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A multigene phylogeny of the eristaline flower flies (Diptera: Syrphidae), with emphasis on the subtribe Criorhinina

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We present the first multigene phylogeny focused on Eristalinae (Diptera: Syrphidae) utilizing a dataset containing 120 flower fly species from across all four subfamilies and representing 13 out of 16 tribes. Eight genes were used in the construction of the phylogeny: mitochondrial cytochrome *c* oxidase subunit I and the nuclear genes 28S ribosomal DNA, Alanylt RNA Synthetase, the carbamoyl phosphate synthase domain of CAD, Period, RNA-binding Protein 15 (*RBP*–15, 5'), Casein Kinase 1 and TULP for a total of ~6.7 kB of data. Eristalinae is recovered as paraphyletic with strong support for the elevation of Cerioidini, Merodontini and Volucellini to subfamilial status. *Deineches*, *Flukea* and *Malometasternum* render Criorhinina paraphyletic with respect to the type genus *Criorhina*. A clade with *Criorhina*, *Matsumyia* and *Sphecomyia* is strongly supported. The generic concept of *Criorhina* is paraphyletic, while *Sphecomyia* is monophyletic and *Matsumyia* is monophyletic but requires expansion. Evidence supports the resurrection of *Romaleosyrphus* and the creation of new genera. **Criorhinina** (**stat. rev.**) is restricted to contain *Criorhina*, *Matsumyia*, *Romaleosyrphus* and *Sphecomyia*. Thirteen changes to the higher classification of Syrphidae are proposed.

ADDITIONAL KEYWORDS: classification – *Criorhina* – Eristalinae – hoverflies.

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INTRODUCTION

Syrphidae is a species-rich and charismatic family of Diptera with over 200 genera and 6200 described species worldwide (Pape & Evenhuis, 2019). Driven in part by mimicry, Syrphidae are incredibly varied in appearance. Some genera, like *Sphegina* Meigen, 1822, are small and slender, resembling sphecid wasps. Others are large, hairy bumblebee mimics, such as *Mallota* Meigen, 1822 or *Criorhina* Meigen, 1822, and there are colourful mimics of vespine wasps like *Spilomyia* Meigen, 1803 or *Sphecomyia* Latreille, 1829 (Waldbauer, 1970; Barendregt *et al.*, 2000; Hippa *et al.*, 2015; Moran & Skevington, 2019). Most syrphid flies, with the exception of some ant inquiline Microdontinae, are commonly encountered visiting flowers where they feed on pollen and nectar. Males are often seen hovering over flowers, or other strategic places, waiting for females to mate with (Skevington *et al.*, 2019). These behaviours have led members of this family to be commonly referred to as hoverflies or flower flies.

Historically, the family Syrphidae has been divided into as few as two (Goffe, 1952; Wirth *et al.*, 1965; Vockeroth, 1969) or as many as 21 subfamilies (Shiraki, 1949). In most contemporary literature, a three-subfamily classification of Eristalinae, Microdontinae and Syrphinae has been accepted (Vockeroth & Thompson, 1987; Vockeroth, 1992; Thompson & Rotheray, 1998). Placement of Pipizinae as sister to Syrphinae (Skevington & Yeates, 2000; Ståhls *et al.*, 2003), or at least as related to it (Cheng, 2000; Mengual 2008), has gained increasing support in recent years, culminating in Mengual (2015) who elevated Pipizini to the subfamily Pipizinae. This elevation and placement was corroborated by Young *et al.* (2016) and Pauli *et al.* (2018).

Monophyly of Eristalinae is supported by several studies and the current classification follows this line of reasoning (Goffe, 1952; Hartley, 1961; Vockeroth, 1992; Thompson & Rotheray, 1998). Despite this, molecular data have repeatedly suggested that monophyly of Eristalinae is not supported (Skevington & Yeates, 2000; Ståhls *et al.*, 2003; Mengual *et al.*, 2015; Young *et al.*, 2016; Pauli *et al.*, 2018).

Traditionally, flower flies are further grouped into 16 tribes within these four subfamilies (Thompson, 1972; Vockeroth, 1992; Mengual *et al.*, 2015; Mengual, 2020): Brachyopini, Callicerini, Cerioidini, Eristalini, Merodontini, Milesiini, Rhingiini, Sericomyiini and Volucellini in Eristalinae; Microdontini and Spheginobacchini in Microdontinae; and Bacchini, Melanostomini, Paragini, Syrphini and Toxomerini in Syrphinae. Pipizinae has no tribal division. These tribal divisions have never been rigorously tested for the entire family and many tribes were not supported by phylogenetic studies using morphological or molecular characters (Hippa & Ståhls, 2005; Mengual *et al.*, 2015; Young *et al.*, 2016).

The present study is part of a long-term plan for a study of the family. This part focuses on the subtribe Criorhinina Williston, 1886. Criorhinina is currently placed within the tribe Milesiini of the subfamily Eristalinae (Thompson, 1972; Hippa, 1978; Rotheray & Gilbert, 1999; Hippa & Ståhls, 2005). Adults are found in a variety of forested habitats (Reemer, 1999; Bartsch *et al.*, 2009; Skevington *et al.*, 2019), while larvae dwell in moist cavities of mature hardwood trees or among rotten tree roots (Rotheray & Gilbert, 2011; Speight, 2020).

Criorhinina currently contains seven genera: *Criorhina*, *Deineches* Walker, 1852, *Flukea* Etcheverry, 1966, *Lycastris* Walker, 1857, *Malometasternum* Shannon, 1927, *Matsumyia* Shiraki, 1949 and *Sphecomyia* (Thompson, 1972, 1975; Hippa & Thompson, 1983; Vockeroth & Thompson, 1989; Pape & Evenhuis, 2019). Although never included in a published manuscript, the genus *Pseudopocota* Mutin & Barkalov, 1995 is informally considered a member of Criorhinina (Pape & Evenhuis, 2019).

As a unit of higher organization, Criorhinina first appeared as the tribe Criorhinini in Williston's (1886) higher classification of Syrphidae. Williston included the genera *Brachypalpus* Macquart, 1834, *Caliprobola* Rondani, 1845, *Crioprora* Osten Sacken, 1878, the type genus *Criorhina*, *Ferdinandea* Rondani, 1844 (as *Chrysoclamys* Rondani, 1856; names of genera and higher levels as mentioned in the publication are indicated in parenthesis), *Merapioidus* Bigot, 1882 and *Pocota* Le Peletier & Audinet-Serville, 1828 in his concept. Also included was the genus *Aneriophora* Stuardo & Cortes 1952, but as a synonym of *Criorhina*.

Although Shannon (1921) only examined New World genera, he represents the next attempt to delimit the Criorhinina (his tribe Criorhini under the subfamily Xylotinae), comprising the genera *Blera* Billberg, 1820 (as *Cynorhina* Williston, 1887), *Criorhina*, *Cynorhinella* Curran, 1922, *Merapioidus* and *Somula* Macquart, 1847. Stackelberg (1930) recognized the relationship between *Criorhina* (as *Pentesilea*, misspelling of *Penthesilea* Meigen, 1800) and *Sphecomyia*, ultimately also including the genus *Blera* (as *Cynorrhina*, misspelling of *Cynorhina*) in his concept for the Criorhinina (as Penthesileini).

Hull (1949) made one of the earliest efforts to create a phylogenetic classification of the Syrphidae. Although Hull did not use phylogenetic methods to assemble his classification, morphological synapomorphies were discussed. Hull's concept of Syrphidae included 12 subfamilies and 25 tribes. He redefined Criorhinina (as Criorhinini) to include the genera *Aneriophora* (as *Eriophora* Phillipi, 1865), *Blera* (as *Cynorhina*), *Criorhina* (as *Criorrhina*, misspelling of *Criorhina*),

Deineches, *Dolichogyna* Macquart, 1842 (as *Nosodepus* Speiser, 1913), *Lycastris* and *Merapioidus*.

Goffe (1952) introduced a two-subfamily arrangement, but only applied it to genera native to the British Isles. Wirth *et al.* (1965) and Thompson (1972) solidified the usage of Goffe's two-subfamily arrangement, reducing the status of most of Hull's subfamilies and tribes. Thus, Hull's subfamily Xylotinae became the tribe Milesiini and the tribe Criorhinini became the subtribe Criorhinina.

The current concept of Criorhinina was first proposed by Thompson (1972), which included the genera *Caliprobola*, *Criorhina*, *Deineches*, *Flukea*, *Lycastris*, *Merapioidus*, *Paratropidia* Hull, 1949 and *Sphecomyia*. In contrast with Hull's concept, Thompson included *Blera* and *Somula* in a separate subtribe, Blerina. Additionally, Thompson transferred *Aneriophora* to the Temnostomina, noting that while *Aneriophora* is reminiscent externally of *Criorhina*, male genitalia resemble those of *Temnostoma* Le Peletier & Audinet-Serville, 1828 (see also: Hippa, 1978). Thompson (1972) was the second author to recognize the relationship between *Sphecomyia* and *Criorhina*, and proposed a segmented phallus in the male genitalia as an important diagnostic character for Criorhinina. Further modifications were presented in Thompson (1975), with *Caliprobola* transferred from Criorhinina to Blerina.

Hippa (1980) synonymized *Paratropidia* with the genus *Orthoprosopa* Macquart, 1850, but did not investigate higher systematics of the genus. Ultimately, Hippa & Thompson (1983) formally transferred *Orthoprosopa* to Tropidiina. Hippa (1978) argued that *Malometasternum* was the sister-group of the genera *Deineches* plus *Flukea*, but limited his reclassifications only to Xylotina (as Xylotini). A formal transfer of *Malometasternum* to Criorhinina appeared in Thompson & Vockeroth (1989).

Mutin & Barkalov (1995), in their erection of the genus *Pseudopocota*, placed it within Xylotini (sensu Hippa, 1978), now Xylotina. Transference of *Pseudopocota* to and from Criorhinina appears to have occurred as part of the collaborative effort to produce a biosystematic database of world Diptera species known as *Systema Dipterorum* (Pape & Evenhuis, 2019). Although infrafamilial classifications are inaccessible in the online database, Thompson recorded his concept of Syrphidae infrafamilial classification in the hardcopy version. An infrafamilial classification of Syrphidae derived from Thompson's unpublished concept incorporating modern knowledge can be found on the Syrphidae Community Website: http://syrphidae.myspecies.info/node/6170. Because this new concept was never formally published, the formal concept of Criorhinina remains the one presented by Thompson (1972) with modifications in Thompson (1975), Hippa & Thompson (1983) and Vockeroth & Thompson (1989). Finally, Skevington *et al.* (2019) proposed the monotypic *Merapioidus* should be treated as a junior synonym of *Criorhina*.

Despite their conspicuous nature, revisionary taxonomic work on Criorhinina is relatively scarce. *Sphecomyia* is the only exception to this (Weisman, 1965; Moran & Skevington, 2019). For *Criorhina*, Nearctic species were last reviewed by Curran (1924). Neither the Neotropical nor the Oriental species have been reviewed. In the Eastern Palaearctic, Russian species were reviewed by Mutin & Barkalov (1999) and Japanese species were reviewed by Shiraki (1968), but these reviews have not been reconciled and undescribed species are known from Japan (Ichige, 2006, 2012). Western Palaearctic species can be considered well studied with the last key found in Van Steenis & Gharali (2016). Most of what is known about the biology, lifestyles and larval habitats of this subtribe derives from five *Criorhina* species: *C. asilica* (Fallén, 1816), *C. berberina* (Fabricius, 1805), *C. floccosa* (Meigen, 1822), *C. ranunculi* (Panzer, 1804) and *C. pachymera* (Egger, 1858) (Rotheray & Stuke, 1998; Speight, 2020).

Eighty-two species of Criorhinina have been described worldwide. In addition, there are many undescribed species noted in collections by contemporary workers (Heikki Hippa unpublished; Katsuyoshi Ichige person. comm.; Kevin Moran unpublished; Jeroen van Steenis unpublished; Chris Thompson unpublished). Including these undescribed species, it is currently estimated that there are at least 140 species of Criorhinina worldwide. Haphazardly introducing additional species descriptions into an already cluttered and confusing literature would only compound the magnitude of disarray. Therefore, the description of these new species is planned in the context of larger reviews of genera or of regional revisions of the fauna, after a new framework of generic concepts has been established.

Generic concepts in the subtribe Criorhinina have never been rigorously tested, with apparent bee mimics traditionally placed in the genus *Criorhina* and apparent wasp mimics in the genus *Sphecomyia*. Ståhls (2006) recovered *Criorhina*, *Matsumyia* and *Sphecomyia* as closely related, and *Matsumyia* as nested within *Criorhina*. Paraphyly within these genera may be more extensive than previously realized. Moran & Skevington (2019) reviewed concepts of *Sphecomyia* and found morphological evidence for multiple origins of wasp mimicry and transferred the species *Criorhina fusca* Weisman, 1964, *Criorhina nasica* Osburn, 1908 and *Criorhina occidentalis* Osburn, 1908 from *Sphecomyia* to *Criorhina*. Penney *et al.* (2012) found a strong positive relationship between mimetic fidelity and

body size. This pressure raises the possibility that these gestalts could be convergent and these genera paraphyletic. In the past, unravelling relationships obscured by convergent evolution would have been a difficult task, but modern molecular techniques provide us with an alternative method for testing generic concepts.

Using eight different molecular markers, the aims of the present study are to: (1) provide the first ever phylogeny of Criorhinina using molecular characters and to test current generic concepts; (2) increase our understanding of Eristalinae relationships at tribal level.

Because no phylogenetic hypothesis encompassing the majority of tribes and subtribes has ever been proposed, the construction of this hypothesis will provide clarity about the vague limits of the Eristalinae subfamily, the tribal and subtribal relationships within, and will help to understand the generic concepts within Criorhinina and its phylogeny. This will enable accurate species placement, lead towards a more stable taxonomic future and encourage comparative ecological studies on the evolution of mimicry (Penney *et al.*, 2012), larval lifestyles, pollination ecology, migration and ancestral biology.

MATERIAL AND METHODS

Taxon sampling

Our dataset contained 120 flower fly species from across all four subfamilies. See Table 1 for the sampled tribes and subtribes. Three Microdontinae species were used as outgroups and to root the tree, because Microdontinae has repeatedly been recovered as sister to the remainder of the Syrphidae (Skevington & Yeates, 2000; Ståhls *et al.*, 2003; Reemer & Ståhls, 2013; Mengual *et al.*, 2015; Young *et al.*, 2016; Pauli *et al.*, 2018), validating the hypothesis first proposed by Thompson (1969). All morphospecies of Criorhinina with discrete cytochrome oxidase c subunit I (*COI*) BINs were included when molecular quality material was available. The Barcode Index Number (BIN; Ratnasingham & Hebert, 2013) System is the clustering algorithm used by BOLD (http://www.boldsystems.org/), which employs graph theoretic methods to generate operational taxonomic units (OTUs) and putative species from COI barcode data without prior taxonomic information (http://www.boldsystems.org/index.php/Public_BarcodeIndexNumber_Home).

Specimens for the study were collected by Malaise trap or hand-collecting, preserved in 95–100% ethanol and placed in a −80 °C freezer until extraction.

Table 1. Tribal and subtribal taxon sampling. Included = ***Bold*** + ***Underline***; Missing = *Italicized*

Eristalinae	Microdontinae	Pipizinae	Syrphinae
Brachyopini	**Microdontini**	_	**Bacchini**
Brachyopina	*Spheginobacchini*	_	**Melanostomini**
Spheginina	_	_	*Paragini*
Callicerini	_	_	**Syrphini**
Cerioidini	_	_	**Toxomerini**
Eristalini	_	_	_
Eristalina	_	_	_
Helophilina	_	_	_
Merodontini	_	_	_
Milesiini	_	_	_
Blerina	_	_	_
Criorhinina	_	_	_
Milesiina	_	_	_
Temnostomina	_	_	_
Tropidiina	_	_	_
Xylotina	_	_	_
Rhingiini	_	_	_
Cheilosiina	_	_	_
Pelecocerina	_	_	_
Psarina	_	_	_
Rhingiina	_	_	_
Sericomyiini	_	_	_
Volucellini	_	_	_

After DNA extraction, specimens have been critical-point dried, mounted, labelled and deposited in the Canadian National Collection of Insects, Arachnids and Nematodes or their respective loan institution as noted in Supporting Information, Appendix S1. For *Sphecomyia metallica* (Bigot, 1882), DNA was extracted from a pinned specimen to allow its inclusion in the dataset. The voucher data and unique identifiers for the specimens used for the phylogenetic study are presented in Supporting Information, Appendix S1.

To cover the genetic diversity of Syrphidae, representatives of all four currently recognized subfamilies were included, along with at least one member of each tribe and subtribe in Eristalinae, and with emphasis on Criorhinina. Sampled taxa come from every different Biogeographic Region, but the majority are Nearctic specimens.

The initial method of specimen identification was typically through *COI* barcodes. Morphological corroboration of identifications was completed by K.M.M, A.D.Y and J.H.S. Through our large-scale barcode efforts, we have achieved coverage of over half of all known species and over 80% of named genera. In many cases this allowed us to identify specimens to the species level or at least place it to genus.

For new and unique BINs, a specimen would be run through identification keys for that genus for a given biotic region. If none were available, species descriptions would be checked or the specimen would be compared to vouchers in the Canadian National Collection of Insects, Arachnids and Nematodes (CNC) collection. For truly unique sequences that had no close relative, the specimen would be identified utilizing generic keys for the corresponding biotic region and then compared with vouchers in the CNC collection.

DNA EXTRACTION

Total DNA was extracted from whole specimens using the DNeasy Tissue kit (Qiagen Inc., Santa Clara, CA, USA) following the manufacturer's protocol.

GENETIC MARKERS

Target genes/loci examined in this study were the mitochondrial gene cytochrome *c* oxidase subunit I (*COI*) divided into: HEB, 5' end; PJ, 3' end. The nuclear genes 28S ribosomal DNA (28S, D4–5 region), Alanylt RNA Synthetase (*AATS*, 5' end), the carbamoyl phosphate synthase domain of CAD (*CAD1*, 5' end), Period (*Period*, C3–C5 region) and three nuclear markers not previously used for syrphid phylogenetics: RNA-binding Protein 15 (*RBP*-15, 5' end), Casein Kinase 1 (*CK1*, 5' end) and TULP (*TULP*, 5' end). These previously unused markers were chosen from single-copy nuclear genes found in Diptera transcriptome data. These three in particular were sampled because of high Sanger sequencing success compared to other nuclear genes like *CAD*. Additionally, they possess a high number of phylogenetically informative sites: *RBP*-15 42%, *CK1* 43%, *TULP* 46%. Primers used in this study are listed in Supporting Information, Appendix S2. Many of the primers used to generate the molecular data for this study are new and were specifically designed for Syrphidae. The 5' end of the mitochondrial *COI* gene, also known as DNA barcode (Hebert *et al.*, 2003a, 2003b), was sequenced for each specimen in order to act as a surrogate voucher and allow linkage of the exemplars to a large molecular dataset being assembled.

POLYMERASE CHAIN REACTION (PCR) AMPLIFICATION

Polymerase chain reaction amplifications were carried out in 25-µL reactions with 15.7 µL ddH_2O, 2.5 µL 10X Ex- Taq PCR buffer (containing 20 mmol/L $MgCl_2$), 0.65 µL 25 mmol/L $MgCl_2$, 1 µL of each 10 µmol/L primer, 2 µL 10 mmol/L dNTPs, 0.15 µL ExTaq HS DNA polymerase (Takara Bio USA, Madison, WI, USA), and 2 µL total DNA extract. Amplification cycles were performed on an Eppendorf ep Gradient S Mastercycler (Eppendorf AG, Hamburg, Germany). Amplification products and negative controls were visualized on 1% agarose electrophoresis gels and purified for bidirectional sequencing using either Clone-Well 0.8 % E-Gels (Invitrogen™, Carlsbad, CA, USA), or an ExoSAP-IT protocol (USB Corp., Cleveland, OH, USA).

DNA SEQUENCING AND EDITING

Sequencing reactions were carried out in a volume of 10 µL using an ABI BigDye Terminator v.3.1 Cycle Sequencing kit (PE Applied Biosystems, Foster City, CA, USA). Sequencing reactions were purified using the ABI ethanol/EDTA/sodium acetate precipitation protocol and analysed on an ABI 3130xl Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA). Sequencing of purified PCR products was performed at the Canadian National Collection of Insects, Arachnids and Nematodes, Agriculture & Agri-Food Canada (Ottawa, ON, Canada).

All sequence chromatograms were edited and contigs formed using SEQUENCHER 5.4.6 (Gene Codes Corp., Ann Arbor, MI, USA).

SEQUENCE ALIGNMENT

MAFFT (Katoh & Standley, 2013) was used to produce preliminary machine-based alignments using the

Auto option, except 28S, which was aligned using the Q-INS-I that considers RNA secondary structure. Afterwards, protein-coding genes were translated to amino acids to ensure there were no stop codons. Alignments were modified, if necessary, by eye using MESQUITE 3.6 (Maddison & Maddison, 2018). Introns were removed from *CK1, Period* and *TULP* prior to analysis. Sequences were submitted to BOLD and uploaded from there to GenBank. For *COI*, the 5' and 3' ends were kept as separate partitions, as coverage of the entire gene was not complete. All sequence data obtained are stored online on the BOLD database (www.boldsystems.org). It is publicly accessible in the Multigene Taxa (MULTI19) dataset available at http://www.boldsystems.org/index.php/Public_SearchTerms?query= DS-MULTI19.

Phylogenetic analysis

A maximum likelihood (ML) tree for a single concatenated matrix was estimated using IQTREE 2.0-rc1 (Nguyen, 2015), an edge-linked partition model, with partition-specific rates. Model selection was performed using ModelFinder (Kalyaanamoorthy, 2017) for each gene, choosing from among the extended selection followed by tree interference. A random seed was chosen.

Models chosen were GTR+F+R7: HEB, GTR+F+R6: PJ,GTR+F+R4:28S,TIM2+F+I+G4:*AATS*,GTR+F+R5: *CAD1*, TIM2+F+R5: *Period*, TPM2+F+I+G4: *RBP-15*, TIM2e+R5: *CK1* and TIM2e+R4: *TULP*. A fast bootstrap analysis using IQTREE was performed with 500 replicates and the above settings.

Bayesian analysis was conducted using MrBayes (Ronquist & Huelsenbeck, 2003) through the CIPRES (Miller *et al.*, 2010) portal. The number of chains was set at six to run for 60 000 000 generations. The sample frequency was set at 2500 and the first 25% of trees were discarded. Bayesian posterior probabilities were summarized in a majority-rule consensus tree.

Tree presentation

Although the dataset was analysed as a whole, most Criorhinina taxa were pruned for the ML (Fig. 1) and Bayesian trees (Fig. 2) in which Eristalinae was the primary focus. This was done to distinguish between the two separate aims of this study. Inclusion of the pruned taxa did not alter the topology of the tree as the resulting tree was identical to a tree produced with these pruned taxa excluded from the starting dataset. A zoomed view of Criorhinina is provided in a separate figure (Fig. 3) with ML values displayed.

Only bootstrap values (BS) and posterior probability (PP) above 90% and 0.9, respectively, were displayed on the presented trees, as anything below is not considered indicative of support using ultra-fast bootstrap and Bayesian analytical methods.

RESULTS

Trimmed alignments contained 120 taxa with 6747 sites (1393 bp *COI*; 1240 bp 28S 4–5 region; 536 bp *AATS* 5' end; 646 bp *CAD1* 5' end; 568 bp *Period* C3-C5 region; 633 bp *RBP-15* 5' end; 1201 bp CK1, 5' end; and 530 bp *TULP* 5' end). The concatenated dataset contained an average of 17% missing data. Maximum likelihood estimation of the present concatenated dataset produced the tree shown in Figure 1, while the Bayesian analysis produced the tree shown in Figure 2. The subfamilies Pipizinae and Syrphinae were resolved as clades and Eristalinae was resolved as paraphyletic. The sister-group relationship of Syrphinae and Pipizinae is supported once more (BS = 100; PP = 1). Within Syrphinae, genera were resolved as in previous analyses (Mengual, 2015, 2020), with *Argentinomyia* Lynch Arribalzaga, 1891 (Melanostomini) as sister to the remainder of the Syrphinae and *Platycheirus* Le Peletier & Audinet-Serville, 1828 (Bacchini) as sister to the members of Syrphini and Toxomerini.

For Eristalinae, the studied members of tribes Cerioidini (*Ceriana* Rafinesque, 1815 and *Sphiximorpha* Rondani, 1850), some of the representatives of Merodontini (*Eumerus* Meigen, 1822, *Nausigaster* Shannon, 1921 and *Merodon* Meigen, 1803) and sampled members of the Volucellini (*Graptomyza* Wiedemann, 1820 and *Copestylum* Macquart, 1846) are recovered as independent lineages outside the remainder of the subfamily. Placement of these clades outside Eristalinae is well supported (BS = 100; PP = 1) rendering the current concept of Eristalinae paraphyletic.

In the ML analysis, Volucellini (*Graptomyza* + *Copestylum*) is resolved as the sister-group of Pipizinae + Syrphinae (BS = 100). In the Bayesian analysis, Volucellini is recovered as sister to *Myolepta*, but this placement is poorly supported.

Merodontini is recovered as a clade of *Merodon* and *Eumerus*. Excluding Microdontinae, this clade is strongly supported (BS = 100; PP = 1) as sister to the remainder of Syrphidae. The concept of the tribe is not monophyletic as a relationship with *Psilota* Meigen, 1822 or *Nausigaster* Shannon, 1921, both currently placed in the Merodontini, is not supported by our results.

Cerioidini is monophyletic (BS = 100; PP = 1) with *Nausigaster* as the sister-group to *Ceriana* (BS = 100; PP = 1). Volucellini is also monophyletic (BS = 100;

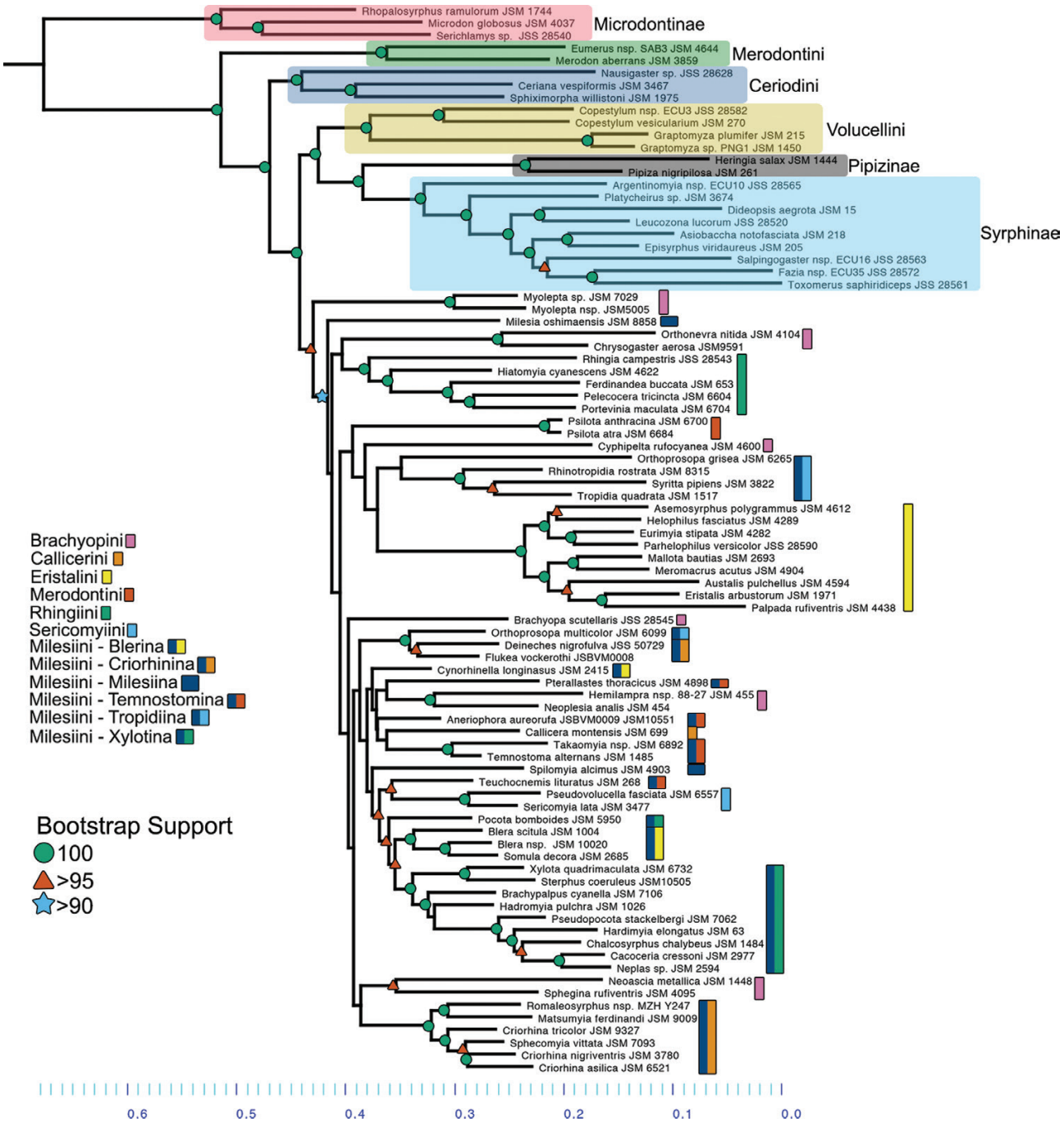

Figure 1. Multigene phylogeny of Syrphidae (ML).

PP = 1), but higher relationships are uncertain with respect to the remainder of Eristalinae and (Pipizinae + Syrphinae).

The monophyly of the remainder of Eristalinae is well supported in the ML analysis (BS ≥ 95) and the clade is placed sister to Volucellini + (Pipizinae + Syrphinae). Within Eristalinae, the tribes Eristalini, Rhingiini and Sericomyiini resolve as monophyletic, while Brachyopini and Milesiini are paraphyletic. Of the subtribes with more than one genus sampled, only Pelecocerina and Spheginina are recovered as monophyletic in the ML analysis, whereas Blerina, Brachyopina, Cheilosiina, Criorhinina, Eristalina, Helophilina, Milesiina, Temnostomina, Tropidiina and Xylotina are paraphyletic. A second area

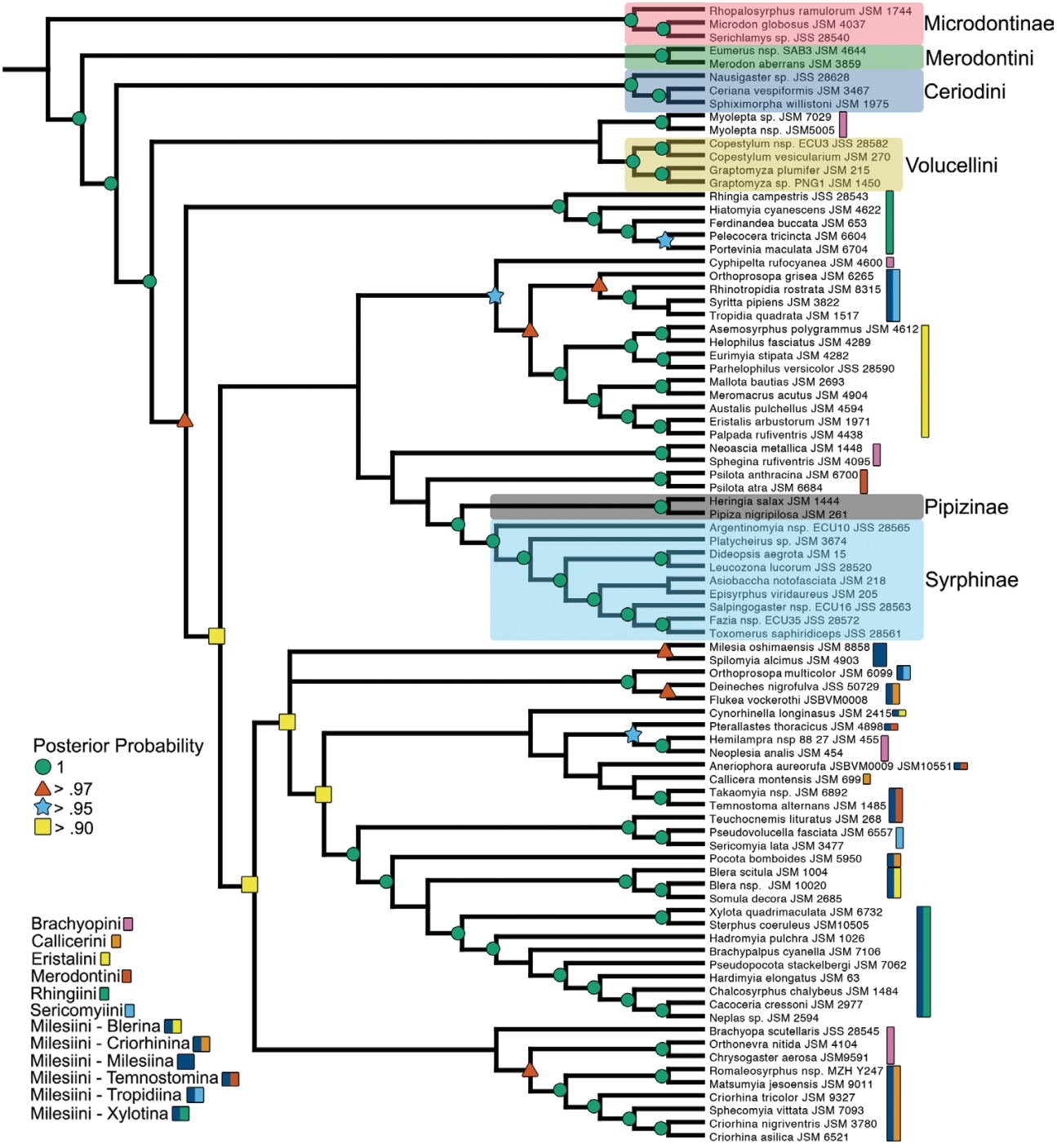

Figure 2. Multigene phylogeny of Syrphidae (Bayesian).

of incongruence between the ML and Bayesian analyses is Bayesian's recovery of a monophyletic Milesiina (*Spilomyia* + *Milesia*) (PP = 0.98).

The tribe Brachyopini is a paraphyletic assemblage of six clades, i.e. *Brachyopa* Meigen, 1822, *Cyphipelta* Bigot, 1859, (*Chrysogaster* Meigen, 1803 + *Orthonevra* Macquart, 1850), (*Hemilampra* Macquart, 1850 + *Neoplesia* Macquart, 1850), *Myolepta* and (*Neoascia* Williston, 1887 + *Sphegina*).

Myolepta is recovered as sister to the rest of Eristalinae in the ML analysis (BS = 100). However in the Bayesian analysis it is recovered as part of a unresolved polytomy with Volucellini. *Cyphipelta* is placed as sister to

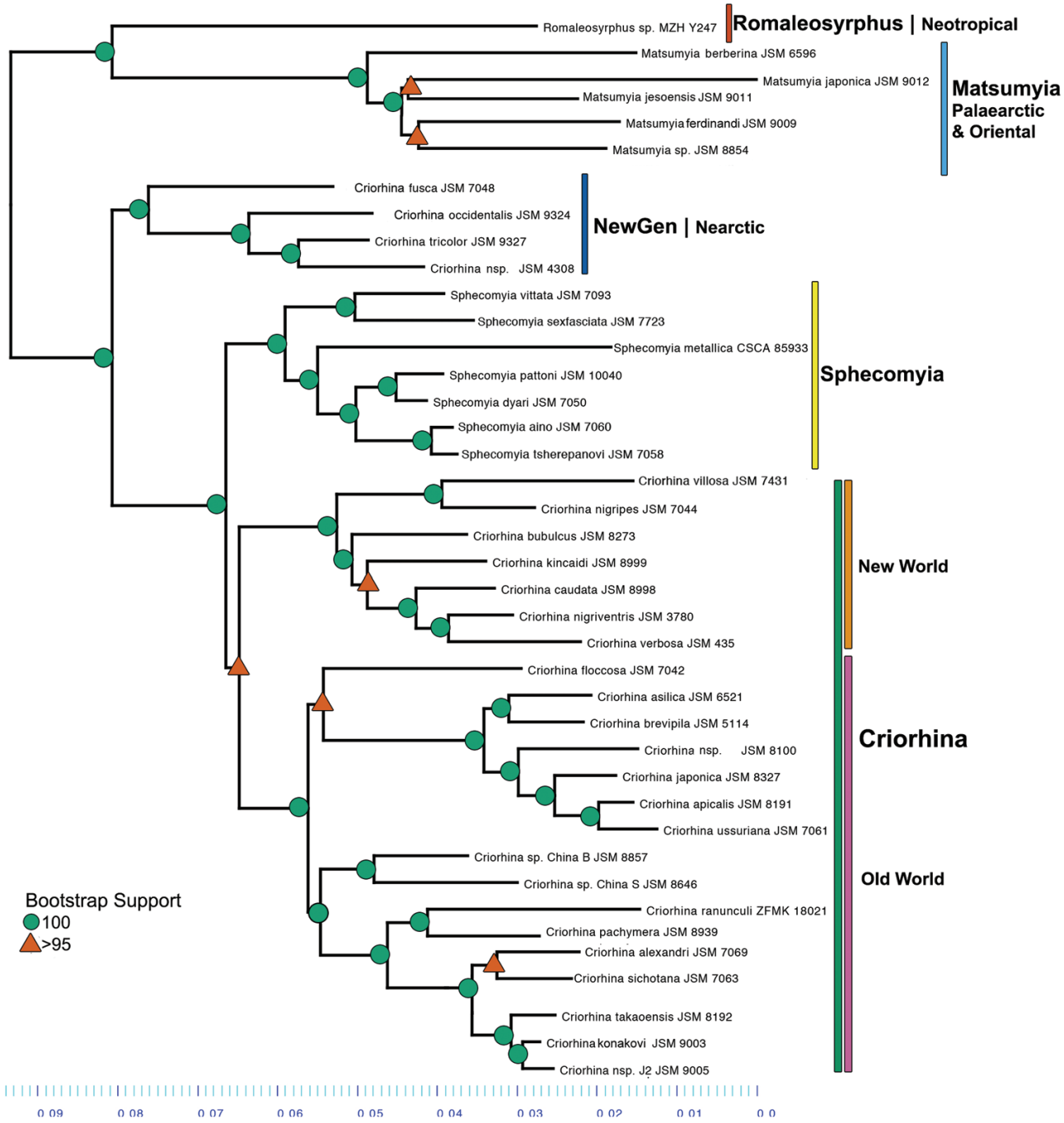

Figure 3. Multigene phylogeny of Criorhinina (Zoomed).(Bars represent monophyletic groups.)

Tropidiina + Eristalini in both the Bayesian and ML analyses, but this position only receives strong support in the former (PP = 0.98).

Further incongruence exists between the two analyses in the placement of *Neoascia* + *Sphegina*. In the ML tree this clade is placed sister to Milesiini, while in the Bayesian analysis it is sister to *Psilota* + (Pipizinae + Syrphinae). Additional incongruence exists as in the ML tree *Chrysogaster* + *Orthonevra* is sister to Rhingiini, but in the Bayesian tree it is recovered as sister to Criorhinina.

The tribe Eristalini is strongly supported as monophyletic (BS = 100; PP = 1), but its two subtribes, Eristalina and Helophilina, are not. *Mallota*, traditionally included in Helophilina, forms a strongly

supported clade with *Meromacrus* Rondani, 1849, traditionally placed in Eristalina (BS = 100; PP = 1).

The current concept of the tribe Milesiini is not monophyletic as the subtribe Tropidiina is recovered as sister to Eristalini. Tropidiina is monophyletic in both analyses, but only well supported in the Bayesian tree (PP = 1), with the exception of *Orthoprosopa multicolor* (Ferguson, 1926), which seems to be a case of mistaken generic placement, because *Orthoprosopa grisea* (Walker, 1835), the type species, is recovered sister to the remaining Tropidiina. *Rhinotropidia* Stackelberg, 1930 is recovered as sister to traditional *Syritta* Le Peletier & Audinet-Serville, 1828 + *Tropidia* Meigen, 1822 (BS = 100; PP = 1).

The tribe Callicerini, as *Callicera* Panzer, 1809, and the tribe Sericomyiini are recovered inside Milesiini. Sericomyiini is recovered as sister to the *Pocota* + (Blerina + Xylotina) clade and this position is well supported (BS ≥ 95; PP = 1). The genus *Teuchocnemis* Osten Sacken, 1875, currently placed in the Temnostomina, is strongly supported as sister to *Sericomyia* Meigen, 1803 (BS ≥ 95; PP = 1).

The subtribe Blerina is rendered paraphyletic by the separation of *Cynorhinella* from the other sampled taxa in this subtribe. The type genus *Blera* is rendered paraphyletic by *Somula decora* Macquart, 1847. Blerina *s.s.* is recovered as sister to the Xylotina *s.s.*, but only in the ML tree is the support significant (BS ≥ 95).

The subtribe Xylotina is monophyletic, except for the recovery of *Pseudopocota* inside Xylotina as sister to the remainder of *Chalcosyrphus* Curran, 1926, and the recovery of *Pocota* as sister to Blerina + Xylotina *s.s.*, as opposed to within the subtribe (BS ≥ 95; PP = 1). *Cacoceria* Hull, 1936 is recovered inside *Chalcosyrphus*, as sister to the subgenus *Neplas* Porter, 1927, in agreement with results obtained by Ståhls (2006). *Hardimyia* is sister to the other sampled subgenera of *Chalcosyrphus*.

Even excluding *Teuchocnemis*, the subtribe Temnostomina is still rendered paraphyletic by the inclusion of *Hemilampra* + *Neoplesia* as sister to *Pterallastes* Loew, 1863, but strong support for this placement is only recovered in the Bayesian analysis (PP = 0.96). The enigmatic *Callicera* is nestled between *Aneriophora* and *Temnostoma* + *Takaomyia* in both analyses but this placement lacks any significant measure of support. Of traditional Temnostomina, only the clade *Takaomyia* Herve-Bazin, 1914 + *Temnostoma* is recovered with high support in both analyses (BS = 100; PP = 1).

Generic relationships of Criorhinina

Deineches and *Flukea* render the subtribe Criorhinina paraphyletic with respect to the type genus *Criorhina*. *Deineches*, *Flukea* and *Orthoprosopa multicolor* form a strongly supported (BS = 100; PP = 1) clade not closely related to *Criorhina*.

In congruence with Ståhls (2006), a relationship between *Criorhina*, *Matsumyia* and *Sphecomyia* is recovered. Most internal relationships of the clade are recovered with bootstrap support and posterior probability values of 100 and 1, respectively (Fig. 3). The one major exception is the node connecting *Specomyia*, Old World *Criorhina*, and the clade containing most New World species *Criorhina*, which only has strong support (BS ≥ 95; PP = 1).

The concept of *Sphecomyia* as presented in Moran & Skevington (2019) is validated, with wasp mimicry having multiple origins inside Criorhinina. *Matsumyia* is recovered as part of a clade containing *Criorhina berberina* and an unnamed Oriental species (Fig. 3). Recovered sister to *Matsumyia s.l.* is the Neotropical species *Romaleosyrphus* sp. (Fig. 3). The species *Criorhina villosa*, formerly placed in the monotypic *Merapioidus* Bigot, 1882, is recovered inside *Criorhina*. Excluding species belonging to *Matsumyia* and *Romaleosyrphus*, *Criorhina* is still rendered paraphyletic, with *Sphecomyia* embedded within the members of the current concept of *Criorhina* (Fig. 3).

DISCUSSION

Eristalinae

The present multigene dataset successfully addressed our primary goal of inferring the phylogenetic relationships within Criorhinina and resolved major questions about the groupings within Eristalinae. Our results resolved the Eristalinae as non-monophyletic but, unfortunately, the selection of genes was unsuccessful in recovering any degree of support, with some exceptions, for most of the tribal and subtribal relationships within what we consider Eristalinae *s.s.*

Although not the focus of this study, placement of groups relative to Microdontinae refutes the hypothesis presented in Rotheray & Gilbert (1999) of a sister-group relationship between Microdontinae, pipizines and syrphines. Instead, our study corroborates Young *et al.* (2016) and Pauli *et al.* (2018) in recovering Merodontini as sister to the remainder of Syrphidae, excluding Microdontinae. In all three analyses, Syrphinae and Pipizinae are both monophyletic and sister to each other, further corroborating Mengual *et al.* (2015).

This analysis provides strong support for the placement of the tribes Cerioidini, Volucellini and parts of Merodontini (including *Merodon*) outside of an otherwise monophyletic Eristalinae. Following the Young *et al.* (2016) analysis using anchored-hybrid

enrichment (AHE) and Pauli *et al.* (2018), which analysed transcriptomes, this is the third molecular study to recover strong support for the placement of Merodontini not only outside of Eristalinae, but also as sister to the remainder of Syrphidae, excluding Microdontinae. Additionally, this analysis finds strong support for the placement of Volucellini outside of Eristalinae; a position first strongly supported in Young *et al.* (2016). The recovery of a potential sister-group relationship between Volucellini and Pipizinae + Syrphinae is intriguing, but poor support is found for this relationship. More critically, this relationship is not recovered in Young *et al.* (2016), who sampled all three clades. It is the authors' opinion that this placement is an artefact of the low number of genes sampled and that the position expressed in Young *et al.* (2016) is likely more accurate.

Neither Young *et al.* (2016) nor Pauli *et al.* (2018) sampled a member of Cerioidini and the placement of this tribe outside Eristalinae is novel. The placement of *Nausigaster* as sister to the remainder of Cerioidini is also novel. A close relationship between *Nausigaster* and *Eumerus* + *Merodon* was not recovered in Ståhls *et al.* (2003), Hippa & Ståhls (2005) or Doczkal & Pape (2009), and neither was it supported by Rotheray *et al.* (1999). Although Mengual *et al.* (2015) recovered *Nausigaster* with other Merodontini (based on a combined dataset ML analysis), this placement lacked support and the authors concluded that the taxon had no consistency in position between the optimization, MAFFT or structural alignments (Mengual *et al.* 2015). Critically, our study recovers high support for a placement as sister to the remainder of Cerioidini. Additionally, Mengual *et al.* (2015) only sampled *COI*, 18S and 28S along with morphology. Our analysis includes both *COI* and 28S, as well as six additional genes. 18S was excluded from this study because the authors found it only 18% informative within the family Syrphidae, with most variation derived from the earliest diverging branches. Finally, one must consider that one or more morphological character(s) used as support for a hypothesized monophyletic clade may instead be plesiomorphic or homoplastic. Considering these factors, the authors are confident that the placement of *Nausigaster* as sister to the remainder of Cerioidini is accurate and will test this in future analyses possessing a higher degree of taxa and gene sampling.

Psilota is not recovered as a close relative of *Eumerus* + *Merodon*, nor does Rotheray & Gilbert (1999) recover a close relationship between *Psilota* and *Eumerus* + *Merodon*. Considering our analysis, along with the fact that Rotheray & Gilbert (1999) used morphological data, it would be reasonable to exclude *Psilota* from Merodontinae, even though its status would be left as *incertae sedis* inside Eristalinae. However, we err on the side of caution and leave its placement unchanged for now.

Young *et al.* (2016) recovered Eristalini as a distinct putative subfamily sister to Pipizinae + Syrphinae. The remainder, which would hypothetically be termed Milesiinae, is resolved as sister to these three clades. However, our study recovers Eristalini inside Eristalinae (BS ≥ 95; PP = 1) similar to Pauli *et al.* (2018), who recovered it as sister to *Syritta*.

Unlike either of the other studies, Pauli *et al.* (2018) recovered Rhingiini as sister to Pipizinae and Syrphinae. A slightly different position is recovered in our Bayesian analysis with Rhingiini placed as sister to the remainder of Eristalinae, as well as Syrphinae and Pipizinae. The placement of Syrphinae + Pipizinae inside traditional Eristalinae in the Bayesian analysis is interesting but no support is recovered for this placement.

The conflict between these three hypotheses is intriguing, as in Young *et al.* (2016) the Eristalini node was the only one lacking full support (68%). Our study appears to suffer from the number of loci analysed, while the limited taxon sampling of Young *et al.* (2016) and Pauli *et al.* (2018) hindered a more accurate recovery of Eristalinae relationships. With such low support recovered for most higher nodes within Eristalinae, it is difficult to draw firm conclusions about tribal and subtribal relationships within this subfamily. Yet, some relationships become obvious.

Brachyopini was recovered as a paraphyletic assemblage of six individual clades, with none of these positions, except *Cyphipelta*, gathering any significant support. It seems likely that Brachyopini is paraphyletic to some degree. This finding is not surprising considering that Mengual *et al.* (2015) also recovered a paraphyletic Brachyopini.

Strong support was recovered for the monophyly of Eristalini, but not for the two subtribes, Helophilina and Eristalina, that compose it. *Mallota*, currently placed in Helophilina, and *Meromacrus*, currently placed in Eristalina, clustered together with strong support. A potential solution is to resurrect the subtribe Mallotina to contain *Mallota* and its relatives. Mengual *et al.* (2015) also recovered paraphyly within these subtribes, with *Mesembrius*, Rondani, 1857 currently placed in Helophilina, resolved as sister to all sampled members of Eristalina. Generic sampling of the Eristalini was limited in scope and paraphyly may be more extensive than revealed, especially considering the findings of Mengual *et al.* (2015), and thus this decision is postponed until a more detailed analysis is conducted.

This is the second molecular study, after Mengual *et al.* (2015), to recover a close relationship between Tropidiina and Eristalini with any degree of support. Still, with only strong support in the Bayesian

analysis, this finding is not actionable. At this time, we do not recommend the elevation of Tropidiina to full tribal status. However, evidence is conclusive that ***Rhinotropidia*** should be resurrected as a monotypic genus (**stat. rev.)**, because the type species *Rhinotropidia rostrata* (Shiraki, 1930) is recovered as sister to the traditional concepts of *Tropidia* and *Syritta*. This taxon is unlike other *Tropidia* species in that it has a concave face in the male.

A second area of incongruence between the ML and Bayesian analyses is Bayesian recovery of a monophyletic Milesiina (*Spilomyia* + *Milesia*) with strong support (PP = 0.98), while likelihood analysis does not support this clade. Potential paraphyly of Milesiina is not surprising, considering Mengual *et al.* (2015) also recovered Milesiina as paraphyletic.

Both tribes Callicerini and Sericomyiini are placed inside the traditional Milesiini. For Callicerini this finding is novel, as in Mengual *et al.* (2015) the tribe was recovered as sister to Volucellini. Mengual *et al.* (2015) also recovered Sericomyiini inside Milesiini, although in their analysis it was recovered, with no support, as sister to the Criorhinina. Of these two subtribes, only the placement of Sericomyiini is especially well supported (BS ≥ 95; PP = 1) as the sister-group to *Pocota* + (Blerina + Xylotina). We find the evidence compelling for the placement of Sericomyiini within Milesiini as the subtribe **Sericomyiina** (**stat. rev.)**. This study marks the first time *Teuchocnemis* is sampled for a molecular phylogeny and we find the evidence persuasive for its placement as sister to the sampled members of Sericomyiina (BS ≥ 95; PP = 1) and thus transfer the genus to Sericomyiina.

Sister to both Blerina and Xylotina subtribes, the subtribe **Pocotina** (**stat. rev.)** is resurrected to contain *Pocota* and the three species it holds. The placement of *Pocota* outside of Xylotina vindicates Hippa (1978), who placed the genus outside the subtribe, although he was uncertain of its affinities inside Milesiini. Our study supports the sister-group relationship between Blerina and Xylotina, first recovered in a molecular analysis in Mengual *et al.* (2015), where the relationship lacked support.

Monophyly of Blerina is maintained by the removal of *Cynorhinella* with the genus left as *incertae sedis* inside the **Milesiini** (**stat. rev.**). With the type genus *Blera* rendered paraphyletic by *Somula decora*, generic concepts in the subtribe should be re-examined in lieu of this finding. Finally, *Pseudopocota* is recovered as the closest relative of *Chalcosyrphus*, validating its current placement in Xylotina. These findings contradict Mengual *et al.* (2015), who recovered a monophyletic Blerina and Xylotina. However, our analysis sampled these subtribes more heavily, with the problematic genera *Cynorhinella*, *Pocota* and *Pseudopocota* not sampled in the previous study.

The recovered relationship of Sericomyiina + (Pocotinia + (Blerina + Xylotina) is interesting as it raises questions about the ancestral face state of the last common ancestor for this group. Syrphids with elongated faces are better adapted to feeding from flowers with long corollas and it is of interest how often this adaptation evolved. Mapping this character trait onto the recovered relationship leads to a ladder of elongate and concave faces: (elongate + (concave) + (elongate + concave)) and only leads to more questions about the character state in the most recent common ancestor for this clade.

The addition of *Sterphus* Philippi, 1865 and *Macrometopia* Philippi, 1865, which we did not sample, to a future analysis may shed light on this question, as members of these genera do not possess concave faces. Thompson (1972) theorized these two genera were the earliest diverging members of Xylotina. However, Hippa (1978) disagreed, considering *Sterphus* more closely related to *Xylota* and *Macrometopia* instead more closely related to *Sericomyia*.

Criorhinina

For the subtribe Criorhinina, *Deineches* and *Flukea* render the subtribe paraphyletic with respect to the type genus *Criorhina*. *Deineches*, *Flukea* and *Orthoprosopa multicolor* form a strongly supported (BS = 100; PP = 1) clade not closely related to *Criorhina* that may need to be elevated to subtribal status. We remove *Deineches* and *Flukea* from Criorhinina and leave them as *incertae sedis* in **Eristalinae** for now (**stat. rev.**). *Orthoprosopa multicolor* is separate from other *Orthoprosopa* species but is left in *Orthoprosopa* for now until the concept of a potential new genus can be more fully explored. Finally, *Pseudopocota*, as stated above, is recovered within Xylotina as the closest relative of *Chalcosyrphus*, validating Mutin and Barkalov's (1995) placement of the genus in the subtribe.

In light of these results, it is necessary to redefine the concept of the subtribe Criorhinina. With *Criorhina* as the type genus, Criorhinina (stat. rev.) is here restricted to contain only *Criorhina*, *Matsumyia* and *Sphecomyia* and any clades that may be extracted from the current concepts of these genera.

A relationship between *Criorhina*, *Matsumyia* and *Sphecomyia*, and most of the internal relationships of the clade, are recovered with bootstrap support values and posterior probability values of 100 and 1, respectively (Fig. 3). Higher nodes were identical between the two analyses. Five major groupings are supported: *Matsumyia* and relatives, an unnamed clade, *Sphecomyia*, Old World *Criorhina* and New World *Criorhina*. New World and Old World *Criorhina* are resolved as sister-groups, with *Sphecomyia* sister

to both, but bootstrap support is only 95. Thus, the relationships among these three clades are not fully resolved with our data.

Excluding species belonging to the *Matsumyia* group of genera, *Criorhina* is still rendered paraphyletic by the genera *Merapioidus* and *Sphecomyia*. Most members of the genus *Criorhina* form a clade sister to *Sphecomyia* and are hereafter referred to as *Criorhina s.s.* Sister to both *Criorhina s.s.* and *Sphecomyia* is a clade of wasp and bumble-bee mimic species endemic to the Nearctic region (Fig. 3). Included in this clade, termed NewGen, are the wasp mimic *Criorhina fusca* and *Criorhina occidentalis* which Moran & Skevington (2019) transferred from Sphecomyia to Criorhina. The concept of *Sphecomyia* as presented in Moran & Skevington (2019) is validated, with wasp mimics not forming a single monophyletic clade.

As a result of this paraphyly, the creation of a new genus is necessary if *Sphecomyia* is to be maintained as a generic concept. This will be done in a separate paper as part of an ongoing global revision of Criorhinina taxonomy (Moran & Skevington in prep.). Based upon the type species *Criorhina asilica*, *Criorhina s.s.* appears to be composed of two clades: Old World and New World, with the genus as a whole placed sister to *Sphecomyia*. *Merapioidus* is placed within the New World clade of *Criorhina s.s.* and should be considered a synonym, as first proposed by Skevington *et al.* (2019). The concept of *Sphecomyia*, as presented in Moran & Skevington (2019), is validated, with wasp mimicry having independent origins in *Criorhina s.l.* and *Sphecomyia*.

In support of Ståhls (2006), our analysis also recovers *Matsumyia* inside *Criorhina*. More extensive taxa sampling, however, reveals *Matsumyia* is part of a clade containing *Criorhina berberina* and an unnamed Oriental species (Fig. 3). The need for a review of Old-World species concepts is clear, especially as Oriental *Criorhina* have never been evaluated with regard to the concept of *Matsumyia*. The concept of *Matsumyia* will require modification to accommodate these additional species (K. Moran unpubl. data).

Recovered sister to *Matsumyia s.l.* is an undescribed Neotropical species of *Romaleosyrphus*. (Fig. 3). Based on a neighbour-joining (NJ)-analysis of *COI* barcodes, and a revision of Neotropical Criorhinina submitted at the same time as this paper (Moran & Skevington, unpubl. data), this species is a member of a clade currently placed in *Criorhina*, which includes *Criorhina villosa* (Bigot, 1882), *Criorhina arctophiloides* (Giglio-Tos, 1892) and several other undescribed species. When describing *R. villosa*, Bigot (1882) erected *Romaleosyrphus* to contain it and assigned it as the type species. The name *Romaleosyrphus* would take precedence over *Matsumyia*, but rather than synonymize these taxa, we hereby resurrect *Romaleosyrphus* as the sister-lineage to ***Matsumyia*** (**stat. rev.**).

CONCLUSION

The results presented in this study corroborate some earlier analyses and hypotheses and delve deeper into the phylogeny and intergeneric relationships of the family Syrphidae using a larger taxon sample as compared to previous studies. Additionally, the study shows that many phylogenetic questions remain. We suggest that relationships within this group are obscured as a result of rapid speciation and that it will require a genomic approach to peel back the fog surrounding their evolutionary history.

Despite not being its focus, our study shows continuing support for both the monophyly of Microdontinae, Pipizinae and Syrphinae, and a sister-group relationship between Syrphinae and Pipizinae. Additionally, our results support the tribal relationships of Syrphinae as hypothesized in Mengual (2020).

We suggest the following nomenclatural changes:

1. *Rhinotropidia* (stat. rev.) is resurrected as a genus sister to *Tropidia* + *Syritta*.
2. Criorhinina is redefined to include only *Criorhina*, *Sphecomyia* and *Matsumyia* (stat. rev.), a resurrected *Romaleosyrphus* (stat. rev.) and an undescribed genus.
3. *Deineches* and *Flukea* are excluded from Criorhinina and left as *incertae sedis* in Milesiini. Although not included in this study, we exclude *Lycastris* and *Malometasternum* from Criorhinina, leaving them as *incertae sedis* in Milesiini. *Malometasternum* shows a close relationship with *Deineches* (based on unpublished 28S and *COI* barcode data). Cytochrome *c* oxidase subunit I barcode data and morphology suggest *Lycastris* should be removed from Criorhinina. We suspect it belongs in the Tropidiina because of the presence of stigmal crossveins (as in *Meropidia* Hippa & Thompson, 1983 and *Calcaretropidia* Keiser, 1971) and a more advanced state of the elongated snout (as in *Paratropidia* Hull, 1949).
4. *Cacoceria* is demoted to a subgenus of ***Chalcosyrphus*** (**comb. nov.**).
5. The following new combination is proposed: *Criorhina berberina* changed to ***Matsumyia berberina*** (Fabricius, 1805) (**comb. nov.**).
6. Transfer of *Pocota* from Xylotina (stat. rev.) to the newly resurrected Pocotina (stat. rev.).
7. Removal of *Cynorhinella* from Blerina (stat. rev.) with the genus left as *incertae cedis* in Milesiini.
8. Placement of Sericomyiini (stat. rev.) within Milesiini as the subtribe Sericomyiina.

9. *Teuchocnemis* is transferred to Sericomyiina.

Despite strong support for this action, the authors do not propose elevating Merodontini, Volucellini and Cerioidini to subfamilial level until an NGS phylogeny (Moran *et al*., in prep.) sampling over 500 taxa and over 1500 genes is published.

The authors have no doubt that Merodontini should be regarded as a valid subfamily, as this position is now well supported by three studies. However, questions remain regarding which taxa are members of the subfamily. We do not sample *Alipumilio* Shannon, 1927, *Austrocheilosia* Thompson, 2008, *Azpeytia* Walker, 1865, *Cepa* Thompson & Vockeroth 2007, *Lyneborgimyia* Doczkal & Pape 2009, *Megatrigon* Johnson, 1898 or *Platynochaetus* Wiedemann, 1830, and as a result they would remain combined with Merodontini. Elevation of the subfamily holds the potential to produce confusion if some of these unsampled genera render Merodontini paraphyletic.

Volucellini is a clearly monophyletic unit now supported in multiple studies as belonging outside Eristalinae. However, higher level relationships remain elusive and it may be premature to elevate the group without a stable understanding of them.

Cerioidini enjoys high support values for higher relationships and is unlikely to create orphan taxa. However, as the finding of Cerioidini as a distinct subfamily is novel and unique to this paper, the authors accept that it may be prudent to wait for confirmation of this finding from future analyses.

The next logical step is to build upon the framework provided by this paper and the earlier AHE study (Young *et al.*, 2016) by increasing the number of target loci and by incorporating a more thorough taxon sampling of the many morphologically diverse groups within Syrphidae. With such a high level of ecological and morphological diversity, a detailed phylogeny of Syrphidae will support future work in fields such as pollination biology and biological control, and will help to answer major challenging questions that remain open, such as the evolution of inquiline–host associations in myrmecophilic flies, the evolution of larval feeding behaviour, the development of perfect and imperfect mimicry, the origin and biogeography of the different taxonomic groups, as well as patterns of migratory behaviour.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Appendix 1. Specimen data.
Appendix 2. Multi-gene primer table.