WILEY-BLACKWELL

Cladistics 31 (2015) 491–508

Cladistics

10.1111/cla.12105

Phylogenetic relationships and taxonomic ranking of pipizine flower flies (Diptera: Syrphidae) with implications for the evolution of aphidophagy

Ximo Mengual[a,*], Gunilla Ståhls[b] and Santos Rojo[c]

[a]*Zoologisches Forschungsmuseum Alexander Koenig, Adenauerallee 160, D-53113 Bonn, Germany;* [b]*Finnish Museum of Natural History, Zoology unit, FI-00014 University of Helsinki, PO Box 17, Helsinki, Finland;* [c]*Departmento de Ciencias Ambientales y Recursos Naturales/Instituto Universitario CIBIO, Universidad de Alicante, Apdo 99. E-03080 Alicante, Spain*

Accepted 12 November 2014

Abstract

The taxonomic rank and phylogenetic relationships of the pipizine flower flies (Diptera: Syrphidae: Pipizini) were estimated based on DNA sequence data from three gene regions (COI, 28S and 18S) and 111 adult morphological characters. Pipizini has been treated as a member of the subfamily Eristalinae based on diagnostic adult morphological characteristics, while the larval feeding mode and morphology is shared with members of the subfamily Syrphinae. We analysed each dataset, both separately and combined, in a total evidence approach under maximum parsimony and maximum likelihood. To evaluate the influence of different alignment strategies of rDNA 28S and 18S genes on the resulting topologies, we compared the topologies inferred from a multiple alignment using fast Fourier transform (MAFFT) program with those topologies resulting from aligning the secondary structure of these rDNA genes. Total evidence analyses resolved pipizines as a sister group of the subfamily Syrphinae. Although the structural alignment and the MAFFT alignment differed in the inferred relationships of some clades and taxa, there was congruence in the placement of pipizines. The homogeneous morphology of the Pipizini clade in combination with their unique combination of characters among the Syrphidae suggest a change of rank to subfamily. Thus, we propose to divide Syrphidae into four subfamilies, including the subfamily Pipizinae stat. rev.

Introduction

Syrphidae (flower flies, hover flies) is one of the most abundant and conspicuous dipteran families with over 6000 described species worldwide (Brown, 2009; Thompson, 2013a). Adults are flower visitors and are important pollinators both in natural ecosystems and agricultural crops (e.g. Pérez-Bañón et al., 2003; Ssymank et al., 2009). Immature stages present a very variable range of feeding modes (including saprophagy, phytophagy, mycophagy, and predation), and thus can be found in a very diverse array of habitats (Rotheray and Gilbert, 1999; Rotheray et al., 2000; Reemer and Rotheray, 2009; Ureña and Hanson, 2010).

Traditionally, the Syrphidae has been classified into three subfamilies, that is, Microdontinae, Eristalinae, and Syrphinae (Thompson and Rotheray, 1998). The first mentioned subfamily comprises taxa with larval associations with ants (for a review see Reemer, 2013). The majority of the Eristalinae larvae are saprophagous in various decaying organic media, and some are phytophagous in various plants (Hövemeyer, 1987; Rotheray, 1993; Stuke, 2000; Grosskopf, 2005; Fischer et al., 2006; Morales and Wolff, 2010). The subfamily Syrphinae contains taxa with predatory larvae that prey on soft-bodied arthropods, mainly Hemiptera (for a review see Rojo et al., 2003), while a few species have recently been shown to develop as miners in

*Corresponding author:
E-mail address: x.mengual@zfmk.de

plants (Nishida et al., 2002; Weng and Rotheray, 2008).

The tribe Pipizini is a well-defined aggregate exhibiting a unique character combination for morphological characters of both immature stages and adults (Thompson, 1972; Rotheray and Gilbert, 1989; Thompson and Rotheray, 1998). Pipizines are distributed worldwide but are absent from the Afrotropical Region, and the greatest diversity is found in the Holarctic Region. A total of eight genera and approximately 180 species are currently recognized (Thompson, 2013a; Vujić et al., 2013): *Claussenia* Vujić & Ståhls, 2013, *Cryptopipiza* Mutin, 1998, *Heringia* Rondani, 1856, *Neocnemodon* Goffe, 1944, *Pipiza* Fallén, 1810, *Pipizella* Rondani, 1856, *Trichopsomyia* Williston, 1888, and *Triglyphus* Loew, 1840.

Pipizini are medium-sized flower flies (5–10 mm) mainly with a black coloured body and with pale abdominal maculae present in some species. Pipizini larvae are predatory, as are those of Syrphinae, but pipizines show a preference for woolly or root aphids with waxy secretions and other gall-forming hemipterans, for example Pemphigidae, Psyllidae, Phylloxeridae, and Adelgidae (Rojo et al., 2003).

The classification of the Pipizini has been a longstanding taxonomic issue, as pipizines share the larval feeding mode with the Syrphinae, but some external morphological characters with the Eristalinae (Thompson, 1972). The typically eristaline adult characters include a pilose postpronotum (bare in Syrphinae) and male abdomen with four unmodified pregenital segments (five in Syrphinae).

Williston (1885) introduced the family-group name Pipizinae for *Pipiza*, together with *Nausigaster* Williston, 1884 and *Paragus* Latreille, 1804. Since then, many authors have considered the tribe Pipizini a member of the subfamily Syrphinae (Verrall, 1901; Efflatoun, 1922; Brunetti, 1923; Glumac, 1960; Hartley, 1961; Wirth et al., 1965) based on the larval biology and male genitalia. Also, studies on the structure and number of chromosomes suggested the inclusion of pipizines into the subfamily Syrphinae, as they shared similar chromosome numbers (Boyes and van Brink, 1972). Pipizini were also treated as members of Eristalinae in many works, however, based on adult characters (Williston, 1887; Curran, 1921; Shannon, 1921, 1922a,b, 1923; Hull, 1949; Goffe, 1952; Dušek and Láska, 1967; Thompson, 1969, 1972; Vockeroth, 1969; Shatalkin, 1975b; Bartsch, 2009; Huang and Cheng, 2012). As members of the subfamily Syrphinae, pipizines have been associated with the genus *Paragus* (Verrall, 1901; Rotheray and Gilbert, 1989). In the subfamily Eristalinae, pipizines were placed in the Rhingiini tribe of similarly dark coloured hover flies, with variable ranks: as a single genus *Pipiza* (Williston, 1887; Shannon, 1921), as different genera within Chilosiinae (= Rhingiini) (Sack, 1932), as subtribe Pipizina within the supertribe Pipizaria (Goffe, 1952), or as a tribe comprising several genera (Curran, 1921; Hull, 1949; Thompson, 1972; Shatalkin, 1975b).

Previous phylogenetic analyses on the family Syrphidae were based either on one character type, that is, immature stages morphology (Rotheray and Gilbert, 1999) or adult morphology (Hippa and Ståhls, 2005), or researchers used a very limited dataset of characters and taxa (Cheng et al., 2000). Ståhls et al. (2003) used for the first time a combined dataset with molecular data and morphological characters of adults and larvae, but their taxon sampling remained limited. They found that Pipizini and Syrphinae were resolved as sister groups under the different character weighting schemes, except in the equal weight maximum parsimony analysis where Pipizini was resolved as sister group to Eristalinae + Syrphinae.

For our analyses we used the majority of the adult morphological characters of Hippa and Ståhls (2005) and three molecular markers, the mitochondrial protein-coding gene cytochrome c oxidase subunit I (COI), the D2–D3 region of the nuclear ribosomal 28S rRNA gene, and a small fragment of the nuclear ribosomal 18S rRNA gene. Aligning rRNA genes, as opposed to protein-coding genes, is not a straightforward task, and the use of the gene's secondary structure has been shown to be useful to establish positional nucleotide homologies for phylogeny reconstruction (Kjer, 1995; Gillespie et al., 2005; Subbotin et al., 2007; Marvaldi et al., 2009; Murienne et al., 2010). Therefore, we decided to contrast the topologies that were inferred based on multiple sequence alignment versus those inferred using the secondary structure of the rRNA genes for evaluating the placement of the Pipizini.

The aims of this study are threefold: (i) to re-address the systematic position and taxonomic ranking of the tribe Pipizini based on morphological and molecular characters; (ii) to elucidate the phylogenetic relationships among pipizines and other syrphid groups; and (iii) to explore the effects of the rRNA gene alignments based on the secondary structure on these phylogenetic relationships. Previous studies provide a model of the secondary structure of the 28S rRNA gene for Syrphidae (Mengual et al., 2012), but for the first time we use the secondary structure of the rRNA 18S gene to align the DNA sequences in order to perform the present analysis.

Material and methods

Taxonomic sampling

Taxa were selected to represent all the 16 recognized tribes of Syrphidae (Bacchini, Paragini, Syrphini,

Toxomerini, Microdontini, Spheginobacchini, Brachyopini, Callicerini, Ceriodini, Eristalini, Eumerini, Milesiini, Pipizini, Rhingiini, Sericomyini, and Volucellini). Several genera of each tribe were included as ingroup, with the exception of Callicerini, Ceriodini, and Sericomyini, and the monogeneric tribes Paragini, Spheginobacchini, and Toxomerini with only one representative each. All eight genera of Pipizini were included in the analysis. A member of the genus *Megaselia* Rondani, 1856 (family Phoridae) was constrained as outgroup. We also included six additional outgroup genera from the families Platypezidae and Pipunculidae, which are the closest families to Syrphidae (Skevington and Yeates, 2000; Wiegmann et al., 2011; Lambkin et al., 2013). A total of 96 taxa were used in the analyses, including 89 ingroup syrphid taxa. Appendix S1 of the online Supporting Information lists the species included in the analysis, the collection data and the GenBank accession numbers.

Adult morphological character coding

We used the morphological character matrix of Hippa and Ståhls (2005) (hereafter HS), which comprises 120 characters for 48 species, although a number of characters were excluded (see below). The matrix by HS includes scorings for characters of many of the taxa used in the present study, but new scorings were added for 62 taxa. Morphological studies were based on specimens present in the collection of the Zoological Museum of the Finnish Museum of Natural History (MZH) and at the Zoological Museum Alexander Koenig (ZFMK). A number of characters were excluded because the coding of the states would require scanning electron images (SEM) and these were not available (e.g. character numbers HS64, HS72, HS78, HS79), and a few other characters were also excluded as not being informative for the present study (e.g. HS5, HS60, HS108, HS109, HS116). As a result, a data matrix of 111 characters for adult morphology was scored for all 96 taxa for which molecular characters were available (see Appendices S2 and S3 of the online Supporting Information).

The coding of HS30 (pilosity of the dorsal part of the propleura) was changed, and in some taxa of Syrphinae the state 1 was coded instead of state 0 for the species with usual soft pile intermixed with shorter and stouter pile. For character HS32 (vestiture of postpronotum) an additional state was added: state 2 = postpronotum setose. The coding of HS43 (pilosity of the anterior part of the mesothoracic anepisternum) was undertaken for all taxa, including cases in which the anterior part of anepisternum is not differentiated as a flat area (coded as inapplicable by HS).

DNA extraction and sequencing

A large fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene, a fragment of the nuclear 18S rRNA gene (variable region V4), and the D2–D3 region of the nuclear 28S rRNA gene were used to performed the analyses. One to three legs, the entire abdomen or the entire specimen, either dry pinned or ethanol preserved, were typically used for DNA extraction. Extractions were carried out using the NucleoSpin Tissue DNA Extraction kit (Machery-Nagel, Düren, Germany) following manufacturer's instructions; samples were resuspended in 50 μL ultrapure water.

Remnants of specimens were preserved and labelled as DNA voucher specimens and deposited at the Zoological Museum of the MZH and at the ZFMK, as listed in Appendix S1.

The DNA primers and PCR amplification protocols for mitochondrial COI, and nuclear 28S and 18S rRNA genes were the same as those described in Mengual et al. (2008a). Amplified DNA was electrophoresed on 1.5% agarose gels for visual inspection of amplified products. The PCR products were enzymatically treated with ExoSap-IT (USB, Cleveland, OH, USA) and then sequenced in both directions using the PCR primers. The sequences were edited for base-calling errors and assembled using Sequence Navigator™ (Version 1.01, Applied Biosystems) and Geneious R6 (Version 6.1.6, Biomatters Ltd).

Sequence alignment

No gap was needed to manually align the sequences of the protein-coding COI gene. A total of 1128 nucleotide characters was obtained for the COI data matrix. We approached the alignment of 18S and 28S rRNA genes in two different ways, in order to also explore the effects of the rRNA alignment using its secondary structure. Initially, both genes were aligned using the multiple alignment using fast Fourier transform (MAFFT) program (Katoh et al., 2005, 2009), version 7, which implements iterative refinement methods (Katoh and Standley, 2013). The E-INS-I strategy was chosen because it is optimized for a small-scale alignment and recommended for sequences with multiple conserved domains and long gaps, such as rRNA genes (Katoh et al., 2009). The aligned small fragment of 18S used in this analysis had a total sequence length of 373 bp including gaps, and the D2–D3 region of 28S a total of 710 bp including gaps.

Second, 18S and 28S rRNA genes were aligned with reference to their secondary structure (e.g. Kjer, 1995, 2004; Kjer et al., 2001; Gillespie et al., 2004). For the 28S gene we implemented the model provided by Mengual et al. (2012). For the 18S gene we generated a

new secondary structure model based on the gene model published by Gillespie et al. (2006). For the first time the secondary structure of 28S and 18S is established and used for sequence alignment for the families Phoridae, Platypezidae, and Pipunculidae, and it is also the first time to provide a secondary structure model for 18S of Syrphidae (Fig. 1). Although we used only a small fragment of 18S in our analysis (variable region V4), this model represents a larger section that includes variable regions V3 and V4, and helices before, after, and between them (approximately 720 bp long without gaps). Regions of expansion and contraction (REC), which are small regions of ambiguous alignment (see Kjer et al., 2009), were not excluded from the phylogeny inference analysis in order to compare the exact same sequence using MAFFT and secondary structure alignment methods. As a result 369 bp were included in the analysis for 18S, and 669 bp for the D2–D3 region of 28S, including gaps.

A pruned, short version of 28S gene aligned using the secondary structure (613 bp) was used only as a test to explore whether regions of ambiguous alignment might bias the analyses results (see Table 1 and section Phylogenetic analyses). A pruned fragment was obtained by deleting the insert between 1c and 1c', the REC between 3c and 3c', the REC between 3d and 3e, the insert between 3e and 3e', and the REC between 3e' and 3d' (see Mengual et al. (2012) for reference).

Phylogenetic analyses

We used two different phylogenetic analysis methodologies, maximum likelihood (ML) and maximum parsimony, to compare the results of the different alignment strategies independently of the methodology used to analyse the data. The ML analyses were performed using the program Garli (genetic algorithm for rapid likelihood inference) Version 2.01.1067 (Zwickl, 2006, 2013), and maximum parsimony analyses using the program TNT (tree analysis using new technology) Version 1.1 (Goloboff et al., 2008). For both datasets, that is, data aligned using MAFFT and data aligned using the secondary structure, several runs using the datasets separately or in combination under ML and maximum parsimony were performed as follows: (i) morphological matrix; (ii) whole D2–D3 region of 28S gene; (iii) D2–D3 region of 28S gene excluding the regions with ambiguous alignment; (iv) all molecular data (COI, 28S and 18S); (v) all molecular data but using the pruned version of 28S; (vi) combined molecular and morphological data; and (vii) combined molecular and morphological data but using the pruned version of 28S (see Table 1).

Analytical runs using the Garli were performed on the Topaz cluster at the National Museum of Natural History, Smithsonian Institution (Washington, DC). Maximum parsimony analyses using TNT were performed on desktop PC, with 8 Core 3.40 GHz i7 and 8 Gb RAM. All trees were drawn with the aid of FigTree Version 1.3.1 (Rambaut, 2009).

Maximum parsimony analyses. Phylogenetic analyses using maximum parsimony were executed using the computer program TNT Version 1.1 and gaps were treated as missing data. The maximum parsimony analysis was carried using TNT search commands with default values for Sectorial Searches, Ratchet, Tree fusing and Drift algorithms. Bremer support values were calculated using TNT. Bootstrap support values were calculated with 1000 replicates.

Maximum likelihood analyses. For ML, the molecular dataset was divided into five partitions: first codon position of COI, second codon position of COI, third codon position of COI, 28S gene, and 18S gene. We determined the best choice of model for each partition using jModelTest 2.1.1 (Darriba et al., 2012) under the Akaike information criterion (AIC), as recommended by Posada and Buckley (2004), and analysed the data under the recommended models. For the morphological dataset we selected the Mkv model of Lewis (2001), which assumes that the data collected contain only variable characters (datatype = standardvariable) as suggested by Zwickl (2013). The TIM3 + I+G model was selected for COI position 1, GTR+I+G for COI position 2 and GTR+G for COI position 3. When MAFFT was used for the alignment, the TIM1 + I+G model was the best fit for 28S and the GTR+I+G model for 18S. These models differ from the the best-fit ones when the secondary structure of the rRNA genes was used: GTR+I+G model for 28S and TVM+I+G model for 18S.

Thirty independent runs (three different runs with the command searchreps = 10) were conducted for each of the analyses performed and the different datasets, using scorethreshforterm = 0.05 and significanttopochange = 0.0001 settings and using the automated stopping criterion, terminating the search when the ln score remained constant for 50 000 consecutive generations. Bootstrap support values were estimated from 1000 replicates using the same independent models in the Garli.

Results

Data

A total of 89 different flower fly species comprised the ingroup, representing 75 different genera. The mitochondrial COI dataset comprised 1128 nucleotide characters, and this gene was only partially obtained

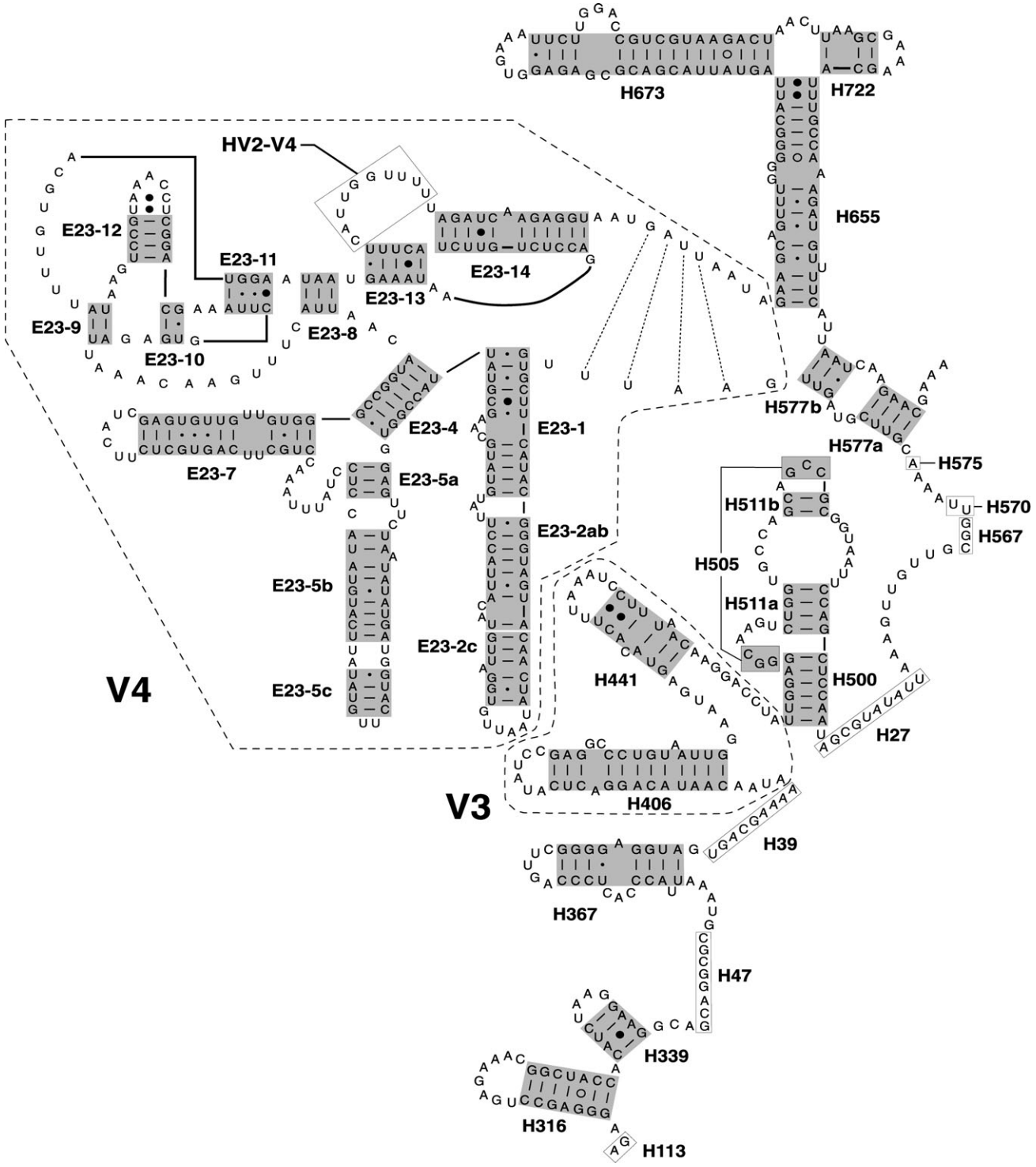

Fig. 1. The secondary structure model of the V3 and V4 variable regions and related core sequence of the SSU 18S nuclear rRNA from the syrphid *Caliprobola speciosa* (Rossi, 1790). Unambiguously aligned regions are in grey and notated following Gillespie et al. (2005). Base-pairing is indicated as follows: standard canonical pairs by lines (C–G, G–C, A–U, U–A); wobble G•U pairs by small black circles (G•U); A•G pairs by open circles (A○G); other non-canonical pairs by large black circles (e.g., U●U).

for six species: *Callicera aurata* (Rossi, 1790), *Copestylum vagum* (Wiedemann, 1830), *Metadon wulpii* (Mik, 1899), *Pipiza luteitarsis* Zetterstedt, 1843, *Pipiza femoralis* Loew, 1866 and *Rohdendorfia alpina* Sack, 1938 (see Appendix S1). Five hundred and seventeen nucleotides were maximum parsimony informative

Table 1
Summary of the different maximum parsimony and maximum likelihood analyses performed using several datasets with the two types of alignment. Morphology = only adult morphological data; 28S = only rRNA 28S gene; DNA = combined dataset with COI, 28S, and 18S sequences; Combined = combined datasets with morphological data and the sequences of COI, 28S, and 18S

	Morphology	28S s.a.	28S m.a.	28S cut s.a.	DNA s.a.	DNA m.a.	DNA cut s.a.	Combined s.a.	Combined m.a.	Combined cut s.a.
Maximum parsimony analyses										
Ingroup monophyly	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES
Syrphoidea	NO	YES	YES	YES	YES	YES	YES	YES	YES	YES
Microdontinae as sister group of the rest of syrphids	YES	NO	YES	NO	YES	YES	NO	YES	YES	YES
Microdontinae monophyly	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES
Syrphinae monophyly	YES	NO	YES	NO	YES	NO	NO	YES	YES	YES
Eristalinae monophyly	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO
Pipizinae monophyly	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES
Sister group of Pipizinae	Syrphinae	Eumerini + Eristalini	*Sphegina*	polytomy	Syrphinae	Syrphinae	Microdontinae + Syrphinae + Eristalinae	Syrphinae	Syrphinae	Syrphinae
Maximum Likelihood analyses										
Ingroup monophyly	NO	NO	YES	NO	YES	YES	YES	YES	YES	YES
Syrphoidea	NO	Pipunculidae inside ingroup	YES	Pipunculidae inside ingroup	YES	YES	YES	YES	YES	YES
Microdontinae as sister group of the rest of syrphids	NO	NO	NO	NO	NO	NO	NO	YES	YES	YES
Microdontinae monophyly	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES
Syrphinae monophyly	NO	NO	YES	NO	YES	YES	YES	YES	YES	YES
Eristalinae monophyly	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO
Pipizinae monophyly	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES
Sister group of Pipizinae	Eristalinae	*Sphegina*	*Spilomyia* + Microdontinae	*Sphegina*	Syrphinae	Syrphinae	Syrphinae	Syrphinae	Syrphinae	Syrphinae

s.a., structural alignment; m.a., MAFFT alignment; cut = pruned version of rRNA 28S gene used.

sites. The mean AT content of the ingroup COI sequences was 71.2%. The uncorrected pairwise sequence divergences for the COI gene between the ingroup taxa ranged from 0.6%, between *Sphaerophoria rueppellii* (Wiedemann, 1830) and *Sphaerophoria scripta* (Linnaeus, 1758), to 19.9% between *Peradon bidens* (Fabricius, 1805) and *Mesembrius peregrinus* (Loew, 1846), and between *Criorhina ranunculi* (Panzer, 1805) and *Pipizella certa* Violovitsh, 1981. The ribosomal D2–D3 region of the 28S rRNA gene was obtained for all ingroup taxa, and the 18S rRNA could not be amplified for five taxa (see Appendix S1).

Combined datasets

Here we present the results of the combined analyses of the three gene regions and the adult morphological characters using maximum parsimony and ML for the two alignments, that is, MAFFT and secondary structure.

The combined maximum parsimony analysis using MAFFT resulted in a single most parsimonious tree of length 10 601 steps, CI = 0.192, RI = 0.452 (Appendix S4, Fig. A15 of the online Supporting Information). The combined maximum parsimony analysis using secondary structure alignments resulted in 28 equally most parsimonious trees of 10 654 steps, CI = 0.192, RI = 0.444 (Appendix S4, Fig. A16), with all the polytomies located inside the subfamily Syrphinae. Both phylogenetic analyses resolved Syrphidae as monophyletic. Within Syrphidae, the subfamilies Microdontinae and Syrphinae were resolved as clades and microdontines placed as sister group of Syrphinae + Eristalinae. Pipizines were resolved as a monophyletic group, sister group of Syrphinae with the genus *Pipiza* as sister group of the rest of pipizines. The subfamily Eristalinae was resolved as non-monophyletic. The maximum parsimony analysis with the MAFFT alignment recovered Eumerini and some members of Brachyopini as sister group of the rest of Eristalinae + Syrphinae. On the other hand, the maximum parsimony analyses using the secondary structure resolved *Neoascia* and *Sphegina* as sister group of Eristalinae + Syrphinae.

The likelihood score for the best maximum likelihood tree, using MAFFT alignment, was –44 998.345073 (Appendix S4, Fig. A17). When the secondary structure alignment was used, the most likely tree score was –45 092.604947 (Fig. 2). Both likelihood trees compare favourably with the maximum parsimony trees (the resulting single tree using MAFFT and the strict consensus tree for the structural alignment) for the higher groupings: Microdontinae, Syrphinae, and pipizines as monophyletic groups (latest two resolved as sister groups) and Eristalinae as polyphyletic, with genera *Neoascia* and *Sphegina* as sister group of (rest of Eristalinae + (Syrphinae + pipizines)).

Results from each of the above four analyses differ below the family level, although some pattern may be seen in all of them, summarized here in Fig. 2. In Syrphinae, for example, the tribe Syrphini was always recovered as divided in two major clades: one group of syrphines including the genus *Paragus* (single genus of the tribe Paragini) and another group of Syrphini including *Toxomerus* (single genus of the tribe Toxomerini). In addition, the tribe Bacchini was never resolved monophyletic and mostly divided into two groups.

Relationships among the genera of Pipizini were very similar across methodologies and different alignments (Fig. 3). The genus *Pipiza* was always recovered as the sister group of the other pipizines, and *Neocnemodon* was always placed as sister group of *Pipizella*. Furthermore, the phylogenetic relationships among eristalines varied among methodologies and alignments. Despite this variation, *Neoascia* and *Sphegina* were always recovered together as the sister group of the rest of Eristalinae, Syrphinae, and pipizines (Fig. 2), except for the MAFFT alignment under maximum parsimony where the tribe Eumerini was placed in this position. Tribes Eumerini, Eristalini, Rhingiini, and Xylotini were recovered as monophyletic groups in all the combined analyses; the Volucellini tribe was also recovered as monophyletic but only in the ML trees.

Discussion

Differences between alignment strategies

Two different alignment strategies were used in the present study for the 28S and 18S rRNA genes. When we compare the inferred topologies using maximum parsimony and ML, we find that the most parsimonious tree, the one with fewer steps, and the phylogram with the best likelihood score were obtained when the MAFFT alignment was used. Both alignments basically differ in length, with the MAFFT alignment being 710 bp long for 28S and 372 bp for 18S, and the structural alignment being 669 bp and 368 bp respectively. Even with shorter global alignments, the analyses using the structural alignment reported longer cladograms and less likely trees (see Results section).

The structural alignment and the MAFFT alignment resolved the major groupings within Syrphidae in a similar way only when a "total evidence" approach was performed, following the terminology of Kluge (1989) (see Fig. 3). Combined analyses with all three molecular markers plus morphology resolved most of the tribes with the same sister-group relationships independently of the optimality criterion or alignment used.

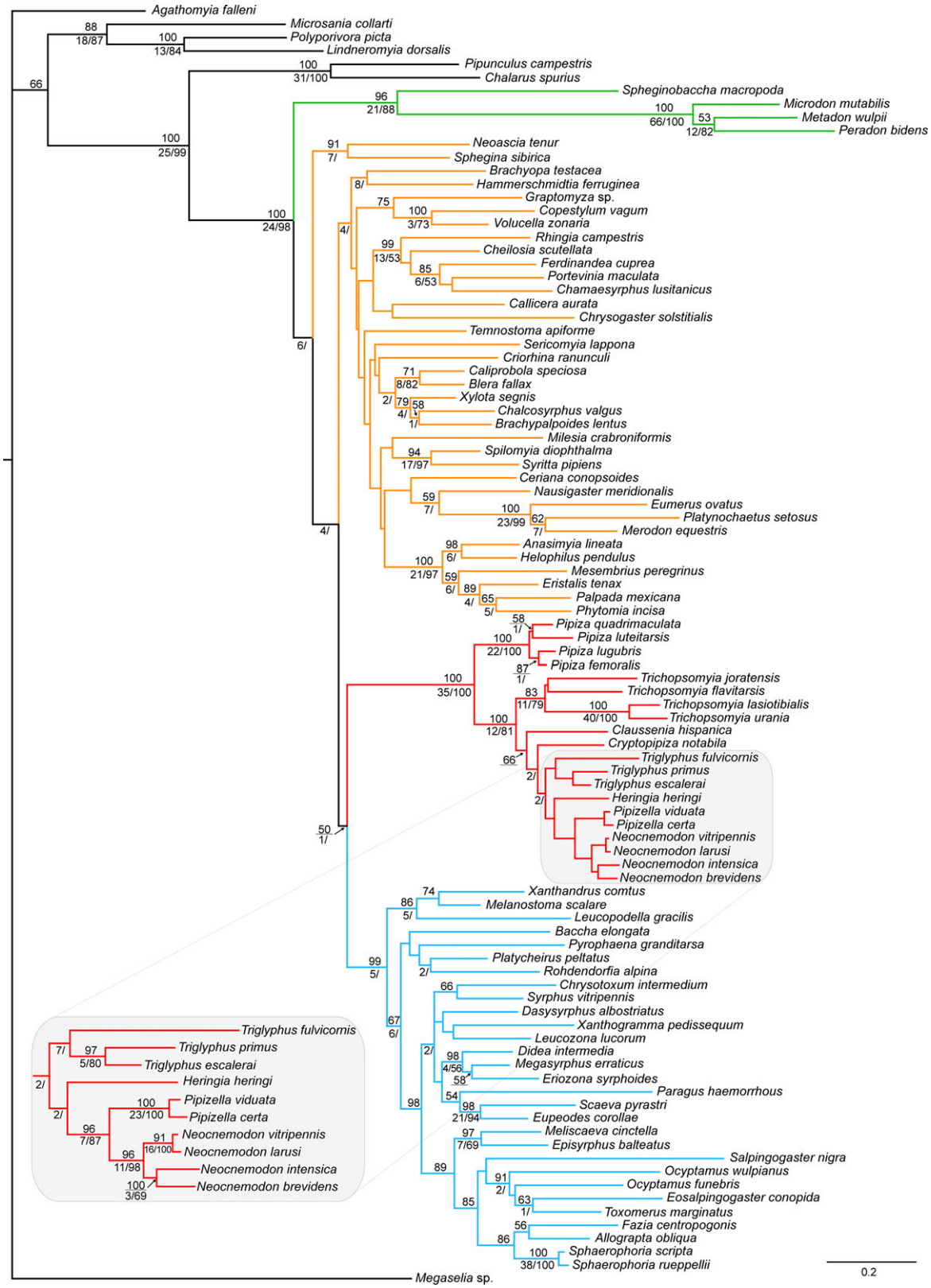

Fig. 2. The ML phylogenetic tree based on the combined dataset using the Garli and the structural alignment for 28S and 18S.. Values of bootstrap support are depicted above the nodes (> 50%); Bremer support values (left) and bootstrap resampling values (right) from maximum parsimony analysis are indicated below each node (> 50%). Legend: black: outgroups; green: Microdontinae; orange: Eristalinae; red: Pipizinae; and blue: Syrphinae.

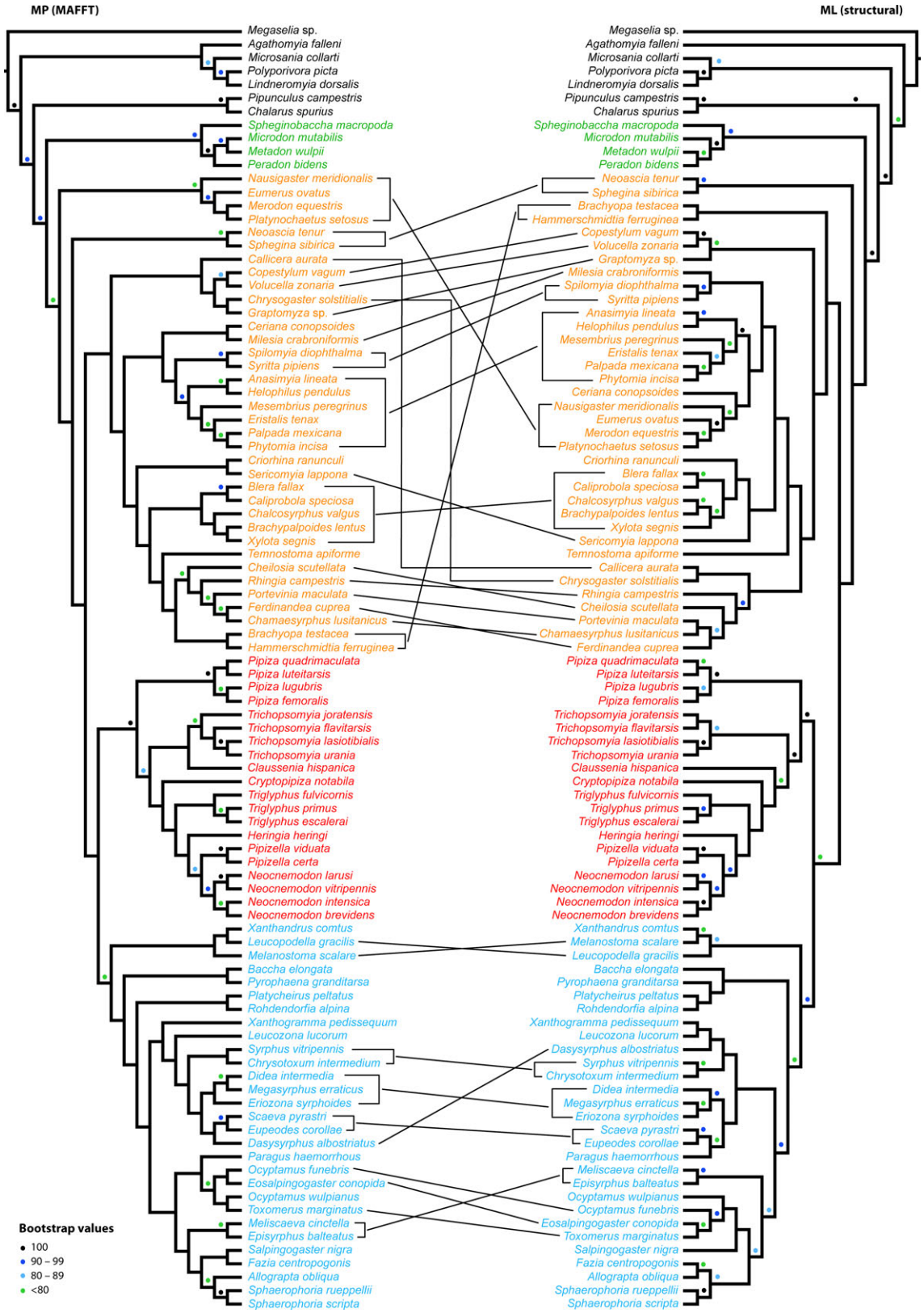

Fig. 3. Topological differences between maximum parsimony cladogram using the MAFFT alignment for 28S and 18S (left) and ML phylogram using the structural alignment for 28S and 18S. Black lines link the same taxon/taxa in both trees; where the taxon is found exactly opposite each other in both trees, no line is present.

Different analyses with only the morphological data, the 28S gene, and the combined molecular markers were performed as indicated in Table 1. Analyses using the same optimality criterion and dataset but different alignments show differences in the major groupings (see Appendix S4). The ML analysis using the three molecular markers resolved pipizines in two different positions: with *Callicera* and *Graptomyza* as sister groups when MAFFT alignment was used, or as sister group of the subfamily Syrphinae with the structural alignment. Maximum parsimony analyses based on the three molecular markers resolved the major groups of Eristalinae, pipizines as sister group of syrphines, and eristalines as non-monophyletic in a different way, with some members of the tribe Bacchini (Syrphinae) within Eristalinae.

Our analyses indicate that the type of alignment has an evident effect on the inferred phylogeny, as MAFFT and structural alignments resolved the syrphid groups in different ways. This effect concerns the alignment of rRNA genes, which have their own evolutionary history and rate of nucleotide substitution. These genes are exposed to numerous deletions and additions of DNA fragments of different size resulting in a crucial length heterogeneity for different taxa, favoured by the absence of a protein-coding process (Giribet and Ribera, 2000; Gillespie et al., 2005; Laurenne et al., 2006). Based on our results and dataset, it seems that the D2–D3 region of the 28S rRNA gene is more similar among members of the tribe Syrphinae than among the eristalines. The sequence length differences found inside the D2–D3 region of 28S between members of the subfamily Eristalinae are larger than between Syrphinae members, and the multiple sequence alignment algorithms in programs such as MAFFT might not be sufficiently sensitive to find and align homologous nucleotide sites due to this sequence length disparity of the 28S, even between closely related taxa.

Also, we observed that regions of expansion and contraction (REC) and inserts in rRNA genes, deleted in the pruned version of the 28S gene, do have a phylogenetic signal and may help resolve some relationships. Inferred phylogenies with the pruned version of 28S resolved major groupings in a different way, especially when maximum parsimony was used as optimality criterion (see Appendix S4).

Syrphidae clades

In agreement with previous studies, the results of our work also hint towards an evolutionary scenario where three subfamilies within Syrphidae are easily distinguishable, although this scenario was not fully recovered (Figs 2 and 3). The subfamily Microdontinae, including *Spheginobaccha*, was always placed as sister group of the remaining ingroup taxa, in agreement with previous results and hypotheses (Hull, 1949; Thompson, 1969, 1972; Shatalkin, 1975a; Skevington and Yeates, 2000; Ståhls et al., 2003; Hippa and Ståhls, 2005; Rotheray and Gilbert, 2008; Reemer and Ståhls, 2013). This position, and its high support values, does not contradict the opinion that microdontines might be considered an independent family (Thompson, 1969, 1972; Speight, 1987), nor does it contradict the taxonomic rank of subfamily within Syrphidae (Reemer and Ståhls, 2013).

Syrphinae was also recovered as monophyletic in all the combined analyses. Results from the present analyses agree broadly with those of Mengual et al. (2008b). The current concept of the tribe Bacchini (Vockeroth, 1992) is polyphyletic and members of this tribe are recovered in two main groups (three groups using maximum parsimony as optimality criterion; Fig. 3) in partial congruence with previous classifications (Hull, 1949; Wirth et al., 1965; Shatalkin, 1975a): one clade with the genera *Xanthandrus*, *Melanostoma* and *Leucopodella*, and another clade with *Baccha*, *Pyrophaena*, *Rohdendorfia* and *Platycheirus*. The tribe Syrphini is resolved into two main groups as in Mengual et al. (2008b), with tribes Paragini and Toxomerini clearly embedded within Syrphini. In our opinion, the tribal classification of Syrphinae needs a better understanding and new morphological characters to define clades within the subfamily. What seems obvious is that there are two main evolutionary lineages within Syrphini, that the tribe Toxomerini is part of a larger lineage, based on molecular and morphological evidence (Mengual et al., 2008a, 2012), and that there is no clear evidence about the possible sister group of the tribe Paragini.

On the other hand, we never recovered Eristalinae as monophyletic in any combined analysis (Figs 2 and 3). Hippa (1978) suggested that the aphidophagous (predatory) and the non-aphidophagous Syrphidae do not represent equivalent divisions. It is not our intention here to discuss in depth the groupings in Eristalinae, as this study was not designed for that purpose, although some notes are given based on our results. Genera *Neoascia* and *Sphegina* were consistently recovered as the sister group of Eristalinae + pipizines + Syrphinae, a position similar to that obtained for these two genera in the cladogram based on morphological characters by Hippa and Ståhls (2005). *Neoascia* and *Sphegina* belong to Brachyopini, subtribe Spheginina, and form a natural group in the words of Thompson (1972) and based on male genitalia characters (Glumac, 1960). In the present study, the subtribe Brachyopina (Brachyopini) was placed in two different positions: as the sister group or first branch within Eristalinae (except Spheginina) in ML analyses or as the sister group of the tribe Rhingiini in the maximum parsimony analyses.

Rhingiini was found monophyletic in all our combined analyses with high bootstrap values, in agreement with previous works (Thompson, 1972; Shatalkin, 1975b; Ståhls et al., 2004). Genus *Rhingia* was always recovered as sister group of the other Rhingiini members in the ML analyses, but *Cheilosia* took this position in the maximum parsimony analyses. We consistently found a clade formed by the genera *Ferdinandea*, *Portevinia* and *Chamaesyrphus* with relatively high bootstrap values. The present results largely agree with results of Ståhls et al. (2004).

Volucellini was recovered in all the combined analyses with relatively high support, and the genus *Graptomyza* was placed as sister group of *Volucella* and *Copestylum*. This arrangement supports the diphyletic origin of the Old World forms of this tribe, which was one of the phylogenetic scenarios suggested by Thompson (1972) and according to the subdivision by Hull (1949). Our results show a difference between maximum parsimony analyses, where the genus *Chrysogaster* (tribe Brachyopini) was recovered as sister group of *Graptomyza*, and ML analyses, where the tribe is monophyletic. Ståhls et al. (2003) included one *Graptomyza* and three species of *Volucella* in their combined analysis using maximum parsimony under optimization alignment, and did not recover the taxa as sister groups in any of the different weighting schemes. The present data are not sufficient to suggest a placement for *Chrysogaster*, but it would not be within Volucellini. There are many good morphological characteristics that support Volucellini as monophyletic as resolved in the ML analyses (see Thompson, 1972; Shatalkin, 1975b).

Another monophyletic tribe always recovered in our combined analyses was Eumerini, as suggested by Thompson (1972). Genus *Nausigaster*, previously referred to its own subfamily (Hull, 1949) or to Microdontinae (Shatalkin, 1975b), was placed as sister group of the remaining members of the tribe. Hull (1949) related *Nausigaster* to Eumerini but pointed out the morphological resemblances between this tribe and Microdontinae. Ståhls et al. (2003) recovered the taxon in various positions under the different weighting schemes. The placement of the tribe varied between ML and maximum parsimony analyses (Fig. 3).

Tribes Ceriodini and Callicerini were represented in our study by only one species each. Ceriodini comprises high-fidelity mimics of wasps and four genera are currently recognized (Thompson, 2013b). *Ceriana* was placed in two different positions dependent upon the methodology used: it was recovered as sister group of Eumerini in ML analyses or it was found as sister group of *Milesia* under maximum parsimony. Results from the combined analyses placed *Callicera* in several positions, although in all of them somehow related to Volucellini and/or Rhingiini (Fig. 3). Only the parsimonious analysis using a structural alignment recovered *Callicera* as sister group of Eristalini. Neither *Ceriana* or *Callicera* show clear affinities to other groups based on male genitalia and morphological characters, as they are either quite unique (Shatalkin, 1975b) or show a mix of derived traits and very primitive ones, as in the case of ceriodines (Thompson, 1972), and increased taxon sampling is vital in future analyses for resolving the placements of these taxa.

The tribe Eristalini was always recovered as monophyletic. This tribe was previously divided in three subtribes, namely Sericomyiina, Helophilina, and Eristaliina (Thompson, 2003). Recently, Skevington and Thompson (2012) recognized Sericomyini as a different tribe comprising four genera, in congruence with previous classifications (Hull, 1949 as subfamily; Glumac, 1960; Wirth et al., 1965; Thompson, 1972; see comments in Shatalkin, 1975b; p. 133; Peck, 1988). Our results mostly agree with this division. *Sericomyia* was never resolved as sister group or close to Eristalini, but in several positions within Eristalinae *sensu lato*.

The tribe Milesiini is a heterogeneous group, best defined by the exclusion of morphological characters (Thompson, 1972). The included members of this tribe were resolved in variable positions in the resulting topologies. The monophyly of the tribe should be tested in future studies including a much larger representation of milesiines.

Finally, Xylotini was recovered as monophyletic in all our combined analyses (as stated by Glumac, 1960; Thompson, 1972; Shatalkin, 1975b; Hippa, 1978), with the genus *Xylota* placed as sister group of *Chalcosyrphus* and *Brachypalpoides* in congruence with the results of Ståhls et al. (2003), except for the maximum parsimony analysis using the MAFFT alignment (Fig. 3).

Results from the present study indicate that single representatives of particular clades, such as *Ceriana*, *Callicera*, *Nausigaster*, and *Neoascia* among others, have no consistency in placement between the optimization (Ståhls et al., 2003), MAFFT or structural alignments. Thus their phylogenetic affinities must be explored separately in studies with particular emphasis on these taxa and broad sampling of many species from these genera. The monophyly of most tribes of Eristalinae needs to be re-evaluated in phylogenetic studies including larger taxon representation.

Types and evolution of predation in Syrphidae

Larval predatory habits are found in the three main lineages of Syrphidae: (i) Microdontinae; (ii) Eristalinae, genera *Volucella* and *Nepenthosyrphus*; and (iii) the clade Pipizinae + Syrphinae. Each of these lineages exhibits very different biological and ecological requirements with distinctive morphological character

adaptations. In the case of Eristalinae, the larval morphology of the few species with zoophagous habits is greatly altered in response to the challenges involved in predation (Rotheray and Gilbert, 2011). Seven species of genus *Volucella* are associated with social wasps and bees but only one species is strictly predatory, the remainder has a mixed saprophagous–predacious feeding mode (Rotheray, 1999). The known larvae of *Nepenthosyrphus* are sit-and-wait aquatic predators in water tanks of pitcher plants. In both cases the predatory way of life probably arises from a different larval feeding mode such as saprophagy (Rotheray, 2003; Rotheray and Gilbert, 2011). Conversely, the monophyletic lineages of the Pipizinae + Syrphinae and Microdontinae are extremely large radiations that exploit two main types of prey: a wide range of soft-bodied arthropods (mainly hemipterans) in the former case, versus the high specialization of the myrmecophagous habits of microdontines. Thorax and mouthparts vary between both groups, and they have different means of gripping and piercing prey and locomotory mechanisms (Rotheray and Gilbert, 1999), which suggest an independent origin for each group of predatory syrphids.

As result of the present study, a common predatory ancestor for Pipizinae and Syrphinae seems plausible, with this particular larval feeding mode on soft-bodied hemipteran evolving only once in the evolution of the Syrphidae. Nonetheless, some interesting differences can be observed when comparing life cycle and feeding habits of both groups.

Compared with Syrphinae, larvae of Pipizinae are related only with three of the five groups of hemipteran sternorrhynchans, that is, Aphidoidea, Phylloxeroidea, and Psylloidea. Moreover, they prey on a narrow range of aphids, adelgids, phylloxerans, and jumping plant lice (Rojo et al., 2003), frequently gall-forming species with waxy secretions and/or subterranean feeding habits (Heiss, 1938; Stubbs and Falk, 2002). However, pipizines have not been reported preying on other sternorrhynchans such as Aleyrodoidea and Coccoidea, which are prey of syrphines in different parts of the world (Rojo et al., 2003). In the first case, gall formation is rare among whiteflies (Byrne, 2005), but waxy secretion, gall-inducing, and subterranean habits are known in many species of scale insects (Gullan and Kosztarab, 1997). Gall-inducers are known from ten coccoid families, but the highest proportion belongs to Eriococcidae (felt scales) and reaches close to 60% of the total if species that produce simple pits or depressions are excluded (Cook and Gullan, 2004). Furthermore, more than 80% of eriococcid gall-inducers around the world occur in Australia (Gullan et al., 2005), predominantly on sclerophyllous plants of the Myrtaceae. The absence of coccidophagous pipizines could be explained by the special characteristics of the scale insect's galls. Most adult female scale insects never leave their galls and their offspring exit via the small apical orifice through which she can eliminate her waste honeydew and also mate with males. Normally a single female is oriented with its head towards the base of the gall and its anal lobes towards the apex with heavily sclerotized anal lobes that act as protection against parasitoids and predators. In contrast, galls induced by aphids, adelgids, phylloxerans, and psyllids are occupied by offspring during their development. These reasons are also valid in the case of syrphines, because only a single eriococcid species has been cited as prey of syrphines until now, that is, the cottony scale *Capulina* aff. *jaboticavae* (Cermeli and Geraud-Pouey, 1997). This gregarious scale insect occurs on the bark of the trunk, branches, and the leaf underside of the Myrtaceae *Psidium guajava* L., but do not induce galls (Cermeli and Geraud-Pouey, 1997).

Due to the fact that some aphidophagous syrphines lay fertile eggs freely on non-infested plants, Chandler (1968a) proposed that predatory flower flies in which the prey is the paramount oviposition stimulus may be termed primarily *aphidozetic* (prey-seeking), whereas those predatory species for which the plant stimulus is dominant may be called primary *phytozetic* (plant-seeking). Pipizinae species feed mostly on gall-forming and root hemipterans, the colonies of which are not exposed at the surface of the plant or soil. Thus, the stimulus for laying fertile eggs might be the presence of the gall or the vegetal tissue malformation, plus the possible release of kairomones by the plant, which could help in the location of the colonies. This has been reported for different species of Pipizinae. Rojo and Marcos-García (1997) detailed the behaviour observed for *Pipiza festiva* Meigen, 1822 (now *Pipiza noctiluca* (Linnaeus, 1758)) and *Heringia heringi* (Zetterstedt, 1843) whose "females laid the eggs on the surface of the galls and the larvae got into the gall by cracks or by the hole formed by the aphid exit." Bergh and Short (2008) indicated a similar behaviour for *Neocnemodon calcarata* (Loew, 1866) and they also observed eggs in the soil where an aphid colony was 2 cm below the soil surface. Females of this species can be found flying and walking near the bases of trees that can exhibit galls on exposed roots.

Pipizinae could be considered phytozetic like some members of the current Bacchini, for example, genera *Melanostoma* and *Platycheirus* that lay batches of eggs in plants free of prey (see Chandler, 1967, 1968a,b; and references therein). Chandler (1968a) hypothesized that the plant location system was the most primitive and the aphid location system gradually replaced it in the evolution of the truly aphidophagous Syrphinae. Unfortunately most of the evidence that Chandler presented, especially facultative phytophagy of phytozetic species, is actually questionable as predatory larvae do

not eat plant tissues (Rotheray and Gilbert, 2011). However, the phytozetic behaviour of pipizines, that is, the gall as stimulus for oviposition and laying eggs on the soil, is genuine. In this sense, we agree with Dušek and Láska (1966) that the narrow oligophagy of pipizines might start with the use of galls as shelters, as some pipizines use larval galleries of poplar clearwing moth, *Paranthrene tabaniformis* (Rottemburg, 1775), and poplar longhorn beetle, *Saperda populnea* (Linnaeus, 1758), as winter shelters (Georgiev et al., 1998, 2004). Secondarily, pipizines using these galls started to prey on waxy aphid inhabitants, which frequently have root-feeding habits. Galls as phytozetic stimulus could also explain the presence of pipizine larvae preying on gall-inducer psyllids (Rojo et al., 1999) and on non-gall forming aphids but that cause important leaf-curling damage (Rojo and Marcos-García, 1997). According to this hypothesis, the pipizine predatory ancestor probably was phytozetic, with the plant (galls) being the stimulus for laying eggs, and their larvae preyed on gall-former hemipterans.

The evolutionary scenario presented by (previous authors) is in agreement with the results obtained in the present study: Pipizinae is the sister group of Syrphinae and the current tribe Bacchini, or some of the groups in which it is divided, resolved as sister group of the remaining Syrphinae. Thus, phytozetic syrphids might have evolved into aphidozetic taxa, which became predatory specialists of Sternorrhyncha (Hemiptera), mainly aphids (Aphidoidea) but also whiteflies (Aleyrodoidea), psyllids (Psyllidae), and scale insects (Coccoidea). Later in the evolution of the Syrphinae, other types of hemipterans and soft-bodied arthropods were also preyed upon and even some species became secondarily phytophagous or pollen feeders (Riley and Howard, 1888; Marín, 1969; Nishida et al., 2002; Weng and Rotheray, 2008; Reemer and Rotheray, 2009; Van Zuijen and Nishida, 2011).

Phylogenetic relationships and ranking of pipizines

In this section we discuss arguments towards revising the taxonomic rank of the tribe Pipizini. The monophyly of the pipizines was always supported by the results of the combined datasets. As cited earlier, pipizines are a well-defined aggregate based on morphological characters of immature stages and adults, larval biology, and male genitalia (Glumac, 1960; Thompson, 1972; Rotheray and Gilbert, 1989; Coovert, 1996; Hippa and Ståhls, 2005). The biology of many Pipizini species is still unknown, but notes about their prey (i.e. Rojo et al., 2003) and studies about their role in biological control of pests can be found in the literature (Rojo and Marcos-García, 1997; Bergh and Short, 2008; and references therein). In contrast to the aphidophagous syrphinae larvae, the pipizines show a preference for woolly or root aphids, usually with waxy secretions, and for gall-forming species (see Heiss, 1938; Dušek and Křístek, 1967; Rotheray, 1993; Stubbs and Falk, 2002). The larvae of Pipizini have long posterior respiratory processes and their spiracular slits are borne on tall carinae. These characters may represent an adaptation of preying on gall and root aphids, to avoid the spiracular slits becoming coated with honeydew and wax secretions, which, in the confined spaces in galls and around roots, tends to build up in large quantities (Rotheray and Gilbert, 1989).

Pipiza was resolved as sister group of the rest of pipizines in all combined analyses (Figs 2 and 3) in congruence with results of Vujić et al. (2013), and as suggested by Thompson (1972) based on the lack of a postanal hood in males, a specialization found only in the pipizines. Curran (1921) and Shatalkin (1975b) also argued this separation of *Pipiza* from the rest of pipizines genera. The other genera were resolved as a clade with high support values and the relationships among them were similar to those recovered by Vujić et al. (2013), with *Neocnemodon* and *Pipizella* as sister groups (Fig. 3).

Based on the present results, it seems clear that there are three well-defined and well-supported clades within the radiation of Syrphidae: Microdontinae, Syrphinae, and pipizines (Fig. 3). In all combined analyses pipizines were placed as sister group of a monophyletic Syrphinae with very high support values, in agreement with previous works (Rotheray and Gilbert, 1999, 2008; Ståhls et al., 2003; Hippa and Ståhls, 2005).

Thompson (1969) placed pipizines as sister group of Syrphinae using a Hennigian argumentation scheme based on the number of abdominal segments and the feeding habits of larvae. Previously, Hartley (1961) assumed that aphidophagous larvae have developed only once during the evolution of Syrphidae and included pipizines into Syrphinae. Coovert (1996) expressed himself in the same line of thought and pointed out that the reduction of the pregenital abdominal segments in pipizines would represent a third line of reduction (parallelism) after microdontines and eristalines, which also have this reduction in number. Furthermore, Coovert (1996) stated that the evolutionary history of the pipizines is an example of discordant evolution (Michener, 1977), where pipizines acquired a predatory larva like syrphines but retained plesiomorphic adult characters. This argument fits well with the phylogenetic relationships recovered in the present study but disagrees with Thompson (1972), who considered Pipizini the most primitive tribe of the eristalines based on several adult morphological characters. Vujić and Glumac (1993) reiterated the same idea of an early separation between pipizines and syrphines.

Karyological data also support this placement for pipizines, with a reduction of chromosome numbers along the evolution of the Syrphidae family. Boyes et al. (1980) reported that four studied members of Microdontinae have 2n = 14. Earlier, Boyes et al. (1971) proposed that the diploid number of chromosomes 2n = 12 was the more primitive chromosome number in the Syrphidae. With some variation and overlapping, most studied species of Eristalinae have 2n = 10 or 12 chromosomes, whereas most species of Syrphinae have 2n = 8 or 10. Boyes and van Brink (1972) reported that 22 out of 24 studied pipizines have 2n = 8 chromosomes, suggesting also the inclusion of pipizines into Syrphinae.

The relative ranking of a group depends on its position in the phylogeny of the whole group (Thompson, 1969), although the ambiguity of the criteria by which taxonomic ranks above the species level are determined is a serious problem in systematics (Hennig, 1966). Nazari et al. (2007) summarized the criteria to formally recognize higher ranks based on other studies (references therein): (i) monophyly; (ii) taxonomic stability; (iii) inclusion of a consistent number of species; (iv) documentation of a decided gap, including genetic distance, separating the higher taxon from another taxon of the same rank; and (v) geological age. Previous and present results corroborate the monophyly of pipizines with different datasets and the group is well defined, so there is stability and a consistent number of species, even if we consider the new intergeneric classification proposed by Vujić et al. (2013) and followed here. Nowadays the molecular distances are no longer used to define ranks, but tree topology and branch support are (Nazari et al., 2007). The tree topology and branch support using two different techniques for alignment and under different optimality criteria for tree searching are congruent in defining a well-supported clade including all the pipizines.

There are more than 100 fossil taxa in Syrphidae recognized within 38 genera, most of them from Eocene and Oligocene epochs (Hull, 1945; Evenhuis, 1994; but see Kovalev, 1979). Interestingly, the majority of the Eristalinae fossils date back from the Eocene (56–34 Ma) and the majority of Syrphinae fossils are from the Chattian age of the Oligocene (28.1–13 Ma), with some older syrphines such as *Asarkina* and *Syrphus* (Eocene: Ypresian to Lutetian) (Evenhuis, 1994). Earliest fossils of pipizines, *Pipiza* (*Pseudopipiza*), are found in Baltic amber, that is, Eocene–Oligocene epochs. Although the fossil record is incomplete, it seems probable that eristalines are older and that syrphine and pipizine lineages appeared very close in time during the evolution of Syrphidae (see Grimaldi and Cumming (1999) and Blagoderov et al. (2002) for more details).

Based on all the evidence mentioned above, we propose to revise the taxonomic rank of the tribe Pipizini to subfamily Pipizinae stat. rev. This is not the first time that pipizines are ranked above tribal level because Williston (1885) originally introduced the family-group name Pipizinae for this clade. Kuznetzov (1987, 1992, 1993) studied the morphology of first instar larvae of syrphids and found a unique state of the posterior respiratory process (prp) for pipizines: the prp consisting of separate tubes positioned on two more or less strongly sclerotized processes on the eighth abdominal segment. This characteristic and others of the immature stages, such as quadrate mandibles and the tip of the anal segment and its transverse bar, prompted him to consider the subfamily rank for Pipizinae (Kuznetzov, 1992), as previously suggested by Rotheray and Gilbert (1989), also based on larval characters of the third instar, and by Borisova (1983, 1984) based on the morphology of the female ovipositor. Moreover, Kuznetzov (1988) studied the egg morphology of syrphids and found a unique feature of pipizines: instead of having complex elements of the chorion ultrastructure to improve the active absorption of oxygen, pipizines have a ribbed egg surface.

Conclusion

Based on morphological and molecular evidence, our results support an evolutionary scenario for Syrphidae where three subfamilies are easily recognized and resolved as monophyletic: Microdontinae, Syrphinae, and Pipizinae stat. rev. Eristalinae is recovered as a non-monophyletic group in the present study. All analyses of combined datasets using different alignment strategies resolve Pipizinae and Syrphinae as sister groups and strongly suggest a common origin of the two groups, with predatory larvae feeding on soft-bodied arthropods. Present results indicate that this kind of predation has evolved only once in the evolution of the Syrphidae.

When small clades are well defined morphologically, molecularly, and phylogenetically, they warrant recognition. The results presented here, the supporting molecular characters, immature stage morphology (egg, first instar larva, third instar larva), adult morphology, female ovipositor, and male genitalia characters lead us to recognize the subfamily Pipizinae stat. rev., and to highlight this unique combination of immature stage morphology, larval biology, and adult morphological characters.

Finally, it is worth mentioning that the structural alignment and the MAFFT alignment resolved the major groupings within Syrphidae in a similar way only when all the molecular markers and morphology were combined. There is an evident effect of the

alignment in the inferred phylogeny and regions with ambiguous alignment may harbour important phylogenetic information, with the MAFFT alignment producing the most optimal topologies as to tree length or likelihood score.

Acknowledgements

The authors thank numerous researchers and collectors who made specimens available for molecular study. We are grateful for logistical support and generous help in collecting material during field work to the followings entities and people: ValBio (Madagascar), INBio (Costa Rica), IVIC, UCV, and UCLA (Venezuela), Universidad del Valle (Colombia), SENASA and Universidad Nacional Agraria La Molina (Peru), Manuel Zumbado, Nancy Carrejo, Carlos Prieto, J. David Nelo, Evelin Arcaya, Graciano Tejada, Yelitza Velasquez and Alexander Rodríguez. We thank CONAGEBIO for permit number 091-2004-OFAU for insect collection for scientific study.

We especially thank Mónica Muñoz-Torres for valuable comments and corrections on a previous version of the manuscript. We are indebted to Matthew Kweskin for solving our doubts when using the Topaz cluster (Smithsonian Institution). This work was partially funded by grant AP2003-3998 of the Spanish Ministry of Education and Science (Ministerio Español de Educación y Ciencia) awarded to X. Mengual.

References

Bartsch, H.. 2009. Tvavingar: Blomfugor (Diptera: Syrphidae: Eristalinae & Microdontinae). ArtDatabanken, Uppsala, Sweden.

Bergh, J.C., Short, B.D., 2008. Ecological and life-history notes on syrphid predators of woolly apple aphid in Virginia, with emphasis on *Heringia calcarata*. Biocontrol 53, 773–786.

Blagoderov, V.A., Lukashevich, E.D., Mostovski, M.B. 2002. Order Diptera Linné, 1758. The True Flies. In: Rasnitsyn, A.P., Quicke, D.L.J. (Eds.), History of Insects. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 227–242.

Borisova, V.G. 1983. Morfologiya yaytseklada i sistema mukh semeystva Syrphidae [= The morphology of the ovipositor and systematics of the flies of family Syrphidae]. In: Skarlato, O.A. (Eds.), Dvukrylyye Nasekomyye, ikh Sistematika, Geograficheskoye Rasprostraneniye i Ekologiya [= Two-Winged Insects, Their Sytematics, Geographic Distribution and Ecology]. Zoological Institute, USSR Academy of Sciences, Leningrad, pp. 7–10 [in Russian].

Borisova, V.G. 1984. Morfologiya yaytsekladov i potentsialnaya plodovitost mukh semeystva Syrphidae (Diptera) v svyazi s ikh ekologiyey [= The morphology of the ovipositors and potential fecundity of the flies of family Syrphidae (Diptera) in connection with their ecology]. PhD thesis, Moscow State University. Moscow [in Russian].

Boyes, J.W., van Brink, J.M., 1972. Chromosomes of Syrphidae VI. The tribe Pipizini. Genetica 43, 321–333.

Boyes, J.W., van Brink, J.M., Boyes, B.C., 1971. Chromosomes of Syrphinae (Diptera: Syrphidae). Misc. Publ. Genet. Soc. Can. 1, 1–158.

Boyes, J.W., van Brink, J.M., Boyes, B.C., Vockeroth, J.R., 1980. Chromosomes of Eristalinae and Microdontinae (Diptera: Syrphidae). Misc. Publ. Genet. Soc. Can. 3, 1–137.

Brown, B.V. 2009. Introduction. In: Brown, B.V., Borkent, A., Cumming, J.M., Wood, D.M., Woodley, N.E., Zumbado, M.A.. (Eds.), Manual of Central American Diptera, Vol. 1. NRC-CNRC Research Press, Ottawa, pp. 1–7.

Brunetti, E.. 1923. Diptera. Pipunculidae, Syrphidae, Conopidae, Oestridae. In: Shipley, A.E. (Eds.), Fauna of British India Including Ceylon and Burma, Vol. III. Taylor & Francis, London.

Byrne, D.N. 2005. Gall-inducing whiteflies (Hemiptera: Aleyrodidae). In: Raman, A., Schaefer, C.W., Withers, T.M. (Eds.), Biology, Ecology, and Evolution of Gall-Inducing Arthropods, Vol.1. Science Publishers, Enfield, pp. 133–141.

Cermeli, M., Geraud-Pouey, F.. 1997. *Capulinia* sp. cercana a *jaboticabae* von Ihring (Homoptera: Coccoidea, Eriococcidae) nueva plaga del guayabo en Venezuela. Agron. Trop. 47, 125–126 [in Spanish].

Chandler, A.E.F., 1967. Oviposition responses by aphidophagous Syrphidae (Diptera). Nature 18, 736.

Chandler, A.E.F., 1968a. Some host-plant factors affecting oviposition by aphidophagous Syrphidae (Diptera). Ann. Appl. Biol. 61, 415–423.

Chandler, A.E.F., 1968b. The relationship between aphid infestation and oviposition by aphidophagous Syrphidae (Diptera). Ann. Appl. Biol. 61, 425–434.

Cheng, X., Lu, J., Huang, C., Zhou, H., Dai, Z., Zhang, G., 2000. Determination of phylogenetic position of Pipizini (Diptera: Syrphidae): based on molecular, biological and morphological data. Sci. China C 42, 146–156.

Cook, L.G., Gullan, P.J., 2004. The gall-inducing habit has evolved multiple times among the eriococcid scale insects (Sternorrhyncha: Coccoidea: Eriococcidae). Biol. J. Linn. Soc. 83, 441–452.

Coovert, G.A. 1996. A revision of the genus *Pipiza* Fallén (Diptera, Syrphidae) of America north of Mexico, with notes on the placement of the tribe Pipizini. Ohio Biol. Surv. Bull. New Ser. 11, i–viii + 68 pp.

Curran, C.H., 1921. Revision of the *Pipiza* group of the family Syrphidae (flower-flies) from north of Mexico. Proc. Calif. Acad. Sci. Ser. 11, 345–393.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9, 772.

Dušek, J., Křístek, J. 1967. Zur Kenntnis der Schwebfliegenlarven (Diptera, Syrphidae) in den Gallen der Pappelblattläuse (Homoptera, Pemphigidae). Z. Angew. Entomol. 60, 124–136 [in German].

Dušek, J., Láska, P. 1966. Occurrence of syrphid larvae on some aphids. In: Hodek, I. (Ed.), Ecology of Aphidophagous Insects: Proceedings of a Symposium Held in Liblice Near Prague, September 27–October 1, 1965. W. Junk Publishers, Hague and Academia, Prague, pp. 37–38.

Dušek, J., Láska, P. 1967. Versuchzum Aufbau eines naturlichen Systems mitteleuropaischer Arten der Unterfamilie Syrphinae (Diptera). Acta Sci. Nat. Brno 1, 349–390 [in German].

Efflatoun, H.C. 1922. A monograph of Egyptian Diptera, Part I. Fam. Syrphidae. Mem. Soc. Ent. Egypte 2, 122 pp., 6 plates.

Evenhuis, N.L. 1994. Catalogue of the Fossil Flies of the World (Insecta: Diptera). Online version. Available from http://hbs.bishopmuseum.org/fossilcat/ (accessed on 17 March 2014).

Fischer, O.A., Mátlová, L., Dvorská, L., Svástová, P., Bartos, M., Weston, R.T., Pavlík, I., 2006. Various stages in the life cycle of syrphid flies (*Eristalis tenax*; Diptera: Syrphidae) as potential mechanical vectors of pathogens causing mycobacterial infections in pig herds. Folia Microbiol. 51, 147–153.

Georgiev, G.Ts., Luvchiev, V.I., Ljubomirov, T., Markova, E.P., Bochev, N.N., 1998. Species of Sphecidae, Syrphidae and Muscidae dwelling larval galleries of poplar clearwing moth (*Paranthrene tabaniformis* Rott.) (Lepidoptera, Sesiidae) in Bulgaria. Acta Zool. Bulgarica 50 , 19–22.

Georgiev, G., Ljubomirov, T., Raikova, M., Ivanov, K., Sakalian, V., 2004. Insect inhabitants of old larval galleries of *Saperda populnea* (L.) (Coleoptera: Cerambycidae) in Bulgaria. J. Pest. Sci. 77, 235–243.

Gillespie, J., Cannone, J., Gutell, R., Cognato, A., 2004. A secondary structural model of the 28S rRNA expansion segments D2 and D3 from rootworms and related leaf beetles (Coleoptera: Chrysomelidae; Galerucinae). Insect Mol. Biol. 13 , 495–518.

Gillespie, J., Yoder, M.J., Wharton, R.A., 2005. Predicted secondary structure for 28S and 18S rRNA from Ichneumonoidea (Insecta: Hymenoptera: Apocrita): impact on sequence alignment and phylogeny estimation. J. Mol. Evol. 61, 114–137.

Gillespie, J., Johnston, J.S., Cannone, J.J., Gutell, R.R., 2006. Characteristics of the nuclear (18S, 5.8S, 28S and 5S) and mitochondrial (12S and 16S) rRNA genes of *Apis mellifera* (Insecta: Hymenoptera): structure, organization, and retrotransposable elements. Insect Mol. Biol. 15 , 657–686.

Giribet, G., Ribera, C., 2000. A review of arthropod phylogeny: new data based on ribosomal DNA sequences and direct character optimization. Cladistics 16, 204–231.

Glumac, S.. 1960. Phylogenetic system of the Syrphid-flies (Syrphidae: Diptera) based upon the male genitalia structure and the type of the larvae with characteristics of the family and tribes. Glasn. Prir. Muz. Beogr. (B) 16: 69–103 [in Serbian, with English summary].

Goffe, E.R., 1952. An outline of a revised classificaton of the Syrphidae (Diptera) on phylogenetic lines. Trans. Soc. Br. Ent. 11, 97–124.

Goloboff, P.A., Farris, J.S., Nixon, K.C., 2008. TNT, a free program for phylogenetic analysis. Cladistics 24, 774–786.

Grimaldi, D.A., Cumming, J.M., 1999. Brachyceran Diptera in Cretaceous ambers and Mesozoic diversification of the Eremoneura. Bull. Am. Mus.Nat. Hist. 239, 1–124.

Grosskopf, G., 2005. Biology and life history of *Cheilosia urbana* (Meigen) and *Cheilosia psilophthalma* (Becker), two sympatric hoverflies approved for the biological control of hawkweeds (*Hieracium* spp.) in New Zealand. Biol. Control 35, 142–154.

Gullan, P.J., Kosztarab, M., 1997. Adaptations in scale insects. Ann. Rev. Entomol. 42, 23–50.

Gullan, P.J., Miller, D.R., Cook, L.G. 2005. Gall-inducing scale insects (Hemiptera: Sternorrhyncha: Coccoidea). In: Raman, A., Schaefer, C.W., Withers, T.M. (Eds.), Biology, Ecology, and Evolution of Gall-Inducing Arthropods, Vol.1. Science Publishers, Enfield, pp. 159–229.

Hartley, J.C., 1961. A taxonomic account of the larvae of some British Syrphidae. Proc. Zool. Soc. Lond. 136, 505–573.

Heiss, E.M., 1938. A classification of the larvae and puparia of the Syrphidae of Illinois exclusive of aquatic forms. Ill. Biol. Monogr. 36, 1–142.

Hennig, W., 1966. Phylogenetic Systematics. University of Illinois Press, Urbana, USA.

Hippa, H., 1978. Classification of Xylotini (Diptera, Syrphidae). Acta Zool. Fenn. 156, 1–153.

Hippa, H., Ståhls, G., 2005. Morphological characters of adult Syrphidae: descriptions and phylogenetic utility. Acta Zool. Fenn. 215, 1–72.

Hövemeyer, K., 1987. The population dynamics of *Cheilosia fasciata* (Diptera, Syrphidae): significance of environmental factors and behavioural adaptations in a phytophagous insect. Oecologia 73 , 537–542.

Huang, C., Cheng, X.. 2012. Diptera: Syrphidae – Fauna Sinica, Insecta Vol. 50. Science Press, Neijing, China [in Chinese].

Hull, F.M., 1945. A revisional study of the fossil Syrphidae. Bull. Mus. Comp. Zool. Harvard 45, 251–355.

Hull, F.M., 1949. The morphology and inter–relationship of the genera of syrphid flies, recent and fossil. Trans. Zool. Soc. Lond. 26 , 257–408.

Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30 , 772–780.

Katoh, K., Kuma, K., Toh, H., Miyata, T., 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Res. 33, 511–518.

Katoh, K., Asimenos, G., Toh, H. 2009. Multiple alignment of DNA sequences with MAFFT. In: Posada, D. (Eds.), Bioinformatics for DNA Sequence Analysis – Methods in Molecular Biology, Vol. 537. Humana Press, New York, pp. 39–64.

Kjer, K.M., 1995. Use of rRNA secondary structure in phylogenetic studies to identify homologous positions: an example of alignment and data presentation from the frogs. Mol. Phylogenet. Evol. 4 , 314–330.

Kjer, K.M., 2004. Aligned 18S and insect phylogeny. Syst. Biol. 53 , 506–514.

Kjer, K.M., Blahnik, R.J., Holzenthal, R.W., 2001. Phylogeny of Trichoptera (Caddisflies): Characterization of signal and noise within multiple datasets. Syst. Biol. 50, 781–816.

Kjer, K.M., Roshan, U., Gillepie, J.G.. 2009. Structural and evolutionary considerations for multiple sequence alignment of RNA, and the challenges for algorithms that ignore them. In: Rosenberg, M.S. (Eds.), Sequence Alignment: Methods, Models, Concepts, and Strategies. University of California Press, Oakland, CA, pp. 105–150.

Kluge, A.G., 1989. A concern for evidence and a phylogenetic hypothesis of relationships among *Epicrates* (Boidae, Serpentes). Syst. Zool. 38, 7–25.

Kovalev, V.G. 1979. The main aspects in the evolution of Diptera Brachycera in the Mesozoic Era. In: Skarlato, O.A., Skufjin, K.V., Narchuk, E.P., Negrobov, O.P., Kandybina, M.N.. (Eds.), Ecological and Morphological Principles of Diptera Systematics (Insecta). Zoological Institute, USSR Academy of Sciences, Leningrad, pp. 35–37. [in Russian. English translation from 1985, Systematics of Diptera (Insecta). Ecological and Morphological Principles. Amerind Publishing Co., New Delhi, viii + 185 pp.]

Kuznetzov, S.Y. 1987. On some plesiomorphic characters of aphidophagous larvae of hover-flies in the subfamily Syrphinae (Diptera, Syrphidae). In: Narchyk, E.P. (Eds.), Dipterous Insects: Systematics, Morphology and Ecology. Zoological Institute, USSR Academy of Sciences, Leningrad, pp. 58–61 [in Russian].

Kuznetzov, S.Y.. 1988. Morphology of the eggs of hover flies (Diptera, Syrphidae). Entomol. Rev. 68: 67–90 [original in Russian, Entomol. Obozr. 67(4), 741–753].

Kuznetzov, S.Y., 1992. The first instar larvae of the subfamily Pipizinae and Eristalinae (Diptera, Syrphidae). Daba un muzejs (Nature and Museum) 4, 24–43.

Kuznetzov, S.Y. 1993. First instar larvae of hover-flies of the subfamily Syrphinae (Diptera, Syrphidae). Entomol. Obozr. 72, 202–221 [in Russian].

Lambkin, C.L., Sinclair, B.J., Pape, T., Courtney, G.W., Skevington, J.H., Meier, R., Yeates, D.K., Blagoderov, V., Wiegmann, B.M., 2013. The phylogenetic relationships among infraorders and superfamilies of Diptera based on morphological evidence. Syst. Entomol. 38 , 164–179.

Laurenne, N.M., Broad, G.R., Quicke, D.L.J., 2006. Direct optimization and multiple alignment of 28S D2–D3 rDNA sequences: problems with indels on the way to a molecular phylogeny of the cryptine ichneumon wasps (Insecta: Hymenoptera). Cladistics 22, 442–473.

Lewis, P.O., 2001. A Likelihood approach to estimating phylogeny from discrete morphological character data. Syst. Biol. 50 , 913–925.

Marín, J.C. 1969. Nota sobre las larvas de *Mesograpta polita* (Say) (Syrphidae, Diptera) en espigas de maíz (*Zea mays* L.) en El Limón, Aragua, Venezuela. Agron. Trop. Rev. Centro Invest. Agron. 19, 335–339 [in Spanish].

Marvaldi, A.E., Duckett, C.N., Kjer, K.M., Gillespie, J.J., 2009. Structural alignment of 18S and 28S rDNA sequences provides insights into phylogeny of Phytophaga (Coleoptera: Curculionoidea and Chrysomeloidea). Zool. Scripta 38 , 63–77.

Mengual, X., Ståhls, G., Rojo, S., 2008a. Molecular phylogeny of *Allograpta* (Diptera, Syrphidae) reveals diversity of lineages and non-monophyly of phytophagous taxa. Mol. Phylogenet. Evol. 49, 715–727.

Mengual, X., Ståhls, G., Rojo, S., 2008b. First phylogeny of predatory hoverflies (Diptera, Syrphidae, Syphinae) using mitochondrial COI and nuclear 28S rRNA genes: conflict and congruence with the current tribal classification. Cladistics 24, 543–562.

Mengual, X., Ståhls, G., Rojo, S., 2012. Is the mega-diverse genus *Ocyptamus* (Diptera, Syrphidae) monophyletic? Evidence from molecular characters including the secondary structure of 28S rRNA Mol. Phylogenet. Evol. 62, 191–205.

Michener, C.D., 1977. Discordant evolution and the classification of allodapine bees. Syst. Zool. 26 , 32–56.

Morales, G.E., Wolff, M., 2010. Insects associated with the composting process of solid urban waste separated at the source. Rev. Bras. Entomol. 54 , 645–653.

Murienne, J., Edgecombe, G.D., Giribet, G., 2010. Including secondary structure, fossils and molecular dating in the centipede tree of life. Mol. Phylogenet. Evol. 57, 301–313.

Nazari, V., Zakharov, E.V., Sperling, F.A.H., 2007. Phylogeny, historical biogeography, and taxonomic ranking of Parnassiinae (Lepidoptera, Papilionidae) based on morphology and seven genes. Mol. Phylogenet. Evol. 42, 131–156.

Nishida, K., Rotheray, G., Thompson, F.C., 2002. First non-predaceous syrphine flower-fly (Diptera: Syrphidae): a new leaf-mining *Allograpta* from Costa Rica. Stud. Dipterol. 9, 421–436.

Peck, L.V. 1988. Family Syrphidae. In: Soós, Á., Papp, L. (Eds.), Catalogue of Palaearctic Diptera, Volume 8. Akadémiai Kiadó, Budapest, pp. 11–230.

Pérez-Bañón, C., Juan, A., Petanidou, T., Marcos-García, M.A., Crespo, M.B., 2003. The reproductive ecology of *Medicago citrina* (Font Quer) Greuter (Leguminosae): a bee–pollinated plant in Mediterranean islands where bees are absent. Plant Syst. Evol. 241, 29–46.

Posada, D., Buckley, T.R., 2004. Model selection and model averaging in phylogenetics: advantages of the AIC and Bayesian approaches over likelihood ratio tests. Syst. Biol. 53, 793–808.

Rambaut, A.. 2009. FigTree: Tree Figure Drawing Tool, Version 1.3.1. Available from http://tree.bio.ed.ac.uk/software/figtree/ (accessed on 17 December 2010).

Reemer, M., 2013. Review and phylogenetic evaluation of associations between Microdontinae (Diptera: Syrphidae) and ants (Hymenoptera: Formicidae). Psyche 2013, 1–9.

Reemer, M., Rotheray, G.E., 2009. Pollen feeding larvae in the presumed predatory syrphine genus *Toxomerus* Macquart (Diptera, Syrphidae). J. Nat. Hist. 43 , 939–949.

Reemer, M., Ståhls, G., 2013. Phylogenetic relationships of Microdontinae (Diptera: Syrphidae) based on parsimony analyses of combined molecular and morphological characters. Syst. Entomol. 38, 661–688.

Riley, C.V., Howard, L.O., 1888. The corn-feeding Syrphus-fly (*Mesograpta polita* Say) (Order Diptera; family Syrphidae). Insect Life 1, 5–8.

Rojo, S., Marcos-García, M.A., 1997. Syrphid predators (Dipt.: Syrphidae) of gall forming aphids (Hom.: Aphididae) in Mediterranean areas: implications for biological control of fruit trees pests. Entomophaga 42, 269–276.

Rojo, S., Pérez-Bañón, C., Marcos-García, M.A., 1999. First observations on the biology of *Scaeva mecogramma* Bigot, 1860 (Diptera, Syrphidae) and notes on some other syrphids preying on psyllids (Hemiptera, Aphalaridae and Triozidae). Volucella, 4, 105–111.

Rojo, S., Gilbert, F., Marcos-García, M.A., Nieto, J.M., Mier, M.P. 2003. A World Review of Predatory Hoverflies (Diptera, Syrphidae: Syrphinae) and Their Prey. CIBIO Ediciones, Alicante.

Rotheray, G.E., 1993. Colour Guide to Hoverfly Larvae (Diptera, Syrphidae) in Britain and Europe. Dipt. Digest 9, 1–156.

Rotheray, G.E., 1999. Descriptions and a key of the larval and puparial stages of north-west European *Volucella* (Diptera, Syrphidae). Studia Dipt. 6 , 103–116.

Rotheray, G.E., 2003. The predatory larvae of two *Nepenthosyrphus* species living in pitcher plants (Diptera, Syrphidae). Studia Dipt. 10 , 219–226.

Rotheray, G.E., Gilbert, F., 1989. The phylogeny and systematics of European predacious Syrphidae (Diptera) based on larval and puparial stages. Zool. J. Linn. Soc. 95, 29–70.

Rotheray, G.E., Gilbert, F., 1999. Phylogeny of Palaearctic Syrphidae (Diptera): evidence from larval stages. Zool. J. Linn. Soc. 127, 1–112.

Rotheray, G.E., Gilbert, F., 2008. Phylogenetic relationships and the larval head of the lower Cyclorrhapha (Diptera). Zool. J. Linn. Soc. 153, 287–323.

Rotheray, G.E., Gilbert, F., 2011. The Natural History of Hoverflies. Forrest Text, Cardigan, UK.

Rotheray, G.E., Zumbado, M., Hancock, E.G., Thompson, F.C., 2000. Remarkable aquatic predators in the genus *Ocyptamus* (Diptera, Syrphidae). Stud. Dipterol. 7, 385–389.

Sack, P., 1932. 31. Syrphidae. In: Lindner, E. (Eds.), Die Fliegen der Palaearktischen Region, Band IV, 6, E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart.

Shannon, R.C., 1921. A reclassification of the subfamilies and genera of North American Syrphidae (Diptera). Bull. Brooklyn Entomol. Soc. 16, 65–72.

Shannon, R.C., 1922a. A reclassification of the subfamilies and genera of North American Syrphidae (Diptera). Bull. Brooklyn Entomol. Soc. 16, 120–128.

Shannon, R.C., 1922b. A reclassification of the subfamilies and genera of North American Syrphidae (Diptera). Conclusions. Bull. Brooklyn Entomol. Soc. 17, 30–42.

Shannon, R.C., 1923. A reclassification of the subfamilies and genera of North American Syrphidae (Diptera). Appendix. Bull. Brooklyn Entomol. Soc. 18, 17–21.

Shatalkin, A.I., 1975a. A taxonomic analysis of the hover flies (Diptera, Syrphidae) I. Entomol. Rev. 54 , 117–125.

Shatalkin, A.I., 1975b. A taxonomic analysis of the hover flies (Diptera, Syrphidae) II. Entomol. Rev. 54 , 127–134.

Skevington, J.H., Thompson, F.C., 2012. Review of New World *Sericomyia* (Diptera: Syrphidae), including description of a new species. Can. Entomol. 144, 216–247.

Skevington, J.H., Yeates, D.K., 2000. Phylogeny of the Syrphoidea (Diptera) inferred from mtDNA sequences and morphology with particular reference to classification of the Pipunculidae (Diptera). Mol. Phylogenet. Evol. 16, 212–224.

Speight, M.C.D., 1987. External morphology of adult Syrphidae (Diptera). Tijdschr. Entomol. 130, 141–175.

Ssymank, A., Kearns, C. 2009. Flies – pollinators on two wings. In: Ssymank, A., Hamm, A., Vischer-Leopold, M. (Eds.), Caring for Pollinators – Safeguarding Agro-Biodiversity and Wild Plant Diversity. Bundesamt für Naturschutz, Bonn, pp. 39–52.

Ståhls, G., Hippa, H., Rotheray, G., Muona, J., Gilbert, F., 2003. Phylogeny of Syrphidae (Diptera) inferred from combined analysis of molecular and morphological characters. Syst. Entomol. 28, 433–450.

Ståhls, G., Vujić, A., Stuke, J.H., Doczkal, D., Muona, J., 2004. Phylogeny of the genus *Cheilosia* and the tribe Rhingiini (Diptera, Syrphidae) based on molecular and morphological characters. Cladistics 4, 1–17.

Stubbs, A.E., Falk, S. 2002. British Hoverflies, an Illustrated Identification Guide, 2nd edn. British Entomological and Natural History Society, Reading, UK, 469 pp.

Stuke, J.-H. 2000. Phylogenetische Rekonstruktion der Verwanschaftsbeziehungen innerhalb der Gattung *Cheilosia* Meigen, 1822 anhand der Larvenstadien (Diptera: Syrphidae). Stud. Dipterol. Suppl. 8, 1–118 [in German].

Subbotin, S.A., Sturhan, D., Vovlas, N., Castillo, P., Tanyi Tambe, J., Moens, M., Baldwin, J.G. 2007. Application of secondary structure model of rRNA for phylogeny: D2–D3 expansion segments of the LSU gene of plant-parasitic nematodes from the family Hoplolaimidae Filipjev, 1934. Mol. Phylogenet. Evol. 43, 881–890.

Thompson, F.C., 1969. A new genus of microdontine flies (Diptera: Syrphidae) with notes on the placement of the subfamily. Psyche 76, 74–85.

Thompson, F.C., 1972. A contribution to a generic revision of the Neotropical Milesiinae (Diptera: Syrphidae). Arq. Zool. 23, 73–215.
Thompson, F.C., 2003. *Austalis*, a new genus of flower flies (Diptera: Syrphidae) with revisionary notes on related genera. Zootaxa 246, 1–19.
Thompson, F.C. 2013a. Family Syrphidae. In: Thompson, F.C., Pape, T. (Eds.), Systema Dipterorum, Version 1.5. Available from http://www.diptera.org (accessed on 17 March 2014).
Thompson, F.C., 2013b. A new Afrotropical ceriodine flower fly with an overview of the group (Diptera: Syrphidae, Ceriodini). Entomol. Mon. Mag. 149, 71–77.
Thompson, F.C., Rotheray, G.E., 1998. Family Syrphidae. In: Papp, L., Darvas, B. (Eds.), Manual of Palaearctic Diptera, Vol. 3. Science Herald, Budapest, pp. 8, 1–139.
Ureña, O., Hanson, P., 2010. A fly larva (Syrphidae: *Ocyptamus*) that preys on adult flies. Rev. Biol. Trop. 58 , 1157–1163.
Van Zuijen, M.P., Nishida, K., 2011. Life history and immature stages of *Allograpta zumbadoi* Thompson, a phytophagous flower fly (Diptera: Syrphidae: Syrphinae). Stud. Dipterol. 17 , 37–51.
Verrall, G.H., 1901. British Flies, Vol. VIII. Platypezidae, Pipunculidae and Syrphidae of Great Britain. Gurney & Jackson, London.
Vockeroth, J.R., 1969. A revision of the genera of the Syrphini (Diptera, Syrphidae). Mem. Ent. Soc. Can. 62, 1–176.
Vockeroth, J.R.. 1992. The Flower Flies of the Subfamily Syrphinae of Canada, Alaska, and Greenland (Diptera: Syrphidae). The Insects and Arachnids of Canada, Part 18. Centre for Land and Biological Resources Research, Ottawa.
Vujić, A., Glumac, S., 1993. Some views on Syrphidae (Diptera) phylogeny. Proc. Nat. Sci., Novi Sad 84, 13–17.
Vujić, A., Ståhls, G., Ačanski, J., Bartsch, H., Bygebjerg, R., Stefanović, A., 2013. Systematics of Pipizini and taxonomy of European *Pipiza* Fallén: molecular and morphological evidence (Diptera, Syrphidae). Zool. Scr. 42 , 288–305.
Weng, J.L., Rotheray, G.E., 2008. Another non-predaceous syrphine flower fly (Diptera: Syrphidae): pollen feeding in the larva of *Allograpta micrura*. Stud. Dipterol. 15 , 245–258.
Wiegmann, B.M., Trautwein, M.D., Winkler, I.S., Barr, N.B., Kim, J.W., Lambkin, C., Bertone, M.A., Cassel, B.K., Bayless, K.M., Heimberg, A.M., Wheeler, B.M., Peterson, K.J., Pape, T., Sinclair, B.J., Skevington, J.H., Blagoderov, V., Caravas, J., Kutty, S.N., Schmidt-Ott, U., Kampmeier, G.E., Thompson, F.C., Grimaldi, D.A., Beckenbach, A.T., Courtney, G.W., Friedrich, M., Meier, R., Yeates, D.K., 2011. Episodic radiations in the fly tree of life. Proc. Natl Acad. Sci. USA 108 , 5690–5695.
Williston, S.W., 1885. On classification of North American Diptera. Bull. Brooklyn Ent. Soc. 7, 129–139.
Williston, S.W.. 1887. Synopsis of the North American Syrphidae. Bull. US Nat. Mus. 31, xxx + 327 pp.
Wirth, W.W., Sedman, Y.S., Weems, H.V. 1965. Family Syrphidae. In: Stone, A., Sabrosky, C.W., Wirth, W.W., Foote, R.H., Coulson, J.R. (Eds.), A Catalog of the Diptera of America North of Mexico. Agricultural Research Service, US Department of Agriculture, Washington, DC, pp. 557–625.
Zwickl, D.J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. PhD Dissertation, University of Texas, Austin, TX.
Zwickl, D.J. 2013. *Garli Version 2.01 and Garli Support Wiki*. Available from https://www.nescent.org/wg_garli/Main_Page and https://code.google.com/p/garli/ (accessed on 17 March 2014).

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Taxa used in the molecular and morphological analyses, including GenBank accession numbers. GenBank accession numbers of newly generated sequences (this project) are in boldface. Subfamilies (–inae), tribes (–ini) and subtribes (–ina) of Syrphidae are indicated.

Appendix S2. List of morphological characters of syrphid adults used in the analyses. Character states and scoring are also indicated.

Appendix S3. Data matrix of morphological characters of syrphid adults (.ss) for Winclada.

Appendix S4. Inferred topologies using different methodologies and datasets as explained in Table 1.

Appendix S5. (a,b) The DNA data matrices (Nexus format) for the combined analyses using different alignment strategies: MAFFT and the secondary structure of the rRNA 28S and 18S genes.