Systematic Entomology (2003) **28**, 433–450

Phylogeny of Syrphidae (Diptera) inferred from combined analysis of molecular and morphological characters

GUNILLA STÅHLS[1], HEIKKI HIPPA[2], GRAHAM ROTHERAY[3], JYRKI MUONA[1] and FRANCIS GILBERT[4]

[1]Finnish Museum of Natural History, University of Helsinki, Finland,
[2]Swedish Museum of Natural History, Stockholm, Sweden,
[3]National Museums of Scotland, Edinburgh, U.K. and
[4]School of Biological Sciences, Nottingham University, Nottingham, U.K.

Abstract. Syrphidae (Diptera) commonly called hoverflies, includes more than 5000 species world-wide. The aim of this study was to address the systematic position of the disputed elements in the intrafamilial classification of Syrphidae, namely the monophyly of Eristalinae and the placement of Microdontini and Pipizini, as well as the position of particular genera (*Nausigaster*, *Alipumilio*, *Spheginobaccha*). Sequence data from nuclear 28S rRNA and mitochondrial COI genes in conjunction with larval and adult morphological characters of fifty-one syrphid taxa were analysed using optimization alignment to explore phylogenetic relationships among included taxa. A species of Platypezidae, *Agathomyia unicolor*, was used as outgroup, and also including one representative (*Jassidophaga villosa*) of the sister-group of Syrphidae, Pipunculidae. Sensitivity of the data was assessed under six different parameter values. A stability tree summarized the results. Microdontini, including *Spheginobaccha*, was placed basally, and Pipizini appeared as the sister-group to subfamily Syrphinae. The monophyly of subfamily Eristalinae was supported. The results support at least two independent origins of entomophagy in syrphids, and frequent shifts between larval feeding habitats within the saprophagous eristalines.

Introduction

Syrphidae (Diptera: Lower Cyclorrhapha) commonly called flower- or hoverflies, comprise more than 5000 described species, one of the most speciose of dipteran families (Thompson & Rotheray, 1998). In contrast to the fairly uniform flower-feeding habits of adult syrphids, larvae are found in a very diverse array of habitats. Those of subfamily Eristalinae are saprophagous in dead wood, coprophagous, phytophagous, aquatic filterfeeders or inquilines in social insect nests, whereas larvae of Microdontinae are inquilines in ants' nests, and larvae of Syrphinae are mostly predaceous on soft-bodied Homoptera.

Correspondence: Dr G. Stahls, Finnish Museum of Natural History, PO Box 17, FIN-00014 University of Helsinki, Finland. E-mail: gunilla.stahls@helsinki.fi

At the beginning of the last century, Syrphidae was divided into 2–20 subfamilies by different authors. A system of three subfamilies (subfamilies Microdontinae, Eristalinae and Syrphinae) was adopted for Syrphidae more than 25 years ago, largely for the sake of convenience (Thompson & Rotheray, 1998). The traditional classification of Syrphidae is based largely on adult characters. In their cladistic study of larval characters, Rotheray & Gilbert (1999) considered all previous estimates of syrphid classification, dealing with a reasonably large section of the family and addressing hypotheses about syrphid phylogenetic relationships based on non-traditional characters (e.g. chromosomes).

Two recent studies address the monophyly of Syrphidae and the systematic position of various clades within the family. Skevington & Yeates (2000) investigated the use of the mitochondrial genes 12S and 16S for developing a phylogeny of superfamily Syrphoidea (Pipunculidae +

Syrphidae). Their combined analysis presented convincing support for this sister-group relationship (bootstrap support of 87%), but support for the monophyly of Syrphidae was weak, and the resolution within Syrphidae was limited. Rotheray & Gilbert (1999) presented a hypothesis of intrafamilial relationships of Syrphidae based on the first comprehensive study of larval morphological characters, including many several outgroup taxa, and concluded Syrphidae was monophyletic. Within the family, their results differ fundamentally from the traditional classification, with few traditional suprageneric taxa recovered. A study by Cheng *et al*. (2000) employed both molecular and morphological data to address the question of the placement of Pipizini, but their small dataset (especially the molecular component) was too limited to elucidate relationships.

The studies of Rotheray & Gilbert (1999) and Skevington & Yeates (2000) both questioned the monophyly of subfamily Eristalinae (previously called Milesiinae). The unique attributes of both the larvae and the adults of the Microdontinae (containing the single tribe, Microdontini) and lack of clear synapomorphies from adult characters to combine it with other Syrphidae have led to many hypotheses of its relationships (Skevington & Yeates, 2000). Thompson (1969, 1972) considered Microdontinae to form a basal, monophyletic group with respect to the rest of Syrphidae, and this view was partly supported by Shatalkin (1975a). Contrary to the traditional monotypic classification of Microdontinae, Shatalkin classified *Sphegina*, *Nausigaster* and *Microdon* into Microdontinae. Thompson (1969) and Speight (1987) discussed raising Microdontinae to familial rank, but were not followed by subsequent authors. In sharp contrast, larval characters placed the Microdontinae within a monophyletic Syrphidae as the sister-group to the Syrphinae + Pipizini, a placement never suggested from studies of adult characters (Rotheray & Gilbert, 1999; this study Fig. 1). The molecular phylogeny of Skevington & Yeates (2000) showed a basal position for

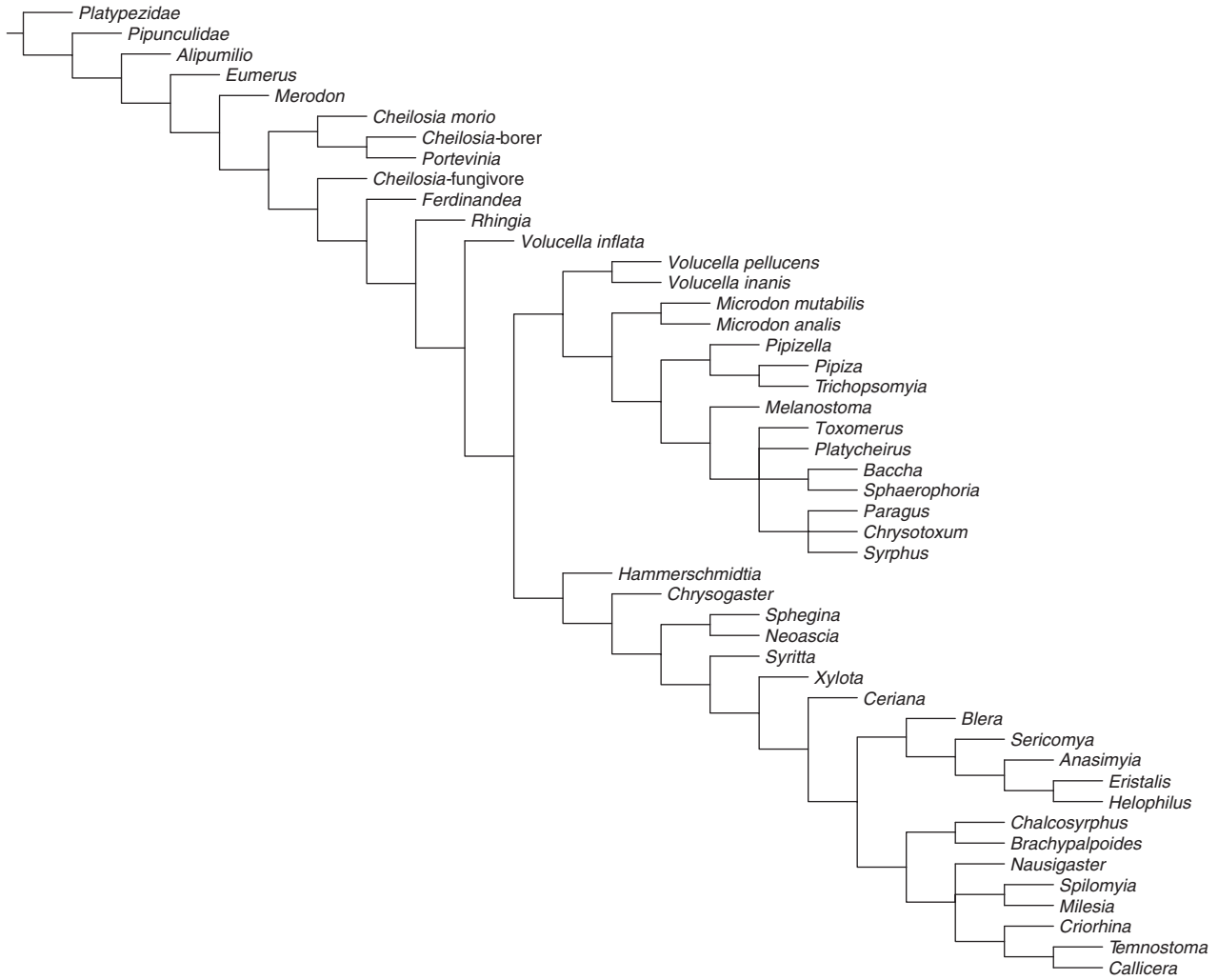

Fig. 1. Strict consensus of sixteen most parsimonious trees inferred from analysis of 187 larval characters (length = 578).

Microdontini, thus supporting the hypothesis proposed by Thompson (1972) and Shatalkin (1975a).

Like the Microdontinae, the classification of Pipizini (geographical distribution world-wide) has been a long-standing problem, the reason being that its members have a syrphine-like larva, but a milesiine-like adult (Thompson, 1972). Vockeroth (1969), Thompson (1972) and Vockeroth & Thompson (1987) referred Pipizini to Eristalinae, whereas Shatalkin (1975a, b) classified them within Cheilosiinae. Rotheray & Gilbert's (1989, 1999) work suggested that Pipizini are the sister-group of Syrphinae. Kuznetzov (1987, 1992) proposed elevation of Pipizini to subfamilial rank, based on scanning electron microscope studies of first-instar larvae of syrphines, pipizines and eristalines. Like the Syrphinae, the pipizines have separated posterior respiratory tubes in L1 larvae, but Kuznetzov claimed that the long sclerotized tubes of the Pipizini were very different from the small non-sclerotized protuberances of the Syrphinae: such an autapomorphy is a weak basis for a change rank. Parsimony analysis of molecular characters of Skevington & Yeates (2000) suggested a weakly supported sister-group relationship between Pipizini (*Triglyphus*) and Milesiini (*Orthoprosopa*). DNA sequences collected by Cheng *et al.* (2000) to test specifically the placement of Pipizini, included four representatives in a study comprising only seven taxa in total. Their parsimony analysis showed that the relationship between Pipizini and Syrphinae (*Metasyrphus corollae*) was closer than the relationship between Pipizini and Eristalinae (*Eumerus strigatus* and *Eristalis tenax*), and hence Pipizini should be transferred to Syrphinae. This supports the earlier hypothesis of Rotheray & Gilbert (1989, 1999) based on morphological characters of the immature stages (Fig. 1).

The phylogenetic position of *Spheginobaccha* (with its Afrotropical and Oriental distribution) has been an enigma. In short, *Spheginobaccha* or its species have been included in six different tribes and in each of the three subfamilies of Syrphidae (Thompson, 1974): for example, using adult characters, Shatalkin (1975a) classified *Spheginobaccha* into the Microdontinae.

The Neotropical genera *Alipumilio* and *Nausigaster* were classified into Eumerini by Thompson (1972), who suggested that they form a special group within the tribe, because they share a unique character state within syrphids (an undifferentiated thoracic mesopleuron). However, Shatalkin (1975a) placed these genera in Microdontinae, referring to the supposed primitive type of aedeagus shared among *Alipumilio*, *Nausigaster*, *Microdon* and *Spheginobaccha*. From larval morphology, the relationships of these taxa are unequivocal (Rotheray *et al.*, 2000): *Alipumilio* is indeed close to *Eumerus*, but *Nausigaster* is distantly related and has the standard morphology of a hook-bearing xylotine larva (Fig. 1).

The aim of this study, as far as classification is concerned, was to address the systematic position of the disputed elements in the intrafamilial classification of Syrphidae, namely the monophyly of Eristalinae and the placement of Microdontini and Pipizini, as well as the position of particular genera (*Nausigaster*, *Alipumilio*, *Spheginobaccha*). In addition, we examined the evolution of the larval feeding modes in the light of the phylogeny recovered. We used three different independent datasets: molecular (this study), and larval (Rotheray & Gilbert, 1999) and adult (H. Hippa & G. Ståhls, unpublished data) morphology. We discovered considerable conflict between phylogenetic hypotheses based on any one of these datatsets, and hence we employ combined analysis (Total Evidence, Kluge, 1989) to evaluate syrphid relationships in light of the diverse and comprehensive dataset we gathered for this purpose. We explored which groupings consistently appeared in the phylogenetic trees under different weighting schemes. Such a sensitivity analysis (*sensu* Wheeler, 1995) gives information on node stability and congruence. We restrict our analyses to this kind of taxonomic congruence within a combined dataset. The result is summarized in a stability tree (*sensu* Schulmeister *et al.*, 2002).

Materials and methods

Taxa and characters

The taxon sampling covered as much taxonomic diversity as possible, representing thirteen of the fourteen recognized syrphid tribes (Thompson & Rotheray, 1998), totalling fifty-one syrphid taxa (Table 1). Most included species have Palaearctic or Holarctic distributions, but a few species are from other biogeographical regions (Table 1). In the molecular and morphological matrices (closely) related taxa were equated. Morphological or molecular data are incomplete for a few species (Table 1). The platypezid *Agathomyia unicolor* was chosen as outgroup, but one representative of the putative sister-group of Syrphidae, Pipunculidae (*Jassidophaga villosa*), was included as well.

The mitochondrial gene cytochrome c oxidase subunit I (COI) (a 1128-bp fragment), and the D2-3 region of the nuclear 28S rRNA gene (*c.* 640-bp fragment) were chosen for sequencing. The dataset of 187 larval morphological characters is that of Rotheray & Gilbert (1989, 1999). The dataset of 122 adult morphological characters was scored from all parts of the syrphid body (H. Hippa & G. Ståhls, unpublished data). Whereas many of these characters are 'traditional', many are novel, including characters of prothoracic sclerites, characters of male and female postabdomen, chaetotaxy of legs and wing and the ultrastructure of hairs and other vestiture on different parts of the body. Morphological datasets are available at www.fmnh.helsinki.fi/users/stahls.

DNA manipulation

Genomic DNA samples were obtained from fresh, dry, frozen or ethanol-preserved larvae or adult flies. DNA extraction, PCR amplification (primers and profiles) of

Table 1. Taxon sampling used in the molecular and morphological analyses, including GenBank accession numbers. Three subfamilies are recognized following Vockeroth (1969), the arrangement of species and genera follows that of Vockeroth (1969) for Syrphinae, and Thompson (1972) and Hippa (1978) for Eristalinae (Milesiinae). AUS: Austria, BOL: Bolivia, CR: Costa Rica, ECU: Equador, FIN = Finland, GER = Germany, ITA: Italy, MAL = Malaysia, RUS = Russia, SPA = Spain, SWE = Sweden, SWI = Switzerland, UK = United Kingdom. NA = not analysed, NS = not submitted to GenBank. Nearctic and Oriental species are indicated.

Species used for molecular characters	Coll. locality	GenBank accessions COI	GenBank accessions 28S	Species used for larval morphol. characters	Species used for adult morphol. characters
Syrphinae					
Bacchini					
Baccha elongata (Fabr.)	FIN: Kangaslampi	AY261707	AY261754	*elongata* (Fabr.), *obscuripennis* Meig.	*elongata* (Fabr.)
Melanostoma scalare Fabr.	FIN: Åland	AY212799	NA	*scalare* Fabr.	*scalare* Fabr.
Platycheirus peltatus (Meig.)	FIN: Somero	AY261706	AY261753	*albimanus* (Fabr.) + multiple Palaearctic repr.	*peltatus* (Meig.)
Paragini					
Paragus haemorrhous Meigen	FIN: Hangö	AY174466	AY261756	*haemorrhous* Meig.	*tibialis* (Fall.)
	SPA: Alicante	AY174470	NA		
Syrphini					
Chrysotoxum intermedium (Meig.)	SPA: Alicante	AY212781	AY261711	*verralli* Collin	*intermedium* (Meig.)
Sphaerophoria scripta (L.)	FIN: Loppi	AY261708	AY261755	*menthastri* (L.), *scripta* (L.)	*scripta* (L.)
Syrphus vitripennis Meigen	GRE: Lesbos	AY212797	AY261728	*ribesii*(L.)	*ribesii(L.), vitripennis* Meigen
Toxomerini (Nearctic and Neotropical)					
Toxomerus marginatus (Say)(Nearctic)	USA: New York;	AY261705	AY261752	No information.	*marginatus* (Say) + other
	USA: Washington DC			NS	NA
Eristalinae					
Pipizini					
Pipiza sp.	FIN: Nousiainen	AY174459	AY261741	*austriaca* Meig., *luteitarsis* (Zett.), *noctiluca* (L.)	*Pipiza* sp.
Pipizella viduata (L.)	SWE: Björnlunda	AY261695	AY261742	*varipes* (Meig.)	*viduata* (L.)
	GER: Thüringen	NS	NS		
Trichopsomyia flavitarsis (Meigen)	SWE: Uppland	AY212798	AY261729	*flavitarsis* (Meigen)	*flavitarsis* (Meigen)
Callicerini					
Callicera rufa Schum.	UK: Edinburgh	NS	NA	*aurata* (Rossi), *rufa* Schum., *spinolae* Rond.	Several species
Rhingiini					
Chamaesyrphus lusitanicus Mik	SWE: Gotland	AY212796	AY261727	NA	*lusitanicus* Mik
Cheilosia illustrata (Harris)	ITA: Alto Adige	AY261693	AY261739	*illustrata* (Harris) + multiple representatives	*illustrata* (Harris) + other
C. convexifrons Stack.	RUS: South Amur	AY261691	AY261737	*morio* Zett., *alaskensis* (N)	*convexifrons* Stack.
C. longula (Zett.)	FIN: Helsinki	AY261692	AY261738	*longula* (Zett.), *scutellata* Fall.	*scutellata* Fall.
Ferdinandea cuprea (Scop.)	SWE: Östhammar	AY261686	AY261732	*cuprea* (Scop.), *nigripes* Ost.-Sack (N)	*cuprea* (Scop.)
	GER: Münster	NS	NS		
Portevinia maculata (Fall.)	SWI: Zürich	AY261696	AY261743	*maculata* Fall.	*maculata* (Fall.)
Rhingia campestris Meig.	FIN: Somero	AY261697	AY261744	*campestris* Meig.	*campestris* Meig.

Brachyopini					
Chrysogaster solstitialis (Fall.)	FIN: Somero	AY212794	NA	*hirtella* Loew, *solstitialis* (Fall.)	*solstitialis* (Fall.)
Hammerschmidtia ferruginea (Fall.)	FIN: Noormarkku	AY261702	AY261749	*ferruginea* (Fall.)	*ferruginea* (Fall.)
Neoascia tenur (Harris)	FIN: Åland	AY212784	AY261714	*geniculata* (Meig.), *meticulosa* Scop., *podagrica* (Fabr.), *tenur* (Harris)	*tenur* (Harris)
Sphegina sibirica Stackelberg	SWE: Upplands-Bro	AY261700	AY261747	*clunipes* (Fall.), *elegans* Schumm., *keeniana* Will. (N), *verecunda* Collin	*sibirica* Stack.
Ceriodini					
Ceriodini sp. (Oriental)	MAL: Poring	AY212791	AY261721	Nearctic and Palaearctic species	Ceriodini sp.
Eumerini					
Alipumilio avispas Vockeroth	BOL: La Paz	AY261709	AY261725	*femoratus* Townsend	*avispas* Vockeroth
Eumerus flavitarsis Zett.	SWE: Upplands-Bro	AY212782	AY261712	*strigatus* Fall., *tuberculatus* Fall.	*tuberculatus* Fall.
Merodon cinereus (Fabr.)	SPA: Alicante	AY212786	AY261716	*equestris* (Fabr.)	*equestris* (Fabr.)
Nausigaster sp.	USA: Arizona	NS	partly, NS	*texana* Curran, *unimaculata* Townsend	spp.
Eristalini					
Anasimyia lineata (Fabr.)	FIN: Åland	AY212785	AY261715	*lineata* (Fabr.), *lunulata* Meigen, *transfuga* (L.)	*lineata* (Fabr.)
Eristalis tenax (L.)	FIN: Åland	AY261703	AY261750	*tenax* L. + multiple other Palaearctic species	*tenax* L.
	SPA: Alicante			NS	NS
Helophilus pendulus (L.)	SWE: Järfälla	AY261704	AY261751	*pendulus* (L.), *hybridus* (L.), *fasciatus* Walker (N)	*pendulus* (L.)
	FIN: Åland	NS	NS		
Sericomyia lappona (L.)	AUS: Imst	AY212795	AY261726	*lappona* (L.), *silentis* Harris	*lappona* (L.)
Milesiini					
Blera fallax (L.)	FIN: Kangaslampi	AY261687	AY261733	*fallax* (L.), *humeralis* (Will.) (N)	*fallax* (L.)
Brachypalpoides lentus (Meig.)	SWE: Uppland	AY212787	AY261717	*lentus* (Meig.)	*lentus* (Meig.)
Chalcosyrphus nemorum (Fabr.)	GER: Münster	AY261701	AY261748	*nemorum* (Fabr.), *metallicus* (Wied.) (N), *metallifera* (Bigot), *violescens* (Meger.) (N)	*nemorum* (Fabr.)
Criorhina asilica (Fall.)	FIN: Nummi-Pusula	AY212789	AY261719	*asilica* (Fall.), *berberina* Fbr., *floccosa* Meig., *ranunculi* Panz.	*asilica* (Fall.)
	UK: Cumbria	NS	NA		
Milesia fuscicosta (Bigot)(Oriental)	MAL: Poring	AY212792	AY261722	*virginiensis* (Drury) (N)	*fuscicosta* (Bigot)
Spilomyia diophthalma (L.)	FIN: Ekenäs	AY261698	AY261745	*alcimus* (Walk.), *citima* Vock., *longicornis* (Loew), *sayi* Goot (all N)	
diophthalma (L.)					
Syritta pipiens (L.)	FIN: Parikkala	AY212783	AY261713	*oceanica* (Macq.) (N),	*pipiens* (L.)

Table 1. Continued.

Species used for molecular characters	Coll. locality	GenBank accessions COI	GenBank accessions 28S	Species used for larval morphol. characters	Species used for adult morphol. characters
				pipiens (L.)	
	SWE: Nynäshamn			NS	NA
Temnostoma vespiforme (L.)	FIN: Hangö	AY261699	AY261746	*alternans* (Loew), *bombylans* (Fabr.), *vespiforme* (L.)	*vespiforme* (L.)
Xylota ignava (Panz.)	SWE: Uppland	AY212790	AY261720	*abiens* (Meig.), *coeruleiventris* Zett., *segnis* (L.), *sylvarum* (L.), *tarda* Meig., *xanthocnema* Collin	*segnis* (L.)
Spheginobacchini					
Spheginobaccha sp. (Oriental)	MAL: Poring	NS	NS	NA	*Spheginobaccha* sp.
Spheginobaccha nr. *macropoda*	MAL: Poring	NS	NS	NA	*Spheginobaccha* nr *macropoda*
Volucellini					
Volucella inanis (L.)	FIN: Somero	AY261690	AY261736	*inanis* (L.)	*inanis* (L.)
V. inflata (Fabr.)	GER: Wörlitz	AY261688	AY261734	*inflata* (Fabr.)	*inflata* (Fabr.)
V. pellucens (L.)		AY261689	AY261735	*bombylans* (L.), *pellucens* (L.), *zonaria* Poda	*bombylans* (L.), *pellucens* (L.)
Graptomyza sp.	MAL: Poring	AY212793	AY261723	NA	*Graptomyza* sp.
Microdontinae					
Microdontini					
Microdon mutabilis (L.)	SWE: Upplands-Bro	AY261694	AY261740	*analis* Mik, *devius* (L.), *mutabilis* (L.)	*mutabilis* (L.)
	RUS: Siberia	NS	NA		
M. analis (Macq.)	FIN: Åland	AY212788	AY261718	*analis* (Macq.), *devius* (L.), *mutabilis* (L.)	*analis* (Macq.)
Microdon sp.	CR: Puntarenas, Osa peninsula	NS	NS	*analis* (Macq.), *devius* (L.), *mutabilis* (L.)	*Microdon* sp.
Ubristes tenuicaudum Curran	ECU: Napo, Julian Sancha Res.	AY261710	NA	*analis* (Macq.), *devius* (L.), *mutabilis* (L.)	*Ubristes* spp.
Outgroup taxa					
Pipunculidae					
Jassidophaga villosa (v. Roser)	SWI: VS Sierre Finges	AY261685	AY261731	Pipunculidae larvae	*Pipunculus* sp.
Platypezidae					
Agathomyia unicolor Oldenberg	UK: Berks	AY261684	AY261730	Platypezidae larvae	*Agathomyia* sp.

the mitochondrial COI gene followed procedures described in Ståhls & Nyblom (2000). The D2–3 region of the nuclear 28S rRNA gene was amplified with primers and PCR profiles described in Belshaw & Quicke (1997) and Campbell *et al*. (1993).

All PCR products were cleaned with the GFX-kit (Amersham Pharmacia Biotech, Little Chalfont, U.K.). The Big Dye Terminator sequencing kit (original and version 2, Applied Biosystems, Foster City, CA) was used for sequencing reactions. Sequencing was carried out on an ABI 377 (Applied Biosystems) sequencer. The PCR primers served also as sequencing primers, and sequences were obtained for both strands. Sequences were inspected and assembled using Sequence Navigator™ (Applied Biosystems), and are available in GenBank under the accession numbers listed in Table 1. When possible, more than one specimen of a species from geographically different areas (Table 1) was used to verify the sequence and detect possible sequence variation.

Direct optimization and simultaneous analysis

The combined data were analysed using direct optimization (optimization alignment), a method described by Wheeler (1996) and implemented in the computer program POY (Gladstein & Wheeler, 1996–2000). The direct optimization is a maximum parsimony algorithm that can process unaligned molecular sequences in addition to morphological and aligned molecular data. Like other heuristic parsimony algorithms, direct optimization strives to find the shortest cladogram by determining the lengths of many different topologies, but unlike other optimization algorithms, it works with unaligned sequences (which may be of unequal length) (Wheeler, 1996; Schulmeister *et al*., 2002).

Direct optimization is the only currently available solution to the problem of including the alignment procedure into the simultaneous analysis framework. A unique scheme of positional homologies is created for each examined topology during the tree search. Hence the length of the shortest combined (simultaneous) analysis cladogram is based on a positional homology scheme generated specifically for this particular topology. The (most parsimonious molecular and co-optimized) positional homologies can be output after the analysis in an implied alignment. In direct optimization, molecular and morphological data are analysed in the same context. Homology statements among nucleotide bases are affected by their co-optimization with morphology (Wheeler, 1996; Schulmeister *et al*., 2002).

Sensitivity analysis and congruence

To examine the sensitivity of a cladogram to the alignment and analysis parameters, and to remove the arbitrariness of the choice of these values, Wheeler (1995) introduced the concept of sensitivity analysis (Schulmeister *et al*., 2002). We explored the results of parsimony analyses by varying the gap cost (insertion–deletion) and change cost (transversion–transition) values, weighting the morphological characters equal to the gap-cost value. We repeated the analyses with six different sets of values for the analysis parameters, and examined under which of the parameter sets a given clade or taxon was recovered as monophyletic. The chosen parameter sets for the sensitivity analysis are the combinations of 'gap cost : transversion cost : transition cost' of 1 : 1 : 1, 2 : 1 : 1, 2 : 2 : 1, 4 : 1 : 1, 4 : 2 : 1, 4 : 4 : 1, weighting the morphological characters equal to the gap cost. This can be graphically represented in a sensitivity plot. In this way, a sensitivity analysis can discern between robust clades (those that appear under most or all of the parameter sets) and less robust clades (those that appear under one or a few of the parameter sets) (Schulmeister *et al*., 2002). We follow Schulmeister *et al*. (2002) and call this a stability tree, a kind of majority-rule consensus tree that summarizes cladograms resulting from repeated analyses of the same data with different parameter values; this distinguishes it from the usual majority-rule consensus tree made from equally most parsimonious trees resulting from a single analysis. In this case, the stability tree shows clades that are present in at least half of the eight combined analyses trees, which can be regarded as relatively stable. This can be regarded as taxonomic congruence, but within a dataset under different weighting schemes. We did not examine the character congruence, commonly performed with the ILD-test (Mickevich & Farris, 1981). This test is used to choose the parameter set (and resulting trees) that maximize the congruence between the datasets and thereby also maximize support. Although many congruent datasets also are the best supported, this is not always the case. Some studies (e.g. Yoder *et al*., 2001) show that lowest incongruence does not necessarily mean highest support (Bremer support). Using the ILD-value as the optimality criterion for choosing the best (or preferred) tree remains ambiguous. For our purpose of evaluating the phylogenetic placements of certain clades, taxonomic congruence within the datasets as presented in a stability tree is sufficient to present the results.

Cladistic analysis

The 28S data were divided into three datasets (using the conserved sequence regions as guide-lines), and the COI into two datasets, speeding up the computations. The morphological characters were treated as unordered. The analyses were performed with a parallel version of POY using eight processors in a UNIX cluster. The command line was as follows: -norandomizeoutgroup -noleading -maxtrees 5 -multibuild 15 -random 20 -treefuse -fuselimit 25 -fitchtrees -slop 5 -checkslop 30 -seed -1 -driftspr -numdriftspr 5 -drifttbr -numdrifttbr 5. The commands used are explained in the POY manual, available online (Janies & Wheeler, 1996–2000; Janies & Wheeler, (2002). In general, the commands refer to known tree-building strategies as implemented in a parallel-processing environment. A few comments are in place, however.

The '-slop' value was set to 5, and the lengths were later tested with '-checkslop 30'. The command '-noleading' prevented the counting of leading and trailing gaps. The '-fitchtrees' option ensures that the trees kept in memory are a random subset of all trees that would have been kept had the tree buffer been larger – a extremely useful option. The commands '-treefuse' and '-fuselimit 25' set the maximum value for donor–recipient treefuses to 25 (see Goloboff, 1999). The commands with 'drift' in their name implement tree-drifting *sensu* Goloboff (1999).

The trees in parenthetical notation were converted into strict or majority-rule consensus trees (or the most parsimonious tree if only one topology was retained) with the program JACK2HEN (available at http://www.cladistics.com).

Results

Sequences

The obtained nucleotide sequences of the mitochondrial gene cytochrome *c* oxidase subunit I (COI) spanned the positions 1776–2904 in COI (numbering is based on *Drosophila yakuba* sequence: Clary & Wolstenholme, 1985), together comprising 1128 nucleotide characters, of which 587 were variable and 440 parsimony informative. There was no evidence of insertions or deletions. Base frequencies were: A 31.7%, C 14.4%, G 14.1% and T 39.6%. The intraspecific variation detected was 0.1–0.3%.

The obtained sequence of the D2–3 region of the nuclear 28S rRNA gene varied between 603 and 640 nucleotides for the species sequenced. An 11-bp insertion present only in six taxa (*Microdon mutabilis* and *M. analis*, *Merodon cinereus*, *Neoascia tenur*, *Milesia fuscicosta* and *Ceriodini* sp.) was removed prior to analyses.

The larval dataset of 187 characters had 132 parsimony informative characters, and the adult dataset of 122 characters had 119 parsimony informative characters.

Larval data

The larval data of Rotheray & Gilbert (1999) with the inclusion of *Alipumilio* and *Nausigaster* (Rotheray *et al.*, 2000) was analysed separately using parsimony analysis with Nona Version 1.8 (Goloboff, 1993. Computer program distributed by the author). This equal weighting analysis resulted in 16 most parsimonious trees (L = 578 steps); the strict consensus of these is shown in Fig. 1. The original dataset of Rotheray & Gilbert (1999) used 85 taxa; the taxon set of the present study with 51 taxa produced an identical topology, and hence the conclusions were not altered.

Adult data

Parsimony analysis using Nona of equally weighted adult dataset with the 122 characters resulted in 59 most parsimonious trees (L = 682 steps). The strict consensus of these is shown in Fig. 2. The clades that are unambiguously supported by the adult characters include subfamily Syrphinae, and tribes Eristalini, Eumerini, Rhingiini, Microdontini and Xylotini, but relationships among all clades remain unresolved.

Molecular data

The molecular data were analysed separately using POY under five different parameter schemes (gap cost : transversion cost : transition cost) 1 : 1 : 1; 2 : 1 : 1; 2 : 2 : 1; 4 : 2 : 1;

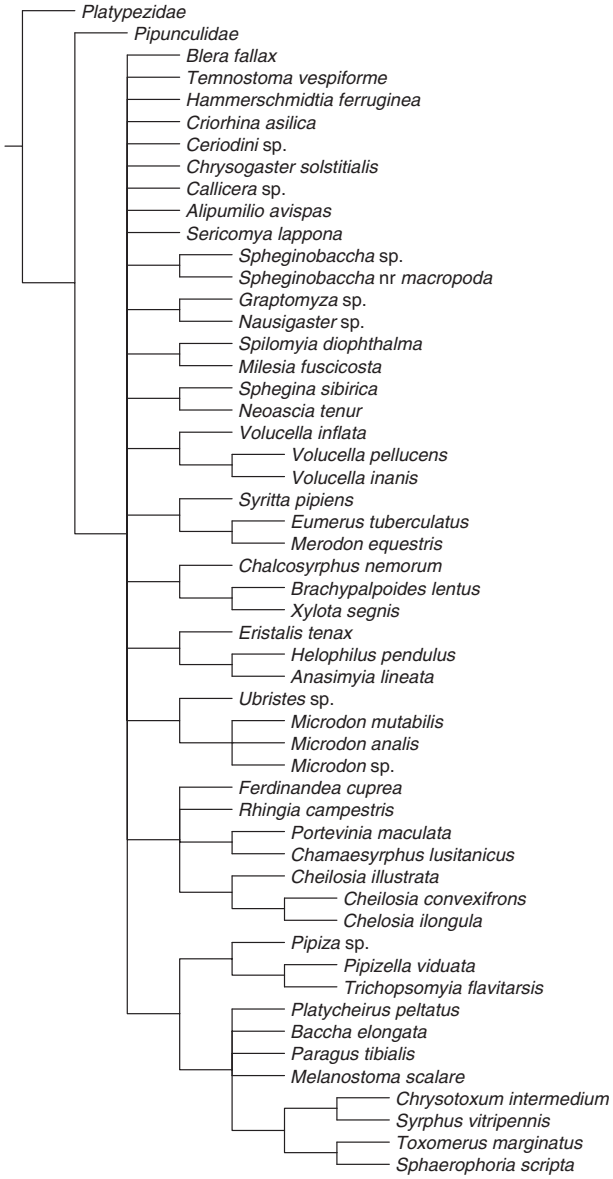

Fig. 2. Strict consensus of 59 most parsimonious trees inferred from analysis of 122 adult characters (length = 682).

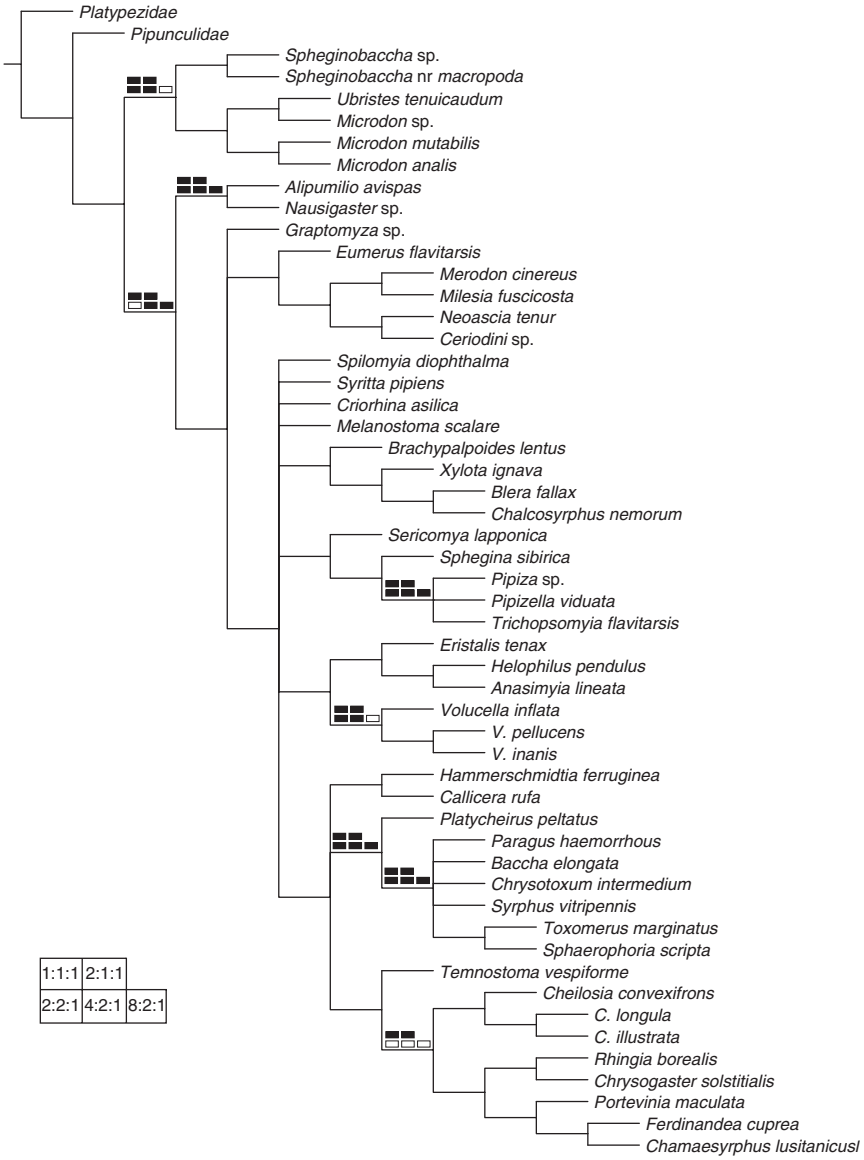

Fig. 3. Stability tree: a majority rule consensus tree (50%) of the trees resulting from combined molecular data under five parameter schemes. The dark fields indicate those parameter sets in which the respective clade came out as monophyletic.

8 : 2 : 1. The results were summarized in a stability tree (50% majority rule consensus tree), Fig. 3. The molecular stability tree supported Microdontini + *Spheginobaccha*, but relationships between and within Syrphinae and Eristalinae remained unresolved. Notably, the Pipizini was never recovered as sister to Syrphinae.

Combined analysis

The trees resulting from the combined analyses with the six different parameter regimes are shown in Figs 4–9. Although these trees have many clades in common, the resolution does vary to some extent between weighting schemes. Four parameter schemes result in nearly identical topologies; 1 : 1 : 1 (1), 2 : 1 : 1 (2), 2 : 2 : 1 (2) and 4 : 2 : 1 (4), whereas the parameter schemes of 4 : 1 : 1 (4) and 4 : 4 : 1 (4) result in topologies that are quite close to the larval phylogeny presented by Rotheray & Gilbert (1999).

The six different parameter regimes resulted in eight most parsimonious trees (Table 2), and the strict consensus tree of these is shown in Fig. 10. The clades that are present in the strict consensus tree have been regarded as monophyletic groups by most authors, but the tree is not very informative. The topologies of the eight trees were summarized in a stability tree (Fig. 11) to extract more information from the results.

Discussion

The most basal syrphid clade of the stability tree is a clade that contains *Spheginobaccha* and Microdontini (Fig. 11).

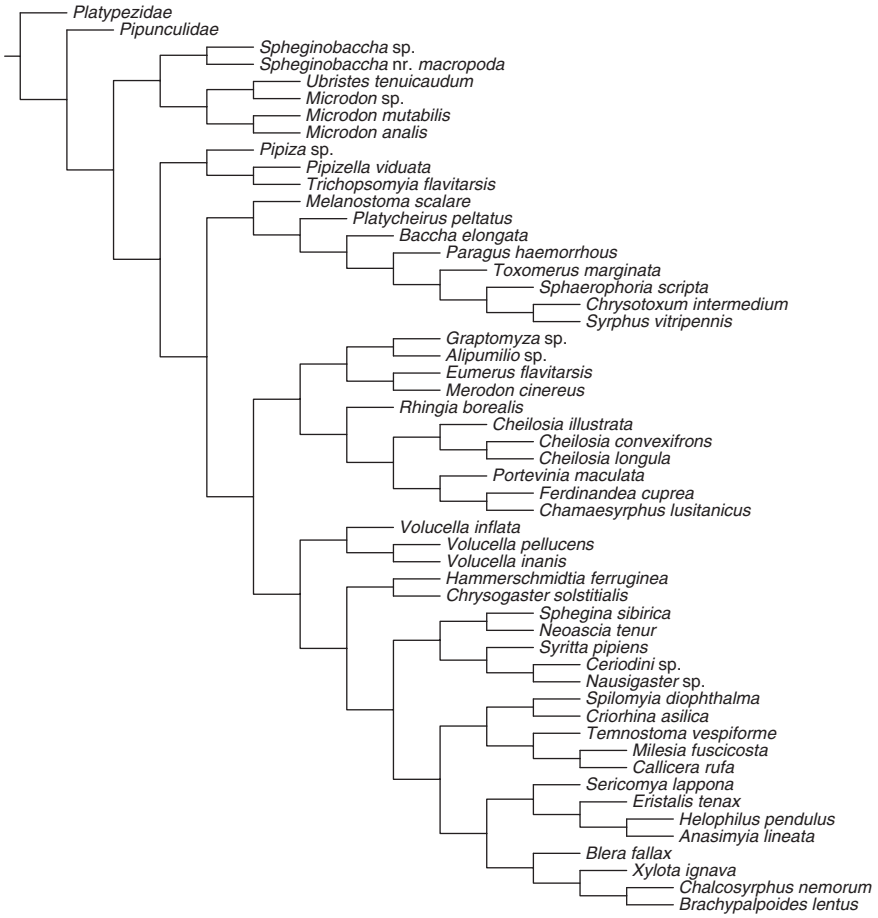

Fig. 4. Combined analysis: parameter scheme 1 : 1 : 1 (1).

This clade is supported in all parsimony analyses under the different parameter schemes. Based on adult characters, Shatalkin (1975a) classified the genus *Spheginobaccha* into Microdontinae, and this result is supported by the present combined analyses. The basal position of the clade is supported in all different parameter regimes, except 4 : 1 : 1 (4) and 4 : 4 : 1 (4) (Figs 7, 9). In his study on Neotropical Microdontinae, Thompson (1969) proposed a basal position of *Microdon* in syrphid evolution. Gilbert *et al.* (1994) and Rotheray & Gilbert (1999) proposed a sister-group relationship of the Microdontini to the Syrphinae + Pipizini, presenting a single clade for syrphids with predatory larvae (Fig. 1). The molecular study of Skevington & Yeates (2000) also suggested a basal placement of Microdontini, both in the separate and combined parsimony analyses of the included molecular data. The basal placement of this subfamily (consisting of *Spheginobaccha* and the Microdontini) was supported by almost all the different parameter schemes in the present analysis, and by the majority of studies cited, suggesting that both the clade and its placement may be very stable. Thompson (1972) and Speight (1987) discussed raising Microdontinae to familial rank, as both the larval and adult morphology differ considerably from that the rest of the syrphids. By contrast, the larval characters scored by Rotheray & Gilbert (1999) supported the traditional classification of the Microdontini within the Syrphidae.

The long-standing dispute of whether the Pipizini is a member of the subfamily Syrphinae (the larval evidence) or the Eristalinae (the traditional evidence from adult morphological characters) was resolved in this study in favour of the larval evidence. Our separate analysis of adult characters recovered the sister-group relationship of Pipizini + Syrphinae (Fig. 2). In the stability tree the tribe was placed as sister-group to Syrphinae (Figs 5–9), or as a separate clade basal to Syrphinae only in the equal weighting scheme (Fig. 4). In the stability tree this sister-group relationship is supported (Fig. 11). The monophyletic clade Pipizini is robustly supported, as it was recovered under all weighting schemes. Placement of Pipizini as an evolutionary lineage separate from Syrphinae was proposed by Goffe (1952) and Thompson (1969). Larval evidence (Rotheray & Gilbert, 1989, 1999) suggested consistently that Pipizini were the sister-group to the rest of Syrphinae, as does the present adult dataset. Cheng *et al.*'s (2000) molecular data also suggested that Pipizini were closer to Syrphinae than to Eristalinae. The molecular study of Skevington & Yeates (2000) placed their single representative of the Pipizini (*Triglyphus fulvicornis*) as sister to Eristalini (*Eristalinus punctulatus*) + Brachyopini (*Cyphipelta rufocyanea*), but

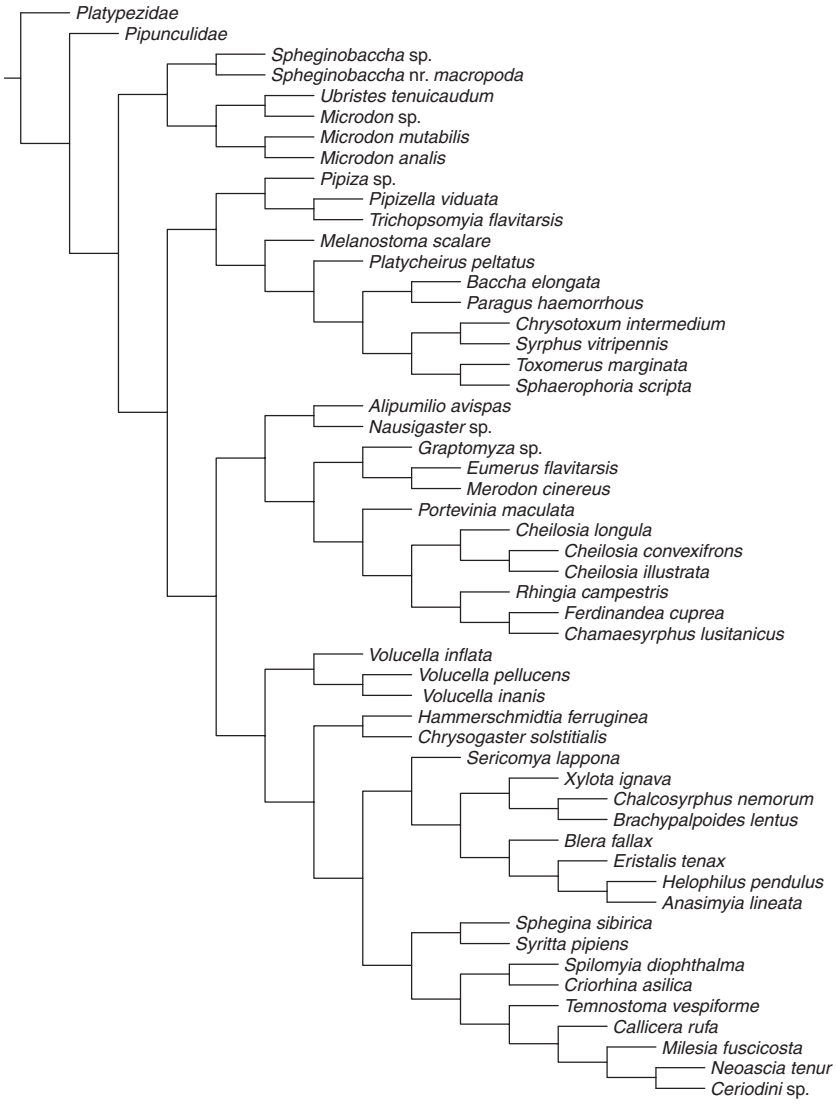

Fig. 5. Combined analysis: parameter scheme 2 : 1 : 1 (2).

branch support was low. Separate analysis of our molecular data never recovered the Pipizini + Syrphinae relationship. In light of our combined analysis, supported by the several studies cited above, Pipizini belongs to subfamily Syrphinae.

Eumerini + Cheilosini form a sister-group relationship under certain weighting schemes. The larvae of members of these two tribes have the same feeding modes being essentially phytophagous, but some are saprophagous. Eumerini always included *Alipumilio*, and under some parameter schemes also *Nausigaster* (Fig. 5). *Graptomyza* was placed in the Eumerini under several weighting schemes (Figs 4–6) as well. *Graptomyza* is classified into the tribe Volucellini (*Volucella* + *Graptomyza* + *Ornidia* + *Copestylum*). Neither our separate analysis of adult or molecular characters nor of the combined analyses suggested a *Graptomyza* + *Volucella* relationship. As the *Graptomyza* data were incomplete (larval data lacking), a definite conclusion about the relationships seems out of place at the moment.

Cheilosia and *Volucella*, and Cheilosiini (Rhingiini), were always monophyletic, and thus these nodes are insensitive to variation in weighting schemes. The hypothesis of Rotheray & Gilbert (1999) suggested that both *Cheilosia* and *Volucella* were non-monophyletic, possibly because multiple larval feeding modes are found within these genera. Adult and molecular characters support the monophyly of these genera.

Most nodes in Eristalinae are sensitive to changes in the different weighting schemes, but some general conclusions may be drawn. For example, *Sericomya lapponica* is placed as sister-group to Eristalini under four weighting schemes. *Blera* (traditionally in the Milesini) appeared as sister-group to Xylotini under five parameter schemes, and as sister-group to the Eristalini once. According to the larval

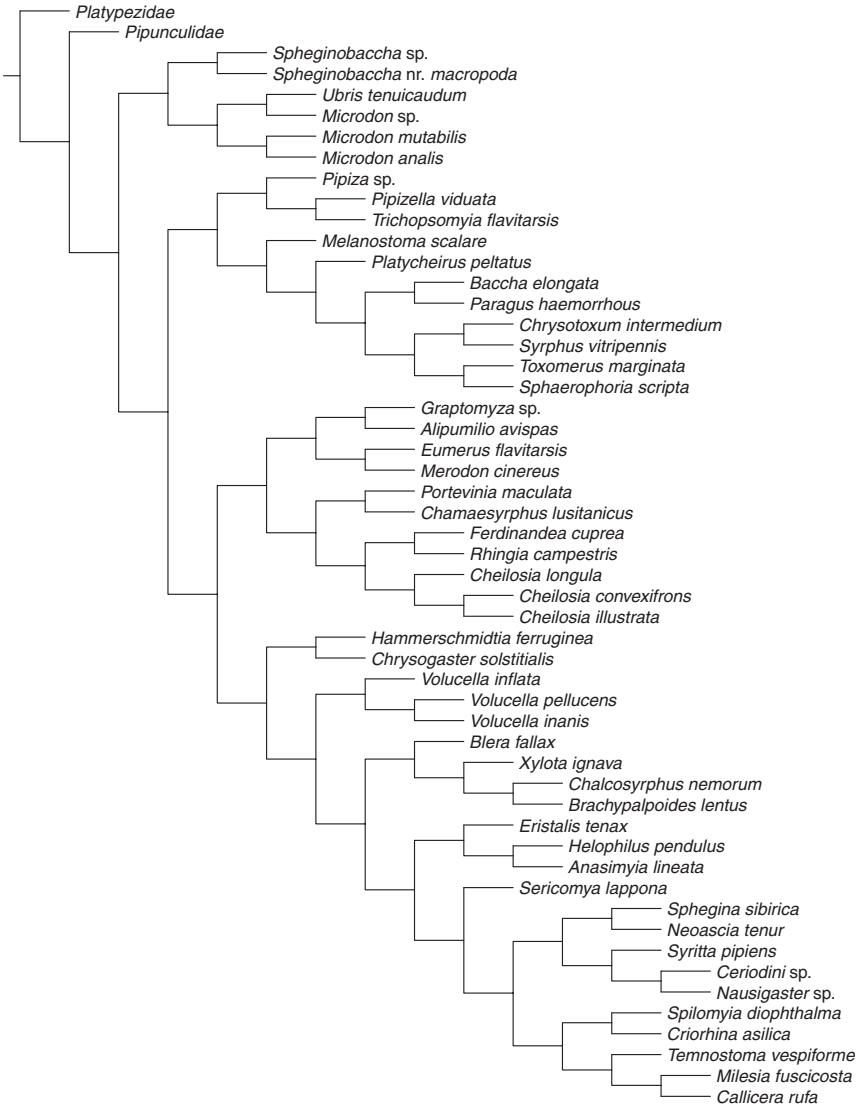

Fig. 6. Combined analysis: parameter scheme 2 : 2 : 1 (2).

characters scored by Rotheray & Gilbert (1999), *Blera* (and *Caliprobola* + *Lejota* not included in the present study) and the Eristalini are the only taxa that exhibit an extended anal segment (long-tailed larvae). In *Blera*, the third ring is extended, whereas in Eristalini the first ring is extended.

Nausigaster was placed as the sister-group to Ceriodini under four parameter schemes, and as sister-group to *Alipumilio* or *Graptomyza* once each. These four taxa are represented in this study by one terminal only. These taxa express a considerable diversity of morphological form, *Alipumilio* and *Nausigaster* being among the most aberrant syrphids that are known. The systematic positions of *Alipumilio* and *Nausigaster* were studied by Rotheray *et al.* (2000), including scoring the character states of *Alipumilio* and *Nausigaster* in the larval dataset. Parsimony analysis (details in Rotheray *et al.*, 2000) suggested *Alipumilio* as the most basal taxon within Syrphidae, immediately followed by *Eumerus*; it also strongly suggested that *Nausigaster* was completely unrelated, placing it within Milesiinae (Fig. 1). The stability tree of the present analyses shows *Alipumilio* + *Graptomyza* as the sister-group to the Eumerini, again close to *Eumerus*, and *Nausigaster* as the sister-group to the included ceriodine species within Milesiini. These placements are largely in agreement with the systematic position proposed by Rotheray *et al.* (2000). *Ceriana* was placed as the sister-group to the milesine genus *Orthoprosopa* by Skevington & Yeates (2000): our results do not support the classification of Ceriodini as a tribe separate from Milesiini.

What are the implications of our results for the evolution of larval feeding habits? Platypezid larvae are associated with fungi and feed on hyphae, fruiting bodies and fungal breakdown products (Ferrar, 1987). Pipunculidae are endoparasitoids of various Homoptera (Ferrar, 1987). By contrast, larvae of Syrphidae have multiple feeding modes

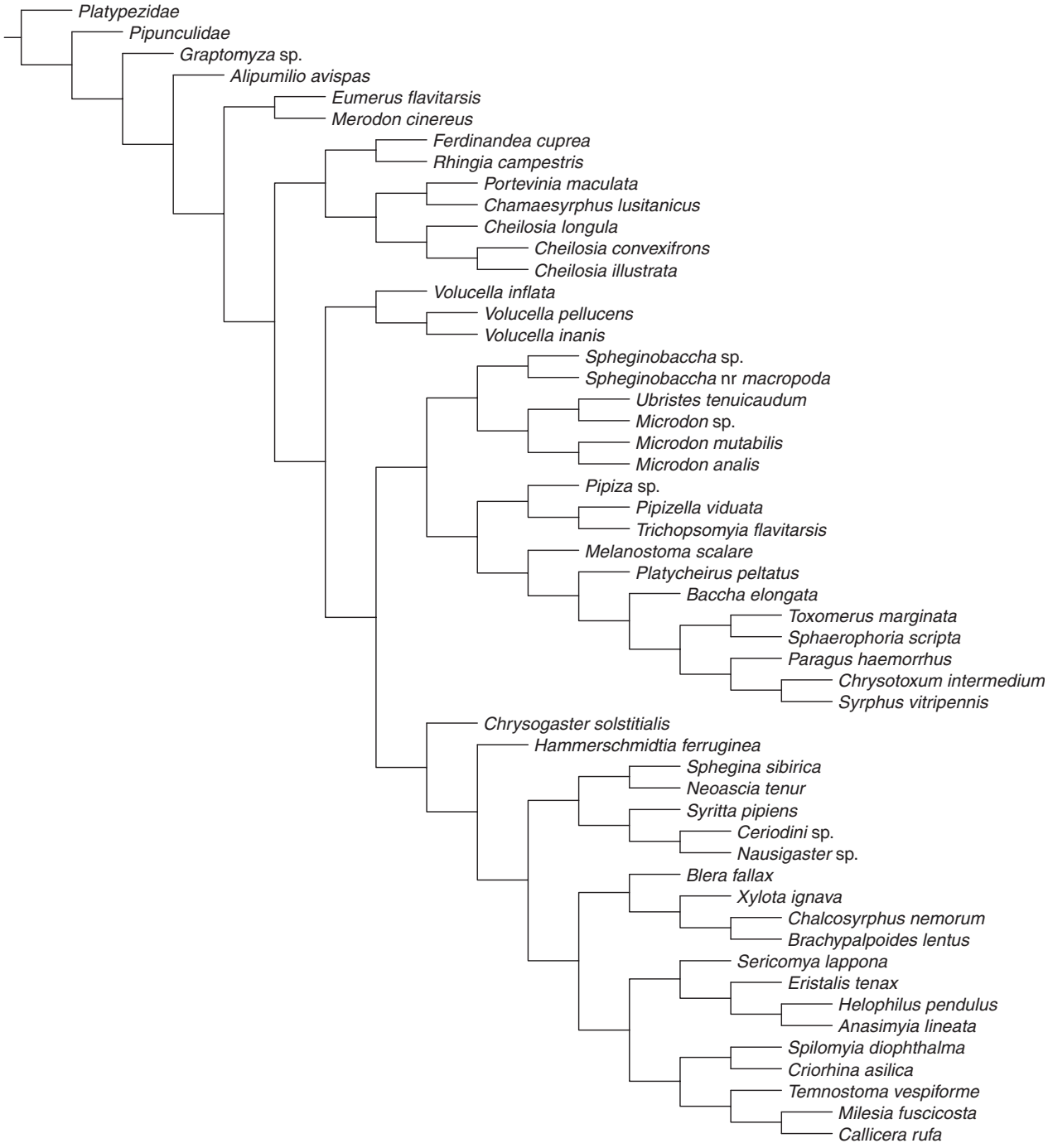

Fig. 7. Combined analysis: parameter scheme 4 : 1 : 1 (4).

involving mycophagy, phytophagy, saprophagy, predation and parasitism. Except for *Cheilosia* and *Volucella*, all genera are true to particular feeding modes, and species within genera occur in the same microhabitat (Rotheray & Gilbert, 1999). Syrphid entomophages (*Microdon*, *Volucella* species, except for *V. inflata*, Pipizini and Syrphinae) exploit two main groups of prey. *Volucella* and *Microdon* prey on larvae of social Hymenoptera, *Volucella* in nests of social aculeates and *Microdon* in ant nests (preying on eggs, larvae and puparia of ants) (see Rotheray & Gilbert, 1999). Pipizini and Syrphinae eat soft-bodied Homoptera. The present results do not support the hypothesis proposed in Rotheray &

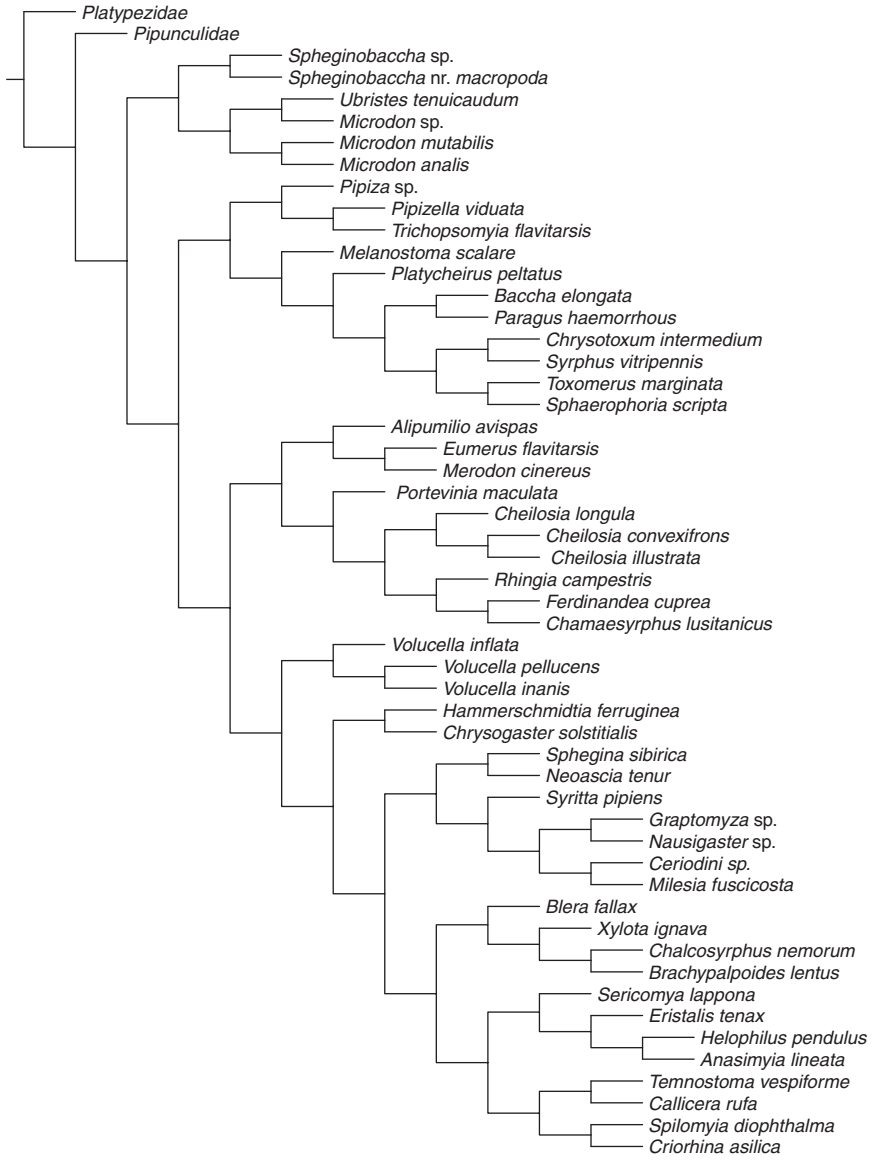

Fig. 8. Combined analysis: parameter scheme 4 : 2 : 1 (4).

Gilbert (1999) that entomophagy in syrphids has a single origin, but rather suggest at least two independent origins. Our results support entomophagy as the basal larval feeding mode, because the two basal lineages constitute taxa with predatory larvae.

Phytophagous larvae are found in Eumerini, saprophagous, phytophagous and mycophagous larvae in Cheilosiini, and in this study Eumerini and Cheilosini clades are basal within Eristalinae. The remaining taxa of Eristalinae are species with saprophagous larvae (except for *Volucella pellucens* and *V. inanis*). The saprophages have a large mouth and a mechanism to filter bacteria suspended in fluids of varying viscosity, but unlike the entomophages, their mouthparts are very uniform (Rotheray & Gilbert, 1999). Saprophages exploit three main habitats: wet decaying vegetation, decaying tree sap and wet decaying heartwood. Rotheray & Gilbert (1999) propose that shifts between these modes are frequent, e.g. larvae of *Hammerschmidtia* (Chrysogasterini), *Sphegina* (Milesiini) and *Chalcosyrphus* (Xylotini) in decaying tree sap, larvae of *Blera* (Xylotini) and *Criorhina* (Milesiini) in wet decaying heartwood. Our results support this hypothesis.

This is the first combined analysis of the phylogenetic relationships of Syrphidae. Although the morphological datasets are smaller than the molecular datasets, they carry some considerable weight in the results because this is 'ensured' by always giving the morphological data the weight of the insertion–deletion event (gap cost). Our study entails a rather broad representation of taxa; future studies will focus on a more thorough taxon sampling of the diverse clades represented by single terminals in this survey. The potential complementary nature of the datasets is

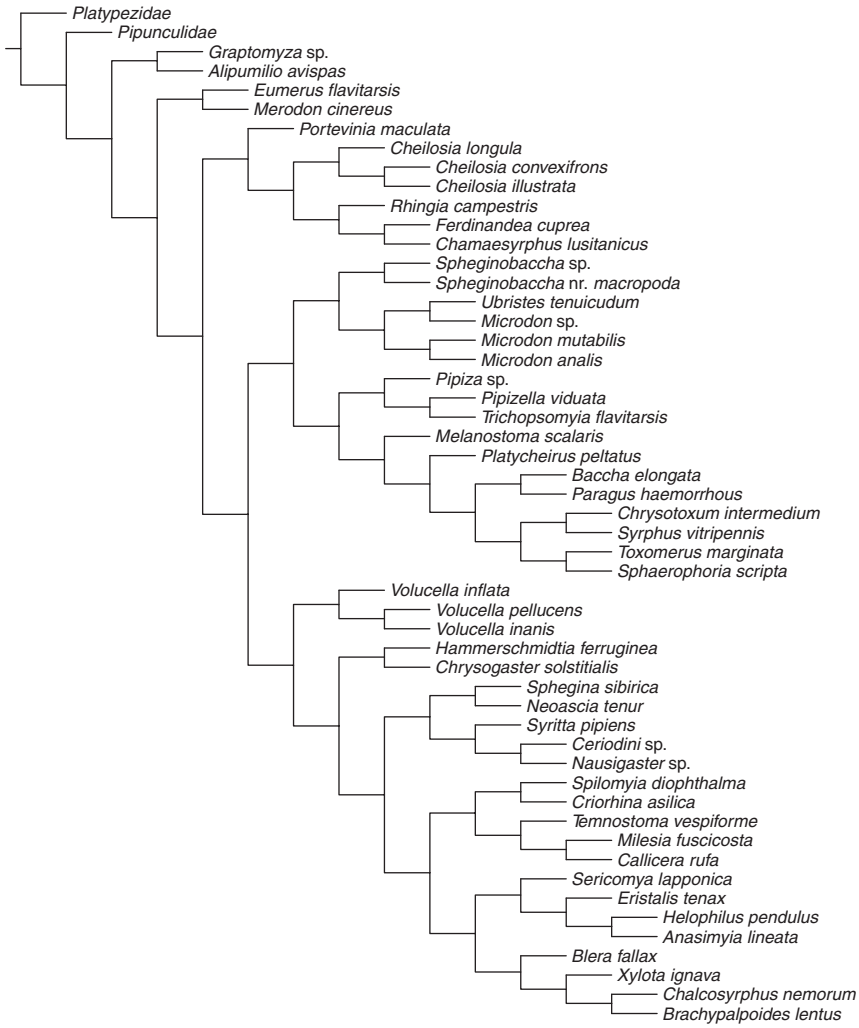

Fig. 9. Combined analysis: parameter scheme 4 : 4 : 1 (4).

the strength of the combined analysis, and results are evaluated in a stability tree. The present classification of Syrphidae into three subfamilies is supported. Placement of Microdontinae as the basal lineage in the family was supported by the majority of the parameter schemes, as well as the inclusion of Pipizini in Syrphinae. The exploration of the intrasubfamilial phylogenetic relationships and stability of clades of both the Syrphinae and the Eristalinae requires a substantial increase in included terminal species.

Table 2. Parameter schemes, number of resulting most parsimonious trees and their lengths (in weighted steps), consistency index (CI) and retention index (RI) for the combined molecular and morphological datasets.

Gap : tv : ts (morph. weight)	No. of trees	Length of combined tree (weighted steps)	CI	RI
1 : 1 : 1 (1)	3	7742	0.16	0.72
2 : 1 : 1 (2)	1	9815	0.22	0.81
2 : 2 : 1 (2)	1	13155	0.22	0.78
4 : 1 : 1 (4)	1	13679	0.28	0.83
4 : 2 : 1 (4)	1	17216	0.19	0.75
4 : 4 : 1 (4)	1	23860	0.19	0.75

Acknowledgements

We are indebted to Ward Wheeler (AMNH) for his help with earlier POY analyses. We thank the following who provided specimens used for the molecular work: A. V. Barkalov, H. Bartsch, P. Chandler, D. Doczkal, F. Dziock, M. A. M. Garcia, N. Laurenne, S. Marshall, B. Merz, M. Metz, S. Rojo, M. Schmaedick, D. Quicke, F. C. Thompson and K. Westman. Financial support from the Niilo Helander Foundation and Carl Cedercreutz Foundation is gratefully acknowledged.

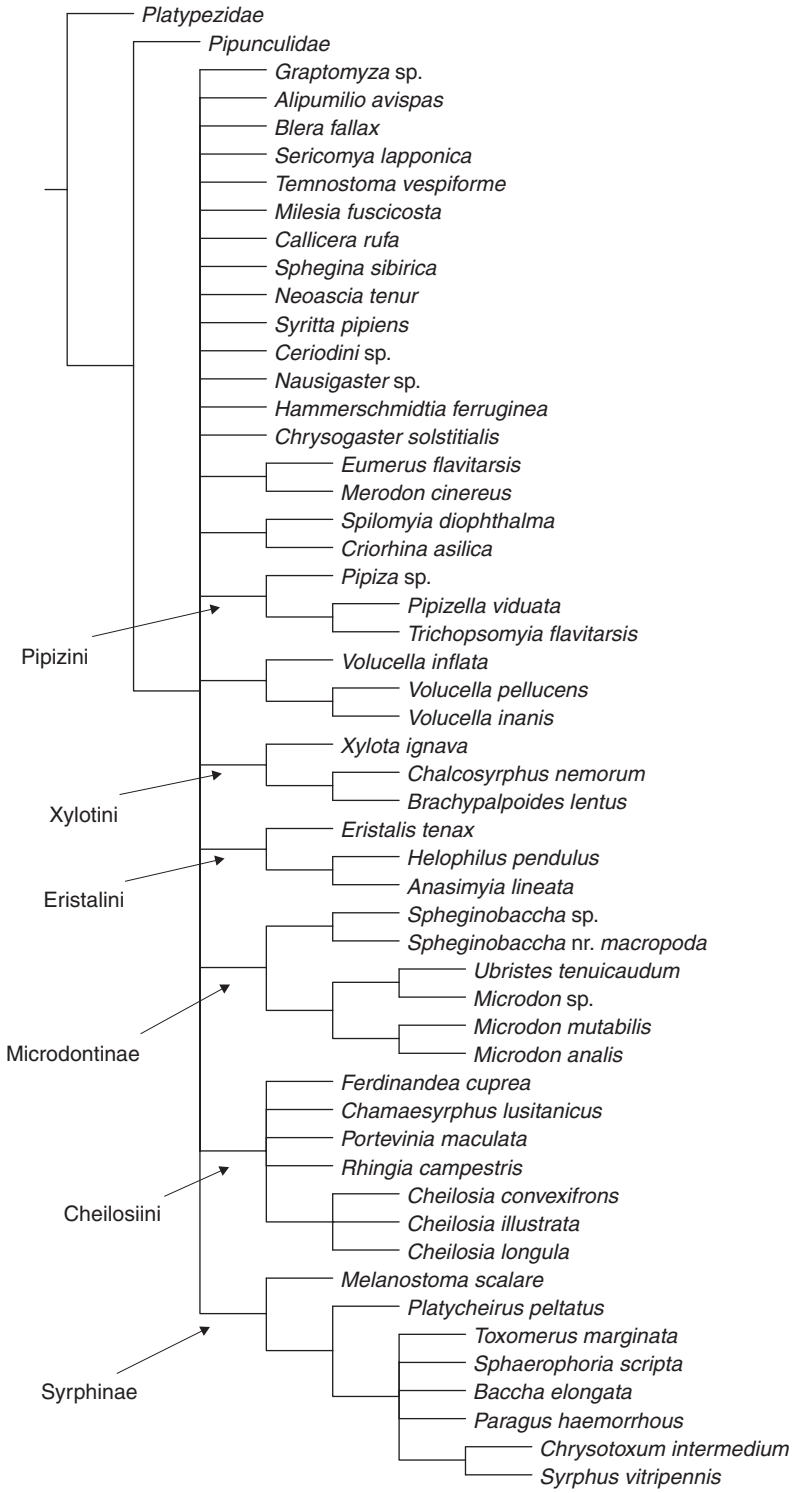

Fig. 10. Strict consensus of eight trees resulting from combined analysis under six different parameter schemes.

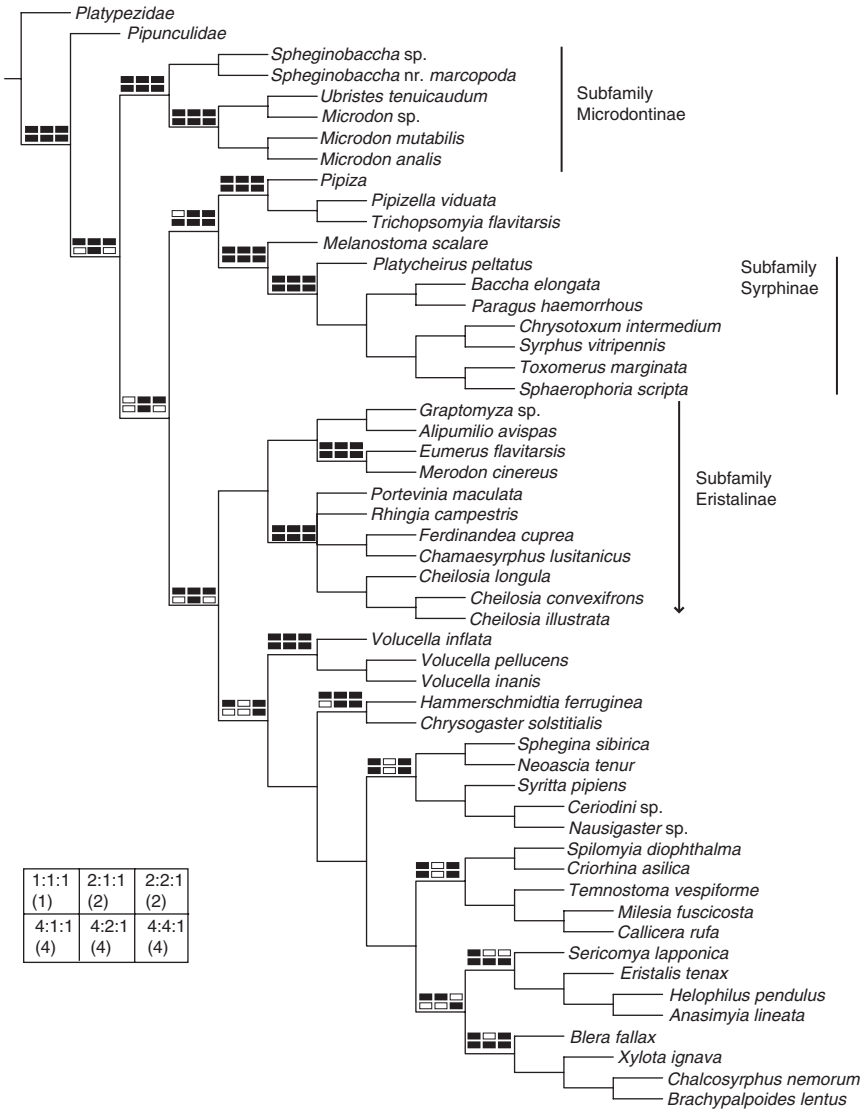

Fig. 11. Stability tree: a majority rule consensus tree (50%) of the trees resulting from all data combined under six parameter schemes. The dark fields indicate those parameter sets in which the respective clade came out as monophyletic.

References

Belshaw, R. & Quicke, D.L.J. (1997) A molecular phylogeny of the Aphidiinae (Hymenoptera: Braconidae). *Molecular Phylogeny and Evolution*, **7**, 281–293.

Campbell, B.C., Steffen-Campbell, J.D. & Werren, J.H. (1993) Phylogeny of *Nasonia* species complex (Hymenoptera, Pteromalidae) inferred from an internal described spacer (ITS2) and 28S rDNA sequences. *Insect Molecular Biology*, **2**, 225–237.

Cheng, X., Lü, 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. *Science in China (Series C)*, **42**, 146–156.

Clary, D.O. & Wolstenholme, D.R. (1985) The mitochondrial DNA molecule of *Drosophila yakuba*: nucleotide sequence, gene organization and genetic code. *Journal of Molecular Evolution*, **22**, 252–271.

Ferrar, P. (1987) *A Guide to the Breeding Habits and Immature Stages of Diptera Cyclorrapha*. Entomonograph 8, Parts 1 and 2. E.J. Brill/Scandinavian Science Press Ltd, Vinderup.

Gilbert, F.S., Rotheray, G.E., Emerson, P. & Zafar, R. (1994) The evolution of feeding strategies. *Phylogenetics and Ecology* (ed. by P. Eggleton & R. Vane-Wright), pp. 378. Academic Press, London.

Gladstein, D.S. & Wheeler, W.C. (1996–2000) POY: phylogeny reconstruction via direct optimization of DNA data. Available at ftp.amnh.org/pub/molecular/poy.

Goffe, E.R. (1952) An outline of a revised classificaton of the Syrphidae (Diptera) on phylogenetic lines. *Transactions Society British Entomology*, **11**, 97–124.

Goloboff, P. (1999) Analyzing large datasets in reasonable times: solutions for composite optima. *Cladistics*, **15**, 415–428.

Hippa, H. (1978) Classification of the Xylotini (Diptera, Syrphidae). *Acta Zoologica Fennica*, **156**, 1–153.

Janies, D. & Wheeler, W.C. (1996–2000) *Documentation for POY*. American Museum of Natural History, New York. ftp.amnh.org/pub/molecular/poy.

Janies, D. & Wheeler, W.C. (2002) *POY Version 3.0: documentation and summary*. American Museum of Natural History, New York. ftp.amnh.pub/molecular/poy.

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

Kuznetzov, S.Yu (1987) On some plesiomorphic characters of aphidophagous larvae of hoverflies in the subfamily Syrphinae (Diptera, Syrphidae). *Dipterous Insects: Systematics, Morphology and Ecology* (Ed. by E.P. Narchyk), pp. 58–61. Zoological Institute, USSR Academy of Sciences, Moscow (in Russian).

Kuznetzov, S.Yu (1992) The first instar larvae of the subfamily Pipizinae and Eristalinae (Diptera, Syrphidae). *Daba en Muzejs*, **4**, 24–43 (in Russian).

Mickevich, M.F. & Farris, S.J. (1981) The implications of congruence in *Menidia*. *Systematic Zoology*, **27**, 143–158.

Rotheray, G.E. & Gilbert, F.S. (1989) The phylogeny and systematics of European predacious Syrphidae (Diptera) based on larval and puparial stages. *Zoological Journal of the Linnean Society*, **95**, 29–70.

Rotheray, G.E. & Gilbert, F.S. (1999) Phylogeny of Palaearctic Syrphidae (Diptera): evidence from larval stages. *Zoological Journal of the Linnean Society*, **127**, 1–112.

Rotheray, G.E., Marcos-Garcia, M.A., Hancock, E.J. & Gilbert, F.S. (2000) The systematic position of *Alipumilio* and *Nausigaster* based on early stages (Diptera, Syrphidae). *Studia Dipterologica*, **7**, 133–144.

Schulmeister, S., Wheeler, W.C. & Carpenter, J.M. (2002) Simultaneous analysis of the basal lineages of Hymenoptera (Insecta) using sensitivity analysis. *Cladistics*, **18**, 455–484.

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

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

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). *Molecular Phylogenetics and Evolution*, **16**, 212–224.

Speight, M.C.D. (1987) External morphology of adult Syrphidae (Diptera). *Tijdschrift voor Entomologie*, **130**, 141–175.

Ståhls, G. & Nyblom, K. (2000) Phylogenetic analysis of the genus *Cheilosia* (Diptera, Syrphidae) using mitochondrial COI sequence data. *Molecular Phylogenetics and Evolution*, **15**, 235–241.

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 Milesinae (Diptera, Syrphidae). *Arquivos de Zoologica*, **23**, 78–215.

Thompson, F.C. (1974) The genus *Spheginobaccha* de Meijere (Diptera: Syrphidae). *Transactions of the American Entomological Society*, **100**, 255–287.

Thompson, F.C. & Rotheray, G.E. (1998) Family Syrphidae. *Manual of Palaearctic Diptera*, **Vol. 3** (ed. by L. Papp & B. Darvas), p. 880. Science Herald, Budapest.

Vockeroth, J.R. (1969) A revision of the genera of the Syrphini (Diptera, Syrphidae). *Memoirs of the Entomological Society of Canada*, **62**, 1–176.

Vockeroth, J.R. & Thompson, F.C. (1987) Family Syrphidae. *Manual of Nearctic Diptera*, 2 (ed. by J.F. McAlpine,), p. 713. Agriculture Canada, Canadian Government Publishing Centre, Hull, Quebec, Canada.

Wheeler, W.C. (1995) Sequence alignment, parameter sensitivity and the phylogenetic analysis of molecular data. *Systematic Biology*, **44**, 321–331.

Wheeler, W.C. (1996) Optimization alignment: the end of multiple sequence alignment in phylogenetics? *Cladistics*, **12**, 1–9.

Yoder, A.D., Irwin, J.A. & Payseur, B.A. (2001) Failure of the ILD to determine data combinability for Slow Loris phylogeny. *Systematic Biology*, **50**, 408–442.

Accepted 23 December 2002