Molecular phylogeny and temporal diversification of *Tanytarsus* van der Wulp (Diptera: Chironomidae) support generic synonymies, a new classification and centre of origin

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**Abstract.** *Tanytarsus* van der Wulp, with 355 currently known valid species, is a comparatively large genus of nonbiting midges (Diptera: Chironomidae). Relationships among *Tanytarsus* and associated genera and among species in the genus have been exceptionally difficult to resolve using morphology or single gene genealogies. Here, the phylogeny of *Tanytarsus* sensu lato is reconstructed based on the combined analysis of five nuclear markers, including both ribosomal (18S) and protein-coding (AATS1, CAD, PGD and TPI) genes. Our results indicate that *Tanytarsus* is paraphyletic with *Caladomyia* Säwedal placed among South American *Tanytarsus*, *Virgatanytarsus* Pinder as part of a Gondwanan clade, and *Corynocera* Zetterstedt within the *Tanytarsus norvegicus* (Kieffer) species group. Based on these results, we synonymize *Caladomyia* and *Virgatanytarsus* with *Tanytarsus*. We propose retaining the older name *Corynocera* until an eventual International Code of Zoological Nomenclature ruling on precedence can be made regarding the more frequent usage of the younger name *Tanytarsus*. As expected, the previously synonymized *Nimbocera* Reiss is confirmed to be a junior synonym of *T. rhabdomantis* (Trivinho-Strixino & Strixino) grouping with other Neotropical *Tanytarsus*. The genus *Sublettea* Roback remains valid outside of *Tanytarsus + Cladotanytarsus* Kieffer. The monophyly of some established species groups is well supported, while other groups are refuted or remain uncertain. Based on a calibrated molecular divergence time analysis, *Tanytarsus* diverged from its sister group *Cladotanytarsus* during the Late Cretaceous to Early Paleogene (61–79 Ma). The genus most likely originated in the Oriental and Palearctic regions.

**Introduction**

Over the last decade, advances in DNA sequencing technologies, bioinformatics and computational biology provided a large amount of molecular data and improved the tools used to analyse them (Goodwin et al., 2016). Some uncertain evolutionary relationships in insects have been resolved by phylogenomics using a large number of genes (Cameron, 2014; Misof et al., 2014), and within Diptera, many molecular phylogenies have been carried out to explore the relationships among families, subfamilies, tribes, genera and species levels (Stähl et al., 2003; Kjer et al., 2006; Kutty et al., 2010; Tachi & Shima, 2010; Cranston et al., 2012; Virgilio et al., 2015; Winterton et al., 2016; Buenaventura & Pape, 2017; Hash et al., 2018). Within Chironomidae, several molecular phylogenies on various groups have been produced, but only one study has attempted to reconstruct the evolutionary history of critical genera in all subfamilies (Cranston et al., 2012). These authors confirm that all sampled subfamilies of Chironomidae, except for Prodiamesinae, are monophyletic, while the tempo of diversification of the family shows a Permian origin with subfamily stem-group origin from the Mid-Late Triassic to the Early Cretaceous.

The genus *Tanytarsus* van der Wulp has 355 currently known valid species worldwide (P. Ashe, personal communication) and is one of the most diverse genera in Chironomidae. The taxonomy and systematics of *Tanytarsus* have received a
considerable amount of attention. Reiss & Fittkau (1971) revised the western Palaearctic *Tanytarsus* and erected 11 species groups based on the morphology of adult males. Likewise, Glover (1973) revised the Australian Tanytarsini, and classified them into five species groups of *Tanytarsus* based on adult male morphology. The Afrotropical fauna was reviewed as part of Freeman’s work on African Chironominae south of the Sahara (Freeman, 1958). The Australian monsoonal *Tanytarsus* were reviewed by Cranston (2000). Species mostly belonging to the *T. eminulus, T. gregarius, T. lugens* and *T. mendax* species groups from Africa, Australia, North America and South and East Asia were revised by Ekrem (2001a, 2001b, 2002, 2004) and Ekrem et al. (2003). The Neotropical fauna has also been described quite extensively (Sublette & Sasa, 1994; Ekrem & Reiss, 1999; Sanseverino & Wiedenbrug, 2000; Sanseverino et al., 2002; Sanseverino, 2006; Sanseverino & Fittkau, 2006; Cranston, 2007; Trivinho-Strixino & Strixino, 2007; Vinogradova et al., 2009; Sanseverino & Trivinho-Strixino, 2010; Gilka & Zakrzewska, 2013; Trivinho-Strixino et al., 2015; Dantas & Gilka, 2017; Trivinho-Strixino & Shimabukuro, 2017), and Sanseverino et al. (2010) suggested the synonymy of the New World genus *Nimbocera* Reiss with *Tanytarsus*. Despite these past efforts, many *Tanytarsus* species remain to be discovered and described. Even in regions considered to be well investigated, species new to science are found on a regular basis (Ghonaim et al., 2004; Ekrem & Halvorsen, 2007; Ekrem & Stur, 2007; Gilka & Pasivirta, 2007; Gilka & Pasivirta, 2008; Gilka & Pasivirta, 2009; Lin et al., 2015, 2017). The increased use of molecular tools in taxonomy has aided the discovery of new cryptic and semicryptic species in *Tanytarsus* (Lin et al., 2015, 2017) and more is to be expected as less well-investigated regions, such as the East Palaearctic and the Oriental regions, are comprehensively explored.

The generic concept of *Tanytarsus* includes species with adults, larvae and pupae similar to those of *Caladomyia* Säwedal, *Corynocera* Zetterstedt, *Sublettea* Roback and *Virgatanytarsus* Pinder (Pinder & Reiss, 1986; Cranston et al., 1989; Sanseverino et al., 2010; Epler et al., 2013). The morphological diagnostic features of these purported genera have not been tested in a phylogenetic framework and it is uncertain if they are compatible with a monophyletic *Tanytarsus* as currently defined.

The genus *Caladomyia* is largely Neotropical with a few species reaching the southern and southwestern USA (Säwedal, 1981; Trivinho-Strixino, 2012). One extinct species is recorded from Eocene Baltic amber (Zakrzewska & Gilka, 2013). Adult males of *Caladomyia* can be separated from other genera by the posteriorly directed bars on the hypopygial anal point (Säwedal, 1981). However, the pupae and larvae of *Caladomyia* cannot be separated from *Tanytarsus*. Hence, we suspect a phylogenetic position of this genus close to or within *Tanytarsus*.

*Corynocera* is Holarctic with a questionable record from New Zealand based on larval subfossil head capsules (Hirvenoja, 1961). The genus includes two described and at least one undescribed species (Epler et al., 2013). The adults are water skaters having comparatively long legs and oar-shaped wings apparently adapted for water surface swarming. These characters are considered diagnostic for adult male *Corynocera*, while wing sheaths without a nose in the pupae (Pinder & Reiss, 1986; Langton, 1991) and the offset central three teeth of the larval mentum are regarded as diagnostic in the immatures. However, the former character is also found in an undescribed species of *Tanytarsus* from Tibet (X.-L. Lin et al., unpublished data) and the latter has been observed in the parthenogenetic *Tanytarsus heliomesonyctios* Langton (Stur & Ekrem, 2011). Disregarding the special morphological (adaptive) characters (autapomorphies) in the adult male, *Corynocera* cannot be separated from *Tanytarsus*.

*Sublettea* was previously regarded as a subgenus of *Tanytarsus* and is recorded from the Oriental and Holarctic regions, including two described and at least two undescribed species (Roback, 1975; Ashe & O’Connor, 1995; Epler et al., 2013). Although the genus was shown as separate from *Tanytarsus* in Cranston et al. (2012), *Tanytarsus* was too sparsely sampled to be confident of this placement.

*Virgatanytarsus* was erected for the *triangularis* group within *Tanytarsus*, with records from the Afrotropical and Palaearctic regions (Pinder, 1982). Pinder’s (1982) generic diagnosis for adult males separates *Virgatanytarsus* from *Tanytarsus* by the anteriorly directed bars on the hypopygial anal point. Pupae are separated by the broad lateral comb on abdominal segment VIII (Pinder & Reiss, 1986), and larvae by posterior parapod claws having numerous small hooklets arranged in multiple rows (Pinder, 1982; Epler et al., 2013). However, all these character states occur in other *Tanytarsus* species (Cranston, 2000; Sanseverino, 2006; Sanseverino & Trivinho-Strixino, 2010). Hence, there are currently no morphological characters that exclusively separate *Virgatanytarsus* from *Tanytarsus*, suggesting the potential synonymy of these genera.

Morphology alone may be insufficient to generate stable phylogenetic hypotheses at the species-level in chironomids because recent species radiation and parallel selection has caused a high level of homoplasy in studied characters (Sæther, 1979). Moreover, lack of larval and pupal data may result in deficient taxon-character matrices. For instance, morphological characters did not support the postulated monophyly of the *Tanytarsus eminulus, gregarius, lugens* and *mendax* groups in unweighted parsimony analyses by Ekrem (2003), and some nodes needed constraints based on unique synapomorphies and/or evidence from molecular data to retrieve monophyletic species groups.

Previous molecular phylogenetic work on *Tanytarsus* has been minimal. Ekrem & Willassen (2004) explored Tanytarsini relationships using a single mitochondrial gene (COI), but did not aim to test outgroup relationships between *Tanytarsus* and morphologically most similar genera, nor was their sampling sufficient to address the evolutionary relationships within the genus.

Here we infer the evolutionary history of *Tanytarsus* sensu lato using multiple nuclear genetic markers from over 130 taxa. Specifically, we address three questions: (i) does *Tanytarsus* represent a monophylum; (ii) what is the phylogenetic relationship among species of *Tanytarsus, Caladomyia, Corynocera, Sublettea* and *Virgatanytarsus*; and (iii) what is the biogeographical history of the genus *Tanytarsus*, including its centre of origin and tempo of diversification?
Material and methods

Taxon sampling

To detect and avoid laboratory contamination and misidentifications, we selected two individuals of each species, unless only one specimen was available. For the phylogenetic analyses, we used a reduced dataset with species represented by one specimen to reduce computing time. After initial phylogenetic analysis, the position of two taxa of Tanytarsus, Tanytarsus cf. riopreto and Tanytarsus shouautumnalis Sasa, were highly unstable in analyses, and were excluded as rogue taxa to improve phylogenetic accuracy (Aberer et al., 2013). Moreover, two initially selected outgroups, Pontomyia natans Edwards and Thienemanniola ploenensis Kieffer, were excluded from the dataset after initial analyses as they were placed well outside of Tanytarsus sensu lato, displaying very long branches in the initial phylogenetic trees. Hence, our final dataset comprised 130 morphospecies, 111 of which represented Tanytarsus sensu stricto, while the remaining 19 species from six genera were considered outgroups or potential members of Tanytarsus sensu lato: five species belong to the genus Caladomyia, four to Cladotanytarsus, one to Corynecora, one to Paratanytarsus Thienemann & Bause, one to Rheotanytarsus Thienemann & Bause, one to Sublettea and six to Virgatanytarsus. In addition, Tanytarsus rhabdomantis (Trivinho-Strixino & Strixino), originally placed in Nimboecera (itself a junior synonym of Tanytarsus) was included to confirm the synonymy of Nimboecera and Tanytarsus. Approximately 99% of all DNA sequences used were generated as part of this study.

A list of all species, specimens, their individual images, georeferences, primers, sequences and other relevant laboratory data of all sequence specimens can be seen online in the Barcode of Life Data Systems (BOLD) (Ratnasingham & Hebert, 2013). Voucher specimens are deposited at the Department of Natural History, NTNU University Museum, Trondheim, Norway, the Department of Natural History, Bergen University Museum, Bergen, Norway, the Department of Natural History, Bergen University Museum, Bergen, Norway, and the College of Life Sciences, Nankai University, Tianjin, China. Specimens were identified morphologically using relevant taxonomic revisions and species descriptions (e.g. Lindeberg, 1963, 1967; Reiss & Fittkau, 1971; Glover, 1973; Sasa, 1980; Sasa & Kawai, 1987; Sublette & Sasa, 1994; Cranston, 2000; Ekrem, 2001a, 2002; Ekrem et al., 2003; Sanseverino, 2006; Cranston, 2007; Vinogradova et al., 2009; Trivinho-Strixino, 2012, Trivinho-Strixino et al., 2015; Dantas & Gilka, 2017).

Gene selection

We selected one ribosomal gene marker (18S) and four protein-coding gene markers, including alanyl-tRNA-synthetase (AATS1), two sections of the CPSase region of carbamoyl-phosphate synthase-aspartate transcarbamoylase-dihydroorotase (CAD1 and CAD4), triose phosphate isomerase (TPI) and 6-phosphogluconate dehydrogenase (PGD). These genes have been used previously to reconstruct phylogenetic relationships among Diptera (Moulton & Wiegmann, 2004; Petersen et al., 2007; Bertone et al., 2008; Su et al., 2008; Ekrem et al., 2010; Gibson et al., 2010; Kutty et al., 2010; Tachi, 2013; Winterton & Ware, 2015). Due to the high mutation rate of the mitochondrial cytochrome c oxidase I (COI) and the documented poor performance in phylogenetic analyses on the genus level in Chironomidae (Ekrem et al., 2010), COI sequences were not included in the analyses.

DNA extraction, PCR amplification, sequencing and alignment

Adult specimens were preserved in 85% ethanol, immatures in 96% ethanol, and they were stored in the dark at 4°C before molecular analyses. Total genomic DNA of specimens was extracted from the thorax and head using either Qiagen® DNA Blood & Tissue Kit or GeneMole DNA Tissue Kit on a GeneMole® instrument (Mole Genetics, Lysaker, Norway) at the Department of Natural History, NTNU University Museum. The standard protocol of the Qiagen® DNeasy Blood & Tissue Kit was used, except that the final elution volume was 100 μL due to the small specimen size. When using the GeneMole DNA Tissue Kit, the standard protocol was followed, except that 4 μL Proteinase K was mixed with 100 μL buffer for overnight lysis at 56°C. The final elution volume was 100 μL. After DNA extraction, the clear exoskeleton was washed with 96% ethanol and mounted in Euparal (Waldeck GmbH & Co. KG, Division Chroma®, Germany) on microscope slides together with the corresponding antennae, wings and legs following the procedure outlined by Sather (1969).

DNA amplifications of selected nuclear genes with primers (Table S1) were carried out using 2.5 μL 10X Takara Ex Taq Buffer (Takara Bio, Japan), 2 μL 2.5 mM dNTP Mix, 0.1 μL Takara Ex Taq HS (Takara Bio), 0.5 μL 25 mM MgCl₂ and 1 μL of each 10 μM primer. The amount of template DNA was adjusted according to the DNA concentration and varied between 2 and 5 μL. ddH₂O was added to make a total of 25 μL for each reaction. Fragments of AATS1, CAD1, CAD4, PGD and TPI were amplified with a touchdown programme: initial denaturation step of 98°C for 10 s, then 94°C for 1 min followed by five cycles of 94°C for 30 s, 52°C for 30 s, 72°C for 2 min and seven cycles of 94°C for 30 s, 51°C for 1 min, 72°C for 2 min and 37 cycles of 94°C for 30 s, 45°C for 20 s, 72°C for 2 min 30 s and one final extension at 72°C for 3 min. A fragment of 18S was amplified with an initial denaturation step of 98°C for 10 s, then 95°C for 3 min followed by 36 subsequent cycles with denaturation at 94°C for 1 min; annealing starting at 57°C and decreased by 2°C every sixth cycle to touchdown at 47°C for 45 s and elongation at 72°C for 1 min; a final additional elongation step at 72°C for 10 min was added in the end. PCR products were visualized on a 1% agarose gel, purified using Illustra ExoProStar 1-Step (GE Healthcare Life Sciences, U.K.)
and shipped to MWG Eurofins (Germany) for bidirectional sequencing using BigDye 3.1 (Applied Biosystems, Foster City, CA, USA) termination. Not all individuals were successfully sequenced for all genes (Table S2).

Sequences were assembled and edited using sequencher 4.8 (Gene Codes Corp., Ann Arbor, MI, USA). The forward and reverse sequences were automatically assembled by the software and contigs were inspected and edited manually. In cases of ambiguity of base calls, we used the appropriate IUPAC code, but replaced the ambiguity symbol ‘N’ with ‘?’ in the data matrices. The sequences names were edited using mesquite 2.7.5 (Maddison & Maddison, 2010). Protein-coding genes were aligned using the muscle algorithm (Edgar, 2004) on amino acids in mega 6 (Tamura et al., 2013). Introns were detected with reference sequences and removed from the alignment using the GT-AG rule (Rogers & Wall, 1980). After removing introns, the codons were aligned. There was no evidence of paralogues in any sequences. For 18S, ambiguous regions were excluded in gblocks v0.91b (Castresana, 2000). The aligned sequences are shown in File S1.

Molecular phylogenetic reconstructions

The level of base substitution saturation for each gene and each position of the protein-coding genes was assessed by using the substitution saturation test of the program DAMBE v.5.5.25 (Xia et al., 2003; Xia & Lemey, 2009; Xia, 2013). We calculated the index of substitution saturation (ISS) of each piece of data and compared it with a critical index of substitution saturation (ISSc), defining a threshold for significant saturation in the data. Saturation is postulated when the ISS value is higher than the ISSc value or not significantly different (Xia, 2013).

All nuclear genetic markers were concatenated using sequencematrix v1.7.8 (Vaidya et al., 2011). To determine the best-fitting nucleotide model for each gene and the concatenated dataset, we used the software PARTITIONFINDER v1.1.1 (Lanfear et al., 2012) under the ‘greedy search’ algorithm based on the Bayesian information criterion (BIC) model metric. During analyses, branch lengths were unlinked to allow the program to estimate them independently for each subset. The best-fitting models were GTR + G + I for 18S and the first two codons for all nuclear protein-coding genes, and TVM + G + I models for the third codon for all genes based on BIC scores for each partition.

Maximum parsimony analyses

Maximum parsimony (MP) phylogenetic trees were reconstructed using paup 4.0b10 (Swofford, 2002) for the concatenated nuclear dataset. All sites were used, and gaps were coded as a fifth character state owing to the fact that gaps may be parsimoniously informative. A heuristic search and the tree–bisection–regrafting (TBR) branch swapping algorithm (Nei & Kumar, 2000) were used to obtain the best MP trees using 100 random replicates and 1000 bootstrap replicates. Maxtrees were set to auto-increase by 100.

Maximum likelihood analyses

Maximum likelihood (ML) phylogenetic analyses for the concatenated nuclear gene dataset was conducted with the software RAxML v8.2.X (Stamatakis, 2006, 2014) using raxmlGUI v1.5b1, with unlinked partitions as selected by PARTITIONFINDER. We used 1000 bootstrap replicates in a rapid bootstrap analysis, the GTR + G + I substitution model and a thorough optimization search for the best-scoring ML tree.

Bayesian inference (BI)

Bayesian tree search was carried out in MRBAYES 3.2.6 (Ronquist et al., 2012). In the Bayesian analyses, datasets were partitioned by gene and codon for the protein-coding genes and by gene for the noncoding gene. Four chains in two runs for 10 million generations, sampled every 100 generations with a burn-in of 0.25, were run with the model selected by PARTITIONFINDER: GTR + G + I for 18S and the first two codons for all nuclear protein-coding genes, and TVM + G + I models for the third codon for all genes. The convergence among the runs and effective sample size (ESS) were monitored using TRACER v1.6 (Rambaut et al., 2014), where we ascertained that the first 25% trees could be discarded as burn-in.

Divergence time estimates

Phylogenetic divergence times were estimated using BEAST v1.8.2 (Drummond et al., 2012). The DNA sequence dataset was partitioned by gene and codon position (except 18S by gene). Similar to Cranston et al. (2012), a separate HKY + G model was applied to each partition. Empirical experience has shown that analysis using the HKY + G model reaches convergence faster than the GTR + G model without significant differences in the results (X.-L. Lin, unpublished observations on a smaller dataset). The uncorrelated lognormal relaxed clock model for among-lineage rate variation was used in conjunction with a Yule speciation model. A lognormal (initial value = 1.0, log(mean) = 0.0, log(stdev) = 1.0, offset = 0)] prior was applied to the ucld.mean parameter (the arithmetic mean of the branch rates). Based on previous dating analyses by Cranston et al. (2012), the outgroup node (including Paratanytarsus, Rheotanytarsus and Sublettea) was calibrated with a normal prior (initial = 68 Ma, mean = 81.5 Ma, stdev = 8 Ma). The analysis was run with a topological constraint on Tanytarsus sensu lato as monophyletic to avoid sampled Cladotanytarsus species being placed in the ingroup. In addition, the internode including all sampled Caladomyia species was calibrated with a normal prior (initial = 34 Ma, mean = 35.6 Ma, stdev = 2 Ma) based on a stem fossil of Caladomyia dated to 37–34 Ma (http://fossilworks .org/?a=taxonInfo&taxon_no=287471) (Zakrzewska & Gilka, 2013). Described Tanytarsus species from amber (e.g. Gilka, 2010, 2011; Gilka et al., 2013; Zakrzewska et al., 2016) were not used for calibration as we could not assign a likely branch based on available morphological characters. The Markov chain
Monte Carlo analyses were run for 40 million generations, sampling trees every 10,000 generations after discarding samples from the first four million generations. TRACER v1.6 was used to examine the BEAST log file and ESSs for each parameter which were all greater than 300. The maximum clade credibility tree with median heights was generated using TREEANNOTATOR v1.8.2 (within the BEAST package) with four million states as burn-in.

**Biogeographic analyses**

To account for phylogenetic uncertainty and uncertainty in area optimization, the event-based method, S-DIVA (Statistical Dispersal-Vicariance Analyses) (Yu et al., 2010), was implemented in RASP v3.2 (Reconstruct Ancestral State in Phylogenies) (Yu et al., 2015). As distributions of outgroups may pose limitations to historical biogeographic analyses (Yu et al., 2015), the outgroup taxa (Paratanytarsus, Rheotanytarsus, Sublettea and Cladotanytarsus) were removed before biogeographic analyses. The geographical distribution of the ancestors was inferred by integrating over all 4001 tree topologies in the sample drawn from the Bayesian MCMC under BEAST. The maximum number of ancestral areas at each node was set to two with extinction (slow); maximum reconstruction (slow) was set to 100 with four steps; and maximum reconstruction for the final tree was set to 1000.

Three different division schemes for zoogeographical regions were compared. Based on Wallace’s traditional zoogeographical regions (Wallace, 1876), six distribution areas were included in the first analysis: (A) Afrotropical region; (B) Neotropical region; (C) Australian region; (D) Oriental region; (E) Palearctic region; and (F) Nearctic region. The second analysis used an updated version of Wallace’s zoogeographical regions (Holt et al., 2013) and hence included nine distribution areas: (A) Afrotropical region; (B) Neotropical region; (C) Australian region; (D) Oriental region; (E) Palearctic region; (F) Nearctic region; (G) Sino-Japanese region; (H) Panamanian region; and (I) Sahara-Arabian region. The third analysis used Bănărescu’s zoogeographical regions (Bănărescu, 1991) for freshwater fauna. As no sampled species are from the Indo-West Pacific, Malagasy and New Zealand regions, only five species distribution areas remained: (A) Ethiopian region; (B) Neotropical region; (C) Australian region; (D) Sino-Indian region; (E) Holarctic region.

**Results and discussion**

**Dataset analyses**

Exclusion of introns and hypervariable regions resulted in a final multigene dataset of 4281 bp, of which 1717 were parsimony-informative. Lengths by loci are: 18S, 933; AATS1, 408; CAD1, 909; CAD4, 846; PGD, 747; TPI, 438. A set of 130 species-level taxa remained, of which 98 were represented by all loci; five lacked 18S data; two lacked AATS1 data; four lacked CAD1 data; 17 lacked CAD4 data; two lacked PGD data; 13 lacked TPI data; 95% of all sequences were obtained successfully. It is demonstrated using simulations (Xi et al., 2016) that species tree estimation under separate models is not impacted when the amount of missing data is low or even high as long as it is randomly distributed. Base composition (A + T) ranges from 50.7% (TPI) to 60.4% (CAD4). Most parsimony-informative characters (> 63%) occurred in the third position of the protein-coding genes (Table S3). The complete results of the substitution saturation tests for all genes and codons indicated that for each partition, ISS values were lower than the ISSc values, suggesting little saturation in base substitution. Hence, we kept all sites in the analyses despite the high variability in third positions of protein-coding markers.

For the Bayesian analyses, the standard deviation of split frequencies was in all cases < 0.01. The log-likelihood values for the best tree of the molecular dataset was −116,480.3372. Both model-based methods (ML and BI) yielded mostly congruent nodes. The nonmodel-based method (MP) yielded mostly congruent internal topology with high support values, but the basal nodes (with low support values) were incongruent with ML and BI results, perhaps because of uncorrected mutational saturation. The results are summarized in Figs 1–4. In general, the BI and ML trees were more robust with more well-supported groups than the MP tree.

**Phylogenetic analyses, classification and biology**

*Relationships of genera in Tanytarsus sensu lato.* Our study confirms the paraphyly of *Tanytarsus* in both model-based analyses. *Tanytarsus* is paraphyletic as *Virgatanytarsus* is placed within a *Tanytarsus* clade (Fig. 1), *Corynocera* is placed within the *Tanytarsus norvegicus* species group (Fig. 2), and *Caladomyia* is placed among South American *Tanytarsus* (Fig. 4). A possible solution for the reclassification of *Tanytarsus* is to divide the group into several smaller genera and keep *Caladomyia, Corynocera* and *Virgatanytarsus* as currently defined. However, our results are inconclusive for the group placement of several species, and we have only sampled about one-third of the described diversity in *Tanytarsus*. Moreover, it might prove difficult to find well-founded morphological diagnostic characters in all associated life stages for many of the groups supported by our molecular data — in particular, those that are in conflict with previously defined groups. We are therefore favouring a reclassification that moves species currently placed in *Caladomyia, Corynocera* and *Virgatanytarsus* to *Tanytarsus* (but see argument with regard to *Corynocera* later).

Based on our result, a monophyletic *Virgatanytarsus* is well supported [Fig. 1; ML bootstrap (MLB) = 74%; posterior probability (PP) = 1] as sister to *Tanytarsus bispinosus* Freeman, which, as seen in *Virgatanytarsus*, has broad lateral combs on pupal abdominal segment VIII but which, in contrast to *Virgatanytarsus*, lacks bars on the adult male anal point. The anteriorly directed bars on hypopygial anal point in the male is not unique to *Virgatanytarsus* and does not separate these species from *Tanytarsus*. Similar features are found in
**Fig. 1.** Maximum likelihood (ML) tree based on the concatenated DNA dataset (18S, AATS1, CAD1, CAD4, PGD, TPI, 4281 characters) of *Tanytarsus* sensu lato. Numbers on branches refer to posterior probabilities over 0.95 + ML bootstrap values over 70%/maximum parsimony bootstrap values over 70%. Clades are labelled with species groups names suggested in this study. [Colour figure can be viewed at wileyonlinelibrary.com].

*Tanytarsus signatus* (van der Wulp) from the Palearctic region, in *T. sp.26XL* (X.-L. Lin et al., unpublished data) from the Oriental region, in *Tanytarsus bifurcus* Freeman from the Afrotropical and Oriental regions, in the Neotropical species *Tanytarsus curvicristatus* Contreras-Lichtenberg, *T. giovannii* Sanseverino & Trivinho-Strixino and *T. pseudocurvicristatus* Trivinho-Strixino, Wiedenbrug & da Silva (Sanseverino & Trivinho-Strixino, 2010; Trivinho-Strixino *et al.*, 2015), and

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Fig. 2. Clade A of the maximum likelihood (ML) tree based on the concatenated DNA dataset (18S, AATS1, CAD1, CAD4, PGD, TPI, 4281 characters) of *Tanytarsus* sensu lato. Numbers on branches refer to posterior probabilities over 0.95 + ML bootstrap values over 70% / maximum parsimony bootstrap values over 70%. Clades are labelled with species groups names suggested in this study. [Colour figure can be viewed at wileyonlinelibrary.com].
also in the Australian species *Tanytarsus liepae* Glover (Glover, 1973). The broad lateral comb on the pupal abdominal segment VIII is also present in *Tanytarsus edwardi* Glover and *T. hardwicki* Cranston from Australia (Cranston, 2000), and in the Neotropical *Tanytarsus riopreto* group (Sanseverino, 2006) in addition to *T. bispinosus* mentioned earlier. Thus, considering both morphological and genetic data, we conclude that no diagnostic differences confidently allow separation of *Virgatanytarsus* from *Tanytarsus* and that these genera should be treated as synonyms. We consider all species formerly placed in *Virgatanytarsus* as members of the *Tanytarsus triangularis* species group.

The genus *Corynocera* is clustered within the *Tanytarsus norvegicus* species group (Fig. 2; MLB = 100%, PP = 1, MP bootstrap (MPB) = 100%). We postulate that the peculiar adult male morphology seen in *Corynocera* species must be an adaptive character evolved within *Tanytarsus* and that it is not diagnostic on a generic level. Treating these as synonyms poses a nomenclatorial challenge, however, as the publication of *Corynocera* (Zetterstedt, 1838) predates that of *Tanytarsus* (Wulp, 1874). According to the rules of the International Code of Zoological Nomenclature (ICZN), *Tanytarsus* should then be listed as a junior synonym of *Corynocera*. On the other hand, *Corynocera* only holds four described species that are comparatively rarely encountered, whereas *Tanytarsus* holds 355, of which many are constantly and widely referred to by taxonomists and ecologists. Hence, following the principle of precedence would not be favouring nomenclatorial stability and a case will be presented to the ICZN to argue for keeping the name *Tanytarsus* for the group. Moreover, as the type species...
Fig. 4. Clade C of the maximum likelihood (ML) tree based on the concatenated DNA dataset (18S, AATS1, CAD1, CAD4, PGD, TPI, 4281 characters) of *Tanytarsus* sensu lato. Numbers on branches refer to posterior probabilities over 0.95 + ML bootstrap values over 70%/maximum parsimony bootstrap values over 70%. Clades are labelled with species groups names suggested in this study. [Colour figure can be viewed at wileyonlinelibrary.com].

of *Corynocera* was not included in our analyses, we are not completely certain that the type species will fall within the *T. norvegicus* species group. Thus, a thorough morphological analyses of all life stages of *Corynocera* and *T. norvegicus* group species should be performed before a formal synonymy.

The monophyletic *Caladomyia* (MLB = 100%, PP = 1, MPB = 99%) cannot be separated from Neotropical and Holarctic *Tanytarsus* genetically (Fig. 4; MLB = 80%, PP = 1), but also no morphological diagnostic characters are found in the immature stages. We regard *Caladomyia* as a junior synonym of *Tanytarsus* and recognize all *Caladomyia* species as members of the monophyletic *Tanytarsus ortoni* species group.

The species *T. rhabdomantis* groups with other Neotropical *Tanytarsus* (Fig. 4) and thus supports the synonymy of...
Nimbocera with Tanytarsus. Cladotanytarsus is recovered as a monophyletic sister group to Tanytarsus; this is consistent with Cranston et al. (2012) but here with wider sampling.

Relationships among and within species groups. Based on the results of the phylogenetic analyses, we propose 11 new monophyletic species groups: the aterrimus, ortoni, curticornis, edwardi, giovanni, heudensis, lestagei, luctuosus, motosuensis, tamakutibasi and thicus species groups (Figs 1–4). These groups, which are well supported in our results and consistent with observed morphological characters, will be discussed elsewhere.

Among previously postulated Tanytarsus species groups, the following are confirmed to be monophyletic with high support values: aculeatus, excavatus, norvegicus, kiche, pallidicornis, signatus, triangularis and the verralli group. The formerly proposed bispinosus, chinyensis, eminusul, gregarius, mcmillani, mendax, lugens and riopreto groups are refuted as previously defined (Table 1; Figs 1–4) and species’ placements are amended according to our results. The relationships between the aculeatus and signatus, (heudensis + pallidicornis) species groups and clades A–C (Fig. 1) remain ambiguous with low support values, possibly due to incomplete taxon sampling and the low number of genetic markers.

The T. signatus species group was proposed by Reiss & Fittkau (1971) as a monotypic group in Europe with the type species T. signatus. Sanseverino (2006) suggested that the South American T. curvicristatus and Australian T. liepae should be included in that group as they also have bars on the anal point in adult males. Despite this morphological character, there is no evidence to support the latter species as belonging to a signatus group. In our molecular analyses, we included T. signatus, T. curvicristatus and an undescribed species that is morphologically similar to T. signatus to explore the relationship among these species. Our result confirms T. signatus as a divergent evolutionary lineage, but T. curvicristatus is genetically very divergent from the signatus lineage. Thus, we believe that T. curvicristatus and its morphologically closely related species (e.g. T. pseudocurvicristatus, also from the Neotropical region) may best be placed in a different species group.

Our results confirm that the aculeatus species group is a distinct lineage that is monophyletic (Fig. 1; MLB = 97%, PP = 1, MPB = 80%) even when including T. palettarius Verneaux, which previously was placed in the chinyensis species group. Re-examination of voucher specimens and comparison of previous descriptions revealed that T. aculeatus Brundin and T. palettarius are morphologically similar in several key characters in the adult males, e.g. both have a long digitus that extends beyond the inner margin of a heart-shaped superior volsella.

The heudensis species complex, previously belonging to the paraphyletic chinyensis species group, is proposed as a new species group in Tanytarsus. The pallidicornis group erected by Reiss & Fittkau (1971) is confirmed monophyletic and sister to the heudensis group (Fig. 1; MLB = 100%, PP = 1, MPB = 100%).

The species placed formerly in the eminusul, gregarius, lugens, mcmillani and mendax species groups (Fig. 2) form a well-supported clade (MLB = 100, PP = 1), which is sister to the norvegicus species group (Fig. 2; MLB = 96%, PP = 1). However, the phylogenetic relationships among the internal groups remain uncertain with low support values, suggesting that these can perhaps be merged into one large monophyletic species group. Morphological characteristics do exist for most subgroups, however, and high branch support for these indicate that a better solution is to revise the group members. For instance, the previously postulated eminusul species group is divided into different clades, indicating that some members should be excluded and transferred to other species groups. Based on our morphological knowledge, species of the lestagei species group (earlier placed in the eminusul group) have well-defined diagnostic characters in adult males such as a strongly angular margin of ventromedian hypopygium pocket, and a comparatively long median volsella with a broad fan of lamellae, while at present no unique synapomorphies can be found in what would be the oscillans species group (T. oscillans + T. unagisempeum). Moreover, the species potentially belonging to the gregarius group are clustered within the lugens species group, suggesting that there is no evolutionary argument to keep these groups separate. Thus, in agreement with Gilka (2000) and Zakrzewska et al. (2018), we suggest that species of the gregarius group should be transferred to an enlarged lugens group to render it monophyletic. The mcmillani group from Afrotropical and Oriental regions, postulated by Ekrem (2003), is paraphyletic with T. spadiceonotatus Freeman separated from the other key taxa, indicating a different evolutionary history. Additionally, T. ovatus Johannsen, placed previously in the mendax group, and an undescribed species similar to T. mcmillani, group in the mcmillani group. The mendax group also came out paraphyletic as three species (T. formosanus Kieffer, T. fuscithorax Skuse and T. pallidulus Freeman) show other sister-group relationships.

The herein postulated motosuensis group includes the morphologically similar T. motosuensis Kawai and T. sp.26XL, both from oligotrophic lakes in the Oriental region. Species of the norvegicus group have been found in oligotrophic lakes in/near the Arctic and on the Qinghai-Tibet Plateau.

Within subtree B (Fig. 3), the aterrimus species group from the Afrotropical and Oriental regions is sister to the tamakutibasi group from the Oriental and Palearctic regions (MLB = 98%, PP = 1, MPB = 87%). The chinyensis species group erected by Reiss & Fittkau (1971) is paraphyletic, as T. palettarius and species within the T. curticornis and T. heudensis complexes are not monophyletic. Based on our molecular phylogeny and agreement in morphological characters, we transfer T. palettarius to the aculeatus species group, erect the curticornis and heudensis species groups, and postulate that T. tamagotoi and T. simantoseteus belong to a reduced chinyensis group. However, there is some uncertainty involved in this as we have not sampled the name-bearing species, T. chinyensis. The curticornis group is probably sister to the remainder of the chinyensis group (MLB = 98%, PP = 1), while the aterrimus + tamakutibasi groups are sister to the chinyensis + curticornis groups (MLB = 69%, PP = 1).
Table 1. Previously proposed species groups in Tanytarsus that are sampled and analysed in the present study.

| Species group | Sensu | Named species included in present analysis | Comment |
|---------------|-------|--------------------------------------------|---------|
| aculeatus group | Reiss & Fittkau (1971) | T. aculeatus Brundin | Confirmed as separate lineage compared with other groups. Only one species originally placed in group |
| bispinosus group | Glover (1973) | T. bispinosus Freeman, T. edwardi Glover, 1973 | Refuted |
| chinyensis group | Reiss & Fittkau (1971) | T. brundini Lindeberg, T. curticornis Kieffer, T. heudensis Goetghhebuer, T. palettaris Verneaux | Refuted and split into several unrelated groups. T. chinyensis was not included in our analyses, thus name-bearing species remain unplaced |
| eminus group | Reiss & Fittkau (1971) | T. ajagos Ekrem, Sublette & Sublette, T. ejuncidaus (Walker), T. eminus (Walker), T. lestagei aggregate of species, T. longitarsis Kieffer, T. medus Reiss & Fittkau, T. okuboi Sasa & Kikuchi, T. oscillans Johannsen, T. pollexus Datta, T. shoudigitatus Sasa, T. striatus Lindeberg, T. tanaundecimus Sasa, T. sunosecondus Sasa | Refuted and split into several groups |
| excavatus group | Reiss & Fittkau (1971) | T. excavatus Edwards, T. nemsonnus Edwards | Confirmed |
| gregarius group | Reiss & Fittkau (1971) | T. gregarius (Kieffer), T. herrmanii Ekrem, Sublette & Sublette, T. inaequulis Goetghhebuer | Refuted and merged with the lugens group |
| mcmillani group | Ekrem (2003) | T. mcmillani Freeman | Refuted. T. spadicornatus does not group with T. mcmillani |
| mendax group | Reiss & Fittkau (1971) (as holochlorus group) | T. aculeatus Brundin, T. denyi Sublette, T. desertor Gilka & Paasivirta, T. formosanus Kieffer (= T. horni Goetghhebuer in Reiss & Fittkau (1971)), T. mendax Kieffer (= T. holochlorus Edwards in Reiss & Fittkau (1971)), T. occultus Brundin, T. ovatus Johannsen, T. volgensis Miseiko, T. wirthi Ekrem, Sublette & Sublette | Confirmed |
| kiche group | Dantas & Gilka (2017) | T. kiche Vinogradova, Riss & Spies | Confirmed as separate lineage; only one previously described species included, which groups with two unnamed species |
Table 1. Continued.

| Species group | Sensu | Named species included in present analysis | Comment |
|---------------|-------|-------------------------------------------|---------|
| *luginens* group | Reiss & Fittkau (1971) | *T. angulatus* Kawai  
*T. bathophilus* (Kieffer)  
*T. lugens* (Kieffer)  
| Refuted and merged with the *gregarius* group |
| *norvegicus* group | Reiss & Fittkau (1971) | *T. anderseni* Reiss & Fittkau  
*T. gracilens* (Holmgren)  
*T. miriforceps* (Kieffer)  
*T. niger* Andersen  
*T. paramiger* Gilka & Paasivirta  
*T. sinuatus* Goetghheber  
*T. sylvaticus* (van der Wulp)  
| Confirmed. Name-bearing species, *T. norvegicus*, not included in the dataset.  
*Tanytarsus paraniger* was allocated to the *norvegicus* group by Gilka & Paasivirta (2008) |
| *pallidicornis* group | Reiss & Fittkau (1971) | *T. buchonis* Reiss & Fittkau  
*T. nigrigrillos* Goetghheber  
*T. pallidicornis* (Walker)  
*T. usmaensis* Pagast  
| Confirmed |
| *recurvatus* group | Reiss & Fittkau (1971) | *T. recurvatus* Brundin  
*T. clivosus* Reiss  
*T. hamatus* Reiss  
*T. hastatus* Sublette & Sasa  
*T. pandus* Sublette & Sasa  
| Confirmed as separate lineage; only one species included in the analysis  
Refuted. The group as defined by Sublette & Sasa (1994) is refuted, but none of the species analysed by us were part of the original group definition by Fittka & Reiss (1973). The inclusion of *T. clivosus*, *T. hamatus*, *T. hastatus* and *T. pandus* in the *rioproto* group (Sublette & Sasa, 1994) was questioned by Sanseverino & Wiedenbrug (2000) and Sanseverino (2006) |
| *rioproto* group | Sublette & Sasa (1994) |  
eg Fittkau & Reiss (1973) |
| *signatus* group | Reiss & Fittkau (1971) | *T. signatus* van der Wulp  
| Confirmed as separate lineage compared to other groups. Only one species previously placed in group |
| *triangularis* group | Reiss & Fittkau (1971) | *T. aboensis* Harrison  
*T. abriscus* Santos Abreu  
| Confirmed. Species in the original description of the *triangularis* group (Reiss & Fittkau, 1971) not included in our analysis, but six species which fit the morphological diagnostics of *Virgatanytarsus* are analysed. Three have previously been described and formally named |
| *verralii* group | Reiss & Fittkau (1971) | *T. debilis* (Meigen)  
*T. innarenis* Brundin  
*T. lactescens* Edwards  
*T. verrallii* Goetghheber  
| Confirmed |
| *Cakodomyia* | Säwedal (1981) | *T. hoefleri* Reiff  
*T. kaplei* Trivinho-Strixino  
*Trivinho-Strixino* (2012) |
| Confirmed as monophyletic group, embedded in *Tanytarsus* |
This clade is sister to clade C (Fig. 1; MLB = 62%, PP = 1), which includes Neotropical Tanytarsus + ortoni + Holartic excavatus, verralli and recurvatus species groups (Fig. 4). The monophyly of the (excavatus + verralli + kiche) clade is well supported (MLB = 100%, PP = 1, MPB = 95%). The newly erected giovannii group, containing three species, is sister to T. curvicristatus (MLB = 76%, PP = 1). The Neotropical riopreto species group as interpreted by Sublette & Sasa (1994) came out paraphyletic as T. rhadomantis and T. obiriciae Trivinho-Strixino & Sonoda, which are both morphologically quite dissimilar, group within the riopreto species group in our tree (Fig. 4). Sublette & Sasa’s (1994) definition of the riopreto group has been questioned (Sanseverino & Wiedenbrug, 2000; Sanseverino, 2006) and our results also support a more narrowly defined group. As we have not sampled the name-bearing species, we cannot conclude which clade should carry the name riopreto.

Divergence time estimation and biogeographic patterns

Tempo of diversification. The oldest known member of the tribe Tanytarsini is Gujaraomyia miripes Gilka & Zakrzewska, recently described from Indian Cambay amber from the early Eocene (c. 52–53 Ma) (Zakrzewska et al., 2018). The oldest known member of the genus Tanytarsus is also found in the Indian Cambay amber (c. 50–52 Ma), but remains to be described (Stebner et al., 2017). Divergence time estimates in our phylogeny of Tanytarsus (Files S2 and S3) indicate that the genus diverged from Cladotanytarsus during the Late Cretaceous and Early Paleogene (61–79 Ma), at least 8 Ma earlier than the oldest known Tanytarsini fossil. There are many autapomorphies in the adult male of Gujaraomyia and its sister-group relationship is therefore uncertain. However, G. miripes Gilka & Zakrzewska shows some affinities with Cladotanytarsus (Zakrzewska et al., 2018). Thus, the coexistence of Tanytarsus and Gujaraomyia in Cambay amber indicate that Tanytarsus evolved earlier than the Ypresian (c. 52 Ma) and perhaps as early as our analyses suggest.

Ancestral area reconstruction. The S-DIVA analyses based on different geographical division schemes yielded similar results (Fig. 5; Files S4 and S5) and proposed 35–61 dispersal and 24–33 vicariance events, but no extinction events to account for the present distribution of Tanytarsus. According to our results, the likely place of origin for Tanytarsus lies in the Old World (Oriental and Paleartic regions) with subsequent dispersal and vicariance events leading to the separation of three major clades (Fig. 5). An early dispersal event during the Palaeocene (65 Ma) led to the Paleartic signatus species group from remaining Tanytarsus, suggesting an origin in Laurasia. Then the ancestral taxon of clade 3 (Fig. 5) evolved in the Oriental region and was isolated from Clade 1 + 2 (Fig. 5) via a vicariance event in the Palaeocene (62 Ma). This is earlier than the collision of the Indian Subcontinent with Asia (55 Ma), but the presence of Australian and south-east African species in this clade indicate a possible Gondwanan origin for the Asian species in clade 3 and is an example of what Sæther & Ekrem (2003) referred to as an Inabrezian distribution. The area of origin of clade 1 lies in the Palearctic region, while that of clade 2 lies in the Neotropical and New World regions.

Within clade 1 (Fig. 5), several species groups (eminulus + gregarius + lugens + mcmillani + mendax + motosuensis + norvegicus) are not recorded in the Neotropical region, and presence outside the Neotropical was probably caused by dispersal events during the Eocene (56 Ma). It is worth mentioning that the monophyletic norvegicus species group is distributed only in/near the Arctic and on the Qinghai-Tibet Plateau of the northern hemisphere. The group probably originated in Laurasia and retained a northern/high latitude distribution by cold adaption. Supporting this are subfossil larval head capsules of Corynocera ambigua Zetterstedt, (a likely member of the norvegicus group) found and dated to 2–3 Ma (Böcher, 1995). This is slightly earlier than the cold Dryas, suggesting that Corynocera survived this extremely cold period in this region. Evidently Corynocera originated during the Middle Miocene (11 Ma) via a vicariance event (e.g. Orogeny in the northern hemisphere). The hypothesized place of origin of the eminulus, gregarius, lugens, mcmillani and mendax species groups in Gondwana (Afrotropical region) (Ekrem, 2003) was supported by our S-DIVA analyses, and subsequent dispersal and vicariance events among these species groups thus explain the observed distribution pattern.

Within clade 2 (Fig. 5), S-DIVA analyses indicate that a vicariance event took place around 55 Ma separating a predominantly Neotropical clade from a predominantly Holarctic clade. Surprisingly, within the Neotropical clade, there is a group of cold-adapted Tanytarsus from the Palaearctic (the excavatus, recurvatus and verralli species groups) that originated about 45 Ma. The result of the S-DIVA analyses indicates that some unknown vicariance events during the Eocene appear to have led to the diversification of the above excavatus, recurvatus and verralli groups. However, it is difficult to imagine how chromo- mids could migrate from the neotropics to the northern Holartic as they are weak fliers. The observed pattern may result from sampling bias and extinction. For instance, the presence of Caladomyi a in Baltic amber indicates a geographically wider distribution of what is now a core Neotropical group. Perhaps a colder climate in the Oligocene reduced the distribution of warm-adapted Tanytarsus, leaving mostly the Neotropical taxa of this group, except for the cold-adapted clade (the excavatus, recurvatus and verralli species groups). Regarding potential sampling bias, the Nearctic region is understudied and may lack North American species in the group. The presence of such Nearctic species could support both a broader ancient range and possible ancient dispersion from South to North America over the Central American Seaway. In a recent study, the isthmus of Panama was found to have formed 10 Ma earlier than the previously estimated 3 Ma (Montes et al., 2015). This can explain the more recent recolonization of southern South America indicated by T. kiche Vinogradova, Riss & Spies in our trees (12–13 Ma). The two, undescribed, closest relatives of T. kiche are both from Costa Rica (north of the Panama Isthmus).
Fig. 5. Hypothesized event-based ancestral area reconstruction of *Tanytarsus* as inferred by Statistical Dispersal-Vicariance Analysis (S-DIVA) analyses based on the updated Wallace’s zoogeographical regions (Holt et al., 2013). Pie diagrams show the ancestral distributions estimated for internal nodes of the phylogeny of *Tanytarsus* by S-DIVA. Blue circles indicate dispersal events, green circles indicate vicariance events. A, Afrotropical region; B, Neotropical region; C, Australian region; D, Oriental region; E, Palearctic region; F, Nearctic region; G, Sino-Japanese region; H, Panamanian region; I, Saharo-Arabian region. [Colour figure can be viewed at wileyonlinelibrary.com].

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Fig. 5. Continued.
Our sampling is biased towards the Palaearctic region. Thus, low representation of African, southern North American and Australian species might hide the true evolutionary and biogeographical history of Tanytarsus. In addition, possible extinction can play an important role in the biogeographical history (Eskov & Lukashevich, 2015), and there are most certainly both extinct and undescribed species in Tanytarsus.

Conclusion

Tanytarsus as currently understood was not resolved as monophyletic in our analyses, as species of Caladomyia, Corynocera and Virgatanytarsus were embedded within Tanytarsus sensu lato. We propose that the best solution for reclassification of Tanytarsus is to synonymize Caladomyia and Virgatanytarsus with Tanytarsus but retain the older Corynocera until an ICZN ruling on precedence of the younger name Tanytarsus can be made. The monophyly of some species groups (aculeatus, aterrimus, ortoni, curticornis, edwardsi, excavatus, giovannii, heusdensis, kiche, lestagei, luctuosus, motorsuensis, norvegicus, pallidicornis, signatus, triangularis, tamakutibasi, thaicus and verralli) is recovered with high support values. However, some previously postulated groups are refuted or remain uncertain, with incomplete sampling. We find that some monophyletic groups can be associated with certain geographical distributions and/or ecology, but details in the biogeographical history are likely to be masked by undersampling and possible extinction in some groups. Tanytarsus probably diverged from its sister group Cladotanytarsus during the Late Cretaceous and Early Paleogene (61–79 Ma) in the Oriental and Palaearctic regions. Additional taxonomic sampling and more genetic data are required to recover the full evolutionary history of the diverse genus in future.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

File S1. The concatenated DNA dataset used for phylogenetic analyses (nexus format).

File S2. BEAST divergence time estimates tree with node age. Nodes on the chronogram represent means of the probability distributions for node ages, with time interval for 95% probability of actual age represented as coloured bars. Timescale units are in Ma, with the estimated age for a divergence given on each node.

File S3. BEAST divergence time estimates tree with 95% height range. Timescale units are in Ma, with the estimated age for a divergence given on each node.

File S4. Hypothesized event-based ancestral area reconstruction of Tanytarsus as inferred by S-DIVA analyses based on Wallace’s traditional zoogeographical regions. Pie diagrams show the ancestral distributions estimated for internal nodes of the phylogeny of Tanytarsus by S-DIVA. Blue circles indicate dispersal events, and green circles indicate vicariance events. A, Afrotropical region; B, Neotropical region; C, Australian region; D, Oriental region; E, Palearctic region; F, Nearctic region.

File S5. Hypothesized event-based ancestral area reconstruction of Tanytarsus as inferred by S-DIVA analyses based on the Bănărescu’s zoogeographical regions. Pie diagrams show the ancestral distributions estimated for internal nodes of the phylogeny of Tanytarsus by S-DIVA. Blue circles indicate dispersal events, and green circles indicate vicariance events. A, Afrotropical region; B, Neotropical region; C, Australian region; D, Oriental region; E, Holarctic region.

Table S1. Overview of gene segments and primer combinations.

Table S2. BOLD sample ID and GenBank accession numbers of specimens in the dataset.

Table S3. Informative sites, and average nucleotide composition in the aligned nuclear gene sequences.

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