



Phylogenomics of Tanypodinae (Diptera: Chironomidae) redefines relationships in Procladiini and Tanypodini and supports a new tribe

Xiu-Ru Xiao^{a,b,1}, Yi-Ni Zhang^{a,b,1}, Hai-Feng Xu^{a,b}, Zhi-Chao Zhang^{a,b}, Yu-Yi Jiang^{a,b}, Yan-Xin Chen^{a,b}, Chen-Hong Li^{a,b}, Bing-Jiao Sun^c, Fabio Laurindo da Silva^d, Xiao-Long Lin^{a,b,*}

^a Engineering Research Center of Environmental DNA and Ecological Water Health Assessment, Shanghai Ocean University, Shanghai 201306, China

^b Shanghai Universities Key Laboratory of Marine Animal Taxonomy and Evolution, Shanghai Ocean University, Shanghai 201306, China

^c China National Environmental Monitoring Centre, Beijing 100012, China

^d Brazilian Agricultural Research Corporation (EMBRAPA), Embrapa Meio-Norte (CPAMN), Av. Duque de Caxias 5650, Teresina, PI 64008-780, Brazil

ARTICLE INFO

Keywords:

Phylogeny

USCOs

Saetheromyiini

Diversification

ABSTRACT

The phylogenetic relationships between Procladiini and Tanypodini (Chironomidae, Tanypodinae) remain unresolved. Here, we recovered an average of 2,432 universal single-copy orthologous genes (USCOs) per species from 29 taxa and used occupancy-filtered USCO datasets to reconstruct their phylogenetic relationships, providing the first USCO-based phylogenomic framework for Tanypodinae. Maximum likelihood and multispecies coalescent analyses recovered consistent and strongly supported topologies. *Djalmabatista* was recovered as a distinct lineage within Procladiini and as sister to *Procladius*, supporting its status as a valid genus. In contrast, *Saetheromyia* was recovered as sister to *Tanypus*, rather than within Procladiini. Independent morphological evidence indicates that *Saetheromyia* exhibits a unique combination of characters absent in other tribes, supporting the establishment of a new tribe, Saetheromyiini Xiao, trib. nov.. Divergence time estimation using fossil calibrations indicates that major Tanypodinae tribes originated in the Early Cretaceous (ca. 123.9–104.0 million years ago, Ma), with the split between Procladiini and Tanypodini in the Late Cretaceous (ca. 96.1–78.2 Ma), followed by diversification of Procladiini from the Late Cretaceous to the Eocene and of Tanypodini primarily during the Miocene. These results provide a robust phylogenomic framework for understanding the evolutionary history and higher classification of Tanypodinae.

1. Introduction

Chironomidae (Diptera), commonly known as non-biting midges, is one of the most widely distributed and biomass-dominant groups of freshwater aquatic insects, comprising over 7,800 described species worldwide (Ashe and O'Connor, 2009; Ferrington, 2008; Pape and Evenhuis, 2023). Their larvae occupy a wide range of aquatic habitats and respond sensitively to environmental changes, making them valuable indicators for ecological monitoring and bioassessment (Mrozińska and Obolewski, 2024). A robust phylogenetic framework is therefore essential not only for understanding the evolutionary history of Chironomidae, but also for improving taxonomic stability and the accuracy of ecological assessments (Czechowski et al., 2020; Martin et al., 2026).

Tanypodinae is the third most species-rich subfamily of Chironomidae and has an almost global distribution, being absent only from

Antarctica (Krosch et al., 2022). The subfamily comprises nine tribes, of which Pentaneurini and Macropelopiini are the most diverse in terms of generic richness (Silva and Ekrem, 2016). However, current knowledge of phylogenetic relationships across Tanypodinae, including among its tribes and several key genera, remains fragmentary, resulting in persistent uncertainty in tribal delimitation and the placement of some problematic taxa.

Procladiini is a highly diverse tribe, including *Procladius*, *Djalmabatista*, *Saetheromyia* and *Lepidopelopia* (Dantas and Hamada, 2018; Silva and Ekrem, 2016). Among these, *Procladius*, *Djalmabatista*, and *Saetheromyia* have been recorded in China and represent important taxa for systematic studies of the tribe (Xiao et al., 2025). Tanypodini, in contrast, comprises the single genus *Tanypus*, with approximately 30 described species (Andersen et al., 2013; Gao et al., 2024) (see Fig. 1).

Within Procladiini, the taxonomic status of *Djalmabatista* remains

* Corresponding author.

E-mail address: lin880224@gmail.com (X.-L. Lin).

¹ These authors contributed equally to this work.



Fig. 1. Photos of representative Procladiini and Tanypodini: (A) larva of *Procladius* sp. 10XL; (B) adult male of *Procladius* sp. 3XL; (C) adult male of *Procladius* sp. 11XL; (D) larva of *Saetheromyia tedoriprima*; (E) adult male of *Saetheromyia tedoriprima*; (F) pupa of *Procladius choreus*; (G) larva of *Tanypus kraatzii*; (H) adult male of *Tanypus* sp. 1XL; (I) adult female of *Tanypus* sp. 2XL. Scale bars: 0.5 mm in (B); 1 mm in (A), (C), (D), (E), (F), (H), (I).

controversial, as it has been treated either as a valid genus or as a sub-genus of *Procladius* (Krosch et al., 2022). The phylogenetic position of *Saetheromyia* has also long been debated. Previous morphological and phylogenetic studies placed *Saetheromyia* within Procladiini, often in association with other genera exhibiting plesiomorphic characters (Silva and Ekrem, 2016). However, a recent mitogenome-based analysis recovered *Saetheromyia* as sister to *Tanypus*, outside Procladiini (Xiao et al., 2025), highlighting persistent uncertainty regarding its placement.

Given the limitations of mitochondrial data in resolving stable phylogenetic relationships (Bonnet et al., 2017), the use of nuclear genomic data is essential to address the issues outlined above. In aquatic insects, the extraction of universal single-copy orthologous (USCOs) from whole-genome sequencing (WGS) data has proven effective for reconstructing robust and well-supported phylogenies (Ge et al., 2024; Godeiro et al., 2023; Lai et al., 2024; Xu et al., 2026; Yu et al., 2022; Zhang et al., 2022). In Chironomidae, recent phylogenomic studies based on nuclear data have resolved relationships across multiple taxonomic levels, providing a robust framework for systematics (Martin et al., 2026; Narayanan Kutty et al., 2018; Xu et al., 2026).

In this study, we aim to resolve the phylogenetic relationships and estimate divergence times of Procladiini and Tanypodini using nuclear genomics data, and to clarify the systematic placement of *Saetheromyia* and *Djalmabatista*, thereby providing a foundation for a stable tribal and generic classification within Tanypodinae.

2. Materials and methods

2.1. Taxon sampling, identification and DNA extraction

All specimens analyzed in this study were collected between 2008 and 2026. Detailed information on the samples and collection metadata is provided in Table S1. Adult specimens were preserved in 85% ethanol, whereas larvae were preserved in 95% ethanol; all specimens were preserved in the dark at -20°C prior to morphological examination and molecular analyses. Specimen were identified by the corresponding author using relevant taxonomic literature (e.g., Baranov et al., 2024; Cranston and Epler, 2013; Fittkau, 1962; Rieradevall and Brooks, 2001; Roback, 2007; Roback, 1980).

Based on previous studies, a total of 29 species were selected as ingroups, including 17 Procladiini species and five Tanypodini species (Krosch et al., 2017; Xiao et al., 2025). In addition, two Clinotanypodini species and five Macropelopiini species were selected as outgroups representing the closest sister groups to Procladiini and Tanypodini (Krosch et al., 2022). The dataset includes a representative sampling of Procladiini, with specimens from three genera: *Djalmabatista*, *Procladius* and *Saetheromyia*.

Genomic DNA was extracted from the whole body, excluding the metasoma in adult specimens and the thorax in larvae, using the DNeasy Blood & Tissue Kit (Qiagen, Germany) and the Universal Genomic DNA Kit (Cwbio, China), following the manufacturers' protocols. All voucher specimens are deposited at the College of Fisheries and Life Science, Shanghai Ocean University, Shanghai, China.

2.2. Library construction, sequencing and assembly of the genome

The Truseq Nano DNA HTS Sample Preparation Kit (Illumina, USA) was employed to construct sequencing libraries. Paired-end sequencing was conducted on the Illumina NovaSeq 6000 platform at Novogene (Tianjin, China), producing 150 bp paired-end reads with a library insert size of 350 bp. For quality control, raw sequencing reads were processed using Trimmomatic v0.32 (Bolger et al., 2014) to remove adapter sequences and low-quality bases. Sequencing quality was assessed using FastQC v0.11.9 (Andrews, 2010).

Genomic assemblies were conducted using the streamlined PLWS v1.0.6 pipeline (<https://github.com/xtmtd/PLWS/>, Zhang et al., 2019)

with BBTools v39.27 (Bushnell, 2014) employed to further refine the data and eliminate low-quality sequences. Genomic contigs were assembled using Minia v3.2.4 (Chikhi and Rizk, 2013) with multiple k-mer strategies (21, 41, 61, 81, 101, 121) to optimize assembly accuracy. Redundant contigs were removed using Redundans v0.13c (Pryszcz and Gabaldón, 2016). Scaffold assembly was conducted with BESST v2.2.8 (Sahlin et al., 2014), and any gaps in the genome were filled with GapCloser v1.12 (Luo et al., 2012), ensuring the continuity of the assembly. Genome assembly statistics were calculated using SeqKit v0.16.0 (Shen et al., 2016) and BBTools. For each sample, clean read yield divided by assembly size was calculated and is reported in place of sequencing depth, as assembly size does not represent an independently estimated genome size and may be influenced by assembly completeness, repeat collapse, contamination, heterozygosity, or uncollapsed haplotypes. To assess the completeness of the assemblies, the BUSCO v5.8.3 (Waterhouse et al., 2018) tool was used, comparing the results against the Diptera reference gene set (diptera_odb10; $n = 3,285$). The WGS data for all 29 species have been deposited in the Genome Sequence Archive (GSA), hosted by the Beijing Institute of Genomics, Chinese Academy of Sciences, China. The dataset is accessible under BioProject number PRJCA014092, with individual sequence accessions listed in Supplementary Table S2.

2.3. Matrix generation and Phylogenetic analyses

The extraction of USCOs and subsequent matrix construction were conducted using BUSCO. To capture a broader set of relatively complete USCOs, we modified the 'length_cutoff' parameter in the control file, increasing it to twice its default value, following previous studies (Ge et al., 2024; Xu et al., 2026; Zhang et al., 2022). This adjustment allows fragmented hits that represent genuine homologous sequences to be reclassified as complete, thereby increasing the number of USCOs available for downstream phylogenetic analyses. After BUSCO extraction, only loci classified as complete and single-copy were retained for downstream analyses; duplicated and fragmented loci were excluded from the primary matrix (Zhang et al., 2019). After extraction, USCO sequences were aligned using MAGUS v0.1.1 (Smirnov and Warnow, 2021), and poorly aligned or unreliable homologous regions were removed using ClipKit v1.1.5 (Steenwyk et al., 2020). To reduce compositional heterogeneity in the alignments, we applied the kpi strategy (-m kpi) and subsequently merged the alignments using Phykit v1.2.1 (Steenwyk et al., 2021).

To ensure high-quality data for phylogenomic analyses, two filtering strategies were employed to minimize systematic biases.

(1) Alignment-based filtering: The redundancy score for each locus was calculated using Phykit, and loci with redundancy scores below 100 were excluded. Compositional heterogeneity was assessed using BaCoCa v1.1 (Kück and Struck, 2014), and loci with a relative composition frequency variability (RCFV) value greater than 0.4 were removed. The 'symtest' function implemented in IQ-TREE v2.0.7 (Minh et al., 2020) was used to test the stationarity, reversibility, and homogeneity (SRH) assumptions, applying a strict p-value cutoff of 0.05. Based on this filtering procedure, three USCO matrices were generated: USCO70, USCO80 and USCO90, corresponding to 70%, 80% and 90% taxon-occupancy, respectively.

(2) Gene tree-based filtering: Gene trees for the USCO70, USCO80 and USCO90 matrices were constructed using the EX_EHO model in IQ-TREE, with 1,000 ultrafast bootstrap (UFBoot2) repetitions. Exceptionally long branches were identified and pruned using TreeShrink v1.3.7 (Mai and Mirarab, 2018), with a threshold set to 0.05 ($-q 0.05$) under the 'all-genes' model. Loci with an average bootstrap support greater than 75 were retained to generate the final matrices (USCO70_abs75, USCO80_abs75 and USCO90_abs75) for subsequent phylogenetic analyses (Table S3).

The phylogenetic relationships were inferred using both Maximum Likelihood (ML) and Multi-Species Coalescent (MSC) methods. For each

USCO matrix, the optimal substitution model and partitioning scheme were selected using ModelFinder (Kalyaanamoorthy et al., 2017) in IQ-TREE, with a loose clustering algorithm ‘rclusterf 10’ (Lanfear et al., 2014). The LG model was applied for ML analyses, as selected by ModelFinder. To account for heterotachy, the General Heterogeneous evolution On a Single Topology (GHOST) model (Crotty et al., 2020) was used in IQ-TREE, configured as ‘-m LG+FO+ H4’. To address the effects of long-branch attraction (LBA), a mixed model (EX_EHO + FO + R) was employed. All ML analyses were supported by 1000 ultrafast bootstrap (UFBoot2) replicates and 1,000 SH-aLRT replicates (Guindon et al., 2010), ensuring robust support for the inferred phylogenetic trees. To account for incomplete lineage sorting (ILS) and reconcile discrepancies between species and gene trees, we applied a weighted ASTRAL-Hybrid approach for MSC analysis, implemented in ASTER v1.15 (Zhang and Mirarab, 2022). Branch support for MSC species trees was assessed using local posterior probabilities (LPPs).

2.4. Divergence time estimation

Divergence times were estimated using the approximate likelihood method implemented in MCMCTree in PAML v4.9j (Yang, 2007). The topology was fixed to the ML tree inferred from the USCO70_abs75 dataset, and the same dataset was used as the sequence input for divergence time estimation. Fossil calibration points were obtained from the Paleobiology Database (PBDB; <https://paleobiodb.org>) and previous studies, following best-practice guidelines (Parham et al., 2012). Two calibration points were applied (Table S4). The root node was constrained to 122–92 Ma based on previous estimates for Chironomidae (Krosch et al., 2017; Krosch et al., 2022), and a fossil calibration was used to constrain the divergence of *Procladius* lineages with a minimum age of 48 Ma (Doitteu and Nel, 2007). Geological time intervals followed the International Chronostratigraphic Chart (Cohen et al., 2013).

The LG substitution model and an independent-rates clock model were used (model = 2, aaRatefile = LG.dat; clock = 2). The Hessian matrix was calculated to obtain the gradient and curvature of branch lengths for the approximate likelihood analysis. Priors for the overall substitution rate and rate variation among lineages were set to $\text{rgene_gamma} = 2 \ 20 \ 1$ and $\text{sigma2_gamma} = 1 \ 10 \ 1$, respectively. For nodes without fossil calibration, a birth-death process prior was specified (BD paras = 1 1 0.1).

Two independent MCMC runs were performed for 450,000 generations each, with samples drawn every 10 generations (45,000 samples per run). The first 50,000 generations (5,000 samples) were discarded as burn-in, leaving 40,000 posterior samples per run for downstream analyses. Convergence and adequate mixing were assessed using effective sample size (ESS > 200) and visual inspection of trace plots in Tracer.

3. Results

3.1. Genome assembly and Matrix construction

We generated genome-scale data for 29 species of Tanypodinae, yielding 1.99–13.64 Gb of read yield per species. As an assembly-relative index, clean read yield divided by assembly size ranged from 17 in *Macropelopia paranebulosa* to 124 in *Tanypus chinensis* among species and is reported here only for comparative purposes. Assembly sizes ranged from 69.60 Mb in *Djalmabatista sinica* to 189.25 Mb in *Clinotanypus yani*. Only *Procladius longistilus*, *Procladius* sp. 3XL, *Procladius* sp. 4XL and *D. sinica* had genome assemblies smaller than 100 Mb. The number of scaffolds ranged from 6,365 to 97,795, and GC content varied between 26.88% and 33.15%. Scaffold N50 and scaffold N90 values ranged from 1.72–36.48 Kb and from 1.11–7.73 Kb, respectively.

The maximum scaffold length ranged from 16.19 to 269.34 Kb, whereas the minimum scaffold length ranged from 1.000–1.003 Kb (Table S2).

USCO completeness assessments showed that complete and single-copy proportions ranged from 38.9% to 95.5% and from 37.8% to 94.7%, respectively, whereas duplicated values ranged from 0.8% to 2.6% and fragmented values from 0.2% to 9.3% (Fig. S1). Across the 29 taxa, an average of 2,432 USCO loci per taxon were recovered. A total of 3,194 USCO loci were recovered, of which 3,167 loci were retained after alignment, filtering and trimming. Degeneracy filtering in Phykit yielded 1,903 loci comprising 460,503 amino acid sites. Subsequent BaCoCa filtering (RCFV < 0.4) retained 1,893 loci (458,980 amino acids), and the SRH test implemented in IQ-TREE further reduced the dataset to 1,880 loci (454,991 amino acids). Based on these results, we constructed three occupancy-filtered matrices—USCO70_abs75, USCO80_abs75 and USCO90_abs75—containing 1,048, 755 and 344 loci, respectively, corresponding to 266,022, 193,570 and 88,840 amino acid sites. Tree-Shrink filtering was then applied to remove long-branch outliers, while retaining the same number of loci in each dataset.

Long-branch outliers were subsequently identified and removed using TreeShrink, while preserving the original number of loci in each matrix. After filtering and applying the 75% occupancy threshold, the final datasets retained a large number of USCO loci and aligned amino acid sites for downstream phylogenomic analyses. Summary statistics for each dataset are provided in Table S3.

3.2. Phylogenetic analyses based on multiple datasets

Phylogenetic reconstructions were conducted using three datasets (USCO70_abs75, USCO80_abs75 and USCO90_abs75) under partitioned, GHOST and EX_EHO models, resulting in a total of nine trees (Files S1–S9). Across all concatenated analyses, highly congruent topologies were recovered, with strong nodal support (SH-aLRT/UFBoot2 = 100/100 for most nodes), indicating robust phylogenetic signal. At the tribal level, all analyses consistently recovered Tanypodinae as a well-supported monophyletic group (LPPs ≥ 0.95 ; SH-aLRT/UFBoot2 ≥ 99). Under the traditional tribal classification, Procladiini and Tanypodini were recovered as paraphyletic, forming a clade sister to Clinotanypodini, which was in turn sister to Macropelopiini. These higher-level relationships were stable across all datasets and inference methods.

At the genus level, all sampled genera were recovered as monophyletic with strong support. *Procladius* formed a distinct clade sister to *Djalmabatista*, whereas *Saetheromyia* was recovered as sister to *Tanypus*. The *Saetheromyia* + *Tanypus* clade was in turn recovered as sister to *Clinotanypus*, and this combined lineage was further sister to *Macropelopia* (Fig. 2). This topology was consistent across all datasets and analytical approaches, indicating a stable phylogenetic placement of *Saetheromyia* as an independent lineage closely related to Tanypodini.

3.3. Divergence time

Trace plots indicated satisfactory convergence of all parameters, with effective sample size (ESS) values exceeding 200. Divergence time analyses suggested that Macropelopiini, Tanypodini, Clinotanypodini and Procladiini originated during the Early Cretaceous (123.87–104.02 Ma; 95% highest posterior density, HPD; Fig. 3). The split between Procladiini and Tanypodini was estimated to have occurred during the Late Cretaceous (96.09–78.18 Ma). Following this split, diversification within Procladiini occurred from the Late Cretaceous to the Eocene (79.51–46.08 Ma), with the origin of *Procladius* dated to 57.66–46.08 Ma. Within Procladiini, *Djalmabatista* diverged at 16.59–11.13 Ma. The split between *Saetheromyia* and *Tanypus* was dated to 77.27–60.62 Ma. Conversely, diversification within *Tanypus* occurred later, at

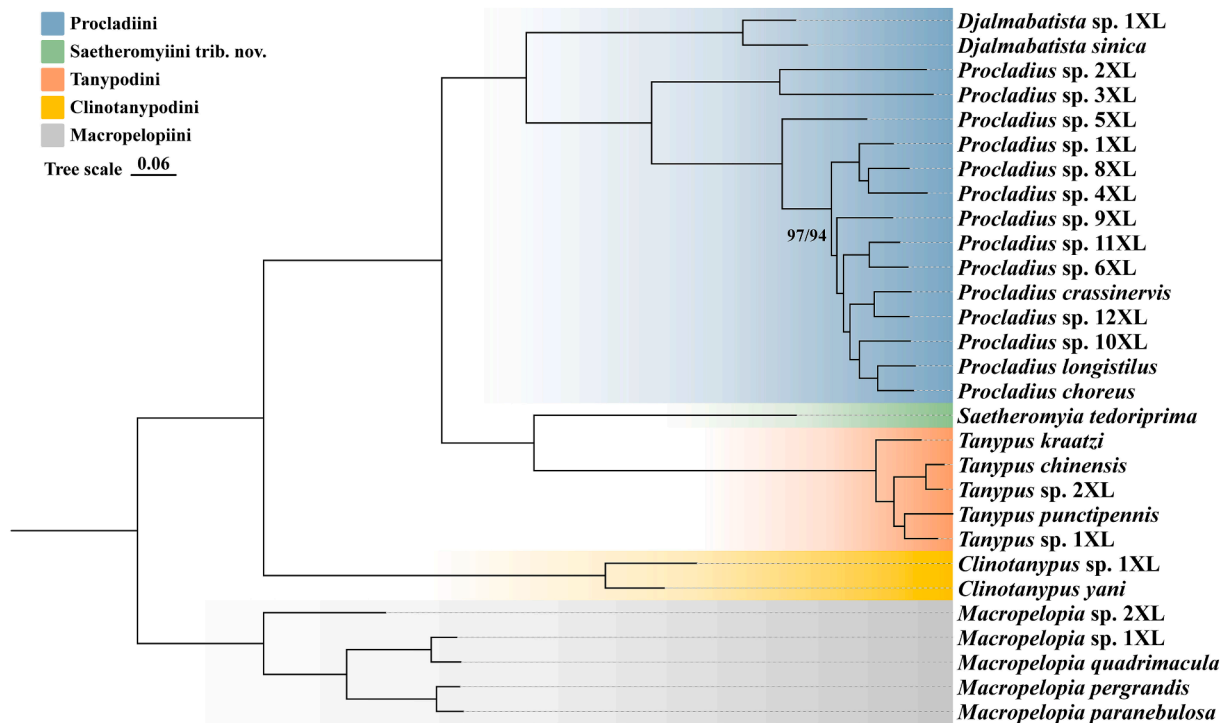


Fig. 2. Phylogenetic tree of Tanypodini and Procladiini inferred from the matrices USCO70_abs75, combined with the partitioning model, GHOST model and EX_EHO model. All nodes unlabeled were supported with maximum values (100/100, UFBoot/SH-aLRT).

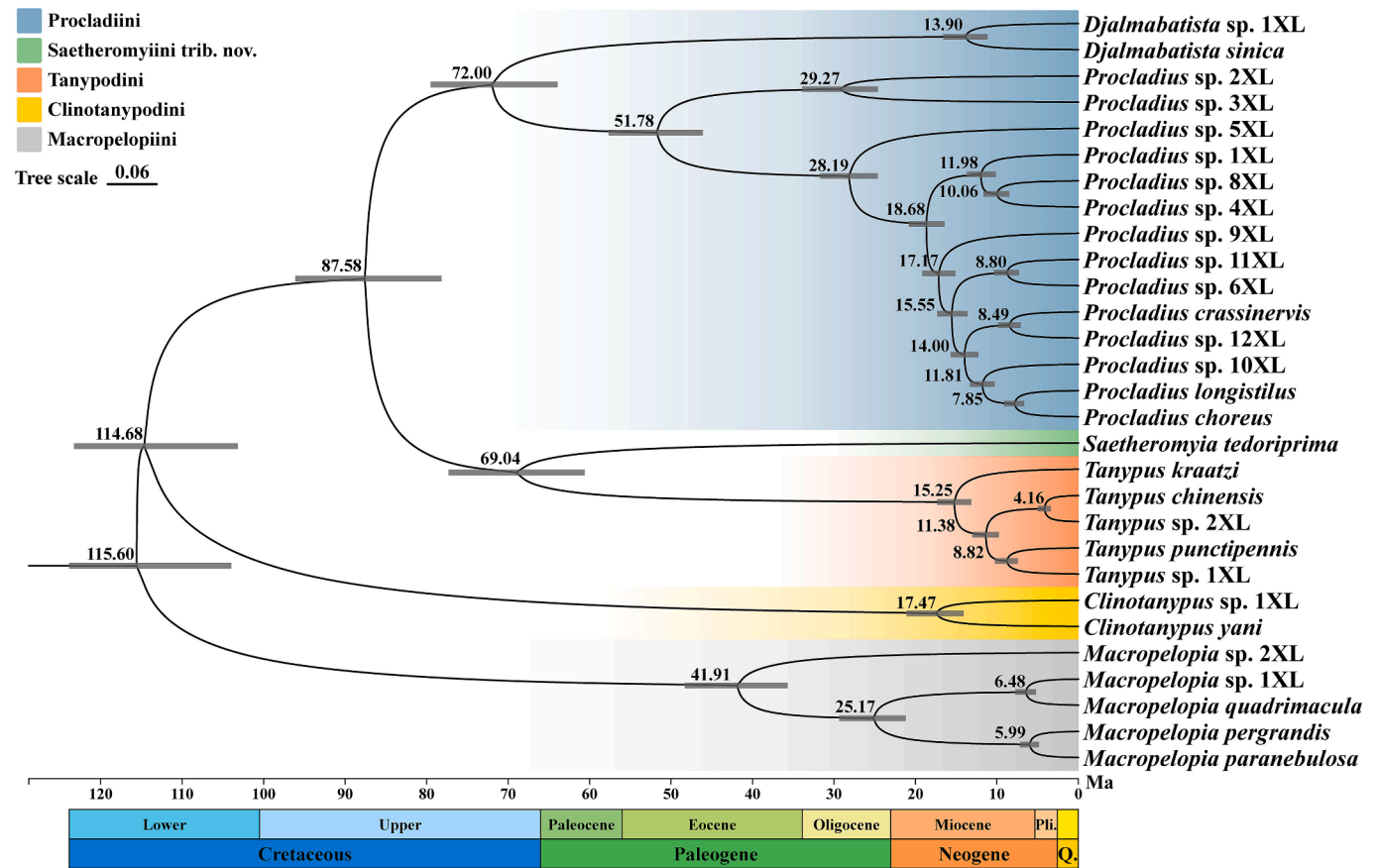


Fig. 3. Phylogeny and divergence time estimation of Tanypodini and Procladiini inferred from the USCO70_abs75 matrix. The chronogram was calibrated using molecular data, with divergence times shown in millions of years ago (Ma). Gray bars at nodes represent the ranges of divergence time estimates. The scale bar indicates 0.1 substitutions per site (branch length).

17.36–13.13 Ma, primarily during the Miocene. These estimates indicate a long and independent evolutionary history for the *Saetheromyia* lineage since the Late Cretaceous.

4. Discussion

4.1. Phylogenetic Position and Tribal Revision of *Saetheromyia*

The phylogenetic placement of *Saetheromyia* has long been uncertain, largely due to its mosaic of morphological characters shared with both *Procladius* and *Tanytus* (Silva and Ekrem, 2016). Morphologically, *Saetheromyia* resembles *Procladius* in having a relatively long distance between veins MCu and FCu ($\geq 0.50 \times \text{Cu}_1$ length), a feature that clearly distinguishes both genera from *Tanytus*, in which this distance is markedly shorter ($\leq$ length of MCu, or $\leq 1/3 \text{ Cu}_1$ length) (Ashe and O'Connor, 2009; Oliver and Dillon, 1989). However, *Saetheromyia* shares with *Tanytus* the presence of a scutal tubercle and the absence of both an inner lobe and an outer process on the gonostylus (Niitsuma, 2007).

Within *Procladius*, subgenera show different character states: *Holotanytus* and *Procladius* lack a scutal tubercle and possess a gonostylus with a distinct outer process, whereas species of *Psilotanytus* may present a scutal tubercle and reduced gonostylar processes. Notably, species previously placed in *Laurotanytus* were later synonymized with *Procladius* (*Psilotanytus*), further demonstrating the morphological variability within *Procladius* and complicating the interpretation of these characters (Dantas and Hamada, 2018; Silva and Ekrem, 2016). This combination of characters has historically challenged the taxonomic placement of *Saetheromyia* (Sasa, 1994).

Our phylogenomic analyses provide independent molecular evidence that clarifies this long-standing issue. *Saetheromyia* is recovered as a well-supported monophyletic lineage sister to *Tanytus*. This relationship is consistently recovered across all datasets and analytical approaches, indicating a stable and robust phylogenetic signal supporting this placement. Re-examination of morphological characters further supports the distinctiveness of *Saetheromyia*. The genus is characterized by a unique combination of traits, including a relatively long distance between veins MCu and FCu, the presence of a scutal tubercle, and the absence of both an inner lobe and an outer process on the gonostylus. This combination of character states is not found simultaneously in any subgenus of *Procladius* (Ashe and O'Connor, 2009; Brodin, 2025; Niitsuma, 2007).

Overall, the concordant evidence from phylogenomics and morphology analyses supports the recognition of a new tribe, *Saetheromyiini* Xiao, **trib. nov.**, to accommodate *Saetheromyia*. This taxonomic decision better reflects the evolutionary history of the group and provides a robust phylogenetic framework for future systematic and evolutionary studies of Tanypodinae.

Systematics

Saetheromyiini Xiao, **trib. nov.**

urn:lsid:zoobank.org:act:E15428EE-91DB-4C66-9164-CB270D29EEB5

Order Diptera Linnaeus, 1758.

Infraorder Nematocera Meigen, 1818.

Family Chironomidae Newman, 1834.

Subfamily Tanypodinae Thienemann & Zavřel, 1916.

Type genus: *Saetheromyia* Niitsuma, 2007.

Type species: *Psilotanytus tedoriprimus* Sasa, 1994. The holotype is deposited in the Department of Zoology, National Science Museum of Nature and Science, Tokyo (NSMT).

Type locality: Ishikawa Prefecture, Japan.

Etymology: The tribal name is derived from the stem of the type genus *Saetheromyia*.

Included taxa: *Saetheromyia tedoriprima* (Sasa, 1994). The species was redescribed in detail by Niitsuma (2007).

Diagnosis: *Saetheromyiini* **trib. nov.** can be distinguished from all other Tanypodinae by the following combination of morphological characters. Adult male: wing vein R_{2+3} divided into R_2 and R_3 ; distance between veins MCu and FCu relatively long; scutal tubercle present; gonostylus without inner lobe and without outer process (Brodin, 2025). Pupa: thoracic horn long and ribbon-like, with a small plastron plate located in the basal quarter; anal lobe subrectangular, with posterior anal macroseta inserted at the posterolateral corner. Larva: ligula with inner lateral teeth bearing outwardly directed apices; basal segment of maxillary palp with a ring organ located basal third (Niitsuma, 2007).

4.2. Tribal and generic relationships

Our phylogenomic analyses based on single-copy nuclear genes provide a robust framework for re-evaluating the tribal and generic relationships of Procladiini and Tanypodini. Previous phylogenetic hypotheses varied considerably depending on the type of data and taxon sampling (Fig. 4). Morphology-based analyses recovered the topology (((*Djalmabatista* + *Saetheromyia*) + *Procladius*) + *Tanytus*) + (*Clinotanytus* + *Macropelopia*), placing *Saetheromyia* within Procladiini (Fig. 4A; Silva and Ekrem, 2016). Molecular studies based on a small number of gene fragments recovered the topology (((*Djalmabatista* + *Procladius*) + *Tanytus*) + *Clinotanytus*) + *Macropelopia* (Fig. 4B; Krosch et al., 2017), whereas more recent datasets recovered (((*Djalmabatista* + *Procladius*) + *Tanytus*) + *Macropelopia*) + *Clinotanytus* (Fig. 4C; Krosch et al., 2022). However, these molecular studies did not include *Saetheromyia*, leaving its tribal affiliation unresolved.

Mitochondrial genome analyses recovered the topology (((((*Saetheromyia* + *Tanytus*) + *Procladius*) + *Djalmabatista*) + *Clinotanytus*) + *Macropelopia*), indicating a close relationship between *Saetheromyia* and *Tanytus* (Tanypodini), but also indicating potential cytonuclear discordance (Fig. 4D; Xiao et al., 2025). Our nuclear genome-based analyses (Fig. 4E), however, recover *Saetheromyia* as an independent lineage sister to Tanypodini rather than grouping with Procladiini, supporting its recognition as a new tribe, *Saetheromyiini* **trib. nov.**. The paraphyly of Procladiini recovered in our analyses results from the traditional tribal classification, which included *Saetheromyia* within the tribe. Once *Saetheromyia* is removed from Procladiini and recognized as a separate tribe, Procladiini is recovered as a well-supported monophyletic lineage comprising *Procladius*, *Djalmabatista*, and their close relatives, and is sister to Tanypodini. Within Procladiini, *Djalmabatista* is recovered as a distinct lineage sister to *Procladius* with strong support, confirming its status as a separate genus rather than a subgenus of *Procladius* (Krosch et al., 2022). Tanypodini remains monogeneric, whereas Clinotanypodini + Macropelopiini form a separate clade sister to Procladiini + *Saetheromyiini* + Tanypodini.

Although our dataset includes representatives from all major lineages from the Chinese fauna, several Tanypodinae genera remain unsampled. Expanding taxon sampling will be important to assess the stability of these revised tribal delimitations and to evaluate whether the proposed framework remains robust when additional genera are included. Nevertheless, our study provides the most comprehensive and robust phylogenetic framework to date for Tanypodinae, reconciling morphological and molecular hypotheses and clarifying the evolutionary history of the subfamily. The recognition of *Saetheromyiini* **trib. nov.** and the confirmation of *Djalmabatista* as an independent genus represent important steps toward a more stable and informative tribal classification within Tanypodinae. This revised classification provides a taxonomy that is consistent with phylogenomic evidence and evolutionary history.

4.3. Divergence time and evolutionary implications

Divergence time estimates indicate that the major lineages of Tanypodinae originated during the Cretaceous, with subsequent diversification of most genera occurring from the Paleogene to the Neogene.

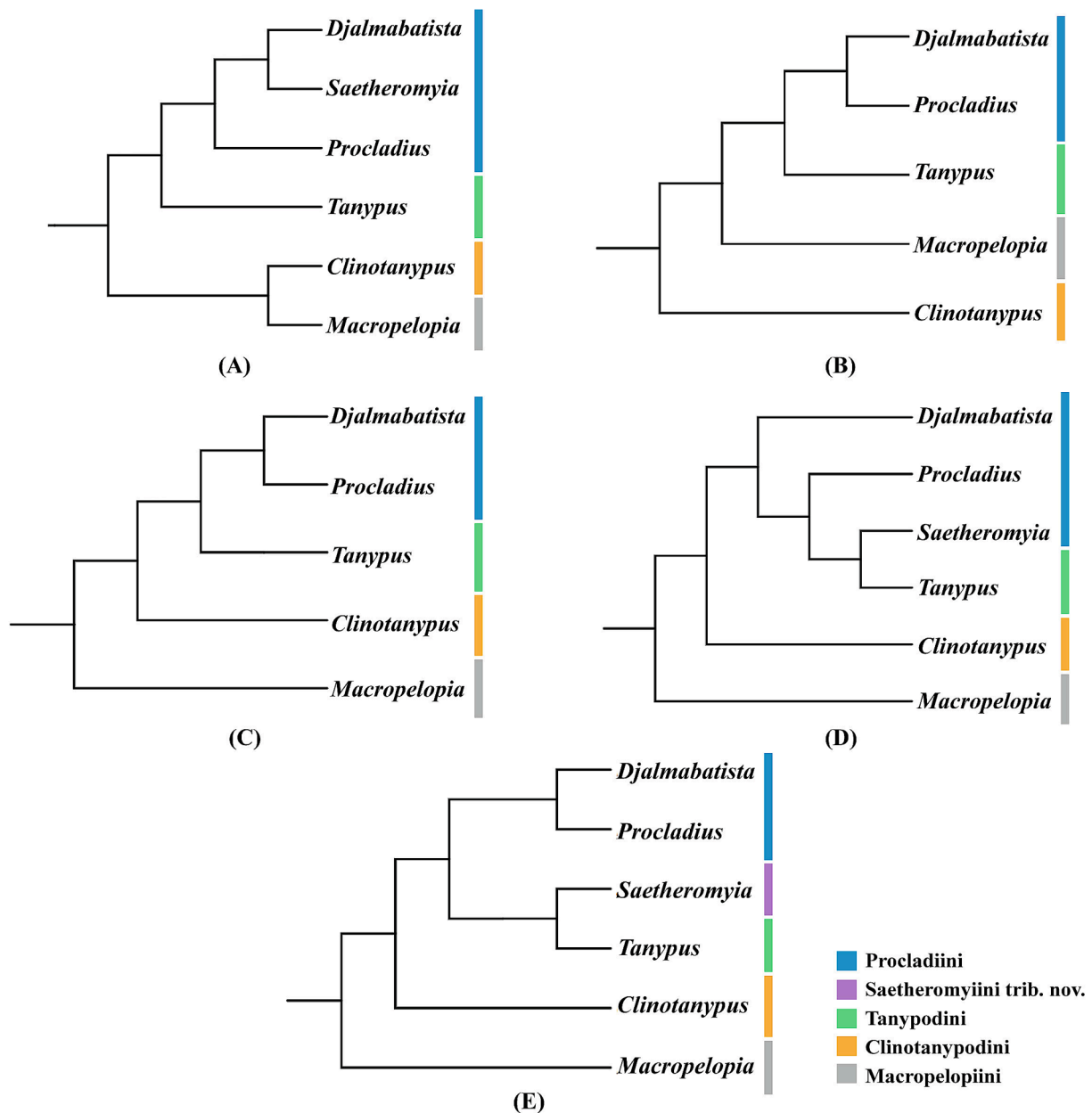


Fig. 4. Hypotheses of phylogenetic relationships among genera of Tanypodini and Procladii. (A) Silva and Ekrem (2016); (B) Krosch et al. (2017); (C) Krosch et al. (2022); (D) Xiao et al. (2025); (E) this study.

This temporal pattern is consistent with previous studies on Tanypodinae that suggested a Late Cretaceous origin followed by Cenozoic diversification, often associated with major geological and biogeographic events (Krosch et al., 2017; Krosch et al., 2022). Biogeographic analyses of *Labrundinia* (Pentaneurini), for example, indicated a Late Cretaceous origin with diversification throughout the Paleogene and Neogene, possibly associated with Gondwanan fragmentation and later regional geological events (Silva and Ekrem, 2016; Silva et al., 2023). Similar temporal patterns recovered in the present study suggest that the diversification of major Tanypodinae lineages may be linked to large-scale historical processes rather than only recent ecological diversification.

The divergence between the *Saetheromyia* + *Tanypus* lineage and Procladii during the Late Cretaceous indicates a long and independent

evolutionary history for *Saetheromyia*, which is consistent with its morphological distinctiveness and supports its recognition as a separate tribe. Recent systematic studies have demonstrated that deeply divergent lineages within Tanypodinae may warrant recognition at tribal rank when supported by multiple lines of evidence. The establishment of Fittkauimyini based on morphological evidence (Silva and Ekrem, 2016) and its subsequent support from molecular phylogenetic analyses (Krosch et al., 2022) illustrates how integrative evidence can justify the recognition of new tribes within the subfamily. In this context, the recognition of *Saetheromyiini* trib. nov. follows a similar systematic framework, as it is supported by phylogenomic, divergence time and morphological evidence.

Together, these results indicate that the higher classification of Tanypodinae is still undergoing refinement, and that phylogenomic

data, combined with morphological and temporal evidence, are essential for establishing a tribal classification that accurately reflects the evolutionary history of the subfamily.

5. Conclusion

This study presents the first genome-scale phylogenetic framework for Tanypodinae based on USCOs data, providing improved resolution of relationships among major lineages of the subfamily. Our phylogenomic results support the recognition of *Saetheromyiini* **trib. nov.**, with *Saetheromyia* forming an independent lineage sister to Tanypodini. Morphological characters, including the relatively long distance between veins MCu and FCu, the presence of a scutal tubercle, and the absence of both an inner lobe and an outer process on the gonostylus, further support the distinctiveness of this lineage. In addition, *Djalma-batista* is confirmed as a valid genus within Procladiini, forming a sister-group relationship with *Procladius*.

Divergence time estimates indicate that the major tribes of Tanypodinae originated during the Lower Cretaceous, followed by subsequent diversification of genera from the Upper Cretaceous to the Miocene. These results suggest that the deep evolutionary history of Tanypodinae played an important role in shaping the present-day diversity and tribal structure of the subfamily.

Despite these advances, the current phylogenetic framework is still limited by incomplete taxon sampling, and future studies incorporating broader geographic sampling and additional genomic data will be important for further refining phylogenetic relationships within Tanypodinae. Overall, this study demonstrates that the integration of phylogenomic, morphological, and temporal evidence provides a robust foundation for a more stable and evolutionarily informed tribal classification of Tanypodinae, highlighting the importance of phylogenomics for resolving deep relationships and stabilizing higher classification in Chironomidae.

Ethics

This work did not require ethical approval from a human subject or animal welfare committee.

Declaration of AI use

We have not used AI-assisted technologies in creating this article.

CRediT authorship contribution statement

Xiu-Ru Xiao: Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Yi-Ni Zhang:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Conceptualization. **Hai-Feng Xu:** Writing – review & editing, Methodology, Conceptualization. **Zhi-Chao Zhang:** Writing – review & editing, Visualization, Conceptualization. **Yu-Yi Jiang:** Writing – review & editing, Visualization, Conceptualization. **Yan-Xin Chen:** Visualization, Investigation, Conceptualization. **Chen-Hong Li:** Writing – review & editing, Investigation, Conceptualization. **Bing-Jiao Sun:** Visualization, Investigation, Conceptualization. **Fabio Laurindo da Silva:** Writing – review & editing, Visualization, Investigation, Conceptualization. **Xiao-Long Lin:** Writing – review & editing, Visualization, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Funding

This work was supported by the National Natural Science Foundation of China (NSFC: 31900344).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We express our sincere gratitude to Shu-Ping Wang, Qian Wang, En-Lou Zhang, Zhen-Yuan Liu, Yu Peng, Shuang Qiu, Yuan-Yuan Han, Mei-Xin Lin, Chao Song, Yuan Yao and Yue Fu for their invaluable assistance with specimen collection. We also acknowledge the Namibian National Commission on Research, Science and Technology (NCRST) and the Ministry of Environment, Forestry, and Tourism for granting permits to collect and export specimens under the Nagoya Protocol. Fabio Laurindo da Silva acknowledges the institutional support of Embrapa. We sincerely thank the two anonymous reviewers for their careful evaluation of this manuscript and their insightful comments, which substantially improved the quality and scientific rigor of this work.

Appendix A. Supplementary data

Supplementary data to this article include tables of sample and sequencing information, genome assembly and filtering statistics, and calibration points, together with a genome assembly evaluation figure and the phylogenetic tree files generated in this study. Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ympev.2026.108693>.

Data availability

The whole-genome sequences of all 29 species are publicly available in the National Genomics Data Center (NGDC), under BioProject ID PRJCA014092 (<https://ngdc.cnpc.ac.cn/bioproject/browse/PRJCA014092>). The raw sequencing data are available in the Genome Sequence Archive (GSA) under accession CRA038138 (<https://ngdc.cnpc.ac.cn/gsa/browse/CRA038138>), and the assembled genomes are available in the Genome Warehouse (GWH, accessions GWHHOGW00000000.1–GWHHOHY00000000.1).

References

- Andersen, T., Ekrem, T., Cranston, P., 2013. The larvae of the Holarctic Chironomidae (Diptera)-Introduction. *Insect Syst. Evol. Suppl.* 66, 7–12.
- Andrews, S., 2010. FastQC: a quality control tool for high throughput sequence data. Babraham Institute, Cambridge, UK. Available online: (accessed on 24 March 2021).
- Ashe, P., O'Connor, J.P., 2009. A world catalogue of Chironomidae (Diptera), Part 1: Buchonomyiinae, Chilenomyiinae, Podonominae, Aphroteniinae, Tanypodinae, Usambaromyiinae, Diamesinae, Prodiamesinae and Telmatogetoninae, 1st ed. *Irish Biogeographical Society*, Dublin, Ireland, pp. 1–445.
- Baranov, V., Lin, X., Hübner, J., Chimeno, C., 2024. Uncovering the hidden diversity of non-biting midges (Diptera, Chironomidae) from central Namibia, using morphology and DNA barcodes. *Afr. Invertebr.* 65, 15–36. <https://doi.org/10.3897/afrinvertebr.65.111920>.
- Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>.
- Bonnet, T., Leblois, R., Rousset, F., Crochet, P.-A., 2017. A reassessment of explanations for discordant introgressions of mitochondrial and nuclear genomes. *Evolution* 71, 2140–2158. <https://doi.org/10.1111/evo.13296>.
- Brodin, Y., 2025. *Procladius* (Diptera, Chironomidae) of Europe and a global view. *Zootaxa* 5591, 1–127. <https://doi.org/10.11646/zootaxa.5591.1.1>.
- Bushnell, B., 2014. BBMap: short read aligner, and other bioinformatic tools. SourceForge.
- Chikhi, R., Rizk, G., 2013. Space-efficient and exact de Bruijn graph representation based on a Bloom filter. *Algorithms Mol. Biol.* 8, 1–9. <https://doi.org/10.1186/1748-7188-8-22>.
- Cohen, K.M., Finney, S.C., Gibbard, P.L., Fan, J.-X., 2013. The ICS international chronostratigraphic chart. *Episodes* 36, 199–204. <https://doi.org/10.18814/epiugs/2013/v36i3/002>.
- Cranston, P., Epler, J., 2013. The larvae of Tanypodinae (Diptera: Chironomidae) of the Holarctic region-Keys and diagnoses. *Chironomidae of the Holarctic Region: Keys and diagnoses. Part 1*, 39–136. <https://doi.org/10.5324/cjcr.v0i26.1656>.

- Crotty, S.M., Minh, B.Q., Bean, N.G., Holland, B.R., Tuke, J., Jermini, L.S., Haeseler, A.V., 2020. GHOST: recovering historical signal from heterotachously evolved sequence alignments. *Syst. Biol.* 69, 249–264. <https://doi.org/10.1093/sysbio/syz051>.
- Czechowski, P., Stevens, M.I., Madden, C., Weinstein, P., 2020. Steps towards a more efficient use of chironomids as bioindicators for freshwater bioassessment: Exploiting eDNA and other genetic tools. *Ecol. Indic.* 110, 105868. <https://doi.org/10.1016/j.ecolind.2019.105868>.
- Doitieu, G., Nel, A., 2007. Chironomid midges from early Eocene amber of France (Diptera: Chironomidae). *Zootaxa* 1404, 1–66. <https://doi.org/10.11646/zootaxa.1404.1.1>.
- Dantas, G.P., Hamada, N., 2018. Immature stages of *Laurotanyus travassosi* Oliveira, Messias Silva-Vasconcelos, 1992 reveal a new synonymy in Tanytopodinae (Diptera: Chironomidae). *Zootaxa* 4444, 462–470. <https://doi.org/10.11646/zootaxa.4444.4.6>.
- Ferrington, L.C., 2008. Global diversity of non-biting midges (Chironomidae; Insecta-Diptera) in freshwater. *Hydrobiologia* 595, 447–455. <https://doi.org/10.1007/s10750-007-9130-1>.
- Fittkau, E.J., 1962. Die Tanytopodinae (Diptera: Chironomidae). (Die Tribus Anatopyniini, Macropelopiini und Pentaneurini), Vol. 6. Walter de Gruyter GmbH & Co KG.
- Gao, S., Wang, C., Tang, Y., Zhang, Y., Ge, X., Zhang, J., Liu, W., 2024. Complete Mitochondrial Genome of *Tanytus chinensis* and *Tanytus kraatzi* (Diptera: Chironomidae): Characterization and Phylogenetic Implications. *Genes* 15, 1281. <https://doi.org/10.3390/genes15101281>.
- Ge, X., Peng, L., Morse, J.C., Wang, J., Zang, H., Yang, L., Sun, C., Wang, B., 2024. Phylogenomics resolves a 100-year-old debate regarding the evolutionary history of caddisflies (Insecta: Trichoptera). *Mol. Phylogenet. Evol.* 201, 108196. <https://doi.org/10.1016/j.ympev.2024.108196>.
- Godeiro, N.N., Ding, Y., Cipola, N.G., Jantarit, S., Bellini, B.C., Zhang, F., 2023. Phylogenomics and systematics of Entomobryodea (Collembola): marker design, phylogeny and classification. *Cladistics* 39, 101–115. <https://doi.org/10.1111/cia.12521>.
- Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. *Syst. Biol.* 59, 307–321. <https://doi.org/10.1093/sysbio/syq010>.
- Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K., Von Haeseler, A., Jermini, L.S., 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat. Methods* 14, 587–589. <https://doi.org/10.1038/nmeth.4285>.
- Krosch, M., Cranston, P., Bryant, L., Strutt, F., McCluen, S., 2017. Towards a dated molecular phylogeny of the Tanytopodinae (Chironomidae, Diptera). *Invertebr. Syst.* 31, 302–316. <https://doi.org/10.1071/IS16046>.
- Krosch, M.N., Silva, F.L., Ekrem, T., Baker, A.M., Bryant, L.M., Stur, E., Cranston, P.S., 2022. A new molecular phylogeny for the Tanytopodinae (Diptera: Chironomidae) places the Australian diversity in a global context. *Mol. Phylogenet. Evol.* 166, 107324. <https://doi.org/10.1016/j.ympev.2021.107324>.
- Kück, P., Struck, T.H., 2014. BaCoCa-A heuristic software tool for the parallel assessment of sequence biases in hundreds of gene and taxon partitions. *Mol. Phylogenet. Evol.* 70, 94–98. <https://doi.org/10.1016/j.ympev.2013.09.011>.
- Lai, Y., Du, S., Li, H., Zheng, Y., Ardila-Camacho, A., Aspöck, U., Aspöck, H., Yang, D., Zhang, F., Liu, X., 2024. Laceywing-specific Universal Single Copy Orthologs designed towards resolution of backbone phylogeny of Neuropterida. *Syst. Entomol.* 50, 309–324. <https://doi.org/10.1111/syen.12657>.
- Lanfear, R., Calcott, B., Kainer, D., Mayer, C., Stamatakis, A., 2014. Selecting optimal partitioning schemes for phylogenomic datasets. *BMC Evol. Biol.* 14, 1–14. <https://doi.org/10.1186/1471-2148-14-82>.
- Luo, R., Liu, B., Xie, Y., Li, Z., Huang, W., Yuan, J., He, G., Chen, Y., Pan, Q., Liu, Y., 2012. SOAPdenovo2: an empirically improved memory-efficient short-read *de novo* assembler. *Gigascience* 1, 1–18. <https://doi.org/10.1186/2047-217X-1-18>.
- Mai, U., Mirarab, S., 2018. TreeShrink: fast and accurate detection of outlier long branches in collections of phylogenetic trees. *BMC Genomics* 19, 23–40. <https://doi.org/10.1186/s12864-018-4620-2>.
- Martin, S.L., La Torre, R., Danneels, B., Tooming-Klunderud, A., Skage, M., Kollias, S., et al., 2026. Haplotype-resolved chromosome-level genome assemblies of four *Diamesa* species reveal the genetic basis of cold tolerance and high-altitude adaptations in arctic chironomids. *Gigascience* 15, giaf160. <https://doi.org/10.1093/gigascience/giaf160>.
- Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., Von Haeseler, A., Lanfear, R., 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol. Biol. Evol.* 37, 1530–1534. <https://doi.org/10.1093/molbev/msaa131>.
- Mrozińska, N., Obolowski, K., 2024. Morphological taxonomy and DNA barcoding: Should they be integrated to improve the identification of chironomid larvae (Diptera)? *Ecohydrol. Hydrobiol.* 24, 1–10. <https://doi.org/10.1016/j.ecohyd.2023.11.007>.
- Narayanan Kutty, S., Wong, W.H., Meusemann, K., Meier, R., Cranston, P.S., 2018. A phylogenomic analysis of Culicomorpha (Diptera) resolves the relationships among the eight constituent families. *Syst. Entomol.* 43, 434–446. <https://doi.org/10.1111/syen.12285>.
- Niitsuma, H., 2007. *Saetheromyia*, a new genus of Tanytopodinae from Japan (Diptera: Chironomidae). In: Andersen, T. (Ed.), *Contributions to the systematics and ecology of Aquatic Diptera—A tribute to Ole A. Caddis Press*, Columbus, Sæther, pp. 219–224.
- Oliver, D.R., Dillon, M.E., 1989. The adult males of Chironomidae (Diptera) of the Holarctic region-key to subfamilies. 74.
- Pape, T., Evenhuis, N., 2023. Systema Dipterorum. *Biodivers. Inf. Sci. Stand.* 7, e111959. <https://doi.org/10.3897/biss.7.111959>.
- Parham, J.F., Donoghue, P.C., Bell, C.J., Calway, T.D., Head, J.J., Holroyd, P.A., Inoue, J. G., Irmis, R.B., Joyce, W.G., Ksepka, D.T., Patane, J.S., Smith, N.D., Tarver, J.E., van Tuinen, M., Yang, Z., Angielczyk, K.D., Greenwood, J.M., Hipsley, C.A., Jacobs, L., Makovicky, P.J., Muller, J., Smith, K.T., Theodor, J.M., Warnock, R.C., Benton, M.J., 2012. Best practices for justifying fossil calibrations. *Syst. Biol.* 61, 346–359. <https://doi.org/10.1093/sysbio/syr107>.
- Pryszcz, L.P., Gabaldón, T., 2016. Redundans: an assembly pipeline for highly heterozygous genomes. *Nucleic Acids Res.* 44, e113. <https://doi.org/10.1093/nar/gkw294>.
- Rieradevall, M., Brooks, S.J., 2001. An identification guide to subfossil Tanytopodinae larvae (Insecta: Diptera: Chironomidae) based on cephalic setation. *J. Paleolimnol.* 25, 81–99.
- Roback, S., 2007. Adults of the Subfamily Tanytopodinae (Pelopinae) in North America (Diptera: Chironomidae). *Academy of Natural Sciences*.
- Roback, S.S., 1980. The immature chironomids of the eastern United States IV. Tanytopodinae-Procladiini. In: *Proceedings of the Academy of Natural Sciences of Philadelphia*, pp. 1–63.
- Sahlén, K., Vezzi, F., Nystedt, B., Lundberg, J., Arvestad, L., 2014. BESST-efficient scaffolding of large fragmented assemblies. *BMC Bioinf.* 15, 1–11. <https://doi.org/10.1186/1471-2105-15-281>.
- Sasa, M., 1994. Additional information on the Chironomidae of Japan, 1994. *Res. Rep. Toyama Pref. Environ. Pollut. Res. Cent.* 1994, 28–67.
- Shen, W., Le, S., Li, Y., Hu, F., 2016. SeqKit: A Cross-Platform and Ultrafast Toolkit for FASTA/Q File Manipulation. *PLoS ONE* 11, e0163962. <https://doi.org/10.1371/journal.pone.0163962>.
- Silva, F.L.D., Ekrem, T., 2016. Phylogenetic relationships of nonbiting midges in the subfamily Tanytopodinae (Diptera: Chironomidae) inferred from morphology. *Syst. Entomol.* 41, 73–92. <https://doi.org/10.1111/syen.12141>.
- Silva, F.L.D., Pinho, L.C., Stur, E., Nihei, S.S., Ekrem, T., 2023. DNA barcodes provide insights into the diversity and biogeography of the non-biting midge *Polypedium* (Diptera, Chironomidae) in South America. *Ecol. Evol.* 13, e10602. <https://doi.org/10.1002/eece3.10602>.
- Smirnov, V., Warnow, T., 2021. MAGUS: multiple sequence alignment using graph clustering. *Bioinformatics* 37, 1666–1672. <https://doi.org/10.1093/bioinformatics/btab992>.
- Steenwyk, J.L., Buida III, T.J., Labella, A.L., Li, Y., Shen, X.-X., Rokas, A., 2021. PhyKIT: a broadly applicable UNIX shell toolkit for processing and analyzing phylogenomic data. *Bioinformatics* 37, 2325–2331. <https://doi.org/10.1093/bioinformatics/btab096>.
- Steenwyk, J.L., Buida III, T.J., Li, Y., Shen, X.-X., Rokas, A., 2020. ClipKIT: a multiple sequence alignment trimming software for accurate phylogenomic inference. *PLoS Biol.* 18, e3001007. <https://doi.org/10.1371/journal.pbio.3001007>.
- Waterhouse, R.M., Seppey, M., Simão, F.A., Manni, M., Ioannidis, P., Kliuchnikov, G., Kriventseva, E.V., Zdobnov, E.M., 2018. BUSCO applications from quality assessments to gene prediction and phylogenomics. *Mol. Biol. Evol.* 35, 543–548. <https://doi.org/10.1093/molbev/msx319>.
- Xiao, X.-R., Chen, M.-H., Li, S.-Y., Guo, B.-X., Zhang, Y., Zhang, Z.-C., et al., 2025. Comparative Mitogenomic Analyses of Tanytopodinae (Diptera: Chironomidae). *Insects* 16, 203. <https://doi.org/10.3390/insects16020203>.
- Xu, H.-F., Zhang, Z.-C., Zhang, D., Baranov, V., Li, C.-H., Lin, X.-L., 2026. Phylogenomics of an early-branching clade of Orthocladinae (Diptera: Chironomidae). *Mol. Phylogenet. Evol.* 214, 108477. <https://doi.org/10.1016/j.ympev.2025.108477>.
- Yang, Z., 2007. PAML 4: phylogenetic analysis by maximum likelihood. *Mol. Biol. Evol.* 24, 1586–1591. <https://doi.org/10.1093/molbev/msm088>.
- Yu, D., Ding, Y., Tihelka, E., Cai, C., Hu, F., Liu, M., Zhang, F., 2022. Phylogenomics of elongate-bodied springtails reveals independent transitions from aboveground to belowground habitats in deep time. *Syst. Biol.* 71, 1023–1031. <https://doi.org/10.1093/sysbio/syaa024>.
- Zhang, C., Mirarab, S., 2022. ASTRAL-Pro 2: ultrafast species tree reconstruction from multi-copy gene family trees. *Bioinformatics* 38, 4949–4950. <https://doi.org/10.1093/molbev/msac215>.
- Zhang, D., Niu, Z.Q., Luo, A.R., Orr, M.C., Ferrari, R.R., Jin, J.F., Wu, Q.T., Zhang, F., Zhu, C.D., 2022. Testing the systematic status of *Homalictus* and *Rostrolalictus* with weakened cross-vein groups within Halictini (Hymenoptera: Halictidae) using low-coverage whole-genome sequencing. *Insect Sci.* 29, 1819–1833. <https://doi.org/10.1111/1744-7917.13034>.
- Zhang, F., Ding, Y., Zhu, C.D., Zhou, X., Orr, M.C., Scheu, S., Luan, Y.X., 2019. Phylogenomics from low-coverage whole-genome sequencing. *Methods Ecol. Evol.* 10, 507–517. <https://doi.org/10.1111/2041-210X.13145>.