs. Spur of fore tibia 38–40 mm long, spurs of mid tibia 38–43 and 33–40 mm long, of hind tibia 39–50 and 36–45 mm long. Apical width of fore tibia 38–40 mm, of mid tibia 40–43 mm, of hind tibia 38–48 mm. Lengths (in mm) and proportions of legs as in Table 2.

Abdomen. Numbers of setae on tergites I–VIII: 14–20, 18–28, 18–34, 22–38, 28–30, 24–30, 18–38, 6–20. Numbers of setae on sternites I–VIII: 6–8, 4–7, 12–16, 21–30, 22–31, 24–38, 32–40.

Genitalia (Fig. 7d, e). Sternite VIII divided, with 50–55 mm long notched floor (Fig. 7e) under vagina; floor with approximately 32–44 setae. Tergite IX with 22 setae; gonocoxite IX with 1–2 setae. Cercus 78–100 mm long, with 42–48 setae. Seminal capsules 65–68 mm long, including 13–18 mm long, collar-shaped neck, 38–40 mm wide. Spermathecal ducts curved. Notum 75–88 mm long. Dorsomesal lobe 71–73 mm long.



FIGURE 4. *Manoa xianjuensis* sp. n., adult female, dorsal view.

TABLE 2. Lengths (in mm) and proportions of legs of *Manoa xianjuensis* sp. n., female (n = 2).

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄
p ₁	680–850	690–750	640–690	290–310	220–250	140–170
p ₂	780–850	710–770	350–400	150–160	110–130	80–90
p ₃	880–950	780–830	480–520	230–250	200 (2)	100–130
	ta ₅	LR	BV	SV	BR	
p ₁	90–120	0.92–0.93	2.71–2.76	2.14–2.32	2.00–3.00	
p ₂	60–80	0.49–0.52	4.28–4.70	4.05–4.26	1.77–2.22	
p ₃	80–90	0.62–0.63	3.43–3.51	3.42–3.46	1.80–2.00	

Immature stages. Unknown.

DNA barcoding analysis. The neighbor joining tree (Fig. 8) based on available DNA barcodes unequivocally associates the adult males and females of *M. xianjuensis* sp. n., and supports differentiation of the new species from the other barcoded Pseudochironomini. In addition, we found two distinct clades of under the name *Riethia stictoptera* Kieffer, 1917 with the maximum intraspecific genetic distance 18.1%, which suggests at least one misidentification in the BOLD data.

Biology and ecology. The new species was collected using a sweep-net close to a small mountain stream in Kuocangshan Nature Reserve in subtropical China. We collected *M. xianjuensis* sp. n. during the rainy season in late May, when the temperature is 15–20°C.

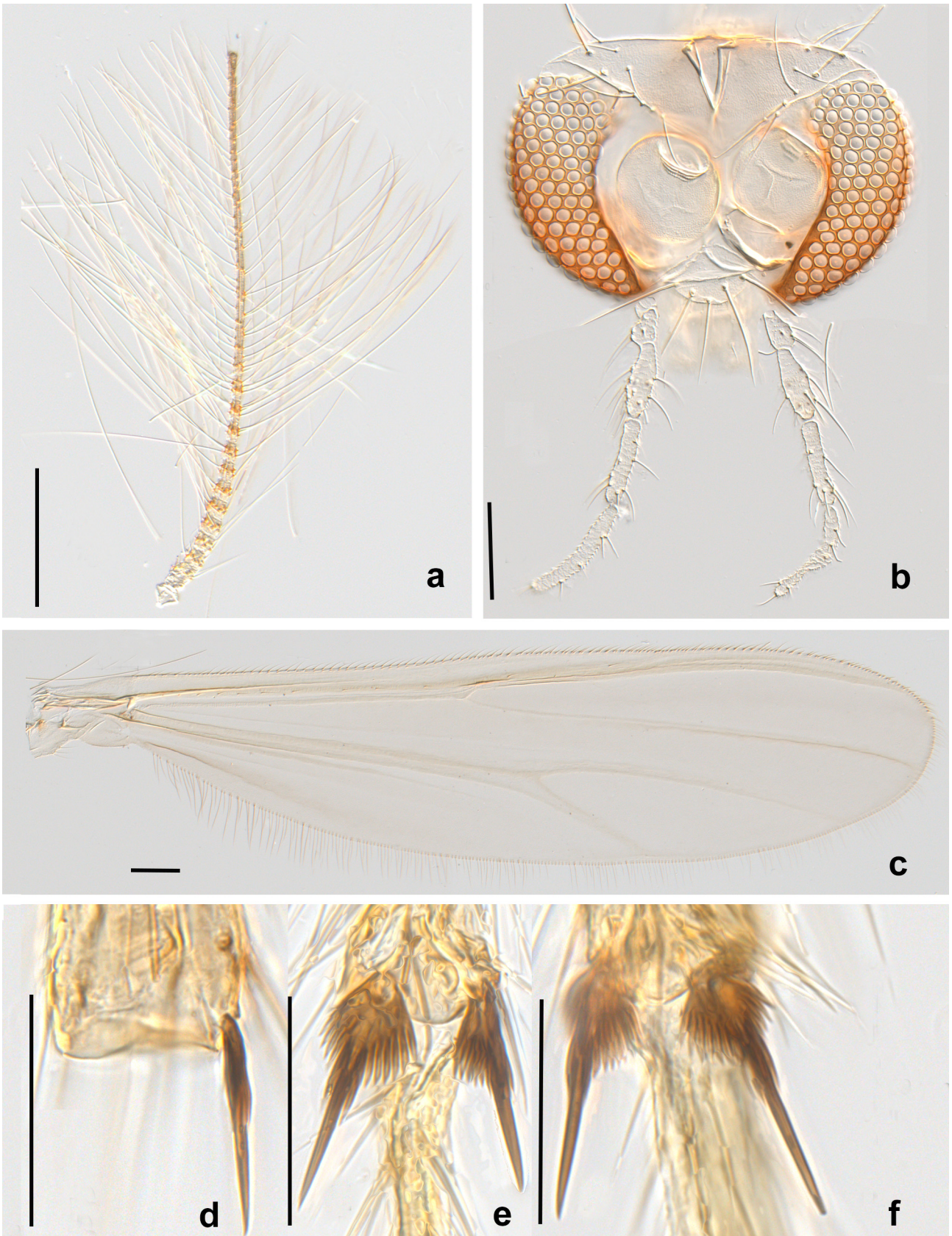


FIGURE 5. *Manoa xianjuensis* sp. n., holotype male: a) antenna, scale bar = 200 μ m; b) head, scale bar = 100 μ m; c) wing, scale bar = 100 μ m; d) apex of front tibia, scale bar = 50 μ m; e) apex of mid tibia, scale bar = 50 μ m; f) apex of hind tibia, scale bar = 50 μ m.

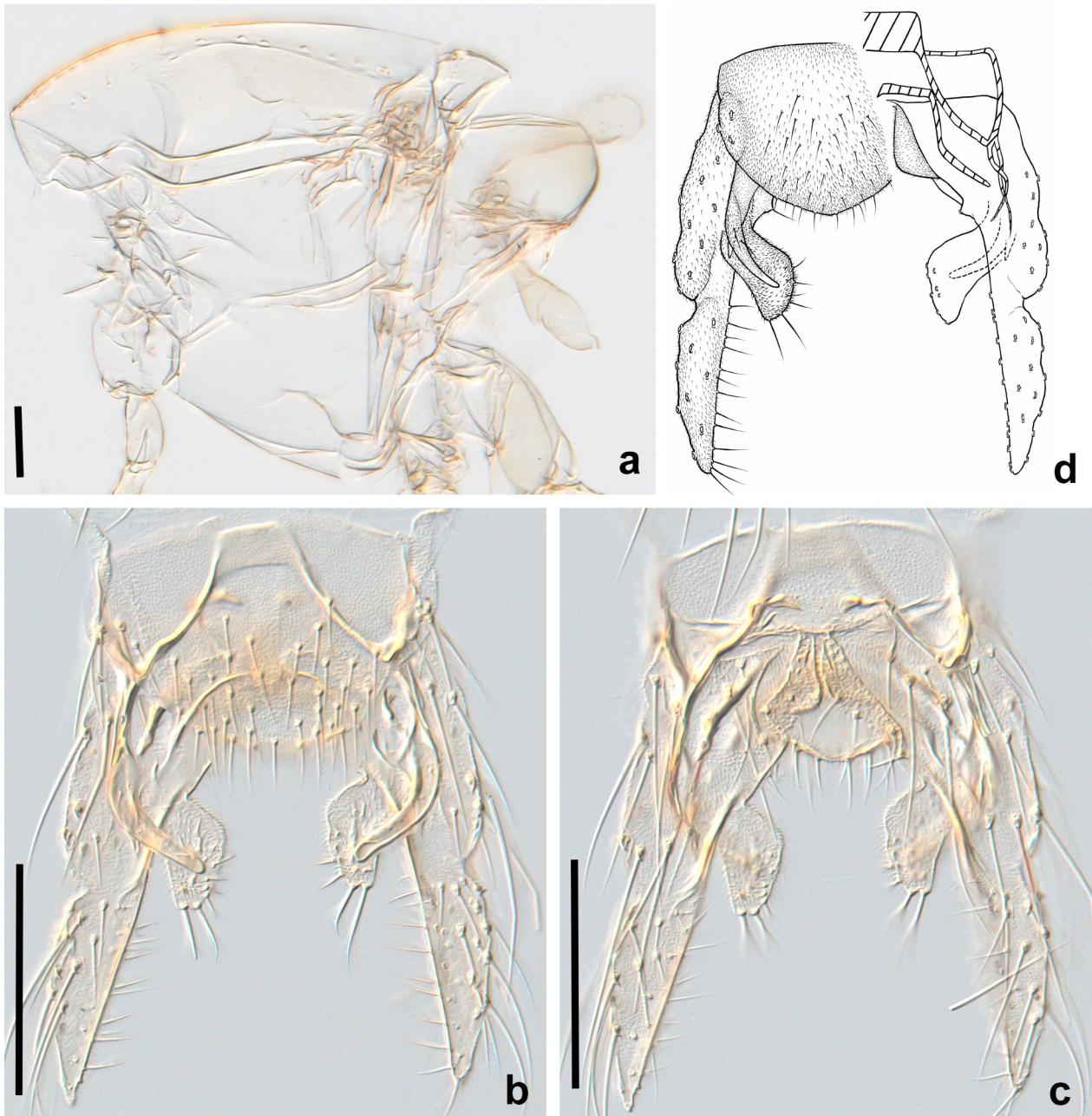


FIGURE 6. *Manoa xianjuensis* sp. n., holotype male: a) thorax; b) hypopygium dorsal view; c) hypopygium ventral view; d) drawing of hypopygium. Scale bars = 100 μ m.

Key to adult males of *Manoa* Fittkau

1. Frontal tubercles present; anteprenotal and supraalar setae absent. Brazil. *M. obscura* Fittkau
- Frontal tubercles absent; anteprenotal and supraalar setae present. 2
2. Pars ventralis and digitus absent; pulvilli and pseudospurs present. North America, Dominican Republic.
 *M. pahayokeensis* Jacobsen
- Pars ventralis and digitus present; pulvilli and pseudospurs absent. 3
3. Wing length 1.81–1.91 mm; AR 1.91–2.19; LR₁ 1.41; wing vein R₁ bare; transverse sternapodeme with oral projections. Tanzania *M. tangae* Andersen & Sæther
- Wing length 1.43–1.50 mm; AR 1.13–1.33; LR₁ 0.96–0.98; wing vein R₁ with 1–3 setae; transverse sternapodeme without oral projections. Oriental China *M. xianjuensis* Qi & Lin sp. n.

Key to adult females of *Manoa* Fittkau

- 1. Frontal tubercles present; anteprenotal and supraalar setae absent. Brazil *M. obscura* Fittkau
- Frontal tubercles absent; anteprenotal and supraalar setae present. 2
- 2. Wing length 1.00–1.16 mm; squama bare. North America. *M. pahayokeensis* Jacobsen
- Wing length > 1.50 mm; squama setose 3
- 3. Wing length 2.00–2.06 mm; wing veins R with 17–20 setae, R₁ with 7–8 setae, R₄₊₅ with 23–29 setae. Tanzania
- Wing length 1.58–1.75 mm; wing veins R with 4 setae, R₁ with 10–12 setae, R₄₊₅ with 11–12 setae. Oriental China
- *M. xianjuensis* Qi & Lin **sp. n.**

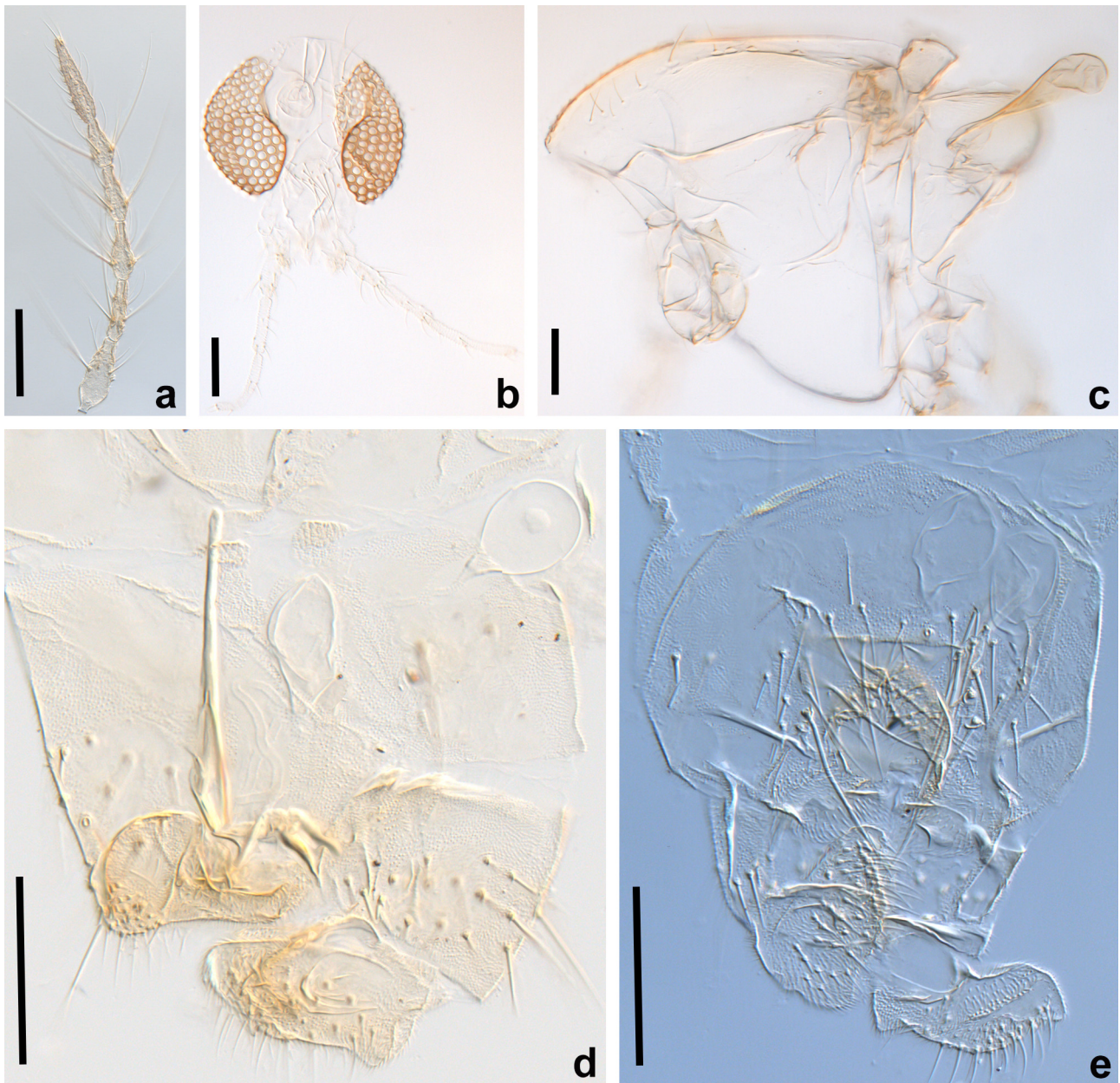


FIGURE 7. *Manoa xianjuensis* **sp. n.**, paratype female: a) antenna; b) head; c) thorax; d) genitalia ventral view; e) genitalia lateral view. Scale bars = 100 μ m.

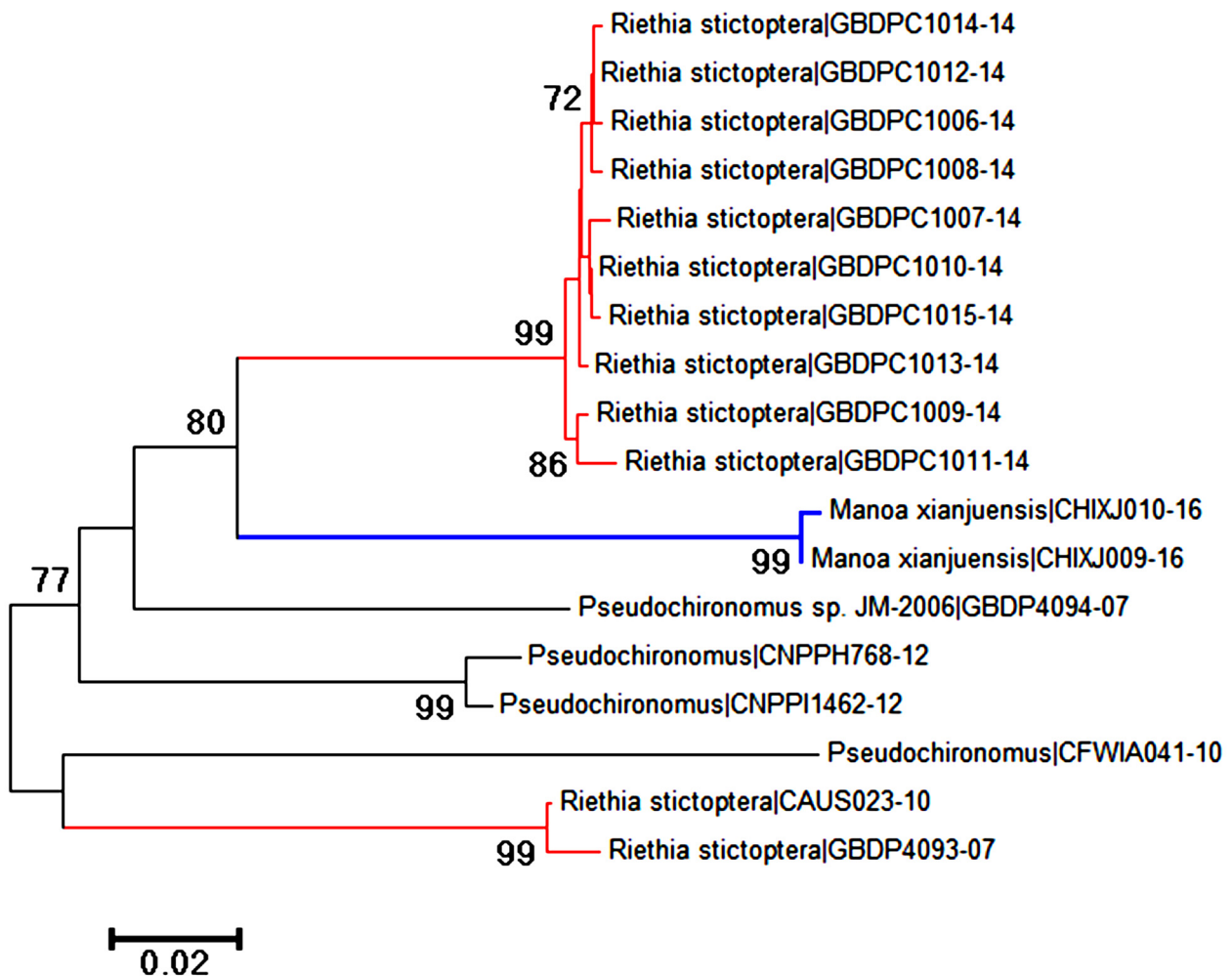


FIGURE 8. Neighbor joining Kimura 2 parameter tree based on available Pseudochironomini DNA barcodes.

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