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Anchored phylogenomics and revised classification of the Miltogramminae (Diptera: Sarcophagidae)

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Abstract

The Miltogramminae (Diptera: Sarcophagidae) includes ~600 species across >40 genera, which constitute ~20% of global Sarcophagidae. While molecular phylogenetic hypotheses have been produced for this group, critical problems persist, including the presence of paraphyletic genera, uncertain relationships between genera, a bias of sampling towards Palaearctic taxa, and low support for many branches. The present study remedies these issues through the application of Anchored Hybrid Enrichment (AHE) to a sample including ~60% of the currently recognised genera (16% of known species) representing all biogeographic regions except the Neotropical. An alignment of 1,281 concatenated loci was analysed with maximum likelihood (RAxML, IQ-TREE), Bayesian inference (ExaBayes) and coalescent-based approaches (ASTRAL, SVDquartets), which resulted in highly supported and concordant topologies, providing unprecedented insight into the relationships of this subfamily of flesh flies, allowing a major update to miltogrammine classification. The AHE phylogenetic hypothesis supports the monophyly of a large proportion of genera. The monophyly of *Metopia* Meigen is restored by synonymy with *Aenigmetopia* Malloch, **syn.n.** To achieve monophyly of *Miltogramma* Meigen, eight species are transferred from *Pterella* Robineau-Desvoidy. The genus *Pterella* is shown to be paraphyletic in its current circumscription, and to restore generic monophyly *Pterella* is restricted to contain only *Pt. grisea* (Meigen). *Erioprocta* Enderlein, **stat.rev.**, is resurrected. The genus *Senotainia* Macquart is reconstructed as paraphyletic. The monotypic genus *Metopodia* Brauer & Bergenstamm is synonymised with *Taxigramma* Macquart, **syn.n.** In light of our phylogenetic hypotheses, a new Miltogramminae tribal classification is proposed, composed of six tribes.

KEYWORDS
Diptera, genomics, hybrid enrichment, phylogenomics, systematics, taxonomy

INTRODUCTION

Flesh flies (Sarcophagidae) are usually divided into three subfamilies based on adult morphology and molecular data (Buenaventura et al., 2020; 2021; Piwczyński et al., 2017; Yan et al., 2021a): Miltogramminae, Paramacronychiinae and Sarcophaginae. The subfamily Miltogramminae is known from ~600 species, divided into more than 40 genera, although many of these are ill-defined morphologically and emerge as non-monophyletic in molecular analyses (Pape, 1996; Piwczyński et al., 2017; Yan et al., 2021a). While comprising fewer species than the Sarcophaginae, miltogrammines display a similar range of first instar morphology and adult sexual dimorphism, and almost as much life history diversity (particularly larval feeding strategies), with species known to be obligate parasites, parasitoids, predators and saprophages, and most commonly hymenopteran kleptoparasites (Pape, 1996; Spofford & Kurczewski, 1990; Szpila, 2010; Xu et al., 2018). The monophyly and circumscription of the Miltogramminae and the phylogenetic placement of this subfamily within the Sarcophagidae have been refined through numerous studies utilising morphology (Pape, 1996; Rohdendorf, 1967; Verves, 1989), multilocus Sanger (Kutty et al., 2010; Piwczyński et al., 2014; Piwczyński et al., 2017; Johnston et al., 2020a, 2020b, 2020c, 2020d; Johnston et al., 2021) and next-generation sequencing data (Kutty et al., 2019; Buenaventura et al., 2020; Buenaventura, 2021; Buenaventura et al., 2021; Yan et al., 2021a). The Anchored Hybrid Enrichment (AHE) phylogeny produced by Buenaventura et al. (2020) is currently the best supported phylogenetic hypothesis for the Sarcophagidae and resolves Miltogramminae together with Paramacronychiinae as sister to Sarcophaginae, in agreement with earlier and more recent molecular studies (Buenaventura, 2021; Buenaventura et al., 2021; Piwczyński et al., 2014, 2017; Yan et al., 2021a, 2021b).

In the first comprehensive study of miltogrammine phylogeny, Rohdendorf (1967) divided the subfamily into four tribes based mostly on the morphology of head, legs and wings, with phylogenetic relationships proposed within two of these. Verves (1989) further developed Rohdendorf's study, and except for the genera *Eumacronychia* Townsend, *Macronychia* Rondani and *Sarcotachina* Portschinsky, which were not considered as miltogrammines (Verves, 1998; Verves & Khrokalo, 2006), he covered the entire subfamily and established a 'phylogenetic' classification with six tribes and 25 subtribes (Table 1). The tree provided by Verves (1989) was a hand-made cladogram based on 46 characters a priori coded into a plesiomorphic versus one or more apomorphic states and as such is largely untestable (Piwczyński et al., 2017). Pape (1996) abandoned the traditional tribal and subtribal system of the Miltogramminae developed by Rohdendorf and Verves and instead replaced species-rich subtribes such as Apodacrina, Hoplacephalina, Miltogrammatina, Opsidiina, Parthomyiina, Phyllotelina, Sphenometopiina and Taxigrammatina with more broadly defined genera.

The Rohdendorf-Verves classification (e.g., Verves & Khrokalo, 2017, 2018, 2020a, 2020b, Barták et al., 2019, Verves et al., 2020a) is rejected by extensive non-monophyly indicated by current molecular studies (Buenaventura et al., 2020; Piwczyński et al., 2017; Yan et al., 2021a), and some of the large genera applied by Pape (1996) have also been resolved as non-monophyletic: *Metopia* Meigen,

TABLE 1 Revised tribal classification of Miltogramminae, with a listing of the genera included in the present study.

Tribe	Genus	Subfamily or tribe sensu Verves (1989)
Eumacronychiini	*Eumacronychia* Townsend	Eumacronychiinae
Sarcotachinini	*Dolichotachina* Villeneuve	Phyllotelini
	Hoplacephala Macquart	Phyllotelini
	Mesomelena Rondani	Metopiaini (as Metopiini)
	Phylloteles Loew	Phyllotelini
	Sphecapatoclea Villeneuve	Phyllotelini
Metopiaini	*Aenigmetopia* Malloch	Miltogrammini
	Metopia Meigen	Metopiaini (as Metopiini)
	Phrosinella Robineau-Desvoidy	Metopiaini (as Metopiini)
Macronychiini	*Amobia* Robineau-Desvoidy	Amobiini
	Macronychia Rondani	Macronychiinae
	Oebalia Robineau-Desvoidy	Oebaliini
	Senotainia Macquart	Miltogrammini (as Miltogrammatini)
Taxigrammini	*Metopodia* Brauer & Bergenstamm	Phyllotelini
	Sphecapatodes Villeneuve	Phyllotelini
	Sphenometopa Townsend	Metopiaini (as Metopiini)
	Taxigramma Macquart	Metopiaini (as Metopiini)
Miltogrammini	*Apodacra* Macquart	Miltogrammini (as –atini)
	Craticulina Bezzi	Miltogrammini (as –atini)
	Eremasiomyia Rohdendorf	Miltogrammini (as –atini)
	Miltogramma Meigen	Miltogrammini (as –atini)
	Protomiltogramma Townsend	Miltogrammini (as –atini)
	Pterella Robineau-Desvoidy	Miltogrammini (as –atini)

Miltogramma Meigen, *Pterella* Robineau-Desvoidy and *Senotainia* Macquart (Buenaventura et al., 2020; Johnston et al., 2020d; Piwczyński et al., 2017).

Piwczyński et al. (2017) produced the first targeted molecular phylogenetic analysis of the Miltogramminae utilising data from one nuclear and three mitochondrial loci from 58 species. The resulting tree divided the subfamily into a non-kleptoparasitic, mainly necrophagous, grade of 'lower' miltogrammines (comprising *Eumacronychia* branching from the base plus a clade including the genera *Dolichotachina* Villeneuve, *Mesomelena* Rondani, *Phylloteles* Loew, *Sarcotachina* and *Sphecapatoclea* Villeneuve) and a clade of higher miltogrammines comprising the remaining genera, which contain mostly kleptoparasitic species. Within the lower miltogrammines, relationships were not fully resolved, as low bootstrap values and conflict between maximum likelihood (ML) and Bayesian inference (BI) trees were found. The dataset used by Piwczyński et al. (2017) was subsequently enlarged by the addition of data from Australian species by Johnston et al. (2020a, 2020b, 2020c, 2020d, 2021), but support values remained low. The difficulty in robustly resolving the 'lower miltogrammines' was somewhat alleviated in the AHE phylogenetic hypothesis of Buenaventura et al. (2020), which provided much better nodal support due to a higher number of loci (~600), but only included 22 miltogrammine taxa. Additionally, this phylogenetic hypothesis could not address the non-monophyly of the lower miltogrammines because *Eumacronychia* was not included in the analysis. A subsequent phylogenetic analysis by Buenaventura (2021) utilised ultraconserved elements (UCEs) and focused mostly on higher level family relationships within the superfamily Oestroidea. This study did not provide a substantial contribution to the phylogeny of miltogrammines, as this taxon was represented by only three species. In a separate study, Buenaventura et al. (2021) used UCEs to explore the evolution of life history traits in Sarcophagidae. As in the previous study, only a few (four) Miltogramminae were included and, as such, little insight was provided into the phylogenetic relationships within this subfamily. Yan et al. (2021a) reconstructed flesh fly evolution from a supertree produced by augmenting a molecular tree with branches from published morphology-based phylogenies. Like previous phylogenetic hypotheses (Buenaventura, 2021; Buenaventura et al., 2020; Piwczyński et al., 2017), this study suffered from low taxon sampling (16 species with transcriptomic data) and, in the case of the supertree approach, few analysed loci (COI and CYTB from GenBank sequences). The mitogenomic study of Yan et al. (2021b) also sampled a small number of Miltogramminae (6 spp.). Notably, none of these molecular studies proposed any formal changes in the classification of the Miltogramminae, except for the transfer of *Sarcotachina* from the subfamily Paramacronychiinae to Miltogramminae (Buenaventura et al., 2020).

The recent publications of Buenaventura (2021), Buenaventura et al. (2020, 2021) and Yan et al. (2021a) highlight the power of data-rich NGS technologies to produce well-supported phylogenies, particularly in situations where previous Sanger-based sequencing technologies had been insufficient (Piwczyński et al., 2017). AHE has gained attention within Diptera phylogenetics, particularly due to the development of an AHE probe set specific for flies (Young et al., 2016). As a result of this probe set, refinement of sequencing techniques and production of several downstream bioinformatic analysis pipelines (arising from the One Thousand Insect Transcriptome Evolution consortium; https://1kite.org), AHE-based phylogenies have been published for several dipteran families to date, including Syrphidae (Young et al., 2016), Acroceridae (Gillung et al., 2018), Bombyliidae (Li et al., 2021), Muscidae (Grzywacz et al., 2021) and, as mentioned, Sarcophagidae (Buenaventura et al., 2020). AHE, using the Diptera probe set, should thus provide a large amount of new data to help resolve miltogrammine phylogeny. Here, we combine the power of large genome-wide gene sampling from AHE together with extensive taxon sampling to address the remaining issues highlighted from previous published phylogenies.

The objective of this study is to provide a greatly expanded AHE-based phylogenetic hypothesis for Miltogramminae, and then use this hypothesis to test the monophyly of most of the larger genera and provide a better revised tribal classification.

MATERIALS AND METHODS

Taxon sampling and DNA extraction

Representative taxa of the global Miltogramminae were selected for DNA extraction to maximise the diversity of genera included in our analyses (Table S1). Total genomic DNA was extracted from the thorax, legs and abdomen of 95% ethanol-preserved specimens using a DNeasy blood and tissue kit (Qiagen, CA, USA). Heads and male genitalia (whole abdomens for females) were retained as vouchers for Australian taxa. For all other taxa, the entire specimen was sacrificed for DNA extraction. The final AHE dataset contained 114 ingroups and 7 outgroups, representing flies from all zoogeographic regions except the Neotropical (photographs for species not illustrated previously are available as Figure S4; illustrations of other species are available in: Johnston et al., 2020a, 2020b, 2020c, 2020d, 2021, Piwczyński et al., 2017 and Szpila & Mielczarek, 2018, Szpila et al. 2023). We used the system of nomenclature proposed by Pape (1996). As part of our testing of previous miltogrammine classifications, the generic and subgeneric names applied by Rohdendorf and Verves (Rohdendorf, 1967, Verves, 1986, 1989, 1994, Verves & Khrokalo, 2017, 2018, 2020a, 2020b, Verves et al., 2020b) were assigned to each species included in this study in Table S1. These generic and subgeneric names are used in the *Results* section, where the tree topology for particular genera, defined sensu Pape (1996), is presented. Non-monophyly of nominal genera was dealt with by new combinations or new generic synonymies as appropriate.

Following extraction, genomic DNA was analysed with Qubit Assay (ThermoFisher Scientific, MA, USA) and gel electrophoresis to confirm DNA concentration and fragmentation. Genomic DNA extracts with insufficient concentration for subsequent AHE capture and sequencing (less than 10 ng/μL) were amplified using the REPLI-g whole genome amplification kit following the manufacturer's instructions (Qiagen, CA, USA).

Anchored hybrid enrichment target capture and sequencing

The general method for sonication, target capture and subsequent sequencing followed Lemmon et al. (2012), with modifications specific to Diptera adapted from Young et al. (2016), Gillung et al. (2018) and Buenaventura et al. (2020).

In short, following extraction and quantification, DNA was sheared into ~300 bp fragments by sonication utilising a Covaris E220 focused ultrasonicator and Covaris micro-tubes (Covaris, MA, USA). DNA libraries were prepared following Meyer and Kircher (2010), including blunt end repair, fragment size selection (using SPRI-select beads; Beckman Coulter, CA, USA) and the addition of indexes. DNA library enrichment was completed using an Agilent Custom SureSelect kit (Agilent Technologies, CA, USA), with probes designed specifically for Diptera (Young et al., 2016) and targeting 559 loci (specific loci sequences available as supplementary material in Young et al., 2016). Enriched DNA was pooled and sequenced as single reads (100 bp) on an Illumina HiSeq 2500 platform at the NCSU Genomic Sciences Laboratory (Raleigh, NC), with up to 48 multiplexed samples per lane in the flow cell. Following sequencing, single reads were assessed for quality using FastQC (Andrews, 2010) and then trimmed with Trimmomatic v.0.36 (Bolger et al., 2014), with the minimum per base sequence quality set to 20 and minimum read length set to 25 bp.

Sequence assembly

Sequence assembly generally followed the bioinformatic pipeline established by the 1KITE consortium (https://1kite.org). De novo assemblies of nucleotide (NT) contigs were carried out using Trinity v.2.4 (Grabherr et al., 2011). A graph-based approach implemented in Orthograph v.0.5.8 (Petersen et al., 2017) was then used to map contigs to known single-copy orthologous genes from a reference set. For this study, we followed Buenaventura et al. (2020) and used the 'Mecopterida' reference set of 3,145 orthologous genes from five reference insect species (Pauli 2018): *Bombyx mori* Linnaeus (Lepidoptera: Bombycidae), *Danaus plexippus* (Linnaeus) (Lepidoptera: Nymphalidae), *Aedes aegypti* (Linnaeus) (Diptera: Culicidae), *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) and *Glossina morsitans* Westwood (Diptera: Glossinidae). Orthograph runs all searches at the amino acid (AA) level and as such all contigs were translated from NT to AA prior to analysis. Following orthology prediction, all terminal stop codons were removed while internal stop codons and 'U' (selenocysteine) were replaced with an 'X' or 'NNN' for AA and NT data, respectively, using a custom Perl script designed by Misof et al. (2014). In the final step, contaminant and non-dipteran sequences were identified using NCBI BLAST and removed from both AA and NT data.

Multiple sequence alignment

Multiple sequence alignments (henceforth referred to as alignments) were created from FASTA files of AAs for each orthologous gene set using MAFFT v.7.237 with the L-INS-i algorithm (Katoh & Standley, 2013), following the procedure by Misof et al. (2014). Outlier removal also followed the general procedure by Misof et al. (2014), with slight modifications as follows: during the first round of outlier removal, outliers were refined and realigned using the 'addfragments' algorithm implemented within MAFFT (Katoh & Frith, 2012), which allows short fragmentary sequences to be aligned to an existing alignment (Buenaventura et al., 2020; Evangelista et al., 2019). Following realignment using 'addfragments', a second round of outlier removal was completed and sequences identified as outliers were removed.

Ortholog reference sequences were also removed from AA alignments and NT data. Data columns containing only 'X' (selenocysteine or missing data) were removed from each AA alignment only. AA alignments were then used as a guide for the alignment of NT sequence data using a modified version of PAL2NAL v.14 (Misof et al., 2014; Suyama et al., 2006). Ambiguously or randomly aligned sections were then identified in AA alignments using Aliscore v.2.2 and subsequently removed. Corresponding codons were also removed from NT alignments using Alicut v.2.3 and custom Perl scripts (Misof & Misof, 2009, Kück & Meusemann, 2010). Following the removal of these regions, NT alignments were realigned using PAL2NAL with AA alignments as a guide.

FASconCAT (Kück & Longo, 2014) was used to concatenate the NT alignment of each locus, resulting in a final alignment with a total of 580,248 bp from 1,281 loci.

Concatenation analyses

ML analysis of the final unpartitioned NT alignment was performed using IQ-TREE v.1.6.10 (Nguyen et al., 2015). All possible substitution models were tested in ModelFinder implemented within IQ-TREE (Nguyen et al., 2015), and the model with the highest corrected Akaike information criterion (AICc) (GTR + FreeRate model with 10 categories and empirical base frequencies '+ R10 + F') was chosen for the final analysis. Node support for this phylogenetic tree was estimated using both 10,000 ultrafast bootstrap replications and SH-Like approximate likelihood ratio tests (Guindon et al., 2010) with 10,000 replicates. To ensure that the resultant phylogenetic tree was not biased by the algorithms and models used for analysis in IQ-TREE, a second ML analysis was also performed in RAxML v.8.0 (Stamatakis, 2014) using the GTR + G + I model [the best available model in RAxML as determined by AICc in ModelFinder] and 1,000 rapid bootstrap iterations implemented in CIPRES Science Gateway (Miller et al., 2010).

BI of NT data was performed in ExaBayes v.1.5 (Aberer et al., 2014) implemented in CIPRES Science Gateway (Miller et al., 2010). Two simultaneous BI analyses were completed using a total of eight Markov Chain Monte Carlo (MCMC) chains (six heated, two cold; temperature = 0.1) run for 1.5 million generations, sampling every 1,000 generations (for a total of 12,000 samples across all runs).

All priors remained in their default state. To confirm run success and effective sampling of the priors, MCMC convergence was confirmed by examining effective sample size (ESS) and potential scale reduction factors (PRSF), ensuring that they were greater than 100 and ~ 1, respectively. As ExaBayes only allows for a single nucleotide substitution model, the default model (GTR + G) was used. A 50% majority consensus tree was then prepared from the resultant trees, with the first 25% of all trees discarded as burn-in.

Coalescent-based analyses

NT alignments were also analysed using two coalescent-based methods, to examine any effects of incomplete linage sorting and gene tree discordance on the species tree. The first coalescent-based analysis was performed using ASTRAL III v.5.6.3 (Zhang et al., 2018). ASTRAL functions by heuristically searching for the tree that shares the greatest number of quartet topologies with the greatest number of input gene trees. Gene trees used for this analysis were prepared using RAxML v.8.0 (GTR + G + I model, 100 bootstrap iterations) for each locus.

A second coalescent-based analysis was performed through the SVDquartets software package implemented in PAUP* (Phylogenetic Analysis Using Parsimony *and other methods; available from http://paup.phylosolutions.com; Chifman & Kubatko, 2014) v.4.a165 (1,000,000 sampled quartets and 1,000 standard bootstrap replicates). SVDquartets analysis differs in approach to ASTRAL using between-taxa single nucleotide polymorphisms from the full concatenated NT alignments to generate quartets. These quartets are then assembled using the QFM algorithm to produce the final species-tree topology. This topology can be tested for uncertainty using non-parametric bootstrapping (Chifman & Kubatko, 2014). It should be noted that, while loci with more than one SNP violate the assumption of independence of sampled SNPs in the SVDquartets method, simulation has shown that multi-locus data provide correct splits under nearly all simulated schemes (Chifman & Kubatko, 2014).

Graphical presentation of data

Two final tree graphics (summarising ML + BI and coalescent-based analyses, respectively) were produced using FigTree v.1.4.3 (available from: http://tree.bio.ed.ac.uk/software/figtree) and edited using Adobe Illustrator® 2022 (available from: https://www.adobe.com/au/products/illustrator). For presentation of the statistical support values, names of analysis and subsequent tests of support have been shortened as follows: IQ-TREE ultrafast bootstrap support = UFBS, IQ-TREE SH-alrt branch support = SH-alrt, RAxML bootstrap support = RBS, ExaBayes posterior probability = PP, ASTRAL quadripartition posterior probability = QP, SVDquartets bootstrap support = SBS. For simplicity, when explaining node support in the phylogenetic trees, high support indicates clades with 90–100% support (for UFBS, SH-alrt, RBS, SBS; or QP, PP = 0.90–1.00), moderate support indicates clades with 75–90% support (QP, PP = 0.75–0.90) and low support indicates clades with 75% or less support (QP, PP = 0.00–0.75).

A note on nomenclature

Within this study we use, where possible, two-letter abbreviations for genera; in cases where two initial letters would not discriminate between generic names, we use the shortest possible unique abbreviation for that genus.

RESULTS

Final alignment

The final nucleotide alignment included 121 taxa (114 ingroup and 7 outgroup taxa) and consisted of 1,281 concatenated loci for a total of 580,240 sites, 10.2% of which were parsimony-informative (59,302 sites). It should be noted that the number of loci included in the alignment is much higher than the targeted 559 loci. This can be attributed to the non-specific amplification of genomic elements during target capture; these additional sequences were then assigned to orthologous genes from the Orthograph reference database.

General phylogenetic results

Both ML (IQ-TREE and RAxML) and BI analyses (ExaBayes) resolved congruent phylogenetic trees (Figure 1), with 81% of nodes resolved with maximum support across all analyses (UFBS, SH-alrt, RBS = 100%; PP = 1.00). In both the ML and BI phylogenetic trees, *Eumacronychia* was placed as sister to all other miltogrammines (Figures 1 and 2) and the six major miltogrammine clades here given tribal rank (Table 1) all resolved as monophyletic with high support. Most of the 12 genera represented by more than one species were resolved as monophyletic, but *Metopia*, *Miltogramma*, *Pterella* and *Senotainia* emerged as either para- or polyphyletic. The coalescent-based analyses (Figure 2) showed variation between methods, with several nodes resolved disparately in ASTRAL and SVDquartets analyses (ASTRAL topology: Figure 2; SVDquartets topology: Figure S1). In both coalescent-based analyses, *Eumacronychia* was placed outside the Sarcotachinini and emerged as polyphyletic with *Eumacronychia* 'sp. 1' sister to all other miltogrammines (QP = 1.00, SBS = 100%) and *Eumacronychia* 'sp. 2' sister to the clade Metopiaini + Macronychiini + Taxigrammini + Miltogrammini (QP = 0.52, SBS = 56.4%). As in the ML and BI analyses, both coalescent-based analyses supported the six main clades of Miltogramminae, given here as tribal rank (Table 1), and resolved the genera *Metopia*, *Miltogramma*, *Pterella* and *Senotainia* as non-monophyletic. Most of the variation between methods was observed in either the backbone of the phylogenetic tree or within the genera *Apodacra* Macquart, *Miltogramma* and *Protomiltogramma* Townsend (Figure 1, marked by red circles; Figure 2). Additionally, in the coalescent-based analysis, *Craticulina*

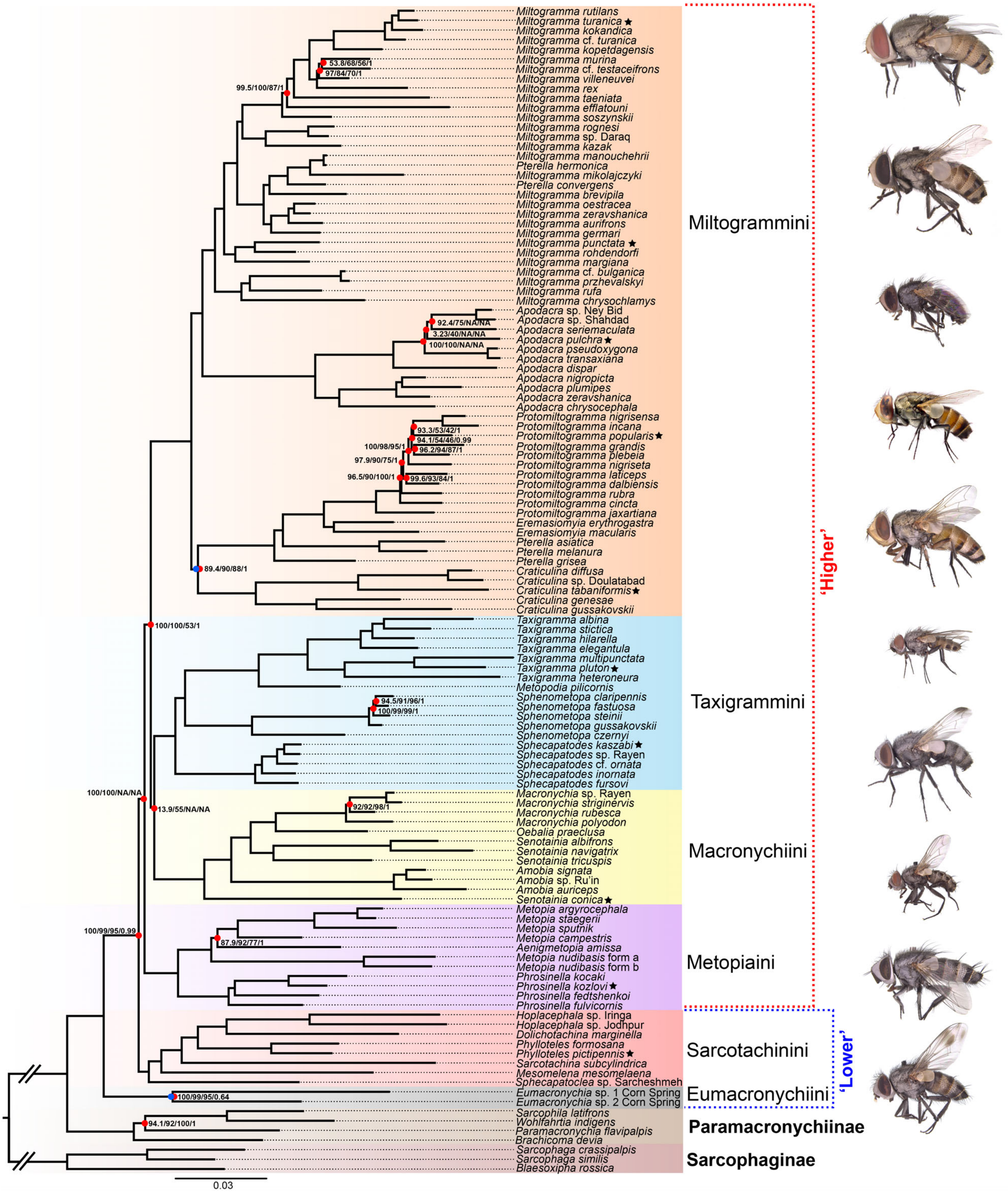

FIGURE 1 Maximum likelihood phylogenetic tree of the Miltogramminae estimated in IQ-tree using GTR + R10 + F substitution model from the AHE nucleotide dataset. Red dots indicate nodes with less than 100% support (or posterior probability = 1) across all analyses. Blue dots indicate major nodes that were not resolved by coalescent-based analysis. Where shown, node support values indicate: (1) IQ-TREE ultrafast bootstrap support; (2) IQ-TREE branch support via SH-aLRT; (3) RAxML bootstrap support; and (4) ExaBayes posterior probability. An 'NA' in the position of a support value indicates clades not resolved in the respective analysis. Branch length scale = 0.03 nucleotide substitutions per site (estimated using the GTR + R10 + F model implemented in IQ-TREE). Species names with stars are represented by a habitus photograph next to their corresponding position on the tree.

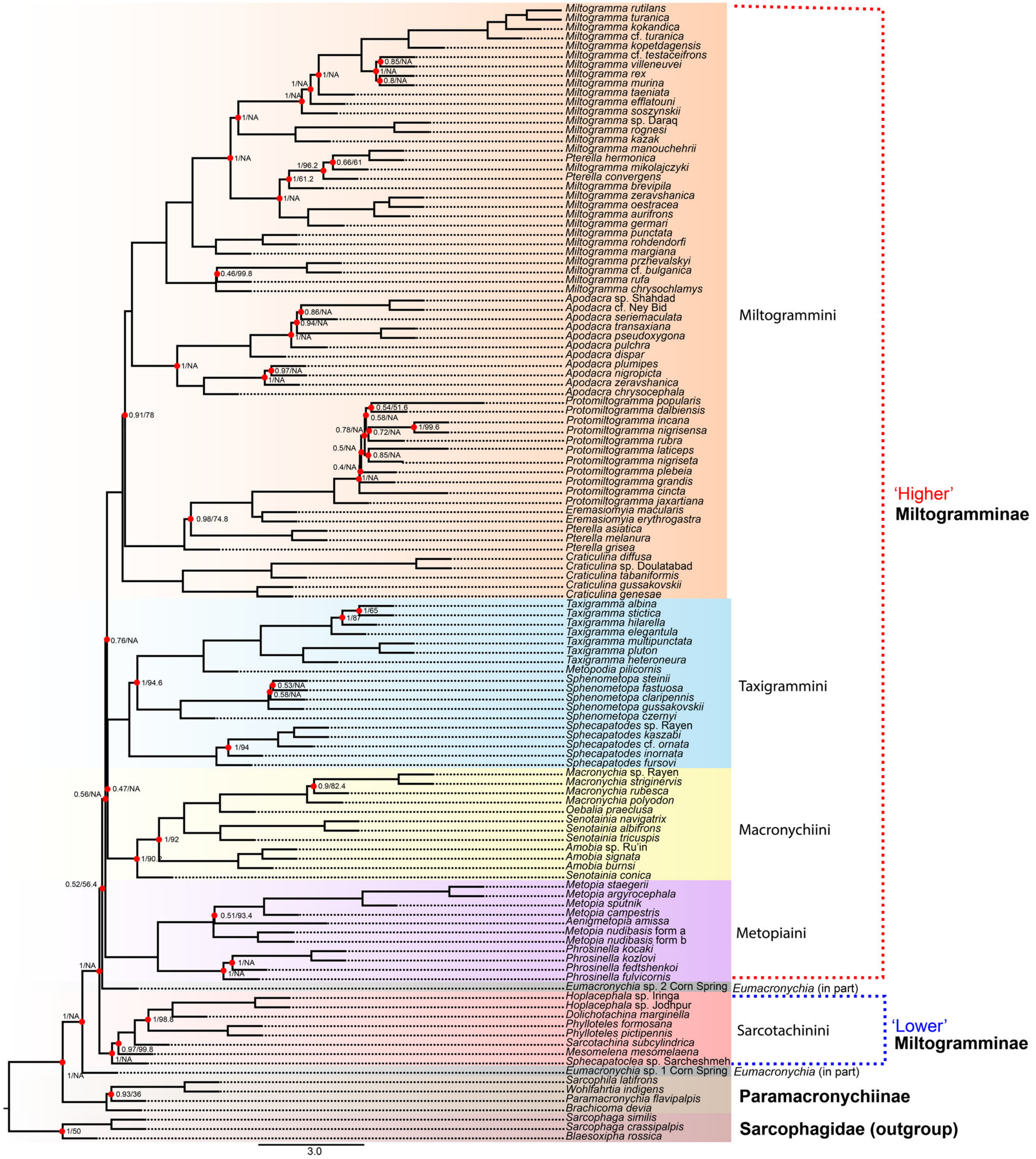

FIGURE 2 Coalescent-based phylogenetic tree of the Miltogramminae estimated in ASTRAL III from the AHE nucleotide dataset. Red dots indicate nodes with less than 100% SVDquartets bootstrap support or ASTRAL quadripartition posterior probability <1. Where shown, node support values indicate: (1) ASTRAL quadripartition posterior probability; (2) SVDquartets bootstrap support. 'NA' indicates clade not resolved in SVDquartets analysis. Branch length scale = 3 coalescent units (as calculated by ASTRAL III).

Bezzi was placed as sister to all other Miltogrammini (QP = 1.00, SBS = 100%) rather than as sister to the clade *Pterella* (in part) + *Eremasiomyia* Rohdendorf + *Protomiltogramma*. While the clades were not resolved identically, the broader phylogenetic relationships in the trees were concordant between the two analyses.

Tribal phylogeny

In the ML and BI analyses, six main clades were recovered, as given in Table 1. Not all clades were resolved with high support in both the ML and BI analyses. In the IQ-TREE analysis (IQ-TREE Topology:

Figure 1), Taxigrammini were resolved as sister to Macronychiini and the two together resolved as sister to Miltogrammini with low support (UFB = 13.9%, SH-alrt = 55%). Alternatively, in the RAxML (RaxML topology: Figure S2) and ExaBayes (ExaBayes topology: Figure S3) analyses, Macronychiini was placed in a clade with Miltogrammini + Taxigrammini with high support (RBS = 96%, PP = 1.00). The coalescent-based analysis failed to resolve a monophyletic *Eumacronychia*, instead splitting the two species to distant positions on the tree (Figure 2).

Generic and subgeneric monophyly

Amobia: Monophyletic and highly supported in all analyses.

Apodacra: Monophyletic. Species of *Apodacra* (*s. str.*) sensu Verves and Khrokalo (2018, 2020a) were present on both branches of the dichotomy for this genus. On one branch, *Apodacra chrysocephala* Rohdendorf was sister to three species of *Apodacra* (*Xerophilomyia* Rohdendorf) (*Ap. nigropicta* (Rohdendorf), *Ap. plumipes* Villeneuve, *Ap. zeravshanica* (Rohdendorf and Verves)) with high support and on the alternative branch species of *Apodacra* (*s. str.*) (*Ap. pulchra* Egger, *Ap. seriemaculata* Macquart) emerged within the species of *Xeromyia* (*Ap. pseudoxygona* Rohdendorf, *Ap. transaxiana* Rohdendorf, *Ap.* sp. Ney Bid, *Ap.* sp. Shahdad). Nodal support for the second clade varied from high to low, or nodes were not resolved in the SVDquartets analysis.

Craticulina: Monophyletic and highly supported in all analyses.

Eumacronychia: Monophyletic in the IQ-TREE analysis, paraphyletic in the SVDquartets analysis.

Hoplacephala: Monophyletic and highly supported in all analyses.

Macronychia: Monophyletic placed as sister to *Oebalia* Robineau-Desvoidy and emerging deeply nested in the Macronychiini with high support. *Macronychia* (*Moschusa*) *polyodon* (Meigen) resolved as sister to the remaining members of the genus and species representing the subgenus *Moschusa* Robineau-Desvoidy formed a paraphyletic grade at the base.

Metopia: Paraphyletic with respect to *Aenigmetopia* Malloch. *Aenigmetopia amissa* Johnston et al. was sister to a clade containing species of *Metopia* subgenera *Anicia* Robineau-Desvoidy and *Metopia* (*s. str.*) (*Me. campestris* (Fallén), *Me. argyrocephala* (Meigen), *Me. sputnik* (Johnston et al.), *Me. staegerii* Rondani) with moderate or high support. *Metopia* (*Chaetanicia* Townsend) *nudibasis* (Malloch) was sister to all other species of *Metopia* + *Aenigmetopia* with high support in all analyses.

Miltogramma: Paraphyletic, *Miltogramma* resolved as a large and highly supported clade, but included two species currently classified as *Pterella* (*Pt. convergens* (Pandellé), *Pt. hermonica* Verves & Khrokalo). Most of the internal nodes were also highly supported. Miltogrammini clade E (Figure 3), the sister to all other clades of Miltogrammini, contained a mixture of species referred sensu Verves (1989) to the genera *Rhynchapodacra* Rohdendorf (*Mi. rufa* (Rohdendorf)) and *Pediasiomyia* Rohdendorf (*Mi. chrysochlamys* (Rohdendorf), *Mi. przhevalskyi* (Rohdendorf), *Mi.* cf. *bulganica*). Clade D grouped together three species from three different genera sensu Verves: *Efflatounomyia* Rohdendorf (*Mi. rohdendorfi* (Tscharykuliev)), *Miltogramma* (*s. str.*) (*Mi. punctata* Meigen), and *Pediasiomyia* Rohdendorf (*Mi. margiana* (Rohdendorf)). Clade C is divided into a branch with four species of *Miltogramma* (subgenus *Dichiracantha* Enderlein) (*Mi. aurifrons* Dufour, *Mi. germari* Meigen, *Mi. oestracea* (Fallén), *Mi. zeravshanica* Rohdendorf), and a branch with a mixture of species from both *Miltogrammidium* Rohdendorf (subgenus *Pseudomiltogramma* Rohdendorf) (*Mi. brevipila* Villeneuve *Mi. mikolajczyki* Szpila & Pape, *Mi. manouchehrii* Szpila & Pape) and *Pterella* (*Pt. convergens*, *Pt. hermonica*). Clade B, containing three species of *Rohdendorfiella* Verves (*Mi. kazak* Pape, *Mi. rognes* Szpila & Pape, *Mi.* sp. Daraq), was sister to a large clade containing a mixture of species of *Anacanthotecum* Rohdendorf (*Mi.* cf. *testaceifrons*), *Miltogramma* (subgenus *Dichiracantha*) (*Mi. villeneuvei* Verves), *Miltogramma* (subgenus *Myochromum* Rohdendorf) (*Mi. murina* Meigen), *Miltogrammidium* (*s. str.*) (*Mi. efflatouni* Rohdendorf, *Mi. kokandica* Rohdendorf, *Mi. rex* Malloch, *Mi. rutilans* Meigen, *Mi. taeniata* Meigen, *Mi. turanica* Rohdendorf, *Mi.* cf. *turanica*, *Mi. soszynskii* Szpila & Pape), and *Miltogrammidium* (subgenus *Pseudomiltogramma*) (*Mi. kopetdagensis* Rohdendorf). Relationships between *Mi. villeneuvei*, *Mi.* cf. *testaceifrons*, and *Mi. murina* received moderate to low support or were not resolved in the SVDquartets analysis (Figure 3). Except for *Rohdendorfiella* Verves, none of the genera and subgenera proposed in Miltogrammatina sensu Verves (1989) and represented in our analysis by more than one species appeared to be monophyletic (Table S2).

Phrosinella: Monophyletic, the Nearctic species *Phr. fulvicornis* (Coquillett) resolved as sister to the Palaearctic clade *Phr. fedtshenkoi* (Rohdendorf) + *Phr. kocaki* Verves & Khrokalo, 2017 + *Phr. kozlovi* (Rohdendorf). All these relationships were highly supported in all analyses.

Phylloteles: Monophyletic and highly supported under all analysis methods.

Protomiltogramma: Monophyletic and highly supported under all analysis methods. Australian species form a clade with support ranging from high to low depending on the analytical approach.

Pterella: Polyphyletic. *Pterella grisea* (Meigen) resolved as sister to a large clade that was divided into *Pt. asiatica* (Rohdendorf) + *Pt. melanura* (Meigen) and *Eremasiomyia* + *Protomiltogramma*. Two other species (*Pt. convergens* and *Pt. hermonica*) were nested separately within *Miltogramma*. All these relationships were highly supported in all analyses.

Senotainia: Polyphyletic, with *Senotainia* (*s. str.*) *conica* (Fallén) resolved as sister to a large clade containing species of *Amobia*, *Oebalia*, *Macronychia*, and the remaining *Senotainia* (*Sen. albifrons* (Rondani), *Sen. navigatrix* (Meijere), *Sen. tricuspis* (Meigen)). *Senotainia* (*s. str.*) *tricuspis* was sister to two species representing the subgenus *Arrenopus* Brauer & Bergenstamm (*Sen. albifrons* and *Sen. navigatrix*). All these relationships were highly supported in all analyses.

Sphecapatodes: Monophyletic, but the topology of the clade does not match the current subgeneric classification. *Sphecapatodes* (subgenus *Turkmenisca* Rohdendorf) *fursovi* (Rohdendorf) was sister to all other species of *Sphecapatodes*, while *Sphecapatodes* (*Tu.*) sp. Rayen

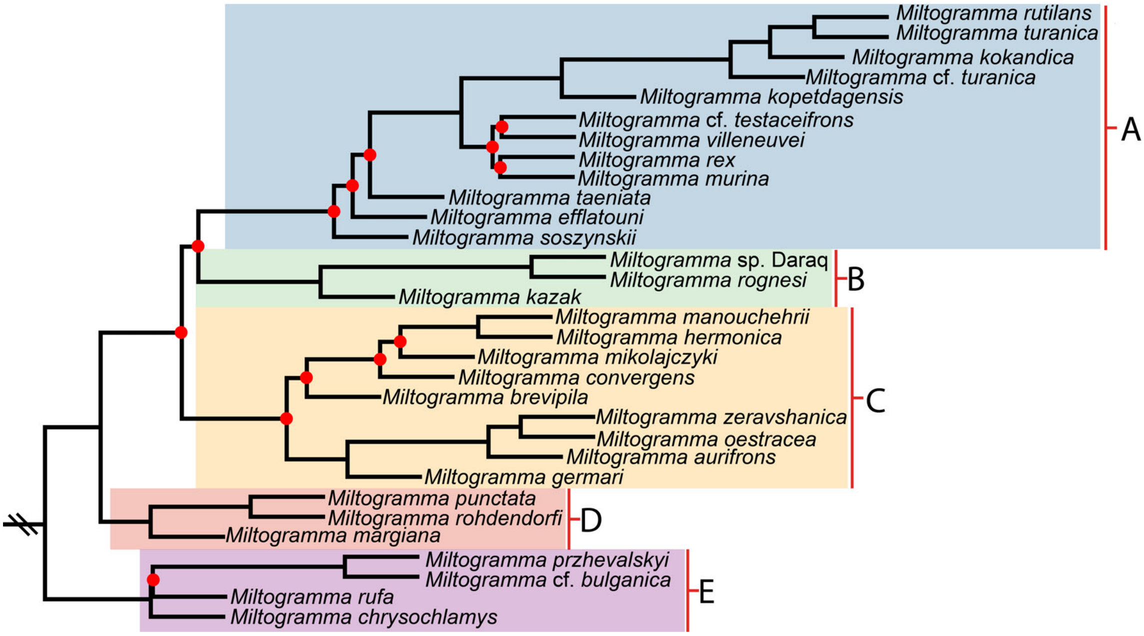

FIGURE 3 *Miltogramma* clades extracted from the maximum likelihood phylogenetic tree of the Miltogramminae estimated in IQ-tree, using GTR + R10 + F substitution model from the AHE nucleotide dataset. Red dots indicate nodes with less than 100% support (or posterior probability = 1.00) across all analyses [Taxon names follow newly proposed combinations].

was nested inside *Sphecapatodes* (*s. str.*) as sister to *Sphecapatodes kaszabi* Rohdendorf & Verves. All relationships were highly supported in all analyses.

Sphenometopa: Monophyletic, but the topology of the clade does not match the current subgeneric classification. *Sphenometopa* (subgenus *Tarsaraba* Rohdendorf) *czernyi* (Strobl) was sister to all other species. *Sphenometopa* (subgenus *Xantharaba* Rohdendorf) *steinii* (Schiner) was nested within *Sphenometopa* (subgenus *Euaraba* Townsend) as sister to a clade *Sphenometopa claripennis* (Villeneuve) + *Sphenometopa fastuosa* (Meigen). Support for nodes of this clade varied from high to low and some nodes were not resolved in SVDquartets analysis.

Taxigramma: Monophyletic and sister to the monotypic genus *Metopodia* Brauer & Bergenstamm and with a topology rejecting the alternative generic classification in three separate genera applied by, for example, Verves (1989) and Verves and Khrokalo (2018). The two clades of *Taxigramma* both included species of *Paragusia* Schiner (*Ta. elegantula* (Zetterstedt), *Ta. multipunctata* (Rondani), and *Ta. pluton* (Verves)). *Taxigramma elegantula* was sister to a clade composed of three species of *Hilarella* Rondani (*Ta. albina* (Zettterstedt), *Ta. heteroneura* (Meigen), and *Ta. stictica* (Meigen)). *Taxigramma heteroneura* was sister to *Ta. multipunctata* + *Ta. pluton*. All relationships were highly supported in all analyses.

Summary of taxonomic actions

The AHE phylogenetic hypothesis supports the monophyly of several genera: *Amobia* Robineau-Desvoidy, *Apodacra* Macquart, *Craticulina* Bezzi, *Hoplacephala* Macquart, *Macronychia* Rondani, *Phrosinella* Robineau-Desvoidy, *Phylloteles* Loew, *Protomiltogramma* Townsend, *Sphenometopa* Townsend, *Sphecapatodes* Villeneuve and *Taxigramma* Perris. The monophyly of *Metopia* Meigen, 1830 is restored by synonymy with *Aenigmetopia* Malloch, 1930, **syn.n.**, resulting in the new combinations *Me. amissa* (Johnston, Wallman, Szpila & Pape, 2020), **comb.n.**, *Me. corona* (Johnston, Wallman, Szpila & Pape, 2020), **comb.n.**, *Me. fergusoni* (Malloch, 1930), **comb.n.**, *Me. kryptos* (Johnston, Wallman, Szpila & Pape, 2020), **comb.n.**, and *Me. pagoni* (Johnston, Wallman, Szpila & Pape, 2020), **comb.n.** To achieve monophyly of *Miltogramma* Meigen, 1830 eight species are transferred from *Pterella* Robineau-Desvoidy, 1975: *Mi. convergens* Pandellé, 1895, **stat.rev.**, *Mi. chaetotarsa* (Rohdendorf & Verves, 1980), **comb.n.**, *Mi. dagestanica* (Rohdendorf, 1975), **comb.n.**, *Mi. hermonica* (Verves & Khrokalo, 2020), **comb.n.**, *Mi. penicillaris* (Rondani, 1865), **comb.n.**, *Mi. secunda* (Rohdendorf, 1975), **comb.n.**, *Mi. trichiosoma* (Rohdendorf, 1927), **comb.n.**, and *Mi. zaisanica* (Verves, 1984), **comb.n.** The genus *Pterella* is shown to be paraphyletic in its current circumscription, and to restore generic monophyly, *Pterella* is restricted to contain only *Pt. grisea* (Meigen, 1824), and *Erioprocta* Enderlein, 1936, **stat.rev.**, is resurrected to contain *Er. melanura* (Meigen, 1824), **comb.n.** and all other species formerly in *Pterella* and with '*Sturmia* spots' in the male, i.e., *Er. asiatica* (Rohdendorf & Verves, 1980), **comb.n.**, *Er. immunita* (Villeneuve, 1923), **comb.n.**, *Er. krombeini* (Verves, 1979), **comb.n.**, *Er. nigrofasciata* (Rohdendorf, 1975), **comb.n.** and *Er. vadoni* (Séguy, 1963), **comb.n.**, and probably also *Pt. abchazica* (Rohdendorf, 1975), **comb.n.**, *Pt. soror* (Rohdendorf, 1975), **comb.n.** and *Pt. zefatica* Verves & Khrokalo, 2020b, **comb.n.**, known only from females. The genus *Senotainia* Macquart, 1846 is reconstructed as paraphyletic, but no action is taken awaiting the phylogenetic position of the type species *Sen. rubriventris* Macquart, 1846. The monotypic genus *Metopodia* Brauer & Bergenstamm, 1891 is synonymised with *Taxigramma* Macquart, 1850, **syn.n.** in agreement with molecular and morphological data, as well as similarity in life history, resulting in the new combination *Ta. pilicornis* (Pandellé, 1895), **comb.n.** A new tribal classification for Miltogramminae is proposed, with six tribes in the phylogenetic topology (Eumacronychiini (Sarcotachinini (Metopiaini (Macronychiini + Taxigrammini) Miltogrammini))).

REVISED CLASSIFICATION AND NEW NOMENCLATURAL ACTS

For the sake of completeness, in this section, we place, to the best of our knowledge, all currently accepted genera of Miltogramminae into our revised tribal classification, including some genera not treated in this paper, and we make explicit all new synonyms, new combinations and revised taxon concepts. For type genera of synonymous nominal family-group and type species of genus-group names, see Sabrosky (1999) and Pape (1996), respectively. [Tribes are ordered according to their sequence on the cladogram in Figure 1.]

Eumacronychiini Townsend, 1908, 64 stat.rev.

Eumacronychiini Townsend 1908: 64. Type genus: *Eumacronychia* Townsend, 1892.

Eumacronychiinae: Verves (1998: 243).

Included genera

Eumacronychia Townsend, *Gymnoprosopa* Townsend.

Biology

Necrophagous in vertebrate carrion; reptile and turtle egg predators.

Sarcotachinini Rohdendorf, 1928 stat.rev.

Sarcotachinini Rohdendorf, 1928: 10. Type genus: *Sarcotachina* Portschinsky, 1881.

Phyllotelina Rohdendorf, 1975: 96, **syn.n.**

Phyllotelini: Rohdendorf (1937: 25), Verves (1989: 120).

Included genera

Alusomyia Villeneuve, *Ambouya* Villeneuve, *Beludzhia* Rohdendorf, *Dolichotachina* Villeneuve, *Hoplacephala* Macquart, *Khowaba* Pape, *Lamprometopia* Macquart, *Medomyia* Rohdendorf, *Mesomelena* Rondani, *Phylloteles* Loew, *Sarcotachina* Portschinsky, *Sphecapatoclea* Villeneuve, *Xiphidiella* Zumpt. Tentative: *Chivamyia* Pape, *Chorezmomyia* Rohdendorf.

Biology

Necrophagous in vertebrate carrion; reptile and turtle egg predators; orthopteran egg predators; trophic relations with termites.

Metopiaini Townsend, 1908, stat.rev.

Metopiini Townsend, 1908: 64 (priority from 1863, Argyridae Robineau-Desvoidy, see Sabrosky, 1999; spelling corrected to Metopiaini based on stem "Metopia-" ICZN 1994). Type genus: *Metopia* Meigen, 1803.

Opsidiina Rohdendorf, 1967: 66, **syn.n.**

Phrosinellina Verves, 1989: 119, **syn.n.**

Aenigmetopiini Verves, 1989: 123, **syn.n.**

Included genera

Euphyto Townsend, *Metopia* Meigen, *Opsidia* Coquillett, *Phrosinella* Robineau-Desvoidy.

Biology

Hymenopteran kleptoparasites utilising invertebrate prey of ground-nesting hosts as hole searchers; also parasitoids of adult soil-nesting bees and predators of liphistiid spiders (Pape, 1996; Schwendinger & Pape, 2000).

Macronychiini Brauer & Bergenstamm, 1889, stat.rev.

Macronychiidae Brauer & Bergenstamm 1889: 76, 117. Type genus: *Macronychia* Rondani 1859.

Macronichiini: Townsend (1931: 317).

Macronichiinae: Townsend (1935: 84).

Senotainiina Rohdendorf, 1930: 9 (priority from 1895, Megerini [sic] Lioy, see Sabrosky, 1999). Type genus: *Senotainia* Macquart, 1846.

Senotainiini: Rohdendorf (1967: 61).

Senotainiina Verves (1989: 122).

Amobiinae Townsend, 1918: 158, **syn.n.**

Amobiini: Verves (1989: 120).

Austrometopiina Rohdendorf, 1967: 61, **syn.n.** [synonymised under Amobiini by Verves (1989: 120, as Australometopiina)].

Nyctellina Rohdendorf 1967: 63, **syn.n.**

Oebaliina Rohdendorf, 1967: 66–69, **syn.n.**

Oebalinii: Verves (1989: 117).

Included genera

Amobia Robineau-Desvoidy, *Macronychia* Rondani, *Oebalia* Robineau-Desvoidy, *Senotainia* Macquart.

Biology

Hymenopteran kleptoparasites, utilising invertebrate prey of soil-nesting and twig-nesting hosts, also mud-daubers and potter wasps; satellites sensu Polidori et al. (2022); one species (*Senotainia tricuspis*) is a true parasitoid of adult social bees (Santini, 1995a, 1995b).

Taxigrammini Rohdendorf, 1967, stat.rev.

Taxigrammatina Rohdendorf 1967: 61 (mandatory change to Taxigrammina due to feminine gender of type genus). Type genus: *Taxigramma* Macquart, 1854.

Hilarellina Rohdendorf, 1967: 66, 67, **syn.n.**
Metopodiina Rohdendorf, 1967: 66, **syn.n.**
Sphenometopina Verves, 1989: 119, [as Sphenometopiina] **syn.n.**

Included genera

Sphecapatodes Villeneuve, *Sphenometopa* Townsend, *Taxigramma* Macquart.

Biology

Hymenopteran kleptoparasites usurping ground-nesting hosts and utilising stored invertebrates; stalkers and lurkers sensu Polidori et al. (2022).

Miltogrammini Lioy, 1864, stat.rev.

Miltogrammini Lioy, 1864: 59. Type genus: *Miltogramma* Meigen, 1803.

Miltogrammini: Rohdendorf (1930: 9).
Miltogrammidae: Brauer & Bergenstamm (1889: 76).
Miltogrammatinae: Enderlein (1928: 6), Rohdendorf (1928: 319).
Apodacrina Rohdendorf, 1967: 63, **syn.n.**
Craticulinina Rohdendorf, 1967: 63, **syn.n.**
Pterellina Rohdendorf, 1967: 63, **syn.n.**

Included genera

Apodacra Macquart, *Chaetapodacra* Rohdendorf, *Craticulina* Bezzi, *Eremasiomyia* Rohdendorf, *Erioprocta* Enderlein, *Miltogramma* Meigen, *Protomiltogramma* Townsend, *Pterella* Robineau-Desvoidy.

Biology

Hymenopteran kleptoparasites utilising both stored pollen (*Miltogramma*) and stored invertebrates (other genera); satellites sensu Polidori et al. (2022).

New generic synonymies

Metopia Meigen, 1803.
  Aenigmetopia Malloch, 1930, **syn.n.**
Miltogramma Meigen, 1803.
  Sogdianomyia Rohdendorf, 1927, **syn.n.**
Taxigramma Macquart, 1850.
  Metopodia Brauer & Bergenstamm, 1891, **syn.n.**

Resurrected as valid genus

Erioprocta Enderlein, 1936, **stat.rev.** (from synonymy with *Pterella* Robineau-Desvoidy, 1863).

New combinations

Erioprocta abchazica (Rohdendorf, 1975), **comb.n.** (from *Pterella* Robineau-Desvoidy, 1863; originally in *Setulia* Robineau-Desvoidy, 1863).

Erioprocta asiatica (Rohdendorf & Verves, 1980), **comb.n.** (from *Pterella* Robineau-Desvoidy, 1863).

Erioprocta immunita (Villeneuve, 1923), **comb.n.** (from *Pterella* Robineau-Desvoidy, 1863; originally in *Setulia* Robineau-Desvoidy, 1863).

Erioprocta krombeini (Verves, 1979), **comb.n.** (from *Pterella* Robineau-Desvoidy, 1863).

Erioprocta melanura (Meigen, 1824), **comb.n.** (from *Pterella* Robineau-Desvoidy, 1863; originally in *Miltogramma* Meigen, 1803).

Erioprocta nigrofasciata (Rohdendorf, 1975), **comb.n.** (from *Pterella* Robineau-Desvoidy, 1863; originally in *Setulia* Robineau-Desvoidy, 1863).

Erioprocta soror (Rohdendorf, 1975), **comb.n.** (from *Pterella* Robineau-Desvoidy, 1863; originally in *Setulia* Robineau-Desvoidy, 1863).

Erioprocta vadoni (Séguy, 1963), **comb.n.** (from *Pterella* Robineau-Desvoidy, 1863; originally in *Setulia* Robineau-Desvoidy, 1863).

Erioprocta zefatica (Verves & Khrokalo, 2020), **comb.n.** (from *Pterella* Robineau-Desvoidy, 1863).

Metopia amissa (Johnston, Wallman, Szpila & Pape 2020), **comb.n.** (from *Aenigmetopia* Malloch, 1930).

Metopia corona (Johnston, Wallman, Szpila & Pape, 2020), **comb.n.** (from *Aenigmetopia* Malloch, 1930).

Metopia fergusoni (Malloch, 1930), **comb.n.** (from *Aenigmetopia* Malloch, 1930).

Metopia kryptos (Johnston, Wallman, Szpila & Pape, 2020), **comb.n.** (from *Aenigmetopia* Malloch, 1930).

Metopia pagoni (Johnston, Wallman, Szpila & Pape, 2020), **comb.n.** (from *Aenigmetopia* Malloch, 1930).

Miltogramma chaetotarsa (Rohdendorf & Verves, 1980), **comb.n.** (from *Pterella* Robineau-Desvoidy, 1863).

Miltogramma convergens Pandellé, 1895, **comb.n.** (from *Pterella* Robineau-Desvoidy, 1863; originally in *Miltogramma* Meigen, 1803).

Miltogramma dagestanica (Rohdendorf, 1975), **comb.n.** (from *Pterella* Robineau-Desvoidy, 1863; originally in *Setulia* Robineau-Desvoidy, 1863).

Miltogramma hermonica (Verves & Khrokalo, 2020a), **comb.n.** (from *Pterella* Robineau-Desvoidy, 1863).

Miltogramma penicillaris (Rondani, 1865), **comb.n.** (from *Pterella* Robineau-Desvoidy, 1863; originally in *Sphixapata* Rondani, 1859).

Miltogramma secunda (Rohdendorf, 1975), **comb.n.** (from *Pterella* Robineau-Desvoidy, 1863; originally in *Sogdianomyia* Rohdendorf, 1927).

Miltogramma trichiosoma (Rohdendorf, 1927), **comb.n.** (from *Pterella* Robineau-Desvoidy, 1863; originally in *Sogdianomyia* Rohdendorf, 1927).

Miltogramma zaisanica (Verves, 1984), **comb.n.** (from *Pterella* Robineau-Desvoidy, 1863).

Taxigramma pilicornis (Pandellé, 1895), **comb.n.** (from *Metopodia* Brauer & Bergenstamm, originally in *Metopia* Meigen, 1803).

DISCUSSION

Improved phylogenetic hypothesis

Our phylogenomic analysis of the Miltogramminae strongly supports the monophyly and interrelationships of the main lineages comprising this diverse section of the family Sarcophagidae. Overall, our various estimates of the phylogeny (e.g., Figure 1) agree with recent molecular phylogenetic hypotheses for the Sarcophagidae (Buenaventura, 2021; Buenaventura et al., 2020; Buenaventura et al., 2021; Kutty et al., 2019; Piwczyński et al., 2017; Yan et al., 2021a) and strongly support a monophyletic Miltogramminae. We retain the biologically meaningful informal division of the subfamily into a grade of mostly necrophagous lower Miltogramminae and a clade of predominantly kleptoparasitic higher Miltogramminae that are associated with solitary aculeate hymenopterans (Buenaventura et al., 2020; Pape, 1996; Piwczyński et al., 2017). The genus *Eumacronychia* appears to be sister to all other miltogrammine genera, further supported by a plesiomorphic shape of the lunule (Pape, 1996), but the exact position of *Eumacronychia* and *Gymnoprosopa* Townsend needs further study, as morphology may indicate that these are sister taxa, with *Eumacronychia* confined to western North America and Central America and *Gymnoprosopa* confined to eastern North America (Pape, 1996). These genera are therefore crucial for understanding ancestral states and early evolutionary processes within the subfamily (Xu et al., 2018). Coalescent-based analyses, which rely on individual gene trees (ASTRAL) or single nucleotide polymorphisms across all loci (SVDquartets), do not convincingly resolve the placement of *Eumacronychia*, as the two sampled species are placed at widely distant positions on the tree. This conflict is likely the result of low coverage for *Eumacronychia*—in particular, for *Eumacronychia* 'sp 2' ('sp 1' 117 loci, 'sp 2' 18 loci), which would consequently reduce the amount of data available for gene tree reconstruction within the coalescent-based analyses (i.e., for 99/117 gene trees containing representatives of *Eumacronychia*, only a single *Eumacronychia* sp. is present). The sparse data for *Eumacronychia* and, in particular, for 'sp 2' do not have a marked effect on the concatenation-based phylogenetic hypotheses (ML and BI), which resolve *Eumacronychia* as monophyletic and as sister to all other miltogrammines. As such, our analyses corroborate *Eumacronychia* as sister to all other Miltogramminae, as also hypothesised by Piwczyński et al. (2017), Buenaventura (2021), Buenaventura et al. (2021) and pending further study of the morphologically similar *Gymnoprosopa* (Yan et al., 2021a). The composition of the subfamily Eumacronychiinae proposed by Verves (1998) was refuted by recent molecular studies, with three out of the six constituent genera resolving deeply nested within Paramacronychiinae (*Goniophyto* Townsend) or Miltogramminae (*Sarcotachina* and *Xiphidiella* Zumpt) with high support (Buenaventura et al., 2020; Piwczyński et al., 2017; Yan et al., 2021a). These placements are also supported by the morphology of both the male terminalia and the first instar larva (Buenaventura et al., 2020; Szpila, 2010). The inclusion of *Eumacronychia* in the subfamily Miltogramminae is fully justified, supported by morphology (Pape, 1996; Xu et al., 2018) as well as molecular data (Piwczyński et al., 2017, Buenaventura et al., 2020, Yan et al., 2021a, present study). Therefore, we propose to classify the genus in a separate tribe, Eumacronychiini, acknowledging its position as sister to all other miltogrammines.

Verves (1998) argued for subfamily status for the genus *Macronychia*, but our analysis resolves the genus as deeply nested within the tribe Senotainiini sensu Rohdendorf (1967), which for reasons of nomenclatural priority henceforth will be known as Macronychiini (cf. above).

Revised tribal system

Neither the phylogenetic topology nor the tribal classification presented by Verves (1989) are congruent with our preferred phylogenetic hypothesis (Figure 1, Table 1).

Verves (1989) split the Miltogramminae into two large clades, one of which was left without definition, the other differentiated by the combination of two ill-defined character states: 'short lower part of head' and a 'proboscis very short'. The length of the proboscis is known to vary considerably within almost every family of calyptrate flies in connection with adaptions to various adult food resources (Marshall, 2012), and this should also be expected in the Miltogramminae, which contain many species with nectar-feeding adults. Flower-visiting species of Miltogramminae with a long proboscis also have the head capsule elongated at the level of the oral margin to accommodate the proboscis when not in use, whereas species feeding on honeydew possess the alternate states (i.e., short proboscis and short head length at level of the oral margin; Karczewski, 1967, Spofford & Kurczewski, 1985, KS pers. obs.). Species with a short proboscis and short head length at the level of the oral margin are present in all tribes of the higher miltogrammines and are usually grouped together with taxa not showing this reduction in proboscis and head length, indicating multiple origins. In contrast to this, an enlargement of the eyes appears to correlate well with our phylogeny. Relatively small eyes can be attributed to taxa belonging to the clades Eumacronychiini and Sarcotachinini as well as some Metopiaini (Polidori et al., 2022, see also Figure S4 and Piwczyński et al., 2017: figure A.1). In comparison, the largest eyes are present in late-branching species of *Miltogramma*.

Our study resolves the tribe Macronychiini as a highly supported clade, a result confirmed by previous molecular studies (Buenaventura et al., 2020; Piwczyński et al., 2017). The tribe Amobiini was placed by Verves (1989) as sister to Phyllotelini sensu Verves (Table 1) based on the shared apomorphic position of the antennal base below the horizontal eye diameter (lower than a half of eye-height). The ambiguity of this character caused by difficulties in the interpretation of its respective states was discussed and illustrated by Szpila et al. (2020), and the relative position of the antennal base needs to be entirely redefined to be used for phylogenetic or taxonomic purposes. It should also be mentioned that Rohdendorf (1967) assigned the subtribes Amobiina and Austrometopiina (genus *Amobia* sensu Pape, 1996) to the tribe Senotainiini, which he defined by the following set of character states: (1) long claws of the male legs (longer than the length of the fifth tarsal segment), (2) long oral margin, (3) high gena, (4) relatively small eyes, and (5) short antenna. These characters (and their respective states) align well with our redefinition of the tribe Macronychiini, which apart from the genera of Rohdendorf's Senotainiini also includes *Oebalia* and *Macronychia*.

Intergeneric relationships and generic monophyly

Our ML and BI analyses of concatenated orthologs provide strong support for the monophyly of most of the established miltogrammine genera (Pape, 1996), with a few noteworthy exceptions.

Our findings corroborate the results of Johnston et al. (2020b, 2020d), where *Aenigmetopia* was nested within *Metopia*, thus rendering the latter paraphyletic. The morphological separation of *Aenigmetopia* and *Metopia* is based primarily upon the presence, in the latter genus, of a unique configuration of fronto-orbital setae with two proclinate and two reclinate setae arranged in separate, partly overlapping rows. This setal configuration has generally been considered an autapomorphic feature of *Metopia*, and its absence in all *Aenigmetopia* is best interpreted as a reversal to the plesiomorphic condition. Restoring the monophyly of *Metopia* requires either a resurrection of *Chaetanicia* (type-species *Ch. sauteri* Townsend, 1933) to accommodate also *Me. nudibasis* (Malloch, 1930) or, as proposed here, broadening its definition by synonymising *Aenigmetopia* with *Metopia*.

The large genus *Miltogramma* emerged as non-monophyletic because of the nested position of two species currently assigned to *Pterella* (Figure 1). *Miltogramma* in the broad sense of Pape (1996) is defined largely by the reduced size of the vibrissa, which is usually of similar length or only slightly longer than the adjacent setae. The species *Pt. convergens* and *Pt. hermonica* possess a vibrissa with a length intermediate between 'typical' *Miltogramma* and 'typical' *Pterella* (i.e., *P. grisea*). A somewhat elongated vibrissa is also observed in *Mi. brevipila*, *Mi. mikolajczyki* and *Mi. manouchehrii* (Piwczyński et al., 2017: figure A.1, Szpila et al. 2023), which form a clade with *Pt. convergens* and *Pt. hermonica*. As such, the current position of these last species within *Miltogramma* requires just a single evolutionary change with regard to vibrissal length. Larval morphology further corroborates the evidence from molecular data and adult morphology in that the first instar of *Pt. convergens* is almost identical to that of *Mi. brevipila* (distinguished only through the length of the anterior part of the labrum, see Szpila, 2010), and it consequently shares several derived character states with *Miltogramma* spp. that are absent from both *Erioprocta* and *Pterella* as redefined here. The same result was obtained for *Pt. convergens* in the molecular phylogenies of Piwczyński et al. (2017) and Buenaventura et al. (2020), although a new combination was not formally proposed in either of those works. A modified chaetotaxy of the male fore tarsus is widely distributed in *Miltogramma* (e.g., Povolný & Verves 1997, Zhang et al., 2015, Piwczyński et al., 2017, Figure S4), and we propose to restore the monophyly of *Miltogramma* by transferring all species of *Pterella* with modified chaetotaxy on the male foretarsus to *Miltogramma*. Apart from *Pt. convergens* and *Pt. hermonica*, this includes *Pt. chaetotarsa* (Rohdendorf & Verves), *Pt. dagestanica* (Rohdendorf), *Pt. penicillaris* (Rondani), *Pt. secunda* Rohdendorf, *Pt. trichiosoma* Rohdendorf, and *Pt. zaisanica* Verves.

The morphology-based definition of *Pterella* given by Pape (1996) and Pape and Szpila (2012) conflicts with the present phylogeny. The species formally included in that genus can be divided into three groups based on sexually dimorphic characters. The first group contains 10 species with modified chaetotaxy on the male foretarsus, which are here transferred to *Miltogramma* (see preceding paragraph). The second group have males with patches of short, black, decumbent bristles laterally on abdominal tergite V ('*Sturmia* spots' sensu Cerretti et al., 2015) and includes *Pt. asiatica* Rohdendorf & Verves, *Pt. immunita* (Villeneuve), *Pt. krombeini* Verves, *Pt. melanura* (Meigen), *Pt. nigrofasciata* (Rohdendorf), and *Pt. vadoni* (Séguy), and probably also *Pt. abchazica* (Rohdendorf), *Pt. soror* (Rohdendorf), and *Pt. zefatica* Verves & Khrokalo, which are known only from females. The two species of *Pterella* with '*Sturmia* spots' included in the present analysis (*Pt. asiatica* and *Pt. melanura*) emerged as sister to the clade *Eremasiomyia* + *Protomiltogramma* in the present analysis. The third group contains *Pterella grisea*, which presents only the sexual dimorphism generally found in the higher miltogrammines (i.e., males with somewhat brighter and more contrastive colours and larger upper eye facets), came out as sister to this clade (Figure 1). To restore monophyletic genera, we propose to transfer all species of *Pterella* with '*Sturmia* spots' in the male to a revalidated *Erioprocta* Enderlein (type species: *Miltogramma melanura* Meigen). This leaves the generic name *Pterella* Robineau-Desvoidy to contain only *Pt. grisea* (Meigen).

Senotainia was non-monophyletic in the phylogenies of Piwczyński et al. (2017) and Buenaventura et al. (2020), where it was divided by *Amobia* into two clades. Our analysis, based on a larger taxon sample, put the two groups of *Senotainia* even farther apart. Analyses of male terminalia and first instar larval morphology appear to support this result, with *Sen. conica* separated from the three other species of the genus included here. However, with no molecular data for the type species, the Nearctic *Sen. rubriventris* Macquart, or for any other Nearctic representatives, the classification and nomenclature of the assemblage of species currently placed in the genus *Senotainia* must be left unsettled.

Adult morphology has historically been considered to support a sister-group relationship between *Sphecapatodes* and *Sphecapatoclea* (Pape, 1996; Verves, 1989), but this conflicts with both larval morphology (Szpila et al., 2017) and molecular data (Piwczyński et al., 2017). The phylogenetic hypothesis presented in this study also rejects the sister-group relationship between *Sphecapatodes* and *Sphecapatoclea* and instead places *Sphecapatoclea* at the base of the Sarcotachinini and *Sphecapatodes* within the Taxigrammini as the sister to *Sphenometopa*. Szpila et al. (2017) noted substantial modifications to the larval morphology of *Sphecapatodes* and suggested the clade *Metopodia* + *Taxigramma* as its sister group, based on shared character states in the larval morphology: (1) simple tip of mouthhook, (2) abdominal segments with fine cuticular ridges only, (3) dorso-lateral surfaces of thoracic and abdominal segments with horizontal rows of spines, (4) reduction of anterior spinose band on anal division, (5) anal tuft with only few spines, and (6) large and conical anal papillae with long apical sensillum.

Miltogrammine life history evolution

Eumacronychia is the earliest branching genus of the Miltogramminae in our phylogenetic hypothesis and therefore provides insight into the possible ancestral life history of all miltogrammines. In their review of the biology of this genus, Xu et al. (2018) argued that its species are generally necrophagous, with females targeting buried vertebrate carrion in sandy areas by larvipositing on the ground above the carrion and the larvae digging down to the food source. Some species have also been recorded as facultative predators of reptile eggs and hatchlings (Andrade et al., 1992; Gámez-Vivaldo et al., 2006; López Barbosa, 1989), and combined with the widespread necrophagy in the Sarcotachinini (Rohdendorf & Verves 1980, McGowan et al., 2001, Szpila et al., 2010), it is considered likely that the ancestral feeding biology of all miltogrammines was some form of necrophagy (Piwczyński et al., 2017; Yan et al., 2021a). An interesting match is seen in the phylogeny of Paramacronychiinae, where Pape (1998) placed the monotypic genus *Galopagomyia* Bischof, known to have been bred from "rotting tortoise eggs" (Lopes 1978), in a dichotomy with the remaining subfamily, although with very weak support. The kleptoparasitism exhibited by most of the higher miltogrammines generally involves larval feeding on food stored by the host for its own progeny, which can include buried/hidden arthropods immobilised by the hymenopteran hosts or pollen.

Within Macronychiini, species of *Senotainia* are predominantly kleptoparasites of ground-nesting digger wasps like most other higher miltogrammines (Spofford & Spofford & Kurczewski, 1990, Povolný & Verves 1997, Verves & Khrokalo 2021), but life habits unique among the Miltogramminae have evolved several times within this tribe. Larvae of *Amobia* develop especially in nests of solitary wasps constructed of small stones and clay, like potter wasps (Hymenoptera: Eumeninae) and mud dauber wasps (Hymenoptera: Sceliphroninae) (Verves et al., 2020a, Verves & Protsenko, 2019). Some species of *Senotainia* (e.g., *Sen. puncticornis* (Zetterstedt), *Sen. trilineata* (Wulp)), a large subset of species of *Macronychia*, and all species of *Oebalia* with known life habits infest nests of aculeate wasps constructed in hollow, dry twigs (Parker & Bohart, 1966, 1968; Rohdendorf 1975, Verves & Khrokalo, 2006). One species of *Oebalia* is known to glue eggs with mature first instar larvae directly to the body of crabronids (Hymenoptera: Crabronidae) (Sanborne 1982). Very few miltogrammines appear to be true parasitoids, but an unidentified Nearctic species of *Macronychia* was stated to be bred from adult tabanids (Thompson, 1978a, 1978b; note that this is in need of confirmation as a search by TP for vouchers in Texas A&M University and the National Museum of Natural History, Washington DC, was unsuccessful). *Senotainia tricuspis* is a well-known parasitoid of adult honey and bumble bees (Hymenoptera: Anthophila) (Haddad et al., 2015; Santini, 1995a, 1995b), but there is only one additional record that could be interpreted as parasitoidism in the Miltogramminae: the generically unplaced '*Chauliooestrus*' *leza* Pape, 1991, which according to label data was bred from adult termites (Pape, 1991; holotype and a female paratype labelled "parasitising *Hodotermes mossambicus* dealates").

From this perspective, the evolution of life histories in the Miltogramminae may have followed a progression from necrophagy in buried vertebrate carrion, as observed in the early-branching *Eumacronychia* spp., to predation of immobilised arthropods deposited in burrows of solitary wasps, and from this to either true insect parasitoidism, like *Senotainia tricuspis* (Meigen) (Piwczyński et al., 2017), or to palynivory, where the hosts are solitary bees, as seen in the recently-branching tribe Miltogrammini. However, this hypothesis of a simple evolutionary shift of life histories in the Miltogramminae, from broad necrophagy to kleptoparasitism, is complicated by the phylogenetic position of the genus *Sphecapatoclea*. This genus is the sister to all other species of Sarcotachinini in both our study and that of Yan et al. (2021a). The life history of this genus is unknown. Szpila et al. (2017) unsuccessfully tried to rear first instar larvae of *Sphecapatoclea* sp. on vertebrate remains under laboratory conditions. The shape of the cephaloskeleton without a dorsal bridge and the tip of labrum bent at a right angle (Szpila et al., 2020) differs from all other Sarcotachinini. The diversity and life histories of the Sarcotachinini remain poorly known in general; among the few available studies are reports of trophic relations with termites, orthopteran oothecae, buried eggs of reptiles and turtles, and vertebrate carrion (Pape, 1996, Szpila et al., 2010, Verves et al., 2020b). [Termite-associated species assigned to *Senotainia* by Zumpt, 1961 appear to belong to the Sarcotachinini (Szpila, Pape, pers. obs.).] There are a few breeding reports for Sarcotachinini (genus *Hoplacephala*), which suggest kleptoparasitism in the nests of digger wasps (Verves et al., 2020b), but these need confirmation.

AUTHOR CONTRIBUTIONS

Nikolas P. Johnston: Conceptualization; investigation; writing – original draft; methodology; validation; visualization; writing – review and editing; software; formal analysis; project administration; data curation. **Thomas Pape:** Conceptualization; funding acquisition; writing – original draft; investigation; methodology; supervision; validation; writing – review and editing; formal analysis; project administration; resources. **Marcin Piwczyński:** Methodology; supervision; data curation; software; formal analysis; funding acquisition; investigation; writing – original draft; conceptualization; writing – review and editing; resources. **James F. Wallman:** Conceptualization; investigation; supervision; validation; writing – review and editing; writing – original draft; methodology; project administration; funding acquisition; resources. **Brian M. Wiegmann:** Investigation; writing – review and editing; methodology; software; formal analysis; supervision; project administration; resources. **Brian K. Cassel:** Investigation; methodology; software; formal analysis; project administration; supervision; resources. **Kamran Akbarzadeh:** Conceptualization; methodology; data curation; resources; investigation. **Krzysztof Szpila:** Conceptualization; investigation; methodology; validation; formal analysis; data curation; supervision; project administration; resources; writing – review and editing; funding acquisition; writing – original draft; visualization.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the Supporting Information of this article, except for molecular data, which are stored on GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and available using the accession numbers provided in the Supporting Information. Other data including Tree files and multiple sequence alignments are available on the online repository FigShare under the following DOI: 10.6084/m9.figshare.22640845.

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SUPPORTING INFORMATION

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

Figure S1. Coalescent-based phylogenetic tree of the Miltogramminae estimated in SVDquartets from the AHE nucleotide dataset.

Figure S2. Maximum likelihood phylogenetic tree of the Miltogramminae estimated in RAxML using GTR + I + G substitution model from the AHE nucleotide dataset.

Figure S3. Bayesian phylogenetic topology of the Miltogramminae estimated in ExaBayes using GTR + I + G substitution model from the AHE nucleotide dataset.

Figure S4. Key traits essential for the identification of the analysed species of Miltogramminae. For each included taxon, indicated by the taxon name in the rightmost panel, images are provided for: (a) habitus, lateral view, (b) head, lateral view, and (c) abdomen. [Taxon names follow newly proposed combinations.]

Table S1. Specimen data and voucher information.

Table S2. *Miltogramma* clades in the context of former classifications.

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