Molecular phylogeny and temporal diversification of *Tanytarsus* van der Wulp (Diptera: Chironomidae) suggest generic synonyms, new classifications and place of origin

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Abstract

Tanytarsus van der Wulp, with 355 currently known valid species, is a comparatively large genus of non-biting 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 ICZN 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 *Tanytarsus* with *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.

Keywords

Diptera; Chironomidae; Bayesian; maximum likelihood; maximum parsimony; nuclear DNA; S-DIVA; biogeography.

Introduction

Over the last decade, advances in DNA sequencing technologies, bioinformatics and computational biology provided large amount of molecular data and improved the tools used to analyze 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 (Ståhls *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.*, 2017). 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 Prodiamesinae, are monophyletic, while the tempo of diversification of the family showing 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 pers. comm.) 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 eleven 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 tropical *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 (2001b; Ekrem, 2001a; Ekrem, 2002; Ekrem, 2004) and Ekrem *et al.* (2003). The Neotropical fauna also has 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; Giłka & Zakrzewska, 2013; Trivinho-Strixino *et al.*, 2015; Dantas & Giłka, 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; Giłka & Paasivirta, 2007; Giłka & Paasivirta, 2008; Giłka & Paasivirta, 2009; Lin *et al.*, 2015; Lin *et al.*, 2017). The increased use of molecular tools in taxonomy has aided the discovery of new cryptic and semi-cryptic species in *Tanytarsus* (Lin *et al.*, 2015; Lin *et al.*, 2017) and more is to be expected as less investigated regions, such as the East Palearctic 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 south-western USA (Säwedal, 1981; Trivinho-Strixino, 2012). One extinct species is recorded from Eocene Baltic amber (Zakrzewska & Giłka, 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 a 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 off-set 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 (Lin et al., unpublished) while 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 Palearctic 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 (COII), 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: 1) Is *Tanytarsus* a monophyletic group? 2) What is the phylogenetic relationship among species of *Tanytarsus*, *Caladomyia*, *Corynocera*, *Sublettea* and *Virgatanytarsus*? 3) What is the biogeographical history of the genus *Tanytarsus*, including its origin and tempo of diversification?

Material and methods

Taxon sampling

To detect and avoid lab contamination and misidentifications, we selected two individuals of each species, except if 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 s.l.*, 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 *Corynocera*, one to *Paratanytarsus* Thienemann & Bause, one to *Rheotanytarsus* Thienemann & Bause, one to *Sublettea* and six belong to *Virgatanytarsus*. In addition, *Tanytarsus rhabdomantis* (Trivinho-Strixino & Strixino), originally placed in *Nimbocera* (itself a junior synonym of *Tanytarsus*) was included to confirm the synonymy of *Nimbocera* and *Tanytarsus*. Approximately 99% of all DNA sequences used were generated as part of this study.

List of all species, specimens, their individual images, georeferences, primers, sequences and other relevant laboratory data of all sequences specimens can be seen online in the publicly accessible full dataset “Molecular phylogeny of *Tanytarsus sensu lato* [DS-PHTAN]”, DOI: dx.doi.org/10.5883/DS-PHTAN and the reduced dataset “Reduced dataset for molecular phylogeny of *Tanytarsus sensu lato* [DS-REDMTAN]” in the Barcode of Life

Data Systems (BOLD) (Ratnasingham & Hebert, 2007; 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 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; Lindeberg, 1967; Reiss & Fittkau, 1971; Glover, 1973; Sasa, 1980; Sasa & Kawai, 1987; Sublette & Sasa, 1994; Cranston, 2000; Ekrem, 2001a; Ekrem, 2002; Ekrem *et al.*, 2003; Sanseverino, 2006; Cranston, 2007; Vinogradova *et al.*, 2009; Trivinho-Strixino, 2012; Trivinho-Strixino *et al.*, 2015; Dantas & Giłka, 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 stored 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 small specimen size. When using 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 on microscope slides together with the corresponding antennae, wings and legs following the procedure outlined by Sæther (1969).

DNA amplifications of selected nuclear genes with primers (Table S1) were carried out using 2.5 µL 10x Ex Taq Buffer, 2 µL 2.5 mM dNTP Mix, 0.1 µL Ex Taq HS, 0.5 µL 25 mM $MgCl_2$ and 1 µl of each 10 µM primer. The amount of template DNA was adjusted according to the DNA concentration and varied between 2-5 µL. ddH_2O 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 program: initial denaturation step of 98°C for 10 s, then 94°C for 1 min followed by 5 cycles of 94°C for 30 s, 52°C for 30 s, 72°C for 2 min and 7 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 1 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 and shipped to MWG Eurofins for bidirectional sequencing using BigDye 3.1 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, Michigan, 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 GT-AG rule (Rogers & Wall, 1980). After removing introns, the codons were aligned. No evidence of paralogues was observed 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 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 the 18S and first two codons for all nuclear protein-coding genes, and TVM + G + I models for the 3rd codon for all genes based on BIC scores for each partition.

Maximum parsimony (MP) 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 resulting from 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 (ML) analyses

Maximum likelihood (ML) phylogenetic analyses for the concatenated nuclear gene dataset was conducted with the software RAxML v8.2.X (Stamatakis, 2006; Stamatakis, 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, data sets were partitioned by gene and codon for the protein-coding genes and by gene for the non-coding gene. Four chains in 2 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 the 18S and first two codons for all nuclear protein-coding genes, and TVM + G + I models for the 3rd 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 (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 s.l.* as monophyletic to avoid sampled *Cladotanytarsus* species being placed in the ingroup. In addition, the inter node 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 & Giłka, 2013). Described *Tanytarsus* species from amber (e.g. Giłka, 2010; Giłka, 2011; Giłka *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 4 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 4 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). Since 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 schema for zoogeographical regions were compared. Based on the traditional Wallace's 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, (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, (I) Sahara-Arabian region. The third analysis used Bănărescu's zoogeographical regions (Bănărescu, 1991) for freshwater fauna. Since 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 hyper-variable regions resulted in a final multigene dataset of 4281 bp, of which 1717 were parsimony informative. Lengths by locus 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 are 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 -116480.3372. Both model-based methods (ML and BI) yielded mostly congruent nodes. The non-model 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* within the *Tanytarsus norvegicus* species group (Fig. 2), and *Caladomyia* 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 favoring a reclassification that moves species currently placed in *Caladomyia*, *Corynocera* and *Virgatanytarsus* to *Tanytarsus* (but see argument with regard to *Corynocera* below).

Based on our result, a monophyletic *Virgatanytarsus* is well-supported (Fig. 1; MLB = 74%; PP = 1) as sister to *T. bispinosus* Freeman, 1961, which as seen in *Virgatanytarsus*, has broad lateral combs on pupal abdominal segment VIII but 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 *Tanytarsus signatus* (van der Wulp, 1859) from the Palearctic region, in *T.* sp.26XL (Lin et al., unpublished) from the Oriental region, in *Tanytarsus bifurcus* Freeman, 1958 from the Afrotropical and Oriental regions, in the

Neotropical species *Tanytarsus curvicristatus* Contreras-Lichtenberg, 1988, *T. giovannii* Sanseverino & Trivinho-Strixino, 2010 and *T. pseudocurvicristatus* Trivinho-Strixino, Wiedenbrug & da Silva, 2015 (Sanseverino & Trivinho-Strixino, 2010; Trivinho-Strixino *et al.*, 2015), and also in the Australian species *Tanytarsus liepae* Glover, 1973 (Glover, 1973). The broad lateral comb on the pupal abdominal segment VIII is present also in *Tanytarsus edwardi* Glover, 1973 and *T. hardwicki* Cranston, 2000 from Australia (Cranston, 2000), and in the Neotropical *Tanytarsus riopreto* group (Sanseverino, 2006) in addition to *T. bispinosus* mentioned above. 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; 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 generic level. Treating these as synonyms poses a nomenclatorial challenge, however, since 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 International Commission on Zoological Nomenclature to argue for the keeping of the name *Tanytarsus* for the group. Moreover, as the type species 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 the Cranston *et al.* (2012) but here with wider sampling.

Relationships among and within species groups. Based on the results from the phylogenetic analyses, we propose eleven new monophyletic species groups: the *aterrimus*-, *ortoni*-, *curticornis*-, *edwardi*-, *giovannii*-, *heusdensis*-, *lestagei*-, *luctuosus*-, *motosuensis*-, *tamakutibasi*- and *thaicus* 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*-, *eminulus*-, *gregarius*-, *mcmillani*-, *mendax*-, *lugens*- and *riopreto* groups are refuted as previously defined (Table 1; Figs 1–4) and species' placements are emended according to our results. The relationships between the *aculeatus*-, *signatus*-, (*heusdensis* + *pallidicornis*) species groups and clades A-C (Fig. 1) remain ambiguous with low support values, possibly due to incomplete taxon sampling and low number of genetic markers.

The *Tanytarsus 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 since they also have bars on the anal point in adult males. Despite this morphological character, no evidence supports that the latter species belongs to a *signatus* group. In our molecular analyses, we included *T. signatus*, *T. curvicristatus* and an undescribed species 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 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. palettaris* Verneaux, 1969 that previously was placed in the *chinyensis* species group. Re-examination of voucher specimens and comparison of previous descriptions revealed that *T. aculeatus* Brundin, 1949 and *T. palettaris* are morphological 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 *heusdensis* 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 *heusdensis* group (Fig. 1; MLB = 100%; PP = 1; MPB = 100%).

The species placed formerly in the *eminulus*-, *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 perhaps can be merged into one large monophyletic species group. Morphological characteristics do exist for most sub-groups, however, and high branch support for these indicate that a better solution is to revise the group members. For instance, the previously postulated *eminulus* species group is divided into different clades, indicating 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 *eminulus* group) have well-defined diagnostic characters in adult males such as a strongly angular margin of ventro-median hypopygial 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. unagiseptimus*). 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 (Giłka, 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 since 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 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. palettaris* and species within the *T. curticornis* and *T. heusdensis* complexes are not monophyletic. Based on our molecular phylogeny and agreement in morphological characters, we transfer *T. palettaris* to the *aculeatus* species group, erect the *curticornis* and *heusdensis* 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 likely sister to the remains of the *chinyensis* group (MLB = 98%; PP = 1), while the *aterrimus* + *tamakutibasi* groups are sister to the *chinyensis* + *curticornis* groups (MLB = 69%; PP = 1).

This *aterrimus* + *chinyensis* + *curticornis* + *tamakutibasi* group clade is sister to clade C (Fig. 1; MLB = 62%; PP = 1), which includes Neotropical *Tanytarsus* + *ortoni*- + Holarctic *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 since *Tanytarsus rhabdomantis* and *T. obiriciae* Trivinho-Strixino & Sonoda, which both are 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 supports a more narrowly defined group. Since 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 know member of the tribe Tanytarsini is *Gujaratomyia miripes* Giłka & Zakrzewska, recently described from Indian Cambay amber from the early Eocene (ca. 52–53 Ma) (Zakrzewska *et al.*, 2018). The oldest known member of genus *Tanytarsus* is also found in the Indian Cambay amber (ca. 50–52 Ma), but remains to be described (Stebner *et al.*, 2017). Divergence time estimates in our phylogeny of *Tanytarsus* (Files S2–3) 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 *Gujaratomyia* and its sister group relationship is therefore uncertain. However, *G. miripes* Giłka & Zakrzewska show some affinities with *Cladotanytarsus* (Zakrzewska *et al.*, 2018). Thus, the co-existence of *Tanytarsus* and *Gujaratomyia* in Cambay amber indicate that *Tanytarsus* evolved earlier than the Ypresian (ca. 52 Ma) and perhaps as early as our analyses suggest.

Ancestral area reconstruction. The S-DIVA analyses based on three different geographical division schemes yielded similar results (Fig. 5; Files S4–5), 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 Palearctic regions) with subsequent dispersal and vicariance events leading to the separation of three major clades (Fig. 5). An early dispersal event during the Paleocene (65 Ma) led to the Palearctic *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 Paleocene (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 Gondwana 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 Palearctic- and Neotropical 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 Palaearctic probably was caused by dispersal events during the Eocene (56 Ma). Worth mentioning is that the monophyletic *norvegicus* species group is distributed only in/near the Arctic and on 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 mid-Miocene (11 Ma) via a vicariance event (e.g. Orogeny in 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 round 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 indicate 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 chironomids could migrate from the neotropics to the northern Holarctic as they are weak fliers. The observed pattern may result from sampling bias and extinction. For instance, the presence of *Caladomyia* is 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 previous 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).

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 s.l.*. 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*-, *edwardi*-, *excavatus*-, *giovannii*-, *heusdensis*-, *kiche*-, *lestagei*-, *luctuosus*-, *motosuensis*-, *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 likely are 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 Palearctic- regions. Additional taxon sampling and more genetic data are required to recover the full evolutionary history of the diverse genus in future.

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Figures

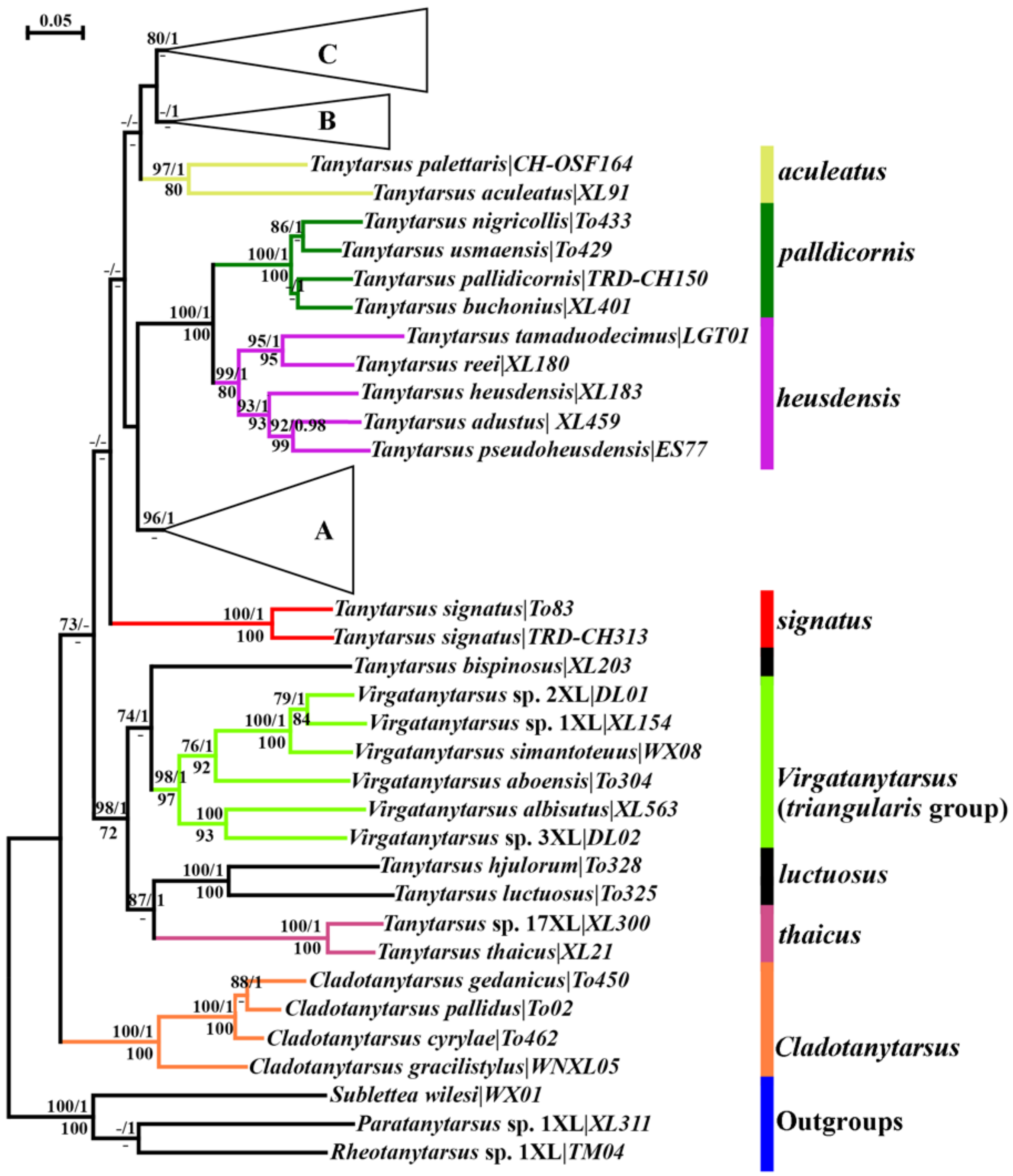


Figure 1. Maximum likelihood tree based on the concatenated DNA dataset (18S, AATS1, CAD1, CAD4, PGD, TPI, 4281 characters) of *Tanytarsus sensu lato*. Numbers on branches refer to posterior probabilities over 0.95 + ML bootstrap values over 70% / MP bootstrap values over 70%. Clades are labelled with species groups names suggested in this study.

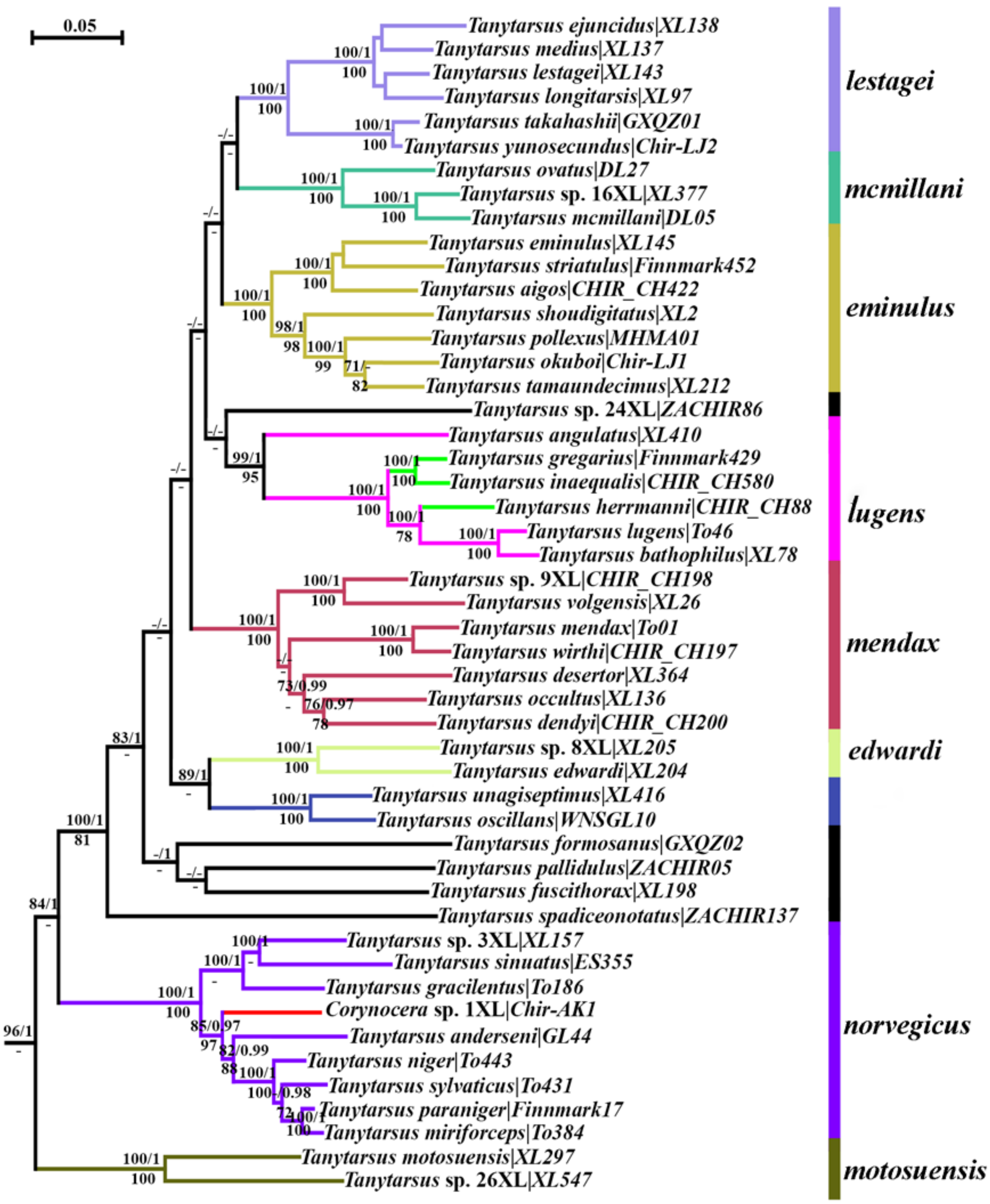


Figure 2. Clade A of the maximum likelihood tree based on the concatenated DNA dataset (18S, AATS1, CAD1, CAD4, PGD, TPI, 4281 characters) of *Tanytarsus sensu lato*. Numbers on branches refer to posterior probabilities over 0.95 + ML bootstrap values over 70% / MP bootstrap values over 70%. Clades are labelled with species groups names suggested in this study.

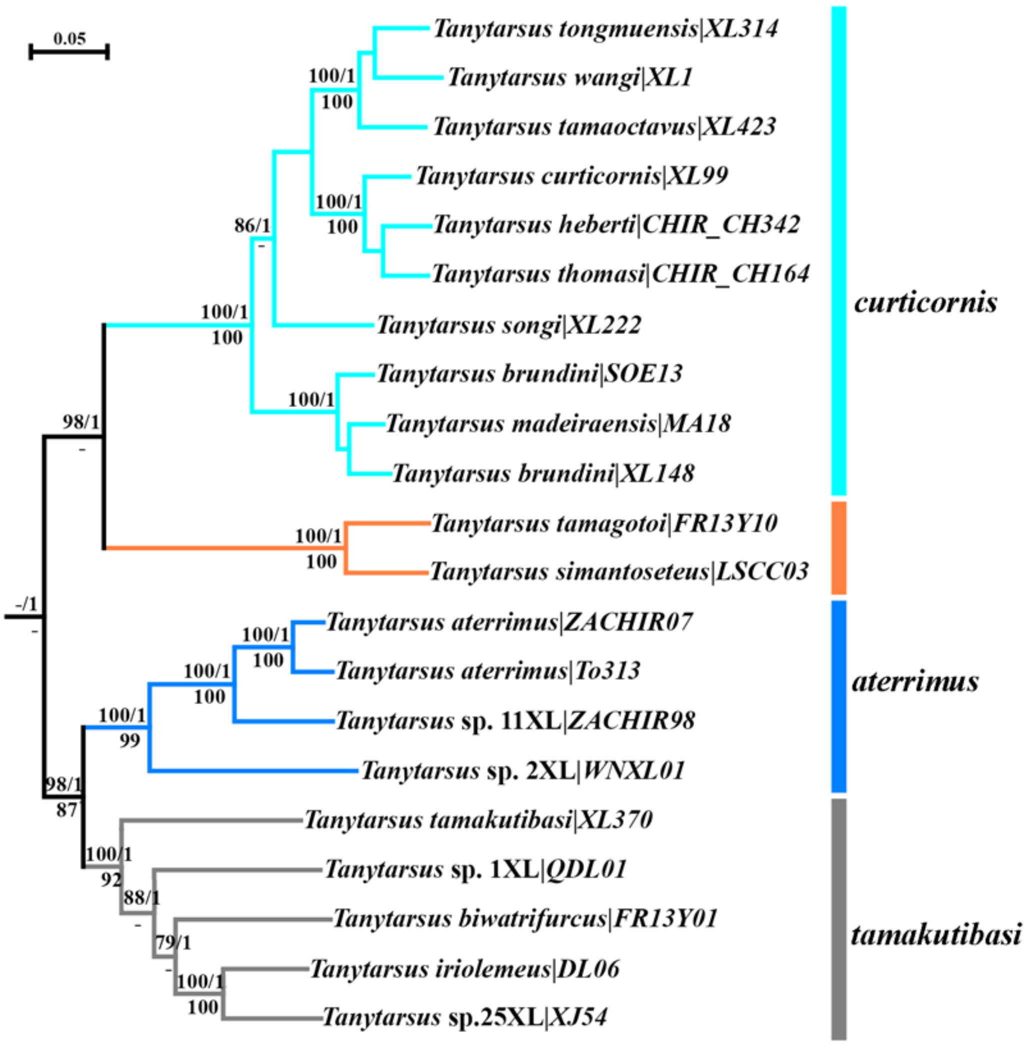


Figure 3. Clade B of the maximum likelihood tree based on the concatenated DNA dataset (18S, AATS1, CAD1, CAD4, PGD, TPI, 4281 characters) of *Tanytarsus sensu lato*. Numbers on branches refer to posterior probabilities over 0.95 + ML bootstrap values over 70% / MP bootstrap values over 70%. Clades are labelled with species groups names suggested in this study.

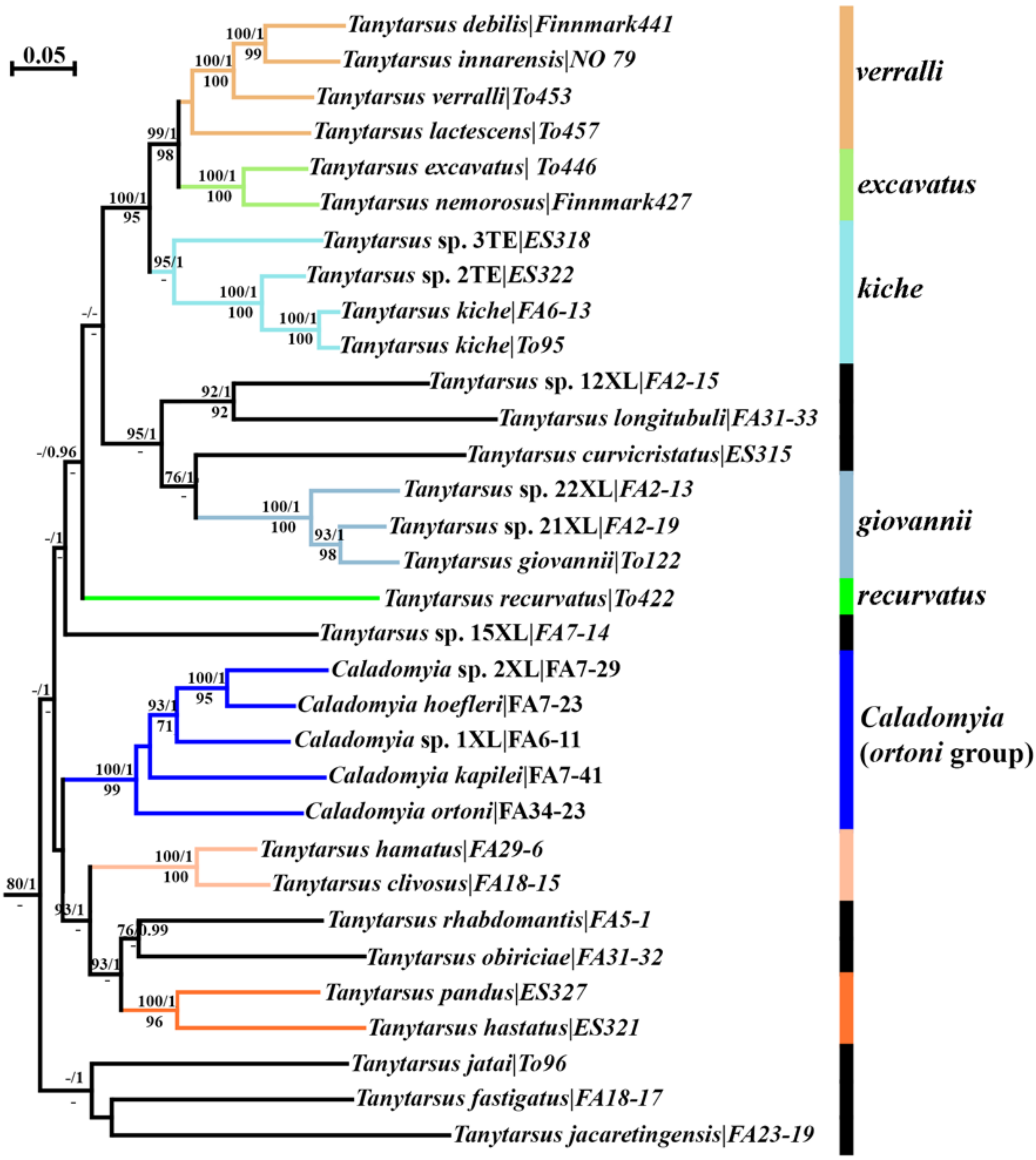
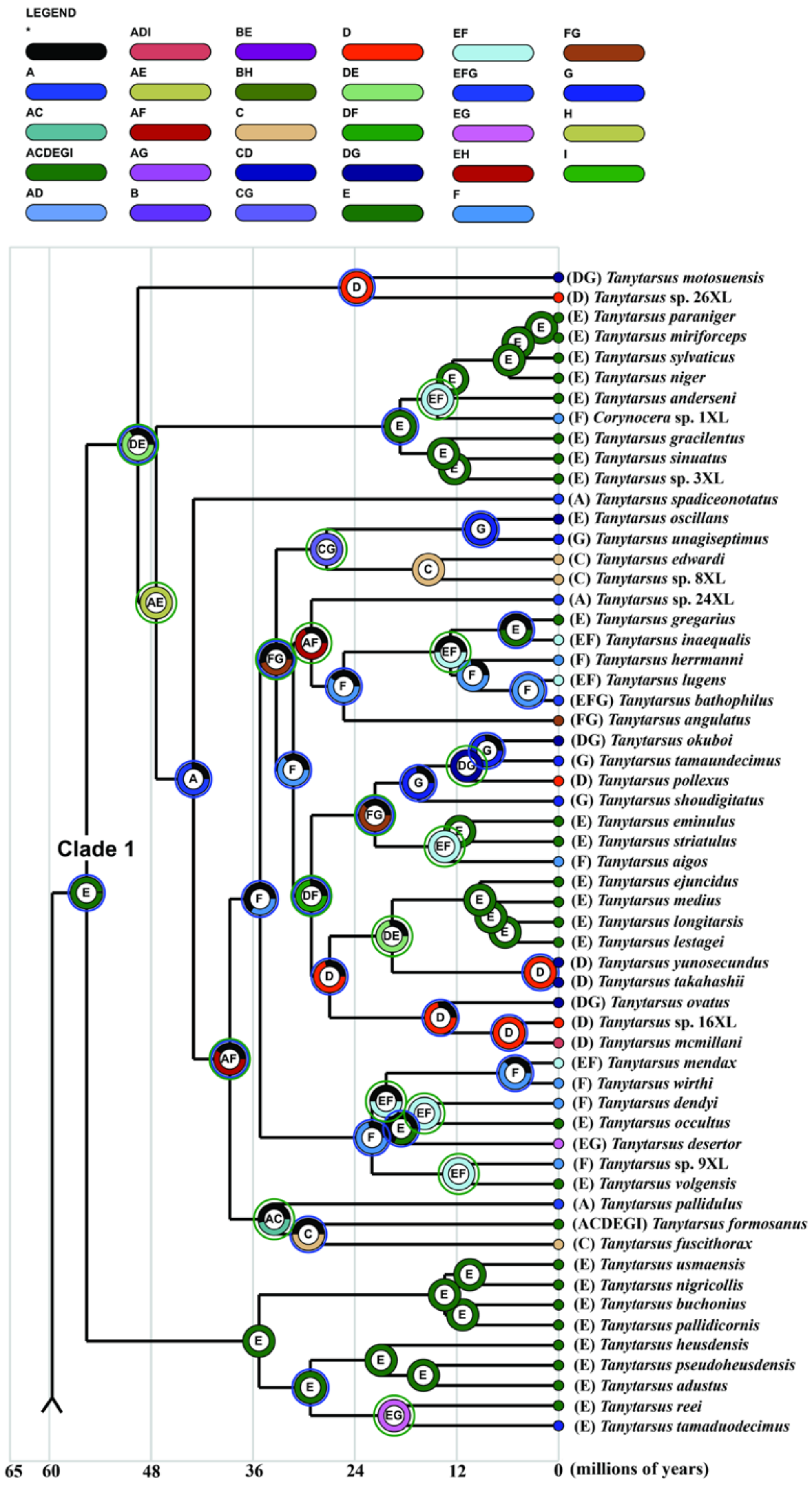


Figure 4. Clade C of the maximum likelihood tree based on the concatenated DNA dataset (18S, AATS1, CAD1, CAD4, PGD, TPI, 4281 characters) of *Tanytarsus sensu lato*. Numbers on branches refer to posterior probabilities over 0.95 + ML bootstrap values over 70% / MP bootstrap values over 70%. Clades are labelled with species groups names suggested in this study.


LEGEND
*
ADI
BE
D
EF
FG
A
AE
BH
DE
EFG
G
AC
AF
C
DF
EG
H
ACDEGI
AG
CD
DG
EH
I
AD
B
CG
E
F
Clade 1
(DG) Tanytarsus motosuensis
(D) Tanytarsus sp. 26XL
(E) Tanytarsus paraniger
(E) Tanytarsus miriforceps
(E) Tanytarsus sylvaticus
(E) Tanytarsus niger
(E) Tanytarsus anderseni
(F) Corynocera sp. 1XL
(E) Tanytarsus gracilentus
(E) Tanytarsus sinuatus
(E) Tanytarsus sp. 3XL
(A) Tanytarsus spadiceonotatus
(E) Tanytarsus oscillans
(G) Tanytarsus unagiseptimus
(C) Tanytarsus edwardi
(C) Tanytarsus sp. 8XL
(A) Tanytarsus sp. 24XL
(E) Tanytarsus gregarius
(EF) Tanytarsus inaequalis
(F) Tanytarsus herrmanni
(EF) Tanytarsus lugens
(EFG) Tanytarsus bathophilus
(FG) Tanytarsus angulatus
(DG) Tanytarsus okuboi
(G) Tanytarsus tamaundecimus
(D) Tanytarsus pollexus
(G) Tanytarsus shoudigitatus
(E) Tanytarsus eminulus
(E) Tanytarsus striatulus
(F) Tanytarsus aigos
(E) Tanytarsus ejuncidus
(E) Tanytarsus medius
(E) Tanytarsus longitarsis
(E) Tanytarsus lestagei
(D) Tanytarsus yunosecundus
(D) Tanytarsus takahashii
(DG) Tanytarsus ovatus
(D) Tanytarsus sp. 16XL
(D) Tanytarsus mcmillani
(EF) Tanytarsus mendax
(F) Tanytarsus wirthi
(F) Tanytarsus dendyi
(E) Tanytarsus occultus
(EG) Tanytarsus desertor
(F) Tanytarsus sp. 9XL
(E) Tanytarsus volgensis
(A) Tanytarsus pallidulus
(ACDEGI) Tanytarsus formosanus
(C) Tanytarsus fuscithorax
(E) Tanytarsus usmaensis
(E) Tanytarsus nigricollis
(E) Tanytarsus buchonius
(E) Tanytarsus pallidicornis
(E) Tanytarsus heusdensis
(E) Tanytarsus pseudoheusdensis
(E) Tanytarsus adustus
(E) Tanytarsus reei
(E) Tanytarsus tamaduodecimus
65 60 48 36 24 12 0 (millions of years)

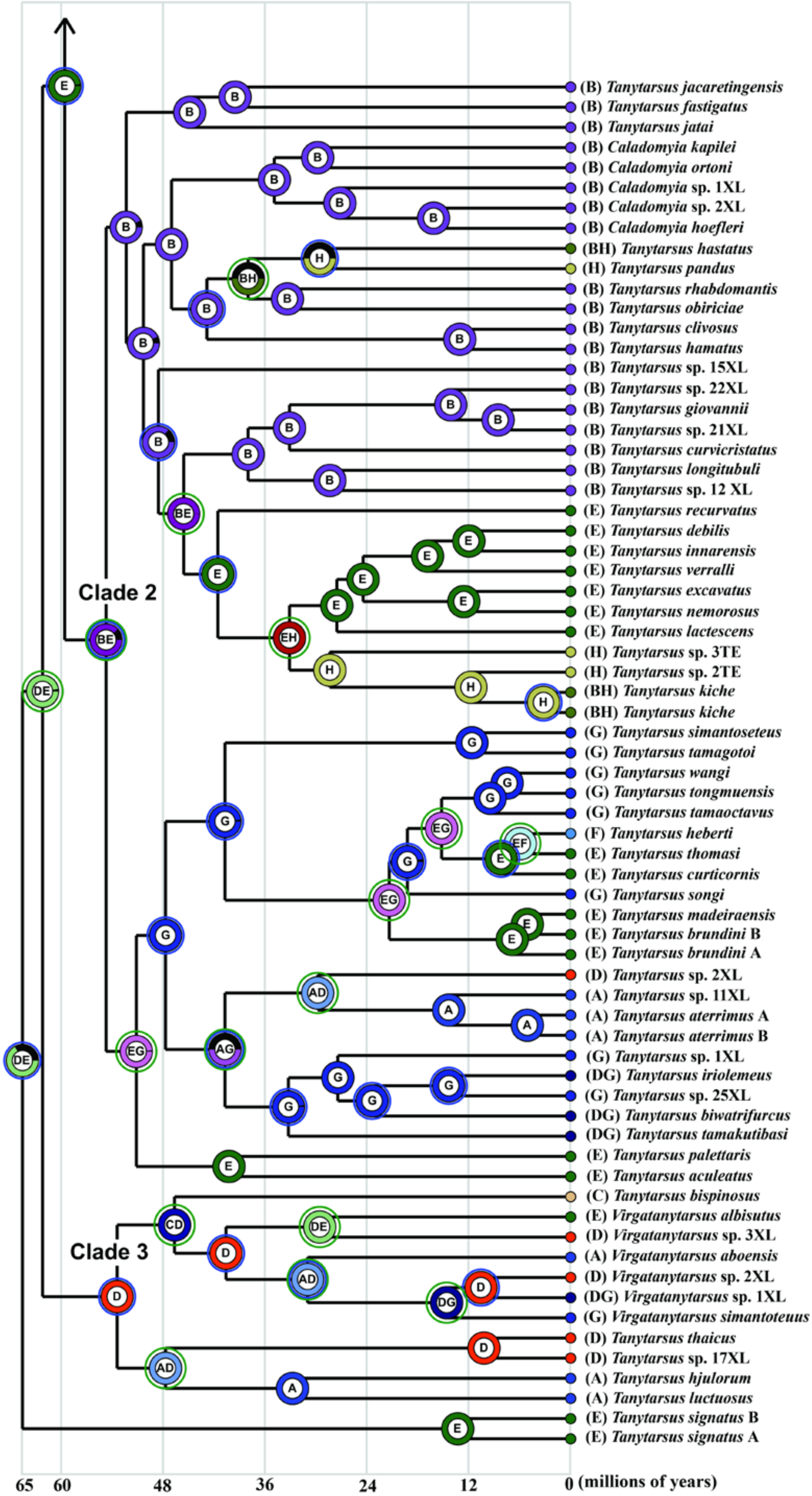

(B) Tanytarsus jacaretingensis
(B) Tanytarsus fastigatus
(B) Tanytarsus jatai
(B) Caladomyia kapilei
(B) Caladomyia ortoni
(B) Caladomyia sp. 1XL
(B) Caladomyia sp. 2XL
(B) Caladomyia hoefleri
(BH) Tanytarsus hastatus
(H) Tanytarsus pandus
(B) Tanytarsus rhabdomantis
(B) Tanytarsus obiriciae
(B) Tanytarsus clivosus
(B) Tanytarsus hamatus
(B) Tanytarsus sp. 15XL
(B) Tanytarsus sp. 22XL
(B) Tanytarsus giovannii
(B) Tanytarsus sp. 21XL
(B) Tanytarsus curvicristatus
(B) Tanytarsus longitubuli
(B) Tanytarsus sp. 12 XL
(E) Tanytarsus recurvatus
(E) Tanytarsus debilis
(E) Tanytarsus innarensis
(E) Tanytarsus verralli
(E) Tanytarsus excavatus
(E) Tanytarsus nemorosus
(E) Tanytarsus lactescens
(H) Tanytarsus sp. 3TE
(H) Tanytarsus sp. 2TE
(BH) Tanytarsus kiche
(BH) Tanytarsus kiche
(G) Tanytarsus simantoseteus
(G) Tanytarsus tamagotoi
(G) Tanytarsus wangi
(G) Tanytarsus tongmuensis
(G) Tanytarsus tamaoctavus
(F) Tanytarsus heberti
(E) Tanytarsus thomasi
(E) Tanytarsus curticornis
(G) Tanytarsus songi
(E) Tanytarsus madeiraensis
(E) Tanytarsus brundini B
(E) Tanytarsus brundini A
(D) Tanytarsus sp. 2XL
(A) Tanytarsus sp. 11XL
(A) Tanytarsus aterrimus A
(A) Tanytarsus aterrimus B
(G) Tanytarsus sp. 1XL
(DG) Tanytarsus iriolemeus
(G) Tanytarsus sp. 25XL
(DG) Tanytarsus biwatrifurcus
(DG) Tanytarsus tamakutibasi
(E) Tanytarsus palettaris
(E) Tanytarsus aculeatus
(C) Tanytarsus bispinosus
(E) Virgatanytarsus albisutus
(D) Virgatanytarsus sp. 3XL
(A) Virgatanytarsus aboensis
(D) Virgatanytarsus sp. 2XL
(DG) Virgatanytarsus sp. 1XL
(G) Virgatanytarsus simantoteuus
(D) Tanytarsus thaicus
(D) Tanytarsus sp. 17XL
(A) Tanytarsus hjulorum
(A) Tanytarsus luctuosus
(E) Tanytarsus signatus B
(E) Tanytarsus signatus A
Clade 2
Clade 3
65
60
48
36
24
12
0 (millions of years)

Figure 5. Hypothesized event-based ancestral area reconstruction of *Tanytarsus* as inferred by 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. The letter 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.

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 to 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) Giłka & Paasivirta (2009)	*T. brundini* Lindeberg *T. curticornis* Kieffer *T. heusdensis* Goetghebuer *T. palettaris* Verneaux	Refuted and split into several unrelated groups. *Tanytarsus chinyensis* was not included in our analyses, thus name-bearing species remain unplaced.
eminulus group	Reiss & Fittkau (1971) Ekrem (2003)	*T. aigos* Ekrem, Sublette & Sublette *T. ejuncidus* (Walker) *T. eminulus* (Walker) *T. lestagei* aggregate of species *T. longitarsis* Kieffer *T. medius* Reiss & Fittkau *T. okuboi* Sasa & Kikuchi *T. oscillans* Johannsen *T. pollexus* Datta *T. shoudigitatus* Sasa *T. striatulus* Lindeberg *T. tamaundecimus* Sasa *T. yunosecundus* Sasa	Refuted and split into several groups.
excavatus group	Reiss & Fittkau (1971)	*T. excavatus* Edwards *T. nemorosus* Edwards	Confirmed.
gregarius group	Reiss & Fittkau (1971) Ekrem (2003)	*T. gregarius* (Kieffer) *T. herrmanni* Ekrem, Sublette & Sublette *T. inaequalis* Goetghebuer	Refuted and merged with the *lugens* group.
mcmillani group	Ekrem (2003)	*T. mcmillani* Freeman *T. spadiceonotatus* Freeman	Refuted. *Tanytarsus spadiceonotatus* does not group with *T. mcmillani*.

mendax group	Reiss & Fittkau (1971) (as *holochlorus* group) Ekrem (2003)	*T. aculeatus* Brundin *T. dendyi* Sublette *T. desertor* Giłka & Paasivirta *T. formosanus* Kieffer (= *T. horni* Goetghebuer 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	Refuted. *Tanytarsus formosanus* does not group with core species in the *mendax* group. *Tanytarsus aculeatus* and *T. ovatus* were suggested as part of the *mendax* group by Ekrem (2003), but this is refuted here. The placement of *Tanytarsus desertor* in the *mendax* group Giłka & Paasivirta (2007) is confirmed.
kiche group	Dantas & Giłka (2017)	*T. kiche* Vinogradova, Riss & Spies	Confirmed as separate lineage; only one previously described species included, which groups with two unnamed species.
lugens group	Reiss & Fittkau (1971) Ekrem (2003)	*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. gracilentus* (Holmgren) *T. miriforceps* (Kieffer) *T. niger* Andersen *T. paraniger* Giłka & Paasivirta *T. sinuatus* Goetghebuer *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 Giłka & Paasivirta (2008).
pallidicornis group	Reiss & Fittkau (1971)	*T. buchonius* Reiss & Fittkau *T. nigricollis* Goetghebuer *T. pallidicornis* (Walker) *T. usmaensis* Pagast	Confirmed.
recurvatus group	Reiss & Fittkau (1971)	*T. recurvatus* Brundin	Confirmed as separate lineage; only one species included in the analysis.
riopreto group	Sublette & Sasa (1994) nec Fittkau & Reiss (1973)	*T. clivosus* Reiss *T. hamatus* Reiss *T. hastatus* Sublette & Sasa *T. pandus* Sublette & Sasa	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 Fittkau & Reiss (1973). The inclusion of *T.*

			clivosus, *T. hamatus*, *T. hastatus* and *T. pandus* in the *riopreto* group (Sublette & Sasa, 1994) was questioned by Sanseverino & Wiedenbrug (2000) and Sanseverino (2006).
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) Kugler & Reiss (1973) Pinder (1982) as *Virgatanytarsus* Cranston & Armitage (1988) as *Virgatanytarsus* Harrison (2004) as *Virgatanytarsus*	*T. aboensis* Harrison *T. albisutus* 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.
verralli group	Reiss & Fittkau (1971)	*T. debilis* (Meigen) *T. innarensis* Brundin *T. lactescens* Edwards *T. verralli* Goetghebuer	Confirmed.
Caladomyia	Säwedal (1981) Reiff (2000) Trivinho-Strixino (2012)	*T. hoefleri* Reiff *T. kapilei* Trivinho-Strixino *T. ortoni* Säwedal	Confirmed as monophyletic group, embedded in *Tanytarsus*.

Supporting information

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

File S2. BEAST divergence time estimates tree with node age. Node 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 millions of years, 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 millions of years, 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 the traditional Wallace'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, green circles indicate vicariance events. The letter 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, green circles indicate vicariance events. The letter 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.

Tanytarsus motosuensis|XL297
Tanytarsus sp. 26XL|XL547
Tanytarsus paraniger|Finnmark17
Tanytarsus miriforceps|To384
Tanytarsus sylvaticus|To431
Tanytarsus niger|To443
Tanytarsus anderseni|GL44
Corynocera sp. 1XL|Chir-AK1
Tanytarsus gracilentus|To186
Tanytarsus sinuatus|ES355
Tanytarsus sp. 3XL|XL157
Tanytarsus spadiceonotatus|ZACHIR137
Tanytarsus oscillans|WNSGL10
Tanytarsus unagiseptimus|XL416
Tanytarsus edwardi|XL204
Tanytarsus sp. 8XL|XL205
Tanytarsus sp. 24XL|ZACHIR86
Tanytarsus gregarius|Finnmark429
Tanytarsus inaequalis|CHIR CH580
Tanytarsus herrmanni|CHIR CH88
Tanytarsus lugens|To46
Tanytarsus bathophilus|XL78
Tanytarsus angulatus|XL410
Tanytarsus okuboi|Chir-LJ1
Tanytarsus tamaundecimus|XL212
Tanytarsus pollexus|MHMA01
Tanytarsus shoudigitatus|XL2
Tanytarsus eminulus|XL145
Tanytarsus striatulus|Finnmark452
Tanytarsus aigos|CHIR CH422
Tanytarsus ejuncidus|XL138
Tanytarsus medius|XL137
Tanytarsus longitarsis|XL97
Tanytarsus lestagei|XL143
Tanytarsus yunosecundus|Chir-LJ2
Tanytarsus takahashii|GXQZ01
Tanytarsus ovatus|DL27
Tanytarsus sp. 16XL|XL377
Tanytarsus mcmillani|DL05
Tanytarsus mendax|To01
Tanytarsus wirthi|CHIR CH197
Tanytarsus dendyi|CHIR CH200
Tanytarsus occultus|XL136
Tanytarsus desertor|XL364
Tanytarsus sp. 9XL|CHIR CH198
Tanytarsus volgensis|XL26
Tanytarsus pallidulus|ZACHIR05
Tanytarsus formosanus|GXQZ02
Tanytarsus fuscithorax|XL198
Tanytarsus usmaensis|To429
Tanytarsus nigricollis|To433
Tanytarsus buchonius|XL401
Tanytarsus pallidicornis|TRD-CH150
Tanytarsus heusdensis|XL183
Tanytarsus pseudoheusdensis|ES77
Tanytarsus adustus|XL459
Tanytarsus reei|XL180
Tanytarsus tamaduodecimus|LGT01
Tanytarsus jacaretingensis|FA23-19
Tanytarsus fastigatus|FA18-17
Tanytarsus jatai|To96
Caladomyia kapilei|FA7-41
Caladomyia ortoni|FA34-23
Caladomyia sp. 1XL|FA6-11
Caladomyia sp. 2XL|FA7-29
Caladomyia hoefleri|FA7-23
Tanytarsus hastatus|ES321
Tanytarsus pandus|ES327
Tanytarsus rhabdomantis|FA5-1
Tanytarsus obiriciae|FA31-32
Tanytarsus clivosus|FA18-15
Tanytarsus hamatus|FA29-6
Tanytarsus sp. 15XL|FA7-14
Tanytarsus sp. 22XL|FA2-13
Tanytarsus giovannii|To122
Tanytarsus sp. 21XL|FA2-19
Tanytarsus curvicristatus|ES315
Tanytarsus longitubuli|FA31-33
Tanytarsus sp. 12XL|FA2-15
Tanytarsus recurvatus|To422
Tanytarsus debilis|Finnmark441
Tanytarsus innarensis|NO 79
Tanytarsus verralli|To453
Tanytarsus excavatus|To446
Tanytarsus nemorosus|Finnmark427
Tanytarsus lactescens|To457
Tanytarsus sp. 3TE|ES318
Tanytarsus sp. 2TE|ES322
Tanytarsus kiche|To95
Tanytarsus kiche|FA6-13
Tanytarsus simantoseteus|LSCC03
Tanytarsus tamagotoi|FR13Y10
Tanytarsus wangi|XL1
Tanytarsus tongmuensis|XL314
Tanytarsus tamaoctavus|XL423
Tanytarsus heberti|CHIR CH342
Tanytarsus thomasi|CHIR CH164
Tanytarsus curticornis|XL99
Tanytarsus songi|XL222
Tanytarsus madeiraensis|MA18
Tanytarsus brundini|XL148
Tanytarsus brundini|SOE13
Tanytarsus sp. 2XL|WNXL01
Tanytarsus sp. 11XL|ZACHIR98
Tanytarsus aterrimus|To313
Tanytarsus aterrimus|ZACHIR07
Tanytarsus sp. 1XL|QDL01
Tanytarsus iriolemeus|DL06
Tanytarsus sp. 25XL|XJ54
Tanytarsus biwatrifurcus|FR13Y01
Tanytarsus tamakutibasi|XL370
Tanytarsus palettaris|CH-OSF164
Tanytarsus aculeatus|XL91
Tanytarsus bispinosus|XL203
Virgatanytarsus albisutus|XL563
Virgatanytarsus sp. 3XL|DL02
Virgatanytarsus aboensis|To304
Virgatanytarsus sp. 2XL|DL01
Virgatanytarsus sp. 1XL|XL154
Virgatanytarsus simantoteuus|WX08
Tanytarsus thaicus|XL21
Tanytarsus sp. 17XL|XL300
Tanytarsus hjulorum|To328
Tanytarsus luctuosus|To325
Tanytarsus signatus|To83
Tanytarsus signatus|TRD-CH313
Cladotanytarsus cyrylae|To462
Cladotanytarsus gedanicus|To450
Cladotanytarsus pallidus|To02
Cladotanytarsus gracilistylus|WNXL05
Sublettea wilesi|WX01
Paratanytarsus sp. 1XL|XL311
Rheotanytarsus sp. 1XL|TM04
9.0
90 80 70 60 50 40 30 20 10 0

Tanytarsus motosuensis|XL297
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Tanytarsus angulatus|XL410
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Tanytarsus tamaundecimus|XL212
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Tanytarsus eminulus|XL145
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Tanytarsus wirthi|CHIR CH197
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Tanytarsus heusdensis|XL183
Tanytarsus pseudoheusdensis|ES77
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Tanytarsus tamaduodecimus|LGT01
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Tanytarsus fastigatus|FA18-17
Tanytarsus jatai|To96
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Tanytarsus thomasi|CHIR CH164
Tanytarsus curticornis|XL99
Tanytarsus songi|XL222
Tanytarsus madeiraensis|MA18
Tanytarsus brundini|XL148
Tanytarsus brundini|SOE13
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Tanytarsus sp. 11XL|ZACHIR98
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Tanytarsus aterrimus|ZACHIR07
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Tanytarsus tamakutibasi|XL370
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Tanytarsus bispinosus|XL203
Virgatanytarsus albisutus|XL563
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Virgatanytarsus aboensis|To304
Virgatanytarsus sp. 2XL|DL01
Virgatanytarsus sp. 1XL|XL154
Virgatanytarsus simantoteuus|WX08
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Tanytarsus sp. 17XL|XL300
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Tanytarsus luctuosus|To325
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Tanytarsus signatus|TRD-CH313
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Cladotanytarsus gedanicus|To450
Cladotanytarsus pallidus|To02
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Sublettea wilesi|WX01
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Rheotanytarsus sp. 1XL|TM04
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LEGEND
*
A
AC
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F
(DE) Tanytarsus motosuensis
(D) Tanytarsus sp. 26XL
(E) Tanytarsus paraniger
(E) Tanytarsus miriforceps
(E) Tanytarsus sylvaticus
(E) Tanytarsus niger
(E) Tanytarsus anderseni
(F) Corynocera sp. 1XL
(E) Tanytarsus gracilentus
(E) Tanytarsus sinuatus
(E) Tanytarsus sp. 3XL
(A) Tanytarsus spadiceonotatus
(D) Tanytarsus oscillans
(DE) Tanytarsus unagiseptimus
(C) Tanytarsus edwardi
(C) Tanytarsus sp. 8XL
(A) Tanytarsus sp. 24XL
(E) Tanytarsus gregarius
(EF) Tanytarsus inaequalis
(F) Tanytarsus herrmanni
(EF) Tanytarsus lugens
(EF) Tanytarsus bathophilus
(EF) Tanytarsus angulatus
(DE) Tanytarsus okuboi
(DE) Tanytarsus tamaundecimus
(D) Tanytarsus pollexus
(DE) Tanytarsus shoudigitatus
(E) Tanytarsus eminulus
(E) Tanytarsus striatulus
(F) Tanytarsus aigos
(E) Tanytarsus ejuncidus
(E) Tanytarsus medius
(E) Tanytarsus longitarsis
(E) Tanytarsus lestagei
(DE) Tanytarsus yunosecundus
(DE) Tanytarsus takahashii
(DE) Tanytarsus ovatus
(D) Tanytarsus sp. 16XL
(AD) Tanytarsus mcmillani
(EF) Tanytarsus mendax
(F) Tanytarsus wirthi
(F) Tanytarsus dendyi
(E) Tanytarsus occultus
(DE) Tanytarsus desertor
(F) Tanytarsus sp. 9XL
(E) Tanytarsus volgensis
(A) Tanytarsus pallidulus
(ACDE) Tanytarsus formosanus
(C) Tanytarsus fuscithorax
(E) Tanytarsus usmaensis
(E) Tanytarsus nigricollis
(E) Tanytarsus buchonius
(E) Tanytarsus pallidicornis
(E) Tanytarsus heusdensis
(E) Tanytarsus pseudoheusdensis
(E) Tanytarsus adustus
(E) Tanytarsus reei
(DE) Tanytarsus tamaduodecimus
(B) Tanytarsus jacaretingensis
(B) Tanytarsus fastigatus
(B) Tanytarsus jatai
(B) Caladomyia kapilei
(B) Caladomyia ortoni
(B) Caladomyia sp. 1XL
(B) Caladomyia sp. 2XL
(B) Caladomyia hoefleri
(B) Tanytarsus hastatus
(B) Tanytarsus pandus
(B) Tanytarsus rhabdomantis
(B) Tanytarsus obiriciae
(B) Tanytarsus clivosus
(B) Tanytarsus hamatus
(B) Tanytarsus sp. 15XL
(B) Tanytarsus sp. 22XL
(B) Tanytarsus giovannii
(B) Tanytarsus sp. 21XL
(B) Tanytarsus curvicristatus
(B) Tanytarsus longitubuli
(B) Tanytarsus sp. 12XL
(E) Tanytarsus recurvatus
(E) Tanytarsus debilis
(E) Tanytarsus innarensis
(E) Tanytarsus verralli
(E) Tanytarsus excavatus
(E) Tanytarsus nemorosus
(E) Tanytarsus lactescens
(B) Tanytarsus sp. 3TE
(B) Tanytarsus sp. 2TE
(B) Tanytarsus kiche B
(B) Tanytarsus kiche A
(DE) Tanytarsus simantoseteus
(DE) Tanytarsus tamagotoi
(D) Tanytarsus wangi
(D) Tanytarsus tongmuensis
(DE) Tanytarsus tamaoctavus
(F) Tanytarsus heberti
(F) Tanytarsus thomasi
(E) Tanytarsus curticornis
(E) Tanytarsus songi
(E) Tanytarsus madeiraensis
(E) Tanytarsus brundini B
(E) Tanytarsus brundini A
(D) Tanytarsus sp. 2XL
(A) Tanytarsus sp. 11XL
(A) Tanytarsus aterrimus A
(A) Tanytarsus aterrimus B
(D) Tanytarsus sp. 1XL
(DE) Tanytarsus iriolemeus
(D) Tanytarsus sp. 25XL
(DE) Tanytarsus biwatrifurcus
(DE) Tanytarsus tamakutibasi
(E) Tanytarsus palettaris
(E) Tanytarsus aculeatus
(C) Tanytarsus bispinosus
(E) Virgatanytarsus albisutus
(D) Virgatanytarsus sp. 3XL
(A) Virgatanytarsus aboensis
(D) Virgatanytarsus sp. 2XL
(DE) Virgatanytarsus sp. 1XL
(DE) Virgatanytarsus simantoteuus
(D) Tanytarsus thaicus
(D) Tanytarsus sp. 17XL
(A) Tanytarsus hjulorum
(A) Tanytarsus luctuosus
(E) Tanytarsus signatus B
(E) Tanytarsus signatus A
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LEGEND
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(D) Tanytarsus motosuensis
(D) Tanytarsus sp. 26XL
(E) Tanytarsus paraniger
(E) Tanytarsus miriforceps
(E) Tanytarsus sylvaticus
(E) Tanytarsus niger
(E) Tanytarsus anderseni
(E) Corynocera sp. 1XL
(E) Tanytarsus gracilentus
(E) Tanytarsus sinuatus
(D) Tanytarsus sp. 3XL
(A) Tanytarsus spadiceonotatus
(D) Tanytarsus oscillans
(D) Tanytarsus unagiseptimus
(C) Tanytarsus edwardi
(C) Tanytarsus sp. 8XL
(A) Tanytarsus sp. 24XL
(E) Tanytarsus gregarius
(E) Tanytarsus inaequalis
(E) Tanytarsus herrmanni
(E) Tanytarsus lugens
(D) Tanytarsus bathophilus
(DE) Tanytarsus angulatus
(D) Tanytarsus okuboi
(D) Tanytarsus tamaundecimus
(D) Tanytarsus pollexus
(D) Tanytarsus shoudigitatus
(E) Tanytarsus eminulus
(E) Tanytarsus striatulus
(E) Tanytarsus aigos
(E) Tanytarsus ejuncidus
(E) Tanytarsus medius
(E) Tanytarsus longitarsis
(E) Tanytarsus lestagei
(D) Tanytarsus yunosecundus
(D) Tanytarsus takahashii
(D) Tanytarsus ovatus
(D) Tanytarsus sp. 16XL
(AD) Tanytarsus mcmillani
(E) Tanytarsus mendax
(E) Tanytarsus wirthi
(E) Tanytarsus dendyi
(E) Tanytarsus occultus
(DE) Tanytarsus desertor
(E) Tanytarsus sp. 9XL
(E) Tanytarsus volgensis
(A) Tanytarsus pallidulus
(ACDE) Tanytarsus formosanus
(C) Tanytarsus fuscithorax
(E) Tanytarsus usmaensis
(E) Tanytarsus nigricollis
(E) Tanytarsus buchonius
(E) Tanytarsus pallidicornis
(E) Tanytarsus heusdensis
(E) Tanytarsus pseudoheusdensis
(E) Tanytarsus adustus
(E) Tanytarsus reei
(D) Tanytarsus tamaduodecimus
(B) Tanytarsus jacaretingensis
(B) Tanytarsus fastigatus
(B) Tanytarsus jatai
(B) Caladomyia kapilei
(B) Caladomyia ortoni
(B) Caladomyia sp. 1XL
(B) Caladomyia sp. 2XL
(B) Caladomyia hoefleri
(BE) Tanytarsus hastatus
(B) Tanytarsus pandus
(B) Tanytarsus rhabdomantis
(B) Tanytarsus obiriciae
(B) Tanytarsus clivosus
(BE) Tanytarsus hamatus
(B) Tanytarsus sp. 15XL
(B) Tanytarsus sp. 22XL
(B) Tanytarsus giovannii
(B) Tanytarsus sp. 21XL
(BE) Tanytarsus curvicristatus
(B) Tanytarsus longitubuli
(B) Tanytarsus sp. 12XL
(E) Tanytarsus recurvatus
(E) Tanytarsus debilis
(E) Tanytarsus innarensis
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(A) Tanytarsus aterrimus B
(D) Tanytarsus sp. 1XL
(D) Tanytarsus iriolemeus
(D) Tanytarsus sp. 25XL
(D) Tanytarsus biwatrifurcus
(D) Tanytarsus tamakutibasi
(E) Tanytarsus palettaris
(E) Tanytarsus aculeatus
(C) Tanytarsus bispinosus
(E) Virgatanytarsus albisutus
(D) Virgatanytarsus sp. 3XL
(A) Virgatanytarsus aboensis
(D) Virgatanytarsus sp. 2XL
(D) Virgatanytarsus sp. 1XL
(D) Virgatanytarsus simantoeuus
(D) Tanytarsus thaicus
(D) Tanytarsus sp. 17XL
(A) Tanytarsus hjulorum
(A) Tanytarsus luctuosus
(E) Tanytarsus signatus B
(E) Tanytarsus signatus A
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Table S1. Overview of gene segments and primer combinations

Gene segment	Oligo name	Oligo sequence (5'-3')	Reference
18S	18S_ai	CCTGAGAAACGGCTACCACATC	(Whiting *et al.*, 1997)
	18S_bi	GAGTCTCGTTCGTTATCGGA	(Whiting *et al.*, 1997)
AATS1	A1-92F	TAYCAYCAYACNTTYTTYGARATG	(Regier *et al.*, 2008)
	A1-244R	ATNCCRCARTCNATRTGYTT	(Su *et al.*, 2008)
CAD1	54F	GTNGTNTTYCARACNGGNATGGT	(Moulton & Wiegmann, 2004)
	405R	GCNGTRTGYTCNGGRTGRAAYTG	(Moulton & Wiegmann, 2004)
	122F	CCACTYATYGGNAAYTATGGNGT	This study
	909R	AAYYTMAATGAYAAYTCNAAYGARGGA	This study
CAD4	787F	GGDGTNACNACNGCNTGYTTYGARCC	(Moulton & Wiegmann, 2004)
	1098R	TTNGGNAGYTGNCCNCCCAT	(Moulton & Wiegmann, 2004)
PGD	PGD-2F	GATATHGARTAYGGNGAYATGCA	(Regier *et al.*, 2008)
	PGD-3R	TRTGIGCNCCRAARTARTC	Brian Cassel (pers. comm.)
	PGD-4R	CNGTCCARTTNGTRTG	Brian Cassel (pers. comm.)
TPI	TPI-111Fb	GGNAAYTGGAARATGAAYGG	(Bertone *et al.*, 2008)
	TPI-275R	CCCANACNGGYTCRTANGC	Brian Cassel (pers. comm.)
	TPI-277R	CDATNGCCCANACNGGYTC	Brian Cassel (pers. comm.)
	TPI-281R	TRNCCNGTNCCDATNGCCCA	Brian Cassel (pers. comm.)

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Table S2. BOLD sample ID and GenBank Accession Numbers of specimens in the dataset.

Species	Sample ID	18S	AATS1	CAD1	CAD4	COI	PGD	TPI
Caladomyia hoefleri	FA7-23	MG785078	MG895495	N/A	MG785765	MG785995	MG785561	MG792414
Caladomyia kapilei	FA7-41	MG785048	MG895465	N/A	MG785739	MG785984	MG785532	MG792386
Caladomyia ortoni	FA34-23	MG785150	MG895570	MG785332	MG785835	MG786025	MG785635	MG792485
Caladomyia sp. 1XL	FA6-11	MG785068	MG895486	MG785249	MG785758	MG785992	MG785552	MG792405
Caladomyia sp. 1XL	FA6-12	MG785084	MG895502	MG785265	MG785772	MG785998	MG785568	MG792421

Caladomyia sp. 2XL	FA7-29	MG785135	MG895554	MG785316	MG785821	MG786018	MG785619	N/A
Cladotanytarsus cyrylae	To462	MG785153	MG895573	MG785335	MG785838	MG786027	MG785638	MG792488
Cladotanytarsus gedanicus	To450	MG785165	MG895586	MG785348	MG785850	MG786030	MG785651	MG792500
Cladotanytarsus gracilistylus	WNXL 05	MG785123	MG895543	MG785304	MG785810	MG786011	MG785608	MG792459
Cladotanytarsus pallidus	To02	N/A	MG895590	MG785352	MG785853	AM398683	MG785655	MG792504
Corynocera sp. 1XL	Chir-AK1	MG785105	MG895524	MG785286	MG785792	MG786004	MG785589	MG792441
Corynocera sp. 1XL	Chir-AK2	MG785184	MG895606	MG785368	MG785868	MG786039	MG785671	MG792517
Paratanytarsus sp. 1XL	XL311	MG785186	MG895610	MG785372	MG785872	MG786041	MG785675	N/A
Pontomyia natans	To509	MG785056	MG895473	MG785236	MG785746	MG785988	MG785540	MG792393
Pontomyia natans	To510	MG785124	MG895544	MG785305	MG785811	MG786012	MG785609	MG792460
Rheotanytarsus sp. 1XL	TM04	MG785070	MG895488	MG785251	MG785759	MG785994	MG785554	MG792407
Sublettea wilesi	WX01	MG785197	MG895622	MG785384	MG785883	MG786049	MG785687	MG792531
Sublettea wilesi	WX02	MG785131	MG895550	MG785312	MG785817	MG786016	MG785615	MG792466
Tanytarsus aculeatus	XL91	MG785204	MG895627	MG785391	MG785889	KT613825	N/A	MG792537
Tanytarsus aculeatus	XL92	MG785089	MG895508	MG785270	MG785777	KT613474	N/A	MG792427
Tanytarsus adustus	XL459	MG648779	N/A	MG648789	N/A	MG678795	MG700386	MG648818
Tanytarsus aigos	CHIR_ CH422	MG785180	MG895602	MG785364	MG785865	KT613712	MG785667	MG792514
Tanytarsus aigos	CHIR_ CH632	MG785101	MG895520	MG785282	MG785788	KT613503	MG785585	MG792438
Tanytarsus anderseni	Finnma rk110	MG785157	MG895578	MG785340	MG785843	HQ551521	MG785643	MG792492
Tanytarsus anderseni	GL44	MG785113	MG895533	MG785294	MG785801	KT613552	MG785598	MG792449
Tanytarsus angulatus	XL410	MG785059	MG895476	MG785239	MG785749	MG785989	MG785542	MG792396
Tanytarsus angulatus	XL411	MG785168	MG895589	MG785351	MG785852	MG786031	MG785654	MG792503
Tanytarsus aterrimus	To313	N/A	MG895503	N/A	N/A	AM084262	MG785569	MG792422
Tanytarsus aterrimus	ZACHI R07	MG785046	MG895463	N/A	N/A	KT613340	MG785530	MG792384
Tanytarsus bathophilus	XL78	MG785062	MG895479	MG785242	MG785752	KT613389	MG785545	MG792399
Tanytarsus bathophilus	XL79	MG785160	MG895581	MG785343	MG785846	KT613670	MG785646	MG792495
Tanytarsus bispinosus	XL203	MG785034	MG895451	MG785216	MG785727	KT613318	MG785518	MG792374
Tanytarsus biwatrifurcus	FR13Y 01	MG785155	MG895575	MG785337	MG785840	KT613649	MG785640	N/A
Tanytarsus biwatrifurcus	NBSM 06	MG785107	MG895526	MG785288	MG785794	KT613537	MG785591	N/A
Tanytarsus brundini	SOE13	N/A	MG648597	MG648544	N/A	AM398769	MG648659	N/A
Tanytarsus brundini	SOE23 9	N/A	MG648561	MG648511	N/A	HQ105357	MG648623	N/A
Tanytarsus brundini	To137	N/A	MG648595	MG648542	MG648672	MG680441	MG648657	N/A
Tanytarsus brundini	XL148	MG648489	MG648578	MG648526	MG648665	KT613637	MG648640	MG648603
Tanytarsus buchonius	XL401	MG785161	MG895582	MG785344	N/A	MG786029	MG785647	MG792496
Tanytarsus brundini	XL403	MG648486	MG648567	MG648516	N/A	MG680424	MG648629	MG648601
Tanytarsus clivosus	FA18-15	MG785067	MG895485	MG785248	MG785757	MG785991	MG785551	MG792404
Tanytarsus curticornis	To82	MG648491	MG648586	N/A	MG648667	AM398770	MG648648	N/A

Tanytarsus curticornis	XL99	MG648484	MG648554	MG648504	MG648662	KT613384	MG648616	N/A
Tanytarsus curvicristatus	ES315	MG785117	MG895537	MG785298	MG785804	JF870774	MG785602	MG792453
Tanytarsus curvicristatus	ES325	MG785158	MG895579	MG785341	MG785844	JF870783	MG785644	MG792493
Tanytarsus debilis	Finnmark441	MG785030	MG895447	MG785213	MG785723	JN265052	MG785514	MG792370
Tanytarsus debilis	Finnmark450	MG785139	MG895558	MG785320	MG785825	JN265060	MG785623	MG792473
Tanytarsus dendyi	CHIR_CH200	MG785190	MG895615	MG785377	MG785876	KT613769	MG785680	MG792524
Tanytarsus dendyi	CHIR_CH513	MG785166	MG895587	MG785349	N/A	KT613685	MG785652	MG792501
Tanytarsus desertor	XL364	MG785192	MG895617	MG785379	MG785878	MG786046	MG785682	MG792526
Tanytarsus edwardi	XL204	MG785081	MG895498	MG785261	MG785768	KT613446	MG785564	MG792417
Tanytarsus ejuncidus	CH-OSF38	MG785202	MG895625	MG785389	MG785887	KT613818	MG785692	MG792535
Tanytarsus ejuncidus	XL138	MG785085	MG895504	MG785266	MG785773	KT613471	MG785570	MG792423
Tanytarsus eminulus	XL145	MG785038	MG895455	MG785220	MG785730	KT613325	MG785522	MG792377
Tanytarsus excavatus	To446	MG785044	MG895461	MG785226	MG785736	KT613334	MG785528	MG792382
Tanytarsus fastigatus	FA18-17	MG785178	MG895600	MG785362	MG785863	MG786036	MG785665	MG792512
Tanytarsus formosanus	GMM01	MG785103	MG895522	MG785284	MG785790	KT613531	MG785587	MG792439
Tanytarsus formosanus	GXQZ02	MG785071	MG895489	MG785252	MG785760	KT613431	MG785555	MG792408
Tanytarsus fuscithorax	XL198	MG785203	MG895626	MG785390	MG785888	KT613820	MG785693	MG792536
Tanytarsus fuscithorax	XL199	MG785141	MG895560	MG785322	MG785827	KT613617	MG785625	MG792475
Tanytarsus giovannii	To122	N/A	MG895607	MG785369	MG785869	MG786040	MG785672	MG792518
Tanytarsus gracilentus	To186	MG785147	MG895566	MG785328	MG785833	MG786022	MG785631	MG792481
Tanytarsus gracilentus	SOD03	MG785146	MG895565	MG785327	MG785832	KT613624	MG785630	MG792480
Tanytarsus gregarius	Finnmark429	MG785151	MG895571	MG785333	MG785836	KT613646	MG785636	MG792486
Tanytarsus hamatus	FA29-6	MG785134	MG895553	MG785315	MG785820	MG786017	MG785618	MG792469
Tanytarsus hamatus	FA29-7	MG785189	MG895613	MG785375	MG785874	MG786043	MG785678	MG792522
Tanytarsus hastatus	ES321	MG785115	MG895535	MG785296	MG785802	JF870779	MG785600	MG792451
Tanytarsus heberti	CHIR_CH342	MG648495	MG648594	MG648541	MG648671	KT613768	MG648656	N/A
Tanytarsus herrmanni	CHIR_CH88	MG785055	MG895472	MG785235	MG785745	KT613360	MG785539	MG792392
Tanytarsus herrmanni	CHIR_CH277	N/A	MG895577	MG785339	MG785842	GU073203	MG785642	MG792491
Tanytarsus heusdensis	XL183	MG648776	MG648795	MG648785	N/A	MG678792	MG700382	MG648816
Tanytarsus heusdensis	XL186	MG648780	MG648799	MG648790	N/A	KT613681	MG700387	MG648819
Tanytarsus hjulorum	To328	MG785043	MG895460	MG785225	MG785735	AM084264	MG785527	MG792381
Tanytarsus inaequalis	CHIR_CH193	MG785208	MG895631	MG785395	MG785893	GU073206	MG785697	MG792541
Tanytarsus inaequalis	CHIR_CH580	MG785092	MG895511	MG785273	MG785780	KT613483	MG785576	MG792430
Tanytarsus innarensis	NO 79	MG785126	MG895545	MG785307	MG785813	KT613579	MG785611	MG792461
Tanytarsus iriolemeus	DL06	MG785106	MG895525	MG785287	MG785793	KT613536	MG785590	MG792442
Tanytarsus iriolemeus	WNSGL15	MG785176	MG895598	MG785360	MG785861	KT613706	MG785663	MG792510

Tanytarsus jacaretingensis	FA23-19	MG785027	MG895444	MG785210	N/A	MG785979	MG785511	MG792368
Tanytarsus jacaretingensis	FA23-20	MG785149	MG895569	MG785331	N/A	MG786024	MG785634	MG792484
Tanytarsus jatai	To96	MG785104	MG895523	MG785285	MG785791	MG786003	MG785588	MG792440
Tanytarsus jatai	To97	MG785121	MG895541	MG785302	MG785808	MG786010	MG785606	MG792457
Tanytarsus kiche	FA6-13	MG785086	MG895505	MG785267	MG785774	MG785999	MG785571	MG792424
Tanytarsus kiche	To95	MG785191	MG895616	MG785378	MG785877	MG786045	MG785681	MG792525
Tanytarsus kiche	To100	MG785174	MG895596	MG785358	MG785859	MG786033	MG785661	N/A
Tanytarsus lactescens	To457	MG785039	MG895456	MG785221	MG785731	KT613328	MG785523	MG792378
Tanytarsus lestagei	XL143	MG785063	MG895480	MG785243	MG785753	KT613395	MG785546	MG792400
Tanytarsus longitarsis	XL97	MG785173	MG895595	MG785357	MG785858	KT613703	MG785660	MG792508
Tanytarsus longitarsis	XL98	MG785200	MG895624	MG785387	MG785885	KT613804	MG785690	MG792533
Tanytarsus longitubuli	FA31-33	MG785079	MG895496	MG785259	MG785766	MG785996	MG785562	MG792415
Tanytarsus longitubuli	FA34-18	MG785054	MG895471	MG785234	MG785744	MG785987	MG785538	MG792391
Tanytarsus luctuosus	To325	MG785088	MG895507	MG785269	MG785776	AM084266	MG785573	MG792426
Tanytarsus luctuosus	To327	MG785096	MG895515	MG785277	MG785784	AM084267	MG785580	MG792434
Tanytarsus lugens	To46	MG785057	MG895474	MG785237	MG785747	KT613369	N/A	MG792394
Tanytarsus lugens	To48	MG785130	MG895549	MG785311	MG785816	KT613591	N/A	MG792465
Tanytarsus madeiraensis	MA18	MG648493	MG648588	MG648535	MG648669	KT613738	MG648650	MG648606
Tanytarsus madeiraensis	MA19	N/A	MG648579	MG648527	N/A	KT613642	MG648641	N/A
Tanytarsus mcmillani	DL05	MG785082	MG895499	MG785262	MG785769	KT613459	MG785565	MG792418
Tanytarsus mcmillani	WZSM02	MG785201	N/A	MG785388	MG785886	KT613806	MG785691	MG792534
Tanytarsus medius	CH-eik124	MG785028	MG895445	MG785211	MG785721	KT613303	MG785512	MG792369
Tanytarsus medius	XL137	MG785094	MG895513	MG785275	MG785782	KT613486	MG785578	MG792432
Tanytarsus mendax	To01	MG785033	MG895450	N/A	MG785726	AM084268	MG785517	MG792373
Tanytarsus mendax	To05	MG785111	MG895531	N/A	MG785799	AM084269	MG785596	MG792447
Tanytarsus miriforceps	Finnmark446	MG785169	MG895591	MG785353	MG785854	JN265056	MG785656	MG792505
Tanytarsus miriforceps	To384	MG785206	MG895629	MG785393	MG785891	GU073207	MG785695	MG792539
Tanytarsus motosuensis	XL297	MG785205	MG895628	MG785392	MG785890	MG786050	MG785694	MG792538
Tanytarsus motosuensis	XL373	MG785037	MG895454	MG785219	MG785729	MG785980	MG785521	MG792376
Tanytarsus nemorosus	Finnmark427	MG785187	MG895611	MG785373	MG785873	JN265049	MG785676	N/A
Tanytarsus nemorosus	Finnmark684	MG785102	MG895521	MG785283	MG785789	JN265097	MG785586	N/A
Tanytarsus niger	To443	MG785122	MG895542	MG785303	MG785809	KT613571	MG785607	MG792458
Tanytarsus nigricollis	To433	MG785136	MG895555	MG785317	MG785822	KT613598	MG785620	MG792470
Tanytarsus obiriciae	FA31-32	MG785125	N/A	MG785306	MG785812	MG786013	MG785610	N/A
Tanytarsus occultus	To301	N/A	MG895500	MG785263	MG785770	MG785997	MG785566	MG792419
Tanytarsus occultus	XL136	MG785119	MG895539	MG785300	MG785806	KT613565	MG785604	MG792455
Tanytarsus occultus	XL231	MG785080	MG895497	MG785260	MG785767	KT613443	MG785563	MG792416
Tanytarsus okuboi	Chir-LJ1	MG785093	MG895512	MG785274	MG785781	KT613485	MG785577	MG792431

Tanytarsus okuboi	TM02	MG785098	MG895517	MG785279	MG785786	KT613492	MG785582	N/A
Tanytarsus oscillans	WNSGL10	MG785167	MG895588	MG785350	MG785851	KT613687	MG785653	MG792502
Tanytarsus ovatus	DL27	MG785058	MG895475	MG785238	MG785748	KT613373	MG785541	MG792395
Tanytarsus ovatus	WNSGL01	MG785185	MG895609	MG785371	MG785871	KT613735	MG785674	MG792520
Tanytarsus palettaris	CH-OSF164	MG785074	MG895491	MG785255	MG785761	JN265004	MG785558	MG792411
Tanytarsus palettaris	CH-OSF165	MG785118	MG895538	MG785299	MG785805	JN265005	MG785603	MG792454
Tanytarsus pallidicornis	TRD-CH150	MG785148	MG895567	MG785329	N/A	KT613639	MG785632	MG792482
Tanytarsus pallidicornis	TRD-CH153	MG785052	MG895469	MG785232	N/A	KT613350	MG785536	MG792389
Tanytarsus pallidulus	ZACHIR05	MG785076	MG895493	MG785257	MG785763	KT613437	MG785560	MG792413
Tanytarsus pandus	ES327	MG785159	MG895580	MG785342	MG785845	JF870785	MG785645	MG792494
Tanytarsus pandus	ES328	MG785087	MG895506	MG785268	MG785775	JF870786	MG785572	MG792425
Tanytarsus paraniger	Finnmark17	MG785162	MG895583	MG785345	MG785847	HQ941598	MG785648	MG792497
Tanytarsus pollexus	MHMA01	MG785045	MG895462	MG785227	MG785737	KT613339	MG785529	MG792383
Tanytarsus pollexus	WNSGL12	MG785083	MG895501	MG785264	MG785771	KT613465	MG785567	MG792420
Tanytarsus pseudoheusdensis	ES77	MG648778	MG648798	MG648788	N/A	MG678794	MG700385	N/A
Tanytarsus pseudoheusdensis	ES79	N/A	MG648796	MG648786	N/A	MG678793	MG700383	N/A
Tanytarsus recurvatus	CHIR_CH450	MG785065	MG895483	MG785246	MG785756	GU073217	MG785549	N/A
Tanytarsus recurvatus	To422	MG785171	MG895593	MG785355	MG785856	MG786032	MG785658	N/A
Tanytarsus reei	XL13	MG648775	MG648793	MG648783	N/A	KT613377	MG700380	N/A
Tanytarsus reei	XL180	MG648781	MG648800	MG648791	N/A	KT613702	MG700388	MG648820
Tanytarsus rhabdomantis	FA2-12	MG785129	MG895548	MG785310	MG785815	MG786015	MG785614	MG792464
Tanytarsus rhabdomantis	FA5-1	MG785154	MG895574	MG785336	MG785839	MG786028	MG785639	MG792489
Tanytarsus riopreto cf.	FA7-42	MG785143	MG895562	MG785324	MG785829	MG786020	MG785627	MG792477
Tanytarsus shouautumnalis	XL307	MG785066	MG895484	MG785247	N/A	MG785990	MG785550	MG792403
Tanytarsus shouautumnalis	XL422	MG785114	MG895534	MG785295	N/A	MG786008	MG785599	MG792450
Tanytarsus shoudigitatus	TM01	MG785090	MG895509	MG785271	MG785778	KT613475	MG785574	MG792428
Tanytarsus shoudigitatus	XL2	MG785110	MG895530	MG785292	MG785798	KT613548	MG785595	MG792446
Tanytarsus signatus	To83	MG785207	MG895630	MG785394	MG785892	MG786051	MG785696	MG792540
Tanytarsus signatus	TRD-CH313	MG785053	MG895470	MG785233	MG785743	MG785986	MG785537	MG792390
Tanytarsus signatus	TRD-CH411	MG785042	MG895459	MG785224	MG785734	MG785983	MG785526	MG792380
Tanytarsus simantoseteus	LSCC03	MG785170	MG895592	MG785354	MG785855	KT613698	MG785657	MG792506
Tanytarsus simantoseteus	XL298	MG785144	MG895563	MG785325	MG785830	MG786021	MG785628	MG792478
Tanytarsus sinuatus	XL55	N/A	MG895614	MG785376	MG785875	MG786044	MG785679	MG792523
Tanytarsus sinuatus	ES355	MG785032	MG895449	MG785215	MG785725	KT613307	MG785516	MG792372
Tanytarsus sinuatus	ES377	MG785077	MG895494	MG785258	MG785764	KT613439	N/A	N/A

Tanytarsus sinuatus	XL438	N/A	MG895529	MG785291	MG785797	MG786006	MG785594	MG792445
Tanytarsus songi	XL222	MG648485	MG648559	MG648509	MG648663	KT613441	MG648621	MG648600
Tanytarsus sp. 1XL	QDL01	MG785138	MG895557	MG785319	MG785824	KT613605	MG785622	MG792472
Tanytarsus sp. 1XL	QDL02	MG785116	MG895536	MG785297	MG785803	KT613556	MG785601	MG792452
Tanytarsus sp. 2TE	ES322	MG785128	MG895547	MG785309	N/A	JF870780	MG785613	MG792463
Tanytarsus sp. 2XL	WNXL 01	MG785035	MG895452	MG785217	N/A	KT613321	MG785519	MG792375
Tanytarsus sp. 2XL	WNXL 03	MG785099	MG895518	MG785280	N/A	KT613500	MG785583	MG792436
Tanytarsus sp. 3TE	ES317	MG785172	MG895594	MG785356	MG785857	JF870775	MG785659	MG792507
Tanytarsus sp. 3TE	ES318	MG785156	MG895576	MG785338	MG785841	JF870776	MG785641	MG792490
Tanytarsus sp. 3XL	XL48	MG785029	MG895446	MG785212	MG785722	KT613305	MG785513	N/A
Tanytarsus sp. 3XL	XL157	MG785137	MG895556	MG785318	MG785823	KT613596	MG785621	MG792471
Tanytarsus sp. 8XL	XL205	MG785133	MG895552	MG785314	MG785819	KT613592	MG785617	MG792468
Tanytarsus sp. 9XL	CHIR_ CH198	MG785047	MG895464	MG785228	MG785738	KT613344	MG785531	MG792385
Tanytarsus sp. 9XL	CHIR_ CH264	MG785132	MG895551	MG785313	MG785818	GU073221	MG785616	MG792467
Tanytarsus sp. 11XL	ZACHI R98	MG785073	N/A	MG785254	N/A	KT613434	MG785557	MG792410
Tanytarsus sp. 11XL	ZACHI R100	MG785199	N/A	MG785386	N/A	KT613802	MG785689	N/A
Tanytarsus sp. 12XL	FA2-15	MG785188	MG895612	MG785374	N/A	MG786042	MG785677	MG792521
Tanytarsus sp. 15XL	FA7-14	MG785127	MG895546	MG785308	MG785814	MG786014	MG785612	MG792462
Tanytarsus sp. 16XL	XL377	MG785100	MG895519	MG785281	MG785787	MG786002	MG785584	MG792437
Tanytarsus sp. 17XL	XL300	MG785041	MG895458	MG785223	MG785733	MG785982	MG785525	MG792379
Tanytarsus sp. 17XL	XL395	MG785108	MG895527	MG785289	MG785795	MG786005	MG785592	MG792443
Tanytarsus sp. 21XL	FA2-19	MG785193	MG895618	MG785380	MG785879	MG786047	MG785683	MG792527
Tanytarsus sp. 22XL	FA2-13	MG785183	MG895605	MG785367	MG785867	MG786038	MG785670	N/A
Tanytarsus sp. 24XL	ZACHI R86	MG785060	MG895477	MG785240	MG785750	KT613378	MG785543	MG792397
Tanytarsus sp. 24XL	ZACHI R87	N/A	MG895481	MG785244	MG785754	KT613396	MG785547	MG792401
Tanytarsus sp. 25XL	XJ54	MG785142	MG895561	MG785323	MG785828	MG786019	MG785626	MG792476
Tanytarsus sp. 26XL	XL547	N/A	MG895568	MG785330	MG785834	MG786023	MG785633	MG792483
Tanytarsus spadiceonotatus	ZACHI R137	MG785181	MG895603	MG785365	N/A	KT613716	MG785668	MG792515
Tanytarsus striatulus	Finnma rk452	MG785036	MG895453	MG785218	MG785728	JN265062	MG785520	N/A
Tanytarsus sylvaticus	To431	MG785164	MG895585	MG785347	MG785849	KT613677	MG785650	MG792499
Tanytarsus sylvaticus	To432	MG785031	MG895448	MG785214	MG785724	KT613306	MG785515	MG792371
Tanytarsus takahashii	GXQZ 01	MG785072	MG895490	MG785253	N/A	KT613432	MG785556	MG792409
Tanytarsus tamaduodecimus	LGT01	MG648777	MG648797	MG648787	N/A	KT613493	MG700384	MG648817
Tanytarsus tamagotoi	FR13Y 10	MG785075	MG895492	MG785256	MG785762	KT613436	MG785559	MG792412
Tanytarsus tamakutibasi	XL237	N/A	MG895608	MG785370	MG785870	KT613731	MG785673	MG792519
Tanytarsus tamakutibasi	XL370	MG785040	MG895457	MG785222	MG785732	MG785981	MG785524	N/A
Tanytarsus tamaoctavus	XL287	MG648494	MG648592	MG648539	MG648670	MG680439	MG648654	MG648607

Tanytarsus tamaoctavus	XL423	MG648492	MG648587	MG648534	MG648668	MG680435	MG648649	MG648605
Tanytarsus tamaundecimus	XL212	MG785163	MG895584	MG785346	MG785848	KT613675	MG785649	MG792498
Tanytarsus thaicus	XL21	MG785051	MG895468	MG785231	MG785742	KT613347	MG785535	
Tanytarsus thomasi	CHIR_ CH164	MG648487	MG648573	MG648521	MG648664	KT613577	MG648635	MG648602
Tanytarsus thomasi	CHIR_ CH167	N/A	MG648583	MG648531	N/A	KT613694	MG648645	N/A
Tanytarsus tongmuensis	XL314	MG648483	MG648551	MG648501	MG648661	MG680417	MG648613	MG648599
Tanytarsus tongmuensis	XL333	MG648488	MG648576	MG648524	N/A	MG680429	MG648638	N/A
Tanytarsus unagiseptimus	QJY02	MG785049	MG895466	MG785229	MG785740	KT613346	MG785533	MG792387
Tanytarsus unagiseptimus	XL416	MG785112	MG895532	MG785293	MG785800	MG786007	MG785597	MG792448
Tanytarsus usmaensis	To62	MG785069	MG895487	MG785250	N/A	MG785993	MG785553	MG792406
Tanytarsus usmaensis	To429	MG785179	MG895601	MG785363	MG785864	KT613711	MG785666	MG792513
Tanytarsus verralli	To452	MG785194	MG895619	MG785381	MG785880	KT613782	MG785684	MG792528
Tanytarsus verralli	To453	MG785091	MG895510	MG785272	MG785779	KT613477	MG785575	MG792429
Tanytarsus volgensis	XL26	MG785064	MG895482	MG785245	MG785755	KT613399	MG785548	MG792402
Tanytarsus wangi	XL1	MG648490	MG648581	MG648529	MG648666	KT613654	MG648643	MG648604
Tanytarsus wirthi	CHIR_ CH197	MG785195	MG895620	MG785382	MG785881	GU073223	MG785685	MG792529
Tanytarsus wirthi	CHIR_ CH411	MG785198	MG895623	MG785385	MG785884	GU073222	MG785688	MG792532
Tanytarsus yunosecundus	Chir-LJ2	MG785061	MG895478	MG785241	MG785751	KT613379	MG785544	MG792398
Tanytarsus yunosecundus	XL259	MG785140	MG895559	MG785321	MG785826	KT613609	MG785624	MG792474
Thienemanniola ploenensis	Chir-TJ1	MG785145	MG895564	MG785326	MG785831	N/A	MG785629	MG792479
Thienemanniola ploenensis	To447	MG785095	MG895514	MG785276	MG785783	MG786000	MG785579	MG792433
Virgatanytarsus aboensis	To304	MG785109	MG895528	MG785290	MG785796	AM398773	MG785593	MG792444
Virgatanytarsus albisutus	XL563	MG785050	MG895467	MG785230	MG785741	MG785985	MG785534	MG792388
Virgatanytarsus simantoteuus	WX08	MG785182	MG895604	MG785366	MG785866	MG786037	MG785669	MG792516
Virgatanytarsus simantoteuus	WX09	MG785152	MG895572	MG785334	MG785837	MG786026	MG785637	MG792487
Virgatanytarsus sp. 1XL	WZSM 01	MG785120	MG895540	MG785301	MG785807	MG786009	MG785605	MG792456
Virgatanytarsus sp. 1XL	XL154	MG785175	MG895597	MG785359	MG785860	MG786034	MG785662	MG792509
Virgatanytarsus sp. 2XL	DL01	MG785196	MG895621	MG785383	MG785882	MG786048	MG785686	MG792530
Virgatanytarsus sp. 3XL	DL02	MG785177	MG895599	MG785361	MG785862	MG786035	MG785664	MG792511
Virgatanytarsus sp. 3XL	DL04	MG785097	MG895516	MG785278	MG785785	MG786001	MG785581	MG792435

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

Gene	Nucleotide position	Informative sites	T(%)	C(%)	A(%)	G(%)	AT(%)	GC(%)
18S	All	136(100%)	28.3	17.2	29.2	25.2	57.5	42.5
AATS1	1st	46(26.3%)	23.7	20.4	23.5	32.4	47.2	52.8
	2nd	24(12.2%)	28.5	15.9	33.6	22.0	62.1	37.9
	3rd	127(64.5%)	35.4	22.2	21.8	20.6	57.2	42.8
	All	197(100%)	29.2	19.5	26.3	25.0	55.5	44.5
CAD1	1st	103(23.3%)	21.1	18.7	30.9	29.3	52.0	48.0
	2nd	52(11.7%)	28.0	18.8	35.3	17.9	63.3	36.7
	3rd	288(65.0%)	37.5	18.6	26.0	17.9	63.5	36.5
	All	443(100%)	28.8	18.7	30.7	21.7	59.5	40.5
CAD4	1st	89(21.7%)	21.7	12.1	32.6	33.6	54.3	45.7
	2nd	53(12.9%)	31.8	17.3	34.9	16.0	66.7	33.3
	3rd	268(65.4%)	34.5	19.9	25.6	20.0	40.1	39.9
	All	410(100%)	29.3	16.4	31.1	23.2	60.4	39.6
PGD	1st	53(17.3%)	20.1	17.5	29.2	33.2	49.3	50.7
	2nd	21(6.9%)	29.7	20.6	30.8	18.9	60.5	39.5
	3rd	232(75.8%)	30.4	28.0	21.1	20.5	51.5	48.5
	All	306(100%)	26.7	22.0	27.1	24.2	53.8	46.2
TPI	1st	54(24.0%)	17.3	14.9	21.0	46.8	38.3	61.7
	2nd	29(12.9%)	31.3	27.9	25.1	15.7	56.4	43.6
	3rd	142(63.1%)	35.3	26.7	22.3	15.7	57.6	42.4
	All	225(100%)	27.9	23.2	22.8	26.1	50.7	49.3