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Systematics of Afrotropical Eristalinae (Diptera: Syrphidae) using mitochondrial phylogenomics

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Abstract

We examined the phylogeny and intrageneric classification of eristaline hoverfly genera from the Afrotropical Region using mitochondrial genomes. Genome skimming was used to obtain (nearly) full mtDNA and nuclear rDNA (18S, 28S) genomes of 120 museum vouchers from eight genera and 98 species. Phylogenetic reconstructions of mitogenomes and mitogenomes + nuclear rDNA yielded comparable phylogenies while that of rDNA only resulted in poorly resolved phylogenies. Phylogenetic analyses focused on six genera and supported the monophyly of the genera *Chasmomma* Bezzi, *Eristalinus* Rondani, *Mesembrius* Rondani and *Syritta* Le Peletier & Serville, whereas *Simoides* Loew was not monophyletic and rendered *Phytomia* Guérin-Méneville paraphyletic. We therefore synonymize *Simoides* with *Phytomia*. Within *Chasmomma*, two species-groups that differ in the colour and the shape of the hind femora (*Chasmomma femoratum* and *Chasmomma nigrum* species-groups) were supported. Within *Eristalinus*, the monophyly of the subgenera *Merodonoides* Curran and *Eristalodes* Mik was supported, but not of the subgenus *Eristalinus* Rondani. Within *Syritta*, the monophyly of three out of the five species-groups tested was rejected. This approach illustrates the importance of integrative and iterative approaches in taxonomy and shows that genomic data may not only clarify the systematic relationships among hoverfly genera and species, but also offer perspectives into the evolution of morphological and ecological variation within the family.

KEYWORDS
Afrotropics, flower fly, genome skimming, hoverfly, Syrphidae, systematics

INTRODUCTION

Hoverflies or flower flies (Syrphidae, Figure 1) constitute a large family of Diptera with approximately 6200 species worldwide (Pape & Evenhuis, 2019). The systematic relationships within this family are actively debated due to taxon ranking instability and discrepancies between morphology-based classifications and molecular phylogenies (Mengual et al., 2015; Moran et al., 2021; Young et al., 2016). Until recently, four subfamilies were recognized within Syrphidae: Eristalinae (with nine tribes), Microdontinae (two tribes), Pipizinae and Syrphinae (five tribes). Based on adult morphology, F. C. Thompson (1969) was the first to suggest that Microdontinae are sister to all other Syrphidae, and was later supported in molecular studies (Mengual et al., 2015; Reemer & Ståhls, 2013; Skevington & Yeates, 2000; Ståhls et al., 2003). Young et al. (2016), used anchored hybrid enrichment (AHE) data to corroborate these results and showed that the subfamilies Syrphinae and Pipizinae are monophyletic sister taxa. In addition, they demonstrated that the Eristalinae are paraphyletic. Moran et al. (2021) examined the phylogeny of Eristalinae using a Sanger approach to sequence one mitochondrial and seven nuclear genes, therein confirming the paraphyly of Eristalinae while providing strong support for Cerioidini, Merodontini

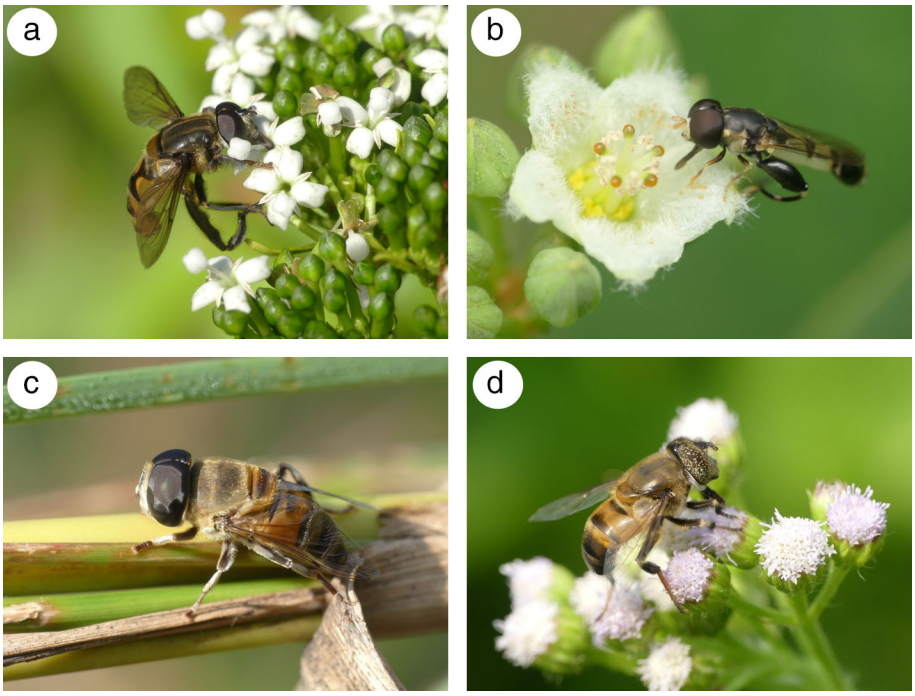

FIGURE 1 Photographs of living Afrotropical hoverflies: (a) *Mesembrius tarsatus* (Bigot, 1883) (Uganda, Mabamba swamps), (b) *Syritta longiseta* Lyneborg & Barkemeyer, 2005 (Uganda, Mabamba swamps), (c) *Phytomia incisa* (Wiedemann, 1830) (Uganda, Mabamba swamps), (d) *Eristalinus* sp. 2 (Uganda, Kibale forest). Photographs © M. Reeme

and Volucellini as subfamilies. This study resulted in a profound generic rearrangement of the subtribe Criorhinina. In contrast, intrageneric relationships within Eristalinae are mostly based on morphology, and studies that have applied molecular phylogenetic tools were restricted to the use of very few gene fragments (De Meyer et al., 2020b; Nedeljković et al., 2013; Pérez-Bañón et al., 2013). Large-scale multigene phylogenetic approaches have not been applied in this group, and intrageneric relationships for these genera should therefore be considered as merely indicative. This also holds for the taxonomic revisions of Eristalinae of the Afrotropical Region, including revisions of *Ceriana* Rafinesque (F. C. Thompson, 2013), *Chasmomma* Bezzi (Kassebeer, 2000), *Eristalinus* Rondani (subgenus *Merodonoides* Curran) (F. C. Thompson, 2019), *Megatrigon* Johnson (Doczkal et al., 2016), (part of) *Merodon* Meigen (Radenković et al., 2011), *Mesembrius* Rondani (Jordaens et al., 2021), *Phytomia* Guérin-Méneville (De Meyer et al., 2020b), *Senaspis* Macquart (De Meyer et al., 2020a) and *Syritta* Le Peletier & Serville (Lyneborg & Barkemeyer, 2005).

Sonet et al. (2019) used genome skimming to obtain full mitochondrial DNA sequences (mitogenomes) of five Afrotropical *Eristalinus* species. Despite studies like this one reporting a growing number of full mtDNA sequences of Diptera, genome skimming has never been applied in Diptera to evaluate its use in mitochondrial phylogenomics at the intrageneric level. This prompted us to further explore the use of mitogenomes for resolving phylogenetic relationships within several hoverfly genera from the Afrotropics. Genome skimming (Crampton-Platt et al., 2016; Straub et al., 2012) is a cost-effective next-generation sequencing technique to assemble full mitochondrial genomes (Le & Gang, 2020; Pu et al., 2017; Yan et al., 2020). Genome skimming has not been used previously in large-scale intrageneric phylogenetic studies in the order Diptera. In support of ongoing taxonomic revisions of Afrotropical Eristalinae, we used genome skimming to explore the intrageneric phylogenetic relationships in selected genera from the region.

The endemic Afrotropical genus *Chasmomma* has only been revised based on morphological characteristics with the distinction of two species-groups (*Chasmomma femoratum* species-group: two species; *Chasmomma nigrum* species-group: three species) that principally differ in the colour and shape of the metafemora (Kassebeer, 2000). No phylogeny has been published on the phylogenetic relationships of the species.

The recent morphological revision of the Afrotropical representatives of the subgenus *Mesembrius* recognized 23 species within the subgenus including six new species (Jordaens et al., 2021). A phylogenetic reconstruction using the mitochondrial cytochrome oxidase subunit I (COXI) barcoding data showed strong support for a species-group with strong sexual dimorphism (12 species) but no support for a species-group with weak sexual dimorphism (11 species). Moreover, only three sister-species relationships (of the 18 species included) were supported.

A recent morphological revision of the Afrotropical representatives of the genus *Phytomia* (De Meyer et al., 2020b) recognized 19 species (including three new species) and the definition of four putative species-groups, though several species remained unplaced (e.g., *Phytomia bullata*, *Phytomia bulligera*, *Phytomia natalensis* and

Phytomia melas species-groups). *Phytomia* is closely related to *Simoides* Loew, which currently comprises six described and two undescribed species (Ssymank et al., 2021). Unambiguous characters to fully separate both sexes of all species are absent, and members of the *P. melas* species-group show a number of transitional characteristics between *Phytomia* and *Simoides* (De Meyer et al., 2020b). Phylogenetic analysis of the COXI barcode region resulted in a mostly unsupported topology and no support for the reciprocal monophyly of *Phytomia* and *Simoides*.

In a revision of *Syritta*, Lyneborg and Barkemeyer (2005) recognized 57 species in 16 species-groups worldwide, with 13 of these species-groups including at least one of the 40 Afrotropical species. However, the phylogenetic relationships within and among species-groups remain largely unknown. The Old-World genus *Eristalinus* Rondani, comprising ca. 75 species, is traditionally divided into five subgenera: *Eristalinus* s.s. with 39 species in the Afrotropics, *Eristalodes* Mik, with 12 Afrotropical species, and three endemic Afrotropical subgenera, *Helophilina* Becker including only a species, *Merodonoides* Curran with seven species, and *Oreristalis* Séguy with four species (Ssymank et al., 2021). Only the taxonomy of the subgenus *Merodonoides* has recently been investigated on morphological grounds (F. C. Thompson, 2019), but subgeneric phylogenetic relationships remain unknown.

The general objective of this study is to provide a well-resolved backbone phylogeny, based on mitogenomes and rDNA, to help clarifying the systematic relationships among, and within, the presumed species-groups of the six above-mentioned Afrotropical Eristalinae genera.

MATERIALS AND METHODS

Taxon sampling

The phylogenies presented in this study are based on 132 Syrphidae mitogenomes (and 18S and 28S rRNA), of which 120 were newly assembled in this study (Table 1). Of all vouchers, 124 were collected from 12 African countries between 2010 and 2019 using a range of methods including Malaise trapping, sweep netting and hand netting. *Microdon* sp. (from Europe and stored at the Zoologisches Forschungsmuseum Alexander Koenig – ZFMK, Bonn, Germany) was used as outgroup to root the trees since Microdontinae is the sister group to the rest of the family (Moran et al., 2021; Young et al., 2016). Afrotropical species were identified by Kurt Jordaens and Marc de Meyer using existing identification keys (provided upon request) and species descriptions and preserved in absolute ethanol at −20°C until DNA extraction. Two mitogenomes of *Eristalis* Latreille (this study) and two Eristalinae and three Syrphinae mitogenomes from the NCBI database were also included as outgroups (Table 2). Overall, the taxon sampling included 74 recognized and 17 undescribed species belonging to seven genera (an overview of voucher collection and classification is provided in Table 1). Vouchers are deposited in the Entomology Collections of the Royal Museum for Central Africa (RMCA, Tervuren, Belgium) (except for *Microdon* sp. that is stored at the ZFMK). Since the RMCA has a strong interest in the Afrotropical Region, the focus in this study is on the Afrotropical species of the genera. Yet, except for *Chasmomma* and *Simoides*, the genera comprise species from other biogeographical regions, especially the Oriental Region, but the species diversity (and thus their presumed origin) is highest in the Afrotropical Region (Ssymank et al., 2021; see also Lyneborg & Barkemeyer, 2005 for *Syritta*). Even though the inclusion of species from other biogeographical regions is required to depict the full picture on the origin and biogeography of the genera, it is unlikely that they will change the phylogenetic relationships among the Afrotropical representatives.

Genome skimming and assemblage

Samples were subjected to low coverage whole-genome sequencing ('genome skimming') following Gillett et al. (2014) and Timmermans et al. (2016) in order to assemble the mitochondrial genome (mitogenome) and two rDNA loci, 18S and 28S. DNA was extracted using a DNeasy Blood and Tissue kit (Qiagen Inc., Hilden, Germany). During genomic library preparation, specimens were either individually indexed or, following Crampton-Platt et al. (2016), pooled by two (to reduce costs), with species pairs chosen to maximize genetic divergence and reduce the risk of chimeric mitogenomic assemblages. For pooled specimens, the two mitogenomes were resolved on the basis of the similarity between the COXI barcode fragment and the Barcode of Life Data System public repository of COXI DNA barcodes. In total, 12 samples were pooled (SI 2). For each sample (both individual and pooled), 100 ng of genomic DNA was normalized in 50 μl ultrapure water. The samples were sonicated to a fragment size of approx. 350 base pairs (bp) using a Covaris S220 Focused-ultrasonicator with Covaris microTUBES. Specimen libraries were prepared with the Illumina Truseq Nano library preparation kit. Total genomic libraries were sequenced on an Illumina HiSeq4000 platform, and the resulting 300 bp paired-end reads were processed using the seed-and-extend method NOVOPlasty (Dierckxsens et al., 2017). Reference DNA fragments (seeds) were taken from the five *Eristalinus* mitogenomes published by Sonet et al. (2019). When NOVOPlasty failed to recover a complete circular mitogenome, SPAdes or a combination of both de novo and/or reference mapping (the latter using the de novo assembly plugin of Geneious and the software BBMap [Bushnell, 2014]) was used to recover the mitochondrial contigs. The rDNA genes 18S and 28S rRNA were recovered using SPAdes (Bankevich et al., 2012) from all individually indexed specimens. Pooled samples were excluded from this analysis to avoid possible artefactual gene assemblages, and because association between nuclear data and the mitogenome was not possible in absence of public reference sequences of 18S and 28S for these species. As in Sonet et al. (2019), a draft annotation of the mitogenomes was obtained using the default settings of MITOS (Bernt et al., 2013), after which annotations were manually checked following the criteria described in Cameron (2014). Both rDNA genes were annotated using RNAmmer (Lagesen et al., 2007).

TABLE 1 Overview of the 132 samples included in this study

Subfamily	Tribe	Subtribe	Genus	No. putative species	No. vouchers
Eristalinae	Eristalini	Eristalina	*Eristalinus*	27	39
			Eristalis	2	3
			Phytomia	10	10
			Simoides	2	2
		Helophilina	*Chasmomma*	6	6
			Helophilus	1	1
			Mesembrius	13	17
	Milesiini	Temnostomina	*Korinchia*	1	1
		Tropidina	*Syritta*	28	48
	Volucellini		*Volucella*	1	1
Microdontinae			*Microdon*	1	1
Syrphinae	Syrphini		*Simosyrphus*	1	1
	Syrphini		*Episyrphus*	1	1
	Syrphini		*Eupeodes*	1	1

Note: Number of putative species in each of the taxonomic groups is shown, as well as the number of vouchers for each group.

Phylogenetic inference

Phylogenetic tree reconstructions were based on 97 complete, circular, mitochondrial genomes (12 retrieved from the NCBI database; 85 from this study), 35 incomplete, linear mitochondrial assemblages and 106 18S-28S rDNA assemblages. Tree reconstructions were obtained for three alignments: (a) mitochondrial data only (i.e., concatenated protein-coding genes [PCGs], mitochondrial rRNA's and tRNA's), (b) nuclear data only (i.e., concatenated 18S and 28S rDNA), and (c) concatenated mitochondrial and nuclear data. Alignments were obtained with the Clustal W (J. D. Thompson et al., 1994) plugin of Geneious Prime 2019.2.3 (https://www.geneious.com) using default parameters. Phylogenetic relationships were evaluated in a Bayesian inference (BI) and maximum likelihood (ML) framework. For BI, PartitionFinder v.2.1.1. (Lanfear et al., 2012) was used to identify the optimal evolutionary model per gene partition (linked branch lengths and greedy search). All Bayesian tree reconstructions were obtained using MrBayes XSEDE v.3.2.6 and relied on the General Time Reversible model (Tavaré, 1986) with an estimated proportion of invariant sites and gamma distributed among-site variation (GTR + I + G) as indicated by PartitionFinder. Starting trees for each chain were random and the default values of MrBayes were chosen for all settings, including prior distributions. MrBayes metropolis coupled Markov Chain Monte Carlo were run for 30 million generations (until the average standard deviation of split frequencies fell below 0.01) with a heating temperature of 0.1. Trees were sampled every 1000 generations, with the first 50% of these discarded as burn-in. ML analysis used RAxML BlackBox (Stamatakis, 2014) for the tree reconstruction, implementing RAxML-HPC v.8 with 1000 bootstraps. Both Bayesian and ML analyses were executed on the CIPRES Science Gateway v.3.3 portal (https://www.phylo.org) (Miller et al., 2010). Consensus trees were visualized in FigTree v.1.4.3 (Rambaut, 2016). Tree nodes with Bayesian posterior probabilities (PP) > 0.95 and bootstrap support >70% were considered as supported.

RESULTS

Genome skimming of the 120 samples processed in this study provided 85 complete, circular, mitochondrial genomes (including the D-loop) (range: 15,274–17,023 bp) and 35 almost-complete linear mitochondrial assemblages (range: 14,517–16,353 bp) (because of variations in the D-loop [AT-rich region] these mitogenomes could not be circulized unambiguously). We obtained 106 nuclear assemblages including 18S rRNA (1273–1990 bp) and/or 28S rRNA (1660–7579 bp) gene fragments (SI 2). Consistent with what has been reported for Syrphidae (Sonet et al., 2019), all mitogenomes included 13 protein-coding genes (PCGs), 2 rRNA (12S, 16S rRNA) and 22 tRNA genes. The order of genes was identical in all samples and start and stop codons were consistent with those reported for invertebrates in general (Cameron, 2014) and Syrphidae in particular (Sonet et al., 2019), except for *Microdon* sp. (AB49479912), which lacked a stop codon for NAD1. Tree topologies provided by different partition schemes (concatenated mitochondrial + nuclear, mitochondrial only, nuclear only) were largely congruent (Table 2) and differences are explained in detail per genus below. The concatenated mitochondrial + nuclear data provided the strongest phylogenetic signal with 88.4% of the nodes supported (i.e., with PP ≥0.95 and >70% bootstrap support [BS]). Yet, trees recovered from the analysis of the mitochondrial dataset only (SI 3) also provided strongly supported topologies (i.e., 85.3% of the nodes supported). Trees recovered from the shorter, and less polymorphic, nuclear data showed fewer (59.3%) supported nodes. Yet, supported nodes were largely in line with those from the mitochondrial and mitochondrial + nuclear phylogenies (Table 2,

TABLE 2 Overview of monophyletic groups recovered in maximum likelihood (ML) and Bayesian (BI) tree reconstructions based on differently concatenated mitochondrial and nuclear loci

		Total evidence (mt + nuclear loci) ML	Total evidence (mt + nuclear loci) BI	mt loci ML	mt loci ML	Nuclear loci ML	Nuclear loci BI
Subfamily	Eristalinae	R	R	R	n/a	n/a	n/a
	Syrphinae	S	S	S	S	n/a	n/a
Tribe	Eristalini	S	S	S	S	S	S
	Syrphini	S	S	S	S	n/a	n/a
	Milesiini	R	R	R	n/a	n/a	n/a
Genus	*Syritta*	S	S	S	S	S	S
	Mesembrius	S	S	S	S	S	S
	Eristalis	n/a	n/a	n/a	n/a	n/a	n/a
	Chasmomma	S	S	S	S	n/a	n/a
	Phytomia	R	R	R	R	n/a	n/a
	Simoides	R	R	R	R	n/a	n/a
	Eristalinus	S	S	S	S	S	S
Eristalinus subgenera	*Merodonoides*	S	S	S	S	S	S
	Eristalinus	R	R	R	R	n/a	n/a
	Eristalodes	S	S	S	S	n/a	n/a
Syritta species-groups	*Syritta pipiens*	S	S	S	S	n/a	n/a
	Syritta nigrifemorata	R	R	R	R	n/a	R
	Syritta lanipes	R	R	S	S	R	n/a
	Syritta bulbus	R	R	R	R	n/a	n/a
	Syritta hirta	R	R	R	R	R	n/a
	Syritta flaviventris	S	S	S	S	S	S
Chasmomma species-groups	*Chasmomma femoratum*	S	S	S	S	n/a	n/a
	Chasmomma nigrum	n/a	S	n/a	S	n/a	n/a
Phytomia species-group	*Phytomia bulligera*	n/a	R	R	R	S	S
	Phytomia natalensis	S	S	S	S	n/a	n/a

Note: Species-groups are defined by previous revisions (see Introduction).
Abbreviations: n/a, not applicable due to insufficient taxon sampling or node support; R, rejected; S, monophyly supported.

Figures 2–4, SI 3). A comparison of tree topologies and node support obtained from the different datasets schemes is provided in Tables 2, SI 3 and SI 4.

Higher phylogenetic relationships

The subfamily Eristalinae was recovered as paraphyletic, with the monophyletic Syrphinae nested within Eristalinae. The tribe Milesiini was not monophyletic, with *Syritta* sister to the tribe Eristalini, while *Korinchia angustiabdomena* (Huo, Ren & Zheng) was sister to Syrphinae+(*Syritta* + Eristalini). Our analyses supported the monophyly of the five focal genera *Syritta*, *Chasmomma*, *Eristalinus*, *Mesembrius* and *Phytomia* (inclusive of *Simoides*) (see Table 2 and below). Within the tribe Eristalini, *Helophilus virgatus* Coquillett and *Mesembrius* were sister to the other Eristalini but there was neither support for the monophyly of the subtribe Helophilina (i.e., *Helophilus* + *Mesembrius* + *Chasmomma*), nor the subtribe Eristalina (i.e., *Eristalis* + *Eristalinus* + *Phytomia* + *Simoides*). Phylogenetic relationships between genera also remained largely unresolved (Figure 5, Table 2, SI 3).

Intrageneric phylogenetic relationships

Chasmomma is divided into two clades, one including the two species of the *C. femoratum* species-group, *Chasmomma laterale* Curran and *C. femoratum* Bezzi, and one including the species of the *C. nigrum*

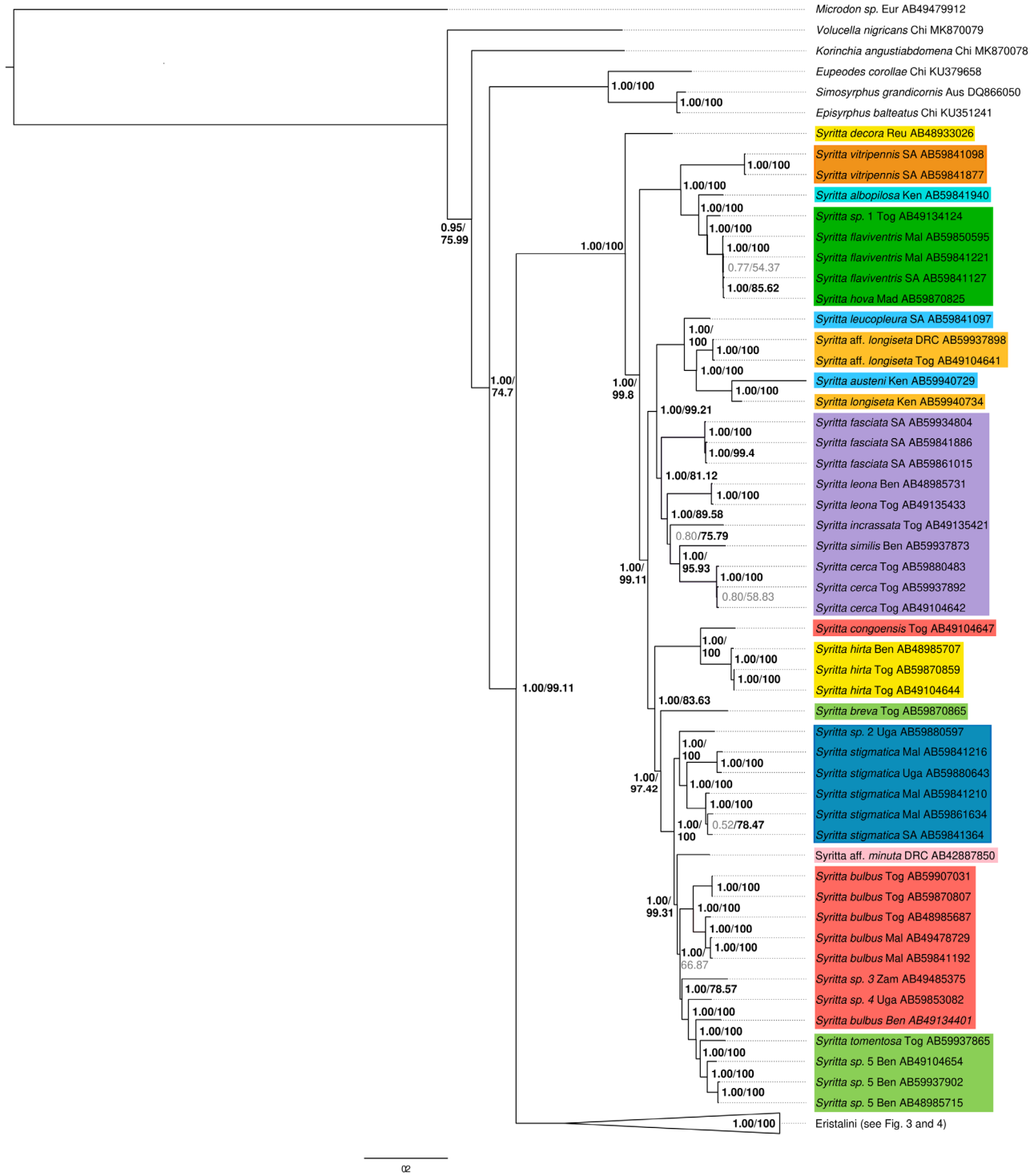

FIGURE 2 Detailed view of phylogenetic relationships within the genus *Syritta* based on the analyses of concatenated mitochondrial (13 PCGs, 22 tRNAs, 2 rRNAs) and nuclear (18S and 28S rRNA) data. Node labelling as in Figure 5. Species-groups as defined by Lyneborg and Barkemeyer (2005) are indicated in the following colours: *Syritta bulbus* = red, *Syritta divergata* = pink, *Syritta flaviventris* = dark green, *Syritta hirta* = yellow, *Syritta lanipes* = light orange, *Syritta nigrifemorata* = light blue, *Syritta oceanica* = cyan, *Syritta pipiens* = purple, *Syritta stigmatica* = dark blue, *Syritta tomentosa* = light green, *Syritta vitripennis* = dark orange

species-group, *Chasmomma albitarsis* Kassebeer, *Chasmomma minutum* Kassebeer, *C. nigrum* Kassebeer and an undescribed *Chasmomma* species. Yet, the latter group was only supported in the Bayesian analysis (Figure 3).

Representatives of the genus *Eristalinus* were resolved in two main clades, the first including species of the subgenus *Merodonoides*: *Eristalinus gymnops* (Bezzi), *Emegametapodus megametapodus* Thompson, and *Emegametapodus myiatropinus* (Speiser), the second including all remaining

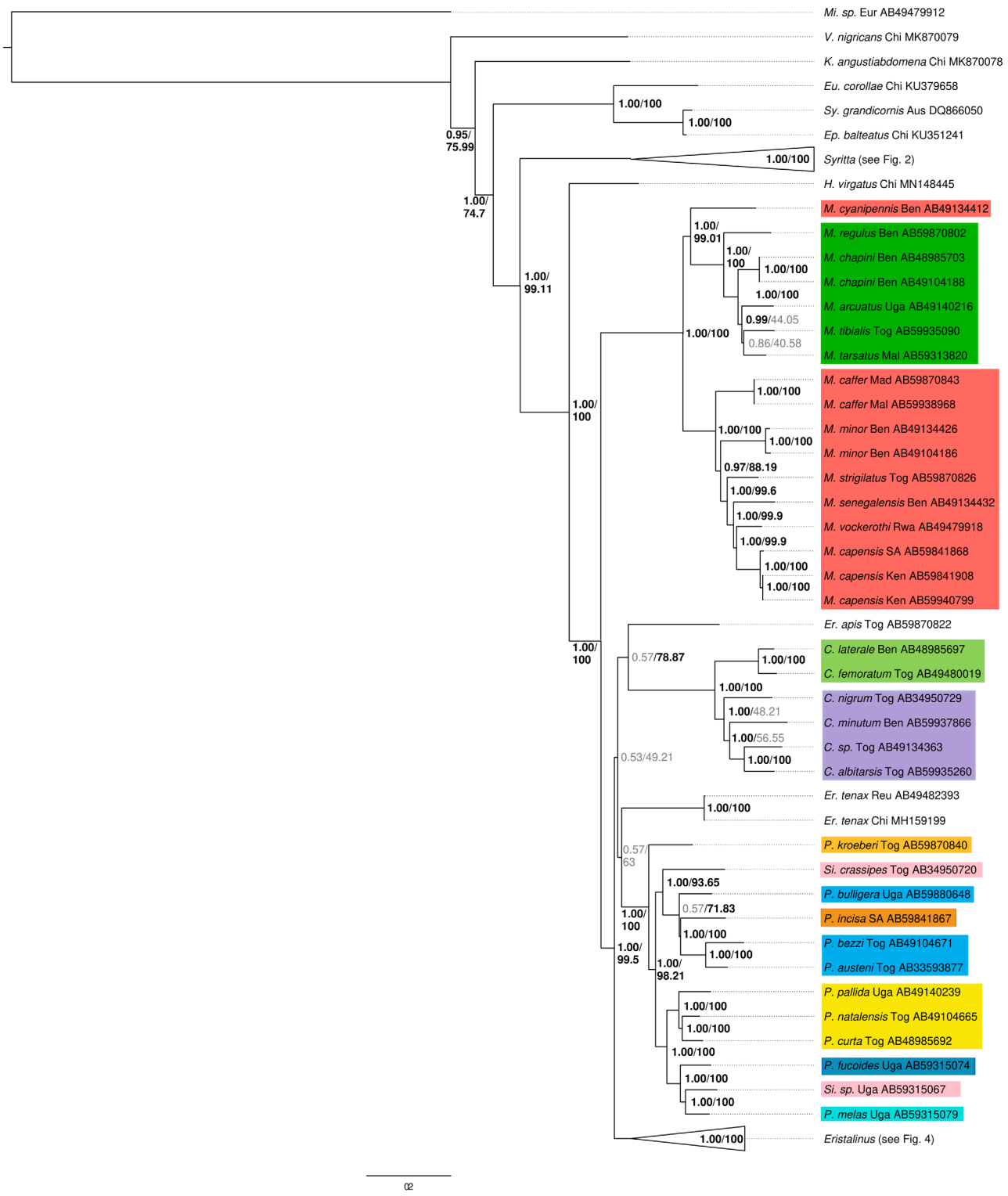

FIGURE 3 Detailed view of phylogenetic relationships within the genera *Mesembrius*, *Chasmomma*, *Eristalis*, *Phytomia* and *Simoides* based on the analyses of concatenated mitochondrial (13 PCGs, 22 tRNAs, 2 rRNAs) and nuclear (18S and 28S rRNA) data. Node labelling as in Figure 5. Species-groups as defined by earlier revisions (see text) are indicated in the following colours: genus *Mesembrius*: species-group with strong sexual dimorphism = dark green, species-group with weak sexual dimorphism = red; genus *Chasmomma*: *Chasmomma femoratum* species-group = light green, *Chasmomma nigrum* species-group = purple; genus *Phytomia*: *Phytomia bullata* species-group = light orange, *Phytomia bulligera* species-group = light blue, *Phytomia natalensis* species-group = yellow, *Phytomia melas* species-group = cyan, genus *Simoides* = pink, unplaced *Phytomia incisa* = dark orange, unplaced *Phytomia fucoides* = dark blue

FIGURE 4 Detailed view of phylogenetic relationships within the genera *Eristalinus* based on the analyses of concatenated mitochondrial (13 PCGs, 22 tRNAs, 2 rRNAs) and nuclear (18S and 28S rRNA) data. Node labelling as in Figure 5. Subgenera as defined by Ssymank et al. are indicated in the following colours: *Merodonoides* = blue, *Eristalinus* = green, *Oreristalis* = orange, *Helophilina* = yellow, *Eristalodes* = red

species (Figure 4). Within this latter clade, *Eristalinus eclarus* (Curran) (subgenus *Eristalinus* s.s.), *Eristalinus aeneus* (Scopoli) (subgenus *Oreristalis*), *Eristalinus smaragdinus* (subgenus *Helophilina*) and *Eristalinus vicarians* (Bezzi) (subgenus *Eristalinus* s.s.) were recovered in more basal positions. The remaining species were divided into two sister clades including seven species of the subgenus *Eristalinus* s.s.: *Eristalinus lineifacies* (Curran), *Eristalinus* aff. *cupreus*, *Eristalinus euzonus* (Loew), *Eristalinus* aff. *euzonus* and two putative new species and 12 species, respectively. In this latter clade,

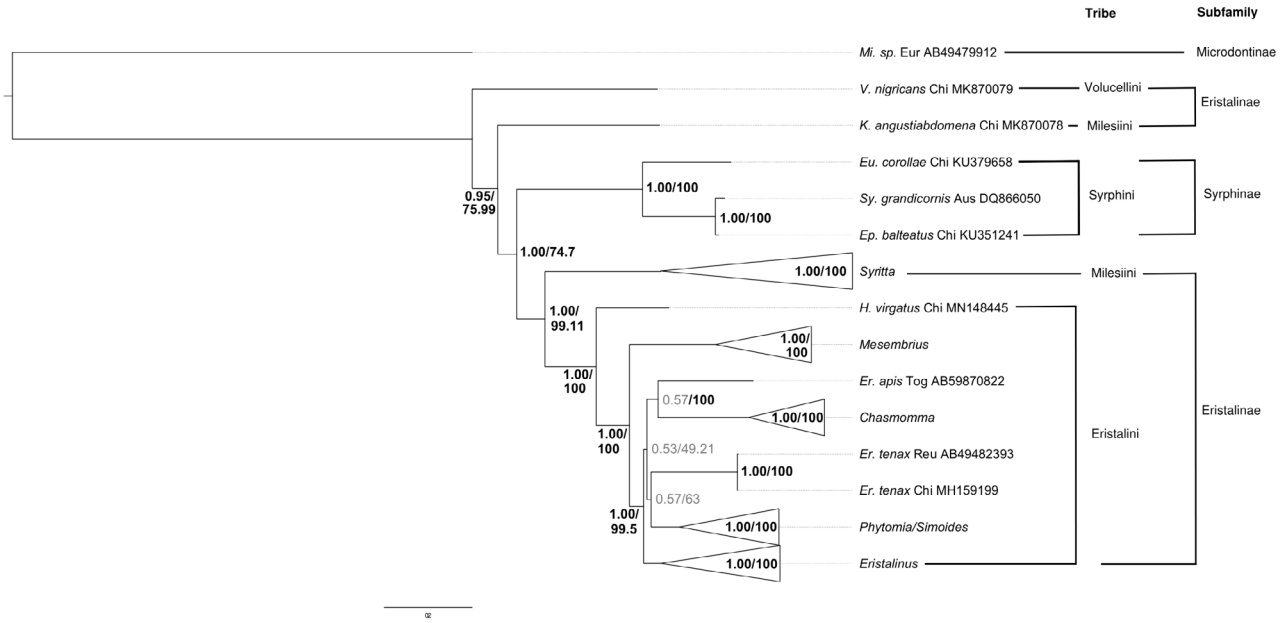

FIGURE 5 Bayesian inference tree with posterior probabilities (PP; in bold when >0.95) and bootstrap support (BS; in bold when >70%) values of the maximum likelihood method are given at nodes as PP/BS (there were no topological differences between both methods): overview of the main clades based on the analyses of concatenated mitochondrial (13 PCGs, 22 tRNAs, 2 rRNAs) and nuclear (18S and 28S rRNA) data. Tribes and subfamilies are indicated with brackets. See Figures 2–4 for detailed node support information

E. vicarians (Bezzi) was sister to the rest of the group comprising the species of the subgenus *Eristalodes* (*Eristalodes quinquelineatus* (Fabricius), *Eristalodes barclayi* Bezzi, *Eristalodes fuscicornis* Karsch, *Eristalodes taeniops* (Wiedemann), an unidentified species and a putative new species), sister to *Eristalodes flaveolus* (Bigot) + *Eristalodes tabanoides* (Jeannicke) and rendered the subgenus *Eristalinus* s.s. paraphyletic. For the subgenus *Eristalodes*, we observed differences in the phylogenies based on different datasets. In the phylogeny of the combined data, the monophyly of the subgenus was supported, with *E. taeniops* versus the remainder of the species as most basal split, while the mtDNA partition showed a polytomy, and monophyly for the subgenus was not supported with the nuclear partition.

The genus *Mesembrius* (Figure 3) consisted of two main clades, one including six species, five of which belonged to the species-group with strong sexual dimorphism (*Mesembrius arcuatus* Jordaens, Goergen & De Meyer, *Mesembrius chapini* Curran, *Mesembrius tibialis* Jordaens, Goergen & De Meyer, *Mesembrius tarsatus* (Bigot), and *Mesembrius regulus* (Hull), with the latter in a basal positions) and *Mesembrius cyanipennis* (Bezzi) with the latter species sister to the rest, the other clade including six species of the species-group with weak sexual dimorphism (*Mesembrius caffer* [Loew], *Mesembrius capensis* [Macquart], *Mesembrius minor* [Bezzi] *Mesembrius senegalensis* [Macquart], *Mesembrius strigatus* [Bezzi] and *Mesembrius vockerothi* Jordaens, Goergen & De Meyer), with the latter sister to the rest. The phylogeny based on the mitogenomes resulted in two polytomies, one with *M. arcuatus* + *M. tarsatus* + *M. tibialis*, one with *M. minor* + *M. caffer* + the monophyletic clade of *M. minor* + *M. caffer* + *M. vockerothi* + *M. senegalensis*. The phylogeny based on nuclear data placed *M. cyanipennis* sister to all other *Mesembrius*, yet, also resulted in a clade with the strong sexually dimorphic species and a clade with the weak sexually dimorphic species excluding *M. cyanipennis*.

The genera *Phytomia* and *Simoides* (Figure 3) were not recovered as reciprocally monophyletic but formed a clade sister to *Phytomia kroeberi* (Bezzi). The remaining species were divided into two clades: one included five species of the genus *Phytomia* (*P. fucoides* Bezzi, *P. melas* Bezzi, and the monophyletic *P. natalensis* species-group of *Phytomia curta* (Loew) + *P. natalensis* (Macquart) + *Phytomia pallida* De Meyer, Goergen & Jordaens) and an undescribed species of the genus *Simoides*. The other clade included *Simoides crassipes* (Fabricius) and four species of the genus *Phytomia*, the latter in which *Phytomia incisa* (Wiedemann) rendered the three species of the *P. bulligera* species-group (*P. bulligera* [Austen], *Phytomia austeni* De Meyer, Goergen & Jordaens, *Phytomia bezzii* Curran) paraphyletic. The phylogeny based on nuclear data resulted in a trichotomy of *P. kroeberi* with the two other clades.

Syritta (Figure 2) consisted of *Syritta decora* Walker (*Syritta hirta* species-group) sister to the rest of the group divided into three main clades. The first included five species of three species-groups (*Syritta flaviventris* species-group: *S. flaviventris* Macquart, *Syritta hova* Lyneborg & Barkemeyer and an undescribed species *Syritta* sp. 7; *Syritta oceanica* species-group: *Syritta albopilosa* Lyneborg & Barkemeyer; *Syritta vitripennis* species-group: *S. vitripennis* Bigot). The second clade included two clades with *Syritta leucopleura* Bigot and *Syritta austeni* Bezzi from the *S. austeni* species-group, and *Syritta longiseta* Lyneborg & Barkemeyer and *Syritta* aff. *longiseta* from the *Syritta lanipes* species-group on the one hand and the species of the *Syritta pipiens* species-group (*Syritta fasciata* (Wiedemann), *Syritta*

leona Lyneborg & Barkemeyer, *Syritta incrassata* Lyneborg & Barkemeyer, *Syritta similis* Lyneborg & Barkemeyer, *Syritta cerca* Lyneborg & Barkemeyer) on the other. By contrast, *S. longiseta* from Kenya (AB59940734) and the two *S.* aff. *longiseta* from the Democratic Republic of the Congo (AB59937898) and Togo (AB49104641) (*S. lanipes* species-group) formed a monophyletic group only when considering the mitochondrial dataset (SI 3), while their monophyly was not supported in both the nuclear and the concatenated mitochondrial and nuclear datasets. The third main clade included a clade with *Syritta congoensis* Lyneborg & Barkemeyer (*Syritta bulbus* species-group) and *S. hirta* Curran (*S. hirta* species-group) sister to a clade with 10 species (*Syritta tomentosa* species-group: *Syritta breva* Lyneborg & Barkemeyer, *S. tomentosa* Lyneborg & Barkemeyer and an undescribed species; *S. bulbus* species-group: *S. bulbus* Walker at two positions in the clade and three undescribed species; *Syritta stigmatica* species-group: *S. stigmatica* Loew and an undescribed species; *Syritta divergata* species-group: *Syritta minuta* Lyneborg & Barkemeyer). Both *S. stigmatica* and *S. bulbus* showed substantial intraspecific variation. In *S. stigmatica* there was substantial variation with an average intraspecific p-distance of 0.04 (range: 0.001–0.057) and within *S. bulbus* an average p-distance of 0.02% (range: 0.014–0.058). These values were considerably higher than intraspecific p-distances in other *Syritta* species (average p-distances = 0.003–0.025).

DISCUSSION

Higher phylogenetic relationships

Based on the sampling of tribes and genera included here, we confirm Eristalinae as a non-monophyletic group (see also, e.g., Mengual et al., 2015; Moran et al., 2021; Young et al., 2016). The monophyly of Milesiini is also rejected, similar to Mengual et al. (2015). Still, our results indicate that genome-skimmed mitogenomes are more suited for understanding intrageneric species relationships rather than higher-level phylogeny in Syrphidae. In combination with recently developed nuclear markers (Moran et al., 2021), full mtDNA data will allow testing of current hypotheses on the subgeneric division and factors that mediate speciation processes such as biogeographical processes, vicariance, hybridization and introgression, among others.

Intrageneric relationships in the genus *Chasmomma*

Chasmomma is an endemic Afrotropical genus with five described and one undescribed species, which can be divided into two species-groups: the *C. femoratum* species-group with two species (*C. femoratum* and *C. laterale*) with a relatively slender metafemur and yellow legs, except for the black-and-yellow metafemur, and the *C. nigrum* species-group with four species (*C. nigrum*, *C. minutum*, *C. albitarsis*, and the undescribed species) with a thickened metafemur and entirely black legs (Kassebeer, 2000). Our phylogenetic analyses showed strong support for the *C. femoratum* species-group, and thus a sister-species relationship between *C. femoratum* and *C. laterale*. The monophyly of the *C. nigrum* species-group was supported in the Bayesian inference (suggesting the following relationship: (*nigrum* + (*minutum* + [*albitarsis* + *C.* sp.])) but not in the ML analysis. Within the *C. nigrum* species-group, the undescribed species was sister to *C. albitarsis* (Figure 3).

Intrageneric relationships in the genus *Eristalinus*

Our phylogenetic analysis revealed new insights in the subgeneric classification of the genus *Eristalinus*. The subgenus *Merodonoides* (with three out of seven Afrotropical species included), was recovered as monophyletic and sister to the remaining subgenera. *E. eclarus* (subgenus *Eristalinus*) was sister to the remaining *Eristalinus* and was not recovered with other species of the subgenus. Moreover, the subgenus *Eristalodes* (with seven species out of 12 Afrotropical species included) is monophyletic and rendered the subgenus *Eristalinus* (with only 11 out of 39 Afrotropical species included) as paraphyletic. Some species, such as *E. vicarians*, showed substantial differentiation in the mtDNA between specimens from West Africa (Togo, Benin) and East Africa (Malawi) (p-distance = 0.0297–0.0305), which hints at phylogeographic structuring. Other species show low differentiation in their mtDNA and nuclear rDNA (e.g., *E. tabanoides* vs. *Eristalodes* aff. *tabanoides*; *E.* aff. *euzonus* and *Eristalodes andersoni*) and this may suggest relatively recent divergences or that the morphological differences are merely intraspecific variation.

The phylogenetic analysis of this genus requires a more substantial sampling but sets a basis for taxonomic revisions of the subgenera. Phylogenetic trees based on mtDNA and nuclear rDNA were sometimes conflicting, suggesting that the relationship among some species might be affected by incomplete lineage sorting or introgression.

Intrageneric relationships in the genus *Mesembrius*

The Afrotropical representatives of the genus *Mesembrius* (Figure 3) are divided into two subgenera, *Mesembrius* s.s. and *Vadonimyia* Séguy. Eleven out of twenty-three species currently recognized in the subgenus *Mesembrius* s.s. were included in this study. The subgenus was recently revised by Jordaens et al. and a preliminary phylogenetic analysis of COXI barcode data resulted in a poorly resolved topology with only three sister-species relationships supported. As expected, the genome-skimmed phylogeny provided much higher node support and resulted in a fully resolved phylogeny. As observed by Jordaens et al. (in press), species with strong sexual dimorphism and little differentiation in male genitalia (i.e., *M. arcuatus*, *M. chapini*, *M. regulus*, *M. tarsatus* and *M. tibialis*) were recovered as a monophyletic group. In contrast though, species showing weak sexual dimorphism and with strongly differentiated male genitalia were also resolved as a monophyletic group. Depending on the phylogenetic analysis, *M. cyanipennis* (a species with weak sexual dimorphism) was sister to the clade of species with strong sexual dimorphism or to the

remainder of *Mesembrius*. These results suggest that strong sexual dimorphism has evolved only once during the radiation of African *Mesembrius*. Additionally, *M. caffer* and *M. minor*, two of the most widespread *Mesembrius* species, were sister to a clade of species with weak sexual dimorphism. Within the clade of strong sexually dimorphic species, *M. regulus* was sister to all other species within the clade. It will be important to include non-Afrotropical species in the phylogenetic analysis. Likewise, the analysis of the species of the subgenus *Vadonimyia*, where males have extraordinarily large terminalia, may shed light on the evolution of this enigmatic Malagasy subgenus, while the phylogenetic analysis of AHE data may shed light on the relationships between *Mesembrius* and putative related genera such as *Helophilus* Meigen.

Intrageneric relationships in the genera *Phytomia* and *Simoides*

We included 14 of the 19 Afrotropical *Phytomia* species and provide further evidence of paraphyly of *Phytomia* by *Simoides*. Indeed, *S. crassipes* was sister to the clade of species of the *P. bulligera* species-group, including *P. incisa*, while an undescribed *Simoides* species was sister to *P. melas* (*P. melas* species-group) and part of a clade with *P. fucoides* (unplaced) and species of the *P. natalensis* species-group. It is therefore very unlikely that adding non-Afrotropical *Phytomia* species will change the status of *Phytomia* relative to *Simoides*. Moreover, the morphological differences between these two genera are subtle, such that it is not possible to unambiguously separate both sexes of all species. De Meyer et al. (2020a, 2020b) showed that eye morphology of males from *P. melas* and *Phytomia poensis* (i.e., the *P. melas* species-group) was slightly deviant from other *Phytomia* species, possibly reflecting transitional changes between the holoptic *Phytomia* and the dichoptic *Simoides*. As suggested by De Meyer et al. (2020b) on the basis of preliminary tree reconstructions, *Phytomia* and *Simoides* cannot be maintained as separate entities and that the morphological differences between both genera (i.e., holoptic eyes in male *Phytomia*, dichoptic eyes in male *Simoides*) at least have evolved twice. We therefore synonymize *Simoides* with *Phytomia*.

Analysis of the mitogenomic data here provided additional information on the systematic relationships within *Phytomia* and suggests that *P. kroeberi* (the only species of the *P. bullata* species-group included) occupies a relatively basal position in the genus. Species from the *P. bullata* species-group (*Phytomia aurigera*, *P. bullata*, *Phytomia serena* and *P. kroeberi*) can be easily differentiated from the other *Phytomia* species on the basis of their well-demarcated wing markings, the presence of a weak or marked protuberance on the metafemur and the well-developed bullae on abdominal tergites II–IV. However, analysis of the three other species is needed to corroborate that these morphological character states represent synapomorphies. Our phylogeny provided strong support for the *P. natalensis* species-group (*P. natalensis*, *P. curta* and *P. pallida*) and shows that the scutum having the anterior third covered by a yellow to yellow-grey pilosity and pollinosity, strongly contrasting with the black posterior part and hyaline wing represent synapomorphies. Monophyly of the *P. bulligera* species-group (*P. bulligera*, *P. austeni*, *P. bezzii* analysed here) is supported given that *P. incisa* (unplaced in De Meyer et al., 2020b) is included, although morphological synapomorphies for this group are unknown. The phylogeny also confirmed the sister-species relationship between *P. bezzii* and *P. austeni*. Similar to *Mesembrius*, *Phytomia* comprises a number of Asian species as well, and analysis including these species will undoubtedly provide an even better insight into taxonomic relationships among all the species.

Intrageneric relationships in the genus *Syritta*

Our phylogeny of the genus *Syritta* adds new insights into the morphology-based subdivision in species-groups proposed by Lyneborg and Barkemeyer (2005). It will provide a useful backbone for future morphological revisions of the Afrotropical *Syritta* species. These results support the monophyly of the *S. flaviventris* species-group (although the non-Afrotropical species *Syritta caboverdensis* Lyneborg & Barkemeyer was not studied) and of the *S. pipiens* species-group (five out of eight species sampled, i.e., the Afrotropical species *Syritta dentata* Lyneborg & Barkemeyer and the non-Afrotropical species *S. pipiens* [Linnaeus] and *Syritta stylata* Lyneborg & Barkemeyer were not included). However, the monophyly of the *S. bulbus* (two out of five species included), *S. hirta* (two out of four species included) and *Syritta nigrifemorata* species-groups (two out of six species included) were not supported, suggesting that the morphological synapomorphies for these groups proposed by Lyneborg and Barkemeyer (2005) are not justified.

One of the most interesting results is that all included species of the *S. vitripennis*, *S. oceanica* and *S. flaviventris* species-groups formed a single clade, suggesting that the secondary reduction (and loss) of the spurious vein may be a synapomorphy for these three species-groups and that it has evolved only once rather than three times independently as suggested by Lyneborg and Barkemeyer (2005).

The remainder of *Syritta* comprised two sister clades, one clade included species from both the *S. lanipes* (one out of three Afrotropical species included) and *S. nigrifemorata* species-groups (two out of six Afrotropical species included). Lack of reciprocal monophyly suggests that the modified metafemur in species of the *S. nigrifemorata* species-group might not be a synapomorphy. However, the *S. nigrifemorata* species-group was strongly supported by the mtDNA, and thus different datasets showed different phylogenies. More comprehensive taxon and data sampling will help to clarify these patterns and to better evaluate the possible role of incomplete lineage sorting or secondary introgression. The other clade comprised species of the *S. pipiens* species-group, a group that seems to be characterized by three synapomorphies related to characters of the male genitalia, and by the more strongly sclerotinized sterna II–IV in the female compared with other *Syritta* (see Lyneborg & Barkemeyer, 2005).

A large clade recovered here includes members of the five species-groups, *S. bulbus*, *S. hirta*, *S. tomentosa*, *S. stigmatica* and *S. divergata* as well as three undescribed species. The species of the *S.*

tomentosa species-group were not monophyletic since *S. breva* was basal in the clade while *S. tomentosa* and an undescribed species rendered the *S. bulbus* species-group as paraphyletic. Lyneborg and Barkemeyer (2005) already doubted the monophyly of the species-group even though they did not provide arguments. *S. stigmatica* (the only species in the *S. stigmatica* species-group) and *S. bulbus* show substantial intraspecific differentiation and are subdivided into several distinct clades that could not be directly related to geography. Interestingly, *S. decora* (of the *S. hirta* species-group) was not recovered with *S. hirta* but was recovered as sister to all other *Syritta*. While Lyneborg and Barkemeyer (2005) pointed out that the male terminalia morphology and pleural patterns of *S. decora* are markedly different compared with the other representatives of this species-group, they did not consider the species as sister to the rest of the genus. Conversely, representatives of the *S. divergata*, *S. bulbus*, *S. tomentosa* and *S. stigmatica* species-groups, which were considered as a basal group by Lyneborg and Barkemeyer (2005) on the basis of morphological characters and geographic distributions, were recovered in relatively derived positions.

As for the genus *Mesembrius*, the inclusion of non-Afrotropical *Syritta* species in the phylogenetic analyses is required to obtain a complete picture of species-groups and their intrageneric relationships.

CONCLUSIONS

We show that the phylogenetic analysis of (nearly) full mtDNA and nuclear rDNA sequences obtained through genome skimming is a powerful tool to resolve intrageneric phylogenetic species relationships in Diptera. These results will provide useful background information to be used in the framework of the ongoing morphological revisions of the hoverfly subfamily Eristalinae. Additionally, these results offer perspectives into the evolution of morphological and ecological variation within these genera, while population genomic approaches using full mtDNA in combination with recently published nuclear markers (Moran et al., 2021) may provide insight into the population genetic (hybridization, introgression) and phylogeographic processes that affect speciation in Afrotropical Syrphidae.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare that are relevant to the content of this article.

AUTHOR CONTRIBUTIONS

Conceptualization: Nele Mullens, Kurt Jordaens, Massimiliano Virgilio, Gontran Sonet. *Data analysis*: Nele Mullens, Gontran Sonet. *Writing of the original draft*: Nele Mullens, Kurt Jordaens. *Data interpretation, reviewing and editing*: Nele Mullens, Gontran Sonet, Massimiliano Virgilio, Georg Goergen, Steven B. Janssens, Marc de Meyer, Kurt Jordaens.

DATA AVAILABILITY STATEMENT

The genetic data produced in this paper are available on GenBank® (www.ncbi.nlm.nih.gov/genbank/). Accession numbers are available as supplementary material.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

Data S1. SI 1: Overview of all 132 hoverfly individuals used in this study with indication (whenever possible) of collecting date and locality. GenBank nos. of samples taken from the NCBI database are shown in bold and with an asterisk.

SI 2: Mitogenome, 18S rRNA and 28S rRNA length (bp = base pairs) obtained through genome skimming of the 132 Syrphidae vouchers considered in this study. Circular mitochondrial genomes are indicated with an asterisk. NA = not applicable.

SI 3: Bayesian Inference (with PP and ML bootstrap values [BT] at the nodes as PP/BT) phylogenetic tree of the mitochondrial partition (13 PCG's, 2 rRNA's and 22 tRNA's).

SI 4: Bayesian Inference (with PP and ML bootstrap values [BT] at the nodes as PP/BT) phylogenety of the nuclear data (18S and 28S rRNA genes). All nodes with posterior probability >0.95 and bootstrap >70% are considered as supported.

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