

A Phylogeny of Aphid Parasitoids (Hymenoptera: Braconidae: Aphidiinae) Inferred From Mitochondrial NADH 1 Dehydrogenase Gene Sequence

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Wasps of the braconid subfamily Aphidiinae are solitary endoparasitoids of aphids. Several aspects of their biology have been the focus of intuitive evolutionary hypotheses which could be tested with a robust phylogeny. Phylogenetic hypotheses have been proposed previously for aphidiines based on morphology, embryology, and DNA sequences. However, many of them are based on a limited number of characters and/or taxa and lack congruence. In addition, many of the inferred phylogenies have not been based upon cladistic analysis. Therefore, a phylogenetic study of Aphidiinae was undertaken, utilizing 465 bp of DNA sequence of the mitochondrial NADH1 dehydrogenase gene. DNA sequences were obtained from 40 taxa, including 14 genera and three outgroups. It is suggested that in agreement with most of the previously proposed phylogenies, the aphidiines, each of the three recognized tribes (Praini, Ephedrini, Aphidiini), and most genera are monophyletic. In contrast to previously proposed phylogenies, the clade of *Praon* + *Dyscritulus* (=Praini), rather than Ephedrini, is basal among the aphidiines. © 1999 Academic Press

INTRODUCTION

Wasps of the braconid subfamily Aphidiinae are solitary endoparasitoids which exclusively parasitize aphids. Approximately 50 genera and 400 species are included in the subfamily (Mackauer and Starý, 1967; Starý, 1988). Aphidiines have often been treated as a separate family, Aphidiidae, because of their specialization on aphids and the presence of other morphological features, such as a flexible suture between the second and third tergites of the gaster. However, recent phylogenetic studies have shown aphidiines to be a lineage within Braconidae (Quicke and van Achterberg, 1990; Wharton *et al.*, 1992). It still remains unclear to which of the many braconid subfamilies the aphidiines are most closely related.

Several aphidiine species have been utilized successfully in biological control programs throughout the world (Carver, 1989; Hughes, 1989). Because of their importance as biological control agents, many aspects of aphidiine biology have been studied [see Starý (1970) for a review of aphidiine biology]. Although aphidiines are a coherent group defined by a number of synapomorphies, significant differences exist in morphology, biology, and behavior among tribes, genera, and species. Because of varied interpretation of these differences, there has been disagreement concerning the phylogenetic placement of some aphidiine taxa. A number of different phylogenies have been proposed previously for aphidiines based on adult and larval morphology (Mackauer, 1961, 1968; Chou, 1984; Gärdenfors, 1986; O'Donnell, 1989; Finlayson, 1990), embryology (Tremblay and Calvert, 1971), and DNA sequences (Belshaw and Quicke, 1997). The most widely accepted classification scheme for aphidiines is that of Mackauer (1961), who divided the subfamily into four tribes: Aclitini, Aphidiini, Ephedrini, and Praini [reductions in rank are necessary here relative to Mackauer's (1961) treatment of the group as a family]. The Aphidiini is the largest of the four tribes and includes a majority of known genera and species; it is subdivided into two subtribes, Aphidiina and Trioxina.

One of the main phylogenetic controversies concerns the basal lineage among extant aphidiines. Determination of the basal lineage is important because of its strong implications for the evolution of the subfamily and certain morphological and life history traits (e.g., venom apparatus, pupation habit, and host utilization). Each of the four recognized tribes mentioned above have been suggested as being basal (see Fig. 1): Ephedrini, based on adult morphology (Mackauer, 1961; Gärdenfors, 1986) and DNA sequences (Belshaw and Quicke, 1997); Praini, based on pupation habit and venom apparatus (Tobias, 1967; Tobias and Kyriak, 1971; Edson and Vinson, 1979); Aclitini, based on morphology and behavior (Chou, 1984); and Trioxina

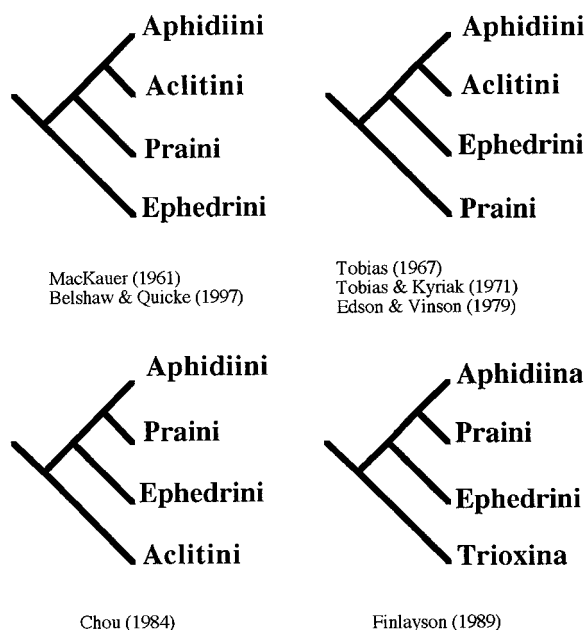


FIG. 1. Hypotheses of relationships among aphidiine tribes as presented in previous studies.

(=Aphidiini), based on final instar larval morphology (Finlayson, 1990). Obviously, only one, if any, of the four proposals can be correct. Making this determination has proven to be problematic, as each of the above proposals is based on a limited number of characters and/or a limited number of taxa.

Belshaw and Quicke (1997) recently proposed a phylogenetic tree for 18 aphidiine species, using DNA sequences from portions of the mitochondrial cytochrome *b* gene (*cyt-b*, 433 bp) and nuclear elongation factor-1 α (EF-1 α , 418 bp) and 28S rRNA (28S D2, 415–506 bp) genes. The tree inferred from *cyt-b* sequences was largely unresolved, that inferred from EF-1 α showed aphidiines to be polyphyletic, and the tree based on 28S rRNA sequence was largely congruent with that proposed by Mackauer (1961). However, with regard to the 28S rRNA analysis, Belshaw and Quicke (1997, p. 289) stated that “the true homology of many aligned positions is questionable.” The ambiguous alignment of the 28S rRNA sequences was due to a large number (61%) of variable characters, missing data, and insertion of numerous gaps throughout, including a 50-bp gap in species of *Ephedrus*.

The ambiguity in the data and the results of Belshaw and Quicke (1997) suggested a need for verifying their results by including a more diverse range of aphidiine taxa and employing DNA sequence from a different gene. Thus, the objectives of the present study were to use the DNA sequence of a portion of the mitochondrial NADH1 dehydrogenase gene, both separately and in conjunction with published sequences of the 28S rRNA D2 variable region (Belshaw and Quicke, 1997), to infer

a phylogenetic tree for Aphidiinae and to compare trees inferred here to those based on other character sets.

MATERIALS AND METHODS

Insects

A list of aphidiine taxa analyzed in this study is presented in Table 1. The insect specimens were dried, frozen, or preserved in alcohol. *Perilitus coccinellae* (Braconidae: Euphorinae) and *Apanteles* sp. and *Cotesia congregatus* (both Braconidae: Microgastrinae) were selected as outgroup taxa.

DNA Extraction and PCR

DNA was extracted from one to five wasps following the procedure outlined by Kambhampati and Smith (1995). Polymerase chain reaction (PCR) amplifications were performed in 50 μ l volume as described by Kambhampati *et al.* (1992). The temperature profile for the amplification of the NADH 1 gene fragment included an initial denaturation step of 95°C for 3 min followed by 35 cycles of 95°C for 30 s, 45–48°C for 1 min, and 72°C for 1 min. A final extension step of 72°C for 7 min was also added. Amplified products (40–48 μ l) were electrophoresed on a 2% low-melting-point agarose gel. The band corresponding to the target PCR product was excised with a sterile razor blade and purified using minicolumns (Wizard PCRpreps, Promega) according to the manufacturer’s instructions.

DNA Cloning and Sequencing

For most taxa, double-stranded PCR products were sequenced directly using cycle sequencing with the *fmol* DNA Sequencing System (Promega) following the manufacturer’s instructions. The reaction mixtures were electrophoresed on preheated 6% polyacrylamide + urea denaturing gels for 7 h with two loadings (second load applied 4 h after the first). In cases in which PCR amplification was weak and/or as a method of rapidly obtaining sequence from fragment ends, the PCR product was cloned into a T-vector (TA Cloning kit, Invitrogen) and the insert was amplified with M13 primers from a single white colony and sequenced with M13 or NADH 1 dehydrogenase primers (see below). Sequences for *Apanteles* sp. and *C. congregatus* were obtained with an ABI 377 automated sequencer, using the PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit FS (Perkin-Elmer) according to manufacturer’s specifications. Both strands of the PCR product were sequenced for all taxa.

Oligonucleotide Primers

The oligonucleotide primers used in this study were based on the mitochondrial sequence of *Apis mellifera* (Crozier and Crozier, 1993). The primer sequences for the amplification of a 465-bp portion of the NADH 1 dehydrogenase gene are: forward (ND1F)- 5'-GAT AAA

TABLE 1

Aphidiine Taxa and Outgroups Used in this Study

Species	Aphid host	Aphid host plant
<i>Aphidius ervi</i>	<i>Acyrtosiphon pisum</i>	<i>Medicago sativa</i>
<i>Aphidius matri- cariae</i>	<i>Diuraphis noxia</i>	<i>Triticum aestivum</i>
<i>Aphidius nigripes</i>	<i>Acyrtosiphon pisum</i>	<i>Medicago sativa</i>
<i>Aphidius picipes</i>	<i>Diuraphis noxia</i>	<i>Triticum aestivum</i>
<i>Aphidius pisivorus</i>	<i>Acyrtosiphon pisum</i>	<i>Medicago sativa</i>
<i>Aphidius smithi</i>	<i>Acyrtosiphon pisum</i>	<i>Medicago sativa</i>
<i>Aphidius sonchi</i>	<i>Hyperomyzus latucae</i>	<i>Sonchus oleraceus</i>
<i>Binodoxys acalephae</i>	<i>Aphis epilobii</i>	<i>Epilobium hirsutum</i>
<i>Binodoxys angelicae</i>	<i>Aphis fabae</i>	<i>Evonymus europaeus</i>
<i>Diaeretiella rapae</i>	<i>Diuraphis noxia</i>	<i>Triticum aestivum</i>
<i>Dyscritulus plani- ceps</i>	<i>Drepanosiphum plantanoides</i>	<i>Acer pseudoplatani</i>
<i>Ephedrus californi- nicus</i>	<i>Acyrtosiphon pisum</i>	<i>Medicago sativa</i>
<i>Ephedrus laevicollis</i>	<i>Chaetosiphon</i> sp.	<i>Rosa</i> sp.
<i>Ephedrus niger</i>	<i>Uroleucon cichorii</i>	<i>Crepis biennis</i>
<i>Ephedrus persicae</i>	<i>Myzus cerasi</i>	<i>Prunus avium</i>
<i>Ephedrus prociphili</i>	<i>Prociphilus</i> sp.	<i>Fraxinus excelsior</i>
<i>Euaphidius setiger</i>	<i>Periphyllus</i> sp.	<i>Acer platanoides</i>
<i>Falciconus pseudo- platani</i>	<i>Drepanosiphum plantanoides</i>	<i>Acer pseudoplatani</i>
<i>Lipolexis gracilis</i>	<i>Aphis chloris</i>	<i>Hypericum perfo- ratum</i>
<i>Lysiphlebus cardui</i>	<i>Aphis fabae</i>	<i>Cirsium arvense</i>
<i>Lysiphlebus melan- driicola</i>	<i>Brachycaudus lych- nidis</i>	<i>Silene alba</i>
<i>Lysiphlebus testa- ceipes</i>	<i>Aphis gossypii</i>	<i>Citrus</i> sp.
<i>Monoctonus pau- lensis</i>	<i>Acyrtosiphon pisum</i>	<i>Medicago sativa</i>
<i>Paramonctonus angustivalvus</i>	<i>Nasonovia cf. nigra</i>	<i>Hieracium cf. syl- vaticum</i>
<i>Pauesia pini</i>	<i>Cinara pruinosa</i>	<i>Picea abies</i>
<i>Pauesia silvestris</i>	<i>Cinara pini</i>	<i>Pinus sylvestris</i>
<i>Pauesia unilachni</i>	<i>Schizolachnus pineti</i>	<i>Pinus sylvestris</i>
<i>Pauesia</i> sp.	<i>Cinara cf. brauni</i>	<i>Pinus nigra</i>
<i>Praon abjectum</i>	<i>Aphis fabae</i>	<i>Chenopodium</i> sp.
<i>Praon barbatum</i>	<i>Acyrtosiphon pisum</i>	<i>Trifolium pratense</i>
<i>Praon bicolor</i>	<i>Eulachnus agilis</i>	<i>Pinus sylvestris</i>
<i>Praon dorsale</i>	<i>Uroleucon jaceae</i>	<i>Centaurea jacea</i>
<i>Praon pequodorum</i>	<i>Acyrtosiphon pisum</i>	<i>Medicago sativa</i>
<i>Praon pubescens</i>	<i>Nasonovia cf. nigra</i>	<i>Hieracium cf. sil- vaticum</i>
<i>Praon volucre</i>	<i>Sitobion fragariae</i>	<i>Rosa</i> sp.
<i>Trioxys betulae</i>	<i>Symydobius oblongus</i>	<i>Betula pendula</i>
<i>Trioxys falcatus</i>	<i>Periphyllus</i> sp.	<i>Acer campestre</i>
Outgroup	Outgroup host	Outgroup subfamily
<i>Perilitus coccinellae</i>	<i>Hippodamia conver- gens</i>	Euphorinae
<i>Cotesia congregata</i>	<i>Manduca sexta</i>	Microgastrinae
<i>Apanteles</i> sp.	Not available	Microgastrinae

TCA AAA/T GGG/T GT-3' (positions 12,622–12,638 of *A. mellifera*) and reverse (ND1R)-5'-CAA CCT TTT AGT GAT GC-3' (13,078–13,095 of *A. mellifera*).

Sequence Alignments and Phylogenetic Inference

DNA sequences were read from autoradiographs into a computer using SEQIN (PC/GENE, Intelligenetics, Inc.) and aligned using CLUSTAL V (Higgins and Sharp, 1989). The aligned sequences were checked and modified as needed. Taxa used in the combined (ND1 + 28S) analysis are presented in Table 2 with 28S rRNA GenBank accession numbers and/or sources.

Phylogenetic analyses were performed using maximum parsimony (MP) and neighbor-joining (NJ) methods using PAUP* ver. 4d62 (written by D. L. Swofford; used with permission) using the multiple equally parsimonious heuristic search option with tree bisection-reconnection and 500 random addition sequence replicates for DNA and 100 random addition sequence replicates for amino acid sequence data. Branch support was assessed by bootstrapping (1000 replications; Felsenstein, 1985) and decay index (Bremer, 1994). The data were also analyzed by the NJ method (Saitou and Nei, 1987) using the Tajima–Nei distance (Tajima and Nei, 1984) and bootstrapped for 1000 replications in PAUP*.

As a measure of heterogeneity among data sets, the incongruence length difference (ILD) test (Mickey and Farris, 1981; Farris *et al.*, 1994) was applied to the combined (ND1 + 28S) data matrix (invariant characters removed; 10,000 randomizations) using the partition homogeneity test option in PAUP* ver. 4d62. Finally, alternative phylogenetic trees, reflecting generally accepted relationships, were interactively constructed and evaluated using MacClade version 3.0 (Maddison and Maddison, 1992).

TABLE 2

Aphidiine/Braconid Taxa for which 28s rRNA Sequences Were Used in a Combined Analysis

Species	GenBank accession number or source
<i>Aphidius ervi</i>	Z83582
<i>Binodoxys acalephae</i>	Z83587
<i>Diaeretiella rapae</i>	Z83583
<i>Dyscritulus planiceps</i>	Z83594
<i>Ephedrus californicus</i>	Z83596
<i>Ephedrus persicae</i>	Z83598
<i>Falciconus pseudoplatani</i>	Z83589
<i>Pauesia unilachni</i>	Z83584
<i>Praon abjectum</i>	Z83591
<i>Praon dorsale</i>	Z83592
<i>Outgroup</i>	
<i>Peristenus</i> sp.	Z97952
<i>Cotesia</i> sp.	Mardulyn and Whitfield (submitted)
<i>Apanteles</i> sp.	Mardulyn and Whitfield (submitted)

The sequences reported in this paper can be obtained from the GenBank database under accession numbers AFO69160–AFO69199. The sequence alignment for the combined data set is available from P.T.S.

RESULTS

The length of the sequenced NADH1 dehydrogenase gene fragment for 37 aphidiine taxa was invariant at 465 bp. The alignment of the DNA sequence resulted in a total of 465 characters. Of the 465 characters, 288 characters (61.5%) were variable and 220 (47.0%) were parsimony informative among the 37 ingroup taxa. The alignment of the translated amino acid sequence resulted in a total of 156 characters. Of the 156 characters, 107 characters (69%) were variable and 80 (51%) were parsimony informative.

The ILD test indicated that the ND1 and 28S data sets were heterogeneous ($P < 0.001$). Nonetheless, the two data sets were used in a combined analysis because both simulation (Cunningham, 1997) and empirical (Remsen and DeSalle, 1998) studies have shown that, although data partitions may be incongruent, combining genes generally improves phylogenetic accuracy. The combined (ND1 + 28S) data set resulted in a total of 975 characters, including gaps. Of the 975 characters, 598 characters (61%) were variable and 449 (46%) were parsimony informative.

Parsimony analysis identified (1) two equally parsimonious trees of 1214 steps when the characters were unweighted (Fig. 2); (2) a single most parsimonious tree with a length of 335.6 steps when the parsimony informative characters were successively weighted (Carpenter, 1988) and assigned a base weight of 1 (Fig. 3); and (3) a single most parsimonious tree of 1218 steps when the ND1 and 28S rRNA data sets were combined for 10 ingroup and 3 outgroup taxa (Fig. 5). In all parsimony trees, the 37 aphidiine taxa included in this study were monophyletic. Taxa in each of the three tribes were also monophyletic, with Praini basal, Ephedrini intermediate, and Aphidiini the most apical lineage (Figs. 2–5). The overall topology of trees derived from analyses based on amino acids (not shown) was concordant with those obtained using the full DNA sequence; however, relationships among congeneric species were generally poorly resolved. Likewise, the topology of the tree inferred using the neighbor-joining method (Fig. 4) was similar to those obtained using parsimony. Many of the relationships were supported in 70–100% of the bootstrap replications and in trees 1–15 steps longer than the most parsimonious tree (Figs. 2–5). Most notable was the basal positioning of Praini, which was supported in 64–91% of the bootstrap replications and in trees that were as much as 5–11 steps longer than the most parsimonious trees (Figs. 2–5). We assessed whether current opinions on aphidiine relationships are compatible with our results

by forcing the monophyletic Ephedrini clade into a basal position on the MP tree and counting the number of extra steps that this implied. A tree with a basal Ephedrini required seven additional steps added to the most parsimonious tree ($L = 1214$ vs 1221).

DISCUSSION

In this paper a phylogenetic analysis of relationships among 37 aphidiine taxa, representing 14 genera and 3 tribes, based on the DNA sequence of a portion of the mitochondrial NADH1 dehydrogenase gene has been presented. The characteristics of the 465-bp portion of insect mtDNA are consistent with those of other insect mtDNA sequences. For example, a bias toward adenine and thymine (82.6% of total) is consistent with the base composition of mtDNA sequences of other insects (Simon *et al.*, 1994), including Hymenoptera (Cameron *et al.*, 1993; Crozier and Crozier, 1993; Ayala *et al.*, 1996; Vest Pederson, 1996). There was also an overall transversional bias, a majority of which were A(↔)T transversions (83.4%), which is consistent with findings from other Hymenoptera (Vest Pederson, 1996; Downton and Austin, 1997).

The parsimony and neighbor-joining trees exhibited variable levels of branch support in the form of bootstrap and decay index (Figs. 2–5). However, the monophyly of each of the tribes, Praini, Ephedrini, and Aphidiini, was generally well supported in all trees. The fact that the relationships among taxa inferred from parsimony and neighbor-joining analyses were nearly identical, with relatively strong branch support, provided confidence in the inferred relationships.

Underestimation of branch lengths due to homoplasy can occur when species have been separated for a long time, which allows for the accumulation of multiple substitutions in the third codon position (Nei, 1987; Irwin *et al.*, 1991). However, none of the methods employed to take homoplasy into account (i.e., successive weighting of the parsimony informative characters and amino acid analysis) resulted in drastic changes in tree topology. The analysis of amino acids resulted in reduced resolution among closely related taxa and in an increase in the number of equally parsimonious trees.

The results of parsimony and neighbor-joining analyses were consistent with the monophyly of the Aphidiinae, as suggested in previous studies (Mackauer, 1961, 1968; Mackauer and Starý, 1967). Because members of the tribe Aclitini were not included in our analysis, the monophyly inferred here must remain subject to further verification. Although members of Aclitini possess several synapomorphies (e.g., wing venation, ovaries, inside pupation, etc.) suggesting a close relationship to the Aphidiini (Takada and Shiga, 1974), other unique and apparently plesiomorphic traits (e.g., several aspects of behavior, genitalia, etc.) imply a more basal

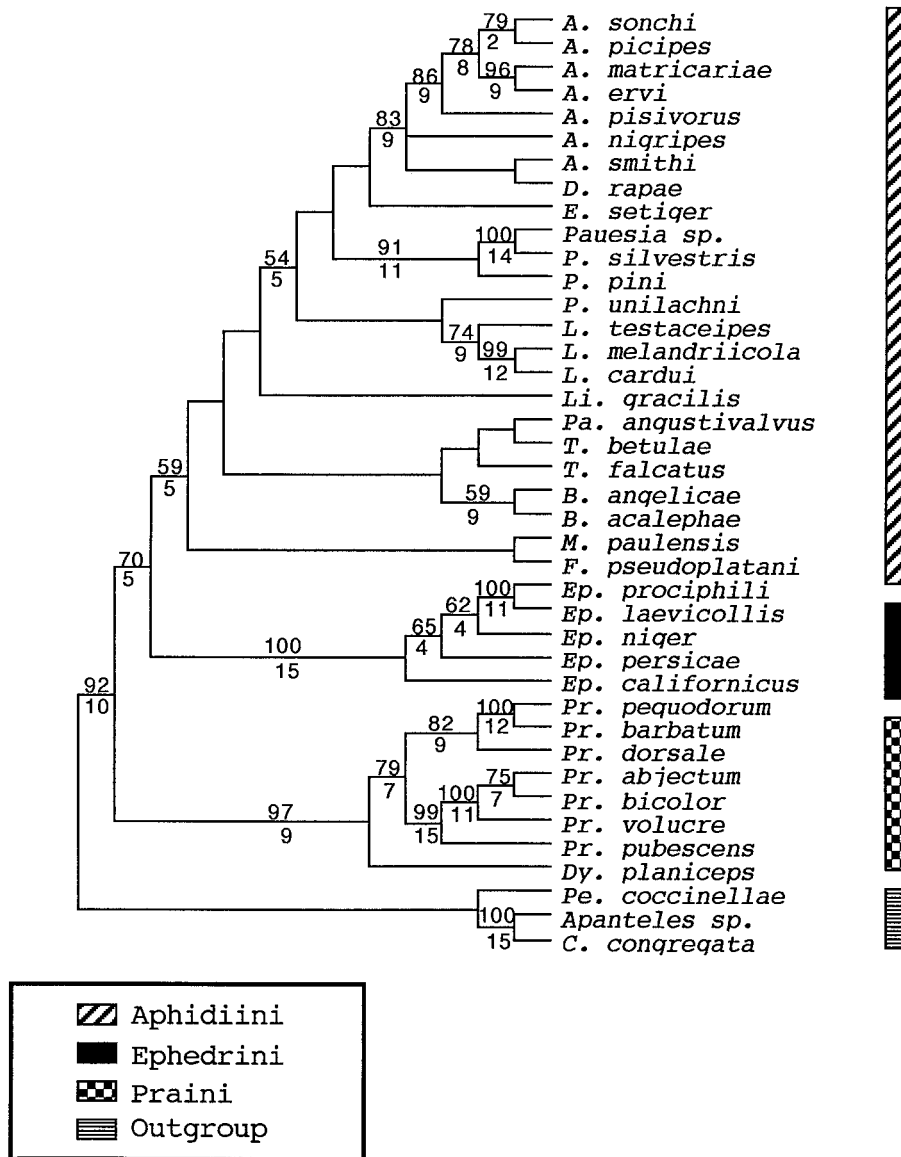


FIG. 2. Strict consensus of 2 equally parsimonious trees based on unweighted parsimony analysis of a 465-bp portion of the mitochondrial NADH 1 dehydrogenase gene for 37 aphidiine taxa. Tree length, 1214; consistency index, 0.43; retention index, 0.66. Numbers above branches are bootstrap values (%) and those below branches are decay indices. Only bootstrap values above 50% are shown.

position within Aphidiinae (Mackauer, 1961). Thus, inclusion of Aclitini in future phylogenetic analyses is critical before conclusions can be made concerning the monophyly and basal clade relationships of the subfamily.

The three tribes included in our study were found to be monophyletic. Previous morphological studies suggested that Ephedrini and Praini are more closely related to each other than either is to Aphidiini (Mackauer, 1961; Mackauer and Stary, 1967; Stary, 1970; O'Donnell, 1989; Finlayson, 1990). Both lineages share several characters which are not exhibited by the Aphidiini, including relatively complete wing venation (Mackauer and Stary, 1967), prolongately oval eggs

(Stary, 1970), 1st-instar larval appendages and mandible shape (O'Donnell, 1989), and cephalic structures of final instar larvae (Finlayson, 1990), but they differ from each other in pupation habit (Stary, 1970), venom apparatus (Edson and Vinson, 1979), and other adult characters (Gärdenfors, 1986).

Based on morphology, Ephedrini has generally been considered to constitute the basal lineage among extant aphidiines (Mackauer, 1961, 1968; Mackauer and Stary, 1967; Gärdenfors, 1986; O'Donnell, 1989) and some recent DNA evidence (Belshaw and Quicke, 1997) supports this view. In contrast, our results indicated that the clade composed of *Praon* + *Dyscritulus* (Praini) is more basal than that of *Ephedrus* (Ephedrini). The

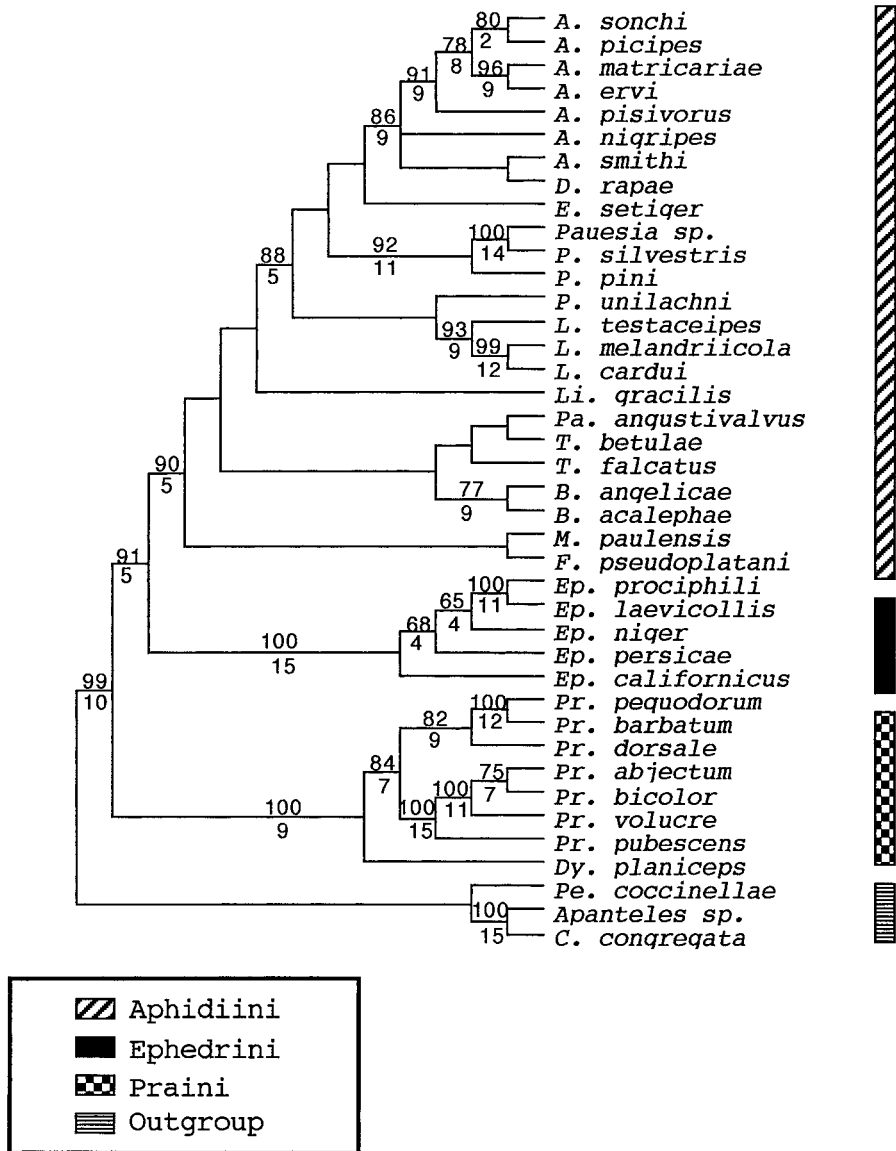


FIG. 3. Single most parsimonious tree based on parsimony analysis and successive weighting of a 465-bp portion of the mitochondrial NADH 1 dehydrogenase gene for 37 aphidiine taxa. Tree length, 335.6; consistency index, 0.66; retention index, 0.80. Numbers above branches are bootstrap values (%) and those below branches are decay indices. Only bootstrap values above 50% are shown.

support for this conclusion (bootstrap, 64–91%; decay index, 5–11) is comparable to that reported for Ephedrini as being basal (bootstrap, 65%; decay index, 5) by Belshaw and Quicke (1997). Moreover, a combined analysis of ND1 and 28S rRNA sequences also recovered Praini as the basal clade (Fig. 5). However, strong conclusions cannot be made based on the combined approach because the two data partitions were found to be incongruent and the analysis is based on a small number of ingroup taxa ($n = 10$). Additional evidence supporting the basal placement of Praini included the additional seven steps in length added to the most parsimonious topology by forcing Ephedrini into the basal ingroup position.

In comparison to previous published topologies (see Fig. 1), the results reported here are most similar to those of Tobias (1967) and Tobias and Kyriak (1971) based on pupation behavior and those of Edson and Vinson (1979) based on venom apparatus morphology. With respect to pupation behavior, aphidiines either pupate inside the dead host (most extant species) or outside and underneath the empty exoskeleton of the host (most members of Praini). Mackauer (1961) proposed that outside pupation in Praini evolved by way of secondary loss of internal pupation, represents an adaptation in response to hyperparasitoids (Mackauer, unpublished), and is not homologous to the external pupation exhibited by species in other braconid subfami-

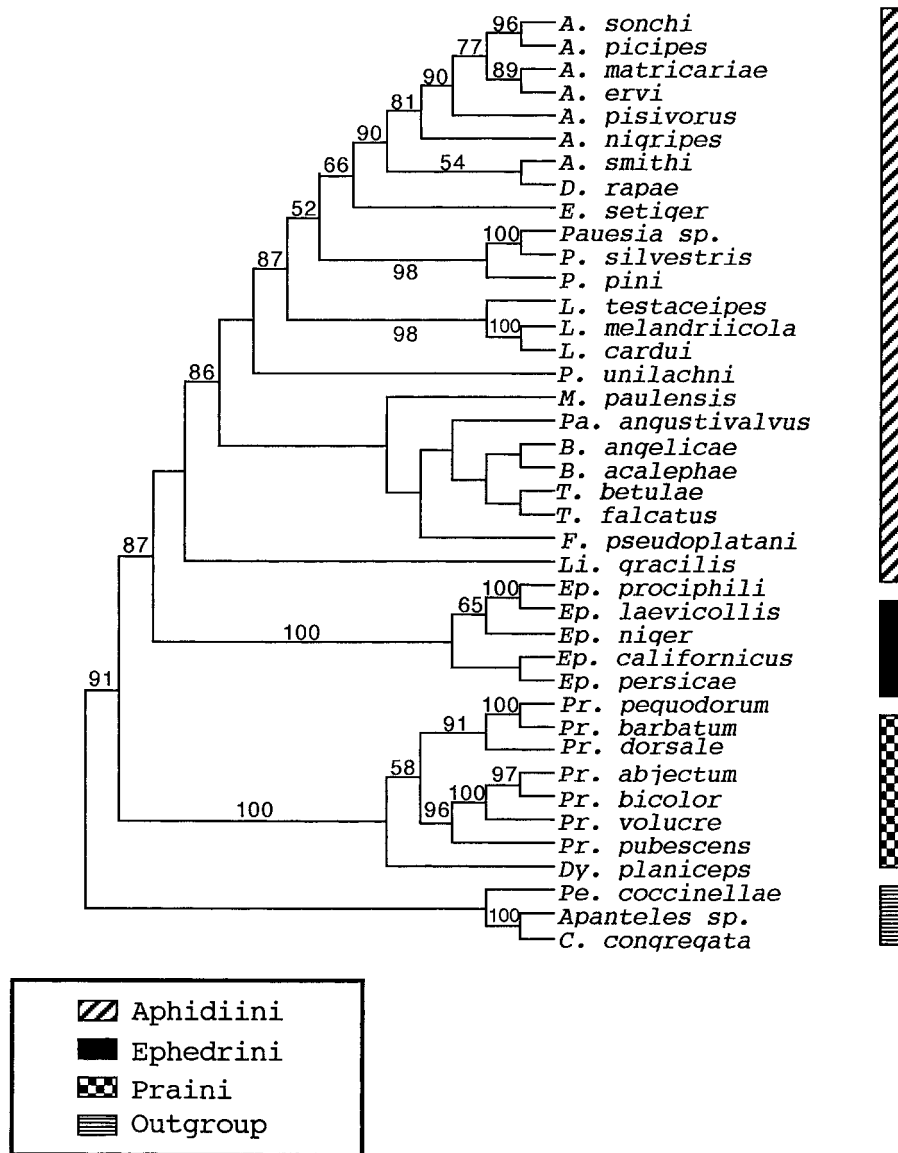


FIG. 4. Phylogenetic analysis using the neighbor-joining method (Saitou and Nei 1987). Numbers above branches are bootstrap values (%).

lies. Central to the issue of pupation behavior is the phylogenetic position of the genus *Areopraon* (a member of Praini), which contains species which pupate internally and others which pupate externally or both depending on the circumstances (Starý, 1970). Morphological evidence suggests that *Areopraon* is basal within Praini. Given that we could not include *Areopraon* in this study, conclusions concerning the evolution of pupation behavior in aphidiines must remain subject to further verification.

Three of the seven genera for which multiple species were included were not monophyletic, namely *Pauesia*, *Aphidius*, and *Trioxys* (see Figs. 2 and 3). Paraphyly in *Pauesia* was due to *P. unilachni* falling outside the other *Pauesia* species. This result was surprising be-

cause members of *Pauesia* are united not only by morphological features (e.g., large central areola of adults) but also by their distinct host range of attacking aphids in the family Lachnidae. However, the separation of *P. unilachni* from the other *Pauesia* species is also supported by the results of Belshaw and Quicke (1997), which showed that *P. unilachni* and *P. juniperorum* were not monophyletic, and by egg morphology. The eggs of *P. unilachni* are club shaped (Völkl and Kraus, 1996), whereas the eggs of all other members of Aphidiini are lemon shaped. Thus, *P. unilachni* may represent either a highly derived or a basal *Pauesia* species. Inclusion of additional species from this genus should provide insight into the monophyly of this genus.

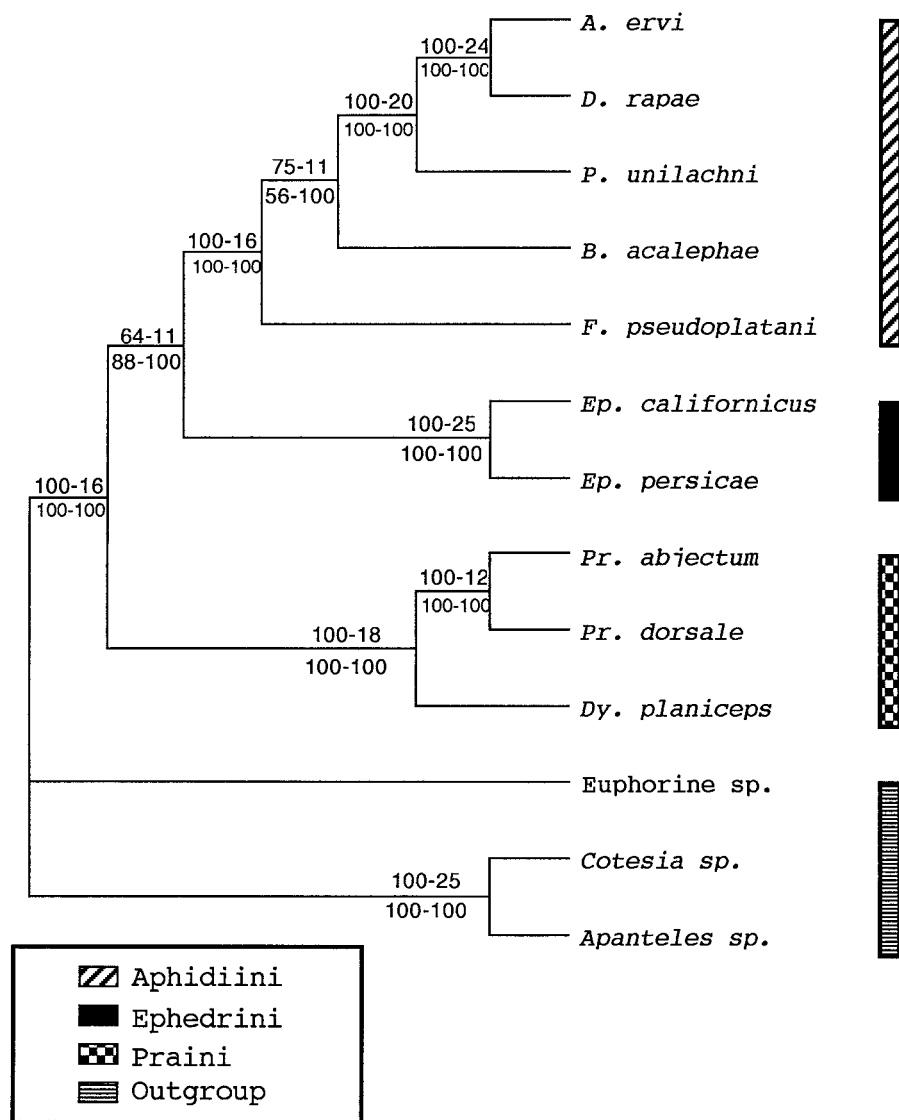


FIG. 5. Combined-evidence single most parsimonious tree for 10 aphidiine taxa. Tree length, 1218; consistency index, 0.66; retention index, 0.64. Numbers above branches are bootstrap values (%) and decay indices and numbers below the branches are bootstrap values (%) for the neighbor-joining tree and successive weighting parsimony analysis. Alignment of 28S rRNA sequences is from Belshaw and Quicke (1997). The alignment was modified to account for dissimilar taxon sampling among data partitions. The outgroup taxa are “hybrids”, as no outgroup taxa were in common among the two data sets. The hybrid outgroup taxa are as follows: (1) Euphorine sp. = (*Peristenus* sp., 28S; Belshaw *et al.*, 1998) + (*Pe. coccinellae*, ND1); (2) *Cotesia* sp. = (*Cotesia* sp., 28S; Mardulyn and Whitfield, unpublished) + (*C. congregata*, ND1); and (3) *Apanteles* sp. = (*Apanteles* sp., 28S; Mardulyn and Whitfield, unpublished) + (*Apanteles* sp., ND1).

Paraphyly of *Trioxys* was due to a sister group relationship between the two *Trioxys* spp. and *Paramo-noctonus angustivalvus*. Similarly, *Aphidius smithi* was a sister of *Diaeretiella rapae* rather than the other *Aphidius* species included in the study. However, we suspect that the paraphyly of both *Aphidius* and *Tri-oxys* was probably a result of our limited taxon sampling.

In summary, our results indicated that the 465-bp portion of the mitochondrial NADH 1 dehydrogenase gene was useful for inferring a phylogeny for the aphidiine taxa included in the study. Although there

has been a considerable amount of work done on the morphology of larval and adult aphidiines, few of these data have been analyzed using cladistic methods. We propose that future phylogenetic studies concentrate on generating a morphological matrix so that these data may be combined with currently available molecular data and analyzed using the “total evidence” approach (Kluge and Wolf, 1993). Differences in results between the present study and that of Belshaw and Quicke (1997) suggest a need for additional data from both these genes to converge on a sufficient overlap in taxon sampling so that these data sets may be com-

bined and analyzed with a more complete data set. We propose that future molecular and/or morphological phylogenetic studies include exemplars from all four recognized tribes and from a more diverse range of aphidiine genera (i.e., *Adialytus*, *Areopraon*, *Diaeretus*, *Lysaphidus*, *Paralipsis*, *Protaphidius*, *Pseudopraon*, *Pseudephedrus*, etc.), as this would prove useful in evaluating the monophyly of the subfamily as a whole, verifying which group constitutes the basal lineage, and in studying the evolutionary pathways of life history traits.

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REFERENCES

- Ayala, F. J., Wetterer, J. K., Longino, J. T., and Hartl, D. L. (1996). Molecular phylogeny of *Azteca* Ants (Hymenoptera: Formicidae) and the colonization of *Cecropia* trees. *Mol. Phylogenet. Evol.* **5**: 423–428.
- Belshaw, R., and Quicke, D. L. J. (1997). A molecular phylogeny of the Aphidiinae (Hymenoptera: Braconidae). *Mol. Phylogenet. Evol.* **7**: 281–293.
- Belshaw, R., Fitton, M., Herniou, E., Gimeno, C., and Quicke, D. L. J. (1998). A phylogenetic reconstruction of the Ichneumonoidea (Hymenoptera) based on the D2 variable region of 28S ribosomal RNA. *Syst. Entomol.* **23**: 109–123.
- Bremer, K. (1994). Branch support and tree stability. *Cladistics* **10**: 295–304.
- Cameron, S. A., Derr, J. N., Austin, A. D., Wooley, J. B., and Wharton, R. A. (1992). The application of nucleotide sequence data to phylogeny of the Hymenoptera: A review. *J. Hym. Res.* **1**: 63–79.
- Carpenter, J. M. (1988). Choosing among multiple equally parsimonious cladograms. *Cladistics* **4**: 291–296.
- Carver, M. (1989). Biological control of aphids. In "Aphids, their Biology, Natural Enemies and Control" (A. K. Minks and P. Harrewijn, Eds.), pp. 141–165. Elsevier, Amsterdam.
- Chou, L.-Y. (1984). The phylogeny of Aphidiidae (Hymenoptera). *J. Agric. Res. (China)* **33**: 437–446.
- Crozier, R. H., and Crozier, Y. C. (1993). The mitochondrial genome of the honeybee *Apis mellifera*: Complete sequence and genome organization. *Genetics* **133**: 97–117.
- Cunningham, C. W. (1997). Can three incongruence tests predict when data should be combined? *Mol. Biol. Evol.* **14**: 733–740.
- Dowton, M., and Austin, A. D. (1997). Evidence for AT-transversion bias in wasp (Hymenoptera: Symphyta) mitochondrial genes and its implications for the origin of parasitism. *J. Mol. Evol.* **44**: 398–405.
- Edson, K. M., and Vinson, S. B. (1979). A comparative morphology of the venom apparatus of female braconids (Hymenoptera: Braconidae). *Can. Entomol.* **111**: 1013–1024.
- Farris, J. S., Källersjö, S. M., Kluge, A. G., and Bult, C. (1994). Testing significance of incongruence. *Cladistics* **10**: 315–319.
- Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using bootstrap. *Evolution* **39**: 783–791.
- Finlayson, T. (1990). The systematics and taxonomy of final instar larvae of the family Aphidiidae. *Mem. Entomol. Soc. Canada* Vol. 152.
- Gärdenfors, U. (1986). Taxonomic and biological revision of Palaearctic *Ephedrus* (Haliday) (Hymenoptera, Braconidae, Aphidiinae). *Entomol. Scand., Suppl.* **27**: 1–95.
- Higgins, D. M., and Sharp, P. M. (1989). Fast and sensitive multiple sequence alignment on a microcomputer. *CABIOS* **5**: 151–153.
- Hughes, R. D. (1989). Biological control in the open field. In "Aphids, their Biology, Natural Enemies and Control" (A. K. Minks and P. Harrewijn, Eds.), pp. 167–198. Elsevier, Amsterdam.
- Irwin, D. M., Kocher, T. D., and Wilson, A. C. (1991). Evolution of the cytochrome *b* gene of mammals. *J. Mol. Evol.* **32**: 128–144.
- Kambhampati, S., and Smith, P. T. (1995). PCR primers for the amplification of four insect mitochondrial gene fragments. *Insect Mol. Biol.* **4**: 233–236.
- Kambhampati, S., Black, W. C., IV, and Rai, K. S. (1992). Random amplified polymorphic DNA of mosquitoes: Techniques, applications and statistical analyses. *J. Med. Entomol.* **29**: 939–945.
- Kluge, A. G., and Wolf, A. J. (1993). Cladistics: What's in a word? *Cladistics* **9**: 183–199.
- Mackauer, M. (1961). Die Gattungen der Familie Aphidiidae und ihre verwandtschaftliche Zuordnung (Hymenoptera: Ichneumonoidea). *Beitr. Entomol.* **11**: 792–803.
- Mackauer, M. (1968). "Hymenopterorum Catalogus. Pars 3, Aphidiidae," Junk, Gravenhage.
- Mackauer, M., and Starý, P. (1967). "World Aphidiidae (Hymenoptera: Ichneumonoidea)," Le Francois, Paris.
- Maddison, W. P., and Maddison, D. R. (1992). "MacClade: Analysis of Phylogeny and Character Evolution. Version 3.0." Sinauer, Sunderland, MA.
- Mickevich, M. F., and Farris, W. M. (1981). The implications of congruence in *Menidia*. *Syst. Zool.* **30**: 351–370.
- Nei, M. (1987). "Molecular Evolutionary Genetics," Columbia Univ. Press, New York.
- O'Donnell, D. J. (1989). A morphological and taxonomic study of first instar larvae of Aphidiinae (Hymenoptera: Braconidae). *Syst. Entomol.* **14**: 197–219.
- Quicke, D. L. J., and van Achterberg, C. (1990). Phylogeny of the subfamilies of Braconidae (Hymenoptera: Ichneumonoidea). *Zool. Verh. Leiden* **258**: 1–95.
- Remsen, J. R., and DeSalle, R. (1998). Character congruence of multiple datasets and the origin of Hawaiian Drosophilidae. *Mol. Phylogenet. Evol.* **9**: 225–235.
- Saitou, N., and Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**: 406–425.
- Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., and Flook, P. (1994). Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann. Entomol. Soc. Am.* **87**: 651–701.
- Starý, P. (1970). "Biology of Aphid Parasites (Hymenoptera: Aphidiidae) with Respect to Integrated Control," Series Entomologica 6, Junk, The Hague.
- Starý, P. (1988). Aphidiidae. In "Aphids, their biology, Natural

- Enemies and Control" (A. K. Minks and P. Harrewijn, Eds.), Vol. 2B, pp. 171–184. Elsevier, Amsterdam.
- Tajima, F., and Nei, M. (1984). Estimation of evolutionary distance between nucleotide sequences. *Mol. Biol. Evol.* **1**: 269–285.
- Takada, H., and Shiga, M. (1974). Description of a new species and notes on the systematic position of the genus *Aclitus* (Hymenoptera: Aphidiidae). *Kontyu* **42**: 283–292.
- Tremblay, E., and Calvert, D. (1971). Embryosystematics in the aphidiines (Hymenoptera: Braconidae). *Boll. Lab. Entomol. Agr. Filippo Silvestri di Portici* **29**: 223–249.
- Tobias, V. I. (1967). A review of the classification, phylogeny and evolution of the family Braconidae (Hymenoptera). *Ent. Obozr.* **56**: 646–659.
- Tobias, V. I., and Kyriak, I. G. (1971). *Areopraon pilosum* Mackauer, 1959, and problems concerning phylogeny and distribution of the family Aphidiidae (Hymenoptera). *Ent. Obozr.* **50**: 11–16.
- Vest-Pederson, B. (1996). A phylogenetic analysis of cuckoo bumblebees (*Psithyrus*, Lepeltier) and bumblebees (*Bombus*, Latreille) inferred from sequences of the mitochondrial gene cytochrome oxidase 1. *Mol. Phylogenet. Evol.* **5**: 289–297.
- Völkl, W., and Kraus, W. (1996). Foraging behaviour and resource utilization of the aphid parasitoid *Pauesia unilachni*: Adaptation to host distribution and mortality risks. *Entomol. Exp. Appl.* **79**: 101–109.
- Wharton, R. S., Shaw, S. R., Sharkey, M. J., Wahl, D. B., Woolley, J. B., Whitfield, J. B., Marsh, P. M., and Johnson, J. W. (1992). Phylogeny of the subfamilies of the Braconidae (Hymenoptera: Ichneumonoidea): A reassessment. *Cladistics* **8**: 199–235.