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Phylogeny of the Syrphoidea (Diptera) Inferred from mtDNA Sequences and Morphology with Particular Reference to Classification of the Pipunculidae (Diptera)

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Sequence data from 420 bp of mitochondrial 12s ribosomal DNA and 490 bp of 16s rDNA were analyzed for 27 species of Syrphoidea (Diptera) and two outgroup taxa. Morphological data for the Pipunculidae were combined with the pipunculid molecular data set. A partition homogeneity test on these data sets revealed no significant incongruence. The pipunculid phylogeny from molecular data closely resembles the published phylogeny based on morphology, with differences only with respect to the Nephrocerinae. There is very strong support for the monophyly of the Pipunculinae and the Chalarinae. The Nephrocerinae are hypothesized to be paraphyletic. Within the Syrphidae, there is support for a monophyletic Syrphinae and Microdontinae, but the Eristalinae are paraphyletic. More data are needed to resolve the eristaline phylogeny.

Key Words: **Pipunculidae; Syrphidae; Syrphoidea; phylogeny; mitochondrial rDNA; 12s gene; 16s gene; molecular systematics; morphology; total evidence.**

INTRODUCTION

The Syrphoidea (Pipunculidae + Syrphidae) are a morphologically and biologically diverse group of flies. The Pipunculidae are exclusively endoparasitoids of Auchenorrhyncha, whereas the Syrphidae exhibit diverse life history strategies as larvae. The Syrphinae are almost exclusively predators (usually of aphids), the Microdontinae are inquilines in the nests of social insects (mostly ants), and the Eristalinae may be saprophagous (most Milesiini), coprophagous (some Rhingiini and Milesiini), mycetophagous (some Rhingiini), phytophagous (most Rhingiini, Merodontini, some Brachyopini), predacious (Pipizini), aquatic filter feeders (mainly Eristalini and some Brachyopini and Milesiini), or specialized inquilines in social insect nests (some Volucellini and Merodontini) (Akre *et al.*, 1988; Duffield, 1981; Gilbert *et al.*, 1994; Stubbs and Falk, 1993; Thompson and Vockeroth, 1989; Van Pelt and Van Pelt, 1972). This extreme variation in life history data is reflected in the morphology of these flies, both as larvae and adults. Homology of structures across the Syrphoidea is often difficult to assess, has led to disagreements about the evolution of the group, and may be the reason that no one has attempted a cladistic analysis that incorporates many of the higher taxa in the superfamily.

Our goal was to investigate the utility of two mitochondrial genes (12s and 16s rDNA) for developing a phylogeny of the Syrphoidea. We were particularly keen to investigate four aspects of the phylogeny which have dogged investigators over the years: (1) the placement of the Nephrocerinae, (2) the placement of the Microdontinae, (3) the monophyly of the Eristalinae, and (4) the monophyly of the Pipunculidae.

History of the Nephrocerinae

The first attempt to construct a phylogeny of the Pipunculidae was by Aczél (1948). His polarity inferences either were based on a few fossil characters or, for the most part, were ad hoc. Despite this, his proposed higher phylogenetic groups were mostly congruent with modern constructs; these included a monophyletic basal Chalarinae, a terminal monophyletic Dorilaini (=Pipunculinae), *Nephrocerus* Zetterstedt + *Protonephrocerus* Collin + Dorilaini as the sister group of the Chalarinae, and *Protonephrocerus* + Dorilaini as the sister group of *Nephrocerus.* He proposed the new tribe Nephrocerini for the genus *Nephrocerus,* and Protonephrocerini for *Protonephrocerus* and *Metanephrocerus* Aczél.

Albrecht (1990) was unable to ascertain the relationship between the Chalarinae, *Nephrocerus, Protonephrocerus,* and the rest of the Pipunculidae. Rafael and De Meyer (1992) produced the first thorough phylogenetic analysis and reclassification of the Pipunculidae. Their data provide good evidence for a basal, monophyletic Chalarinae and a terminal, monophyletic Pipunculinae, while refuting Aczél's (1948) concept for the Protonephrocerini. In contrast, evidence is provided for a monophyletic *Nephrocerus* + *Proto-*

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nephrocerus (Nephrocerinae) sister group of the Pipunculinae (cf. Fig. 3).

The Microdontinae

Larvae of Microdontinae are predators upon the brood of ants (Akre *et al.*, 1988). As such, they are markedly different from any other syrphid larvae. Their flattened, disk-shaped larvae and the slug-like larval locomotion led to their misidentification in the past as molluscs and coccoids (Wheeler, 1908). The adults are also morphologically and behaviourally divergent from other Syrphidae. These unique attributes and a lack of clear synapomorphies with other Syrphidae have led to many hypotheses of relationship. Rondani (1856–1857) first proposed this subfamily as the Microdoninae, and soon after Lioy (1864) combined the Microdoninae with *Psarus* Latreille and *Chrysotoxum* Meigen in the Psariti. Williston (1886) then placed the tribe Microdontini in the Syrphinae. Verrall (1901) again separated the Microdontinae into its own subfamily and removed *Psarus* and *Chrysotoxum* from it. Hull (1949) suggested that the Microdontinae were in a monophyletic group that included what he called the Eumerinae and the Nausigasterinae. Genera from the latter subfamilies are now treated as part of the Eristalinae. In partial accordance with this, Goffe (1952) and Wirth *et al.* (1965) placed the Microdontinae in what we now call the Eristalinae (=Sphixinae of Goffe, =Milesiinae of Wirth *et al.*). Most recent works (e.g., Knutson *et al.*, 1975; Smith and Vockeroth, 1980; Thompson and Vockeroth, 1989; Thompson *et al.*, 1976) treat the Microdontinae as a separate subfamily. Thompson (1969, 1972) considered the Microdontinae to form a basal, monophyletic group with respect to the rest of the Syrphidae. A recent hypothesis by Rotheray and Gilbert (1999) places the Microdontinae in a terminal position as the sister group of the Syrphinae + Pipizini.

The Eristalinae

From the preceding examination of the Microdontinae it is clear that the Eristalinae has also had a mixed history. It has been divided into over 20 different subfamilies (Goffe, 1952), but is now represented by just one. Williston (1886) was the first to comment on the frustrating dearth of generic or group characters available within the Syrphidae. Specific characters are often excellent, but characters used to elucidate higher taxa often have unclear homologies or clearly exhibit homoplasy.

Monophyly of the Pipunculidae

The Pipunculidae has been considered a monophyletic group since its inception, but there have been few efforts to find support for this notion. Rafael and De Meyer (1992) suggested five synapomorphies for the Pipunculidae, but only one of these is incontrovertible (ovipositor forming a piercer-like structure). Two of the characters are larval (larvae are endoparasites of Homoptera (=Auchenorrhyncha), and larvae possess a chitinized postspiracular plate) and only a few Pipunculidae have documented life histories and larval stages (for example, the life histories of all Nephrocerinae are unknown). Enlarged anterior ommatidia are found in all female Chalarinae and Pipunculinae, but are not distinct in *Nephrocerus*. The fifth synapomorphy given for the Pipunculidae is rather vague and could describe any of a number of Syrphidae, particularly some of the Pipizini (head mainly occupied by large compound eyes). Given these problems, we feel that it is prudent to test the monophyly of the Pipunculidae by examining alternative data sets (i.e., mitochondrial DNA) across as many syrphid and pipunculid higher taxa as possible.

MATERIALS AND METHODS

Specimen Acquisition

With five exceptions, samples were collected into absolute ethanol by the authors. Chris Thompson collected the two species of *Toxomerus* Macquart into absolute ethanol. Mike Irwin and Don Webb collected *Protonephrocerus chiloensis* Collin into 70% ethanol. *Nephrocerus daeckei* Johnson and *Verrallia virginica* Banks were sequenced from pinned specimens donated by Steve Marshall (DEBU).

At least one species from each subfamily and most tribes of syrphids and pipunculids were sampled. The only pipunculid tribe not sampled was the Pipunculini. Several unsuccessful attempts were made to sequence *Pipunculus viduus* Cresson. Two species from closely related families within the lower Cyclorrhapha were used to root the tree (a platypezid and a phorid). The 29 taxa sequenced are listed in Appendix 1. When possible, at least one additional fly from the same collection lot was pinned as a voucher and the remaining body and/or genitalia of at least one of the sequenced individuals were retained. The only exception was with *Protonephrocerus chiloensis*. Two headless specimens have been retained for future molecular work and will be maintained as vouchers in the first author's personal collection (JSPC). A specimen from another collection locality has also been designated as a voucher and will be kept in a recognized museum (DEBU). Only one extant species of *Protonephrocerus* is known, so this should not pose a problem. Voucher specimens are deposited in the University of Queensland Insect Collection (UQIC, St. Lucia, Queensland, Australia; G. Daniels), the United States National Museum (USNM, Washington, DC, USA; F. C. Thompson), the University of Guelph (DEBU, Guelph, Canada; S. A. Marshall), and in Peter Chandler's personal collection (PCPC) as indicated in Appendix 1.

Three specimens of each species were sequenced whenever possible to control against sequencing and contamination errors and to discover intraspecific sequence variation. Fewer than three specimens were sequenced in the following cases: *Protonephrocerus chiloensis, Orthoprosopa griseus* (Walker), and *Toxomerus geminatus* (Say) (only two specimens of each were available); *Collinias* Aczél and *Jassidophaga* Aczél (two specimens of one species and one specimen of a closely related species were sequenced for both of these genera; these genera have not been revised in Australia and species limits were not well established prior to sequencing); and *Verrallia virginica* (DNA could be recovered from only one specimen).

DNA Extraction

Initially, nucleic acid extractions were adapted from a CTAB protocol similar to that used by Graham *et al.* (1994). The entire fly was removed from absolute ethanol, dried on a kimwipe in a laminar flow for 5 min, and then ground into a fine powder using a mortar and pestle. A volume of 200–800 μl of 2% CTAB buffer was then added (amount of CTAB buffer added depended on the amount of tissue; 200 μl was used with small pipunculids like *Chalarus* Walker and up to 800 μl with large syrphids like *Dideopsis* Matsumura). [CTAB: 2% hexadecyltrimethylammonium bromide, Sigma Aldrich, Castle Hill, New South Wales, Australia; CTAB buffer, pH 8.0: 2% CTAB, 1.4 M NaCl, 200 mM EDTA, 100 mM Tris–HCl, pH 8.0]. This was mixed gently by inversion until the ground material was completely resuspended and then incubated for 30 min at 65°C. To pellet cellular debris this mixture was centrifuged at 13,000*g* for 5 min. The supernatant was then transferred to a sterile microcentrifuge tube and 0.5 vol of chloroform/iso-amyl alcohol (24:1) was added before mixing gently by inversion several times. To separate the aqueous phase from the organic phase this mixture was centrifuged at 13,000*g* for 1 min. The supernatant was then removed and the chloroform extraction was repeated once or twice as necessary. After removal of the upper aqueous phase, an equal volume of PEG precipitation buffer was added and the mixture was vortexed gently by hand before incubation at room temperature for 10 min. [PEG precipitation buffer: 30% PEG8000, 30 mM $MgCl_2$]. After centrifuging at 13,000*g* for 10 min at room temperature the supernatant was removed and the DNA was quickly washed twice with 250 μl cold (−20°C) 70% ethanol. The pellet was then dried at 65°C for 30 s. Resuspension of the pellet in 100 μl of 1× TE buffer, pH 8.0, with RNase (10 μg/ml) was achieved by gently vortexing the tubes followed by incubation at 65°C for 20 min [1× TE buffer, pH 8.0: 10 mM Tris–HCl, 1 mM EDTA, pH 8.0]. The resultant solution was stored at 4°C.

Grinding entire specimens was necessary to produce an adequate yield of DNA with CTAB, so we switched to Chelex 100 extractions modified from Walsh *et al.* (1991). Chelex extraction from heads produced adequate yields of DNA; legs were less consistent. The heads were removed from the specimens in absolute ethanol, then dried on kimwipes in the laminar flow for 5 min, and placed in the freezer at −85°C for at least 10 min. A plastic pestle and a microcentrifuge tube were placed on dry ice for 10 min. The specimen was then placed in the chilled tube and ground into a fine powder while still on dry ice. Then 0.5 mL of boiling Chelex was added to the ground tissue and the skewer was washed in the molten Chelex. The lid was then closed and the tube vortexed gently by hand and placed on a heater block at 100°C for 15 min. As the tube was heated, the lid was opened to release pressure, and the tube was vortexed gently every 3 min. The microcentrifuge tubes were placed in a −20°C freezer for 5 min to cool the stock and then centrifuged for 5 min at 13,000*g*. The samples were stored at 4°C.

12s and 16s PCR Amplification

The 5′ end of 12s small ribosomal subunit was amplified using mtD-35 (SR-J-14233) and mtD-36 (SR-N-14588) and the 5′ end of 16s large ribosomal subunit was amplified using mtD-32 (LR-J-12887) and mtD-34 (LR-N-13398) (Simon *et al.*, 1994). These regions were amplified using standard PCR procedures (e.g., Kocher *et al.*, 1989). The PCR mixture was optimized as follows: 2.5 μl 10× PCR buffer (Boehringer Mannheim), 5.7 μl RO H_2O, 0.5 μl dNTP (10 mM), 2.0 μl of each primer (10 μM), 2.5 μl PVP40 (polyvinylpyrrolidone M.W. 40,000; Sigma, Sydney), 0.3 μl *Taq*, 1.5 μl $MgCl_2$ (1.5 mM), and up to 8.0 μl of template (the difference was made up with RO H_2O as required). Amplification was achieved using the following program: 1 cycle of 92°C (3 min), 45°C (45 s), 72°C (45 s); 39 cycles of 92°C (45 s), 45°C (45 s), 72°C (45 s); 1 cycle of 72°C (2 min), 25°C (2 min). The resulting product was typically impure and produced poor sequences. We isolated the desired band by gel purification. To accomplish this, 80 μl of PCR product was run out on 1.5% agarose gel electrophoresis in 1× TAE buffer. Bands were excised from the gels, applied to GenElute spin columns, centrifuged for 10 min at 12,000*g*, then PEG precipitated, and resuspended in 9 μl of RO H_2O. The resulting product produced clean, virtually error-free sequences every time.

Sequencing

DNA sequences were amplified by PCR and single-stranded DNA was sequenced by the dideoxy chain-termination method (Sanger *et al.*, 1977) using an Applied Biosystems Inc. automatic sequencer (ABI 377). A standard terminator PCR mixture was used (8.0 μl of big dye terminator mix, 3.2 μl of the appropriate

primer (1 μM), and 8.8 μl of RO H_2O and template). Amplification was achieved using the following program: 25 cycles of 96°C (10 s), 50°C (5 s), 60°C (4 min); 1 cycle of 25°C (2 min). For each specimen studied, sequence was obtained from both strands. The considerable overlap of strands (~65% for 12s, ~80% for 16s) allowed confident assessment of nucleotide identity for all sequences analyzed. Sequences were deposited in GenBank under the Accession Nos. AF154680 to AF154825.

Data Analysis

Initial alignment of the sequences was conducted using SeqEd (Myers and Kececioglu, 1992), but refinement was made manually. Alignment was relatively straightforward and only 6 bp of 16s could not be satisfactorily aligned. These bases were removed. The alignments that we used for our analyses were lodged in the EMBL Nucleotide Sequence Database (Alignment Nos. DS41242 and DS41243).

Parsimony analysis was performed with PAUP* (Swofford, 1999) and replicated with PAUP (Swofford, 1993). Character polarity was based on outgroup comparison (Nixon and Carpenter, 1993). Phoridae and Platypezidae were defined as outgroups for all analyses. The heuristic search procedure was used with stepwise addition and 25 random replications for most analyses. A branch and bound search was used when analyzing the pipunculid data alone. The heuristic search option was used with tree bisection–reconnection branch swapping, MULPARS, and random addition of taxa. All multistate characters were treated as nonadditive. Genetic variations discovered by sequencing three specimens of each species were treated as polymorphisms and not as missing data. Gaps were treated as a fifth base.

Evidential support for different clades was assessed using the nonparametric bootstrap (BS; 1000 replicates using the same parameters described for our initial parsimony analysis) (Felsenstein, 1985) and branch support (BrS) (Bremer, 1994). The latter was calculated with the program AutoDecay 3.0.3 (Eriksson and Wikström, 1996). Branch support indicates the number of extra steps from the most-parsimonious solution at which clade(s) fail to be resolved as successively longer trees are examined. A high numerical value for a clade indicates good support. Tree measures such as the consistency index (CI) and the retention index (RI) were used to evaluate the fit of the data to the tree. Character evolution was examined using the program MacClade (Maddison and Maddison, 1992).

All analyses were performed for each gene separately and then on the combined data sets after performing a test for homogeneity (Farris *et al.*, 1994) using PAUP*.

RESULTS AND DISCUSSION

Separate Analyses—Syrphoidea

The uncorrected pairwise sequence divergence among taxa for 12s rDNA ranged from 2.7 to 20.4%. A considerable difference in sequence divergence exists between the Syrphidae and the Pipunculidae. Within the Syrphidae the average sequence divergence is about 8%, whereas within the Pipunculidae it is over 12%. Average proportions of A:T:G:C are 40:38:9:13. This ratio is consistent among all of the species included and is representative of the ratio observed in other Dipteran taxa, including Drosophilidae, Tephritidae, and Culicidae (Beard *et al.*, 1993; Clary and Wolstenhome, 1985; Han and McPheron, 1997).

Analysis of the 420 12s rDNA sites yielded 153 parsimony-informative characters. Twelve equally parsimonious trees were discovered (tree statistics in Table 1). A strict consensus of these 12 trees is shown in Fig. 1. Molecular data from 12s rDNA do not resolve the relationships between the subfamilies of Pipunculidae, and the positions of *Nephrocerus* and *Protonephrocerus* are equivocal. The scope of the Pipunculinae and Chalarinae essentially mirror the hypothesis of Rafael and De Meyer (1992). The only exception is that Rafael and De Meyer (1992) suggested that *Eudorylas* + *Tomosvaryella* is the sister of *Collinias*, and *Eudorylas* + *Tomosvaryella* + *Collinias* is the sister of *Cephalops*. Within the Syrphidae, there is support for a monophyletic Syrphinae and Microdontinae and a paraphyletic Eristalinae. None of these findings are surprising, but some authors would argue against the basal position of the Microdontinae (e.g., Hippa, 1998; Rotheray and Gilbert, 1999). This issue will be explored in more detail in the discussion of the combined analysis. Platypezidae occupies an unorthodox but poorly supported position as the sister group of the Syrphidae.

The uncorrected sequence divergence among taxa for 16s rDNA ranged from 3.4 to 16.7%. The difference in the average sequence divergence between the Syrphidae and the Pipunculidae is similar to that observed for 12s. Average proportions of A:T:G:C are 40:36:8:15.

Analysis of the 490 16s rDNA sites yielded 142 parsimony-informative characters. Twenty-three equally parsimonious trees were discovered (tree statistics in Table 1). The strict consensus of these trees contains relatively little resolution, even less if poorly supported (i.e., BS $<$ 50%) clades are collapsed. Within the Pipunculidae, support for the subfamilies Pipunculinae and Chalarinae is still reasonable (BrS 2, 9; BS 64, 93, respectively); however, the monophyly of the Nephrocerinae, the Syrphinae, and even the Pipunculidae are ambiguous.

Combined Analysis—Syrphoidea

A partition homogeneity test on the 12s and 16s data suggests that these data sets are not significantly in-

TABLE 1

Tree Statistics

	Syrphoidea: 12s analysis (Fig. 1)	Syrphoidea: 16s analysis	Syrphoidea: 12s and 16s combined analysis (Fig. 2)	Pipunculidae: morphological data set* (cf. Fig. 3)	Pipunculidae: 12s and 16s (Fig. 3)	Pipunculidae: combined 12s, 16s, and morphology (Fig. 4)	Pipunculidae: combined analysis including fossils (cf. Fig. 4)
CI	0.45	0.41	0.42	0.74	0.55	0.57	0.57
CI excluding uninformative characters	0.40	0.35	0.37	0.66	0.47	0.49	0.49
RI	0.59	0.49	0.53	0.79	0.52	0.57	0.58
Tree length	622	662	1294	112	812	932	932
Parsimony-informative characters	153	142	295	56	241	304	303
Constant characters	213	294	510	34	565	599	599
Variable, parsimony-uninformative characters	54	54	105	27	104	124	125
Total number of characters	420	490	910	117	910	1027	1027
No. of taxa	29	29	29	12	15	15	17
No. of most-parsimonious trees	12	23	6	1	1	1	3

* The data set from Rafael and De Meyer (1992) is reduced to include only the genera for which we collected molecular data. They did not include outgroups with their analysis, so three outgroups are added here. Reducing the number of taxa to be analyzed and adding outgroups produced a single tree of topology identical to that of their published tree.

congruent ($P = 0.62$). The data sets were thus combined and analysis yielded six most-parsimonious trees (tree statistics in Table 1). A strict consensus of the six trees is shown in Fig. 2. Clade support is consistently higher than for either independent analysis, and the phylogeny contains more resolution. Within the Pipunculidae, the positions of *Nephrocerus* and *Protonephrocerus* are still unclear. Supports for the Pipunculinae and Chalarinae are particularly good (BrS 12, 14, respectively; BS 99 for both clades). There is also moderate support for a monophyletic Pipunculidae (BrS 3; BS 73).

Support within the Syrphidae remains poor. Relaxation of parsimony by a single step (i.e., BrS = 1) collapses almost every clade. Only the Syrphinae + Eristalinae has significant support (BrS 4; BS 92). The basal position of the Microdontinae is supported in all six most-parsimonious trees, but both bootstraps and branch support are very low (54 and 1, respectively). Combining these data with alternative data sets that are currently being explored (e.g., Hippa, 1998; Rotheray and Gilbert, 1999; Ståhls-Mäkelä *et al.*, 1998) may lead to a more robust syrphid phylogeny. Addition of more taxa to this data set and exploration of other genes may also contribute to our understanding of the evolution of the Syrphidae. In the meantime, results from this analysis are not robust enough to challenge current syrphid taxonomy.

Separate Analyses—Pipunculidae

To include the morphological data of Rafael and De Meyer (1992) in our analysis we removed the syrphids from our molecular data set. The characters in the morphological data set are not useful for separating lineages of hover flies, so it was decided to leave only one species of hover fly (*Eristalinus punctulatus* (Macquart)), the phorid, and the platypezid in our analysis for outgroup comparison.

The matrix assembled by Rafael and De Meyer (1992) was reduced to include only the pipunculid exemplars that we used in our molecular analysis. Branch and bound analysis resulted in a single tree (cf. Fig. 3) of topology identical to that of their tree that included all of the pipunculid genera. As mentioned above, our combined 12s + 16s data set was also condensed to include only the pipunculids plus three outgroups. Branch and bound analysis found a single tree (Fig. 3). Other than the position of *Nephrocerus* and *Protonephrocerus*, the molecular tree and the morphological tree are identical. The molecular data place these taxa in separate lineages, whereas the morphological data align them as sister genera (BS 3). To-

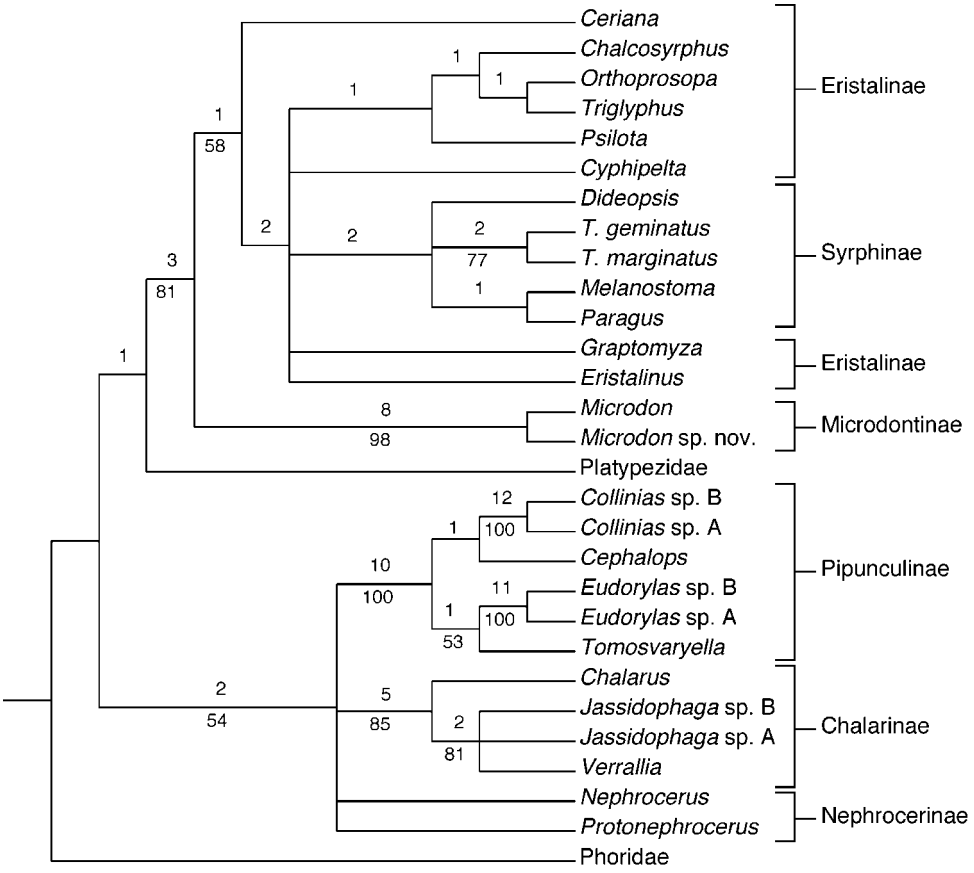

FIG. 1. Consensus tree of syrphoid relationships inferred from 12s rDNA data alone. Numbers below the branch are bootstrap values; numbers above the branch are branch supports.

gether they comprise the Nephrocerinae, the putative sister group to the Pipunculinae.

Combined Analysis—Pipunculidae

Given that reduced taxon sampling had little effect on tree topology among the shortened pipunculid trees, we decided to combine the data and analyze them together. A partition homogeneity test on the 12s, 16s, and morphological data (three partitions) suggests that these data sets are not significantly incongruent ($P = 0.38$). Analysis of this combined data yielded one most-parsimonious tree (Fig. 4; tree statistics in Table 1). Support for the Pipunculinae and Chalarinae is overwhelming (BrS 23, 26; BS 100, 100, respectively). These subfamilies are unarguably monophyletic, and the tree topology within these lineages is entirely congruent with the phylogeny proposed by Rafael and De Meyer (1992). The position of *Nephrocerus* and *Protonephrocerus* between the basal Chalarinae and the terminal Pipunculinae essentially reflects the hypothesis of Rafael and De Meyer (1992). The difference from their analysis is that these two genera apparently do not form a monophyletic group.

There is only one extant species of *Protonephrocerus*, but two fossil species are thought to be closely related (*P. florissantius* Carpenter and Hull and *Metanephrocerus collini* (Carpenter and Hull)). *Metanephrocerus* is a monotypic genus that was erected by Aczél (1948) to include a species originally described in *Protonephrocerus*. We coded these fossils from descriptions in the literature and added them to our previous analysis in an effort to test the monophyly of the *Protonephrocerus* lineage. Forty-six characters were coded for *P. florissantius* and 45 were coded for *M. collini*. The morphological character matrix is presented in Appendix 2. With only 45 to 46 characters coded of the combined data set total of 1027, a loss of resolution in the resulting tree was expected. Three equally parsimonious trees identical in topology to Fig. 4 were recovered from this analysis. Support for many lineages is dramatically reduced due to the inclusion of so much missing data in the analysis. Branch support was affected much more than bootstrap values by the inclusion of these missing data. This novel aspect of measures of clade support will be explored further in another paper (J. H. Skevington, C. L. Lambkin, and D. K. Yeates, unpublished). Despite the reduced support for many

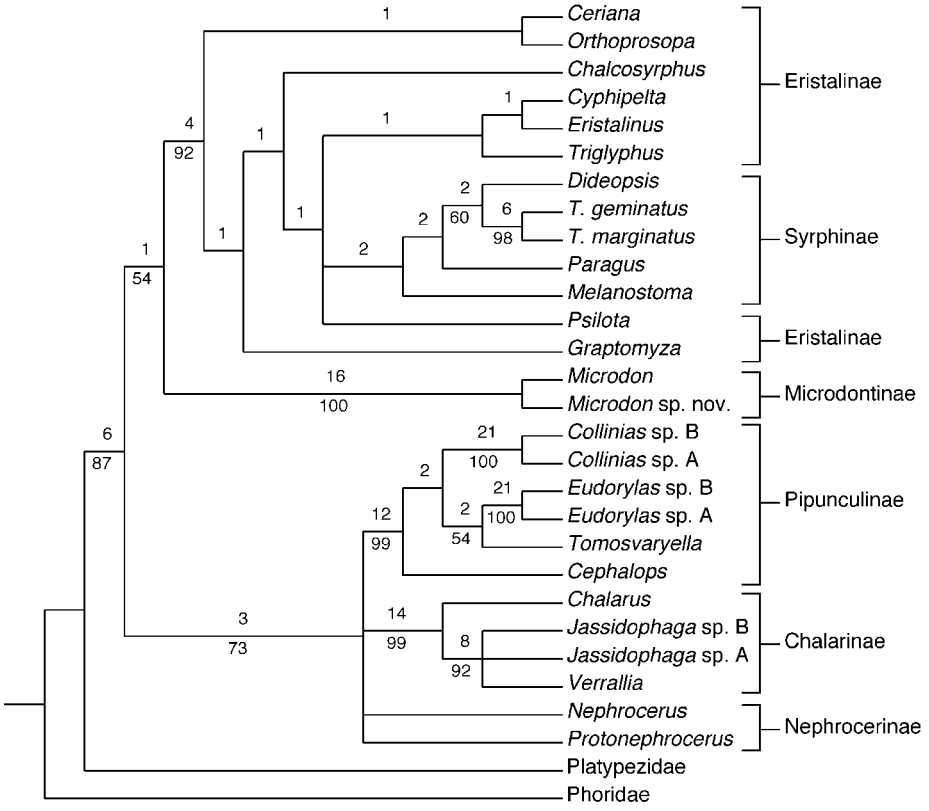

FIG. 2. Consensus tree of syrphoid relationships inferred from all molecular data (combined 12s and 16s data). Numbers below the branch are bootstrap values; numbers above the branch are branch supports.

lineages, there is still support for a lineage that includes both *Metanephrocerus* and *Protonephrocerus*. Only the relationships among the species of *Metanephrocerus* and *Protonephrocerus* were equivocal.

The position of *Protonephrocerus* + *Metanephrocerus* as the sister group to the Pipunculinae renders the Nephrocerinae paraphyletic and suggests that the subfamilial taxonomy of the Pipunculidae needs modification. We feel that inclusion of *Protonephrocerus* and *Metanephrocerus* within a redefined Pipunculinae would weaken this decisively monophyletic lineage. Erection of a new subfamily to include *Protonephrocerus chiloensis, P. florissantius,* and *M. collini* would leave *Nephrocerus* as the sole genus in the subfamily Nephrocerinae. This action should be considered if additional data are discovered which support our hypothesis. Our present results do not provide enough evidence to validate this action. Hypotheses based on data from both genes alone and from the combined 12s and 16s data set give no indication that *Nephrocerus* should be considered the sister taxon of the *Protonephrocerus* clade + the Pipunculinae and that the Nephrocerinae as outlined by Rafael and De Meyer (1992) are paraphyletic. Only when combined with the morphological data (Fig. 4) is there an indication of paraphyly, albeit with very limited evidence (BrS 2; BS 55). Although the addition of fossil data to our matrix conveys some additional support for our hypothesis of a paraphyletic Nephrocerinae, missing data and equivocal character interpretations limit their utility.

No ecological data or larval data are available for *Nephrocerus, Protonephrocerus,* or *Metanephrocerus.* These data, plus data from other sources are needed to further test our hypothesis of relationships presented here. The discovery and character coding of additional well-preserved fossils, particularly males of *M. collini* and *P. florissantius,* would be an excellent test of our hypothesis.

CONCLUSIONS

12s and 16s rDNA have proven to provide particularly useful data for analysis of relationships within the Pipunculidae. Expansion of this analysis to include exemplars from all existing genera of pipunculids would greatly strengthen our hypothesis of big-headed fly relationships. There have been several challenges to the generic monophyly of several pipunculid genera and these genes would also provide an excellent independent test of the monophyly of these taxa. Thus, expansion of this analysis to include several species of *Cephalops/Cephalosphaera, Jassidophaga/Verrallia,*

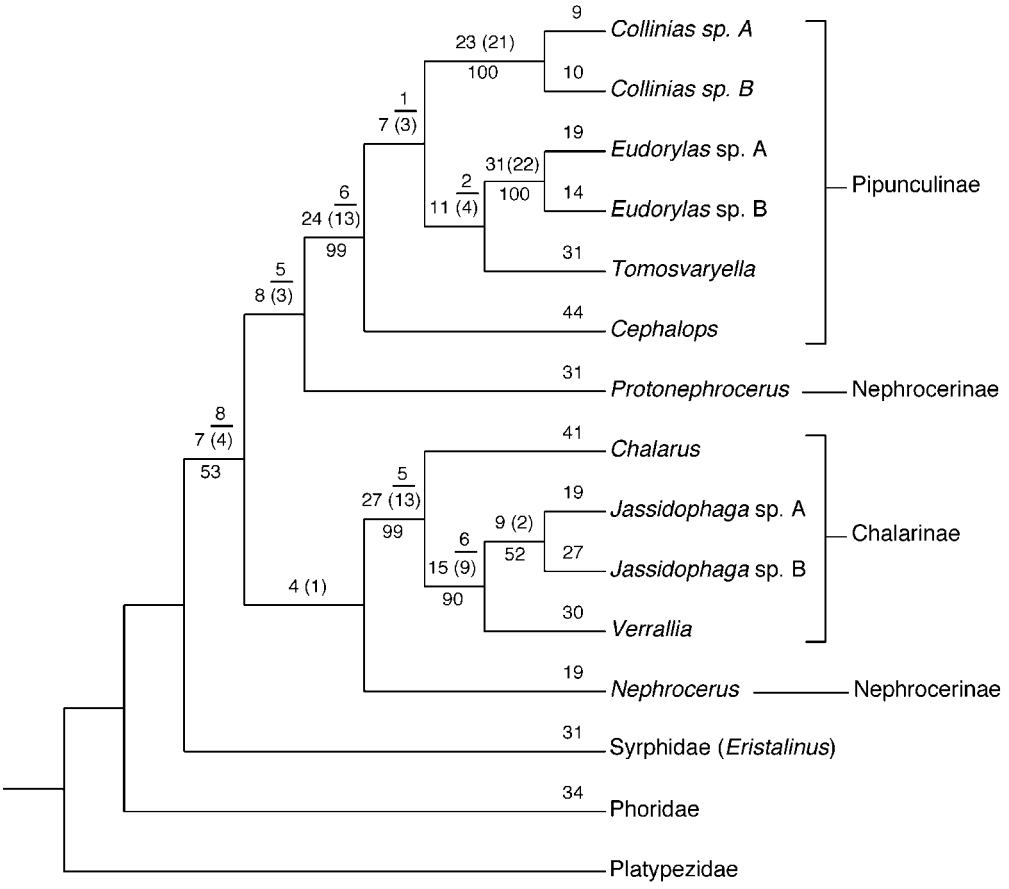

FIG. 3. Most parsimonious tree of pipunculid relationships inferred from molecular data alone (combined 12s and 16s data). Numbers below the branch are bootstrap values; numbers above the branch refer to the number of characters that change unambiguously on the branch and branch supports are in parentheses. This tree is very similar to the most-parsimonious tree based on the morphological data of Rafael and De Meyer (1992). Branch supports for the latter are included above the branch supports for the molecular tree.

Claraeola/Eudorylas s.l., *Claraeomorpha/Dorylomorpha*, and *Pipunculus/"Parapipunculus"* is recommended. With one exception, each of these pairs of genera can be separated morphologically only by the presence or absence of an M_2 wing vein. Recently, Rafael and De Meyer (1992) discovered an additional morphological character to differentiate *Verrallia* and *Jassidophaga* (presence of ventral warts on the femora in *Jassidophaga*). However, *Verrallia* can still be defined only by the presence of M_2, leaving the possibility open that it is paraphyletic with respect to *Jassidophaga*.

Unlike the excellent resolution that 12s and 16s rDNA provided within the Pipunculidae, these genes provided poor resolution of the relationships between hoverflies. There is weak support for a monophyletic Syrphinae and a basal Microdontinae, together with a paraphyletic Eristalinae. Traditional morphological phylogenetic analysis of the Syrphidae has been piecemeal at best and proposed relationships have been obscured by homoplasy. An equally large amount of homoplasy manifested itself in the 12s and 16s data. Exploration of different molecular and morphological data sets may resolve some of these problems with homoplasy. Fortunately, there is currently a great deal of interest in the phylogeny of the Syrphidae and some fundamental questions surrounding their evolution. Rotheray and Gilbert (1989) published a larval phylogeny of the Syrphinae and have recently expanded their data set to include most Palaearctic hover flies (Rotheray and Gilbert, 1999). Gilbert *et al.* (1994) investigated the evolution of feeding strategies within the Eristalinae. Ståhls-Makela (1998) and Ståhls-Makela *et al.* (1998) are currently collecting molecular data for a higher phylogeny of the Syrphidae, and Hippa (1998) has prepared an adult morphological data set to help unravel syrphid evolution. We hope that all of this interest in the group culminates in a robust phylogeny and encourages others to investigate this fascinating group of flies. When these data are all gathered and published, a single combined analysis of all of the data should provide our best estimate of syrphid phylogeny to date. Clarification of monophyly of and relationships among subfamilies and tribes will lead to the ability to

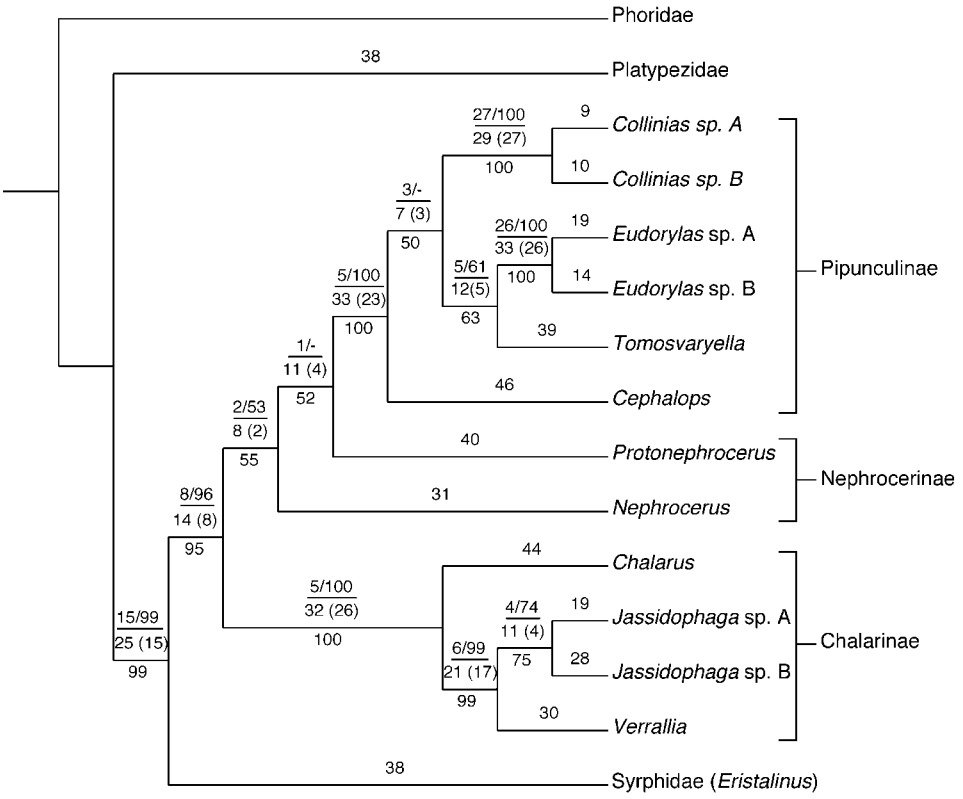

FIG. 4. Most-parsimonious tree of pipunculid relationships inferred from combined molecular (12s and 16s) and morphological data. Numbers below the branch are bootstrap values (1000 replicates); numbers above the branch refer to the number of characters that change unambiguously on the branch and branch supports are in parentheses. The topology of this tree does not change after adding fossils to the data set; however, the large amount of missing data radically alters some support indices. Branch supports and bootstrap values for the latter tree are included above the branch supports for the nonfossil tree to illustrate this effect (in the form BrS/BS).

examine lower taxonomic levels and erect phylogenetic classifications for them as well. This process will lead to a classification with much greater predictive value for other related studies.

APPENDIX 1

Voucher Information

Phoridae: Metopininae: Metopinini:

Megaselia sp. A. AUSTRALIA: QLD: Brisbane, 2♀♀, 11.xi.1997, in house [on old ice cream], S. Winterton, JSS 4421-2 (UQIC); Brisbane, St. Lucia, [University of Queensland], in lab, 1♀, 10.xi.1997, J. Skevington, JSS 4423 (UQIC).

Pipunculidae: Chalarinae:

Chalarus sp. A. AUSTRALIA: QLD: Brisbane, Mt. Coot-tha, 27°29′ S, 152°57′ E, 170 m, 1♂, 8.xi.1997, hilltop, J. & A. Skevington, head used for DNA extraction, JSS 935 (UQIC); Carnarvon National Park, Mount Moffatt Summit, 25°03′35″ S, 148°02′38″ E, 1097 m, 3♂♂, 29.xi.1997, hilltop, J. Skevington, head used for DNA extraction, JSS 3671, 3951-2 (UQIC).

Jassidophaga sp. A. AUSTRALIA: QLD: Carnarvon National Park, Mount Moffatt Summit, 25°03′35″ S, 148°02′38″ E, 1097 m, 1♂, 2.xii.1997, hilltop, J. Skevington & C. Lambkin, JSS 1850 (UQIC).

Jassidophaga sp. B. AUSTRALIA: QLD: Scrub Road, Brisbane Forest Park, 27°25′06″ S, 152°50′14″ E, 1♂, 3–10.x.1997, Malaise trap, S. Winterton, N. Power & J. Skevington, head used for DNA extraction, JSS 1266 (UQIC).

Verrallia virginica Banks. CANADA: ON: Lambton Co., Port Franks, Watson Property near L-lake, 2♀♀, 12–15.vii.1996, Malaise trap, J. Skevington, JSS 3652-3 (DEBU).

Nephrocerinae:

Nephrocerus daeckei Johnson. CANADA: ON: Lambton County, Pinery Provincial Park, Burley Campground, 1♂, 7–14.vi.1995, Malaise trap in wet meadow, J. Skevington, 2 legs used for DNA extraction, JSS 942 (DEBU); same data, 1♂, 15–19.vi.1995, JSS 944 (UQIC); Lambton County, Port Franks, Watson Prop-

erty near L-lake, 1♀, 8–10.vi.1996, Malaise trap, head and 2 legs used for DNA extraction, JSS 945 (DEBU).

Protonephrocerinae:

Protonephrocerus chiloensis Collin. CHILE: (IX), Malleco, P. N. Nahuelbuta, Pichinahuel, 1♂, 16–20.xii.1993, G. & M. Wood, 4 legs used for DNA extraction, JSS 945 (DEBU); Santiago Province: la Plata, 5 km west La Rinconado de Maipú, 400 m, 33°49′84″ S, 70°90′42″ W, 2♀♀, 24.ix.–7.xii.1997, G. Barrie, M. I. Irwin, Malaise trap in cyn bottom, JSS 4485-6 (JSPC).

Pipunculinae: Cephalopsini:

Cephalops cochleatus De Meyer & Grootaert. AUSTRALIA: QLD: Undara Volcano National Park, Bluff, 770 m, 2♂♂, 11.vi.1997, hilltop, J. & A. Skevington, body used for PCR, only genitalia remaining (dissected) as voucher, JSS 528, 530 (UQIC); 14.6 km E[ast] Lakeland Downs, 270 m, 15°46′ S, 144°57′ E, 2♂♂, 8.vi.1997, J. & A. Skevington, open, dry savanna (range land), hilltop, JSS 537, 540 (UQIC).

Microcephalopsini:

Collinias sp. A. AUSTRALIA: QLD: Scrub Road, Brisbane Forest Park, 27°25′ S, 152°50′ E, 1♀, 10–17.x.1997, Malaise trap, S. Winterton, N. Power, head used for DNA extraction, JSS 3681 (UQIC).

Collinias sp. B. AUSTRALIA: QLD: Bribie Island, QDPI Fisheries site, 27°03′ S, 153°11′ E, 1♀, 19–26.ix.1997, S. Winterton, N. Power, Malaise trap in heathland–*Acatia* regrowth, JSS 3950 (UQIC).

Eudorylini:

Eudorylas sp. A. AUSTRALIA: QLD: Undara Volcano National Park, Bluff, 770 m, 4♂♂, 11.vi.1997, hilltop, J. & A. Skevington, body used for PCR, only genitalia remaining (dissected) as voucher, JSS 675-7, 679 (UQIC).

Eudorylas sp. B. AUSTRALIA: QLD: Undara Volcano National Park, Bluff, 770 m, 2♂♂, 11.vi.1997, hilltop, J. & A. Skevington, body used for PCR, only genitalia remaining (dissected) as voucher, JSS 668, 674 (UQIC).

Tomosvaryellini:

Tomosvaryella sp. A. AUSTRALIA: QLD: Brisbane, Mount Coot-tha, 27°29′ S, 152°57′ E, 170 m, 2♂♂, 16.viii.1997, hilltop, J. & A. Skevington & C. Lambkin, head used for DNA extraction, JSS 3948-9 (UQIC).

Platypezidae: Platypezinae:

Lindneromyia argentifascia Chandler. AUSTRALIA: QLD: Carnarvon National Park, Mount Moffatt Section, Mount Moffatt Summit, 25°03′35″ S, 148°02′38″ E, 1097 m, 3♂♂, 20.i.1998, hilltop, J. & A. Skevington & S. Winterton, JSS 2766 (PCPC), JSS 2767-8 (UQIC).

Syrphidae: Microdontinae:

Microdon sp. Nov. AUSTRALIA: SA: Flinder's Ranges National Park, 2♂♂, 10–11.x.1997, Malaise trap, J. & A. Skevington, S. Winterton, JSS 1234 and 1238 (UQIC); Flinder's Ranges National Park, Dingley Dell Campground, 1♂, 10.x.1997, Malaise trap, J. & A. Skevington, S. Winterton, JSS 3692 (USNM).

Microdon variegatus (Walker). AUSTRALIA: QLD: 8 Mile Plains, Brisbane, 1♀, 3.x.1987, R. Gerrits, hind leg used for DNA extraction, JSS 1237 (UQIC); Carnarvon National Park, Mount Moffatt Section, 25°03′52″ S, 148°01′00″ E, 1♂, 29.xi.1997, Malaise trap, J. Skevington & C. Lambkin, JSS 3664 (UQIC); same data, 1♀, 30.xi.1997, JSS 3665 (USNM); Carnarvon National Park, Mount Moffatt Section, 25°03′49″ S, 148°01′57″ E, 1♀, 29.xi.1997, JSS 3666 (UQIC); Ferny Grove, 1♂, 23.iv.1989, D. Logan, head used for DNA extraction, JSS 3955 (UQIC).

Syrphinae: Bacchini:

Melanostoma apicale Bigot. AUSTRALIA: QLD: Mount Lewis near Julatten, 16°34′ S, 145°17′ E, c. 1000 m, 2♂♂, 30.v.1997, sweep, meadow in rainforest, J. & A. Skevington, JSS 4404 in USNM, JSS 4405 (UQIC).

Toxomerini:

Toxomerus geminatus (Say). USA: 1♂, no data, JSS 4477 (UQIC).

Toxomerus marginatus (Say). USA: 1♀, no data, JSS 4480 (UQIC).

Paragini:

Paragus (*Pandasyopthalmus*) *politus* Wiedemann. AUSTRALIA: QLD: Daintree National Park, near Cape Tribulation, 2♂♂, 5.vi.1997, rainforest opening along road, J. & A. Skevington, JSS 3683 (UQIC), JSS 4411 (USNM).

Syrphini:

Dideopsis aegrota (Fab.). AUSTRALIA: QLD: 2.4 km down Cow Bay Road near Daintree Nat[ional] Park, 16°12′ S, 145°26′ E, 1♀, 3–5.vi.1997, J. & A. Skevington, Malaise trap in opening beside rainforest, JSS 4406 (USNM); Daintree National Park, Cape Tribulation Section, 1♀, 4.vi.1997, rainforest opening along road, J. & A. Skevington, JSS 1242 (UQIC).

Eristalinae: Pipizini:

Triglyphus fulvicornis Bigot. AUSTRALIA: NSW: Warrumbungle National Park, Observatory, 1♂, 17.x.1997, J. Skevington, hilltop, JSS 4407 (USNM); Warrumbungle National Park, 1♀, 19.x.–3.xi.1997, J. Skevington, S. Winterton, Malaise trap [dry creek bed in *Eucalyptus* forest], JSS 4408 (UQIC).

Volucellini:

Graptomyza plumifer Ferguson. AUSTRALIA: QLD: Tamborine Mountain, Palm Grove Trail, 27°56′ S, 153°12′ E, 3♀♀, 29.iii.1998, J. & A. Skevington, at sap wounds on dying tree, JSS 4409, 4419 (USNM), JSS 4410 (UQIC).

Eristalini:

Eristalinus (*Lathyrophthalmus*) *punctulatus* (Mac-

quart). AUSTRALIA: QLD: Koonchera Dune near Koonchera Waterhole, 26°41′ S, 139°30′ E, 2♂♂, 2.ix.1997, J. & A. Skevington, JSS 4416 (USNM), JSS 4417 (UQIC).

Brachyopini:

Cyphipelta rufocyanea Walker. AUSTRALIA: QLD: Brisbane, Mount Coot-tha, 27°29′16″ S, 152°57′02″ E, 2, 19.iv.1998, hilltop, 170 m, J. Skevington, JSS 4414 (USNM), JSS 4415 (UQIC).

Merodontini:

Psilota rubra Klocker. AUSTRALIA: SA: Adelaide, Belair National Park, 35°00′ S, 138°38′ E, 2♂♂, 4.x.1997, J. & A. Skevington, C. Lambkin, S. Winterton, hilltop, JSS 4412 (USNM), JSS 4413 (UQIC).

Cerioidini:

Ceriana (*Monoceromyia*) sp. A. AUSTRALIA: QLD: Brisbane, 5.ii.1998, T. A. Heard, from colony of *Trigona carbonaria*, JSS 4403 (UQIC), JSS 4418 (USNM).

Milesiini:

Orthoprosopa griseus (Walker). AUSTRALIA: QLD: 6 km E. of Dunwich, North Stradbroke Island, 27°30′ S, 153°27′ E, 1♂, 4.iv.1987, G. Daniels, JSS 3668 (UQIC); same data, 1♂, 15.iii.1986, JSS 3669 (USNM).

Chalcosyrphus (*Hardimyia*) *elongatus* (Hardy). AUSTRALIA: QLD: Scrub Road, Brisbane Forest Park, 27°25′06″ S, 152°50′14″ E, 1♂, 1♀, 3–10.x.1997, Malaise trap, S. Winterton, N. Power, J. Skevington, JSS 1244 (USNM), JSS 1245 (UQIC); 1, same data, 24–31.ix.1997 JSS 3691 (UQIC).

APPENDIX 2

Morphological Character Coding for Pipunculid Analyses (Characters Follow Rafael and De Meyer (1992))

	1 1 1 1 1 1 1 1 1 1 2 2 2 2 2 2 2 2 2 2 3 3 3 3 3 3 3 3 3 3 4 4 4 4 4 4 4 4 4 4 5 5 5 5 5 5 5 5 5 5 6 6 6 6 6 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4
Collinias sp. A	1 1 ? ? 1 1 1 1 1 1 1 1 1 1 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 0 0 1 0 0 0 0
Collinias sp. B	1 1 ? ? 1 1 1 1 1 1 1 1 1 1 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 0 0 1 0 0 0 0
Cephalops	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0/1 0 0 1 1 1 0/1 1 1 1 0 0 0/1 1 1 0 0/1
Chalarus	1 1 1 1 1 0 0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1 0 0 0 0 0 0 0 ? 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
Jassidophaga sp. A	1 1 1 1 1 0 0 0 0 0 0 0 0 0 1 1 1 1 0 0 0 1 1 1 1 1 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
Jassidophaga sp. B	1 1 1 1 1 0 0 0 0 0 0 0 0 0 1 1 1 1 0 0 0 1 1 1 1 1 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
Verrallia	1 1 1 1 1 0 0 0 0 0 0 0 0 0 1 1 1 1 0 0 0 1 1 1 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
Nephrocerus	1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
P. chiloensis	1 1 1 1 1 1 1 1 1 1 1 1 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 1 0 0 0 0 0 0 0 0 0 0 0 1 1 1 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
P. florissantius	1 1 ? ? ? 1 ? 1 1 1 ? ? 1 ? ? ? ? 0 0 0 0 ? ? ? ? ? 0 ? 0 1 ? ? 0 0 0 ? ? 0 ? ? ? 0 ? 0 1 1 ? 1 ? ? ? 0 ? ? 0 ? 0 0 ? ? ? ? 0 ?
Metanephrocerus	1 1 ? ? ? ? ? 1 1 1 ? ? 1 ? ? ? ? 1 0 0 0 ? ? ? ? ? 0 ? 0 1 ? ? 0 0 ? ? ? 0 ? ? 0 0 ? 0 1 1 ? 1 ? ? ? 0 ? ? 0 ? 0 0 ? ? ? ? 0 ?
Eudorylas sp. A	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 1 0 0 1 0 1 0 0 1 0 0 1 1 1 0/1 1 1 1 0 0 1 0 0 0 0
Eudorylas sp. B	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 1 0 0 1 0 1 0 0 1 0 0 1 1 1 0/1 1 1 1 0 0 1 0 0 0 0
Tomosvaryella	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 1 1 1 0 0 1 0 0 1 0 0 1 1 1 1 1 1 1 0 0 0/1 0 0 0 0
Syrphidae (*Eristalinus*)	0 0 0 0 0 0 0 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 ? 0 0 1 0 0 0 0 0 0 0 1 1 0 0 0 0 0 0 1 1 0 0 0 0 0 0 1 1 0 ? ? 1 1 0 0 0 0 0 ?
Phoridae (*Megacelia*)	0 0 0 0 0 0 0 0 0 0 1 0 1 1 ? 0 0 0 0 1 0 0 0 0 0 0 ? 0 0 ? 0 0 0 0 0 0 0 1 0 0 0 0 1 0 1 1 1 0 0 0 0 0 1 0 ? 0 0 ? ? 0 0 1 0 0 0 ?
Platypezidae (*Lindneromyia*)	0 0 0 0 ? 0 0 0 0 0 1 0 1 0 0 ? 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 ? 0 0 0 1 1 0 0 1 1 1 0 0 0 0 1 0 0 0 0 ? 1 0 0 1 0 0 0 0

	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 6 6 6 6 6 7 7 7 7 7 7 7 7 7 7 8 8 8 8 8 8 8 8 8 8 9 9 9 9 9 9 9 9 9 9 0 0 0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7
Collinias sp. A	1 0 1 1 1 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0/1 1 0/1
Collinias sp. B	1 0 1 1 1 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0/1 1 0/1
Cephalops	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0/1 0 0 0 0/1 1 0/1
Chalarus	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
Jassidophaga sp. A	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
Jassidophaga sp. B	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
Verrallia	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
Nephrocerus	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
P. chiloensis	0 0 0 0 0 0 0 0 0 0 0 0 ? 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
P. florissantius	? 0 ? ? 0 ? ? ? 0 0 0 0 ? ? 0 0 ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? 0 0 0 ? ? 0 ? ? ? 0 ? 0 ? ? 0 ? 0 0 ? ? ?
Metanephrocerus	? 0 ? ? 0 ? ? ? 0 0 0 0 ? ? 0 0 ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? 0 0 0 ? ? 0 ? ? ? 0 ? 0 ? ? 0 ? 0 0 ? ? ?
Eudorylas sp. A	0 0 1 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 1
Eudorylas sp. B	0 0 1 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 1
Tomosvaryella	0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 1 0/1 0/1 1 1 0 0 0 0 0 0 0 1 1 1
Syrphidae (*Eristalinus*)	0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 ? ? 0 0 ? 0 0 0 0 0 0 0 0 0 ? 0 0 0 0 1 0 ? 0 0 0 0 0 0 0 0 0 0 0 ? 0 0 0
Phoridae (*Megacelia*)	0 0 1 0 ? 0 0 0 0 0 0 0 0 0 0 ? 1 ? ? 0 0 ? 1 0 0 0 0 0 0 0 0 ? 0 0 0 ? 1 0 ? 0 0 0 0 1 0 0 0 0 ? 0 ? ? 0 0 0
Platypezidae (*Lindneromvia*)	0 0 0 0 1 0 0 0 0 0 0 0 0 0 1 ? 1 ? 0 0 1 0 1 0 0 0 0 0 0 0 0 0 0 1 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 ? 0 0 0

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