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DNA barcodes and morphology reveal unrecognized species in Chironomidae (Diptera)

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Abstract

For over a decade, DNA barcoding has proven an effective modern tool in taxonomy, evolutionary biology and biodiversity research. Many new species have been discovered and described with DNA barcodes as part of their diagnostic features. Using DNA barcodes, we uncovered a number of potential species within the *Tanytarsus curticornis* and *Tanytarsus heusdensis* species complexes (Diptera: Chironomidae) and detected morphological differences *a posteriori* that support the description of new species. Unusually large intraspecific divergence in COI *p*-distance (up to 10%) was observed for two species complexes. In total, eight species new to science are described and figured: *T. adustus* **sp. n.**, *T. heberti* **sp. n.**, *T. madeiraensis* **sp. n.**, *T. pseudoheusdensis* **sp. n.**, *T. songi* **sp. n.**, *T. thomasi* **sp. n.**, *T. tongmuensis* **sp. n.** and *T. wangi* **sp. n.**. *Tanytarsus reei* and *T. tamaoctavus* are redescribed, and *T. tusimatneous* is listed as a new junior synonym of *T. tamaduodecimus*. The diagnostic characters of the remaining species of the complexes are discussed. Keys to males and pupae are given.

Keywords

COI sequences; Tanytarsini; new species; key

ZooBank: http://zoobank.org/44BE75EA-B2E8-42F1-9ACA-709F7157969B

Introduction

Over the past decade, the use of short standardized genetic markers for species identification, so-called DNA barcoding (Hebert et al. 2003a, b), has proven effective in biodiversity assessments (Hajibabaei et al. 2016; Lee et al. 2016) and taxonomic revisions (Hausmann et al. 2016; Miller et al. 2016). DNA barcoding has aided our understanding of species community compositions, food webs and genetic variation within species (Baker et al. 2016; Littlefair & Clare 2016; Roslin & Majaneva 2016) and is an important and useful asset in biosecurity (Ashfaq & Hebert 2016;

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Hodgetts et al. 2016) and biomonitoring of freshwater ecosystems (Brodin et al. 2013; Carew et al. 2013). DNA barcodes can uncover cryptic species diversity (Macher et al. 2016; Witt et al. 2006; Yang et al. 2012), and indicate species boundaries with additional morphological and ecological data. For example, Hebert et al. (2004) discovered ten different species in the *Astraptes fulgerator* species complex when combining DNA barcodes, host plant records and larval morphology. DNA barcode have also been used efficiently to detect hidden species diversity in Chironomidae (Anderson et al. 2013; Carew et al. 2011; Lin et al. 2015; Silva & Wiedenbrug 2014; Song et al. 2016; Stur & Ekrem 2015).

The genus *Tanytarsus* van der Wulp, 1874 (Diptera: Chironomidae) is the most species-rich genus of the tribe Tanytarsini in the subfamily Chironominae with more than 400 accepted species names worldwide (Roskov Y. et al. 2017). Larvae of *Tanytarsus* are eurytopic, occurring in all types of freshwater and some even in marine or terrestrial environments (Epler et al. 2013). The genus was erected by van der Wulp (1874) with *T. signatus* (van der Wulp, 1859) as the type species, and various species groups and species have been revised over the last few decades (Cranston 2000; Ekrem 2001, 2002, 2004; Ekrem et al. 2003; Giłka & Paasivirta 2009; Glover 1973; Lindeberg 1963, 1967; Reiss & Fittkau 1971; Sanseverino 2006).

In general, morphological identification at species-level in Chironomidae relies strongly on characters of adult males, especially the hypopygium in combination with a range of morphological measurements, counts and ratios. Morphological determination of some *Tanytarsus* species groups can be extremely challenging. Additionally, there are many unknown and cryptic species in *Tanytarsus* and it is difficult to associate the immature stages with adults through rearing since it is time-consuming and not always successful. DNA barcodes can overcome issues of rearing to associate different life stages.

The *Tanytarsus curticornis* Kieffer, 1911 and *T. heusdensis* Goetghebuer, 1923 species complexes have previously been placed in the *T. chinyensis* species group (Giłka & Paasivirta 2009; Reiss & Fittkau 1971), based on morphology. However, this species group is unsupported (polyphyletic) in results from molecular phylogenetic analyses of *Tanytarsus* based on five protein coding and one ribosomal nuclear markers (Lin et al. in prep.). Therein, *Tanytarsus chinyensis*, *T. curticornis* and *T. heusdensis* are found in three different clades that are not each other's closest relatives. As a consequence, we treat species morphologically extremely similar to *T. curticornis* and *T. heusdensis* in the adult male stage as members of these species complexes, respectively. By this characterization, there are eight hitherto known species in the *T. curticornis* species complex. Six of these species are known from the Holarctic region: *Tanytarsus brundini*, Lindeberg, 1963, *T. curticornis* Kieffer, 1911, *T. ikicedeus* Sasa & Suzuki, 1999, *T. neotamaoctavus* Ree, Jeong & Nam, 2011, *T. salmelai* Giłka & Paasivirta, 2009, *T. tamaoctavus* Sasa, 1980; and two that are Afrotropical: *Tanytarsus congus* Lehmann, 1981, *T. pseudocongus* Ekrem, 1999 (Edwards 1929; Ekrem 1999, 2001; Giłka & Paasivirta 2009; Kieffer 1911; Lehmann 1981; Lindeberg 1963; Ree et al. 2011; Reiss & Fittkau 1971; Sasa 1980). The *T. heusdensis* complex includes four species occurring in the Palaearctic region: *T. heusdensis* Goetghebuer, 1923, *T. reei* Na & Bae, 2010, *T. tamaduodecimus* Sasa, 1983, *T. tusimatneous* Sasa & Suzuki, 1999 (Albu 1980; Giłka & Paasivirta 2009; Goetghebuer 1923, 1928; Lindeberg 1970; Na & Bae 2010; Reiss & Fittkau 1971; Sasa 1983; Sasa & Ichimori 1983; Sasa & Suzuki 1999a; Sasa & Suzuki 1999b; Storå 1939).

The morphological similarity between species in the *T. curticornis* and *T. heusdensis* species complexes has likely led to misidentifications and underestimation of species diversity in the past. Generally, DNA barcode data can give a good estimation of species diversity in *Tanytarsus* (Lin et al. 2015) and should, together with morphology, be used in description of closely related species. Here we review species in the *T. curticornis* and *T. heusdensis* species complexes, diagnose and describe them based on morphology and DNA barcodes, and provide keys to adult males and pupae.

Materials and methods

We examined the nominal types of *T. heusdensis*, *T. ikicedeus*, *T. manlyensis*, *T. neotamaoctavus*, *T. reei* and *T. tamaduodecimus*. Additional collections were made in Canada, China, Czech Republic, Germany and Norway, using standard insect collecting techniques such as Malaise traps, sweep nets and light traps. Adult specimens were preserved in 85% ethanol, immatures in 96% ethanol, and stored dark at 4°C before morphological and molecular analyses. Genomic DNA of most specimens was extracted from the thorax and head using Qiangen DNA Blood and Tissue Kit at the Department of Natural History, NTNU University Museum following the standard protocol, except that lysis was done overnight and the final elution volume was 100 μl. After DNA extraction, the cleared exoskeleton was mounted in Euparal on microscopy slides together with the corresponding wings, legs and antennae following the procedures outlined by Sæther (1969).

The 658 bp (barcode fragment) of the cytochrome *c* oxidase subunit 1 (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 $MgCl_2$, 0.2 μl Takara Ex Taq HS, 1 μl 10 μM of each primer, 2 μl template DNA and 14.3 μl ddH_2O. Amplification cycles were performed on a Bio-Rad C1000 Thermal Cycler and followed a program with an initial denaturation step of 95°C for 5 min, then 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 and shipped to MWG Eurofins for bidirectional sequencing using BigDye 3.1 termination. Sequences were assembled and edited using the software Sequencher 4.8 (Gene Codes Corporation). Sequence information was uploaded on BOLD 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 of *T. curticornis* and *T. heusdensis* species complexes using the Kimura 2-Parameter (K2P) model were calculated in MEGA 6 (Table S1–2). The neighbor joining tree was constructed using the K2P substitution model, 1000 bootstrap replicates and the "pairwise deletion" option for missing data in MEGA 6.

List of all species, specimens, their individual images, geo-references, primers, sequences and other relevant laboratory data of all sequenced specimens can be seen online in the publicly accessible datasets "*Tanytarsus curticornis* species complex [DS-TANYSC]", DOI: dx.doi.org/10.5883/DS-TANYSC, and "*Tanytarsus heusdensis* complex [DS-HEUSDEN]", DOI: dx.doi.org/10.5883/DS-HEUSDEN in the Barcode of Life Data Systems (BOLD) (Ratnasingham & Hebert 2007, 2013).

The morphological terminology and abbreviations follow Sæther (1980) except for the vannal fold (here called postcubitus), "lamellate setae" of median volsella (here called "lamellae"), "sensillae basiconicae" on the anal point (here called "spinulae") and "filamentous setae (LS)" in pupal exuviae (here called "taeniae") (Langton 1994). Measurements are given as ranges followed by the mean, when four or more specimens are measured, followed by the number of specimens measured (n) in parentheses. Digital photography were taken with a resolution of 300 dpi using a Leica DFC420 camera mounted on a Leica DM6000 B compound microscope using bright field or Nomarski DIC light settings and the software Leica Application Suite 4.8. The types from China are deposited at the College of Life and Sciences, Nankai University, Tianjin, China, and the remaining types of new species described herein are deposited at the Department of Natural History, NTNU University Museum, Trondheim, Norway or The Canadian National Collection of Insects, Arachnids & Nematodes.

Institute acronyms used in the text include: ANIC, Australian National Insect Collection, Australia; BDN, Department of Biology, Nankai University, now College of Life Science, Nankai University, Tianjin, China; BMNH, Natural History Museum, London, England; CNC, Canadian National Collection of Insects, Arachnids & Nematodes, Ottawa, Ontario, Canada; KU, Entomological Research Institute, Korea University, Seoul, Korea; LUOMUS, Finnish Museum of Natural History, Helsinki, Finland; NSMT, National Museum of Nature and Science, Tokyo, Japan; NTNU-VM, Department of Natural History, NTNU University Museum, Trondheim, Norway; RBINS, Royal Belgian Institute of Natural Sciences, Bruxelles, Belgium; UMSP, University of Minnesota Insect Collection, Department of Entomology, University of Minnesota, United States of America; YU, College of Medicine, Yonsei University, Seoul, Korea; ZSM, Zoologische Staatssammlung München, Munich, Germany; ZMBN, Natural History Collections, University Museum of Bergen, Norway.

Results

DNA barcode analyses

The neighbor joining tree (Figure 1) based on available COI DNA barcodes of species in the *T. curticornis* species complex revealed nine distinct genetic clusters, of which six could not be associated morphologically or genetically to any previously described species. The neighbor joining tree (Figure 2) based on available COI DNA barcodes of five *T. heusdensis* species complex presents five distinct genetic clusters suggesting two species new to science. In the *T. curticornis* species complex, the minimum interspecific genetic distance is 8% between *T. tongmuensis* **sp. n.** and *T. wangi* **sp. n.**, and the maximum intraspecific genetic distance 10% in *T. thomasi* **sp. n.**. In the

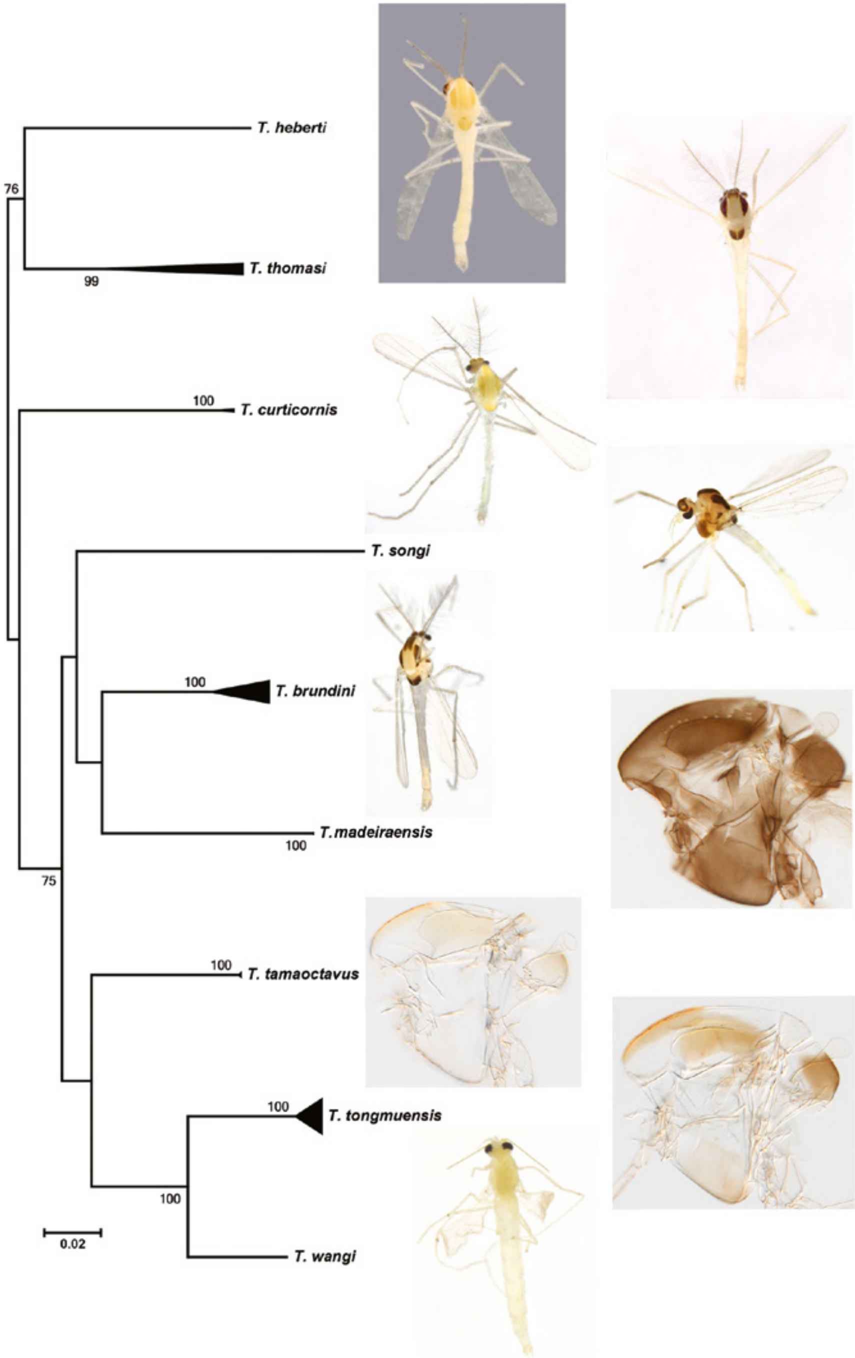

Fig. 1. Neighbor joining tree for nine morphospecies of the *Tanytarsus curticornis* species complex based on K2P distances in DNA barcodes. Numbers on branches represent bootstrap support (>70%) based on 1000 replicates; scale equals K2P genetic distance; figures show the full body or thorax of adult males of each corresponding morphospecies.

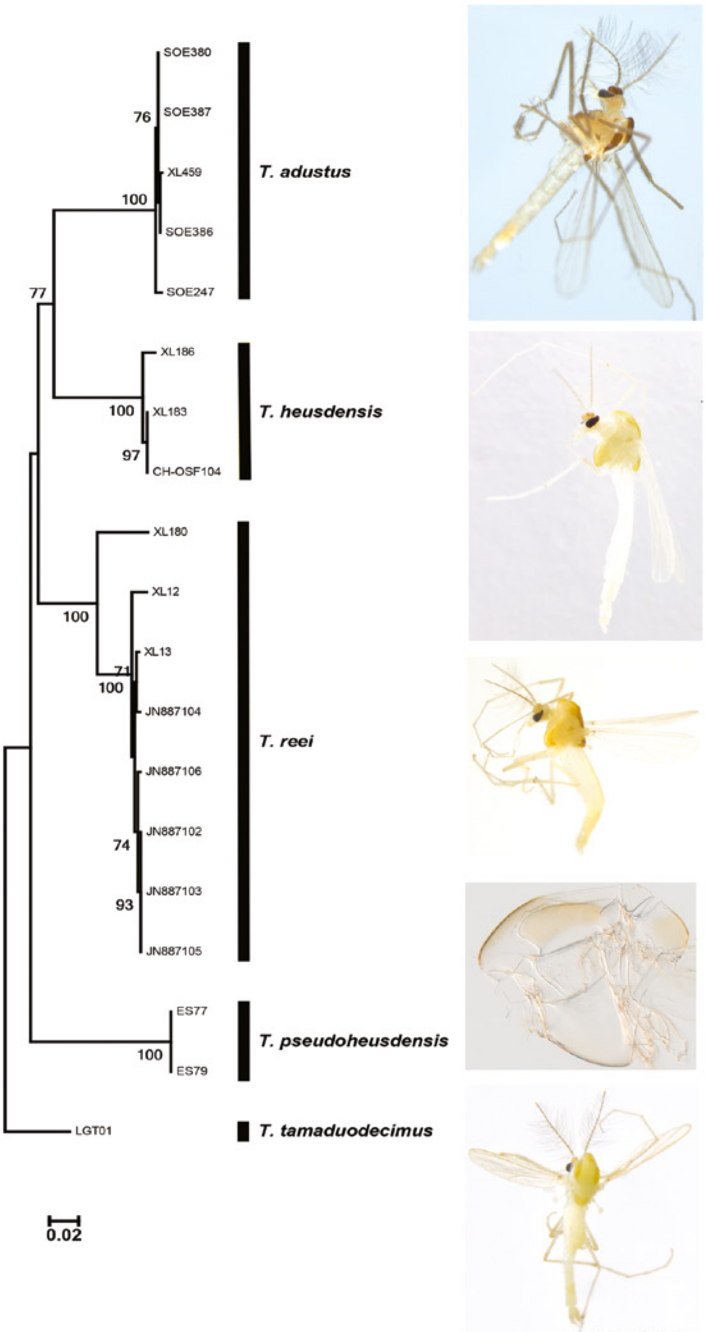

Fig. 2. Neighbor joining tree for five species of the *Tanytarsus heusdensis* species complex based on K2P distances in DNA barcodes. Numbers on branches represent bootstrap support (>70%) based on 1000 replicates; scale equals K2P genetic distance; figures show the full body or thorax of adult males of each corresponding morphospecies.

T. heusdensis species complex, the minimum interspecific genetic distance is 16% between *T. heusdensis* and *T. adustus* **sp. n.**, and the maximum intraspecific genetic distance is 8% in *T. reei* between the Chinese and German populations. Due to the observed deep intraspecific divergence, we extended the dataset with multiple nuclear genes, on a subset of taxa to explore the species boundaries within these two species complexes using different analytical tools. The results are generally consistent with the pattern observed with DNA barcodes and completely in the accordance with the morphological concepts and named species described here (Lin et al. in prep.).

Taxonomy

The *Tanytarsus curticornis* species complex

The species of the *T. curticornis* complex are grouped together based on a number of morphological characters in the adult males: the anal tergal bands are of V-type and widely separated medially; a few or no small median setae are present near the base of anal point; a few spinulae are present between anal crests; superior volsella is generally oval, often with straight or concave median margin, bearing two anteromedian and 4–5 dorsal setae; digitus extends beyond the inner margin of the superior volsella, always with apical pear-shaped lobe (Figure 16C); small seta present on stem of digitus (except in *T. neotamaoctavus*); stem of median volsella short.

Descriptions

Tanytarsus brundini Lindeberg, 1963

Figures 3–4

Tanytarsus brundini Lindeberg, 1963: 127, figure 7, 10; Reiss & Fittkau 1971: 98, figures 4–5; Giłka & Paasivirta 2009: 35, figures 1, 5–9, 19, 22, 25. Holotype ♂ (LUOMUS), Finland, Puruvesi, 9.VI.1962, leg. B. Lindeberg. [Not examined]

Material examined

Belgium: 1♂ (RBINS), Chiny, 17–22.VIII.1933, leg. M. Goetghebuer. Czech Republic: 1♂ (NTNU-VM: 148235, BOLD Sample ID: XL148), Southern Bohemia, České Budějovice, near River Vltava, 48.977°N, 14.470°E, 387 m a.s.l., 17.VIII.2014, Sweep net, leg. X.L. Lin. China: 2♂♂ (BDN: 21476, 21507), Xinjiang Uyghur Autonomous Region, Aketao County, Kalakule Lake, 18.VIII.2002, leg. N. Tang. Norway: 2♂♂ (NTNU-VM: 136337, BOLD Sample ID: Finnmark239; NTNU-VM: 136338, BOLD Sample ID: Finnmark240), Finnmark, Lebesby, Eastorjavri, Lake at outflow, 70.4427°N, 27.3485°E, 260 m a.s.l., 28.VII.2010, leg. T. Ekrem; 1♂ (ZMBN: chi23847, BOLD Sample ID: To137), Hordaland, Dalseid, Vaksdal, Bolstadfjorden, 60.6552°N, 5.8038°E, 100 m a.s.l., 31.V.2003, Sweep net, leg. E. Stur & T. Ekrem; 1♂ (NTNU-VM: 200130, BOLD Sample ID: TRD-CH137), Sør-Trøndelag, Klæbu, Selbusjøen, near Bjørkly, 63.2744°N, 10.5613°E, 157 m a.s.l., 25.IX.2014, leg. E. Stur. Portugal: 1♂ (UMSP), Vita de Ponte River & Reservoir, 17.IV.1984, leg. E.J. Fittkau. Ukraine: 6♂♂ (NTNU-VM: 148236, BOLD Sample ID: XL402; NTNU-VM:

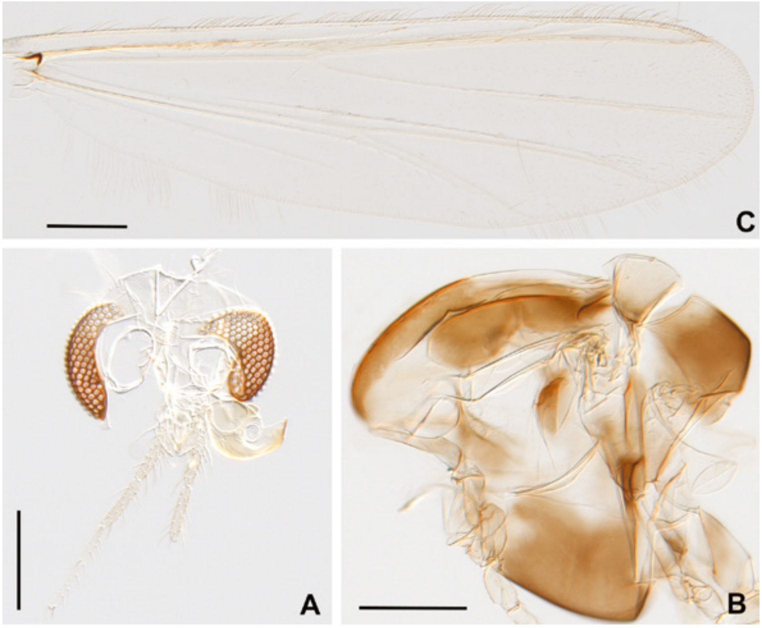

Fig. 3. *Tanytarsus brundini*, male: A) head; B) thorax; C) wing. Scale bar = 200 µm.

148237, BOLD Sample ID: XL403; NTNU-VM: 148238, BOLD Sample ID: XL404; NTNU-VM: 148239, BOLD Sample ID: XL405; NTNU-VM: 148240, BOLD Sample ID: XL407), Krym, Sevastopol, near Chornaya River, 44.4861°N, 33.8136°E, 253 m a.s.l., 12.X.2013, Sweep net, leg. V. Baranov.

Diagnosis

Tanytarsus brundini can be separated from other *Tanytarsus* species by the following combination of characters: anal tergal bands of male hypopygium of V-type and widely separated medially; anal point coniform, tapering to rounded apex, with 4–5 spinulae between anal crests; superior volsella oval with concave inner margin; digitus with pear-shaped apical lobe; median volsella very short, lamellae not reaching tip of digitus; gonostylus straight or slightly curved, with more or less parallel margins tapering to widely rounded apex; larval antennal pedestal with a medially curved, acute spur; pupal segment V–VIII with 1, 1, 3, 5 lateral taeniae respectively; chaetae densely distributed on apical half of pupal thoracic organ.

Remarks

Tanytarsus brundini is regarded as widely distributed in the western Palaearctic region. However, most records should be reevaluated considering the results presented in this review.

Distribution

Northwest China, Europe.

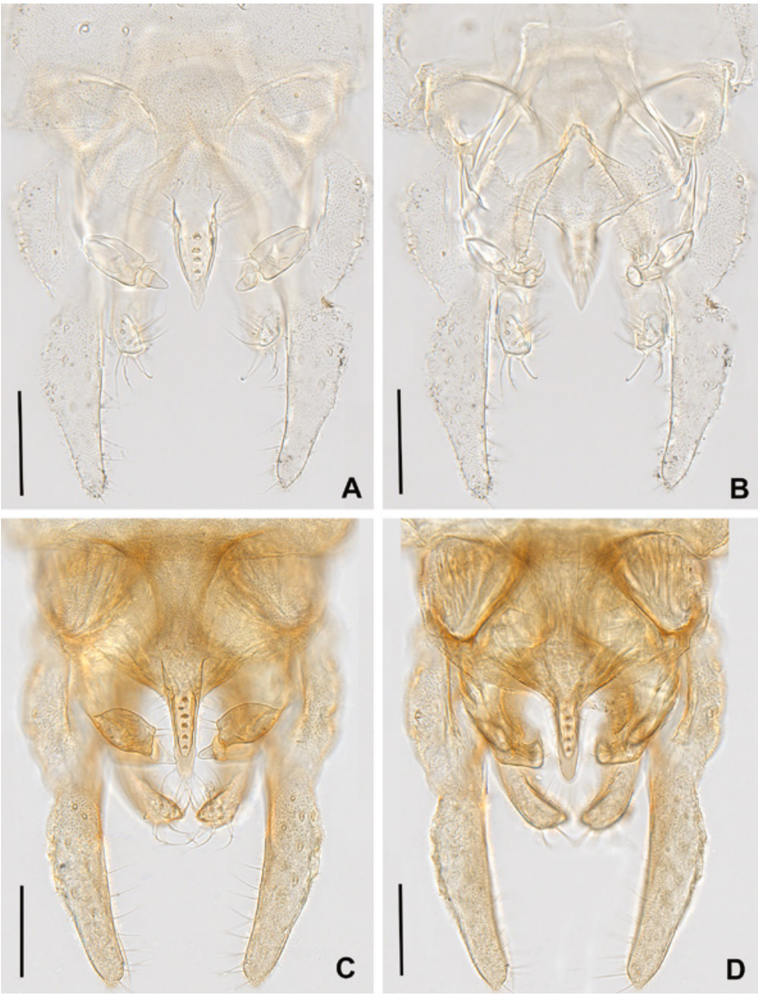

Fig. 4. *Tanytarsus brundini*, male: A) hypopygium dorsal view (Chiny, Belgium); B) hypopygium ventral view (Chiny, Belgium); C) hypopygium dorsal view (Portugal); D) hypopygium ventral view (Portugal). Scale bar = 50 μm.

Tanytarsus congus Lehmann, 1981

Tanytarsus congus Lehmann, 1981: 48, figures 88–91; Ekrem 2001: 8, figures 5–6. Holotype ♂ (ZSM) Zaire, Kinsangani, Simisimi-stream, 24.III.1975. [Not examined]

Diagnosis

Tanytarsus congus can be separated from other *Tanytarsus* species by the following combination of characters in the adult male: AR 0.60–0.80; $LR_1 > 3.30$; spinulae in single row between well-developed anal crests; superior volsella oval with dorsolateral, small patch of microtrichia, two apical setae where one is sitting on small ventral projection, digitus with swollen apex reaching beyond superior volsella at its median posterior

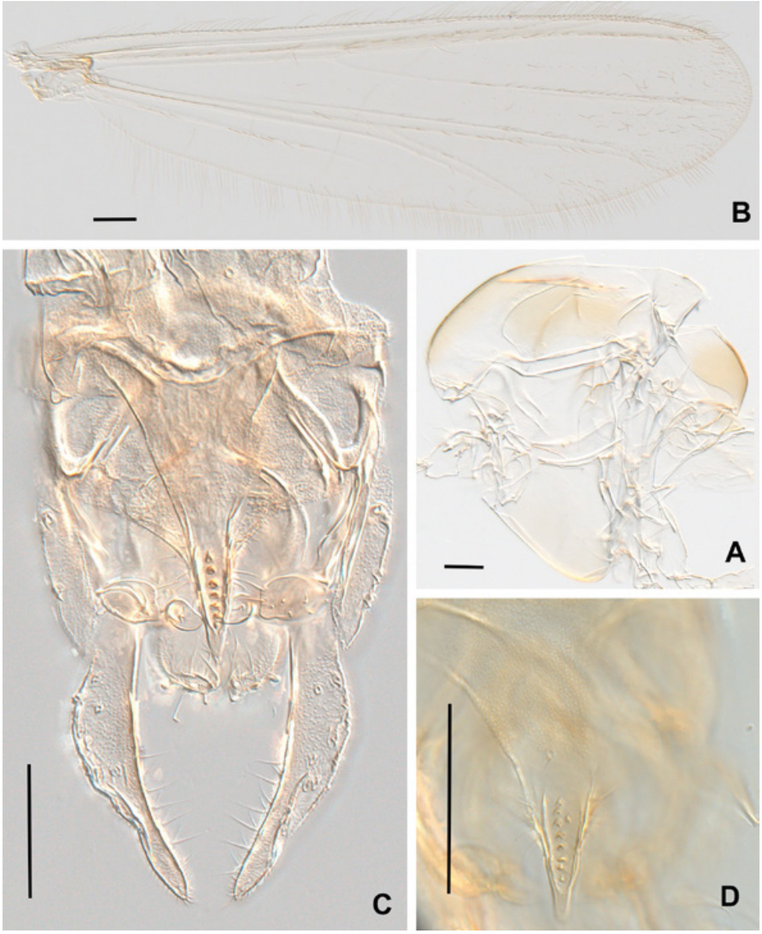

Fig. 5. *Tanytarsus curticornis*, male: A) thorax, scale bar = 100 µm; B) wing, scale bar = 100 µm; C) hypopygium (Trondheim, Norway); D) partial anal tergite showing one median seta present (Aust-Agder, Norway).

margin, carrying one seta placed at c. 1/2 length of digitus; median volsella relatively short with two setiform and three distal pectinate lamellae; inferior volsella S-shaped [For further description see Ekrem (2001) and Lehmann (1981)].

Distribution

Ghana, Senegal, Democratic Republic of the Congo.

Tanytarsus curticornis Kieffer, 1911

Figure 5

Tanytarsus curticornis Kieffer, 1911: 52; Lindeberg 1963: 127, figures 5, 8; Reiss & Fittkau 1971: 100, figure 7; Giłka & Paasivirta 2009: 38, figures 10–14, 20, 23, 26.

Tanytarsus (*Tanytarsus*) *curticornis* Kieffer, 1911: Edwards 1929: 415.

Material examined

Norway: 1♂ (ZMBN: chi23826, BOLD Sample ID: To82), Aust-Agder, Valle, Flåni, Sandnes, 59.1457°N, 7.5394°E, 273 m a.s.l., 29.VI.2001, leg. T. Ekrem; 1♂ (NTNU-VM: 148241, BOLD Sample ID: XL99), Sør-Trøndelag, Trondheim, Lian Lake, 63.400°N, 10.317°E, 219m, 31.VIII.2014, Sweep net, leg. X.L. Lin.

Diagnosis

Tanytarsus curticornis can be separated from other *Tanytarsus* species by the following combination of characters: pupal segment V-VIII with 2, 2, 3, 5 lateral taeniae respectively; chaetae distributed scarcely on apical half of thoracic horn; adult male ground colour of thorax, scutellum, halteres (Figure 5A), legs and abdomen yellowish green; antennal pedicel, tentorium, scutal stripes, postnotum and sternum yellowish green to pale brown; wing membrane pale, with C, M and radial veins slightly darker; frontal tubercles usually absent; third palpomere shorter than fourth; wing membrane under M_{3+4}, Cu_1 and An partially free of macrotrichia (Figure 5B); 1/4 proximal section of R_{4+5}, proximal half of Cu and neighboring false vein bare; gonostylus with slight constriction in distal part or regularly tapered to slender apex; anal tergite with single strong basilateral seta on each side; median setae absent or rarely one present (one Norwegian specimen); anal point with 4–10 spinulae, with blunt, widely rounded or slightly abrupt apex; superior volsella oval with straight median margin (Figure 5C); pear-shaped apical lobe of digitus roundish, broadly conical, stout in comparison with relatively small superior volsella; median volsella very short, inner margin of coxite above median volsella more or less straight; inferior volsella short, with broadly rounded apex.

Remarks

One of the Norwegian specimens (BOLD Sample ID: To82) possesses one median tergite seta in the adult male (Figure 5D). This is regarded as an aberrant or rare feature for *T. curticornis*.

Distribution

Palaearctic region.

Tanytarsus heberti **sp. n.**

Figures 6–8

ZooBank: http://zoobank.org/55AC9FBC-5A5E-4844-B66A-4B8B2084E46C

Type material

Holotype ♂ (NTNU-VM: 148242, BOLD Sample ID: CHIR_CH342), Canada, Manitoba, Churchill, 12 km S Churchill, Goose Creek Marina, 58.6610°N, 94.1650°W, 5 m a.s.l., 17.VII.2007, Malaise trap, leg. T. Ekrem & E. Stur. Paratypes:

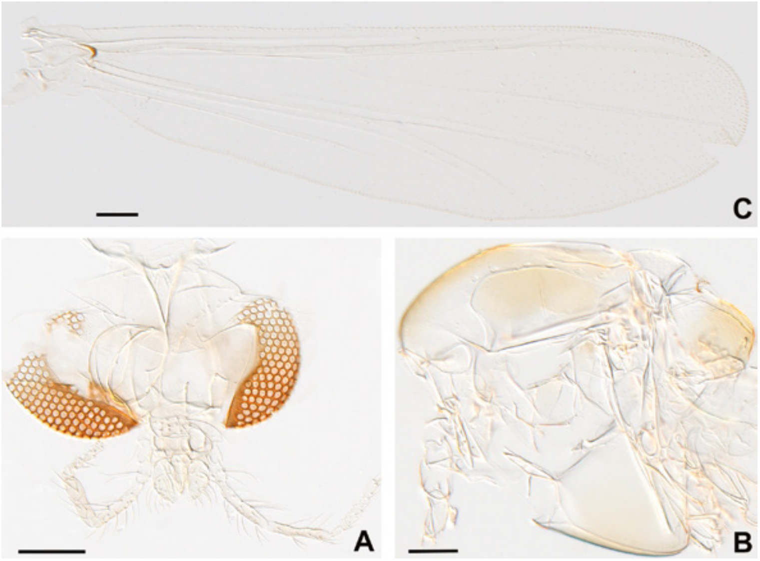

Fig. 6. *Tanytarsus heberti* **sp. n.**, holotype male: A) head; B) thorax; C) wing. Scale bar = 100 μm.

Fig. 7. *Tanytarsus heberti* **sp. n.**, holotype male: A) hypopygium dorsal view; B) hypopygium ventral view. Scale bar = 50 μm.

1♂ (CNC, BOLD Sample ID: BIOUG18216-G06), 2♀♀ (CNC, BOLD Sample ID: BIOUG18225-G07; CNC, BOLD Sample ID: BIOUG18226-C03), Canada, Manitoba, Wapusk National Park, 58.723°N, 93.46°W, 2 m a.s.l., 27.VII.2014, leg. D. Iles.

Diagnosis

The adult male can be distinguished from known species of *Tanytarsus* by the following combination of characters: body mostly yellow with pale brown stripes on scutum, postnotum, preepisternum (Figure 6B); AR 0.71; wing vein Cu with 0–2 setae,

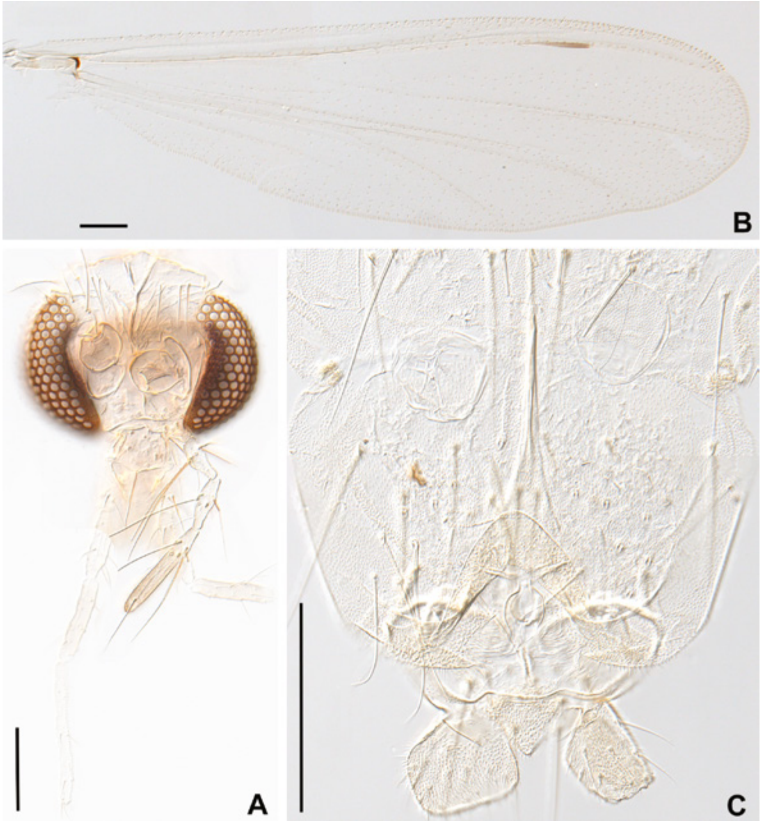

Fig. 8. *Tanytarsus heberti* **sp. n.**, paratype female: A) head; B) wing; C) genitalia. Scale bar = 100 μm.

cell m with one seta; anal point bearing six spinulae placed in regular row between anal crests; superior volsella roundish slightly elongated with straight inner margin, bearing two anteromedian and 4–5 dorsomedian setae; digitus stout, including one pear-shaped apical lobe with rounded apex and enlarged tubercle bearing a single seta; stem of median volsella short, 4–5 μm long, located above superior volsella, bearing three falciform lamellae; gonostylus comparatively thin, broadest at middle, tapering to a blunt apex.

Etymology

Named after Paul D. N. Hebert, for his continued support of Chironomidae DNA barcoding; noun in genitive case.

Description

Adult male (n = 2). Total length 2.38–2.47 mm. Wing length 1.60–1.63 mm. Total length/wing length 1.48–1.52.

Colouration. Head, legs and abdomen yellow; antenna brown. Thorax ground colour yellow with light brown stripes anteriorly on scutum, laterally under parapsidal suture, postnotum and on preepisternum.

Head (Figure 6A). Antenna with 13 flagellomeres, ultimate flagellomere 350 μm long. AR 0.71. Frontal tubercles absent. Temporal setae 8–9. Clypeus with 10–13 setae. Tentorium 125 μm long, 28 μm wide. Palpomere lengths (in μm): 33–35, 35–43, 93–103, 98–100, 103. Third palpomere shorter than fourth palpomere, bearing 2 sensilla clavata distally.

Thorax (Figure 6B). Dc 6–7; Ac 6–7; Pa 1; Scts 4. Halteres with 3–4 setae.

Wing (Figure 6C). VR 1.29–1.39. Brachiolum with one seta, Sc bare, R with 14–17 setae, R_1 with 16–17 setae, R_{4+5} with 15–26 setae, M_{1+2} with 44–45 setae, M_{3+4} with 19–26 setae, false vein with 33–39 setae, Cu with 0–2 setae, Cu_1 with 16–18 setae, PCu with 4–14 setae, An with 23–27 setae, remaining veins bare. Cell r_{4+5} with c. 115–120 setae, m with one seta, m_{1+2} with c. 90–95 setae, m_{3+4} with 46–53 setae, cu+an with 6–45 setae, remaining cells bare. Anal lobe strongly reduced.

Legs. Fore leg bearing single tibial spur, 20–23 μm long. Combs of mid tibia 21 μm wide with 25 μm long spur, and 23 μm wide with 20 μm long spur; combs of hind tibia 15 μm wide with 28 μm long spur, 11 μm wide with 20 μm long spur. Tarsomere 1 of mid leg with 2 sensilla chaetica distally. Lengths (in μm) and proportions of legs as in Table 1.

Hypopygium (Figure 7). Tergite IX 88–95 μm long, with two median setae and small microtrichia-free area around base of anal point. Lateral tooth absent. Anal tergal bands of V-type, widely separated, ending at mid-length of tergite. Anal point 48–63 μm long, tapering to narrow, rounded apex, microtrichia absent between anal crests, four lateral setae on each side of anal point; six spinulae in regular row between anal crests. Transverse sternapodeme 45–50 μm long. Phallapodeme 80–85 μm long. Gonocoxite 123–130 μm long. Gonostylus 100–105 μm long, curved inwards, comparatively thin, broadest at middle, tapering to a narrowly rounded apex. Superior volsella roundish, slightly elongated, with straight inner margin, bearing two anteromedian and 4–5 dorsomedian setae. Digitus stout, with two lobes, one apical, pear-shaped with rounded apex, one enlarged tubercle bearing single seta. Stem of median volsella short, 4–5 μm long, located anterior to superior volsella, bearing three apparently falciform lamellae, 10 μm long. Inferior volsella slightly curved, 70–75 μm long, bearing 10–12 strong apical setae. HR 1.17–1.30, HV 2.35–2.38.

Adult female (n = 2). Total length 1.68–1.72 mm. Wing length 1.45–1.63 mm. Total length/wing length 1.03–1.19.

Table 1. Lengths (in μm) and proportions of legs for *Tanytarsus heberti* **sp. n.**, adult male

	fe	**ti**	**ta_1**	**ta_2**	**ta_3**	**ta_4**	**ta_5**	**LR**	**BV**	**SV**	**BR**
p_1	660	380	-	-	-	-	-	-	-	-	-
p_2	770	710	-	-	-	-	-	-	-	-	-
p_3	670	530	290	170	110	85	65	0.55	3.47	4.14	3.50

Colouration. As male.
Head (Figure 8A). Antenna with 4 flagellomeres, flagellomere length (in µm): 70–75, 53–55, 50–65, 98–128. AR 0.57–0.66. Frontal tubercles absent. Temporal setae 5–7. Clypeus with 10–14 setae. Tentorium 100 µm (2) long, 13–15 µm wide. Palpomere lengths (in µm): 25–38, 30–38, 65–88, 100 (1), 168 (1).
Wing (Figure 8B). VR 1.22–1.27. Brachiolum with 1 seta, Sc bare, R with 15–16 setae, R_1 with 16–18 setae, R_{4+5} with 28–37 setae, M_{1+2} with 34–38 setae, M_{3+4} with 39–43 setae, false vein with 62–70 setae, Cu with 13–20 setae, Cu_1 with 23–55 setae, PCu with 28–54 setae, An with 22–40 setae, remaining veins bare. Cell r_{4+5} with c. 180–200 setae, m with 7–9 setae, m_{1+2} with c. 110–130 setae, m_{3+4} with 50–60 setae, cu+an with 70–80 setae, remaining cells bare. Anal lobe strongly reduced.
Thorax. Dc 7–10; Ac 6–8; Pa 1; Scts 4. Halteres with 4 setae.
Legs. Tibial armature as in male (all tarsomeres broken).
Genitalia (Figure 8C). Tergite IX slightly triangular; sternite VIII with 14–16 setae; vaginal floor large, covering about half of vaginal opening ventrally; gonapophysis VIII single lobe with long posteromedially directed microtrichia; gonocoxapodeme strongly curved; coxosternapodeme well-developed with obvious anterior and posterior lobes. Notum including rami 135–158 µm long, notum alone c. 50–88 µm long. Seminal capsules semi-circular, 43–60 µm long, 43–45 µm wide, with 105–130 µm long spermathecal ducts. Postgenital plate triangular. Cercus 53–55 µm long, with 13–18 setae.
Immatures unknown.

Remarks

The new species resembles *T. brundini* in the adult male, but can be separated from this species by having less setae on the wing vein Cu, apparently falciform lamellae of the median volsella, and more than 20% divergence in partial COI sequences (Figure 1).

Tanytarsus ikicedeus **Sasa & Suzuki, 1999**

Figure 9

Tanytarsus ikicedeus Sasa & Suzuki, 1999: 149, figures 4a–m; Yamamoto & Yamamoto 2014: 354. Holotype ♂ (NSMT Type no. 357: 18), Japan, Nagasaki Prefecture, Iki Island, Satofure, 27.III.1998, leg. H. Suzuki. [Examined]

Diagnosis

Tanytarsus ikicedeus can be separated from other *Tanytarsus* species by the following combination of characters: scutal stripes and postnotum yellowish brown, other scutal portions and scutellum pale, abdomen and legs yellow; frontal tubercles absent; AR 0.90–1.00; third palpomere shorter than fourth; wing membrane covered with macrotrichia from ¼ of wing length to tip; LR_1 2.09–2.29; tarsomere 1 of mid leg with 4 sensilla chaetica; anal tergite with 2 median setae near base of anal point; anal point triangular, with nipple on apex; spinulae in single row between well-developed anal

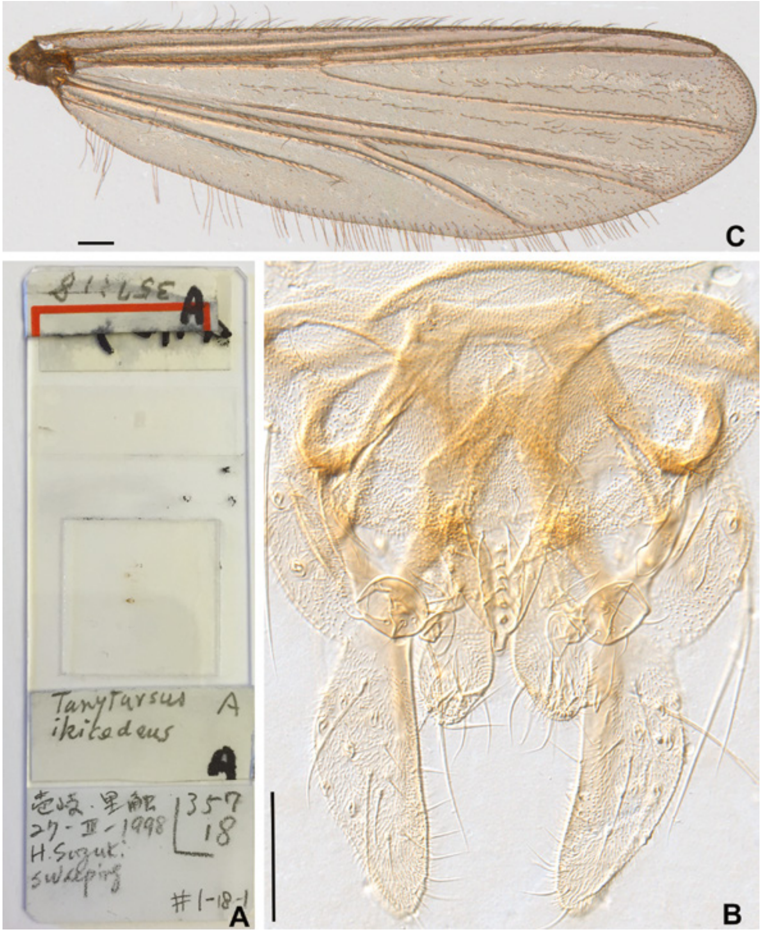

Fig. 9. *Tanytarsus ikicedeus*, holotype male: A) microscope slide; B) wing, dry mounted, scale bar = 50 μm; C) hypopygium, scale bar = 50 μm.

crests; superior volsella oval, bearing 2 anteromedian and 4 dorsal setae; digitus stout with pear-shaped apical lobe, 1 digitus-seta located on cylindrical tubercle at base; median volsella small, with one pectinate lamella; gonostylus with broadly rounded apex.

Distribution

Japan.

Tanytarsus madeiraensis **sp. n.**

Figures 10–13

ZooBank: http://zoobank.org/7B3793A2-2D6A-4891-AEDC-3B8E1AFAADBF

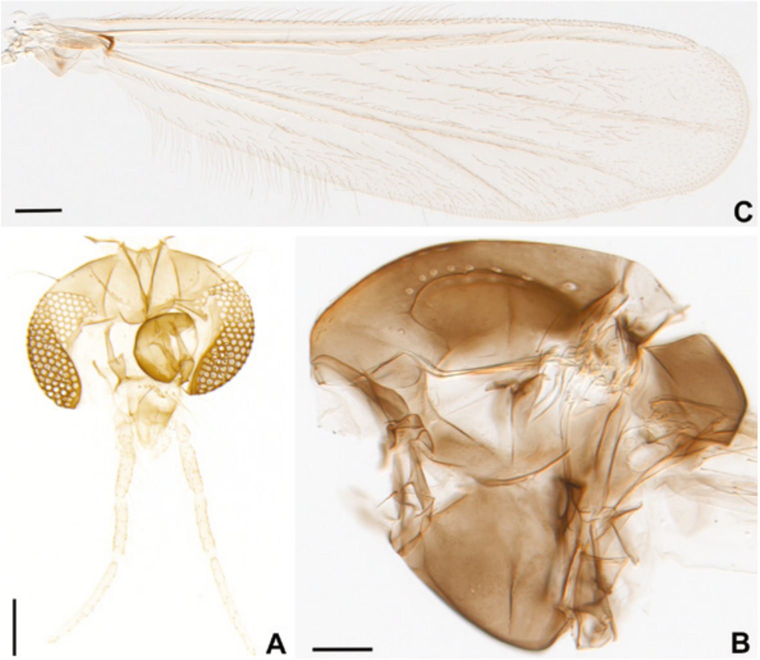

Fig. 10. *Tanytarsus madeiraensis* **sp. n.**, A) holotype male head; B) paratype male thorax; C) holotype male wing. Scale bar = 100 μm.

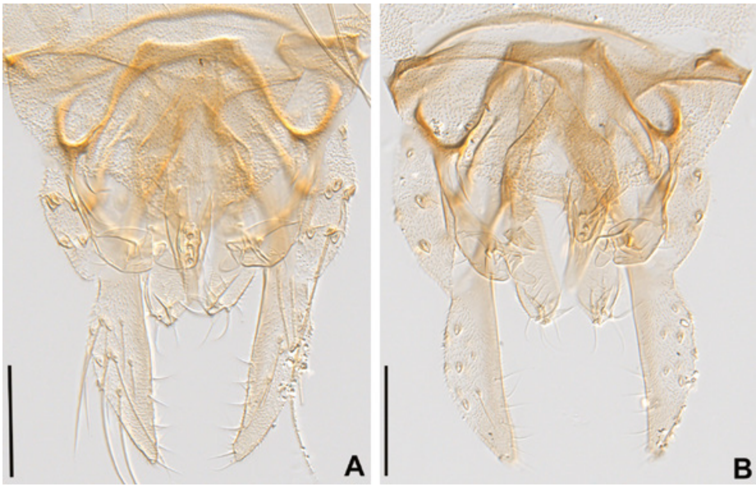

Fig. 11. *Tanytarsus madeiraensis* **sp. n.**, male: A) hypopygium dorsal view; B) hypopygium ventral view showing median volsella and digitus. Scale bar = 50 μm.

Type material

Holotype ♂, Pe, L (NTNU-VM: 145314, BOLD Sample ID: MA19), Portugal: Madeira, Rabaçal, Levada pond, 32.7340°N, 17.0580°W, 1463 m a.s.l., 06.VIII.2006, Rearing, leg. T. Ekrem. Paratype ♂, Pe, L (NTNU-VM: 145313, BOLD Sample ID: MA18) as holotype.

Fig. 12. *Tanytarsus madeiraensis* **sp. n.**, paratype larval head capsule, scale bar = 100 µm.

Diagnosis

Tanytarsus madeiraensis can be distinguished from known species of *Tanytarsus* by the following combination of characters in the adult male: head, thorax, legs and abdomen of adult male entirely dark brown to black (Figure 10B); AR 0.47–0.49; frontal tubercles absent; third palpomere shorter than fourth; wing membrane covered with macrotrichia; LR_1 1.91–2.09; tarsomere 1 of mid leg without sensilla chaetica; three median setae placed near the base of anal point; anal tergal bands of V-type, well separated, ending at mid-length of tergite; digitus stout with pear-shaped apical lobe, digitus-seta located on cylindrical tubercle at base of digitus; stem of median volsella short and cylindrical, located anterior to superior volsella, with one simple lamella. Pupal thoracic horn slender, arising from spherical base, tapered, terminating in fine apical section, chaetae distributed on distal 2/3; spines of tergites III–VI in anterior, small, elongated patches; segments V–VIII with 1, 2, 3, 5 lateral taeniae respectively; anal lobe with two long taeniate dorsal setae; fringe with 22–23 uniserial taeniae; posterolateral comb of segment VIII 20–23 µm wide, with 4–6 large outermost spines and 6–7 small inner spines; male genital sacs extend beyond anal lobe. Larval mental and mandibular teeth brown-black; AHR 0.20–0.30; AAR 0.58; antenna shorter than

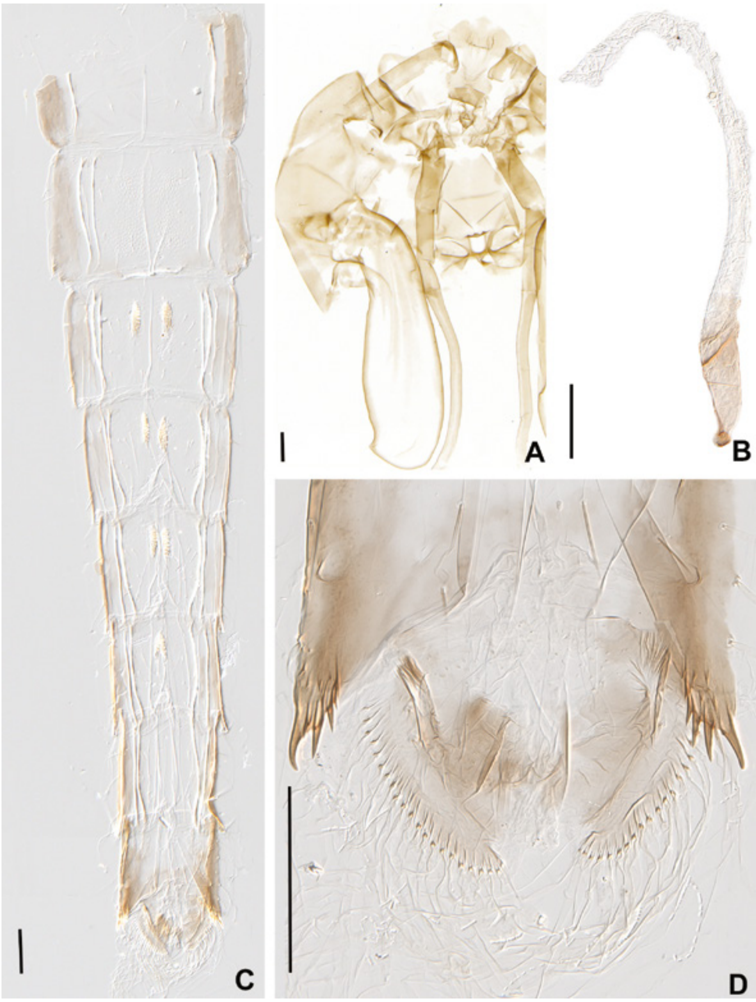

Fig. 13. *Tanytarsus madeiraensis* **sp. n.**, holotype pupal exuviae: A) cephalothorax; B) thoracic horn; C) abdomen; D) segment VIII showing posterolateral comb. Scale bar = 100 µm.

head length, 5-segmented, only first and second antennal segments well sclerotized, AR 2.23; LOR 3.43; MVR 0.90; labrum with SI pectinate and chaetae plumose; SII with small points, on pedestal; clypeal seta S3 long, simple; premandible with 4 teeth in addition to lateral spine; premandibular brush well-developed.

Etymology

Named after the type locality, Madeira; adjective in nominative case.

Description

Adult male (n = 2). Total length 2.12–2.54 mm. Wing length 1.37–1.38 mm. Total length/wing length 1.54–1.84.

Colouration. Head, thorax, legs and abdomen entirely dark brown to black.
Head (Figure 10A). Antenna with 13 flagellomeres, ultimate flagellomere 235–240 μm long. AR 0.47–0.49. Frontal tubercles absent. Temporal setae 8–11. Clypeus with 14–16 setae. Tentorium 100–105 μm long, 25–30 μm wide. Palpomere lengths (in μm): 27–28, 33–43, 90–95, 105–115, 175–180. Third palpomere with 2 sensilla clavata distally.
Thorax (Figure 10B). Dc 8–9; Ac 8–9; Pa 1. Scts 4. Halteres with 4 setae.
Wing (Figure 10C). VR 1.25–1.30. Brachiolum with 1 seta, Sc bare, R with 21–27 setae, R_1 with 16–17 setae, R_{4+5} with 34–45 setae, M_{1+2} with 44–60 setae, M_{3+4} with 23 setae, false vein with 60–63 setae, Cu with 18–20 setae, Cu_1 with 14 setae, PCu with 44–52 setae, An with 31–32 setae, remaining veins bare. Cell r_{4+5} with c. 160–180 setae, m with 10–13 setae, m_{1+2} with c. 100–130 setae, m_{3+4} with 50–60 setae, cu+an with 50–60 setae, remaining cells bare. Anal lobe strongly reduced.
Legs. Fore leg bearing single tibial spur, 30–35 μm long. Combs of mid tibia 15–18 μm wide with 19–28 μm long spur, and 16–18 μm wide with 13–15 μm long spur; combs of hind tibia 20–30 μm wide with 30 μm long spur, 18–20 μm wide with 20 μm long spur. Tarsomere 1 of mid leg without sensilla chaetica. Pulvilli absent. Lengths (in μm) and proportions of legs as in Table 2.
Hypopygium (Figure 11). Tergite IX 75–77 μm long, with three median setae at base of anal point. Lateral tooth absent. Anal tergal bands of V-type, well separated, ending at mid-length of tergite. Anal point 45–53 μm long, with 3–4 lateral setae on each side, with blunt apex; three spinulae placed in regular row between anal crests. Transverse sternapodeme 40 μm long. Phallapodeme 58–70 μm long. Gonocoxite 103–113 μm long. Gonostylus with straight inner margin, 78–85 μm long, comparatively broad, tapering to pointed apex. Superior volsella roundish with straight to concave median margin, bearing 2 anteromedian and 4 dorsal setae. Digitus stout with pear-shaped apical lobe, digitus-seta located on cylindrical tubercle at base of digitus. Stem of median volsella short and cylindrical, located anterior to superior volsella, 12–14 μm long, with one simple lamella. Inferior volsella slightly curved, 60–63 μm long, with 9–10 apical setae. HR 1.31–1.32, HV 2.72–2.99.
Larva (n = 2, head capsules). Head Capsule (Figure 12). Length 265–565 μm, width 215–470 μm, length/width 1.20–1.23; mental and mandibular teeth dark brown. Antennal pedestal 80–113 μm long, 46–78 μm wide, with a well-developed spur 28–40

Table 2. Lengths (in μm) and proportions of legs for *Tanytarsus madeiraensis* **sp. n.**, adult male

	fe	**ti**	**ta_1**	**ta_2**	**ta_3**	**ta_4**
p_1	650–670	340–350	670–710	340–350	270–280	230
p_2	650	460–500	300	140–150	100	60
p_3	680	600–650	410–430	250	210–220	130–140

	ta_5	**LR**	**BV**	**SV**	**BR**
p_1	100	1.91–2.09	1.79	1.39–1.52	2.50–2.72
p_2	50–60	0.60–0.65	3.81–4.14	3.70–3.83	3.57–3.82
p_3	70–80	0.63–0.72	2.55–2.56	2.98–3.24	2.70–2.81

µm long, 6–10 µm wide. AHR 0.20–0.30; AAR 0.58. Antenna shorter than head length, 5-segmented, only first and second antennal segments well sclerotized. Antennal segment length (in µm): 138, 32, 17, 10, 3; AR 2.23. Antennal blade 48 µm long. Lauterborn organ (LO) stems + stylus 103 µm long; LOR 3.43. Mandible 95–175 µm long, 37–68 µm wide, 1 ventral apical tooth, 3 ventral inner teeth, 1 dorsal, pale apical tooth present; two outer mandibular setae present; seta subdentalis thick and curved, 32–53 µm long, reaching beyond apex of dorsal tooth; seta interna well-developed with 4 main branches. Mentum 68–115 µm wide, with 11 teeth; median tooth with small lateral notches; ventromental plate 75–128 µm wide, MVR 0.90. Labrum with S I pectinate and chaetae plumose; S II with small points, on pedestal; clypeal seta S3 long, simple. Labral lamella pectinate. Pecten epipharyngis consisting of three plumose lobes. Premandible 46–83 µm long, with 4 teeth in addition to lateral spine; premandibular brush well-developed. Maxilla with two long and one short lacinial chaetae, palp normally developed, postoccipital margin darkly pigmented, postoccipital plate well-developed, pale.

Pupal exuviae (n = 2). Length 2.80–2.81 mm, pale-light yellow, with darker pigmentation on cephalothorax, basal ¼ of thoracic horn and laterally on abdomen.

Cephalothorax (Figures 13A–B). Length 780–780 µm. Cephalic area with thin frontal setae, 63–88 µm long. Antepronotals comprising one median and one lateral, each weakly developed, taeniate setae, 25–45 µm long; three precorneals similarly shaped, arranged in triangle, one stouter and darker than remaining two, 25–58 µm long. Thoracic horn c. 545–550 µm long, 44–45 µm wide, arising from spherical base, tapered, terminating to fine apical section, chaetae distributed on distal 2/3. Two relatively closely situated pairs of Dc, 155–170 µm between pairs. Dc 1 and 2 short (10–15 µm), Dc 3 and 4 longer (18–35 µm), one thick, one thin. Nose of wing sheath well-developed.

Abdomen (Figures 13C–D). Tergite I bare. Shagreen on tergite II as in Figure 13C; pedes spurii B on tergite II weakly developed; hook row 125–130 µm long. Spines of tergites III–VI 3–13 µm long, in anterior, elongated patches. Segment I with 2 D setae; segment II with 3 D, 3–4 V and 3 L setae; segment III with 5 D, 3–4 V and 3 L setae; segment IV with 5 D, 4 V and 3 L setae; segment V with 5 D, 4 V, 2 L setae and 1, 70–75 µm long lateral taeniae; segment VI with 5 D, 4 V, 2 L setae and 2, 48–52 µm long lateral taeniae; segment VII with 5 D, 3 V, 1 L setae and 3, 53–70 µm long lateral taeniae; segment VIII with 1 D, 1 V and 5, 63–78 µm long lateral taeniae; anal lobe with two 50–60 µm long taeniate dorsal setae; fringe with 22–23 uniserial taeniae. Posterolateral comb (Figure 13D) of segment VIII 20–23 µm wide, with 4–6 large outermost spines and 6–7 small inner spines. Genital sacs of male extending beyond anal lobe.

Female unknown.

Remarks

The new species resembles *T. salmelai* in the adult male, but can be separated from this species by the absence of sensilla chaetica on tarsomere 1 of mid leg, dense setation on the wing membrane and relative straight inferior volsella. The new species also resembles *T. brundini* in the adult male, but can be separated from this species by the darker body colour, lower AR, and more than 13% mean divergence in partial COI sequences (Figure 1).

Tanytarsus neotamaoctavus Ree, Jeong & Nam, 2011

Figure 14

Tanytarsus neotamaoctavus Ree, Jeong & Nam, 2011: 254, figure 7. Holotype ♂ (YU no. CH-6758), Korea, Jeollabuk-do, Muju-gun, Muju-eup, Dangsan-ri, 22.V.2009, leg. K.Y. Jeong [Not examined]; Paratypes 2♂♂ (YU no. RCH-6757, RCH-6763) as holotype [Examined].

Diagnosis

Tanytarsus neotamaoctavus can be separated from other *Tanytarsus* species by the following combination of characters in the adult male: body mostly yellow with darker scutal stripes and post notum; AR 0.80–0.82; third palpomere longer than fourth; LR_1 = 2.51–2.61; anal point robust with narrow, rounded apex; digitus twisted, without digitus-seta; median volsella tiny (absent in the original description).

Remarks

Based on the original description (Ree et al. 2011), *T. neotamaoctavus* is closely related to *T. tamaoctavus* in the shape of hypopygium, e.g. median volsella absent. The

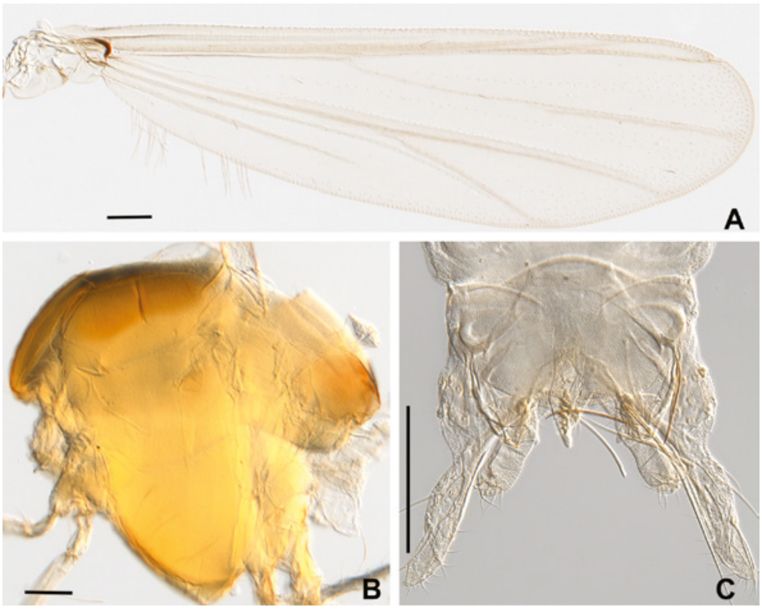

Fig. 14. *Tanytarsus neotamaoctavus*, paratype male: A) wing; B) thorax; C) hypopygium. Scale bar = 100 µm.

two species can be separated from each other by the slightly higher AR (0.80–0.82) and stout anal point in *T. neotamaoctavus*, opposed to the lower AR (0.65) and slender anal point in *T. tamaoctavus*. After examination of the paratypes of *T. neotamaoctavus*, we discovered the presence of a tiny median volsella hidden by the superior volsella. The median volsella was noted as absent in the original description; digitus seta absent.

Distribution

South Korea.

Tanytarsus pseudocongus Ekrem, 1999

Tanytarsus pseudocongus Ekrem, 1999: 56, figure 2; Ekrem 2001: 22, figures 30–31. Holotype ♂ (ZMBN: no.305), Ghana, Western Region, Ankasa Game Production Reserve, Malaise trap, 7.–11.XII.1993. [Not examined]

Diagnosis

Tanytarsus pseudocongus can be separated from other *Tanytarsus* species by the following combination of characters: AR < 0.45; LR_1 < 2.90; spinulae in single row between well-developed anal crests; superior volsella oval with a few microtrichia between 4–5 dorsal setae and 2 median setae where 1 is sitting on a small ventral projection; digitus with a swollen apex reaching beyond superior volsella at its median posterior margin, carrying 1 seta basally; median volsella relatively short with 3 distal, feathery lamellae in addition to 2 simple lamellae (Ekrem 1999).

Distribution

Ghana, Nigeria.

Tanytarsus salmelai Giłka & Paasivirta 2009

Tanytarsus salmelai Giłka & Paasivirta, 2009: 32, figures 3, 4, 15–18, 21, 24, 27. Holotype ♂ Finland, Arcto-Alpine ecoregion, Aksonjunni, 36 km south of Nuorgam, Utsjoki, 2.VII.2007, leg. J. Salmela. [Not examined].

Diagnosis

Tanytarsus salmelai can be separated from other *Tanytarsus* species by the following combination of characters: colour dark, with wing 1.15–1.50 mm long and AR 0.44–0.51; frontal tubercles always present; third palpomere longer than fourth; Anal lobe strongly reduced; membrane brownish, sparse macrotrichia apically; LR_1 1.52–1.59; tarsomere 1 of mid leg with 3 sensilla chaetica; anal point slender, with narrowed and strongly elongated apex; superior volsella oval with straight median margin, bearing two anteromedian setae; digitus stout, with apical pear-shaped lobe and single seta at base; stem of median volsella short, with group of short pectinate lamellae; inferior volsella parallel-sided, with square apex and darkly pigmented dorsomedian, subapical ridge.

Distribution

Finland.

Tanytarsus songi **sp. n.**

Figures 15–16

ZooBank: http://zoobank.org/94A5BA86-6A64-47BA-9AD7-4C005E3AE41C

Type material

Holotype ♂ (BDN & BOLD Sample ID: XL222), China: Hebei, Handan, Shexian, Kuangmenkou, 36.518°N, 113.703°E, 670 m a.s.l., 14.X.2014, Sweep net, leg. C. Song.

Diagnosis

The adult male can be distinguished from known species of *Tanytarsus* by the following combination of characters: body mostly brown with darker scutal stripes, postnotum,

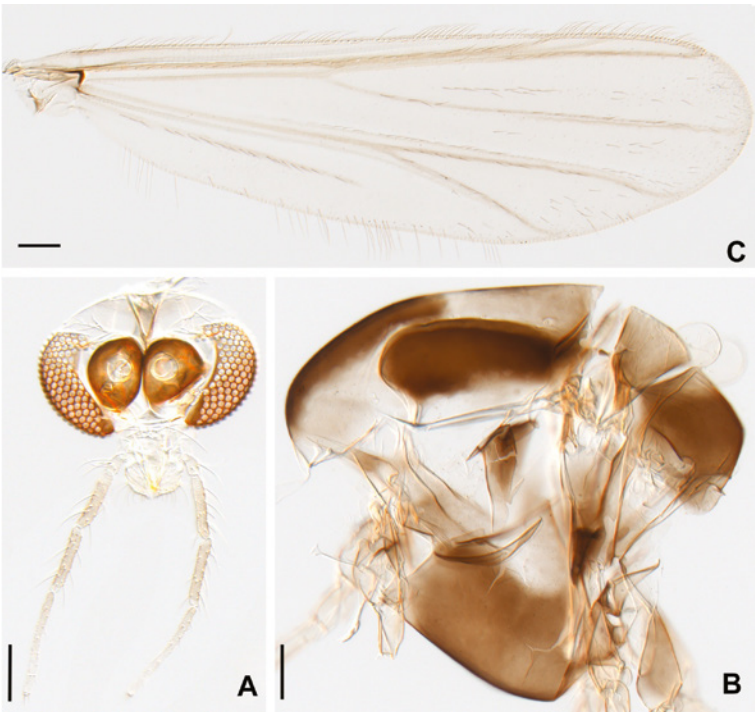

Fig. 15. *Tanytarsus songi* **sp. n.**, holotype male: A) head; B) thorax; C) wing. Scale bar = 100 µm.

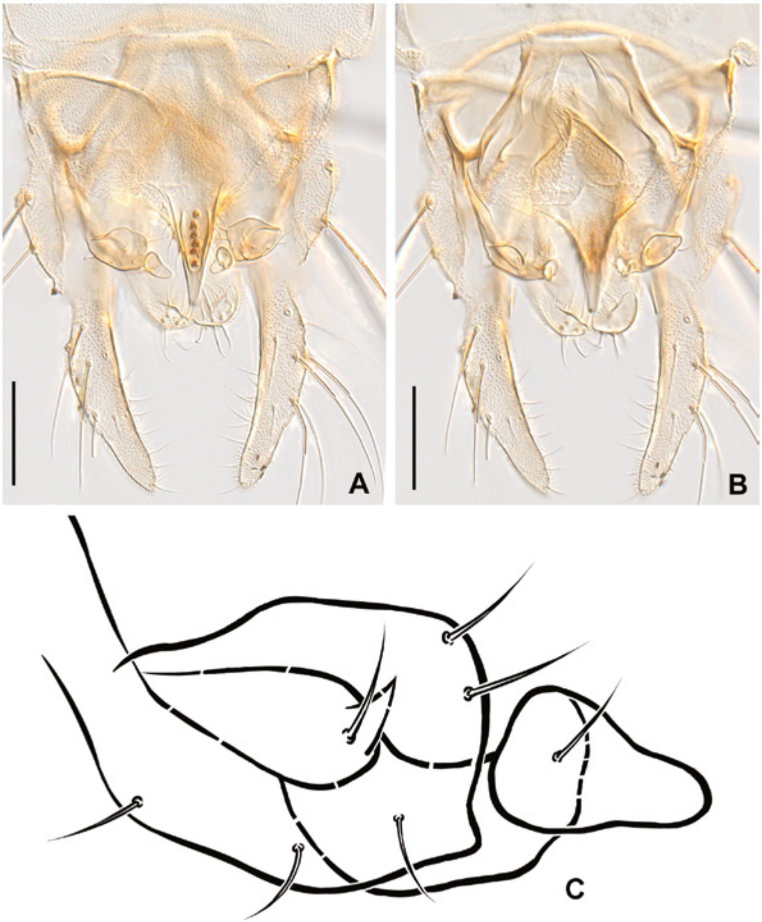

Fig. 16. *Tanytarsus songi* **sp. n.**, holotype male: A) hypopygium dorsal view, scale bar = 50 µm; B) hypopygium ventral view, scale bar = 50 µm; C) superior volsella with digitus.

epimeron II, median anepisternum II and preepisternum (Figure 15B); frontal tubercles absent; third palpomere shorter than fourth; wing vein Cu and cell m bare, vein PCu with 1–3 setae; tarsomere 1 of mid leg with four sensilla chaetica distally; anal tergite without median seta; superior volsella almost square with straight median margin, bearing two anteromedian and four dorsal setae; digitus stout, with apical pear-shaped lobe, digitus-seta located on cylindrical tubercle at base of digitus; stem of median volsella short, located underneath superior volsella, bearing group of short pectinate lamellae; gonostylus comparatively narrow, slightly curved inwards, with rounded apex.

Etymology

Named after Chao Song, the collector of the material; noun in genitive case.

Description

Adult male (n = 1). Total length 2.68 mm. Wing length 1.60 mm. Total length/wing length 1.72.
Colouration. Thorax ground colour brown with dark brown stripes anteriorly on scutum, laterally under parapsidal suture, postnotum and on preepisternum; dark brown median anepisternum II, epimeron II. Head and legs brown. Abdomen yellow.
Head (Figure 15A). Antenna lost. Frontal tubercles absent. Temporal setae 8. Clypeus with ten setae. Tentorium 115 μm long, 28 μm wide. Palpomere lengths (in μm): 45, 35, 108, 118, 180.
Thorax (Figure 15B). Dc 7; Ac 12; Pa 1; Scts 4. Halteres with three setae.
Wing (Figure 15C). VR 1.20. Brachiolum with 1 seta, Sc bare, R with 21 setae, R_1 with 22 setae, R_{4+5} with 33 setae, M_{1+2} with 50 setae, M_{3+4} with 26 setae, false vein with 49 setae, Cu_1 with 22 setae, PCu with three setae, An with 23 setae, remaining veins bare. Cell r_{4+5} with c. 140 setae, m_{1+2} with c. 60 setae, m_{3+4} with 48 setae, cu+an with 36 setae, remaining cells bare. Anal lobe strongly reduced.
Legs. Fore leg bearing single tibial spur, 18 μm long. Combs of mid tibia 13 μm wide with 23 μm long spur, and 10 μm wide with 18 μm long spur; combs of hind tibia 25 μm wide with 25 μm long spur, 13 μm wide with 23 μm long spur. Tarsomere 1 of mid leg with four sensilla chaetica distally. Lengths (in μm) and proportions of legs as in Table 3.
Hypopygium (Figure 16). Tergite IX 65 μm long. Median seta absent. Small microtrichia-free area surrounding base of anal point. Lateral tooth absent. Anal tergite bands of V-type, widely separated medially, ending at half length of tergite. Anal point 50 μm long, triangular, tapering to rounded apex, four lateral setae on each side; six spinulae placed in regular row between anal crests. Transverse sternapodeme 45 μm long, with slightly developed oral projections. Phallapodeme 75 μm long. Gonocoxite 110 μm long. Gonostylus slightly curved, 95 μm long, with rounded apex. Superior volsella almost square, with straight median margin, slightly elongated and medially directed, bearing two anteromedian and four dorsal setae. Digitus (Figure 16C) stout, with apical pear-shaped lobe, digitus-seta located on cylindrical tubercle at base of digitus. Stem of median volsella short, located underneath superior volsella, bearing group of short pectinate lamellae. Inferior volsella slightly curved, 55 μm long, bearing ten strong apical setae. HR 1.16, HV 2.82.
Female and immatures unknown.

Table 3. Lengths (in μm) and proportions of legs for *Tanytarsus songi* **sp. n.**, adult male

	fe	ti	ta_1	ta_2	ta_3	ta_4	ta_5	LR	BV	SV	BR
p_1	690	340	-	-	-	-	-	-	-	-	-
p_2	700	500	290	140	100	70	50	0.58	4.13	4.14	5.71
p_3	770	650	-	-	-	-	-	-	-	-	-

Remarks

The new species resembles *T. curticornis* in the adult male, but can be separated from this species by having less setae on the wing veins Cu and PCu and more than 16% K2P-divergence in partial COI sequences (Figure 1). Previous records of *T. curticornis* from the eastern Palaearctic region might be *T. songi*.

***Tanytarsus tamaoctavus* Sasa, 1980**

Figures 17–18

Tanytarsus tamaoctavus Sasa, 1980: 23, figures 23–25; Na 2004: 30, figure 17; Na et al. 2010: 61, 62; Sasa & Kikuchi 1995: 51, 138; Yamamoto & Yamamoto 2014: 359. Holotype ♂ (NSMT Type no. A 43:51), Japan, Tokyo, Minamiasakawa River, 17.VIII.1979, leg. M. Sasa. [Type material lost, not examined]

Material examined

China: 1♂ (BDN & BOLD Sample ID: XL423), Guangdong, Guangzhou, Conghua, Guifeng Mountain, 22.5416°N, 113.0120°E, 68 m a.s.l., 29.V.2015, Light trap, leg. H.Q. Tang; 1♂ (BDN: GSF3), Guangdong, Guangzhou, Conghua, Guifeng Mountain, 22.5416°N, 113.0120°E, 68 m a.s.l., 19.IV.2015, Sweep net, leg. H.Q.

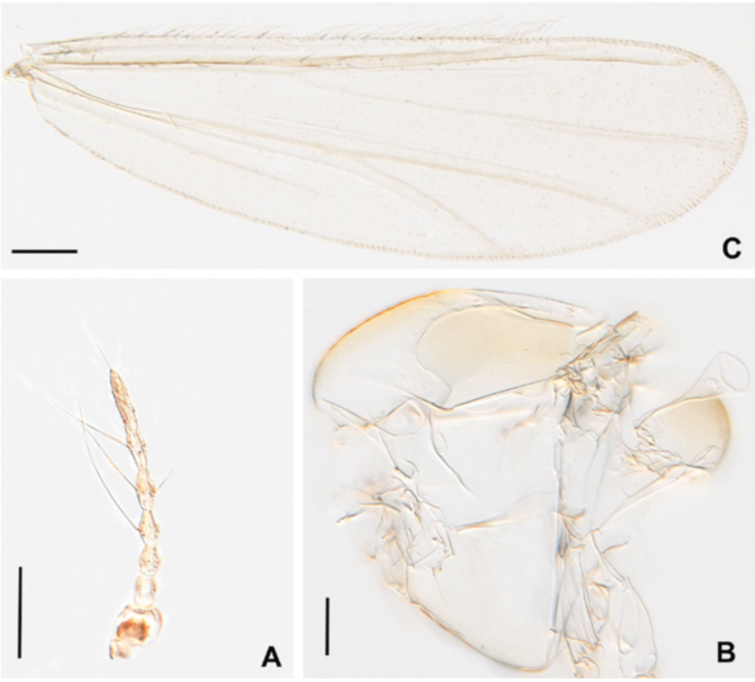

Fig. 17. *Tanytarsus tamaoctavus*, male from China: A) gynandromorph female antenna; B) thorax; C) wing. Scale bar = 100 µm.

Fig. 18. *Tanytarsus tamaoctavus*, male from Guangdong, China: A) hypopygium dorsal view; B) hypopygium ventral view showing median volsella and digitus-seta. Scale bar = 50 μm.

Tang; 2♂♂ (BDN & BOLD Sample ID: XL351; BDN: G5A9), Zhejiang, Jinhua, Pan'an, Dapanshan National Nature Reserve, 28.9728°N, 120.5250°E, 700 m a.s.l., 19.VII.2012, leg. X.L. Lin; 2♂♂ (BDN & BOLD Sample ID: XL287; BDN: I4A144), Zhejiang, Quzhou, Kaihua, Suzhuangzheng, 29.1749°N, 118.1340°E, 300 m a.s.l., 15–17.IV.2011, Light trap, leg. X.L. Lin. Korea: 1♂ (YU no.A-354), Gyeonggi-do, Gapyeong, Jeokmok-ri, Garim, Gapyeongcheon, 5.VI.2004, Light trap, leg. Y.J. Bae.

Diagnosis

Tanytarsus tamaoctavus can be separated from other *Tanytarsus* species by the following combination of characters in the adult male: body mostly yellow with brown stripes on scutum, postnotum and preepisternum (Figure 17B); AR 0.62–0.70; frontal tubercles absent; third palpomere equal or slightly longer than fourth; wing membrane covered with macrotrichia; LR_1 2.37–2.75; tarsomere 1 of mid leg with 2–4 sensilla chaetica (16 in female); two median setae near the base of anal point; anal tergal bands of V-type, well separated, ending at mid-length of tergite; anal point robust compared to size of hypopygium, with blunt apex; superior volsella roundish, bearing 2 antero-median and 4–5 dorsal setae; digitus stout with pear-shaped apical lobe, digitus-seta located on cylindrical tubercle at base of digitus; median volsella tiny and hyaline (absent in the original description). Pupa can be separated by having a long, narrow thoracic horn, tapered to fine apical section, chaetae distributed on distal 2/3; tergite II with a pair of spine patches surrounded by shagreen; small spines of tergite III–VI in elongated patches anteriorly; segments V–VIII with 0, 1, 3, 5 lateral taeniae; fringe with 30–34 uniserial taeniae; posterolateral comb of segment VIII bearing one large spine and 8–12 small accessory spines; male genital sacs extending beyond anal lobe.

Description

Adult male (n = 4). Total length 2.08–2.50 mm. Wing length 1.13–1.30 mm. Total length/wing length 1.78–1.82.

Colouration. Head, legs and abdomen yellow. Thorax ground colour yellow with pale brown stripes anteriorly on scutum, laterally under parapsidal suture, basally on scutellum, dorsally on postnotum and on preepisternum.

Head. Antenna usually with 13 flagellomeres, ultimate flagellomere 260–340 μm long (gynandromorphy with female-like antenna with 5 flagellomeres found in Zhejiang, China, Figure 17A; normally developed *T. tamaoctavus* female antenna has 4 flagellomeres). AR 0.60–0.69. Frontal tubercles 3–5 μm long, 3–4 μm wide. Temporal setae 5–9. Clypeus with 9–20 setae. Tentorium 105–118 μm long, 23–28 μm wide. Palpomere lengths (in μm): 25–30, 25–25, 78–110, 78–108, 138–190. Third palpomere equal or a little longer than fourth palpomere, bearing 2 sensilla clavata distally.

Thorax (Figure 17B). Dc 6–8; Ac 4–10; Pa 1; Scts 2–6. Halteres with 3–4 setae.

Wing (Figure 17C). VR 1.18–1.28. Brachiolum with 1 seta, Sc bare, R with 13–20 setae, R_1 with 15–23 setae, R_{4+5} with 10–30 setae, M_{1+2} with 18–29 setae, M_{3+4} with 16–26 setae, false vein with 29–52 setae, Cu with 2–13 setae, Cu_1 with 13–14 setae, PCu with 10–24 setae, An with 16–22 setae, remaining veins bare. Cell r_{4+5} with c. 81–120 setae, m with 0–7 setae, m_{1+2} with c. 40–82 setae, m_{3+4} with 32–47 setae, cu+an with 21–51 setae, remaining cells bare. Anal lobe strongly reduced.

Legs. Fore leg bearing single tibial spur, 10–20 μm long. Combs of mid tibia 10–16 μm wide with 20–25 μm long spur, and 10–15 μm wide with 13–15 μm long spur; combs of hind tibia 15–18 μm wide with 15–28 μm long spur, 15–20 μm wide with 15–25 μm long spur. Tarsomere 1 of mid leg with 2–4 sensilla chaetica. Pulvilli absent. Lengths (in μm) and proportions of legs as in Table 4.

Hypopygium (Figure 18). Tergite IX 60–65 μm long, with two median setae near the base of anal point. Lateral tooth absent. Anal tergal bands of V-type, well separated, ending at mid-length of tergite. Anal point robust compared to size of hypopygium, 30–42 μm long, with 3–4 lateral setae on each side, blunt apex; 6–8 spinulae placed irregularly between anal crests. Transverse sternapodeme broad, 40–55 μm long. Phallapodeme 48–75 μm long. Gonocoxite 88–100 μm long. Gonostylus 73–75 μm long. Superior volsella roundish, bearing 2 anteromedian and 4–5 dorsal setae. Digitus stout with pear-shaped apical lobe, digitus-seta located on cylindrical tubercle at base of digitus. Stem of median volsella short, located underneath superior volsella, 4 μm long,

Table 4. Lengths (in μm) and proportions of legs for *Tanytarsus tamaoctavus*, adult male

	fe	ti	ta_1	ta_2	ta_3	ta_4
p_1	540–640	255–300	620–780	300–390	250–300	180–250
p_2	485–620	405–440	235–250	100–120	60–90	35–50
p_3	505–650	505–570	360–410	195–240	180–230	105–140

	ta_5	LR	BV	SV	BR
p_1	90–110	2.43–2.75	1.64–1.73	1.14–1.28	2.88–3.13
p_2	35–40	0.57–0.59	4.44–4.89	3.79–4.24	3.57–5.45
p_3	60–70	0.72–0.88	2.38–2.55	2.73–3.14	3.63–6.38

with group of subulate lamellae. Inferior volsella slightly curved, 50–60 µm long, with 9–11 apical setae. HR 1.17–1.33, HV 2.77–2.33.

Remarks

According to Sasa's original description, *T. tamaoctavus* is closely related to *T. brundini* and *T. curticornis* based on the shape of adult male hypopygium, but can be separated from the latter two species by the absence of median volsella in the adult male, presence of a pair of spine patches surrounding by shagreen on pupal tergite II and presence of 0, 1, 3, 5 lateral taeniae on pupal segments V–VIII respectively. Unfortunately, the type material of *T. tamaoctavus* was lost when Sasa's collection was transferred from Toyama to NIES in September, 2000 (T. Kobayashi pers. comm.). Na (2004) recorded and redescribed *T. tamaoctavus* from Korea and based the identification partly on the absence of median volsella in the adult male hypopygium. Here, we redescribe the species based on the available Chinese specimens and find no significant difference in morphology between the Chinese and Korean specimens and the holotype description except for the putative absence of the median volsella. After examination of specimens from China and Korea and observations in a new collection of the species from Japan (K. Kawai pers. comm.), the presences of a tiny median volsella underneath (and hidden by) the superior volsella can be confirmed in all populations. Thus, the apparent absence noted in the original description likely is due to an error. A slight morphological variation between populations is recorded for the pupa where Chinese specimens have 1, 2, 3, 5 lateral taeniae on segments V-VIII respectively (H.Q. Tang pers. comm.). In general, *T. tamaoctavus* is difficult to differentiate morphologically from the presumed sibling species in Asia (see diagnostic characters and key), but the species is well supported as a distinct genetic lineage based on mitochondrial and nuclear markers (Lin et al. in prep.). Through associations of adult life stages with DNA barcodes we found aberrant sexual characters in two adults from Zhejiang Province, China (female type bodies with male hypopygium).

Distribution

China, Japan, Korea.

Tanytarsus thomasi **sp. n.**

Figures 19–20

ZooBank: http://zoobank.org/CC3512A3-6ADC-4C42-85EE-91F46ED9050C

Type material

Holotype: ♂ (NTNU-VM: 148251, BOLD Sample ID: CHIR_CH166), Canada, Manitoba, Churchill, Ramsey Creek, 58.73054°N, 93.78007°W, 13 m a.s.l., 14.VIII.2006, Netting, leg. J. Knopp. Paratypes: 4♂♂, (NTNU-VM: 148248, BOLD Sample ID: CHIR_CH124; NTNU-VM: 148249, BOLD Sample ID:

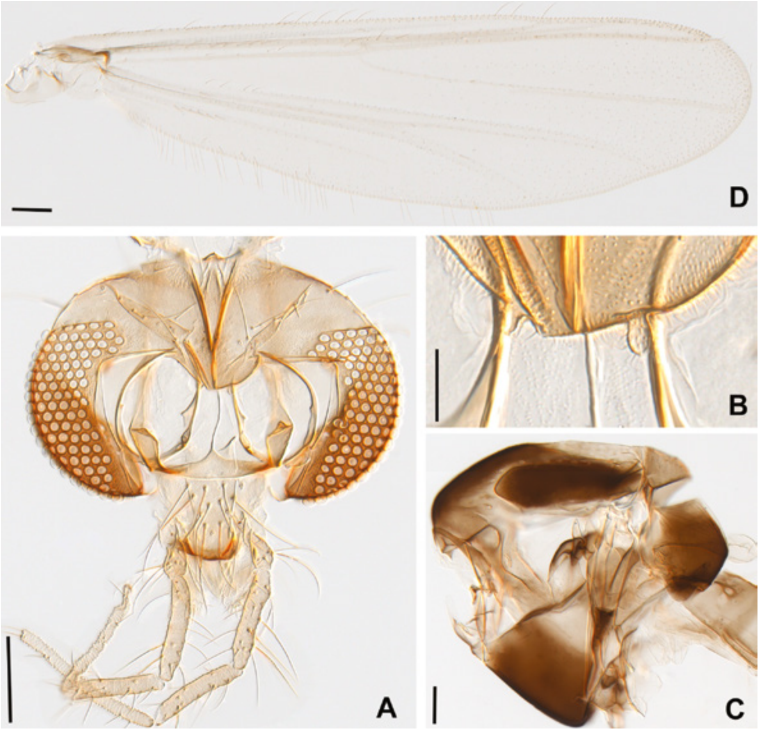

Fig. 19. *Tanytarsus thomasi* **sp. n.**, male: A) holotype head, scale bar = 100 μm; B) holotype partial head showing frontal tubercles, scale bar = 20 μm; C) paratype thorax, scale bar = 100 μm; D) paratype wing showing four unusual setae presenting in the cell r, scale bar = 100 μm.

CHIR_CH164; NTNU-VM: 148250, BOLD Sample ID: CHIR_CH165; NTNU-VM: 148252, BOLD Sample ID: CHIR_CH167) as holotype; 1♂, (NTNU-VM: 148253, BOLD Sample ID: CHIR_CH224), Canada, Manitoba, Churchill, Ramsey Creek, 58.73054°N, 93.78007°W, 13 m a.s.l., 15–17.VIII.2006, Malaise trap, leg. T. Ekrem & E. Stur; 1♂, (NTNU-VM: 136760, BOLD Sample ID: Finnmark666), Norway, Finnmark, Sør-Varanger, Tormajavri, 69.26996°N, 29.11843°E, 146 m a.s.l., 20.VI.2010, leg. T. Ekrem & E. Stur.

Diagnosis

The adult male can be distinguished from known species of *Tanytarsus* by the following combination of characters: body mostly brown, darker scutal stripes, postnotum and preepisternum; AR 0.71–0.94; frontal tubercles present; third palpomere longer than fourth; wing membrane covered with macrotrichia; LR_1 1.85–2.02; tarsomere 1 of mid leg with 2–3 sensilla chaetica; 2–4 median tergite setae near base of anal point; anal tergal bands of V-type, well separated, ending at mid-length of tergite; digitus stout with pear-shaped apical lobe, digitus-seta located on cylindrical tubercle at base of digitus; stem of median volsella short, with 2–3 pectinate lamellae.

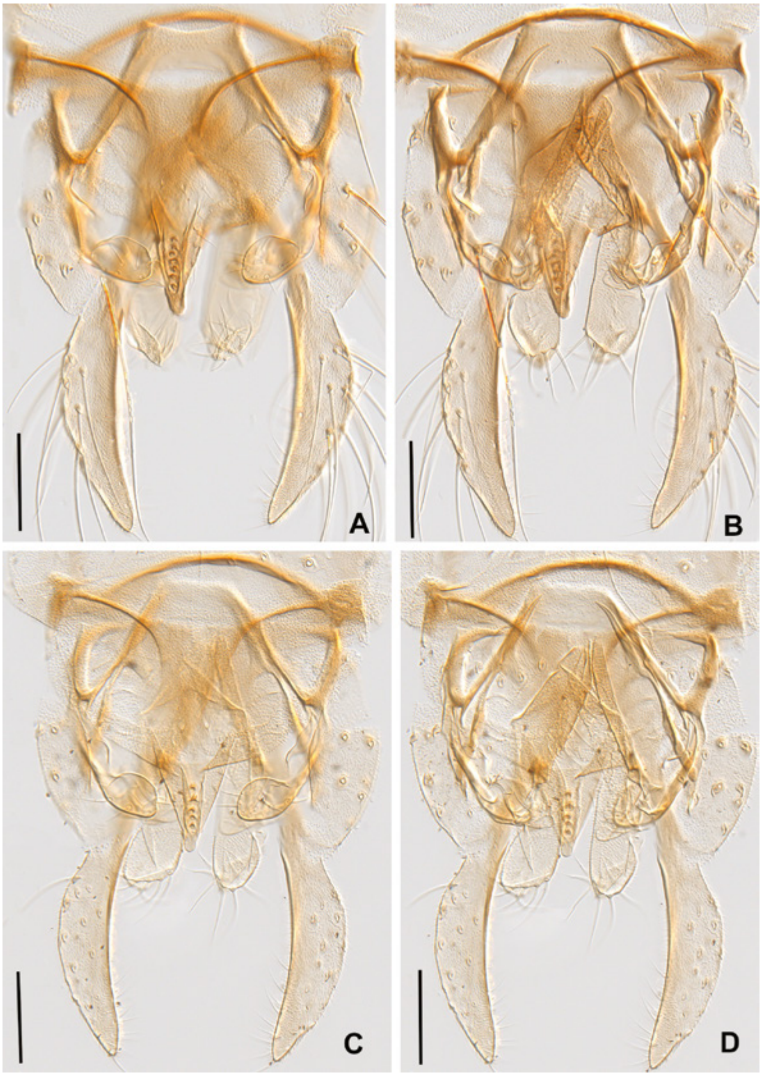

Fig. 20. *Tanytarsus thomasi* **sp. n.**, male: A) holotype hypopygium dorsal view; B) holotype hypopygium ventral view; C) paratype hypopygium dorsal view; D) paratype hypopygium ventral view. Scale bar = 50 µm.

Etymology

Named after Thomas Stur Ekrem for good company in the field; noun in genitive case.

Description

Adult male (n = 4). Total length 2.58–3.11, 2.77 mm. Wing length 1.60–1.95, 1.71 mm. Total length/wing length 1.56–1.68, 1.60.

Colouration. Head, legs and abdomen brown. Thorax ground colour brown with dark brown stripes anteriorly on scutum, laterally under parapsidal suture, on postnotum and on preepisternum; dark brown median anepisternum II, epimeron II.

Head (Figures 19A-B). Antenna with 13 flagellomeres, ultimate flagellomere 355–470, 398 µm long. AR 0.71–0.94, 0.79. Frontal tubercles cylindrical (Figure 20b), 8–10, 9 µm long, 4–5, 4 µm wide. Temporal setae 8–11, 9. Clypeus with 12 (n = 4) setae. Tentorium 105–140, 123 µm long, 23–39, 27 µm wide. Palpomere lengths (in µm): 26–38, 33; 35–38, 36; 100–130, 114; 100–123, 111; 135–193, 168. Third palpomere always longer than fourth palpomere, bearing 2 sensilla clavata distally.

Thorax (Figure 19C). Dc 8–9, 9; Ac 7–9, 8; Pa 1; Scts 4–6, 6. Halteres with 2–5, 4 setae.

Wing (Figure 19D). VR 1.15–1.20, 1.17. Brachiolum with 1 (n = 4) seta, Sc bare, R with 17–21, 19 setae, R_1 with 15–19, 17 setae, R_{4+5} with 21–38, 27 setae, M_{1+2} with 41–50, 46 setae, M_{3+4} with 22–28, 25 setae, false vein with 53–60, 56 setae, Cu with 4–17, 10 setae, Cu_1 with 14–17, 15 setae, PCu with 19–42, 27 setae, An with 20–28, 24 setae, remaining veins bare. Cell r with 4 setae (only found in the specimen CHIR_CH165), r_{4+5} with c. 145–180, 160 setae, m with 3–8, 5 setae, m_{1+2} with c. 140–150, 143 setae, m_{3+4} with 43–80, 55 setae, cu+an with 12–96, 63 setae, remaining cells bare. Anal lobe strongly reduced.

Legs. Fore leg bearing single tibial spur, 18–28, 23 µm long. Combs of mid tibia 13–15, 14 µm wide with 20–25, 22 µm long spur, and 12–14, 13 µm wide with 10–18, 14 µm long spur; combs of hind tibia 14–25, 18 µm wide with 25–28, 27 µm long spur, 13–25, 16 µm wide with 16–28, 22 µm long spur. Tarsomere 1 of mid leg with 2–3, 2 sensilla chaetica. Pulvilli absent. Lengths (in µm) and proportions of legs as in Table 5.

Hypopygium (Figure 20). Tergite IX with 2–4, 3 median setae near the base of anal point. Lateral tooth absent. Anal tergal bands of V-type, well separated, ending at mid-length of tergite. Anal point strong, triangular with blunt apex, a few microtrichia placed near base, 49–65, 57 µm long, with 3–4, 3 lateral setae on each side; 5–7, 6 spinulae in single row between well-developed anal crests. Transverse sternapodeme 38–50, 44 µm long. Phallapodeme 80–108, 97 µm long. Gonocoxite 120–125, 123 µm long. Gonostylus 110–120, 113 µm long, curved, with rounded apex. Superior volsella oval, bearing 2 anteromedian and 5 dorsal setae. Digitus stout with pear-shaped apical

Table 5. Lengths (in µm) and proportions of legs for *Tanytarsus thomasi* **sp. n.**, adult male

	fe	**ti**	**ta_1**	**ta_2**	**ta_3**	**ta_4**
p_1	700–790, 740	390–420, 405	720–850, 778	390–450, 413	310–340, 325	250–270, 262
p_2	690–800, 723	550–600, 565	290–350, 315	170–190, 178	130–140, 135	90–100, 94
p_3	780–910, 817	740–760, 750	450–480, 467	280–290, 283	250–280, 262	170–180, 175

	ta_5	**LR**	**BV**	**SV**	**BR**
p_1	110–130, 113	1.85–2.02, 1.90	1.65–1.84, 1.74	1.42–1.53, 1.48	1.67–3.33, 2.65
p_2	70–80, 74	0.53–0.58, 0.55	3.14–3.65, 3.36	3.94–4.31, 4.13	3.75–5.25, 4.62
p_3	90–100, 94	0.61–0.65, 0.63	2.32–2.54, 2.45	3.21–3.38, 3.26	5.00–5.35, 5.30

lobe, digitus-seta located on cylindrical tubercle at base of digitus. Stem of median volsella very small, with 2–3, 2 pectinate lamellae. Inferior volsella slightly curved, 75–78, 76 μm long, with 11–13, 12 apical setae. HR 1.00–1.12, 1.09, HV 2.34–2.59, 2.44.
Female and immatures unknown.

Remarks

In one specimen (BOLD Sample ID: CHIR_CH165), cell r has four setae which is unusual for *Tanytarsus*. *Tanytarsus thomasi* is similar to *T. ikicedeus* Sasa & Suzuki in the adult male hypopygium, but can be separated from the latter species by having frontal tubercles, third palpomere longer than fourth, a median volsella with 2–3 pectinate lamellae and comparatively lower AR (0.71–0.94) and LR_1 (1.85–2.02). Future DNA data on *T. ikicedeus* from Japan will reveal if these species also can be separated genetically. *Tanytarsus thomasi* has unusually high intraspecific K2P-divergence in partial COI sequences (up to 10%), but can be differentiated from other species within the *T. curticornis* complex by more than 15% mean K2P-divergence (Figure 1).

***Tanytarsus tongmuensis* sp. n.**

Figures 21–23

ZooBank: http://zoobank.org/29EDBBEF-E9EA-4AA9-9D2F-67BAAD8F9C9A

Type material

Holotype: ♂ (BDN & BOLD Sample ID: XL323), China, Fujian, Nanping, Wuyi Mountian, Tongmu, 27.745°N, 117.677°E, 800 m a.s.l., 29.IV.2015, leg. Q. Wang. Paratypes: 5♂♂ (BDN & BOLD Sample ID: XL346–349), 1♀ (BDN & BOLD Sample ID: XL350) as holotype; 16♂♂ (BDN & BOLD Sample ID: XL314–317, XL319, XL324, XL326, XL327, XL329–335, XL337, XL345), 1♀ (BDN & BOLD Sample ID: XL337), China, Fujian, Nanping, Wuyi Mountian, Tongmu, 27.7452°N, 117.6778°E, 720 m a.s.l., 27–28.IV.2015, leg. Q. Wang.

Diagnosis

The adult male can be distinguished from known species of *Tanytarsus* by the following combination of characters: body mostly pale brown with dark scutal stripes, postnotum and preepisternum; AR 0.66–0.74; frontal tubercles absent; third palpomere longer than fourth; wing length 1.63–1.73 mm; wing membrane covered with macrotrichia; LR_1 2.38–2.72; tarsomere 1 of mid leg with 4–5 sensilla chaetica; 1–5 median setae near the base of anal point; anal tergite bands of V-type, well separated, ending at mid-length of tergite; anal point robust, with rounded apex; digitus stout with pear-shaped apical lobe, one digitus-seta located on cylindrical tubercle at base of digitus; stem of median short, with 2 subulate lamellae.

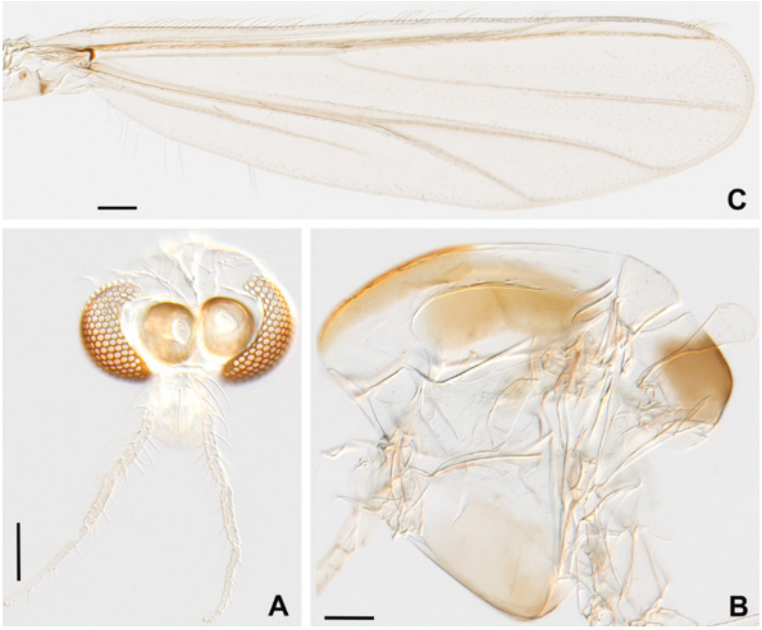

Fig. 21. *Tanytarsus tongmuensis* **sp. n.**, holotype male: A) head; B) thorax; C) wing. Scale bar = 100 µm.

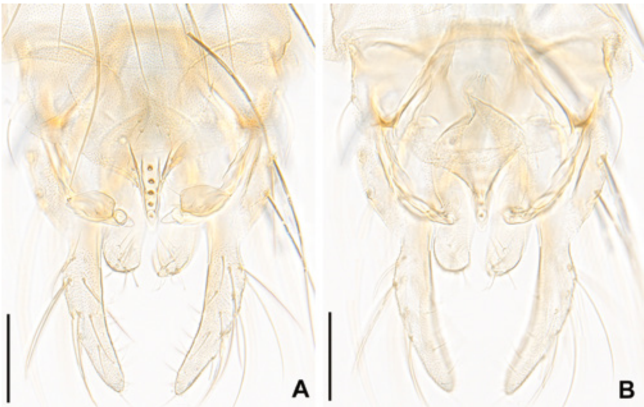

Fig. 22. *Tanytarsus tongmuensis* **sp. n.**, holotype male: A) hypopygium dorsal view; B) hypopygium ventral view. Scale bar = 50 µm.

Etymology

Named after the type locality, Tongmu; adjective in nominative case.

Description

Adult male (n = 8). Total length 2.42–2.77, 2.63 mm. Wing length 1.63–1.73, 1.68 mm. Total length/wing length 1.49–1.65, 1.08.

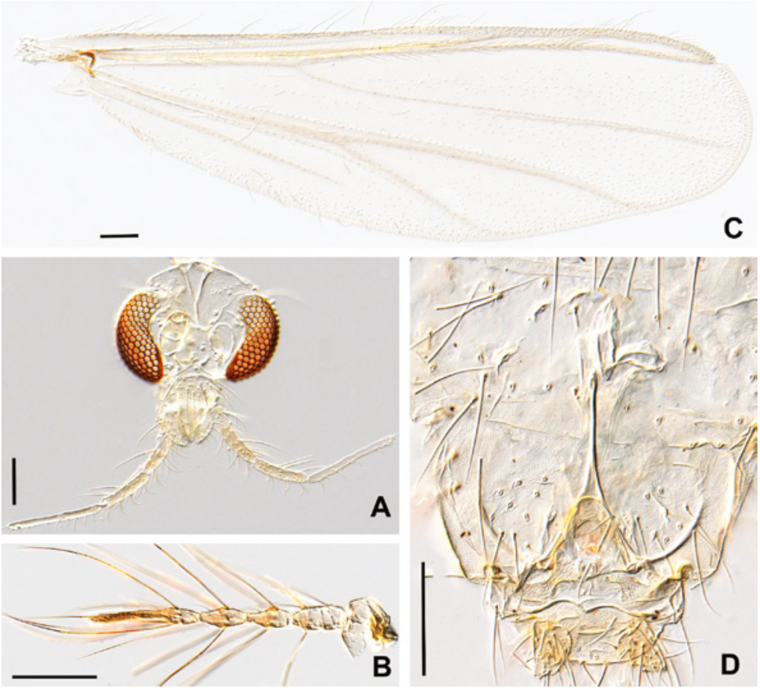

Fig. 23. *Tanytarsus tongmuensis* **sp. n.**, paratype female: A) head; B) antenna; C) wing; D) genitalia. Scale bar = 100 μm.

Colouration. Thorax ground colour pale brown with dark stripes anteriorly on scutum, laterally under parapsidal suture, on postnotum and paler on preepisternum; pale brown median anepisternum II, epimeron II. Head and legs pale brown. Abdomen yellow.

Head (Figure 21A). Antenna with 13 flagellomeres, ultimate flagellomere 350–360, 355 μm long. AR 0.66–0.74, 0.69. Frontal tubercles absent. Temporal setae 7–9, 8. Clypeus with 10–12, 11 setae. Tentorium 120–125, 123 μm long, 28–45, 30 μm wide. Palpomere lengths (in μm): 28–33, 30; 31–35, 33; 110–125, 116; 105–120, 113; 185–215, 198. Third palpomere always longer than fourth palpomere, bearing 2 sensilla clavata distally.

Thorax (Figure 21B). Dc 6–8, 7; Ac 8–10, 9; Pa 1; Scts 2–4, 3. Halteres with 2–3, 3 setae.

Wing (Figure 21C). VR 1.13–1.25, 1.18. Brachiolum with 1 seta, Sc bare, R with 16–26, 20 setae, R_1 with 19–25, 22 setae, R_{4+5} with 32–38, 34 setae, M_{1+2} with 38–55, 46 setae, M_{3+4} with 25–34, 29 setae, false vein with 51–80, 70 setae, Cu with 7–12, 10 setae, Cu_1 with 15–17, 16 setae, PCu with 23–28, 25 setae, An with 21–27, 25 setae, remaining veins bare. Cell r_{4+5} with c. 90–160, 130 setae, m with 4–5, 4 setae, m_{1+2} with c. 110–120, 115 setae, m_{3+4} with 60–65, 63 setae, cu+an with 59–99, 75 setae, remaining cells bare. Anal lobe strongly reduced.

Table 6. Lengths (in μm) and proportions of legs for *Tanytarsus tongmuensis* **sp. n.**, adult male

	fe	ti	ta_1	ta_2	ta_3	ta_4
p_1	690–790, 745	290–370, 343	790–930, 830	410–450, 428	340–370, 356	280–300, 285
p_2	700–760, 733	540–550, 545	280–350, 313	100–160, 136	100–110, 106	60–70, 65
p_3	750–820, 780	710–730, 718	470–520, 496	270–300, 288	250–270, 259	160–190, 182

	ta_5	LR	BV	SV	BR
p_1	110–130, 119	2.38–2.72, 2.47	1.65–1.76, 1.70	1.26–1.30, 1.28	2.50–3.25, 2.86
p_2	40–50, 45	0.56–0.65, 0.60	4.18–4.67, 4.35	3.68–4.13, 3.89	2.70–4.38, 3.60
p_3	85–95, 89	0.68–0.71, 0.69	2.36–2.37, 2.36	3.04–3.06, 3.05	3.75–5.33, 4.56

Legs. Fore leg bearing single tibial spur, 20–30, 25 μm long. Combs of mid tibia 20–23, 21 μm wide with 20–28, 26 μm long spur, and 15–20, 18 μm wide with 13–20, 16 μm long spur; combs of hind tibia 20–25, 22 μm wide with 23–30, 26 μm long spur, 16–25, 19 μm wide with 12–25, 17 μm long spur. Tarsomere 1 of mid leg with 4–5, 4 sensilla chaetica. Pulvilli absent. Lengths (in μm) and proportions of legs as in Table 6.

Hypopygium (Figure 22). Tergite IX with 1–5, 3 median setae near base of anal point. Lateral tooth absent. Anal tergal bands of V-type, well separated, ending at mid-length of tergite. Anal point strong, long, almost parallel-sided, with blunt apex, microtrichia absent in between crests, 43–75, 60 μm long, with 4–5, 4 lateral setae on each side; 4–6, 5 spinulae placed in single row between well-developed anal crests. Transverse sternapodeme narrow, 33–45, 39 μm long. Phallapodeme 58–85, 73 μm long. Gonocoxite 90–100, 95 μm long. Gonostylus 85–100, 93 μm long, comparatively thin, slightly medially curved, with rounded apex. Superior volsella medially directed, oval with short, straight inner margin, bearing 2–3, 2 anteromedian and 4–5, 5 dorsal setae. Digitus stout with pear-shaped apical lobe, digitus-seta located on cylindrical tubercle at base of digitus. Stem of median volsella short, located underneath superior volsella, 4 μm long, with 2 subulate lamellae. Inferior volsella slightly curved, 63–68, 65 μm long, with 9–11, 10 apical setae. HR 1.00–1.06, 1.04, HV 2.71–2.85, 2.79.
Adult female (n = 2). Total length 1.98–2.24 mm. Wing length 1.63–1.75 mm. Total length/wing length 1.13–1.38.
Colouration. As male.
Head (Figures 23A-B). Antenna with 4 flagellomeres (Figure 23B), ultimate flagellomere 125–135 μm long, AR 0.72–0.76. Frontal tubercles absent. Temporal setae 5–8. Clypeus with 18 setae. Tentorium 110 μm long, 15 μm wide. Palpomere lengths (in μm): 38, 28–30, 103–125, 115–118, 195–203.
Thorax. H 5–6; Dc 8; Ac 8–10; Pa 1; Scts 4. Halteres with 3 setae.
Wing (Figure 23C). VR 1.25–1.40. Brachiolum with 1 seta, Sc bare, R with 14–22 setae, R_1 with 20–24 setae, R_{4+5} with 43–61 setae, M_{1+2} with 59–80 setae, M_{3+4} with 30–40 setae, false vein with 100–114 setae, Cu with 24–27 setae, Cu_1 with 14 setae, PCu with 33–57 setae, An with 28–37 setae, remaining veins bare. Cell r_{4+5} with c.

Table 7. Lengths (in μm) and proportions of legs for *Tanytarsus tongmuensis* **sp. n.**, adult female

	fe	ti	ta_1	ta_2	ta_3	ta_4
p_1	700–730	360–380	910	410–420	340–350	280–281
p_2	660–690	500–520	280–300	140–142	100–103	60–70
p_3	720–760	660–700	420	240–250	220–230	130–150
	ta_5	**LR**	**BV**	**SV**	**BR**	
p_1	120–121	2.39–2.53	1.68–1.76	1.16–1.22	3.38–4.29	
p_2	50–60	0.56–0.58	4.00–4.19	4.03–4.14	3.70–4.00	
p_3	80–100	0.60–0.64	2.61–2.65	3.29–3.48	4.40–5.00	

240–260 setae, m with 7–16 setae, m_{1+2} with c. 190–200 setae, m_{3+4} with 90–100 setae, cu+an with 160–170 setae, remaining cells bare. Anal lobe strongly reduced.
Legs. As male, except 12–13 sensilla chaetica distally on mid tarsomere 1. Lengths (in μm) and proportions of legs as in Table 7.
Genitalia (Figure 23D). Sternite VIII with 28–30 setae; vaginal floor small, covering c. 1/4 of vaginal opening ventrally; gonapophysis VIII single lobe with long posteromedially directed microtrichia; gonocoxapodeme strongly curved; coxosternapodeme well-developed with obvious anterior and posterior lobes. Notum including rami 128–168 μm long, notum alone c. 60–83 μm long. Seminal capsules ovoid, large, 65–68 μm long, 38–40 μm wide, with 125–130 μm long spermathecal ducts. Postgenital plate subtriangular. Cercus 50–53 μm long, 45–49 μm wide, with 18–30 setae.
Immatures unknown.

Remarks

Tanytarsus tongmuensis is similar to *T. tamaoctavus* and *T. neotamaoctavus* in the adult male, but can be separated from the latter two species by having stout, almost parallel-sided anal point. DNA barcoded *T. tongmuensis* specimens are more than 13% different from specimens of *T. tamaoctavus* in partial COI sequences (Figure 1).

Tanytarsus wangi **sp. n.**

Figures 24–25

ZooBank: http://zoobank.org/7C758369-CADA-416B-917A-C3B46D684433

Type material

Holotype ♂ (BDN: L7A23), China, Zhejiang, Lishui, Qinyuan, Baishanzu Nature Reserve, 27.756°N, 119.194°E, 1564 m a.s.l., 24–26.VII.2012, Light trap, leg. X.L. Lin; Paratypes: 2♂♂, 1♂ (BDN & BOLD Sample ID: XL1) as Holotype; 1♂ (BDN: 2630), China, Fujian, Nanping, Wuyishan Mountain, Sangang, 117.680°N, 27.748°E, 934 m a.s.l., Light trap, leg. X.H. Wang.

Fig. 24. *Tanytarsus wangi* **sp. n.**, male: A) paratype head; B) holotype antenna; C) paratype thorax; D) paratype wing. Scale bar = 100 µm.

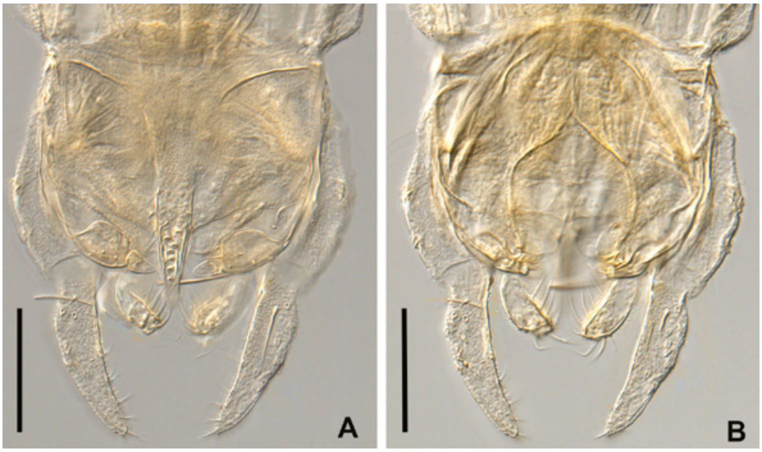

Fig. 25. *Tanytarsus wangi* **sp. n.**, male: A) holotype hypopygium dorsal view; B) holotype hypopygium ventral view. Scale bar = 50 µm.

Diagnosis

The adult male can be distinguished from known species of *Tanytarsus* by the following combination of characters: body pale yellow; AR low (0.43–0.49); frontal tubercles absent; third palpomere equal or longer than fourth; wing membrane covered with macrotrichia; tarsomere 1 of mid leg with two sensilla chaetica; anal point almost parallel-sided, evenly tapered distally, with rounded apex, bearing 5–7 spinulae irregularly placed between anal crests and 3–4 lateral setae; 2–4 median setae near the base of anal point; superior volsella roundish with slightly concave inner margin, bearing two anteromedian setae, two dorsal setae and two lateral setae; digitus with pear-shaped apical lobe, bearing one seta at base; stem of median volsella short, bearing three falciform lamellae; gonostylus short, slightly curved inwards, tapered to pointed apex.

Etymology

Named after Xin-Hua Wang, for his outstanding contribution to the knowledge of Chironomidae in China; noun in genitive case.

Description

Adult male (n = 3). Total length 1.95–2.63 mm. Wing length 0.92–1.15 mm. Total length/wing length 1.89–2.28.
Colouration. Head, thorax, legs and abdomen pale yellow.
Head (Figure 24A). Antenna with 13 flagellomeres, ultimate flagellomere 190–200 µm long. AR 0.43–0.49. Frontal tubercles absent. Temporal setae 6–8. Clypeus with 12–14 setae. Tentorium 88–100 µm long, 20–25 µm wide. Palpomere lengths (in µm): 25–35, 25–30, 88–93, 80–93, 145–160. Third palpomere equal or longer than fourth, bearing 2 sensilla clavata distally.
Thorax (Figure 24B). Dc 3–6; Ac 3–7; Pa 1; Scts 2–4. Halteres with 3–4 setae.
Wing (Figure 24C). VR 1.35–1.37. Brachiolum with 1 seta, Sc bare, R with 14–18 setae, R_1 with 13–18 setae, R_{4+5} with 15–22 setae, M_{1+2} with 22–33 setae, M_{3+4} with 19–23 setae, false vein with 50–80 setae, Cu with 12–12 setae, Cu_1 with 11–14 setae, PCu with 22–24 setae, An with 19–22 setae, remaining veins bare. Cell r_{4+5} with c. 50–60 setae, m with 2–5 setae, m_{1+2} with c. 50–80 setae, m_{3+4} with 41–50 setae, cu+an with 27–36 setae, remaining cells bare. Anal lobe strongly reduced.
Legs. Fore leg bearing single tibial spur, 10–25 µm long. Combs of mid tibia 13–18 µm wide with 18–25 µm long spur, and 18–23 µm wide with 13–18 µm long spur; combs of hind tibia 20–26 µm wide with 20–28 µm long spur, 17–25 µm wide with 13–18 µm long spur. Tarsomere 1 of mid leg with 2 sensilla chaetica. Lengths (in µm) and proportions of legs as in Table 8.
Hypopygium (Figure 25). Tergite IX 66–70 µm long, with 2–4 median setae near the base of anal point. Lateral tooth absent. Anal tergite bands of V-type, well separated, ending at mid-length of tergite. Anal point almost parallel-sided, 33–53 µm long, with 3–4 lateral setae on each side, with blunt apex; 6–7 spinulae placed irregularly between anal crests. Transverse sternapodeme 30–40 µm long. Phallapodeme 48–68 µm long. Gonocoxite 85–100 µm long. Gonostylus 58–80 µm long, slightly curved inwards, tapered to pointed apex. Superior volsella roundish with concave median margin, bearing 2 anteromedian and 4 dorsal setae. Digitus stout with pear-shaped apical lobe, one digitus-seta located on cylindrical tubercle at base of digitus. Stem of median volsella short, located underneath superior volsella, 5 µm long, with three falciform lamellae. Inferior volsella weakly curved, 60–68 µm long, with 8–10 apical setae. HR 1.10–1.47, HV 2.44–3.75.

Table 8. Lengths (in μm) and proportions of legs for *Tanytarsus wangi* **sp. n.**, adult male

	fe	ti	ta_1	ta_2	ta_3	ta_4
p_1	370–500	225–265	610–705	285–330	200–260	170–220
p_2	410–500	350–400	225–240	105–110	60–73	35–50
p_3	410–540	350–395	320–350	180–210	140–200	85–120

	ta_5	LR	BV	SV	BR
p_1	100–105	2.65–2.84	1.56–1.68	0.93–1.09	2.25–3.43
p_2	35–40	0.60–0.64	4.15–4.30	3.49–3.78	3.57–4.71
p_3	65–75	0.73–0.85	2.14–2.36	2.47–2.91	2.00–6.25

Female and immatures unknown.

Remarks

The new species is most similar to *T. tamaoctavus* in the adult male, but can be separated morphologically from this species by having paler body colour and a lower AR. They separate by more than 12% K2P-divergence in partial COI sequences (Figure 1).

Key to adult males of the *Tanytarsus curticornis* complex

1. Digitus with apical pear-shape lobe (Figure 16C), extending beyond inner margin of superior volsella; anal tergal bands of V-type, widely separated medially; superior volsella generally oval, often with straight or concave median margin; spinulae present between anal crests; stem of median volsella short 2
– Not as above .... Not keyed
2. Microtrichia present on superior volsella; lamellae of median volsella reaching tip of the digitus. Afrotropical region. 3
– Microtrichia absent on superior volsella; stem of median volsella short, lamellae of median volsella never reaching tip of digitus. Other zoogeographical regions. ... 4
3. AR < 0.45; LR_1 < 2.90; seta on digitus placed basally. Ghana, Nigeria .... *T. pseudocongus* Ekrem
– AR > 0.60; LR_1 > 3.30; seta on digitus placed at half-length. Ghana, Senegal, Democratic Republic of the Congo. .... *T. congus* Lehmann
4. Median setae on anal tergite absent (Figures 5C; 16A) 5
– Median setae on anal tergite present (Figure 22A) 6
5. Wing cell m and vein Cu bare (Figure 15A). Palaearctic China. .... *T. songi* **sp. n.**
– Wing cell m and vein Cu setose (Figure 5A). Palaearctic. ..... *T. curticornis* Kieffer
6. Wing membrane with macrotrichia in distal half of cell r_{4+5} and sometimes in apical part of cell m_{1+2}. Finland. .... *T. salmelai* Giłka & Paasivirta
– Wing membrane almost entirely covered with macrotrichia 7
7. Thorax entirely dark brown (Figure 10B); tarsomere 1 of mid leg without sensilla chaetica. Madeira. .... *T. madeiraensis* **sp. n.**
– Thorax pale yellow, or with dark brown scutal stripes; tarsomere 1 of mid leg with sensilla chaetica 8

8. Body pale yellow, thorax with pale brown scutal stripes (Figure 24C); AR 0.43–0.53. Oriental China. .... *T. wangi* **sp. n.**
– Body dark yellow, thorax with dark brown scutal stripes; AR > 0.60 9
9. Anal point with pointed apex 10
– Anal point blunt apex 11
10. Anal point (Figure 7A) comparatively long, reaching the apex of inferior volsella; median volsella bearing three falciform lamellae (Figure 7B). Canada. .... *T. heberti* **sp. n.**
– Anal point (Figures 4A, C) comparatively short, not reaching the apex of inferior volsella; median volsella bearing pectinate lamellae (Figure 4B). Palaearctic. .... *T. brundini* Lindeberg
11. Frontal tubercles (Figure 19B) present; LR_1 1.85–2.02. Canada. .... *T. thomasi* **sp. n.**
– Frontal tubercles absent; LR_1 2.37–2.84 12
12. AR 0.90–1.00; anal point triangular, with nipple-shaped apex (Figure 9B). Japan. .... *T. ikicedeus* Sasa & Suzuki
– AR 0.60–0.80; anal point not as above 13
13. Wing length > 1.50 mm; anal point robust 14
– Wing length < 1.30 mm (Figure 17C); anal point slender. China, Japan, Korea. .... *T. tamaoctavus* Sasa
14. Wing length 1.63–1.73 mm; AR 0.66–0.74; digitus bearing seta (Figure 22B). Oriental China. .... *T. tongmuensis* **sp. n.**
– Wing length 1.50 mm; AR 0.82; digitus without seta (Figure 14C). Korea. .... *T. neotamaoctavus* Ree, Jeong & Nam

Key to known pupae of the *Tanytarsus curticornis* complex

1. Cephalic tubercles shallow mounds; thoracic horn with chaetae shorter than the diameter of thoracic horn; segment VIII with five lateral taeniae; tergites with small anterior spines in patches 2
– Without the above combination of characters .... Not keyed
2. Tergite II with a pair of spine patches surrounded by shagreen 3
– Only shagreen present on Tergite II 4
3. Segments V–VII with 2, 2, 3 lateral taeniae. Palaearctic. ..... *T. curticornis* Kieffer
– Segments V–VII with 0, 1, 3 lateral taeniae. China, Japan. .. *T. tamaoctavus* Sasa
4. Thoracic horn c. 600 µm long; segments V–VII with 1, 1, 3 lateral taeniae; chaetae distributed densely on apical half of thoracic horn. Palaearctic. .... *T. brundini* Lindeberg
– Thoracic horn c. 540–550 µm long; segments V–VII with 1, 2, 3 lateral taeniae; chaetae distributed on distal 2/3 of thoracic horn. Madeira. .... *T. madeiraensis* **sp. n.**

The *Tanytarsus heusdensis* species complex

In general, the species of the *T. heusdensis* species complex have the following morphological characters in adult males: the anal tergite bands are of V-type, widely separated;

median setae of the anal tergite are comparatively long, placed near the base of anal point; anal point is stout, apically pointed, with several spinulae between well-developed crests; superior volsella is sub-circular, with concave inner margin, bearing 1–2 anteromedian and 5–7 dorsal setae; digitus is long and usually swollen apically, extending well beyond the median margin of the superior volsella; stem of the median volsella is shorter than its lamellae.

Descriptions

Tanytarsus adustus **sp. n.**

Figures 26–28

ZooBank: http://zoobank.org/82241A10-3EA9-4210-9774-30913BC4204D

Type material

Holotype ♂, (NTNU-VM: 124470, BOLD Sample ID: SOE247), Norway: Sør-Trøndelag, Røros kommune, Sølendet, Springbrook A2, 62.6903°N, 11.8416°E, 763 m a.s.l., 3.VIII.2006, Malaise trap, leg. O. Frengen. Paratypes: 1♂ (NTNU-VM: 148234, BOLD Sample ID: XL459), Norway, Sør-Trøndelag, Røros kommune, Sølendet, Springbrook C2, 62.69°N, 11.842°E, 763 m a.s.l., 7.VIII.2006, Malaise trap, leg. O. Frengen; 1♂ (ZMBN: chi23817), Luxemburg, Oesling, W Clervaux, Helokrene-E3, Emergency trap, 50.05°N, 5.988°E, 455 m a.s.l., 18.V.1999, leg. I. Schrankel; 2♀♀ (NTNU-VM: 124617, BOLD Sample ID: SOE380; NTNU-VM: 124623, BOLD Sample ID: SOE386; NTNU-VM: 124624, BOLD Sample ID: SOE387):

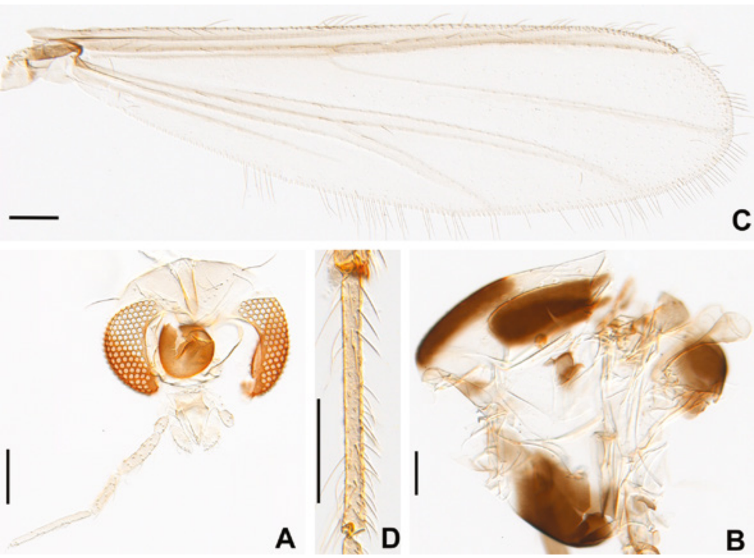

Fig. 26. *Tanytarsus adustus* **sp. n.**, male: A) holotype head; B) paratype thorax; C) paratype wing; D) paratype tarsomere 1 of mid leg showing the absence of sensilla chaetica. Scale bar = 100 µm.

Fig. 27. *Tanytarsus adustus* **sp. n.**, holotype male: A) hypopygium dorsal view; B) hypopygium ventral view. Scale bar = 50 μm.

Norway, Sør-Trøndelag, Røros kommune, Sølendet, Springbrook A1–2, 62.6903°N, 11.8416°E, 763 m a.s.l., 22.VI-3.VII.2006, Malaise trap, leg. O. Hanssen.

Diagnosis

The adult male can be distinguished from known species of *Tanytarsus* by the following combination of characters: body entirely brown with darker scutal stripes on the thorax; AR 0.56–0.62; wing cell m bare; tarsomere 1 of mid leg without sensilla chaetica; LR_1 1.76–1.84; 6–10 median setae near the base of anal point; anal point triangular, tapered to pointed, attenuate apex; anal crests fused posteriorly at c. half length of anal point; superior volsella sub-circular, with slightly concave inner margin, bearing 2 anteromedian and 5–6 dorsal setae; digitus long, with blunt apex, extending well beyond inner margin of superior volsella; transverse sternapodeme with weak oral projections; gonostylus widest at mid length, slightly curved inwards, with rounded apex.

Etymology

The specific name is from Latin '*adustus*', adjective referring to the brown body colouration.

Description

Adult male (n = 3). Total length 1.95–2.26 mm. Wing length 1.30–1.50 mm. Total length/wing length 1.50–1.57.
Colouration. Thorax ground colour brown with dark brown stripes anteriorly on scutum, laterally under parasidal suture, postnotum, preepisternum; and median anepisternum II. Head, legs and abdomen brown. Head, legs and abdomen brown. Halteres and wing pale brown.

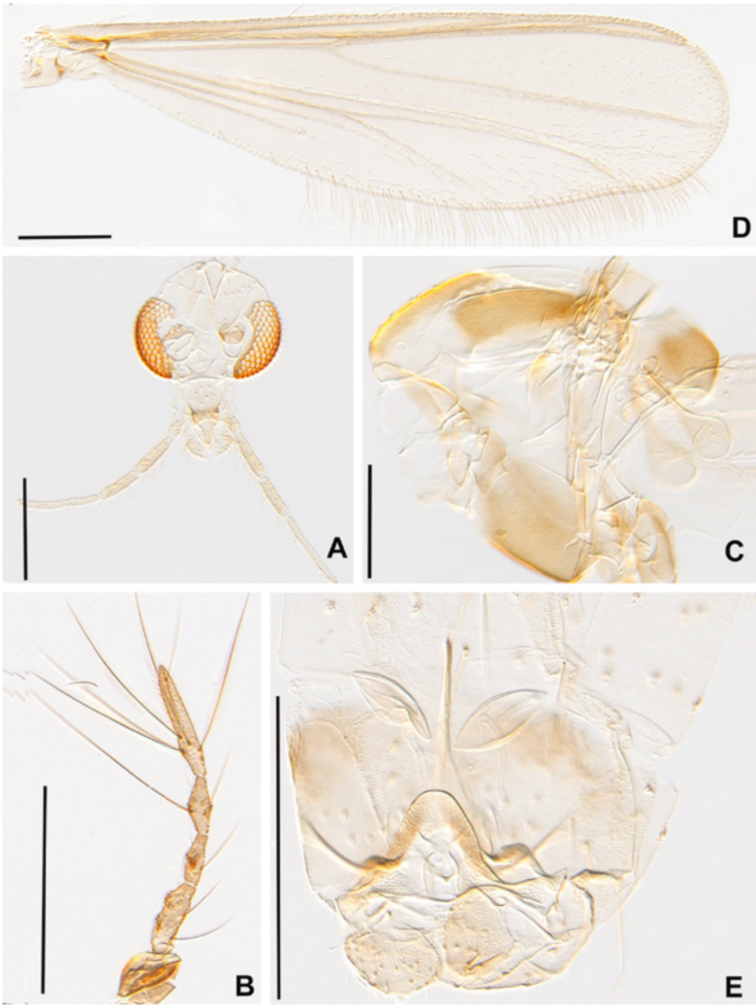

Fig. 28. *Tanytarsus adustus* **sp. n.**, paratype female: A) head; B) antenna; C) thorax; D) wing; E) genitalia. Scale bar = 200 μm.

Head (Figure 26A). Antenna with 13 flagellomeres, ultimate flagellomere 250–310 μm long. AR 0.56–0.62. Frontal tubercles absent. Temporal setae 9–11. Clypeus with 8–10 setae. Tentorium 105–115 μm long, 20–30 μm wide. Palpomere lengths (in μm): 28–33, 28–40, 100–125, 80–105, 153–185.
Thorax (Figure 26B). Dc 7–8; Ac 6–9; Pa 1; Scts 4. Halteres with 4–5 setae.
Wing (Figure 26C). VR 1.08–1.17. Brachiolum with 1 seta, Sc bare, R with 16–24 setae, R_1 with 13–18 setae, R_{4+5} with 25–33 setae, M_{1+2} with 40–50 setae, M_{3+4} with 25–44 setae, false vein with 36–54 setae, Cu with 7–18 setae, Cu_1 with 12–16 setae, PCu with 16–19 setae, An with 14–22 setae, remaining veins bare. Cell r_{4+5} with c. 80–90 setae, m bare, m_{1+2} with c. 55–110 setae, m_{3+4} with 27–55 setae, cu+an with 31–72 setae, remaining cells bare. Anal lobe strongly reduced.

Table 9. Lengths (in μm) and proportions of legs for *Tanytarsus adustus* **sp. n.**, adult male

	fe	**ti**	**ta_1**	**ta_2**	**ta_3**	**ta_4**
p_1	610–720	320–390	590–690	320–390	270–310	190–240
p_2	590–680	460–530	240–300	130–160	90–110	60–80
p_3	640–740	600–670	350–420	210–250	200–240	110–150
	ta_5	**LR**	**BV**	**SV**	**BR**	
p_1	90–120	1.76–1.84	1.73–1.75	1.58–1.61	2.01–2.11	
p_2	50–70	0.52–0.57	3.61–3.91	4.00–4.38	3.11–3.22	
p_3	60–90	0.58–0.62	2.51–2.74	3.36–3.54	3.11–4.00	

Legs. Fore leg bearing single tibial spur, 15–20 μm long. Combs of mid tibia 20–25 μm wide with 25–26 μm long spur, and 10–20 μm wide with 18–23 μm long spur; combs of hind tibia 14–23 μm wide with 28–30 μm long spur, 20–36 μm wide with 20–28 μm long spur. Tarsomere 1 of mid leg without sensilla chaetica (Figure 26D). Lengths (in μm) and proportions of legs as in Table 9.

Hypopygium (Figure 27). Tergite IX 73–75 μm long, with 6–10 median setae near the base of anal point. Lateral tooth absent. Anal tergite bands of V-type, widely separated, ending at mid-length of tergite. Anal point triangular, tapering to pointed, attenuate apex, 40–45 μm long, with 4–6 lateral setae on each side; 4–6 spinulae placed regularly between anal crests which fused posteriorly at c. half length of anal point. Transverse sternapodeme 43–48 μm long, with weak oral projections. Phallapodeme 70–95 μm long. Gonocoxite 110–120 μm long. Gonostylus widest in middle, slightly curved inward, 88–108 μm long with rounded apex. Superior volsella sub-circular, with slightly concave inner margin, bearing 2 anteromedian and 5–6 dorsal setae. Digitus long, with blunt apex, extending beyond anterior margin of superior volsella. Stem of median volsella 10–15 μm long, bearing 3 setae and 3–5 subulate lamellae. Inferior volsella weakly curved, 75–88 μm long, bearing 9–11 apical setae. HR 1.07–1.28, HV 2.09–2.22.

Adult female (n = 3). Total length 1.53–1.56 mm. Wing length 1.30–1.50 mm. Total length/wing length 1.02–1.515.

Colouration. Same colour pattern as in male, but paler.

Head (Figures 28A-B). Antenna with 4 flagellomeres (Figure 28B), ultimate flagellomere 105–113 μm long, AR 0.51–0.62. Frontal tubercles absent. Temporal setae 8. Clypeus with 10 setae. Tentorium 113 μm long, 10–13 μm wide. Palpomere lengths (in μm): 28–29, 30–33, 98–100, 83–93, 158–170.

Thorax (Figure 28C). H 1; Dc 6–8; Ac 6–9; Pa 1; Scts 4. Halteres with 4–5 setae.

Wing (Figure 28D). VR 1.11–1.20. Brachiolum with 1 seta, Sc bare, R with 14–17 setae, R_1 with 13–14 setae, R_{4+5} with 25–26 setae, M_{1+2} with 33–37 setae, M_{3+4} with 19–29 setae, false vein with 40–53 setae, Cu with 14–17 setae, Cu_1 with 12–14 setae, PCu with 23–28 setae, An with 16–19 setae, remaining veins bare. Cell r_{4+5} with c. 120–145 setae, m with 4–5 setae, m_{1+2} with c. 130–140 setae, m_{3+4} with 50–68 setae, cu+an with 83–88 setae, remaining cells bare. Anal lobe strongly reduced.

Table 10. Lengths (in µm) and proportions of legs for *Tanytarsus adustus* **sp. n.**, adult female

	fe	ti	ta_1	ta_2	ta_3	ta_4
p_1	570–620	320–370	610–640	310–340	250–290	200–210
p_2	560–600	440–510	240–250	120–140	90–100	50–70
p_3	600–630	600–670	330–370	190–220	180–200	120–120
	ta_5	**LR**	**BV**	**SV**	**BR**	
p_1	90–100	1.73–1.91	1.73–1.76	1.46–1.55	1.44–2.86	
p_2	40–60	0.49–0.55	3.68–4.13	4.17–4.44	2.00–2.38	
p_3	70–80	0.57–0.59	2.66–2.70	3.46–3.58	1.44–1.88	

Legs. As male, except five sensilla chaetica distally on mid tarsomere 1. Lengths (in µm) and proportions of legs as in Table 10.
Genitalia (Figure 28E). Tergite IX slightly triangular; sternite VIII with 22 setae; vaginal floor small, covering c. 1/4 of vaginal opening ventrally; gonapophysis VIII single lobe with long posteromedially directed microtrichia; gonocoxapodeme strongly curved; coxosternapodeme well-developed with obvious anterior and posterior lobes. Notum including rami 140–150 µm long, notum alone c. 95–100 µm long. Seminal capsules ovoid, large, 50–63 µm long, 30–40 µm wide, with 155–193 µm long spermathecal ducts. Postgenital plate subtriangular. Cercus 50–58 µm long, 35–45 µm wide, with 21–31 setae.
Immatures unknown.

Remarks

The new species resembles *T. heusdensis* in the adult male hypopygium, but can be separated from the latter species by having an entirely brown body with darker scutal stripes on the thorax, a lower AR and by lacking sensilla chaetica on ta_1 of mid leg. The new species also separates from *T. heusdensis* by more than 16% divergence in partial COI sequences (Figure 2).

***Tanytarsus heusdensis* Goetghebuer, 1923**

Figure 29

Tanytarsus heusdensis Goetghebuer, 1923: 118, figure 11; Albu 1980: 250, figure 168; Reiss & Fittkau 1971: 101, figures 8–9; Lindeberg 1970: 311. Holotype ♂ (RBINS 18.073), leg. M. Goetghebuer [Examined].

Tanytarsus gotchi Goetghebuer, 1928: 143, figure 11. Holotype ♂ (BMNH 235909), England, Surrey, Richmond-Upon-Thames, 29.VII.1914, leg. D.H. Gotch [Not examined].

Tanytarsus heusdensis var. *kuusamoensis* Storå, 1939: 30, figure 22. [Not examined].

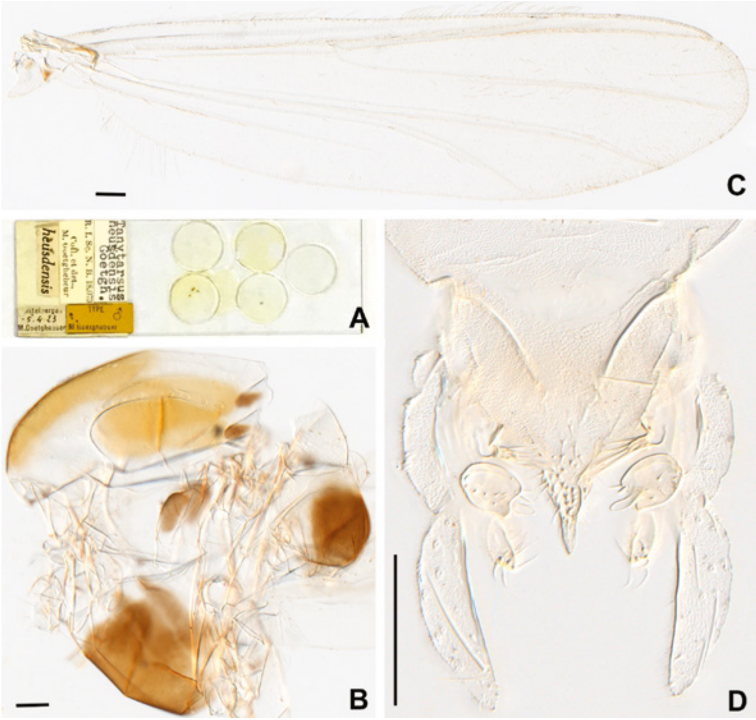

Fig. 29. *Tanytarsus heusdensis*. A) holotype microscope slide; B) male thorax of Norwegian specimen; C) holotype male wing; D) holotype male hypopygium. Scale bar = 100 µm.

Additional material examined

Germany: 2♂♂ (NTNU-VM: 148243, BOLD Sample ID: XL183; NTNU-VM: 148244, BOLD Sample ID: XL186), Bavaria, Pfaffenhofen, Scheller-Mühle, Ilm River, 430 m a.s.l., 48.53°N, 11.509°E, 27.IV.2003, Sweep net, leg. E. Stur; Norway: 2♂♂ (NTNU-VM: 143621, BOLD Sample ID: CH-OSF102; NTNU-VM: 143623, BOLD Sample ID: CH-OSF104), Oslo, S Nordstrand, Ljanselva, Liadalen, 59.8481°N, 10.7927°E, 2.VI.2010, leg. G. Søli & M. Steinert.

Diagnosis

Tanytarsus heusdensis can be distinguished from known species of *Tanytarsus* by the following combination of characters: body mostly yellow with light brown stripes on anterior scutum, postnotum, median anepisternum II and preepisternum (Figure 29B); front tubercles absent; AR 1.02–1.10 (n = 3); wing length > 2 mm; wing membrane covered with macrotrichia; LR_1 1.89–1.92 (n = 3); tarsomere 1 of mid leg with 3–4 sensilla chaetica; anal tergite bands of V-type, widely separated, diagonally pointing towards base of anal point; anal point widened basally, tapering to pointed apex, bearing several spinulae placed irregularly between posteriorly fused anal crests; superior volsella circular with concave inner margin bearing 1–2 anteromedian and 5–7 dorsal setae; digitus thumb-like, tapered to blunt apex, extending beyond inner margin of superior volsella, with one digitus-seta; stem of median volsella about two times longer than wide, lamellae longer than stem, narrow from the base to near the tip, where it splits into several fine points in the form of a crown; transverse sternapodeme with strong oral projections.

Remarks

Tanytarsus heusdensis is widely recorded in the Palearctic region, but its distribution should be revised considering the new, morphologically similar species described here. *Tanytarsus gotchi* was described by Goetghebuer (1928) using few characters and a simple illustration. Reiss & Fittkau (1971) synonymized *T. gotchi* with *T. heusdensis* after examining both holotypes, arguing that the colouration and remaining diagnostic characters fit within the diagnosis of *T. heusdensis*. Storå (1939) described *T. heusdensis* var. *kuusamoensis* having a pale body with dark mesonotal stripes and an AR of about 1.00. The similar species *T. adustus* **sp. n.** and *T. pseudoheusdensis* **sp. n.** have an AR of 0.56–0.62 and entirely brown body and an AR of 0.59–0.67 and mostly yellow body, respectively. Thus, we are convinced that neither *T. gotchi* nor *T. heusdensis* are conspecific with the new species we describe and regard them as junior synonyms of *T. heusdensis*.

Distribution

Palaearctic region.

***Tanytarsus pseudoheusdensis* sp. n.**

Figures 30–31

ZooBank: http://zoobank.org/B675A50B-7143-431F-B925-AF6EDADAC05D

Type material

Holotype ♂ (NTNU-VM: 148246, BOLD Sample ID: ES79), Germany: Bavaria, Nationalpark Berchtesgaden, Herrenrointquelle 312c, 47.58°N, 12.975°E, 1230 m a.s.l., 12.VII.2005, Sweep net, leg. F. Eder & A. Schellmoser. Paratypes: 1 ♂ (NTNU-VM: 148245, BOLD Sample ID: ES77) as holotype; 1 ♂ (NTNU-VM: 93565) Germany, Bavaria, Nationalpark Berchtesgaden, Herrenrointquelle 312c, 47.58°N, 12.975°E, 1230 m a.s.l., 23.VIII-06.IX.2005, leg. F. Eder.

Diagnosis

The adult male can be distinguished from known species of *Tanytarsus* by the following combination of characters: body mostly yellow with pale brown stripes anteriorly on scutum, on postnotum, ventrally on preepisternum, median anepisternum II and

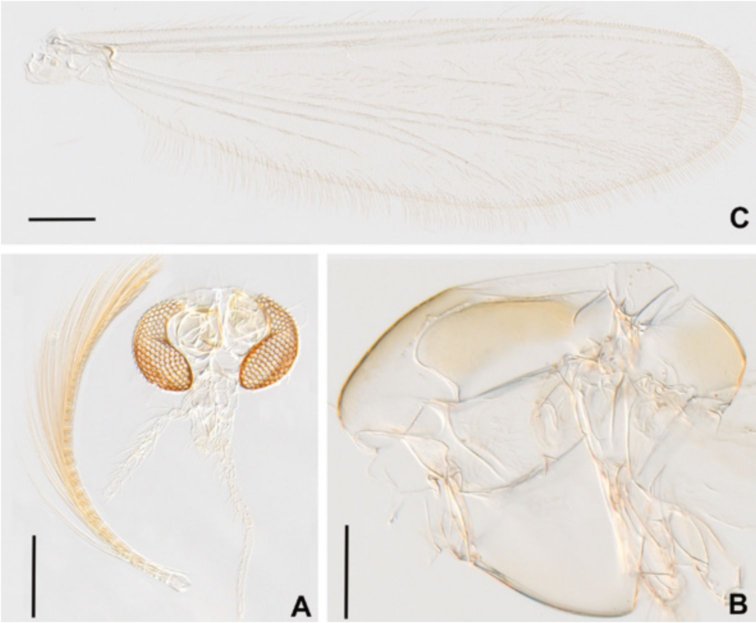

Fig. 30. *Tanytarsus pseudoheusdensis* **sp. n.**, holotype male: A) antenna and head; B) thorax; C) wing. Scale bar = 200 µm.

Fig. 31. *Tanytarsus pseudoheusdensis* **sp. n.**, holotype male: A) hypopygium dorsal view; B) hypopygium ventral view. Scale bar = 50 µm.

epimeron II (Figure 30B); AR 0.59–0.67; wing cell m with 22 setae; LR_1 1.84–2.07; tarsomere 1 of mid leg with 2–3 sensilla chaetica distally; anal tergite with ten median setae near base of anal point; lateral tooth absent; anal tergite bands of V-type, well separated, ending at mid-length of tergite; anal point with digitiform distal end, crests fused posteriorly at half length; 4–6 spinulae placed in irregular row between anal crests; gonostylus with more or less straight inner margin, with rounded apex; superior volsella roundish, slightly elongated and medially directed, with concave inner margin, bearing 2 anteromedian and 5–6 dorsal setae; digitus long, with blunt apex, extending well beyond median margin of superior volsella; median volsella short, barely longer than wide, bearing 3–5 subulate lamellae and a few simple setae; transverse sternapodeme with strong oral projections.

Etymology

The specific name, '*pseudoheusdensis*', reflects the similarities in morphology to *T. heusdensis*.

Description

Adult male (n = 3). Total length 2.85–2.93 mm. Wing length 1.93–1.95 mm. Total length/wing length 1.46–1.52.
Colouration. Thorax ground colour yellow with pale brown stripes anteriorly on scutum, laterally under parapsidal suture, on postnotum and ventrally on preepisternum; pale brown median anepisternum II, epimeron II. Legs and abdomen pale yellow.
Head (Figure 30A). Antenna with 13 flagellomeres, ultimate flagellomere 325–375 μm long. AR 0.59–0.67. Frontal tubercles absent. Temporal setae 10–13. Clypeus with 11–12 setae. Tentorium 108–128 μm long, 25–45 μm wide. Palpomere lengths (in μm): 25–35, 33–35, 120–123, 120–123, 200–213.
Thorax (Figure 30B). Dc 7–9; Ac 9–10; Pa 1; Scts 4–6. Halteres with 3–4 setae.
Wing (Figure 30C). VR 1.12–1.13. Brachiolum with 1 seta, Sc bare, R with 28–34 setae, R_1 with 28–29 setae, R_{4+5} with 27–37 setae, M_{1+2} with 40–50 setae, M_{3+4} with 30–36 setae, false vein with 60–70 setae, Cu with 19 setae, Cu_1 with 18–22 setae, PCu with 44–47 setae, An with 30–35 setae, remaining veins bare. Cell r_{4+5} with c. 200–210 setae, m with 22 setae, m_{1+2} with c. 120–140 setae, m_{3+4} with 90–100 setae, cu+an with 110–115 setae, remaining cells bare. Anal lobe strongly reduced.
Legs. Fore leg bearing single tibial spur, 25 μm long. Combs of mid tibia 20–25 μm wide with 25 μm long spur, and 15–25 μm wide with 15–25 μm long spur; combs of hind tibia 35 μm wide with 25 μm long spur, 20–36 μm wide with 30–38 μm long spur. Tarsomere 1 of mid leg with 2–3 sensilla chaetica distally. Lengths (in μm) and proportions of legs as in Table 11.

Hypopygium (Figure 31). Tergite IX 75–100 μm long, with 10 median setae near the base of anal point. Lateral tooth absent. Anal tergal bands of V-type, well separated, ending at mid-length of tergite. Anal point 48–53 μm long, with 4 lateral setae on each side, digitiform distal end; 4–6 spinulae placed in irregular row between anal crests which are fused posteriorly at half length of anal point. Transverse sternapodeme 50–53 μm long, with strong oral projections. Phallapodeme 90–105 μm long. Gonocoxite 133–135 μm long. Gonostylus with more or less straight inner margin, 118–128 μm long, with rounded apex. Superior volsella roundish with concave median margin,

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Table 11. Lengths (in μm) and proportions of legs for *Tanytarsus pseudoheusdensis* **sp. n.**

	fe	**ti**	**ta_1**	**ta_2**	**ta_3**	**ta_4**
p_1	800–860	460–490	900–990	460–480	380–400	180–310
p_2	800–820	610	380–390	200	130–140	80–90
p_3	900–930	810–840	590	345–360	280–300	190–200
	ta_5	**LR**	**BV**	**SV**	**BR**	
p_1	140–150	1.84–2.07	1.67–1.76	1.36–1.44	2.00–2.21	
p_2	70–75	0.60–0.63	3.64–3.73	3.67–3.71	3.88–4.01	
p_3	90–100	0.70–0.73	2.40–2.58	2.90–3.00	6.88–6.91	

slightly elongated and medially directed, bearing 2 anteromedian and 5–6 dorsal setae. Digitus long, with blunt apex, extending well beyond median margin of superior volsella. Stem of median volsella stout, barely longer than wide, bearing 3–5 subulate lamellae and a few simple setae. Inferior volsella weakly curved, 65–90 μm long, bearing 8–10 strong apical setae. HR 1.04–1.14, HV 2.23–2.48.
Female and immatures unknown.

Remarks

The new species is most similar to *Tanytarsus heusdensis* in the adult male, but can be separated morphologically from this species by having a lower AR and a median volsella bearing subulate lamellae. They also separate by more than 20% K2P-divergence in partial COI sequences (Figure 2).

Tanytarsus reei **Na & Bae, 2010**

Figures 32–33

Tanytarsus reei Na & Bae, 2010: 35, figure 2. Holotype ♂ (KU No. SWU-CHI-A-218), Korea, Gyeonggi-do, Namyangju, Sinwol-ri, Wangsukcheon, Imsonggyo, 14.V.2003, leg. K.B. Na & Y.J. Bae; Paratype ♂ (KU No. SWU-CHI-A-217), same as holotype [Examined].

Additional specimens examined

1♂ (NTNU-VM: 148247, BOLD Sample ID: XL180), Germany, Bavaria, Pfaffenhofen, Scheller-Mühle, Ilm River, 48.53°N, 11.509°E, 430 m a.s.l., 27.IV.2003, Sweep net, leg. E. Stur. China: 2♂♂ (BDN & BOLD Sample ID: XL12; BDN & BOLD Sample ID: XL13), Henan, Zhengzhou, Gongyi, Yungou Village, 34.564°N, 113.035°E, 760 m a.s.l., Sweep net, leg. S.L. Li; 1♂ (BDN: 21374), Heilongjiang Province, Shangzhi, Maoer Mountain, 25.VII.2003, Light trap, leg. J. Li.

Diagnosis

The adult male can be distinguished from known species of *Tanytarsus* by the following combination of characters: body mostly yellow-green with brown stripes on

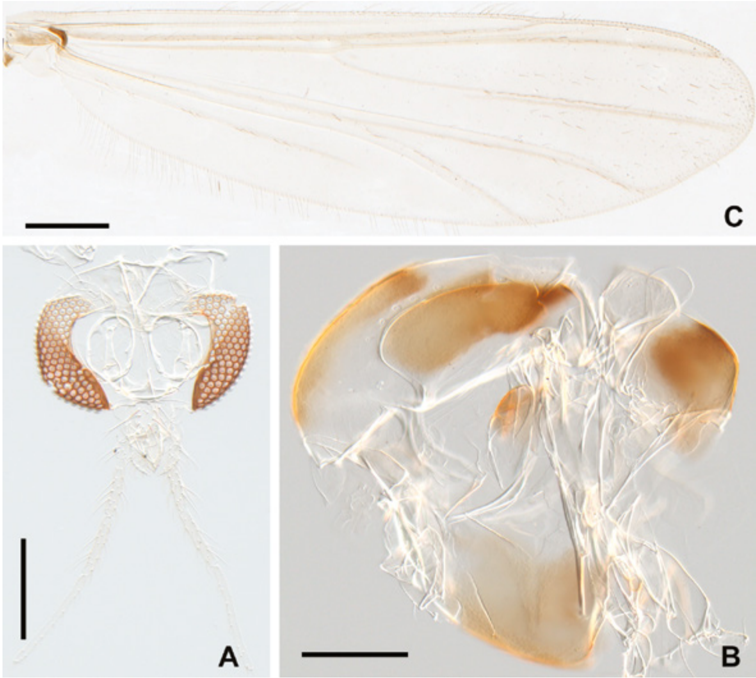

Fig. 32. *Tanytarsus reei*, male: A) head; B) thorax; C) wing. Scale bar = 200 µm.

Fig. 33. *Tanytarsus reei*, male: A) hypopygium dorsal view; B) hypopygium ventral view. Scale bar = 100 µm.

scutum, postnotum, preepisternum and median anepisternum II (Figure 32B); wing length 1.58–2.18 mm; wing membrane with comparatively few setae: vein Cu bare, vein PCu with 0–4 setae, cell m bare, cu+an with 3–7 setae; frontal tubercles small; AR 0.90–1.00; superior volsella sub-circular with concave inner margin, bearing 2 anteromedian and 5–7 dorsal setae; digitus long, with slightly swollen blunt apex, extending beyond median margin of superior volsella; median volsella short, slightly longer than wide, bearing several simple lamellae; transverse sternapodeme with strong oral projections.

Description

Adult male (n = 3). Total length 2.38–3.2 mm. Wing length 1.58–2.18 mm. Total length/wing length 1.47–1.53.
Colouration. Thorax ground colour brown with dark stripes anteriorly on scutum, laterally under parapsidal suture, on postnotum and preepisternum; pale brown median anepisternum II, epimeron II. Head, legs and abdomen yellow, slightly darker tarsi.
Head (Figure 32A). Antenna with 13 flagellomeres, ultimate flagellomere 430–495 µm long. AR 0.96–1.01. Frontal tubercles small, 5–8 µm long, 4–5 µm wide. Temporal setae 9–12. Clypeus with 12–14 setae. Tentorium 125–138 µm long, 30–43 µm wide. Palpomere lengths (in µm): 28–38, 28–50, 115–125, 105–128, 203–230.
Thorax (Figure 32B). Dc 7–9; Ac 6–7; Pa 1; Scts 2–6. Halteres with 2–6 setae.
Wing (Figure 32C). VR 1.12–1.13. Brachiolum with 1 seta, Sc bare, R with 17–23 setae, R_1 with 14–22 setae, R_{4+5} with 18–21 setae, M_{1+2} with 32–40 setae, M_{3+4} with 17–22 setae, false vein with 12–28 setae, Cu bare, Cu_1 with 14–19 setae, PCu with 0–4 setae, An with 20–35 setae, remaining veins bare. Cell r_{4+5} with c. 110–150 setae, m bare, m_{1+2} with c. 85–130 setae, m_{3+4} with 23–50 setae, cu+an with 3–7 setae, remaining cells bare. Anal lobe strongly reduced.
Legs. Fore leg bearing single tibial spur, 20–25 µm long. Combs of mid tibia 16–28 µm wide with 28–30 µm long spur, and 16–25 µm wide with 18–23 µm long spur; combs of hind tibia 24–40 µm wide with 28–30 µm long spur, 20–25 µm wide with 26–28 µm long spur. Tarsomere 1 of mid leg with 6–8 sensilla chaetica. Lengths (in µm) and proportions of legs as in Table 12.
Hypopygium (Figures 33A-B). Tergite IX 70–80 µm long, with 5–8 median setae near the base of anal point. Lateral tooth present. Anal tergal bands of V-type, well separated, ending at mid-length of tergite. Anal point with convex margins in basal part, parallel sided distal part, rounded apex, 38–51 µm long, with 3–4 lateral setae on each

Table 12. Lengths (in µm) and proportions of legs for *Tanytarsus reei* Na & Bae

	fe	**ti**	**ta_1**	**ta_2**	**ta_3**	**ta_4**
p_1	690–880	350–480	810–1010	410–540	340–420	280–350
p_2	680–870	530–670	310–420	180–220	100–160	70–110
p_3	750–950	700–900	490–650	290–380	250–300	180–200

	ta_5	**LR**	**BV**	**SV**	**BR**
p_1	120–150	2.10–2.31	1.61–1.65	1.28–1.35	2.36–4.00
p_2	60–100	0.57–0.63	3.32–3.71	3.67–4.00	4.31–5.78
p_3	90–130	0.58–0.70	2.40–2.48	2.85–3.00	3.88–4.02

side; 6–7 spinulae placed between anal crests which are fused posteriorly at 2/3 length of anal point. Transverse sternapodeme 48–65 µm long, with strong oral projections. Phallapodeme 102–105 µm long. Gonocoxite 93–150 µm long. Gonostylus with inner margin more or less straight, slightly curved inward, 120–125 µm long. Superior volsella sub-circular with concave inner margin, bearing 2 anteromedian and 5–7 dorsal setae. Digitus long, with slightly swollen blunt apex, extending beyond median margin of superior volsella. Stem of median volsella 8–13 µm long, barely longer than wide, bearing several simple lamellae. Inferior volsella weakly curved, 55–93 µm long, bearing 10–11 apical setae. HR 0.74–1.20, HV 1.90–2.56.
Female and immatures unknown.

Remarks

After re-examination of the holotype, we found a few median setae placed near the base of the anal point that were not mentioned in the original description (Na & Bae 2010). *Tanytarsus reei* is here redescribed based on additional material from China and Germany. The species is most similar to *T. heusdensis* in the adult male hypopygium, but can be separated from latter species by having bare wing vein Cu, a sub-circular superior volsella and a median volsella bearing simple lamellae. The two species also separate by more than 17% K2P divergence in partial COI sequences (Figure 2).

Distribution

China, Korea, Germany.

***Tanytarsus tamaduodecimus* Sasa, 1983**

Figures 34–35

Tanytarsus tamaduodecimus Sasa, 1983: 21, figure 17; Sasa & Kikuchi 1995: 51, figure 45E; Yamamoto & Yamamoto: 359. Holotype ♂ (NSMT Type no. 069: 061) Japan, Hikawa, Station C, 12.VI.1981, leg. M. Sasa & A. Shirasaka [Examined].

Tanytarsus tusimatneous Sasa & Suzuki, 1999: 33, figure 40; Yamamoto & Yamamoto: 360. Holotype ♂ (NSMT Type no. 354: 095) Japan, Tsushima, Mitsushima, Sumokawa, 35.III.1998, leg. H. Suzuki [Examined]. **syn. n.**

Tanytarsus tusimatpequeus Sasa & Suzuki, 1999: 35, figure 42; Yamamoto & Yamamoto: 360. Holotype ♂ (NSMT Type no. 353: 094) Japan, Tsushima, Izuhara, Uchiyama, 24.III.1998, leg. H. Suzuki [Not examined].

Additional material examined

China: 1♂ (BDN & BOLD Sample ID: LGT01), Shaanxi, Louguantai National Forest Park, Light trap, 34.0629°N, 108.3296°E, 600 m a.s.l., 25.VII.2013, leg. Q. Wang.

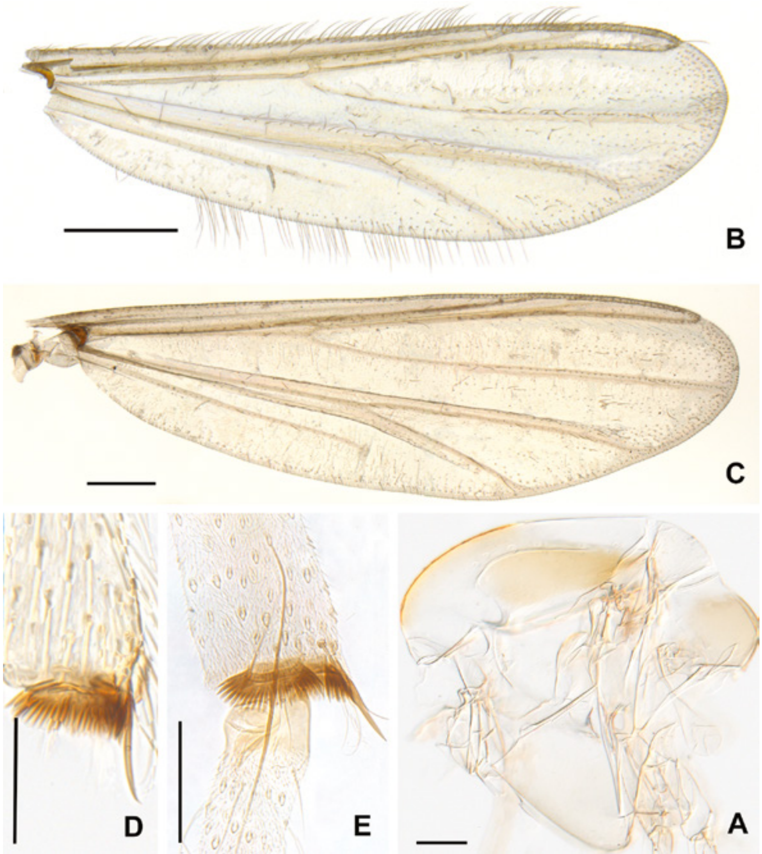

Fig. 34. *Tanytarsus tamaduodecimus*, male: A) thorax of Chinese specimen, scale bar = 100 µm; B) holotype wing, dry mounted, scale bar = 200 µm; C) wing of *Tanytarsus tusimatneous* **syn. n.**, holotype, dry mounted, scale bar = 200 µm; D) hind spur of Chinese specimen, scale bar = 50 µm; E) hind spur of *Tanytarsus tusimatneous* **syn. n.**, holotype, scale bar = 50 µm.

Diagnosis

The adult male can be distinguished from known species of *Tanytarsus* by the following combination of characters: body mostly yellow-green with pale brown stripes on scutum, postnotum, preepisternum and median anepisternum II (Figure 34A); AR 0.55–0.71; frontal tubercles minute; wing cell m bare; LR_1 2.24–2.55; only one comb on hind tibia bearing spur; ten median setae placed irregularly near the base of anal point; anal tergite bands of V-type, moderately widely separated medially; anal point triangular, tapering to pointed, attenuate, pigmented apex; several spinulae between anal crests which are fused posteriorly at 2/3 length of anal point; superior volsella subcircular, with concave inner margin, bearing two anteromedian and 5–7 dorsal setae; digitus curved, with blunt apex, extending beyond median margin of superior volsella, bearing one digitus-seta at base; stem of median volsella short, as long as wide, bearing 4–6 subulate lamellae; transverse sternapodeme with strong oral projections.

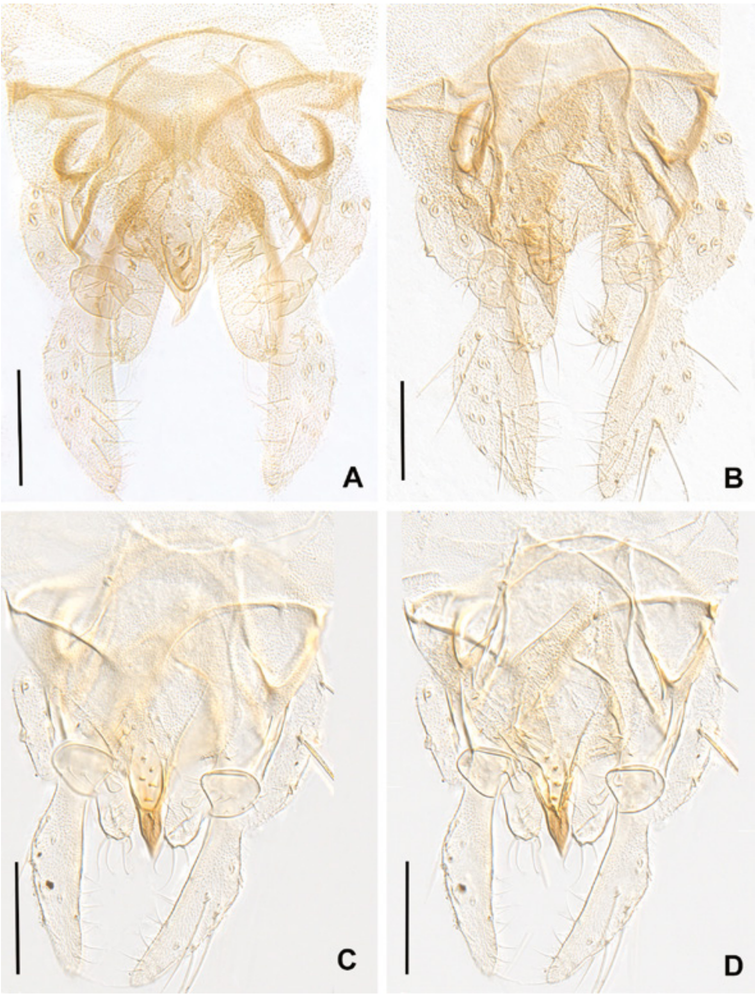

Fig. 35. *Tanytarsus tamaduodecimus*, male: A) holotype hypopygium; B) hypopygium of *Tanytarsus tusimatneous* **syn. n.**; C) dorsal view of hypopygium of Chinese specimen; D) ventral view of hypopygium of Chinese specimen. Scale bar = 50 µm.

Remarks

We examined the holotypes of *T. tamaduodecimus* and *T. tusimatneous* and compared the observed morphology with the original descriptions of *T. tamaduodecimus* and *T. tusimatneous* (Sasa 1983; Sasa & Suzuki 1999a). The two species are similar in all characters considered diagnostic, e.g. anal point tapering to a pointed, attenuate apex combined with the presence of a single spur on the hind tibia (unusual for *Tanytarsus*) and we therefore treat *T. tusimatneous* as a junior synonym of *T. tamaduodecimus*. The small differences observed in the hypopygia (Figure 35) are considered to be due to intraspecific variation and/or differences due to the slide mount. DNA barcode data support *T. tamaduodecimus* as a separate species from other species in the *T. heusdensis* species complex (Figure 2).

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Distribution

China, Japan.

Key to adult males of the *Tanytarsus heusdensis* species complex

1. Hind tibia bear one spur only (Figures 34D-E). China, Japan. .... *T. tamaduodecimus* Sasa
– Hind tibia bear two separate spurs, one on each comb 2
2. Wing vein Cu bare (Figure 32C). China, Germany, Korea. ..... *T. reei* Na & Bae
– Wing vein Cu setose 3
3. Body entirely brown; wing length < 1.5 mm; tarsomere 1 of mid leg without sensilla chaetica (Figure 26D); anal crests fused posteriorly, reaching the apex of anal point (Figure 27A). Norway. .... *T. adustus* **sp. n.**
– Body mostly yellow; wing length > 1.8 mm; tarsomere 1 of mid leg with sensilla chaetica; anal crests not as above 4
4. Gonostylus straight (Figure 31); AR 0.59–0.67. Germany. .... *T. pseudoheusdensis* **sp. n.**
– Gonostylus curved inward (Figure 29D); AR 1.02–1.10. Palaearctic region. .... *T. heusdensis* Goetghebuer

Discussion

DNA barcodes and morphospecies

In general, our results demonstrate that COI is an effective tool for species delimitation and recognition of cryptic species. However, a fixed threshold of COI sequences is not appropriate for all taxonomic groups. In insects for instance, a 2% threshold provides effective identification at the species level of Ephemeroptera (Schmidt et al. 2015; Webb et al. 2012; Zhou et al. 2010), Lepidoptera (Zahiri et al. 2014) and Trichoptera (Zhou et al. 2016), while a 2.2% threshold appears appropriate for Heteroptera (Knebelsberger et al. 2014), 2.5% for aquatic beetles (Monaghan et al. 2005), and >3% for various dipteran groups (Nzelu et al. 2015; Renaud et al. 2012). Furthermore, an average threshold of 4–5% appears appropriate for most Chironomidae (Lin et al. 2015; Meier et al. 2015), even if a 7% threshold has been reported for closely related species in this family (Carew & Hoffmann 2015).

In this study, unusual deep intraspecific K2P-divergences in DNA barcodes are observed. The 10% maximum intraspecific divergence in the *T. curticornis* species complex and 8% maximum intraspecific divergence in the *T. heusdensis* species complex are much higher than the average intraspecific divergences (0.9%–2.32%) previously reported in Chironomidae (Ekrem et al. 2007; Lin et al. 2015; Silva & Wiedenbrug 2014; Sinclair & Gresens 2008; Song et al. 2016). We nevertheless refrain from describing the divergent lineages as separate species since their morphology is consistent and regarded as conspecific. Moreover, nuclear markers show little or no divergence (Lin et al. in prep.), indicating that other explanations for the large divergence in

COI are likely. *Wolbachia* infections can lead to divergent mitochondrial lineages within insect species (Smith et al. 2012; Whitworth et al. 2007), but this bacterial endoparasite has not yet been found in Chironomidae. Although other endosymbionts might cause similar patterns, we are not aware of such documentation in Chironomidae and regard incomplete lineage sorting (Ballard & Whitlock 2004; Heckman et al. 2007; Willyard et al. 2009) a better explanation for the observed differences in DNA barcodes.

Taxonomy

Tanytarsus species have traditionally been subdivided into several species groups to aid in identification, species revisions, and classification. New molecular and morphological data show that the *T. curticornis* and *T. heusdensis* species complexes are distinct from the *T. chinyensis* species group (Lin et al. in prep.) and that characters previously used to define the *chinyensis* group (Giłka & Paasivirta 2009; Reiss & Fittkau 1971) have evolved several times within *Tanytarsus*. Thus, new sets of characters were needed to define the *T. curticornis* and *T. heusdensis* species complexes. This proved to be challenging, but we believe the suggested characters will hold as diagnostic features for species in these two complexes. Two candidate species for the here investigated species groups remain to be sampled for DNA before exclusion can be confirmed, but *T. manlyensis* Glover, 1973 and *T. trifidus* Freeman, 1958 have a S-shaped digitus, without pear-shaped apical lobe. Thus, even if these are superficially similar to species in the *T. curticornis* complex, they do not have the most characteristic feature of this complex.

Traditionally, in taxonomy of the Tanytarsini, some morphological features (e.g. body colouration and wing setation) have been ignored and often treated as intraspecific variation dependent on emergence period and temperature (Lindeberg 1960; Lindeberg 1963). However, there seems to be a constant interspecific variation in pigmentation for several of the *Tanytarsus* species treated here. For instance, *T. madeiraensis* **sp. n.** and *T. thomasi* **sp. n.** of the *T. curticornis* species complex have been regarded as *T. brundini* based on the high morphological similarity in the adult male hypopygium. However, these species have quite different colouration, and also show additional morphological differences when examined more closely (see key and diagnostic characters above). A potential additional species very similar to *T. brundini* was reported by Lindeberg (1963) from Finland. We have not been able to sample fresh material from this population for DNA comparison and are unable to separate it morphologically from *T. brundini*. In the *T. heusdensis* species complex, *T. adustus* **sp. n.** and *T. pseudoheusdensis* **sp. n.** have been misidentified as *T. heusdensis* in previous barcode studies due to the similar shape in the adult male hypopygium (Ekrem et al. 2010). Upon re-examination of the barcode voucher specimens, these genetically distinct semi-cryptic species also can be separated from the nominal *T. heusdensis* by differences in body colouration and wing setation.

Immature life stages are important in biodiversity assessments of chironomids and biological monitoring (Anderson et al. 2013; Ekrem et al. 2010; Ferrington et al. 1991; Kranzfelder et al. 2015; Raunio et al. 2007). In particular chironomid pupal exuviae might provide important characters to help resolve taxonomic issues for cryptic species complex. Unfortunately, there are few species with associated and described immature life stages in the *T. curticornis* and *T. heusdensis* complexes, but those known display characters that appear to be species specific. For instance, morphological features of pupa of *T. madeiraensis* **sp. n.** (Figure 13) will separate this species from others in the *T. curticornis* complex with known pupae. Since it is time-consuming to rear, the immature stages can be associated with adults using DNA barcodes.

Conclusion

Our study has revealed strong concordance between morphological species concepts and DNA barcodes. After reviewing the *T. curticornis* and *T. heusdensis* species complexes using DNA barcodes, we have detected and described eight new species, one new junior synonym, several misidentifications, and provided keys to adult male and known pupae. In the future, a comprehensive sampling of these species, in particular the immature life stages, is required to provide more morphological characters and expand the DNA barcode database for chironomids. With the rapid development and lowered costs of modern molecular techniques, integrative taxonomy combining morphological and molecular data should be more available to Chironomidae taxonomists. This will certainly provide the scientific community more accurate measures of the diversity in one of the most abundant and species rich groups of aquatic insects.

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Table S1. Kimura 2-parameter pairwise genetic distances of the *Tanytarsus curticornis* species complex.

SOE239_*Tanytarsus brundini*											
Finnmark240_*Tanytarsus brundini*	0.04										
XL402_*Tanytarsus brundini*	0.04	0.02									
Finnmark239_*Tanytarsus brundini*	0.05	0.02	0.03								
XL148_*Tanytarsus brundini*	0.05	0.03	0.03	0.02							
TRD-CH137_*Tanytarsus brundini*	0.04	0.01	0.02	0.02	0.02						
To137_*Tanytarsus brundini*	0.05	0.02	0.03	0.02	0.02	0.02					
XL405_*Tanytarsus brundini*	0.05	0.03	0.01	0.03	0.04	0.03	0.04				
SOE248_*Tanytarsus brundini*	0.03	0.03	0.04	0.03	0.04	0.03	0.03	0.04			
XL403_*Tanytarsus brundini*	0.05	0.02	0.01	0.02	0.02	0.02	0.03	0.02	0.04		
SOE13_*Tanytarsus brundini*	0.00	0.04	0.04	0.05	0.05	0.04	0.05	0.05	0.03	0.05	
XL404_*Tanytarsus brundini*	0.04	0.02	0.01	0.03	0.03	0.02	0.03	0.02	0.04	0.01	0.04
SOE05_*Tanytarsus brundini*	0.00	0.04	0.04	0.05	0.05	0.04	0.04	0.05	0.03	0.04	0.00
XL407_*Tanytarsus brundini*	0.05	0.02	0.00	0.02	0.03	0.02	0.03	0.01	0.04	0.01	0.05
To82_*Tanytarsus curticornis*	0.16	0.16	0.16	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.16
XL99_*Tanytarsus curticornis*	0.16	0.17	0.16	0.17	0.17	0.17	0.17	0.17	0.16	0.17	0.16
CHIR_CH342_*Tanytarsus heberti*	0.20	0.20	0.20	0.21	0.21	0.21	0.21	0.20	0.20	0.21	0.20
MA18_*Tanytarsus madeiraensis*	0.14	0.13	0.13	0.14	0.14	0.13	0.14	0.14	0.13	0.14	0.13
MA19_*Tanytarsus madeiraensis*	0.14	0.13	0.13	0.14	0.14	0.13	0.14	0.14	0.13	0.14	0.13
XL222_*Tanytarsus songi*	0.16	0.16	0.17	0.17	0.17	0.17	0.17	0.17	0.16	0.17	0.16
XL423_*Tanytarsus tamaoctavus*	0.13	0.14	0.14	0.16	0.15	0.16	0.16	0.15	0.14	0.14	0.13
XL351_*Tanytarsus tamaoctavus*	0.13	0.14	0.14	0.16	0.15	0.16	0.16	0.15	0.14	0.14	0.13
XL287_*Tanytarsus tamaoctavus*	0.13	0.14	0.14	0.16	0.15	0.15	0.15	0.15	0.14	0.14	0.13
CHIR_CH165_*Tanytarsus thomasi*	0.16	0.16	0.16	0.17	0.17	0.17	0.17	0.17	0.17	0.16	0.16
CHIR_CH224_*Tanytarsus thomasi*	0.16	0.16	0.16	0.17	0.17	0.17	0.16	0.17	0.17	0.16	0.16
Finnmark666_*Tanytarsus thomasi*	0.16	0.17	0.17	0.18	0.17	0.18	0.17	0.18	0.17	0.17	0.16
CHIR_CH164_*Tanytarsus thomasi*	0.18	0.18	0.17	0.18	0.18	0.18	0.17	0.17	0.17	0.17	0.18
CHIR_CH167_*Tanytarsus thomasi*	0.16	0.16	0.16	0.17	0.17	0.17	0.16	0.16	0.17	0.16	0.16
CHIR_CH124_*Tanytarsus thomasi*	0.17	0.17	0.17	0.17	0.17	0.18	0.16	0.17	0.17	0.17	0.17
CHIR_CH166_*Tanytarsus thomasi*	0.16	0.16	0.16	0.17	0.17	0.17	0.17	0.17	0.17	0.16	0.16
XL329_*Tanytarsus tongmuensis*	0.16	0.18	0.16	0.18	0.18	0.18	0.18	0.17	0.16	0.16	0.16
XL314_*Tanytarsus tongmuensis*	0.16	0.18	0.16	0.18	0.18	0.18	0.18	0.17	0.16	0.16	0.16
XL324_*Tanytarsus tongmuensis*	0.16	0.18	0.16	0.18	0.18	0.18	0.18	0.17	0.16	0.16	0.16
XL317_*Tanytarsus tongmuensis*	0.17	0.18	0.17	0.18	0.19	0.18	0.18	0.17	0.17	0.17	0.17
XL333_*Tanytarsus tongmuensis*	0.16	0.18	0.16	0.18	0.18	0.18	0.18	0.17	0.16	0.16	0.16
XL330_*Tanytarsus tongmuensis*	0.16	0.18	0.16	0.18	0.18	0.18	0.18	0.17	0.16	0.16	0.16
XL344_*Tanytarsus tongmuensis*	0.17	0.18	0.17	0.18	0.19	0.18	0.18	0.17	0.17	0.17	0.17
XL323_*Tanytarsus tongmuensis*	0.16	0.18	0.16	0.18	0.18	0.18	0.18	0.17	0.16	0.16	0.16
XL319_*Tanytarsus tongmuensis*	0.16	0.18	0.16	0.18	0.18	0.18	0.18	0.17	0.16	0.16	0.16
XL334_*Tanytarsus tongmuensis*	0.16	0.18	0.16	0.18	0.18	0.18	0.18	0.17	0.16	0.16	0.16
XL331_*Tanytarsus tongmuensis*	0.17	0.18	0.17	0.18	0.19	0.18	0.18	0.17	0.17	0.17	0.17
XL346_*Tanytarsus tongmuensis*	0.16	0.18	0.16	0.18	0.18	0.18	0.18	0.17	0.16	0.16	0.16
XL326_*Tanytarsus tongmuensis*	0.16	0.18	0.16	0.18	0.18	0.18	0.18	0.17	0.16	0.16	0.16
XL345_*Tanytarsus tongmuensis*	0.16	0.17	0.16	0.18	0.18	0.17	0.18	0.16	0.16	0.16	0.16
XL315_*Tanytarsus tongmuensis*	0.16	0.18	0.16	0.18	0.18	0.18	0.18	0.17	0.16	0.16	0.16
XL348_*Tanytarsus tongmuensis*	0.16	0.18	0.16	0.18	0.18	0.18	0.18	0.17	0.16	0.16	0.16
XL332_*Tanytarsus tongmuensis*	0.16	0.18	0.16	0.18	0.18	0.18	0.18	0.17	0.16	0.16	0.16
XL349_*Tanytarsus tongmuensis*	0.16	0.18	0.16	0.18	0.18	0.18	0.18	0.17	0.16	0.16	0.16
XL347_*Tanytarsus tongmuensis*	0.16	0.18	0.16	0.18	0.18	0.18	0.18	0.17	0.16	0.16	0.16
XL335_*Tanytarsus tongmuensis*	0.16	0.18	0.16	0.18	0.18	0.18	0.18	0.17	0.16	0.16	0.16
XL327_*Tanytarsus tongmuensis*	0.16	0.18	0.16	0.18	0.18	0.18	0.18	0.17	0.16	0.16	0.16
XL316_*Tanytarsus tongmuensis*	0.16	0.18	0.16	0.18	0.18	0.18	0.18	0.17	0.16	0.16	0.16
XL1_*Tanytarsus wangi*	0.16	0.15	0.16	0.17	0.17	0.16	0.17	0.17	0.16	0.16	0.16

0.04															
0.01	0.04														
0.16	0.16	0.16													
0.17	0.16	0.16	0.01												
0.20	0.20	0.20	0.17	0.18											
0.14	0.13	0.14	0.16	0.16	0.20										
0.14	0.13	0.14	0.16	0.16	0.20	0.00									
0.16	0.16	0.17	0.19	0.20	0.23	0.19	0.19								
0.14	0.13	0.14	0.15	0.16	0.19	0.16	0.16	0.15							
0.14	0.13	0.14	0.15	0.16	0.19	0.16	0.16	0.15	0.00						
0.14	0.13	0.14	0.15	0.16	0.19	0.16	0.16	0.15	0.00	0.00					
0.16	0.16	0.16	0.16	0.15	0.15	0.20	0.20	0.19	0.17	0.17	0.17				
0.16	0.16	0.16	0.16	0.15	0.15	0.20	0.20	0.20	0.17	0.17	0.17	0.02			
0.16	0.16	0.17	0.16	0.16	0.16	0.20	0.20	0.19	0.17	0.17	0.17	0.02	0.02		
0.17	0.18	0.17	0.15	0.16	0.16	0.21	0.21	0.21	0.18	0.18	0.18	0.09	0.10	0.10	
0.16	0.16	0.16	0.15	0.15	0.15	0.20	0.20	0.19	0.16	0.16	0.15	0.03	0.03	0.03	0.10
0.16	0.17	0.17	0.15	0.15	0.16	0.20	0.20	0.19	0.16	0.16	0.16	0.03	0.03	0.04	0.09
0.16	0.16	0.16	0.16	0.15	0.15	0.20	0.20	0.19	0.17	0.17	0.17	0.00	0.02	0.02	0.09
0.16	0.16	0.16	0.18	0.19	0.19	0.18	0.18	0.20	0.14	0.14	0.14	0.20	0.19	0.20	0.20
0.16	0.16	0.16	0.18	0.19	0.19	0.18	0.18	0.20	0.14	0.14	0.14	0.20	0.19	0.20	0.20
0.16	0.16	0.16	0.18	0.19	0.19	0.18	0.18	0.20	0.14	0.14	0.14	0.20	0.19	0.20	0.20
0.17	0.16	0.17	0.18	0.18	0.19	0.18	0.18	0.20	0.13	0.13	0.13	0.20	0.19	0.20	0.20
0.16	0.16	0.16	0.18	0.19	0.19	0.18	0.18	0.20	0.14	0.14	0.14	0.20	0.19	0.20	0.20
0.16	0.16	0.16	0.18	0.19	0.19	0.18	0.18	0.20	0.14	0.14	0.14	0.20	0.19	0.20	0.20
0.17	0.16	0.17	0.18	0.18	0.19	0.18	0.18	0.20	0.13	0.13	0.13	0.20	0.19	0.20	0.20
0.16	0.16	0.16	0.18	0.19	0.19	0.18	0.18	0.20	0.14	0.14	0.14	0.20	0.19	0.20	0.20
0.16	0.16	0.16	0.18	0.19	0.19	0.18	0.18	0.20	0.14	0.14	0.14	0.20	0.19	0.20	0.20
0.16	0.16	0.16	0.18	0.19	0.19	0.18	0.18	0.20	0.14	0.14	0.14	0.20	0.19	0.20	0.20
0.17	0.16	0.17	0.18	0.18	0.19	0.18	0.18	0.20	0.13	0.13	0.13	0.20	0.19	0.20	0.20
0.16	0.16	0.16	0.18	0.19	0.19	0.18	0.18	0.20	0.14	0.14	0.14	0.20	0.19	0.20	0.20
0.16	0.16	0.16	0.18	0.19	0.19	0.18	0.18	0.20	0.14	0.14	0.14	0.20	0.19	0.20	0.20
0.16	0.16	0.16	0.18	0.19	0.20	0.18	0.18	0.20	0.14	0.14	0.14	0.20	0.19	0.20	0.20
0.16	0.16	0.16	0.18	0.19	0.19	0.18	0.18	0.20	0.14	0.14	0.14	0.20	0.19	0.20	0.20
0.16	0.16	0.16	0.18	0.19	0.19	0.18	0.18	0.20	0.14	0.14	0.14	0.20	0.19	0.20	0.20
0.16	0.16	0.16	0.18	0.19	0.19	0.18	0.18	0.20	0.14	0.14	0.14	0.20	0.19	0.20	0.20
0.16	0.16	0.16	0.18	0.19	0.19	0.18	0.18	0.20	0.14	0.14	0.14	0.20	0.19	0.20	0.20
0.16	0.16	0.16	0.18	0.18	0.19	0.18	0.18	0.20	0.13	0.13	0.13	0.20	0.19	0.20	0.19
0.16	0.16	0.16	0.18	0.19	0.19	0.18	0.18	0.20	0.14	0.14	0.14	0.20	0.19	0.20	0.20
0.16	0.16	0.16	0.18	0.19	0.19	0.18	0.18	0.20	0.14	0.14	0.14	0.20	0.19	0.20	0.20
0.16	0.16	0.16	0.18	0.19	0.19	0.18	0.18	0.20	0.14	0.14	0.14	0.20	0.19	0.20	0.20
0.16	0.16	0.16	0.18	0.19	0.20	0.16	0.16	0.18	0.12	0.12	0.12	0.18	0.16	0.17	0.18

(Continued)

Table S1. *(Cont.)*

CHIR_CH124_*Tanytarsus thomasi*	0.00										
CHIR_CH166_*Tanytarsus thomasi*	0.03	0.03									
XL329_*Tanytarsus tongmuensis*	0.18	0.19	0.20								
XL314_*Tanytarsus tongmuensis*	0.18	0.19	0.20	0.00							
XL324_*Tanytarsus tongmuensis*	0.18	0.19	0.20	0.00	0.00						
XL317_*Tanytarsus tongmuensis*	0.18	0.18	0.20	0.02	0.02	0.02					
XL333_*Tanytarsus tongmuensis*	0.18	0.19	0.20	0.00	0.00	0.00	0.02				
XL330_*Tanytarsus tongmuensis*	0.18	0.19	0.20	0.00	0.00	0.00	0.02	0.00			
XL344_*Tanytarsus tongmuensis*	0.18	0.18	0.20	0.02	0.02	0.02	0.00	0.02	0.02		
XL323_*Tanytarsus tongmuensis*	0.18	0.19	0.20	0.00	0.00	0.00	0.02	0.00	0.00	0.02	
XL319_*Tanytarsus tongmuensis*	0.18	0.19	0.20	0.00	0.00	0.00	0.02	0.00	0.00	0.02	0.00
XL334_*Tanytarsus tongmuensis*	0.18	0.19	0.20	0.00	0.00	0.00	0.02	0.00	0.00	0.02	0.00
XL331_*Tanytarsus tongmuensis*	0.18	0.18	0.20	0.02	0.02	0.02	0.00	0.02	0.02	0.00	0.02
XL346_*Tanytarsus tongmuensis*	0.18	0.19	0.20	0.00	0.00	0.00	0.02	0.00	0.00	0.02	0.00
XL326_*Tanytarsus tongmuensis*	0.18	0.19	0.20	0.00	0.00	0.00	0.02	0.00	0.00	0.02	0.00
XL345_*Tanytarsus tongmuensis*	0.18	0.19	0.20	0.00	0.00	0.00	0.02	0.00	0.00	0.02	0.00
XL315_*Tanytarsus tongmuensis*	0.18	0.19	0.20	0.00	0.00	0.00	0.02	0.00	0.00	0.02	0.00
XL348_*Tanytarsus tongmuensis*	0.18	0.19	0.20	0.00	0.00	0.00	0.02	0.00	0.00	0.02	0.00
XL332_*Tanytarsus tongmuensis*	0.18	0.19	0.20	0.00	0.00	0.00	0.02	0.00	0.00	0.02	0.00
XL349_*Tanytarsus tongmuensis*	0.18	0.19	0.20	0.00	0.00	0.00	0.02	0.00	0.00	0.02	0.00
XL347_*Tanytarsus tongmuensis*	0.18	0.19	0.20	0.00	0.00	0.00	0.02	0.00	0.00	0.02	0.00
XL335_*Tanytarsus tongmuensis*	0.18	0.19	0.20	0.00	0.00	0.00	0.02	0.00	0.00	0.02	0.00
XL327_*Tanytarsus tongmuensis*	0.18	0.19	0.20	0.00	0.00	0.00	0.02	0.00	0.00	0.02	0.00
XL316_*Tanytarsus tongmuensis*	0.18	0.19	0.20	0.00	0.00	0.00	0.02	0.00	0.00	0.02	0.00
XL1_*Tanytarsus wangi*	0.16	0.17	0.18	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08

0.00													
0.02	0.02												
0.00	0.00	0.02											
0.00	0.00	0.02	0.00										
0.00	0.00	0.02	0.00	0.00									
0.00	0.00	0.02	0.00	0.00	0.00								
0.00	0.00	0.02	0.00	0.00	0.00	0.00							
0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00						
0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00					
0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00			
0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08

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Table S2. Kimura 2-parameter pairwise genetic distances of the *Tanytarsus heusdensis* species complex.

SOE380_*Tanytarsus adustus*																		
SOE247_*Tanytarsus adustus*	0.01																	
SOE386_*Tanytarsus adustus*	0.00	0.01																
XL459_*Tanytarsus adustus*	0.00	0.01	0.00															
SOE387_*Tanytarsus adustus*	0.00	0.01	0.00	0.00														
XL183_*Tanytarsus heusdensis*	0.16	0.16	0.16	0.16	0.16													
XL186_*Tanytarsus heusdensis*	0.17	0.17	0.17	0.17	0.17	0.01												
CH-OSF104_*Tanytarsus heusdensis*	0.16	0.16	0.16	0.16	0.16	0.00	0.01											
ES79_*Tanytarsus pseudoheusdensis*	0.21	0.21	0.21	0.21	0.21	0.20	0.21	0.20										
ES77_*Tanytarsus pseudoheusdensis*	0.21	0.21	0.21	0.21	0.21	0.20	0.21	0.20	0.00									
JN887106_*Tanytarsus reei*	0.17	0.18	0.18	0.18	0.17	0.17	0.18	0.17	0.20	0.20								
XL180_*Tanytarsus reei*	0.19	0.19	0.19	0.19	0.19	0.17	0.17	0.17	0.21	0.21	0.07							
JN887104_*Tanytarsus reei*	0.18	0.19	0.19	0.19	0.18	0.17	0.18	0.17	0.20	0.20	0.01	0.07						
JN887105_*Tanytarsus reei*	0.18	0.18	0.18	0.18	0.18	0.17	0.18	0.17	0.20	0.20	0.00	0.08	0.01					
XL13_*Tanytarsus reei*	0.18	0.18	0.18	0.19	0.18	0.17	0.18	0.17	0.20	0.20	0.01	0.08	0.00	0.01				
JN887103_*Tanytarsus reei*	0.18	0.18	0.18	0.18	0.18	0.17	0.18	0.17	0.20	0.20	0.00	0.08	0.01	0.00	0.01			
JN887102_*Tanytarsus reei*	0.18	0.18	0.18	0.18	0.18	0.17	0.18	0.17	0.20	0.20	0.00	0.08	0.01	0.00	0.01	0.00		
XL12_*Tanytarsus reei*	0.18	0.18	0.18	0.18	0.18	0.17	0.18	0.17	0.21	0.21	0.02	0.08	0.02	0.02	0.02	0.02	0.02	
LGT01_*Tanytarsus tamaduodecimus*	0.18	0.18	0.18	0.18	0.18	0.17	0.18	0.17	0.19	0.19	0.16	0.17	0.16	0.16	0.16	0.16	0.16	0.16