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The Afro–Oriental Genus *Yaeprimus* Sasa et Suzuki (Diptera: Chironomidae: Chironomini): Phylogeny, New Species and Expanded Diagnoses

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Abstract: Expanded generic diagnoses of all life stages of *Yaeprimus* Sasa et Suzuki, 2000 (*Lunditendipes* Harrison, 2000, **syn. n.**) are given. *Yaeprimus tropicus* **comb. n.** is redescribed as an adult based on type material. Additionally, a new species *Y. balteatus* **sp. n.** from Oriental China is described based on the adult male and pupa. The phylogenetic position of *Yaeprimus* within Chironomini and the validity of the new species are explored based on concatenated five genetic markers (*18S*, *28S*, *CAD1*, *CAD4*, and *COI-3P*) through both mixed–model Bayesian inference and maximum likelihood methods. The results strongly support *Yaeprimus* as sister to *Imparipecten* Freeman, 1961, which counters a previously proposed systematical position based solely on morphology.

Keywords: *Yaeprimus*; *Lunditendipes*; synonymy; new species; phylogeny; Asia; Africa

1. Introduction

Yaeprimus Sasa et Suzuki, 2000, was established based on adult males of *Yaeprimus isigaabeus* Sasa et Suzuki [1] collected from Ishigaki Island in Japan. Subsequently, it was revised in detail of all stages by Yamamoto and Yamamoto [2] based on reared associated material. The genus was stated to show close relationship to some *Lauterborniella* related genera, such as *Apedilum* Townes, *Zavreliella* Kieffer and *Paralauterborniella* Lenz which are characterised in the larva by having a six-segmented antenna and alternate Lauterborn organs (*Microtendipes* group sensu Cranston et al. [3]), although the adult male of *Yaeprimus* lacks the median volsella or enlarged superior volsella base characteristic of the group.

Simultaneously, Harrison [4] described four new Chironomidae genera from South Africa, amongst which males of *Lunditendipes* Harrison shared similar fore tibia and anal tergite setae with the Asian *Yaeprimus*. Noticeably, an important diagnostic character that appears to distinguish *Lunditendipes* from *Yaeprimus* is the absence of basal setae of the superior volsella according to Harrison's original description. However, this is a flawed observation according to the examination of the types materials deposited in ABM by Helen Barber-James. Actually, those specimens bear two inner basal setae clearly arising from tubercle-like setigers and two heavily sclerotized concavities each containing two strong setae in tergite IX, thus resembling *Y. isigaabeus* [5]. The same character states have been observed also on material deposited in ZSM, which were collected from Kruger

National Park in north–eastern South Africa and identified as *Lunditendipes* by Martin Spies [6]. The two species of *Y. isigaabeus* and *L. tropicus* show considerable similarity justifying our assessment that they are congeneric, despite differences in the anal tergite band and the shape of gonostylus.

During a survey of rural areas in Guangzhou, a distinct male adult was reared from stream sediment, which conformed largely to the generic diagnosis of *Yaeprimus* but its color pattern on the abdomen and legs clearly differs from that of the type species *Y. isigaabeus*. Subsequently, similar specimens collected by the NKU Chironomidae group in Hainan were allocated into the above unknown species after a thorough comparison [7]. Here we confirmed it as new to science and described it based on adult males and pupae.

The systematic position of *Yaeprimus* has received little attention since Yamamoto and Yamamoto's morphological revision [8]which remains somewhat uncertain, and therefore a more integrated taxonomic work is needed to detect the placement of *Yaeprimus* within tribe Chironomini. Here, we conducted a phylogenetic inference based on five genetic loci (*18S*, *28S*, *CAD1*, *CAD4*, and *COI–3P*) to test the Yamamoto's hypothesis and to explore the boundary of the *Microtendipes* group. Additionally, some possible placement and dubious features are discussed.

2. Materials and Methods

2.1. Morphology

Fieldwork was conducted using several classical methods for chironomids collection [9–11]. Adults were caught by light trap and sweeping nets along the aquatic sites. Pupal exuviae were sampled using dip nets (mesh size 250 μm). All samples were preserved in the field with 85% ethanol, then transferred to the laboratory for sorting under a stereomicroscope. Thorax of adults were sampled for DNA extraction, after that, each cleared exoskeleton was mounted permanently in Euparal on microscopic slides with corresponding parts following standard procedures [12]. Identifications were made under a compound microscope with reference to a range of identification tools and published papers [1,2,13,14]. Morphological terminology and abbreviations mainly followed Sæther [15] except the superior volsella. Here the superior volsella base and superior volsella digitus proposed by Cranston [16] are adopted. Measurements were taken according to Epler [17] and given as ranges and followed by average value. The number of observed specimens was recorded in parentheses if it differed from the number (n) stated at the beginning of the description.

2.2. Molecular Work

DNA was extracted using MAGEN® (Beijing, China)Tissue DNA kit in the Molecular Lab of Institute of Groundwater and Earth Science, Jinan University, and QIAGEN® (Hilden, Germany) DNA Blood and Tissue kits at the Tianjin Agricultural University. Standard protocols were followed except for the lysis time and final elution volumes, and all the samples were lysed overnight at 55 °C and eluted with 40 μL of eluent. Universal primers were adopted following previous studies (Table A1). Processes for gene fragments amplification were followed as previous studies except for slight moderation of annealing temperature [18–20]. Polymerase chain reaction (PCR) products were electrophoresed in 1.0% agarose gel, then shipped to Majorbio Company, Guangzhou for purification and bidirectional sequence. One mitochondrial gene (*COI-3P*), two ribosomal genes (*18S* and *28S*), and two sections of the nuclear protein-coding gene (*CAD1* and *CAD4*) were chosen as in Cranston's work to match the comprehensive dataset of Chironomidae. Additionally, the standard barcode, one fragment of the mitochondrial gene (*COI-5P*) proposed by Hebert [21] was sequenced to explore cryptic species and calculate generic distance.

Forward and reverse sequences were assembled automatically and manually edited with Sequencher 4.8 (Gene Codes Corp.). Alignment of the sequences used Muscle algorithm [22] on nucleotides in MEGA X [23]. Some ambiguous bases were eliminated based on the results of alignments and trace file, while the remnants were adopted and showed in the International Union of Pure and Applied Chemistry (IUPAC) code. For protein-coding genes, introns were excised using the GT–AG rule [24] and an amino acid alignment was used as a guide to elucidate exon/intron

boundaries. For *18S* and *28S* rDNA, ambiguous regions were excluded with GBlocks v0.91b using default setting except allowing half gap positions within the final blocks [25,26]. All selected genes except for the standard barcode (*COI–5P*) were concatenated with PhyloSuite v1.1.14 [27] to implement the maximum likelihood and Bayesian inference. In case of missing gaps, they were filled up by "?" to ensure that all sequences were in the same length. The optimal models for each subset were selected by Partition Finder 2 [28] based on the Bayesian information criterion (BIC) and corrected Akaike information criterion (AICc). The best scheme was as follows: GTR+I+G for the *18s*, *28s* and the first two codons for all protein-coding sequence, GTR+G for the third codon of COI–3P and GTR+I+G for third codon of *CAD1*and *CAD4*. Maximum likelihood (ML) phylogenetic analysis was conducted using IQ–TREE 1.6.8 [29] with 1000 bootstrap replicates in a rapid bootstrap analysis and a "greedy search" for the best-scoring ML tree. Bayesian inference was performed in MrBayes v3.2.6 [30]. During the processes, Markov chain Monte Carlo (MCMC) iterations were run with four chains on two runs for 10 million generations, sampled every 100 generations with a burn-in of 0.25. Convergence among the runs was monitored using Tracer v1.6 [31], with the first 25% trees discarded as burn-in. The final average standard deviation of split frequencies was 0.003. Both analyses were completed using the best fitting scheme selected by Partitionfinder. Two species of tribe Tanytarsini were selected as outgroups for this has been considered to be the nearest neighbor of the tribe Chironomini.

In total, 235 sequences of 51 specimens were added to the molecular dataset, 112 of which were downloaded from GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and five of which retrieved from BOLD (Table A2). Forty species were chosen to stand for four complexes, two groups and some ambiguous genera within Chronomini referring to a previous study [3]. Members of *Microtendipes* were enlarged particularly to test the Yamamoto hypothesis. Specimens with less than three markers were excluded from the dataset of concatenated genes. List of all species, specimens, individual images, georeferences, primers, sequences, trace files and other relevant laboratory data of sequenced specimens can be seen online through the publicly accessible dataset "*Yaeprimus*" on the BOLD website (www.boldsystems.org) [32,33].

2.3. Mapping

The distribution map (Figure 1) was made using ArcGis™ software [34], with all possible GPS locations of 17 sites implanted into the vector of World Map (http://www.vectorworldmap.com). For older specimens without GPS data, estimates were made from the finest available detail (e.g., city/country) available from either specimens or publications.

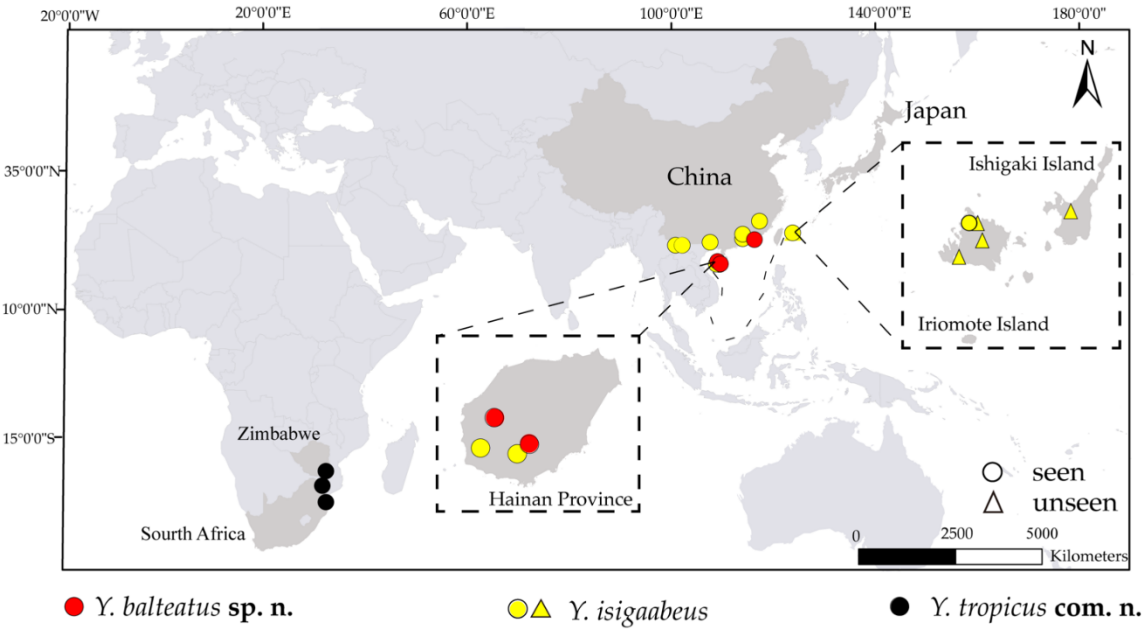

Figure 1. Distribution map of *Yaeprimus*.

2.4. Abbreciation

Institution: ABM, Albany Museum, South Africa; EJNU, Institute of Groundwater and Earth Science, Jinan University, China; NKU, College of Life, Nankai Universtiy, China; ZSM, Zoologische Staatssammlung Muenchen, Germany.

Terminology: Lifer stage, F, female; L, larva; M, male; P, pupa. Morphylogy, T II–X, tergite II to X; Ta_1–Ta_5, tarsus 1 to tarsus 5. Molecular, BS, bootstrap support; PP, posterior possibility.

3. Results

3.1. Molecular Analysis

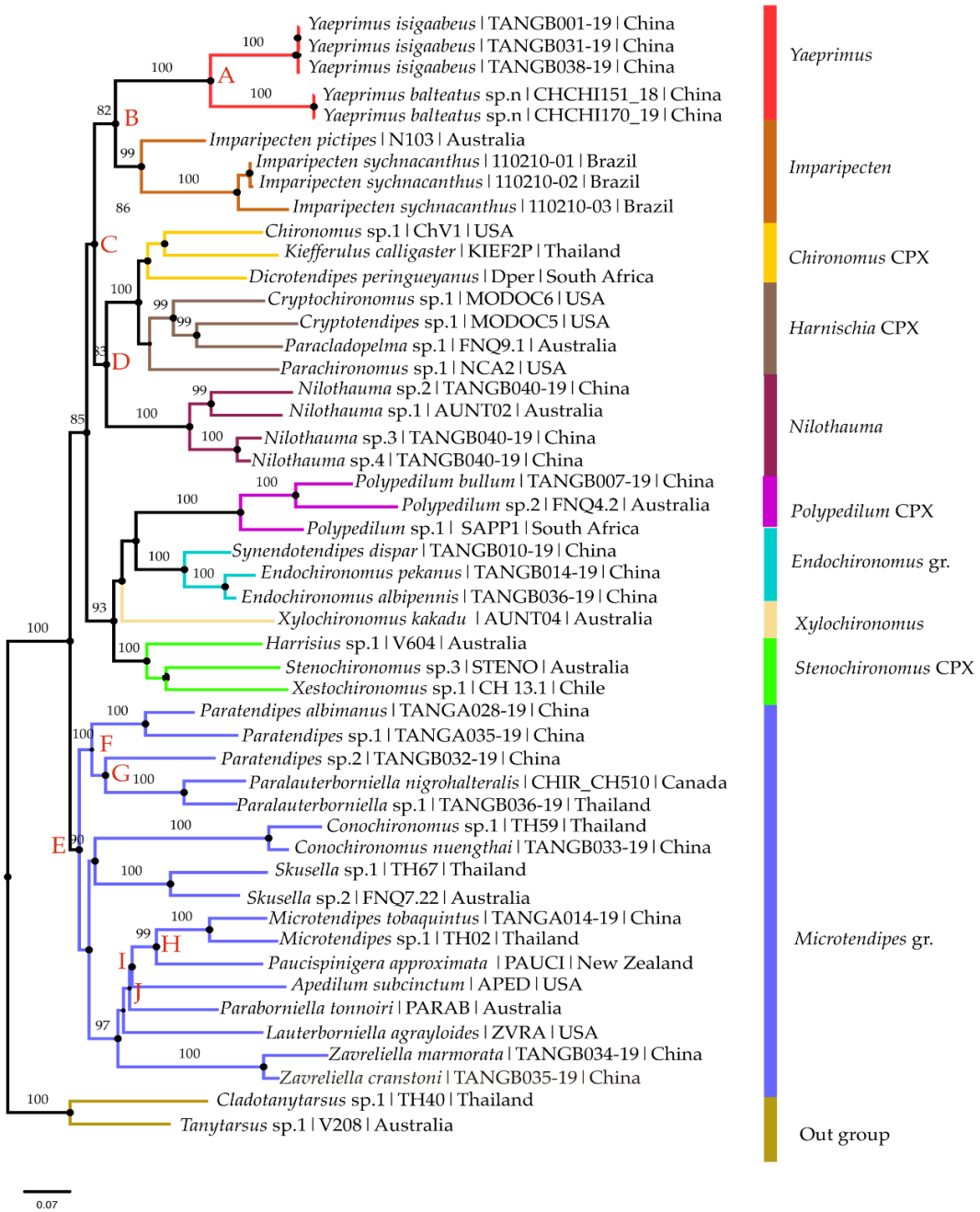

Figure 2. Maximum likelihood tree based on concatenated gene (*18S*, *28S CAD1*, *CAD4*, *COI–3P*) fragments. Nodes are labeled by bootstrap support (BS), only BS > 0.8 are shown.

3.1.1. DNA Barcode

Three COI DNA barcodes of new species from adult males clustered into the same BIN (BOLD:ADH0469), with a maximum intraspecific pairwise genetic distance of 0.64%, and 11.38% divergence to the nearest BIN (BOLD:ACT7861). The nearest neighbor (Sample ID: BIOUG28352–C08) from Guanacaste in Costa Rica, was unidentified in BOLD.

3.1.2. Phylogenetic Analysis

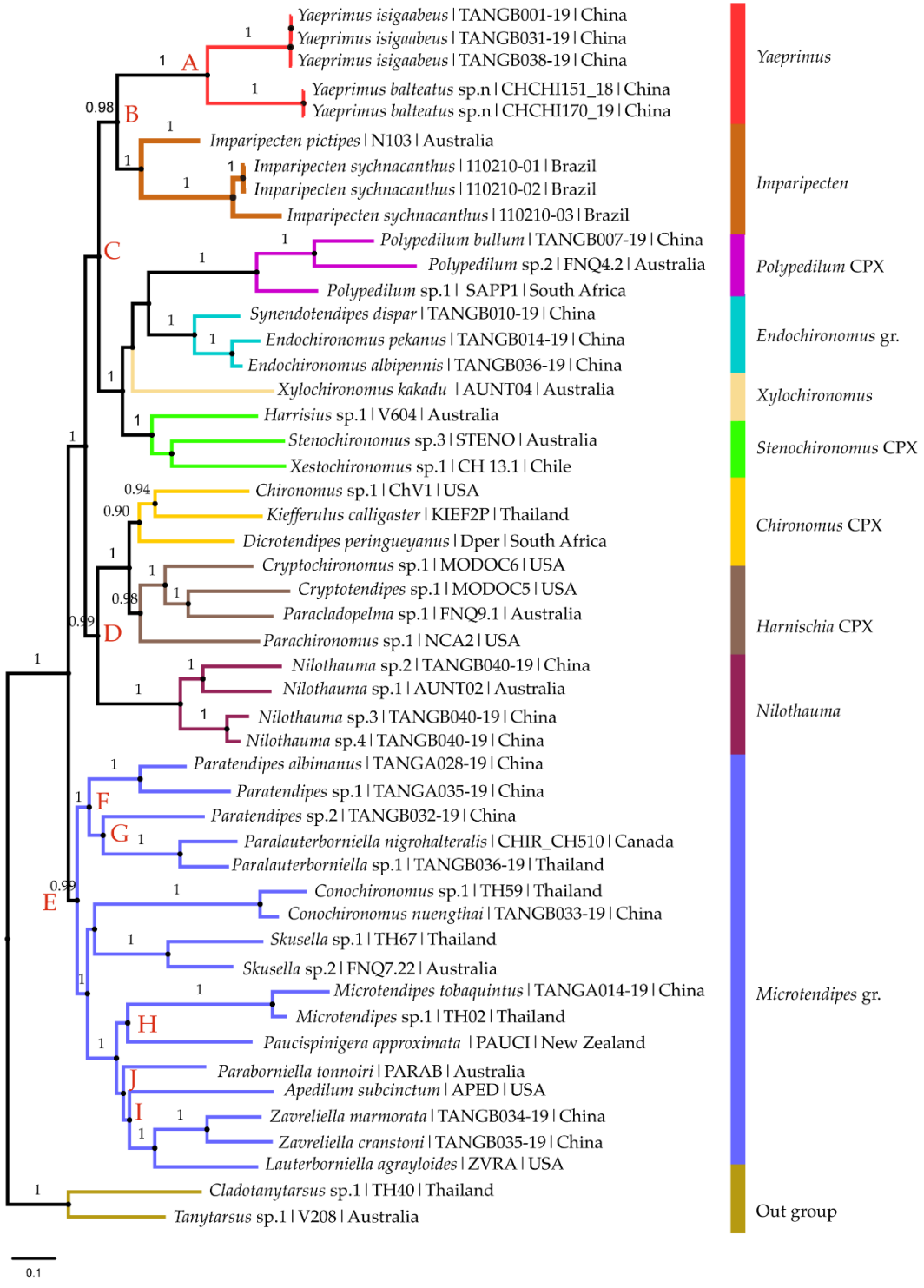

Figure 3. Bayesian inference tree based on concatenated genes (*18S, 28S CAD1, CAD4, COI–3P*) fragments. Nodes are labeled by posterior possibilities (PP), only PP > 0.90 are shown.

The structure of the maximum likelihood (ML) tree was basically similar with that of the Bayesian inference (BI) tree except for some weakly supported clades. As expected, both approaches strongly supported (node A, BS = 100, PP = 1) that *Y. isigaabeus* and new species group together. *Yaeprimus* together with *Imparipecten* Freeman forms a new clade in both trees (node B, BS = 82, PP = 0.98), then the clade is sister to the assemblage of *Chironomus* complex, *Harnischia* complex and *Nilothauma* Kieffer (node C) in ML tree, while shifts to the assemblage of *Polypedilum* Kieffer, *Endochironomus* Kieffer and *Stenochironomus* Kieffer (node C) in BI tree, but either connections to *Chironomus* or *Polypedilum* clades without support. The positions of remaining genera mostly conform to previous results [3]. Besides *Yaeprimus*, something interesting has been discovered in our study. After the inclusion of Chinese populations, *Nilothauma* is verified as sister to the *Chironomus* complex + *Hanischia* complex (node D, BS = 83, PP = 0.99), which showed some tendency in previous work [3] but without robust support. In a *Microtendipes* group (Node E, BS = 90, PP = 0.99), the Chinese populations of *Paratendipes* (node G) are paraphyletic although lack of support. Saether's hypothesis [35], based on characters of female adults that *Patatendipes* is sister to *Microtendipes* + *Nilothauma,* is rejected in both analyses. Our results show that *Patatendipes* (node F, BS = 100, PP = 1) is close to *Paralauterborniella* Lenz, while *Microtendipes* is close to Australian *Paucispinigera* Freeman (Node H, BS = 99). The positions of *Apedilum* Townes (node I) and *Paraborniella* Freeman (node J) vary within acceptable range between two analyses, tough both nodes are weakly supported (Figures 2 and 3).

3.2. Morphology

3.2.1. Generic Diagnosis Emendation

***Yaeprimus* Sasa et Suzuki**

Yaeprimus Sasa et Suzuki, 2000 (M).

Yaetertius Sasa et Suzuki, 2000 (M), Yamamoto and Yamamoto, 2000.

Lunditendipes Harrison, 2000: 224 (M), **syn. n**., type species: *Lunditendipes tropicum* Harrison by original designation.

Yaeprimus Sasa et Suzuki; Yamamoto and Yamamoto, 2000 (M, F, P, L).

Type species: *Yaeprimus isigaabeus* Sasa and Suzuki, 2000: 4, by original designation. Other included species: *Yaeprimus tropicus* (Harrison, 2000), **comb. n.**; *Yaerpimus balteatus* **sp. n.**

Three species conformed to most generic diagnosis given by Yamamoto and Yamamoto [2], except for the following emendations.

Male

Head. Frontal tubules small, hemispheric.

Thorax (Figure 4D, Figure 8A). With a distinct scutal tubercle (*Y. balteatus* **sp. n.**) or flat hump (median protuberance) (*Y. isigaabeus*, *Y. tropicus* **comb. n.**), if the latter, smoothly curved in the middle. Humeral pits present.

Legs (Figure 4F, Figure 8B,C). Tibial combs of mid and hind tibiae nearly or completely fused; if separated narrowly (*Y. balteatus*, *Y. tropicus*), the large (inner) comb usually bears 0–2 straight spurs, the small (outer) comb always has a long apically hooked spur. If fused (*Y. isigaabeus*), there is only one hooked spur, arising more proximally on the outer surface of the comb base. The number of spurs per tibia variable even within a single specimen. Pulvilli present.

Abdomen (Figure 5A). Anterolateral areas of first segment slightly sclerotized with two distinct patches bearing several concentrated setae; T II–VIII with two rows of regular transverse setae centrally. Tergite VIII slightly tapered anteriorly (Figure 4G).

Hypopygium. Tergite IX with a regular row of median anal seta, usually grouped laterally, arising from the distinct pigmented oval field (Figure 4H) or blank oval pits (Figure 8F). Anal tergite band absent or weak. Superior volsella (Figure 6C,D, Figure 8F) with bare base, 1–3 basal setae, arising from the distinct tubercle base, digitus slender distally or with a slightly elongated ventro–lateral ledge distally (hooked), covering a partial or the whole width of digital apex, lacking any outer seta on digitus. Gonostylus was normal (Figure 4H) or reduced (Figure 8F), with several distal-medial setae of different sizes, the seta on the distal-inner corner being the longest and thickest.

Pupa

Tergite spinulation of III–V split into anterior and posterior patches or completely fused. All spinules were nearly uniform sized. Conjunctival bands present on T III and T IV, continuous or medially interrupted. Posterolateral corner of T VIII with 'comb' of 1 main tooth and 3–4 small side teeth. Taeniae pattern (Figure 7A) on A IV–VIII, 4, 4, 4, 4, 4. Uniserial fringe with 20–30 taeniae. Dorsal seta of anal lobe present.

Distribution. *Yaeprimus* was known only from two small Japanese islands for *Y. isigaabeus*. Our study has expanded the genus distribution to south China, South Africa, and Zimbabwe. All specimens have been collected from subtropical and tropical regions.

Remarks. The integrated systematical work of *Yaeprimus* has not been conducted completely before this study. The previous suggested phylogenetic placement was based solely on selected distinctive morphological character states of three life stages, rather than through a formal data matrix. Previously-argued conclusions were not fully reliable lacking rigorous parsimony analysis. Some important characters were ignored, for example, the anteriorly tapered tergite VIII, the inner setal arrangement of gonocoxite, the condition of humeral pit, and the abdominal setae and the pulvilli status. The current inclusion of an additional two species expands the variation within the genus, complicating the generic diagnosis. The main morphological differences among the three species are summarized in Table 1.

Table 1. The main morphological differences on male adults of three *Yaeprimus* species.

Features of male	***Y. balteatus* sp. n.**	***Y. isigaabeus***	***Y. tropicus* com. n.**
Scutum	hump, with tubercle	hump, without tubercle	normal, without tubercle
Tibial comb	separated	fused	separated
Anal tergite band	absent	absent	reduced
Anal median setae	5–6 setae, one row	two pairs, grouped	two pairs, grouped
TIX oval concavity	absent	present	present
Gonostylus	reduced	reduced	normal

3.2.2. Description of Species

Yaeprimus balteatus Han et Tang, **sp. n.**

Material examined (all collected H.Q. Tang, deposited EJNU, unless stated otherwise). Holytype [EJNU–Ershan150910001], male, CHINA: Guangdong Province, Guangzhou City, Ershan county, 23°14′ N, 113°26′ E, 10.ix.2015, light trap; Paratype, 1 male[EJNU–Ershan150320001] and 1 reared pharate female [EJNU–Ershan150521001] as previous, except 20.iii.2015 (emerged 21.v.2015); 1 male [EJNU– Shantou 151014001], Guangdong Province, Shantou City, Chaonan District, Jinxi Reservoir Scenic spot, Fengzai Village, 23°10′ N 116°18′ E, alt. 210 m, 14.x.2016, light trap (ZSM); 1 male[NKU–XL1460], Hainan Province, Changjiang County, Bawangling National Nature Reserve, 19°07′ N, 109°05′ E, 13.iii.2016, light trap, B.J. Sun (NKU); 2 males[NKU–XL1509, NKU–XL1510], Hainan Province, Shuiman County, Wuzhi Mountain., 18°45′ N, 109°36′ E, sweep net, 2.iii.2016, C. Song (NKU).

Etymology. The new name '*balteatus*', derived from Latin (meaning belted), referring to the color bands on the abdomen and leg.

Male (n = 5–6) (Figures 4–6)

Total length, 2.50–2.95, 2.70 (5) mm. Wing length, 1.30–1.60, 1.45 (5) mm.

Coloration (Figures 4A,B and 5A). Generally brown with some pattern in legs and abdomen. Legs were yellow, except for dark brown all femur and complete mid tibia, and pale brown apex of fore and hind tibia. Fore-tarsus with brown apex and gradually brown in Ta_2–Ta_5, others tarsi all pale yellow (Figure 4A,B for colorful photo). Anterolateral corners of A I, posterior half of A III and A V, and almost entire A VI–VIII dark brown. A IX was dark brown, the hypopygium with brown gonocoxite and pale yellow gonostylus.

Head. Antenna (Figure 4C) with pale brown (approximal and distal) or brown (middle) flagellomeres, with almost dark plume. Flagellomere 1–12, 340–390, 358; flagellomere 13, 540–640, 570; AR 1.57–1.64, 1.60 (4). Palpomere lengths (in μm): 25–30, 28 (3); 25–35, 30 (3); 115–125, 120, (3); 100–124, 111 (3); 180–200, 190. Temporals were 10–12, 11. Clypeus had 14–17, 15 (5) setae. The diameter of cephalic tubercle 5 μm, bearing 3–5 small setae.

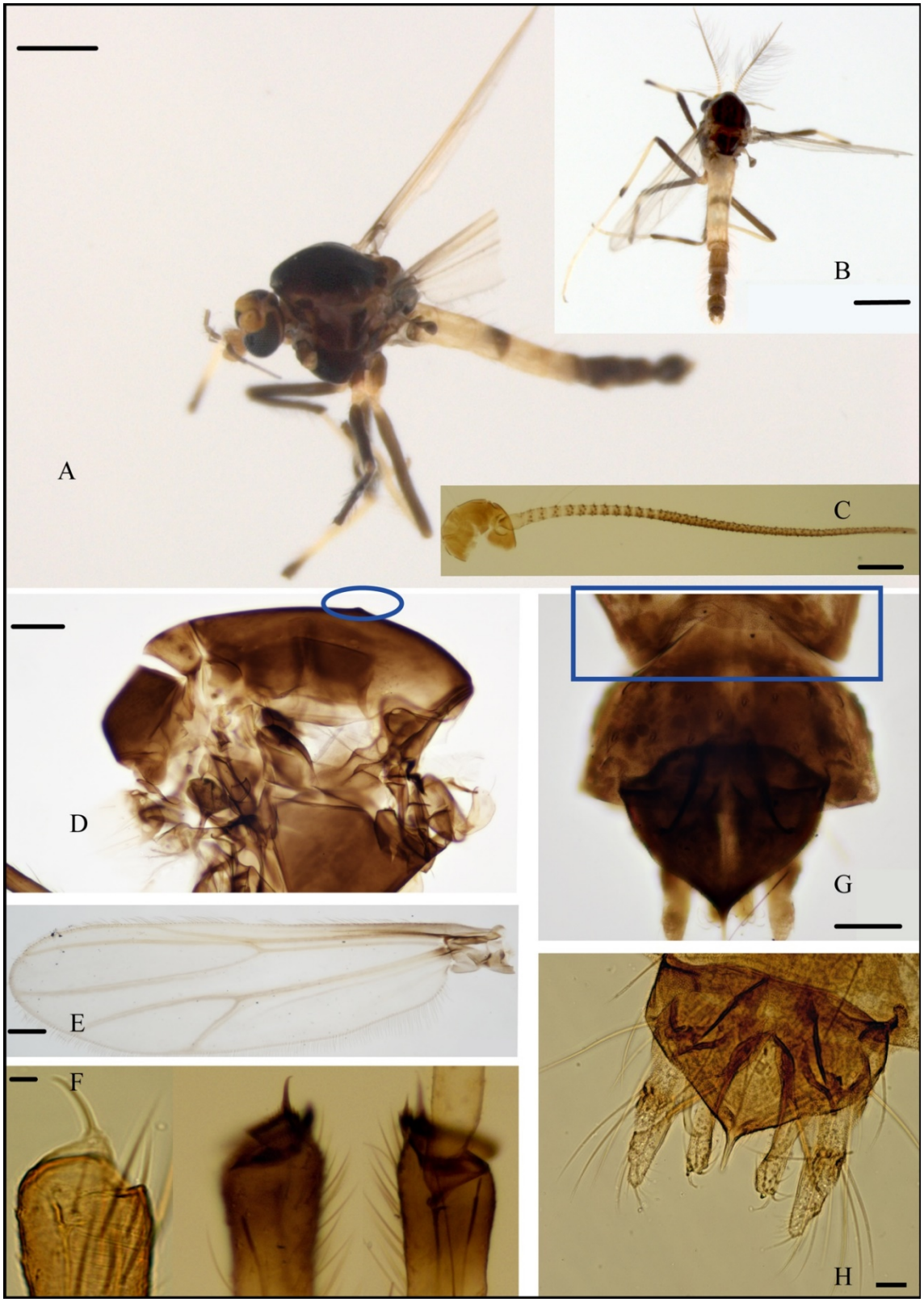

Figure 4. *Yaeprimus balteatus* **sp. n.**, male. Photos: (**A**) lateral view; (**B**) dorsal view; (**C**) antenna; (**D**) thorax; (**E**) wing; (**F**) tibia; (**G**) T VIII; (**H**) hypopygium. Scale bar: A, B, 500 μm; D–H, 100 μm.

Thorax (Figure 4D). Antepronotals 1–2; dorsocentrals 6–9, 6, usually alternately accessorized with 3–5 tiny pits; acrostichals 10–16, 14 (3) arranged in robust two rows, ending in anterior 1/3 before the hump; prealars 3–4, 3, supraalars 1. Scutellum had 7–8 setae, in single row. Tiny trans-oval humeral pits present.

Wing (Figure 4E). VR 1.09–1.22, 1.16 (5); R without seta; R_1 with 0–1 seta; R_{4+5} with 1–2 setae in extreme apex. Squama bare.

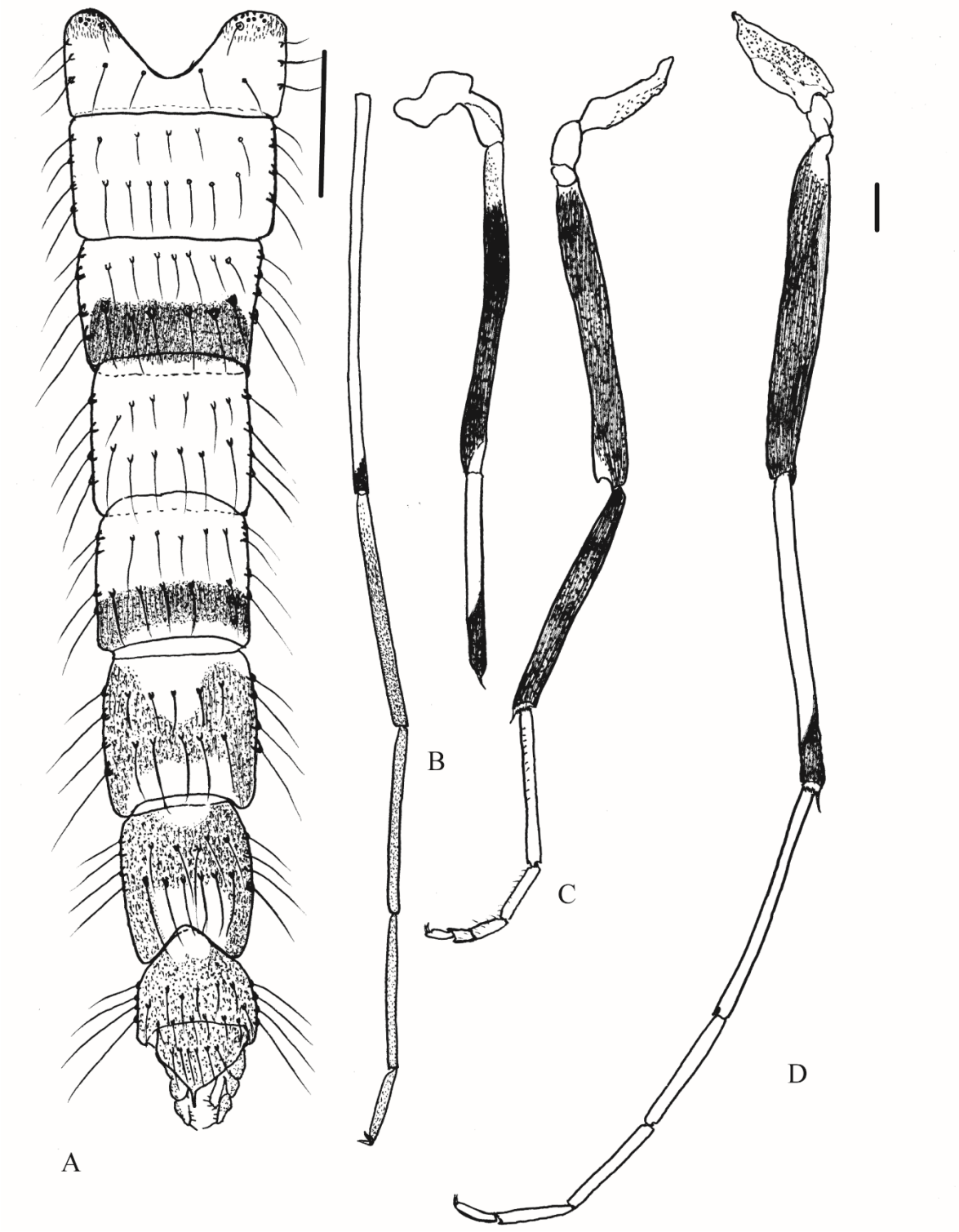

Figure 5. *Y. balteatus* **sp. n.**, male. Illustration. (**A**) abdomen; (**B**) fore legs; (**C**) mid legs; (**D**) hind legs. Scale bar: A, 250 μm; B–D, 100 μm.

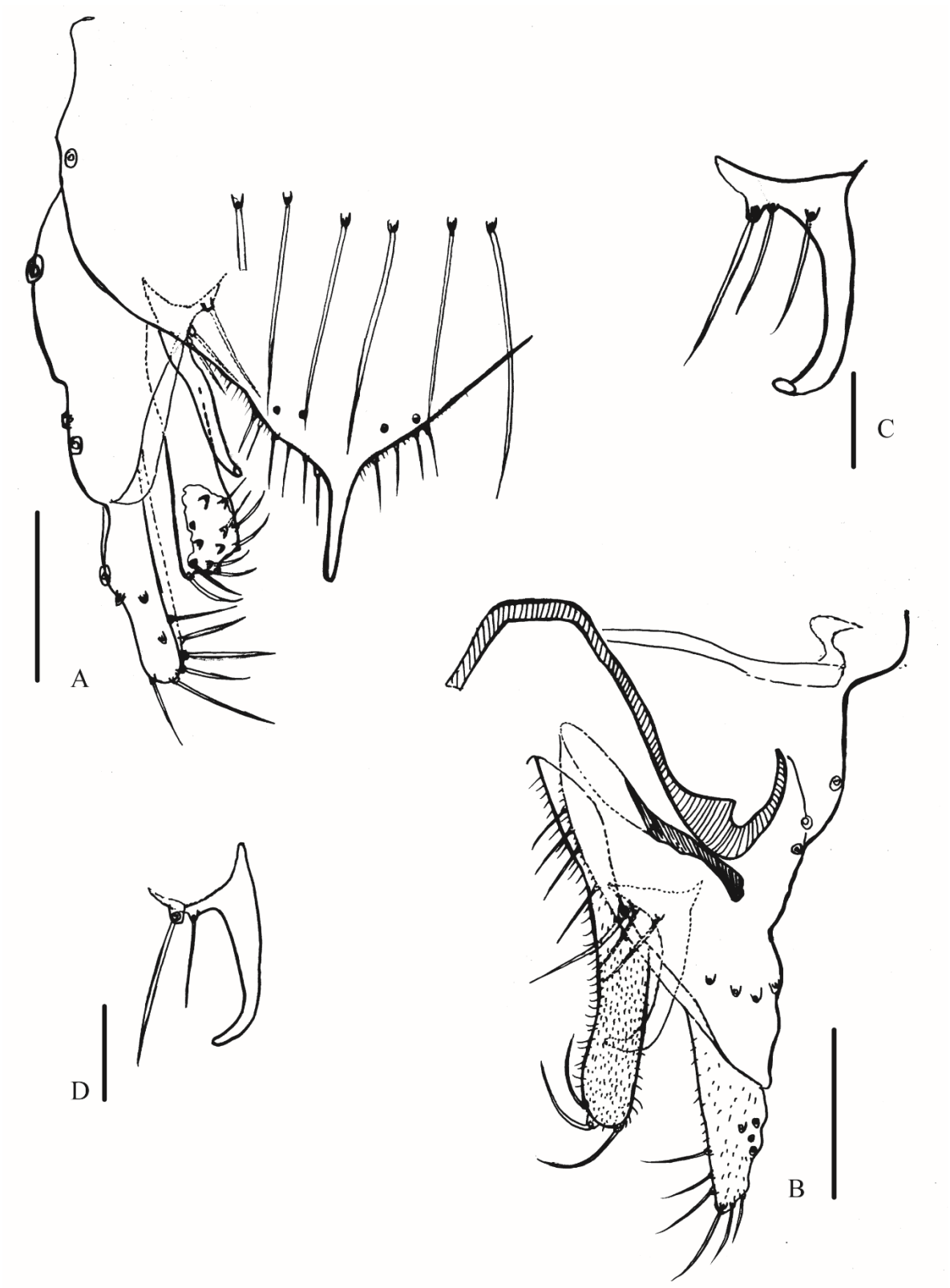

Figure 6. *Y. balteatus* **sp. n.**, male. Illustration: (**A**) hypopygium, dorsal view; (**B**) hypopygium, ventral view; (**C**,**D**) superior volsella. Scale bar: A–D, 100 μm.

Legs (Figures 4F and 5B–D). Fore tibia with a conical scale bearing a slender, distal-hooked spur, 25–30 (2) μm long. Mid tibia with two separated combs, one bearing a distal-hooked spur, 23–37, 28 (3) μm long, another comb with even comb teeth, unspurred; hind tibia with two separated combs, the small one bearing a distal-hooked spur, 25–40, 35 μm (5) long, and the large one bearing 1–2 straight spurs, 12.5–20, 16.5 (5) long. LR_1 1.30–2.19 (2); LR_2 0.58–0.71, 0.65 (3); LR_3 0.77–0.83, 0.80 (3).

BV_1 1.44–2.28(2); BV_2 5.31–5.88, 5.54(3); BV_3 2.44–2.61; 2.54 (3). SV_1 1.30–2.12(2); SV_2 3.20–4.06, 3.54 (3); SV_3 2.59–2.70, 2.63(3). BR_1 2.25–2.50(2); BR_2 3.57–4.00, 3.78 (3); BR_3 3.2–5.36, 4.25(3).

Hypopygium (Figures 4H and 6A,B). T IX with a row of 4–6 setae, arising from fairly large microtrichia-free pit, without distinct lateral group. Distal margin with 3–5 setae both in the dorsal and ventral surfaces. Gonocoxite 115–132, 126 (4) μm long. Gonostylus relatively short, 35–48, 43 (4) μm long, distal portion with 6–10 inner–toward setae, the longest one about 25 long. Superior volsella (Figure 6C,D) with a base without microtrichia, bearing 2–3 inner setae from the tubercle base, distal digitus long and slender, distal curved inwardly, without inner seta. Inferior volsella slightly bullous distally, with 13–16 setae. HR 2.79–3.29, 2.97 (4); HV 5.58–7.14, 6.37 (4).

Pupa (n = 1) (Figure 7)

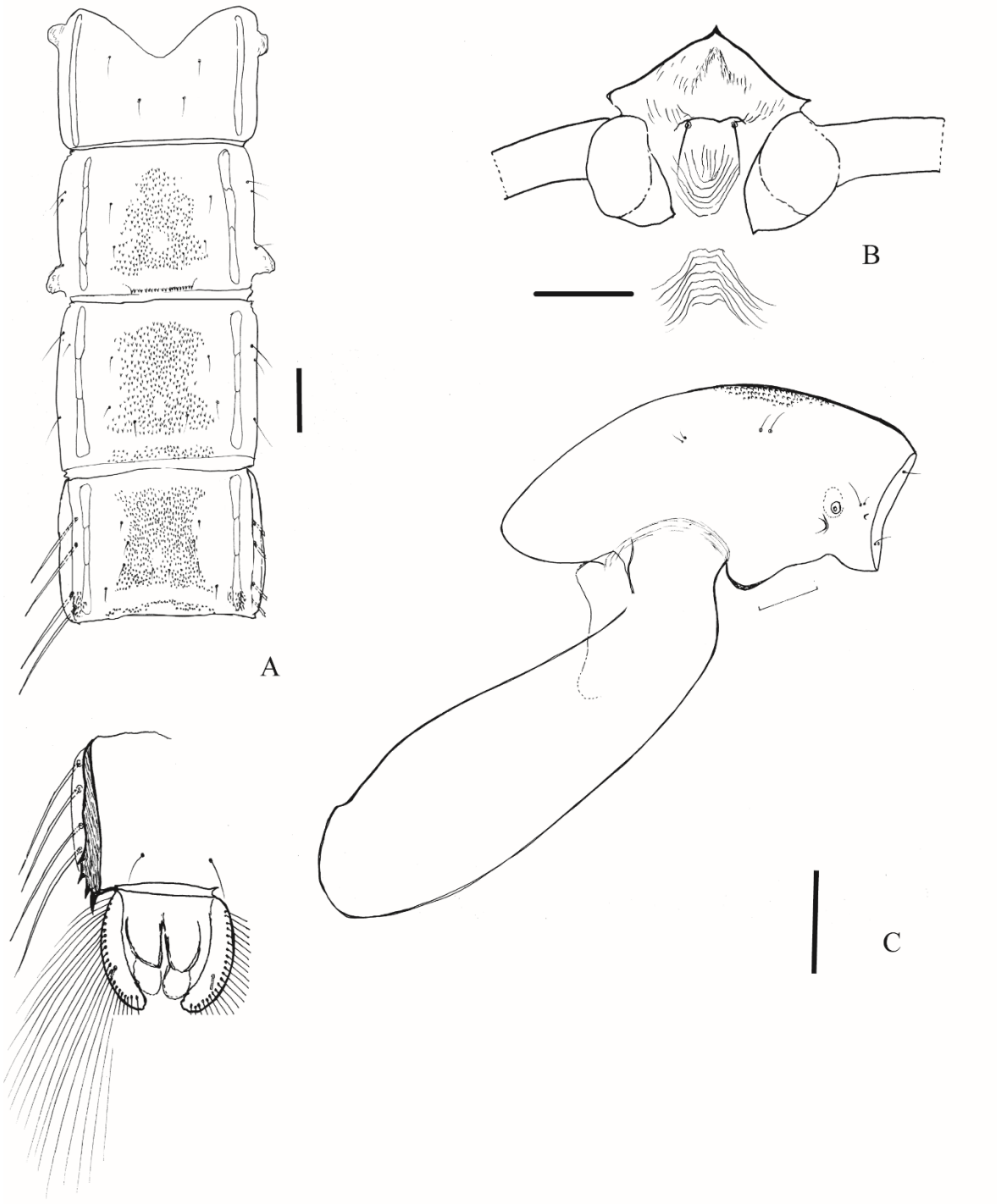

Figure 7. *Y. balteatus* **sp. n.**, pupa. IllustrationL (**A**) abdomen, dorsal view; (**B**) cephalothorax; (**C**) thorax, lateral view. Scale bar: A–C, 100 μm.

Total length ca. 3.0 mm. Cephalic tubercle absent (Figure 7B), frontal setae small, 40 μm long, subequal to the gap between two frontal setae. The thoracic setation as in Figure 7C, thoracic horn invisible. The abdomen (Figure 7A) with dense spinulations in T II–IV, no clear delimitation between the anterior patch and median patch. Continuous conjunctival spinule bands present in T III and IV. The tergite II hook row continuous, short, 30% of the width of segment II, comprising ca. 20 hooks. A V–VII distorted. Comb VIII with one main tooth and three small accessory teeth. The anal lobe 140 μm long and 150 μm wide, with 20–24 taeniae, dorsal seta present.

Remarks. The new species shares the same comb pattern with *Y. tropicus* and reduced gonostylus with *Y. isigaabeus*. It can be distinguished from the other two species by the distinct scutal tubercle and anal median tergal seta arising from the common pale pit rather than sclerotized concavities. For pupa, the new species can be separated from *Y. isigaabeus* by the fused sub-rectangular spinulations and small point-free area in the middle area of T II–IV and conjunctives continuously.

Distribution: China (Guangdong and Hainan).

Yaeprimus isigaabeus Sasa et Suzuki

Yaeprimus isigaabeus, Sasa and Suzuki, 2000: 4; Yamamoto and Yamamoto, 2011: 228.

Yaetertius iriojekeus Sasa et Suzuki, 2000: 18, synonymized by Yamamoto and Yamamoto, 2011.

Material examined (all collected H.Q. Tang, deposited EJNU unless stated otherwise): 1 male, 1 female, CHINA: Fujian Province., Zhangzhou City, Nanjing County, a stream in Huboliao National Nature Reserve, 26°31′ N 117°18′ E, 15.xi.2012; 6 males, China: Guangdong Province, Guangzhou City, Zengcheng District, Shuimei County, Lan stream, 23°21′ N, 113°58′ E, alt. 148, 29.xi.2018, light trap; 1 Pe, Guangdong Provinve, Guangzhou City, Conghua District, Xinlian village, 23°47′ N, 113°59′ E, alt. 240 m, hand net, 18.x.2014; 1 male, CHINA: Hainan Province, Baoting County, Xian'an Shilin scenic spot, 18°36′ N 109°25′ E, alt. 602 m, 14.ii.2015; 2 Pe, Guangxi Province, Congzuo City, Detian waterfall, 22°51′ N, 106°44′ E, alt. 380 m, 24.ii.2012, W. Xia and C.B. Duan; 1 male, CHINA: Yunnan Province, Xishuangbanna Prefecture, Jinghong City, Mengyang County, Xishuangbanna Prefecture, Wild Elephant Valley, 22°10′ N, 100°51′ E, 900 m asl., sweep net; 1Pe, Yunnan Province, Xishuangbanna Prefecture, Jinghong City, Menglun Town, Luosuo River, 21°55′ N 101°17′ E, 22.iii.2019.

Additional compared specimens: 2 males, JAPAN: 2 males, Iriomote Island, Funaura, one slide 24. iii. 2000, another 19.xi.2001, M. Yamamoto.

Conforms mostly to Yamamoto and Yamamoto [2], with the following supplementation and emendation:

Male

Total length 1.8–3.2 mm, wing length 1.1–1.8 mm. AR 1.15–1.50. LR_1 1.57–2.36. Distinct humeral pit present.

Anal tergite with two pairs or three pairs of median setae, arising from a heavily pigmented field, grouped laterally. Superior volsella base with 1–2 inner seta, without microtrichia, digitus with a basal inner seta, and distal elongated, with a ventro–lateral ledge apically.

Pupa

Cephalic tubercle absent, frontal setae reduced, subequal to the gap between two setae. Dorsal seta of anal lobe present.

Distribution. Japan (Ishigaki Island and Iriomote Island); China (Fujian, Guangdong, Hainan, Guangxi, Yunnan).

Yaeprimus tropicus **comb. n.**

Lunditendipes tropicum Harrison 2000: 224

Material examined (all observed by Helen Barber–James, confirmed with authors by the shared photos).

Holotype (CCA. 40G). M, Zimbabwe: Lower of Lundi River, 21°20′ S 32°15′ E, 25.iv.1962, A.D. Harrison; two paratype (GEN. 265AL; GEN. 268AL), Zimbabwe: Ndumu Game Rivers, KwaZulu–Natal, 26°53′ S 32°18′ E, 19.xi.1959, A.D. Harrison.

This species has been described by Harrison [4]: some emendations and additional characters are given here.

Male (n = 4) (Figure 8)

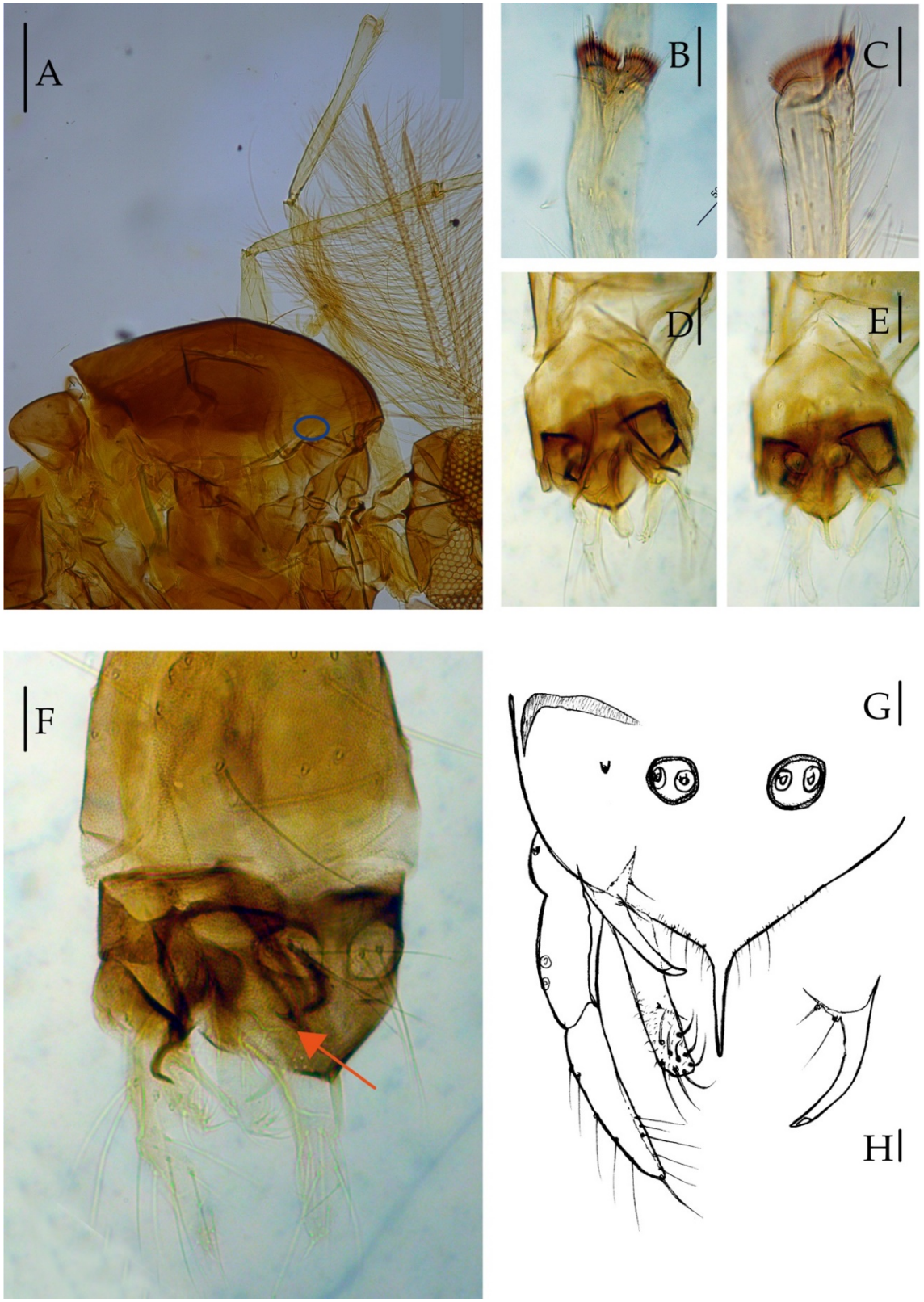

Figure 8. *Yaeprimus tropicus* **comb. n.**, male. (**A**) Thorax, head, lateral view; (**B**) mid tibia; (**C**) hind tibia; (**D**) hypopygium, ventral view; (**E**), hypopygium, dorsal view; (**G**), hypopygium, illustration, dorsal view; (**H**) superior volsella. Scale bar: A, 200 µm; B–F, 100 µm. A, (**F**) Holotype (CCA. 40G]); B–C: Paratype (GEN. 268AL); D–E, G: Paratype (GEN. 265AL).

AR 1.2–1.5, LR 2.1–2.3; thorax (Figure 8A) slight hump, without scutal tubercle, small pale humeral pit present. Mid (Figure 8B) and hind tibia (Figure 8C) with two separated combs, the small combs with long–hooked spurs, the large comb without spur in the mid tibia, with 1–2 outstanding straight spurs in the hind tibia. Pulvilli present. Abdomen II–VIII with two regular rows of setae, T VII (Figure 8D–F) slightly tapered anteriorly. Location of anal tergite median setae (Figure 8D–G) as that in *Y. isigaabeus*, two pairs of strong setae arising from the heavily pigmented areas, with variation one side two setae, another side three. Apart from those two pigmented areas, an isolated additional seta may present, arising directly from the cuticle. Superior volsella basal (Figure 8H) with two inner basal setae arising from tubercles, digitus bare, with a weak ventro–lateral ledge apically. Gonostylus is not reduced, normal (Figure 8D–G).

Remarks. *Y. tropicus* was characteristic by having a normal gonostylus and a weak tergal band.

Distribution. Zimbabwe (Lundi River); South Africa (KwaZulu–Natal).

4. Discussion

It is a great challenge to include *Y. tropicus* and *Y. balteatus* into a single genus since the two species show great divergence comparing to species *Y. isigaabeus*, especially in the often-diagnostic tibial comb pattern. Although molecular results well support (BS = 100, PP = 1) the great affinity between *Y. balteatus* and *Y. isigaabeus* (see red clade), the monotype of *Yaeprimus* could not be validated until the availability of molecular data of *Y. tropicus*. Here, the reasons why we allocate the three species into one genera are as follows. *Y. isigaabeus* and *Y. balteatus* lack tergite bands and bear a relatively short gonostylus, whereas *Y. tropicus* has a weak tergite band and normal gonostylus. The divergence is distinct yet can be also observed in other genera as well. For example, *Pontomyia* Edwards also contain two kinds of gonostylus, reduced in *P. oceana* Tokunaga, while, normal in other two species [36]. More examples can also be found in *Chironomus* (*C. crassiforceps* Kieffer) [10], *Polypedilum* (*P. minimus* Lin et al.) [37], Riethia (*R. phengari* Cranston) [16], *Sticotochironomus* (*S. crassiforceps* Kieffer) [10] and *Orthocladius* v. d. Wulp (*O. brevistylus* Yamamoto, Yamamoto et Tang) [38]. Actually, the shortened gonostylus has been assumed to relate to the convergent mating behavior since a range of species sharing this character has been found in some extremely habitats, such as marine, karst cave and alpine fauna [39]. Variation on tergite IX band from normal to absent can be treated as a continuously varying trait in a single genus because such divergence can also be found in *Apedilum* [40–42], *Paralauterborniella* [8] and *Beardius* [43].

The conical scale bearing a slender apical spur in the fore tibia is an important character state allowing us to allocate the three species into one genus, but the differences in mid and hind tibial combs are noteworthy. The two different patterns of tibial comb in *Yaeprimus* seem to represent two different evolutional trends. The pattern of fused comb with one curved spur will go to some non–core *Microtendipes* group such as *Nilodosis* Kieffer and *Kribiocosmus* Kieffer. Separated combs with 1–2 hooked spurs are typical in the core members of *Microtendipes* group and in the *Polypedilum* complex. Normally, species bearing two different kinds of tibial combs cannot be allocated into one genus, but some special cases can be also found in *Parachironomus* Lenz [6] and *Synendotendipes* Grodhaus [44].

The scutal tubercle can also be treated as a synapomorphy. That continual variation can also be found in some tanypods, like *Procladius* Skuse [45], *Coffmania* Hazra and Chaudhuri [46], and some orthoclads, such as *Parakiefferiella* Thienemann [47] and *Rheosmittia* Brundin [48], and also in *Demicryptochironomus* Lenz [49].

Our molecular analysis indicates that *Yaeprimus* is sister to *Imparipecten* and distant from the *Microtendipes* group. *Yaeprimus* shares similar characters with *Imparipecten,* such as the superior volsella formed as a digitus in male adults, alternate apically-located Lauterborn organ in the larval antenna, and pattern of pupal taeniae of T V–VIII is 4, 4, 4, 4, yet the two genera can be easily separated in all life stages. The conflict between Yamamoto's hypothesis and our molecular analysis is likely a result of some subjective weighting of some morphological characters, such as female genitalia and larval antenna. Actually, some emphasized characters by Yamamoto and Yamamoto are common in a broader range of genera.

In larvae, alternate Lauterborn organs in *Yaeprimus* share the synapomorphy with most members in the *Microtendipes* group. But this trend is not constrained to this group, as similar Lauterborn organ pattern can be also found in *Polypedilum nubifer* group [50], *Imparipecten* [51] and *Sticotochironomus*. Yamamoto and Yamamoto [2] misinterpreted that larva bears a five-segmented antenna with two large Lauterborn organs on segment two which led them to regard it as an apomorphic condition.

Pupa of *Yaeprimus* shows apparent similarity to *Paralauterborniella* both in tergal spinulation and taeniae pattern. The two genera mainly differ in the condition of cephalic tubercle, which is absent in *Yaeprimus* while present in *Paralauterborniella* [9]. In this case, other pupal characters should be evaluated to balance the conflict between molecular analysis and morphology.

Meanwhile, we should notice that the position of clade *Yaeprimus* plus *Imparipecten* is unstable in both trees, which may be caused by an insufficient sample. Given the morphological divergence between *Imparipecten* and *Yaeprimus*, we hypothesized that there were still some other unknown genera showing great affinity with the above clade, linking the two genera and establishing their position within Chironomini.

In conclusion, we redefine genus *Yaeprimus* based on morphological and molecular evidence. Currently, there are three species included in the emended genus. Our molecular result supports *Yaeprimus* is close to *Imparipecten* rather than to the *Microtendipes* group, but some uncertainty remains due to limitations in sampling. To bridge the gap between morphologic and molecular results, more relevant genera are in demand for further studies.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Primers used for polymerase chain reaction amplification and sequencing.

Gene	Name	Sequence	Reference
18S rDNA	18S	CCTGAGAAACGGCTACCACATC	Whiting et al. (1997) [52]
18S rDNA	18S	GAGTCTCGTTCGTTATCGGA	Whiting et al. (1997) [52]
28S rDNA	S3660	GAGAGTTMAASAGTACGTGAAAC	Morse and Normark (2006) [53]
28S rDNA	A335	TCGGAAGGAACCAGCTACTA	Whiting et al. (1997) [52]
CAD1	54F	GTNGTNTTYCARACNGGNATGGT	Moulton and Wiegmann (2004) [54]
CAD1	405R	GCNGTRTGYTCNGGRTGRAAYTG	Moulton and Wiegmann (2004) [54]
CAD4	787F	GGDGTNACNACNGCNTGYTTYGARCC	Moulton and Wiegmann (2004) [54]
CAD4	1098R	TTNGGNAGYTGNCCNCCCAT	Moulton and Wiegmann (2004) [54]
COI-5P	LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer et al. (1994) [55]
COI-5P	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. (1994) [55]
COI-3P	S2183	CAACATTTATTTTGATTTTTTGG	Simon et al. (1994) [56]
COI-3P	A3014	TCCAATGCACTAATCTGCCATATTA	Simon et al. (1994) [56]

Table A2. List of analyzed specimens with corresponding Genbank ID/ Bold ID and accession number.

Species Name	ID	28S	COI–3P	18S	CADIV	CADI	COI–5P
Apedilum subcinctum	APED	HQ440708	HQ440869	HQ440558	HQ440416	HQ440230	N
Chironomus sp. 1	ChV1	HQ440719	HQ440883	HQ440572	HQ440428	HQ440256	N
Cladotanytarsus sp. 1	TH40	HQ440720	HQ440884	HQ440573	HQ440429	HQ440257	N
Conochironomus tobaterdecimus	TH59	HQ440724	HQ440888	HQ440577	HQ440433	HQ440261	N
Cryptochironomus sp. 1	MODOC6	HQ440729	HQ440893	HQ440582	HQ440438	HQ440266	N
Cryptotendipes sp. 1	MODOC5	HQ440730	HQ440894	HQ440583	HQ440439	HQ440267	N
Dicrotendipes peringueyanus	Dper	HQ440732	HQ440896	HQ440585	HQ440440	HQ440270	N
Harrisius sp. 1	V604	HQ440754	HQ440916	HQ440603	HQ440456	HQ440288	N
Imparipecten pictipes	N103	HQ440759	HQ440921	HQ440608	HQ440461	HQ440294	N
Imparipecten sychnacanthus	110210-01	MH131689	MH602431	N	MH602428	MH558540	N
Imparipecten sychnacanthus	110210-02	MH131690	MH602432	N	MH602429	MH558541	N
Imparipecten sychnacanthus	110210-03	MH131691	MH602433	N	MH602430	MH558542	N
Kiefferulus calligaster	KIEF2P	HQ440763	HQ440924	HQ440611	HQ440464	HQ440298	N
Lauterborniella agrayloides	ZVRA	HQ440766	HQ440927	HQ440613	HQ440467	HQ440301	N
Microtendipes sp. 1	TH02	HQ440776	HQ440937	HQ440622	N	N	N
Nilothauma sp. 1	AUNT02	HQ440782	HQ440945	HQ440629	HQ440481	HQ440316	N
Paucispinigera approximate	PAUCI	HQ440806	HQ440969	HQ440649	HQ440500	HQ440338	N
Paraborniella tonnoiri [#]	PARAB	HQ440789	HQ440952	HQ440789	HQ440485	N	N
Parachironomus sp. 1	NCA2	HQ440791	HQ440954	HQ440635	HQ440486	HQ440323	N
Paracladopelma sp. 1	FNQ9.1	HQ440793	HQ440956	HQ440637	HQ440488	HQ440325	N
Polypedilum sp. 1	SAPP1	HQ440813	HQ440977	HQ440657	HQ440506	HQ440346	N
Polypedilum sp. 2	FNQ4.2	HQ440815	HQ440979	HQ440659	HQ440508	HQ440349	N
Skusella sp. 1	TH67	HQ440831	HQ440994	HQ440672	N	HQ440367	N
Skusella sp. 2	FNQ7.22	HQ440832	HQ440995	HQ440673	HQ440525	HQ440368	N
Stenochironomus sp. 3	STENO	HQ440837	HQ441000	HQ440677	HQ440529	HQ440372	N
Tanytarsus sp. 1	V208	HQ440846	HQ441009	HQ440686	HQ440537	HQ440382	N
Xestochironomus sp. 1	CH 13.1	HQ440861	HQ441023	HQ440700	HQ440549	HQ440401	N
Xylochironomus Kakadu	AUNT04	HQ440863	HQ441025	HQ440702	HQ440551	HQ440403	N
Conochironomus nuengthai *	TANGB033-19	Y	Y	Y	N	N	Y
Endochironomus albipennis *	TANGB036-19	Y	N	Y	Y	Y	Y
Endochironomus pekanus *	TANGB014-19	Y	Y	Y	Y	Y	Y
Microtendipes tobaquintus *	TANGA014-19	Y	Y	Y	Y	Y	Y
Nilohtauma sp. 4 *	TANGB042-19	Y	Y	N	Y	N	Y
Nilothauma sp. 2 *	TANGB040-19	Y	Y	N	Y	Y	Y
Nilothauma sp. 3 *	TANGB041-19	Y	Y	N	Y	N	Y
Paralauterborniella nigrohalteralis *	CHIR_CH510	Y	Y	Y	Y	Y	N
Paratendipes albimanus *	TANGA028-19	Y	Y	Y	Y	Y	Y
Paratendipes alpinus *	TANGA035-19	Y	Y	N	Y	Y	Y
Paratendipes sp. 2 *	TANGA032-19	Y	N	Y	Y	Y	Y
Polypedilum bullum	TANGB007-19	Y	Y	Y	Y	N	Y
Polypedilum bullum *	TANGB007-19	Y	N	N	Y	Y	Y
Syendotendipes dispar *	TANGB010-19	Y	Y	Y	Y	Y	Y
Yaeprimus balteatus sp. n. *	CHCHI170-19	Y	Y	Y	Y	Y	N
Yaeprimus balteatus sp. n. *	CHCHI151-18	Y	Y	N	N	Y	N
Yaeprimus balteatus sp. n. *	CHCHI185-19	Y	N	N	N	N	N
Yaeprimus isigaabeus *	TANGB031-19	Y	Y	N	N	N	Y
Yaeprimus isigaabeus *	TANGB001-19	Y	Y	Y	Y	N	Y
Yaeprimus isigaabeus *	TANGB038-19	Y	Y	N	Y	N	Y
Yaeprimus isigaabeus *	TANGB032-19	Y	Y	Y	Y	N	N
Zavreliella cranstoni *	TANGB035-19	N	Y	Y	Y	N	Y
Zavreliella marmorata *	TANGB034-19	N	Y	Y	N	Y	Y

* sequences retrieved from Bold, 'Y' means available, 'N' means not available. Accession number of [#] sequence was renewed by Cranston for the same entries listed for *Paraborniella* as for *Paralauterborniella* in Table A1 from their work in 2012.

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