# 4 Phylogenetic relationships of Microdontinae (Diptera: Syrphidae) based on parsimony analyses of combined molecular and morphological characters

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**Abstract.** The intrasubfamilial classification of Microdontinae Rondani, 1845 (Diptera: Syrphidae) has been considered a challenge ever since the name was first used. Although 59 genus group names are available, still more than 300 out of more than 400 valid species names are classified in the single genus *Microdon* Meigen. The present paper is part of a project aimed at resolving the supraspecific taxonomy and classification of the subfamily. This paper presents the results of a phylogenetic analysis of molecular data as well as the results of a combined analysis of molecular and morphological characters. The morphological dataset is described and discussed in Chapter 3. The molecular dataset contains 96 taxa (87 ingroup), and five sequence fragments of three molecular markers: the mitochondrial COI-gene and the nuclear ribosomal RNA genes 18S and 28S. Analysis of molecular data only resulted in poorly resoved trees. Addition of 174 morphological characters to the dataset resulted in strongly resolved trees. Part of the resolution was lost again when the dataset was supplemented with 93 taxa for which only morphological data were available. Based on a discussion of the problem of missing data, the tree resulting from the analysis of the combined analysis of 96 taxa for which molecular data are available was chosen as the preferred tree. Based on this tree the major implications for the classification of Microdontinae are discussed. The Microdontinae are recovered as the sister group of all other Syrphidae, and the genus *Spheginobaccha* is recovered as the sister group to all other Microdontinae. These results corroborate those of other recent studies, but now for the first time employing a large set of microdontine taxa. The genus *Microdon* is clearly polyphyletic.

## Introduction

The Microdontinae are a subfamily of Syrphidae (Diptera) with a worldwide distribution. The vast majority of more than 400 described species occurs in the tropics, of which approximately 170 in the neotropics. With a little more than 50 species known from the Palaearctic and Nearctic regions together, the group is relatively poorly represented in temperate regions. This partly explains why the taxonomy of the group has so far received little attention compared to several other groups of Syrphidae.

Morphological variation within Microdontinae is large, arguably larger than in many families of Diptera Cyclorrhapha. So far, 59 genus group names (minus misspelled names) have been proposed for the taxa in this subfamily (Cheng & Thompson 2008). Nevertheless, still more than 300 out of approximately 400 valid species names are currently classified in the single genus *Microdon* Meigen, 1803. This apparent taxonomic indecisiveness seems to result not so much from a lack of morphological variation, but rather from an excess of it. Several authors have commented on this paradoxical combination of a wealth of morphological diversity and a scarceness of group-defining characters (Bezzi 1915, Curran 1940, Shannon 1927).

Ever since Rondani (1845) introduced the family group name Microdonellae, this group has been recognized as distinct from other Syrphidae, albeit under different spellings and taxonomic rankings. Only occasionally genera were included which are nowadays considered to belong to other subfamilies (Lioy 1864, Shatalkin 1975a, b, Williston 1886). The placement of the group relative to other Syrphidae, however, has been far from stable. For instance, the group has variously been treated as a tribe within the subfamily Syrphinae (Williston 1886), a subtribe within the tribe Volucellini (Goffe 1952), a family (Thompson 1972) and a subfamily (Ståhls et al. 2003). A more detailed history of the classification of Microdontinae is given in Chapter 5.

The most recent advocates of a family status for Microdontinae are Thompson (1972) and Speight (1987, 2010), based on the 'basal' relationship of Microdontinae with other Syrphidae as inferred by Thompson

(1969) from a Hennigian argumentation scheme of characters considered of critical importance. Speight (1987) found additional morphological differences between Microdontinae and other Syrphidae, which he considered to support the family status of the group as first proposed by Thompson (1972). Several recent studies have confirmed this sister-group relationship (Skevington & Yeates 2000, Ståhls et al. 2003, Rotheray & Gilbert 2008), but most recent authors see no necessity to raise the rank of the group to family level and consider the group as a subfamily of the Syrphidae (Cheng & Thompson 2008, Ståhls et al. 2003). Still, however, certain authors prefer to rank the group as a family (Speight 2010).

The classification of the genus *Spheginobaccha* Meijere, 1908 has received special attention of several authors. Its phylogenetic position has shifted between different subfamilies of Syrphidae (for review see Thompson 1974). The first to include it in the Microdontinae was Hull (1949), after which Thompson (1969) excluded it, and Shatalkin (1975a) included it again. Ståhls et al. (2003) placed it into the Microdontinae, based on a phylogenetic analysis of a combination of morphological and molecular data, which recovered the genus as the sister-group of all other Microdontinae.

Previous phylogenetic hypotheses relied on only a few taxa of Microdontinae, e.g. two in Skevington & Yeates (2000), six in Ståhls et al. (2003) and Hippa & Ståhls (2005). These numbers do little justice to the large morphological diversity of the group, so relationships within the Microdontinae remain completely unaddressed. In addition, the present authors felt the need to confirm the supposed sister-group relationship of *Spheginobaccha* and the other Microdontinae. An extended taxon set representing as many genus groups (whether previously recognized or not) as possible, could potentially provide evidence for refuting or supporting this sister-group relationship. For instance, the genera *Aristosyrphus* Curran, 1941, *Eurypterosyrphus* Barretto & Lane, 1947 and *Mixogaster* Macquart, 1841 have certain characters in common with *Spheginobaccha*, such as a hypandrium with apical part consisting of two separate lobes, an unfurcate aedeagus and characters of wing venation (see Chapter 3). For a better understanding and for establishing the position of *Spheginobaccha*, it was thus necessary to include these taxa in the analyses.

The present paper analyzes a combination of morphological and molecular characters of a large set of microdontine taxa. Although the characters of the immature stages of a few taxa of Microdontinae have previously been used for phylogenetic analyses (Rotheray & Gilbert 2008, Ståhls et al. 2003), the number of taxa for which characters of the immature stages could be obtained is considered too small to be used for the present analyses.

Objects of the present paper are:

- to test the sister-group relationship of *Spheginobaccha* with the other Microdontinae;
- to elucidate the phylogenetic relationships within the Microdontinae;
- to discuss the implications of the phylogenetic hypothesis for the classification of Microdontinae;
- to discuss the question wether Microdontinae are to be treated as a separate family or not.

## Material & Methods

### Note on names: disclaimer

Many of the species names used in this paper are combined with genus group names with which they have not been used before. Some of the generic and specific names have not at all been used previously. The justifications for the new combinations, as well as descriptions of new genera and species, can be found in Chapter 5. None of the names and combinations in the present paper are published for purposes of zoological nomenclature. This is a disclaimer with reference to article 8.2 of the International Code of Zoological Nomenclature, 4th edition (ICZN 1999).

### Ingroup taxa and specimens

The starting point for the selection of taxa to include in the ingroup were the genus group names of Microdontinae as listed by Cheng & Thompson (2008). At least one species, preferably the type species, of all these genus groups was included in the combined analysis, whereas in the molecular analysis as many of these taxa as possible were included, depending on availability for molecular analyses. Exceptions to this general rule are objective or otherwise obvious synonyms (e.g. *Aphritis* Macquart, *Colacis* Gistel, *Holm-*

*bergia* Lynch Arribalzaga) and taxon names which are based only on immature stages (e.g. *Ceratoconcha* Simroth, *Nothomicrodon* Wheeler) (for more information on these names and synonymies see Cheng & Thompson 2008). In many cases more than one species per genus group was included. In addition, many new or little known species were included which had not been previously assigned to one of the existing genus groups, or were merely lumped under the generic name *Microdon*, despite their morphological peculiarities. The taxon set contains 35 species new to science, partly belonging to new genera. Descriptions of most of these taxa can be found in Chapters 5 and 6. For the genus *Spheginobaccha*, six species were included, representing all three species groups recognized by Thompson (1974).

The list of specimens used for DNA extraction, including locality and collection data as well as GenBank accession numbers, is given in Appendix 1. This table also indicates whether the morphological characters were scored from the DNA vouchers or from another specimen. In all cases, except one, morphological and molecular characters are based on specimens of the same species. The only exception is *Rhopalosyrphus ramulorum* Weems & Deyrup, 2003 in the DNA dataset: for this species, morphological characters are based on a specimen of the closely related *R. guntheri* (Lynch Arribalzaga, 1891). The complete list of specimens used for constructing the morphological matrix can be found in Chapter 3.

The specimens used for DNA extraction originate from a wide variety of sources and collection methods. Fresh material (< 1 year old) collected directly into ethanol was scarcely available, so for many taxa older material (up to about 10 years), sometimes preserved dry, was used. Because of this, DNA extraction and PCR results differed strongly among the taxa and among the genetic markers that were sequenced (see Results).

**Outgroup**

The parsimony analyses are rooted on *Chalarus* cf. *spurius* (Fallén, 1816) (Diptera: Pipunculidae). Pipunculidae have been recovered as the sister-group of Syrphidae in a number of recent studies (Rotheray & Gilbert 2008, Skevington & Yeates 2000, Yeates et al. 2007). The genus *Chalarus* Walker, 1834 is a presumed basal taxon in pipunculid phylogeny (Rafael & De Meyer 1992, Skevington & Yeates 2000). The outgroup includes another pipunculid, *Nephrocerus lapponicus* Zetterstedt, 1838, as well as a selection of taxa from the syrphid subfamilies Syrphinae and Eristalinae, which together form the putative sister of Microdontinae (Ståhls et al. 2003). Taxa were selected from a broad range of tribes: Chrysogasterini (*Neoascia tenur*), Eristalini (*Eristalis tenax*), Merodontini (*Merodon equestris*), Pipizini (*Pipiza noctiluca*), Syrphini (*Melanostoma scalare, Syrphus vitripennis*), Xylotini (*Xylota segnis*). Locality and collection data are given in Appendix 1.

**Morphological data**

The morphological data used in this paper are based on Chapter 3, in which 174 character statements are described. A phylogenetic analysis of the morphological dataset is also given in Chapter 3.

**Choice of molecular markers**

For the molecular dataset, five sequence fragments of three molecular markers were used: the mitochondrial COI-gene and the nuclear ribosomal RNA genes 18S and 28S. Primer information and combinations are given below and in table 1.

The molecular markers were chosen based on results of previous studies on Syrphidae. A combination of mitochondrial COI and nuclear 28S sequences with morphological characters yielded good results in the study on intrafamilial relationships of Syrphidae of Ståhls et al. (2003). The 18S gene was used by Mengual et al. (2008) and proved to be informative for reconstructing deeper branches in the study of relationships within the subfamily Syrphinae.

**DNA extraction**

For most specimens, two or three legs were used for DNA extraction. In a few cases the entire thorax or the abdomen was used. Prior to extractions, ethanol preserved samples were rinsed in distilled water.

DNA extractions were done using the NucleoSpin® Tissue extraction kit, following the manufacturer's protocol, eluting the DNA into 50 µl of elution buffer. For some very small specimens NucleoSpin® Tissue XS was used, which involves the same extraction procedures, except for some differences in the quantities of buffers and washing liquids.

**Table 1.** List of primers.

| Gene | Primer name (nickname) | Sequence 5' – 3' | Source |
|---|---|---|---|
| Cytochrome c oxidase subunit I (COI) | LCO-1490 | GGTCAACAAATCATAAAGATATTG- | Folmer et al. 1994 |
| | HCO-2198 | TAAACTTCAGGGTGACCAAAAAATCA | Folmer et al. 1994 |
| | C1-S-1718 (Beet) | GGAGGATTTGGAATTGATTAGTTCC | Simon et al. 1994 |
| | C1-J-2183 (Jerry) | CAACATTTATTTTGATTTTTTGG | Simon et al. 1994 |
| | TL2-N-3014 (Pat) | TCCAATGCACTAATCTGCCATATTA | Simon et al. 1994 |
| | C1-N-2735 (Inger) | AAAATGTTGAGGGAAAAAATGTTA | Lunt et al. 1996 |
| 18S | 1F | TACCTGGTTGATCCTGCCAGTAG | Whiting Lab |
| | b7.0 | ATTTRCGYGCCTGCTGCCTTCCT | Whiting Lab |
| | 2F | AGGGTTCGATTCCGGAGAGGGAGC | Whiting Lab |
| | b3.9 | TGCTTTRAGCACTCTAA | Whiting Lab |
| | b2.9 | TATCTGATCGCCTTCGAACCTCT | Whiting Lab |
| 28S | 2F | AGAGAGAGTTCAAGAGTACGTG | Belshaw et al. 2001 |
| | 3DR | TAGTTCACCATCTTTCGGGTC | Belshaw et al. 2001 |

## PCR

For all gene fragments, PCR amplifications were done using 4-8 µl of DNA-extract, suspended in a total volume of 25 µl reaction mix also containing 2.5 µl of 10X Buffer II, 2 µl mM $MgCl_2$, 4 µl 200 mM dNTP, 0.25 µl of Taq DNA polymerase, ultrapure water (volume dependent on volume of DNA-extract) and 1 µl each of two primers (at 10 pmol/ µl). The primers used for the amplified gene fragments are listed in Table 1. The following combinations were used: COIa: LCO+HCO or the smaller fragment Beet+HCO; COIb: Jerry-Pat or the smaller fragment Jerry+Inger; 18S: the full fragment 1F+b3.9 or the two overlapping fragments 1F+b7.0 and 2F+b2.9; 28S: F2+3DR. For many samples, attempts to amplify larger gene fragments (e.g. LCO+HCO and Jerry+Pat for COI, or 1F+b3.9 for 18S) failed. For this reason, only the smaller fragments were amplified (e.g. Beet+HCO for COI, or 1F+b7.0 for 18S).

For all amplifications, the following thermocycler profile was used: (step 1) 2 min. at 95 °C, (step 2) 1 min. at 94 °C, (step 3) 30 sec. at 49 °C, (step 4) 2 min. at 72 °C, (step 5) repeat steps 2-4 for 30 times, (step 6) 7 min. at 72 °C, (step 7) cool down for some minutes at 4 °C.

The PCR products were visualized by running 4 µl PCR product on a 1.5% agarose gel. PCR products were treated with ExoSapIt prior to sequencing reactions. Sequencing electrophoresis was done in the sequencing laboratory of the Institute for Molecular Medicine, University of Helsinki, Finland, with an ABI3730xl DNA Analyzer.

Sequences of forward- and reverse primers were assembled and edited in Sequence Navigator (version 1.01, Applied Biosystems). For the outgroup taxon *Chalarus spuriae* (MZH_Y800), the COIb sequence was not available, fow which reason the sequences of this taxon were combined with the COIb sequence of *Chalarus* spec. (MZH_Y0038).

## Alignment

The mitochondrial DNA sequences of the (protein coding) COIa and COIb gene fragments were aligned manually by their codon positions. Sequences of the 18S and 28S ribosomal RNA genes were aligned separately using MAFFT version 6 (Katoh & Toh 2008, Katoh et al. 2002, 2009). This program offers a number of different algorithms, several of which have

been demonstrated to perform very well compared to those of other programs (e.g. ClustalW, DIALIGN-T, T-COFFEE) for multiple sequence alignment (Golubchik et al. 2007, Rosenberg 2009). The algorithm used in the present study was E-INS-i. Based on the information in Katoh & Toh (2008) and Katoh et al. (2009), this algorithm was considered to be most suitable for the ribosomal DNA sequences under study, as it was developed for dealing with sequences with considerable length variation.

### Analyses

Analyses of molecular datasets and of the combined datasets were performed using the parsimony program TNT (Tree Analysis using New Technologies) version 1.1, October 2010 (Goloboff et al. 2008) with gaps treated as missing data and morphological characters treated as non-additive. All matrices were analyzed using a combination of all four 'new technology' heuristic search methods of TNT, under their default parameters: sectorial search, parsimony ratchet, tree-drifting and tree-fusing (see e.g. Giribet 2005 and Goloboff et al. 2008 for explanations on commands).

*Molecular data*

All molecular markers were first analyzed separately. Sequences of taxa with remarkable placements (e.g. ingroup taxa in the outgroup) were scrutinized for possible errors in the sequences, e.g. because of copy-paste errors in the datafiles or contamination during DNA extraction or amplification. A small number of suspect or erroneous sequences have subsequently been omitted from further analyses.

One matrix integrating the data of all three different markers (in five fragments) was constructed, which contained 96 taxa and 2808 columns of nucleotide data. The TNT search for this matrix was stopped after the shortest length was found 50 times, after which the trees found were subjected to TBR branch swapping under default parameters. The same analysis was also done with exclusion of COIb fragment of COI, in order to evaluate topological difference resulting from exclusion on the COIb dataset, in which data is missing for 46 of the 96 taxa.

*Combined data*

Molecular and morphological datasets were merged using the *dmerge* command in TNT.

Two combined matrices were constructed: one containing only the 96 taxa for which both molecular and morphological data are available ('subset'), the other containing 189 taxa, including 93 taxa for which only morphological data are available ('total set'). Both matrices include 2808 molecular and 174 morphological characters. The TNT searches for these matrices were stopped after the shortest length was found 100 times (subset) or 10 times (total set), after which the trees found were subjected to TBR branch-swapping.

*Measures of support and stability*

Bremer support values were calculated by TBR branch swapping based on the strict consensus trees. This was done in TNT using the 'Bremer supports' option under the 'Trees' menu, examining trees up to 100 steps longer than the most parsimonious trees. Jackknife values and GC frequency differences (Goloboff et al. 2003) were calculated in TNT, using 1000 replicates and a removal probability of 36%. GC values indicate the difference between the frequency in which nodes are retrieved in the jackknife replicates and the frequency of the most frequent contradictory group. So, in contrast with normal jackknife-values, the GC values are informative for the amount of contradictory information in the dataset. In case these values are equal, there are no contradictory groups which are supported by the data.

## Results

### PCR amplification and obtained sequences

Appendix 1 indicates which fragments could be amplified for each sample. Total success rates for the different fragments were as follows: COIa - (84%); COIb (52%); 18a (94%); 18Sb (66%); 28S (66%).

### Analysis of molecular data

The 'new technology' search of the dataset including all DNA fragments resulted in an initial number of 109 most parsimonious trees of length 8109. TBR branch swapping based on these trees resulted in 1722 equally parsimonious trees of length 8109. The strict consensus of these trees is given in figure 1. Parsimony analysis of the dataset without the COIb

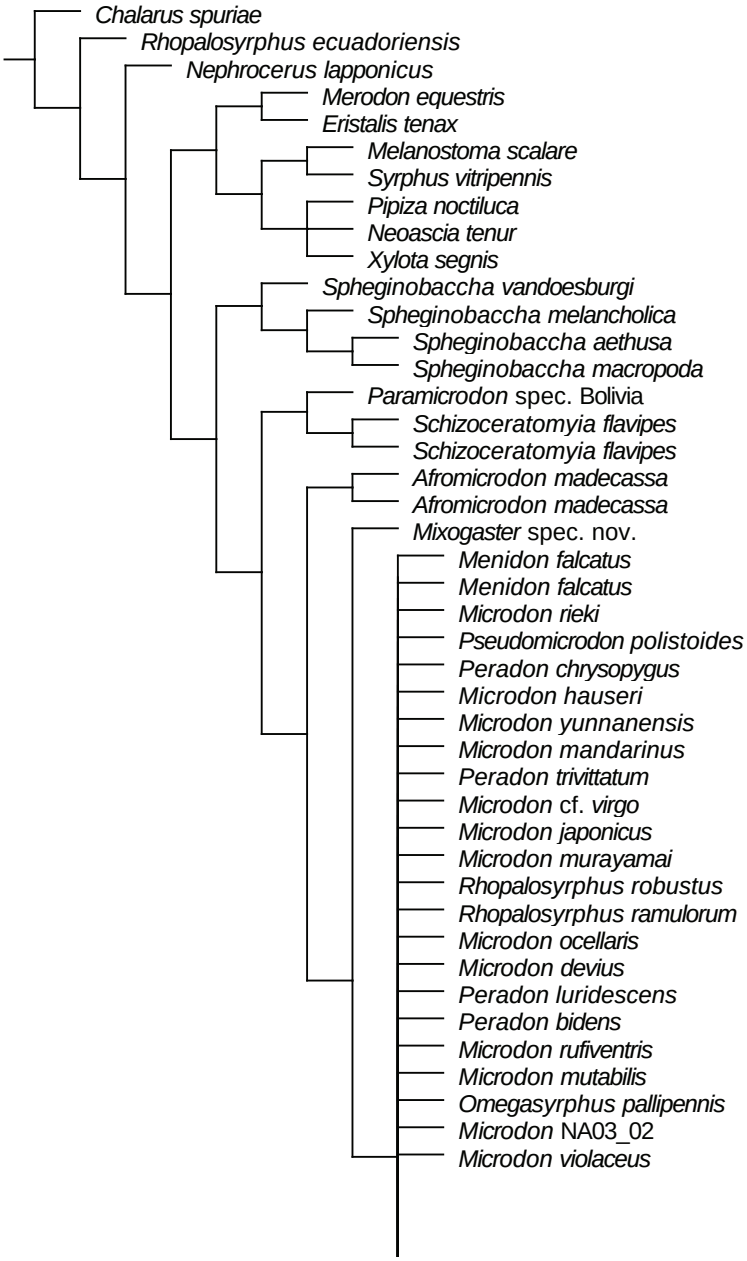


**Fig. 1.** Molecular analysis (all five DNA fragments): strict consensus of 1722 most parsimonious trees of length 8109.
*Continued on next page.*

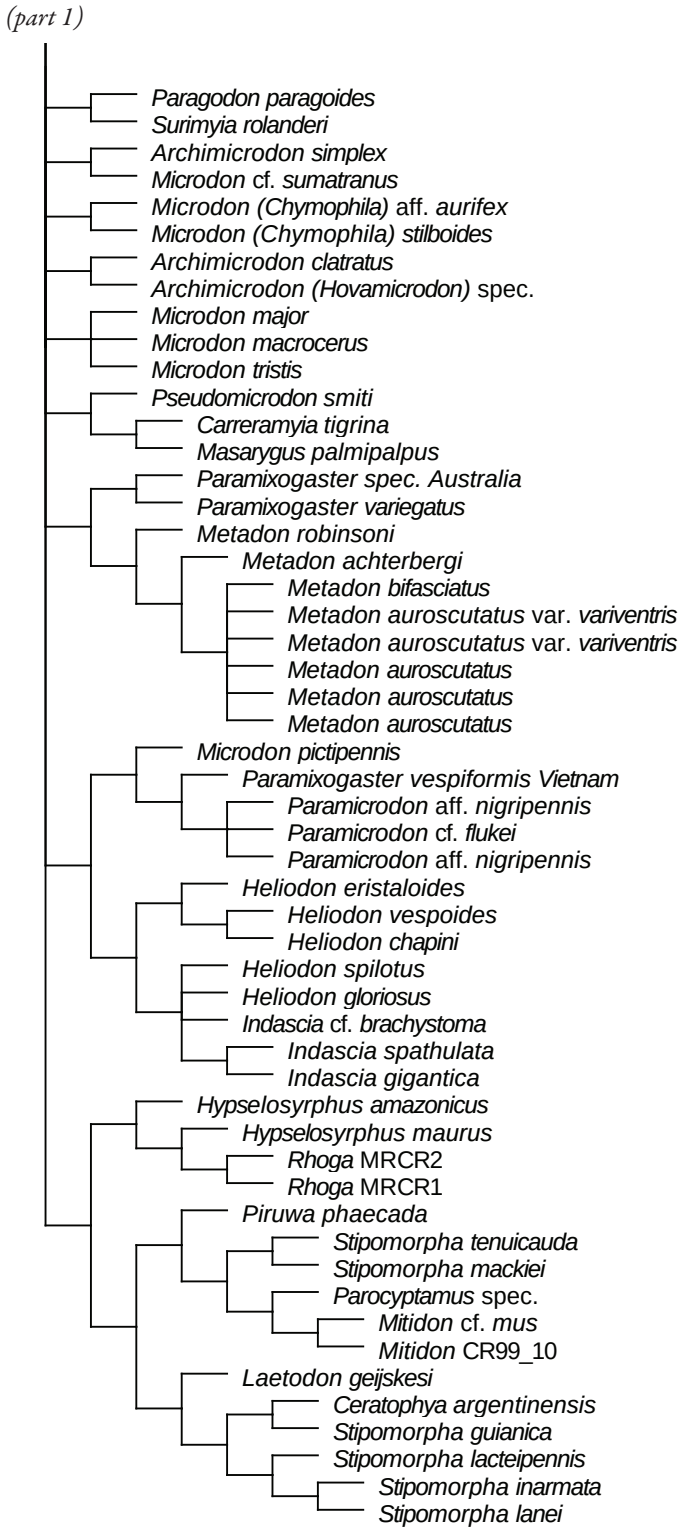


**Fig. 1 part 2.** Continued from previous page.

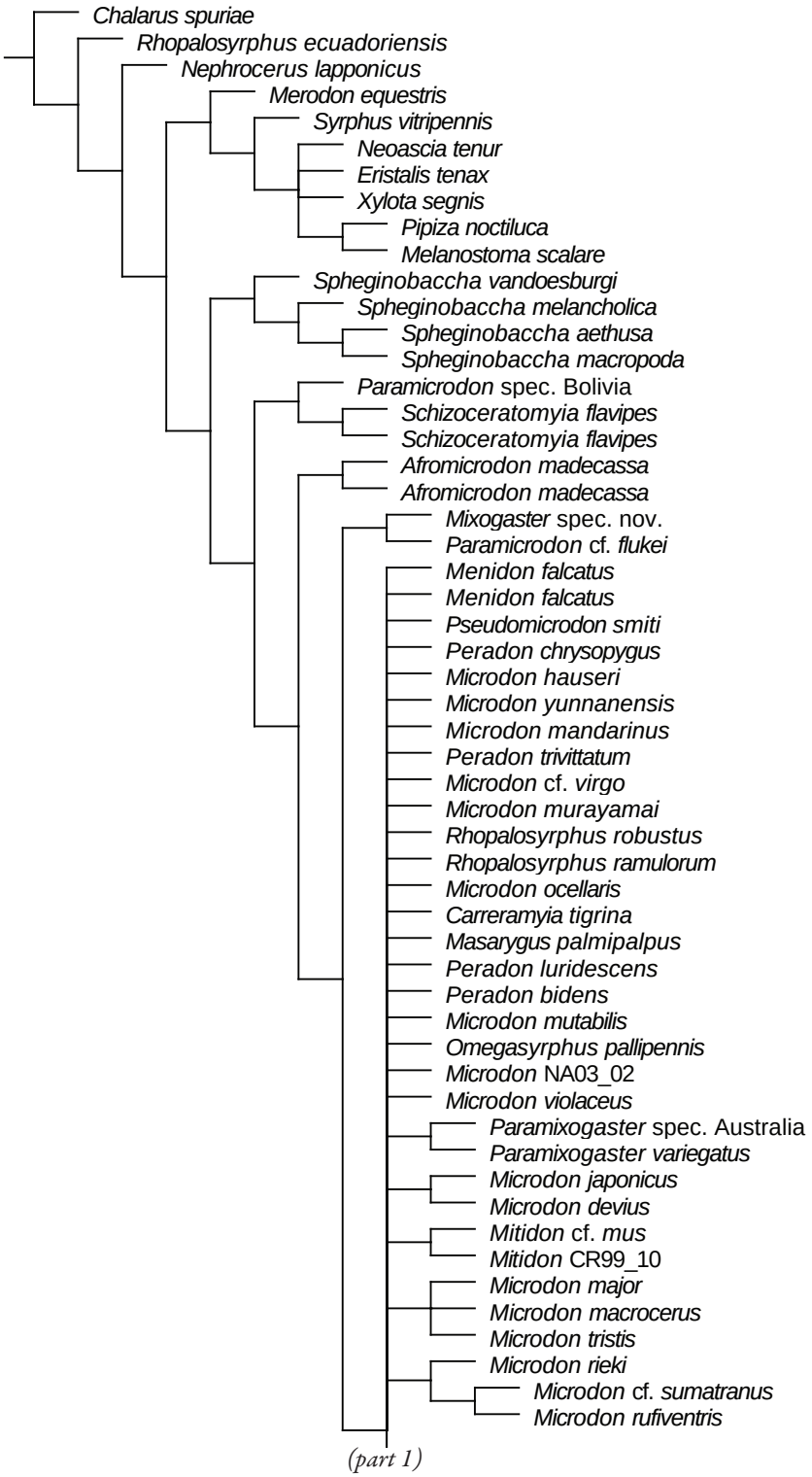


**Fig. 2.** Molecular analysis (all DNA fragments except Jerry + Pat): strict consensus of 88 most parsimonious trees of length 5133. *Continued on next page*.

*(part 1)*

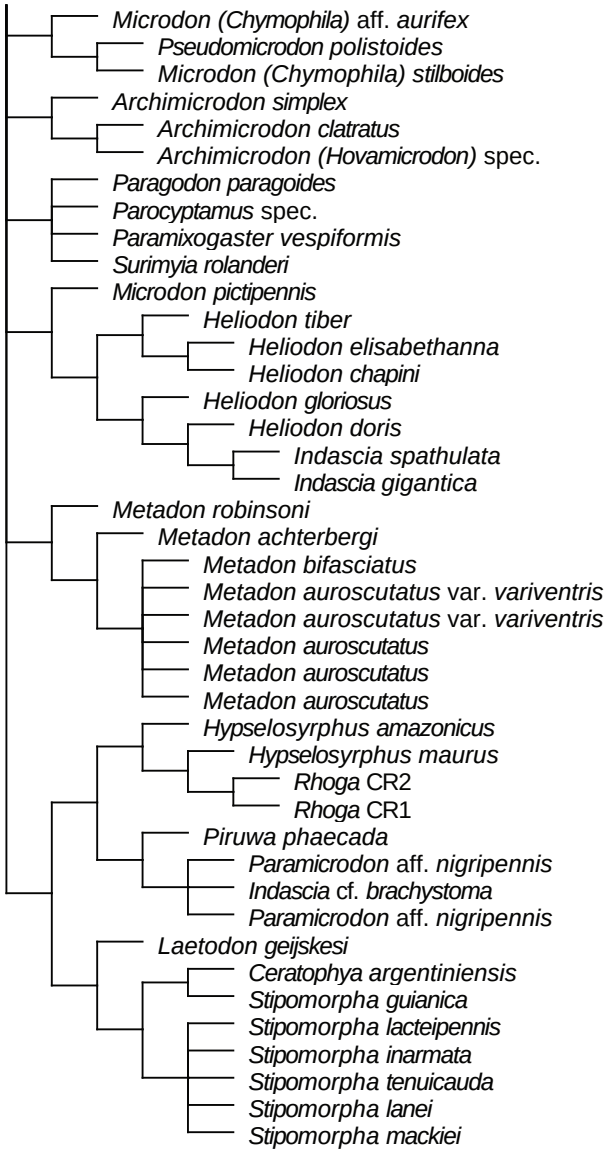


**Fig. 2 part 2.** Continued from previous page.

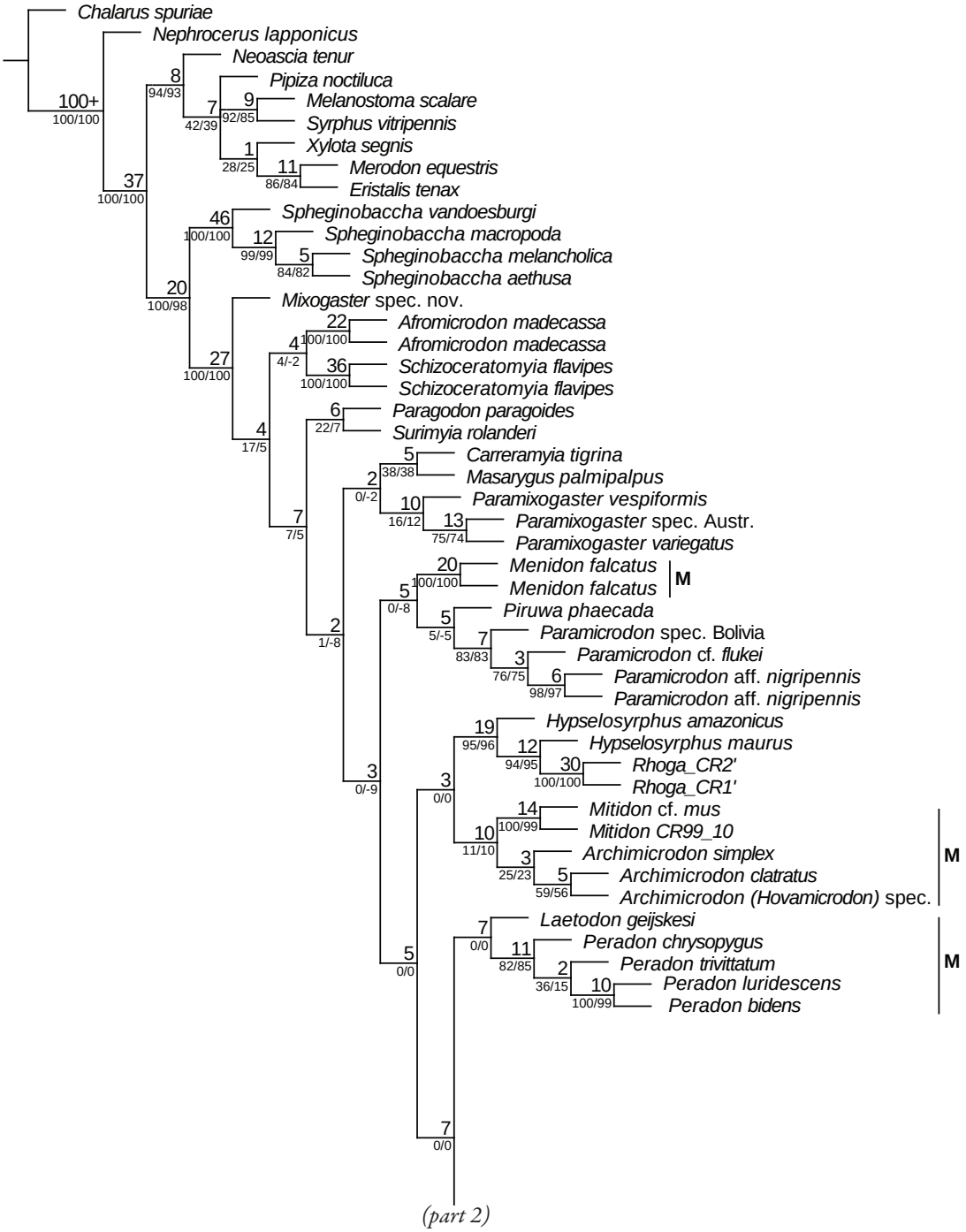


**Fig. 3.** Combined analysis (DNA and morphology), subset of 96 taxa: strict consensus of eight trees of length 9442. Branch values indicate Bremer support (above branch), Jackknife values (left) and GC frequency differences (right). Vertical lines marked 'M' indicate taxa included in the genus *Microdon* by previous authors. *Continued on next page*.

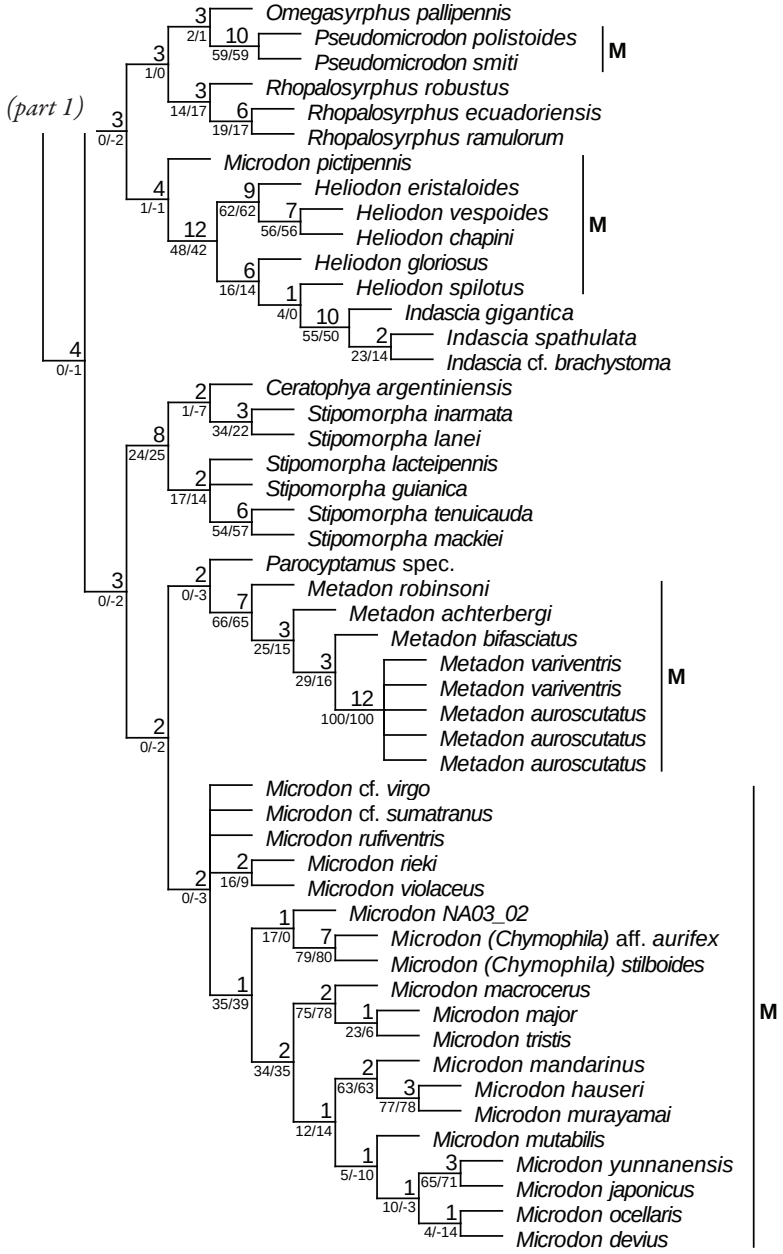


**Fig. 3 part 2.** Continued from previous page.

(Jerry + Pat) sequence resulted in 88 trees of length 5133. The strict consensus is given in figure 2.

### Analysis of combined data

The 'new technology' search of the subset of taxa resulted in eight trees of length 9442. The subsequent TBR based on these trees found no additional trees. The strict consensus is given in figure 3.
The 'new technology' search of the total set of taxa resulted in 26 trees of length 10.542. These trees were found in 10 hits of the shortest length, after a search of 70 hours. The strict consensus of the first four hits, which resulted in 11 trees, was compared with the strict consensus of all 26 trees; they were identical, indicating that the last six hits had no effect anymore on the strict consensus. The subsequent TBR branch swapping based on the 26 trees resulted in 10.000 most parsimonious trees of length 10541. The strict consensus of these was again subjected to sectorial searches and tree fusing, which resulted in 20 trees of length 10.541. The strict consensus of those trees is only slightly different from the previous strict consensus: only three SPR-moves were required to transform the first tree into the other. As a final result, the strict consensus of these two strict consensus trees, which can be regarded as the strict consensus of 10.020 trees of length 10.541, is given in figure 4.

## Discussion

### Evaluation of trees

The two strict consensus trees based on the analyses of molecular data only (both with and without the COIb) are poorly resolved. The majority of taxa are resolved within a large polytomy of Microdontinae, within which only a few small clades are recovered. Apart from this large polytomy, a few genera are placed in separate clades at relatively basal positions: *Spheginobaccha*, *Schizoceratomyia*, *Afromicrodon*, *Mixogaster* and a species of *Paramicrodon*. Overall, there does not seem to be much difference between the molecular tree with the COIb fragment included and the one in which this fragment is excluded. The basal part of the tree is more or less the same, while several small differences can be seen in the large polytomous part. Remarkably, the ingroup taxon *Rhopalosyrphus* spec. nov. (Y1089) is placed among the outgroup taxa in both trees. For this taxon, only the COIa sequence was obtained. When the COIa fragment was analyzed separately, the taxon was not placed in the outgroup, but as sister to another *Rhopalosyrphus* species. This suggests that the sequence is correct, but apparently the lack of additional data causes it to get an unexpected position when all fragments are analyzed simultaneously.
The addition of morphological characters to the dataset clearly adds a lot of resolution to the trees. Especially the combined analysis of the subset of 96 taxa results in a strict consensus with many resolved clades (fig. 3). Part of this resolution is lost again when the 93 taxa with morphological characters only are included in the analysis (fig. 4).
Following the reasoning of Kluge (1989) concerning the philosophy of *total evidence* in phylogenetic analyses, the results obtained from a combination of morphological and molecular data are to be preferred over those obtained from either morphological or molecular data only. The present paper presents the results of two of such combined analyses: one including only the 96 taxa for which both types of data are available (subset), and one in which 93 additional taxa are included for which only morphological characters are available (total set). As the results of both analyses are incongruent at many points, this raises the issue of which results are to be regarded as most reliable. This issue is linked directly to the problem of *missing data*, because the combined matrix of the total set of taxa contains many empty cells (Table 2).
Opposing forces need to be considered concerning the effect of missing data. Although adding taxa with many missing characters can potentially improve the quality of the phylogenetic analyses, e.g. by reducing the effect of long branch attraction (Wiens 2006), it can also decrease the performance of the analyses in terms of accuracy, error and branch supports (Prevosti & Chemisquy 2010). This effect can be mitigated by including more characters to the dataset, whereas adding more taxa with missing characters is not beneficial or even detrimental. The positive effect of adding more characters appears not to be negatively affected by the presence of missing entries. Prevosti & Chemisquy (2010) argue that this implies that there is no reason to exclude characters just because many of their cells are empty. As long as the overall number of characters in a taxon is high enough, the infer-

**Table 2.** Percentages of missing data for different partitions of the data analyzed in the present paper.

| | Subset of 96 taxa | Total set of 189 taxa |
|---|---|---|
| Morphological characters only (n = 174) | 4% | 4% |
| Molecular characters only (n = 2808) | 26% | n.a. |
| Morphological and molecular characters combined (n = 2982) | 25% | 59% |

red phylogeny will be accurate. This is corroborated by the results of other authors (Wiens 2006, Wiens & Moen 2008, Wolsan & Sato 2010). The question as to how many characters are enough is not easy to answer, as this relates to the amount on contradictory information (homoplasy) in the dataset, as well as to issues like branch lengths, taxon sampling and the distribution pattern of missing entries in the datamatrix. Even with the results of several simulations and empirical studies available, there is no recipe for determining the effect of missing data for a single dataset. In the simulations of Wiens (2006), datasets of 200 characters reached an accuracy of well over 90% for missing data proportion up to 50%, while for datasets of 2000 characters this level of accuracy was reached even with more than 80% of missing data. Prevosti & Chemisquy (2010) analyzed a large number of real (not simulated) morphological datasets, in which the total percentage of missing data (empty cells) varied between 0 and 54%. For datasets with around 15% of missing data, they found median accuracy values between 0.28 and 0.50 and median error rates around 0.50. In contrast, Wolsan & Sato (2010) reported very good cladistic performance of a dataset with 62.7% missing entries, and showed that even taxa with around 95% missing entries were accurately placed. However, their dataset contained almost 28.000 characters; a tenfold of the number in the present dataset.

In the present total set of 189 taxa, 93 taxa are included for which only the 174 morphological characters are present, while all 2808 molecular characters are missing. Considering the results of the studies mentioned above, it seems that the results for the total set of taxa cannot be considered reliable. Therefore, in the following discussion of implications for the classification of Microdontinae, the tree based on the combined analysis of the subset of 96 taxa (fig. 3) is our preferred tree. The results of the combined analysis of the total set of 189 taxa (fig. 4) will only be considered as far as they do not contradict the results of the subset. The results of the morphological analysis (Chapter 3) will also be taken into account.

## Implications for the classification of Microdontinae

*Family groups*

At present, only two tribes are recognized within the Microdontinae: Spheginobacchini Thompson, 1972, which includes only the genus *Spheginobaccha*, and Microdontini Rondani, 1845, including all remaining taxa (Cheng & Thompson 2008). The only other proposed family group names are Masarygidae of Brèthes (1908) and Ceratophyini of Hull (1949), which have not been used by other authors since their introduction. Hull (1949) wrote: "Perhaps two tribes should be recognized. The first would be the Microdonini distinguished by (...), and secondly the Ceratophyani (...)." Sabrosky (1999) argued that this name is unavailable, as it was only casually mentioned within in a short diagnosis of a group, not as a formal proposal of a new group name. However, this can be regarded as a "conditional proposal" of a new name. As this conditionally proposed name was published before 1961, there seems to be no formal reason for considering this name unavailable (ICZN 1999: art. 15.1).

Recognition of additional tribes could be useful for making the subfamily more 'manageable' in taxonomic, biogeographic and evolutionary studies and discussions. However, for introducing new family group names (or changing the status of available ones), we feel that the clades under consideration should be sufficiently "reliable". In the present study, the Bremer support and jackknife values in fig. 3 could be used as an aid in assessing the reliability of clades. For most of the larger clades, these values are low. The smaller clades for which these values are higher, are here – subjectively – considered to be of generic level, rather than of family-group level. Because of this, and also

because of the considerations on missing data as discussed in the previous paragraph, the introduction of new tribal names or reinstating available family group names based on the present phylogenetic hypotheses is deemed unjustified.

An exception could be the genus *Mixogaster*, which was recovered as sister to all Microdontinae excluding *Spheginobaccha* with high Bremer support (27) and jackknife value (100). Based on morphology, this genus is also considered to be aberrant enough from the other ingroup taxa to warrant tribal rank. However, as noted in Chapter 3 (see also introduction), this genus has certain possibly important characters in common with the genera *Aristosyrphus* and *Eurypterosyrphus*, which are not represented in the molecular dataset. Before assigning tribal rank to any of these groups, their phylogenetic affinities should be reliably resolved.

Having said this, several of the smaller clades in the presented phylogenies have relatively high support and stability values. Some of these indicate affinities between species and genus groups which have not before been suggested previously. These groupings will be discussed in a separate paper (Chapter 5), which gives descriptions and diagnoses for all genus group names, whether or not previously recognized.

*Spheginobaccha*

The position of *Spheginobaccha* as a sister to all other Microdontinae was recovered in all analyses: based on morphology only (Chapter 3), based on DNA only and based on the combined data, both for the subset and the total set of taxa. Support values are high (fig. 3). These results thus corroborate the results of Ståhls et al. (2003). While their ingroup only included Oriental species of this genus, the present analyses also include representatives of the two African species groups. The African taxa are placed as sisters to the Oriental taxa.

*Microdon*

Over the years, the genus *Microdon* has served as a 'dustbin' for taxa of which taxonomical affinities were not clear enough to place them into any of the other available genus group names. Even though several taxa were placed into other genera, subsequent authors have often considered those genera as subgenera of *Microdon*. The present analyses contain many species of *Microdon* s.l. As can be seen in fig. 3 (taxa previously classified in *Microdon*, or representatives of these taxa, are indicated with an 'M') this group is polyphyletic and its representatives are scattered over different parts of the tree. Although the exact positions of these groups may change in future analyses when more taxa and more molecular data are included, these results provide sufficient basis for subdividing *Microdon* into different monophyletic units. This will be done in Chapter 5, in which discussions and morphological diagnoses will be included and new generic names will be introduced. The names proposed in that paper are already used in the present paper, but not for nomenclatorial purposes (see disclaimer in Material and Methods).

*Remaining genera*

Genus group names are available for most of the clades recovered by the analyses, although for many of the included species these names have not previously been used in the present combinations. Besides, some species are placed in new genera. Discussions about the applications of existing genus group names, the introduction of new genus group names, and the classification of species into the genus groups, are the subjects of a separate paper, published more or less in parallel (Chapter 5).

**Family affairs**

The present results support the sister-group relationship of Microdontinae and other Syrphidae, as originally proposed by Thompson (1969) and subsequently by other authors (Hippa & Ståhls 2005, Skevington & Yeates 2000, Ståhls et al. 2003, Rotheray & Gilbert 2008). Our results are based on a wide representation of taxa: representatives of all valid genus groups are included, as well as taxa from all major biogeographic regions. In addition, both character sets (molecular and morphological) are larger than in previous analyses. Therefore, the results can be regarded as additional support for this sister-group relationship. The results can not, however, be regarded as compelling evidence. The setup of the analysis was not designed to test this relationship explicitly. For that test, a much larger set of Syrphidae taxa would be necessary. Preferably, also more taxa of related groups of 'lower' Cyclorrhapha should be included, such as Phoridae and Platypezidae.

According to Speight (2010), the presumed sister-

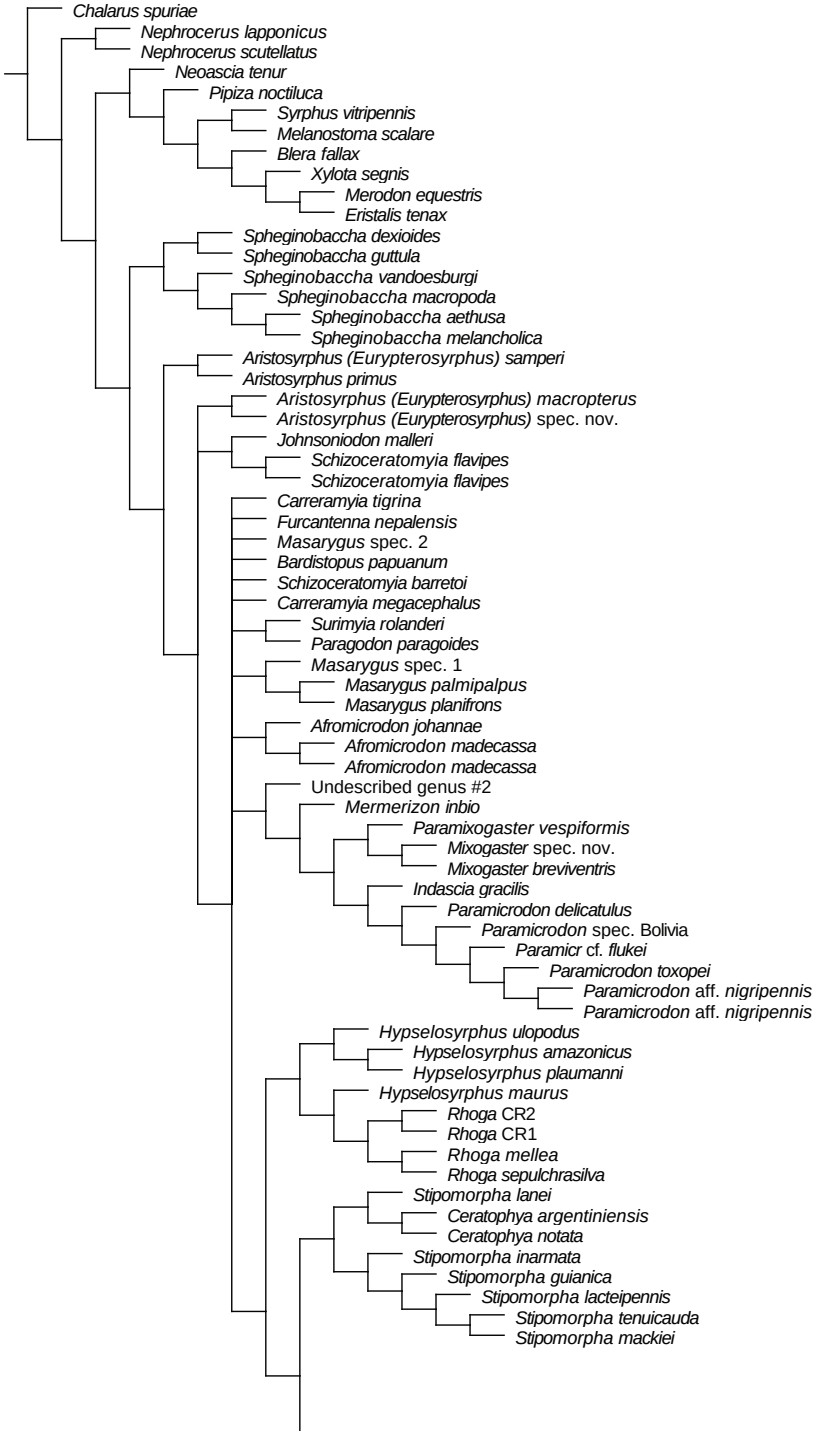


**Fig. 4.** Combined analysis (DNA and morphology), total set of 189 taxa: strict consensus of 10.020 trees of length 10.541. *Continued on next page.*

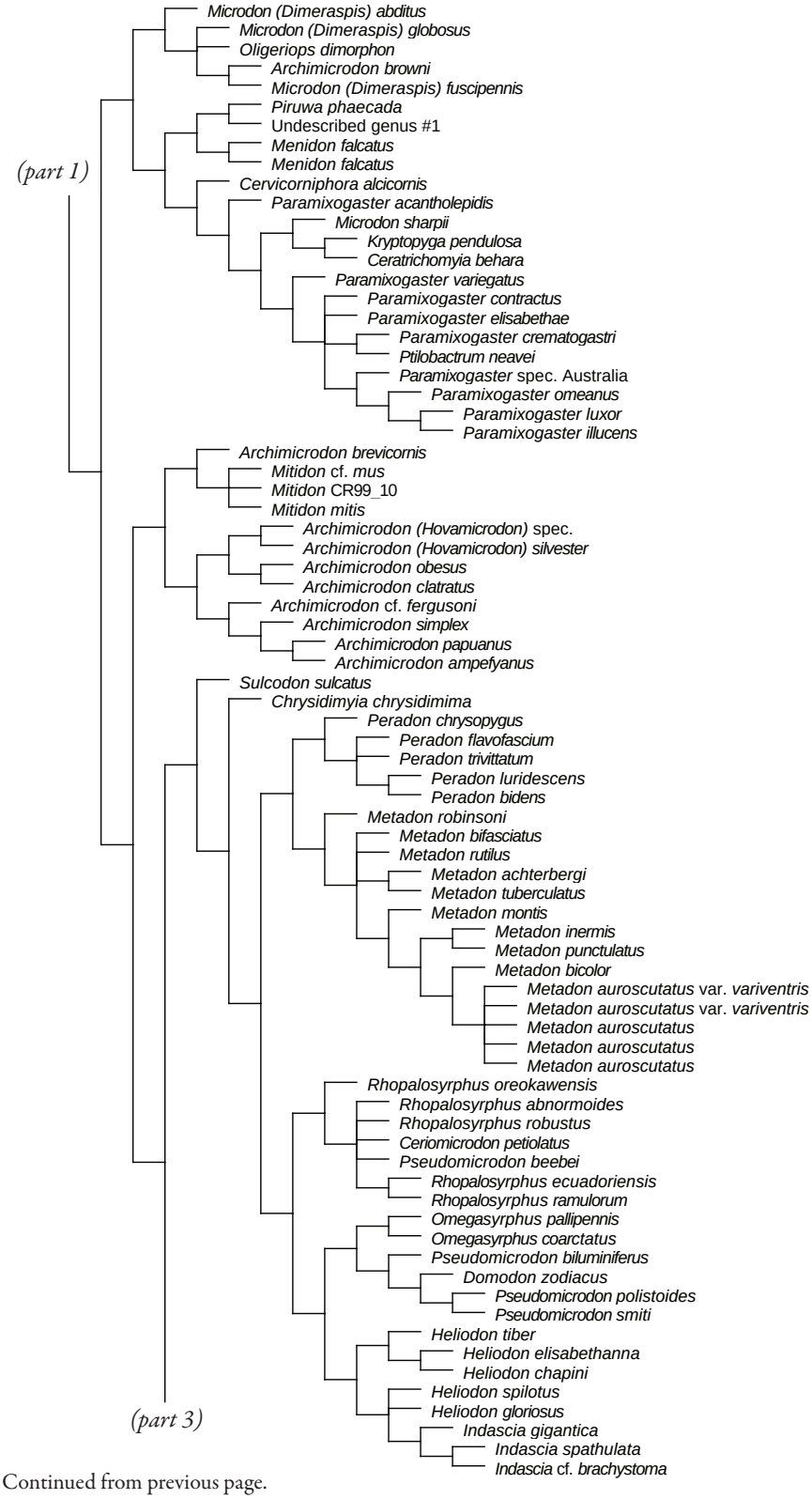


**Fig. 4 part 2.** Continued from previous page.

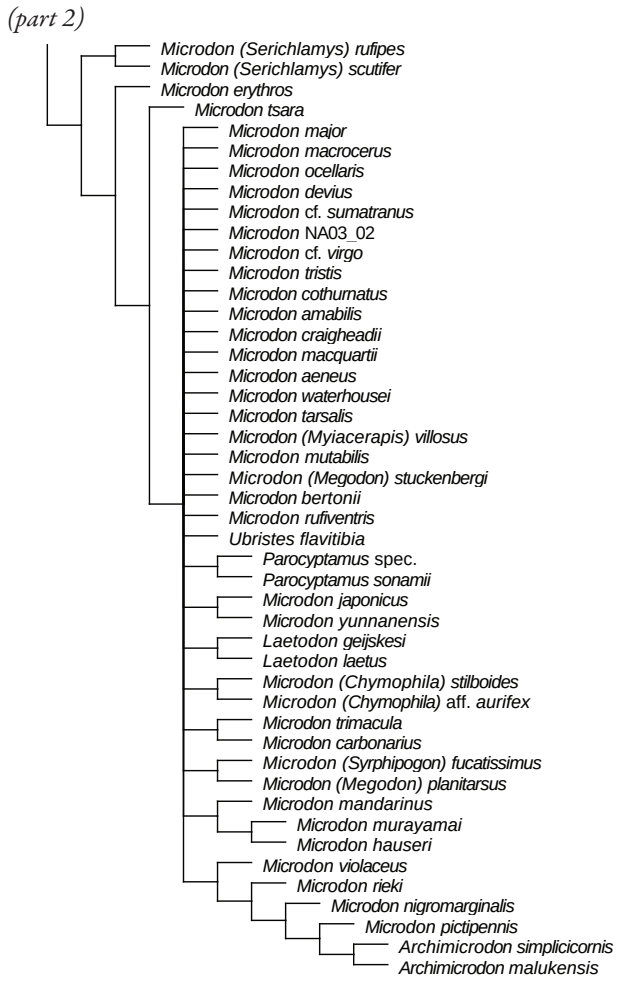


**Fig. 4 part 3.** Continued from previous page.

group relationship between Microdontinae and other Syrphidae "more-or-less reduces the issue of the correct placement of *Microdon* and allied genera to a matter of personal preference". We advocate, however, that in this case, in which available evidence does not demand the classification to be changed, it is preferable to adopt a conservative attitude.

## Acknowledgements

Specimens of most species of Microdontinae are very rarely collected, and fresh specimens required for DNA-analysis are even harder to obtain. Therefore, we owe our gratitude to all entomologists who were so kind to share their material, either from their personal collections or from those of their institutions: Kees van Achterberg (RMNH), Brian V. Brown, Jean A. Cerda, K.-D. Dijkstra, Tim Faasen, Stephen Gaimari (CSCA), Aniel Gangadin, Martin Hauser, Stephen A. Marshall, Ximo Mengual, Chris Raper, Jeff Skevington (CNC), John T. Smit, Villu Soon, Jens-Hermann Stuke, Rob de Vries (RMNH), Shaun Winterton.

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## Appendix 1: DNA voucher specimens

**Morphology: 1** = same specimen used for morphological matrix (Chapter 3); 2 = different specimen of same species used for morphological matrix; 3 = specimen of closely related species used for morphological matrix.
**MZH_code:** voucher code Finnish Museum of Natural History, Helsinki.
**COL:** The following acronyms are used to indicate entomological collections: CNC = Canadian National Collection, Ottawa; INBIO = Instituto Nacional de Biodiversidad, Santo Domingo, Costa Rica; MZH = Finnish Museum of Natural History, Helsinki; RMNH = Netherlands Centre for Biodiversity Naturalis, Leiden.
The last five columns indicate which sequences were included in the molecular data matrix.

| Morphology | MZH_code | Taxon | Country | SEX | LEG | COL | COL_BeetHCO | COL_JP_edited2 | 18S_1Fb7 | 18S_2Fb29 | 28S_F23DR |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | Y1106 | *Afromicrodon madecassa* (Keiser, 1971) | Madagascar | ♂ | Mengual, X. | MZH | Y | | | Y | |
| 2 | Y0379 | *Afromicrodon madecassa* (Keiser, 1971) | Madagascar | ♂ | Mengual, X. | MZH | Y | | | | Y |
| 1 | Y0778 | *Archimicrodon clatratus* (Keiser, 1971) | Madagascar | ♀ | Mengual, X. | MZH | Y | | Y | | |
| 1 | Y1092 | *Archimicrodon simplex* (Shiraki, 1930) | China | ♂ | Blank, Liston, Taeger | RMNH | Y | | Y | Y | Y |
| 1 | Y0378 | *Archimicrodon* (*Hovamicrodon*) spec. | Madagascar | ♀ | Mengual, X. | MZH | Y | Y | Y | | |
| 1 | Y0803 | *Carreramyia tigrina* Reemer | Peru | ♀ | Smit, J.T. | RMNH | Y | Y | Y | | |
| 1 | Y1008 | *Ceratophya argentinensis* Reemer | Argentina | ♀ | Ekrem, T. | RMNH | Y | | Y | Y | Y |
| 2 | Y0800 | *Chalarus spurius* (Fallén, 1816) | Finland | | Ståhls,, G. | MZH | Y | Y | Y | Y | Y |
| 2 | Y0688 | *Eristalis tenax* (Linnaeus, 1758) | Canada | | Steenis, W. van | MZH | Y | Y | Y | Y | Y |
| 1 | Y0907 | *Heliodon chapini* (Hull, 1941) | Thailand | ♀ | Patikhom Tumtip | MZH | Y | | Y | Y | Y |
| 1 | Y1074 | *Heliodon doris* Reemer | Thailand | ♂ | Bunlu Sapsiri | RMNH | | | Y | Y | Y |
| 1 | Y1062 | *Heliodon elisabethanna* Reemer | Thailand | ♀ | | | Y | Y | Y | Y | |
| 2 | Y0906 | *Heliodon gloriosus* (Hull, 1941) | Thailand | ♀ | Patikhom Tumtip | MZH | Y | Y | Y | Y | |
| 1 | Y1072 | *Heliodon tiber* Reemer | Vietnam | ♀ | C. van Achterberg & R. de Vries | RMNH | Y | Y | Y | Y | |
| 1 | Y0801 | *Hypselosyrphus amazonicus* Reemer | Peru | ♂ | Smit, J.T. | RMNH | Y | Y | Y | Y | Y |
| 1 | Y1078 | *Hypselosyrphus maurus* Reemer | Peru | ♀ | Smit, J.T. | RMNH | Y | | Y | | Y |
| 1 | Y0825 | *Indascia* cf. *brachystoma* (Wiedemann, 1824) | Thailand | ♂ | Janteab, L. | RMNH | | Y | Y | | |
| 1 | Y0909 | *Indascia gigantica* Reemer | Thailand | ♂ | Y. Areeluck | RMNH | | | Y | Y | Y |
| 1 | Y1100 | *Indascia spathulata* Reemer | Vietnam | ♂ | Achterberg, C. van & R. de Vries | RMNH | | | Y | Y | Y |
| 1 | Y0806 | *Laetodon geijskesi* (van Doesburg, 1966) | Peru | ♀ | Mengual, X. | MZH | Y | Y | Y | Y | Y |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Y0802 | *Masarygus palmipalpus* Reemer, spec. nov. | Peru | ♂ | Smit, J.T. | RMNH | | Y | Y | | |
| 2 | Y0594 | *Melanostoma scalare* (Fabricius, 1794) | Italy | | Kehlmaier, C. | MZH | Y | Y | Y | Y | Y |
| 2 | Y1324 | *Menidon falcatus* (Williston, 1887) | Costa Rica | ♂ | Reemer, M. | RMNH | Y | Y | Y | Y | Y |
| 2 | Y1325 | *Menidon falcatus* (Williston, 1887) | Peru | ♀ | Smit, J.T. | RMNH | Y | | Y | Y | Y |
| 2 | Y0690 | *Merodon equestris* Meigen, 1822 | Finland | | Meikäläinen, M.. | MZH | Y | Y | Y | Y | Y |
| 1 | Y1086 | *Metadon achterbergi* Reemer | Vietnam | ♀ | Achterberg, C. van & R. de Vries | RMNH | Y | | Y | Y | Y |
| 2 | Y0780 | *Metadon auroscutatus* (Curran, 1928) | Thailand | | | MZH | Y | Y | | | |
| 2 | Y0905 | *Metadon auroscutatus* (Curran, 1928) | Thailand | ♂ | Budsawong | MZH | Y | | Y | Y | Y |
| 1 | Y1082 | *Metadon auroscutatus* (Curran, 1928) | Thailand | ♀ | Patikom Tumtip | RMNH | Y | | Y | Y | Y |
| 1 | Y1083 | *Metadon auroscutatus* var. *variventris* (Curran, 1928) | Thailand | ♂ | Tawit Jaruphan & Orawan Budsawong | RMNH | Y | | | | Y |
| 1 | Y1084 | *Metadon auroscutatus* var. *variventris* (Curran, 1928) | Thailand | ♀ | Tawit Jaruphan & Orawan Budsawong | RMNH | Y | | Y | Y | Y |
| 1 | Y1094 | *Metadon bifasciatus* Matsumura, 1916 | China | ♂ | Blank, Liston, Taeger | RMNH | | | Y | Y | |
| 1 | Y1085 | *Metadon robinsoni* (Curran, 1928) | Vietnam | ♀ | Achterberg, C. van & R. de Vries | RMNH | | | Y | Y | Y |
| 1 | Y1077 | *Microdon* aff. *virgo* Curran, 1940 | Peru | ♀ | Smit, J.T. | RMNH | | | Y | | |
| 1 | Y0910 | *Microdon* cf. *sumatranus* van der Wulp, 1892 | Thailand | ♂ | Somchai Chachumnan & Saink Singtong | RMNH | Y | Y | Y | Y | |
| 1 | Y1010 | *Microdon devius* (Linnaeus, 1761) | Netherlands | ♂ | Smit, J.T. | MZH | Y | Y | Y | Y | Y |
| 1 | Y1096 | *Microdon hauseri* Reemer | China | ♂ | 28-12-2009 | RMNH | Y | | Y | Y | Y |
| 1 | Y1070 | *Microdon japonicus* Yano, 1915 | Japan | ♂ | Kawashima, Itsuro | RMNH | Y | | Y | | |
| 1 | Y1071 | *Microdon macrocerus* Hironaga & Maruyama, 2004 | Japan | ♂ | Komatsu Takashi | RMNH | Y | | Y | | Y |
| 1 | Y1323 | *Microdon major* Andries, 1912 | Netherlands | ♂ | Reemer, M. | RMNH | Y | Y | Y | Y | Y |
| 1 | Y1093 | *Microdon mandarinus* Reemer | China | ♂ | Blank, Liston, Taeger | RMNH | Y | | Y | Y | Y |
| 1 | Y1069 | *Microdon murayamai* Hironaga & Maruyama, 2004 | Japan | ♂ | Komatsu Takashi | RMNH | Y | | Y | | |
| 2 | Y0150 | *Microdon mutabilis* Linnaeus, 1758 | United Kingdom | | Hewitt, S.M. | MZH | Y | | Y | | Y |
| 1 | S0298 | *Microdon* NA03-02 Thompson, in prep. | USA | ♀ | Hauser, M. | MZH | Y | Y | Y | Y | Y |
| 2 | Y1058 | *Microdon ocellaris* Curran, 1924 | USA | ♂ | Cumming, J.M. & J. Skevington | CNC | | | Y | Y | |
| 1 | Y1320 | *Microdon pictipennis* Macquart, 1850 | Australia | ♀ | Winterton, S.L. | RMNH | Y | Y | Y | Y | Y |
| 1 | Y1321 | *Microdon rieki* Paramonov, 1957 | Australia | ♀ | Winterton, S.L. | RMNH | Y | Y | Y | Y | Y |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | Y0577 | *Microdon rufiventris* (Rondani, 1848) | Surinam | ♂ | Reemer, M. | MZH | Y | Y | Y | Y | Y |
| 2 | Y1059 | *Microdon tristis* Loew, 1864 | Canada | ♀ | Skevington, J. | CNC | | | Y | Y | |
| 2 | S0292 | *Microdon violaceus* Macquart, 1842 | Chile | | Irwin, M.E. & E.I. Schlinger | MZH | Y | Y | Y | Y | Y |
| 1 | Y1095 | *Microdon yunnanensis* Reemer | China | ♂ | Blank, Liston, Taeger | RMNH | | | Y | Y | |
| 2 | Y1079 | *Microdon* (*Chymophila*) aff. *aurifex* Wiedemann, 1830 | Fench Guyana | ♀ | Cerda, J.A. | RMNH | Y | | Y | | Y |
| 1 | Y1064 | *Microdon* (*Chymophila*) *stilboides* Walker, 1849 | Thailand | ♀ | Pongpitak & Sathit | RMNH | Y | Y | Y | | Y |
| 1 | Y0369 | *Mitidon* cf. *mus* (Curran, 1936) | Colombia | ♂ | Mengual, X. | MZH | Y | | Y | | Y |
| 1 | S0264 | *Mitidon* CR_99 Thompson in prep. | Costa Rica | ♂ | Marshall, S.A. | INBIO | Y | Y | Y | Y | Y |
| 2 | Y1065 | *Mixogaster* spec. nov. | USA | ♂ | Godwin, W. | CNC | Y | | Y | | Y |
| 2 | Y0578 | *Neoascia tenur* (Harris, 1780) | Finland | | Haarto, A. | MZH | Y | Y | Y | Y | Y |
| 2 | Y0065 | *Nephrocerus lapponicus* Zetterstedt, 1838 | Finland | | Jakovlev, J. | MZH | Y | Y | Y | Y | Y |
| 1 | S0356 | *Omegasyrphus pallipennis* (Curran, 1925) | USA | ♀ | Hauser, M. | MZH | Y | Y | Y | | |
| 2 | Y1314 | *Paragodon paragoides* Thompson, 1969 | Costa Rica | ♀ | Porras, W. & A. Rojas | RMNH | Y | Y | Y | | Y |
| 1 | Y0781 | *Paramicrodon* aff. *nigripennis* (Sack, 1922) | Thailand | ♂ | Katae Sanog & Buakaw Adnafai | MZH | Y | Y | | | |
| 2 | Y1063 | *Paramicrodon* aff. *nigripennis* (Sack, 1922) | Thailand | ♀ | Katae Sanog & Buakaw Adnafai | RMNH | Y | | Y | | |
| 2 | Y0804 | *Paramicrodon* cf. *flukei* Curran, 1936 | Peru | ♀ | Smit, J.T. | RMNH | | Y | Y | Y | |
| 1 | Y1102 | *Paramicrodon* spec. Bolivia | Bolivia | ♂ | Cline, A.R. | RMNH | | | Y | | |
| 2 | Y1057 | *Paramixogaster* cf. *variegatus* (Walker, 1852) | Australia | ♂ | Skevington, J. & M. Mathieson | CNC | Y | Y | Y | Y | Y |
| 1 | Y1322 | *Paramixogaster* spec. Austr. | Australia | ♀ | Monteith & Turco | RMNH | Y | Y | Y | | Y |
| 1 | Y0721 | *Paramixogaster vespiformis* (de Meijere, 1908) | Vietnam | ♂ | C. van Achterberg & R. de Vries | RMNH | Y | Y | Y | Y | |
| 1 | Y1104 | *Parocyptamus* spec. | Thailand | ♂ | Stuke, J.-H. | RMNH | | | Y | Y | |
| 2 | Y0578 | *Peradon bidens* (Fabricius, 1805) | Surinam | ♂ | Reemer, M. | MZH | Y | Y | Y | Y | Y |
| 1 | Y1317 | *Peradon chrysopygus* (Giglio-Tos, 1892) | Costa Rica | ♂ | Reemer, M. | RMNH | Y | Y | Y | Y | Y |
| 2 | Y0579 | *Peradon luridescens* (Walker, 1857) | Surinam | ♂ | Reemer, M. | MZH | Y | Y | Y | Y | Y |
| 2 | Y1080 | *Peradon trivittatum* Curran, 1925 | French Guyana | ♀ | Cerda, J.A. | RMNH | Y | | Y | | |
| 2 | Y1045 | *Pipiza noctiluca* (Linnaeus, 1758) | Sweden | | Johansson, N. | MZH | Y | Y | Y | Y | Y |
| 1 | Y0805 | *Piruwa phaecada* Reemer | Peru | ♀ | Smit, J.T. | RMNH | Y | Y | Y | Y | Y |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Y1319 | *Pseudomicrodon polistoides* Reemer | Peru | ♀ | Smit, J.T. | RMNH | Y | Y | Y | Y | Y |
| 1 | Y1318 | *Pseudomicrodon smiti* Reemer | Peru | ♂ | Smit, J.T. | RMNH | Y | | Y | Y | Y |
| 1 | Y1315 | *Rhoga* CR1 | Costa Rica | ♀ | Reemer, M. | INBIO | Y | Y | Y | | Y |
| 1 | Y1316 | *Rhoga* CR2 | Costa Rica | ♀ | Reemer, M. | INBIO | Y | Y | Y | | Y |
| 1 | Y1089 | *Rhopalosyrphus ecuadoriensis* Reemer | Ecuador | ♂ | Tishechkin, A. | RMNH | Y | | | | |
| 2 | Y1060 | *Rhopalosyrphus ramulorum* Weems & Deyrup, 2003 | USA | ♂ | Skevington, J. | CNC | Y | Y | Y | Y | |
| 1 | Y1066 | *Rhopalosyrphus robustus* Reemer | French Guyana | ♀ | Morvan, O. | CNC | Y | | Y | | Y |
| 2 | Y0718 | *Schizoceratomyia flavipes* Carrera, Lopes & Lane, 1947 | Surinam | ♂ | Gangadin, A. & K.-D.B. Dijkstra | MZH | Y | Y | Y | Y | Y |
| 2 | Y0719 | *Schizoceratomyia flavipes* Carrera, Lopes & Lane, 1947 | Surinam | ♂ | Gangadin, A. & K.-D.B. Dijkstra | MZH | Y | Y | Y | Y | |
| 1 | Y1087 | *Spheginobaccha aethusa* (Walker, 1849) | Vietnam | ♀ | Achterberg, C. van & R. de Vries | RMNH | Y | | Y | Y | Y |
| 2 | S0155 | *Spheginobaccha macropoda* (Bigot, 1883) | Malaysia | | Quicke, D. & N. Laurenne | MZH | Y | | Y | Y | Y |
| 1 | Y1091 | *Spheginobaccha melancholica* Hull, 1937 | Vietnam | ♂ | Achterberg, C. van & R. de Vries | RMNH | Y | | Y | Y | Y |
| 2 | G0423 | *Spheginobaccha vandoesburgi* Thompson, 1974 | Malaysia | | Quicke, D. & N. Laurenne | MZH | Y | | Y | Y | Y |
| 2 | Y0581 | *Stipomorpha guianica* (Curran, 1925) | Surinam | ♂ | Reemer, M. | MZH | Y | Y | Y | Y | |
| 1 | Y1088 | *Stipomorpha inarmata* (Curran, 1925) | French Guyana | ♂ | Keijo Sarv | RMNH | | | Y | | |
| 2 | Y1090 | *Stipomorpha lacteipennis* Shannon, 1927 | Bolivia | ♂ | Cline, A.R. | RMNH | Y | | Y | Y | Y |
| 2 | Y1009 | *Stipomorpha lanei* (Curran, 1936) | Peru | ♀ | Faasen, T. | RMNH | Y | | Y | | Y |
| 2 | Y0580 | *Stipomorpha mackiei* (Curran, 1940) | Surinam | ♀ | Reemer, M. | MZH | Y | Y | Y | | |
| 2 | Y1061 | *Stipomorpha tenuicauda* (Curran, 1925) | French Guyana | ♂ | Morvan, O. | CNC | Y | | Y | Y | |
| 2 | Y0381 | *Surimyia rolanderi* Reemer, 2008 | Surinam | ♂ | Reemer, M. | MZH | Y | Y | Y | | Y |
| 2 | S0053 | *Syrphus vitripennis* Meigen, 1822 | Greece | | Rojo, S. & C. Perez | MZH | Y | Y | Y | Y | Y |
| 2 | Y0030 | *Xylota segnis* (Linnaeus, 1758) | Spain | | Ståhls. G. | MZH | Y | Y | Y | Y | Y |

*Her name was Magill, and she called herself Lil, but everyone knew her as Nancy.*

John Lennon & Paul McCartney 1968, Rocky Raccoon