Eur. J. Entomol. **102**: 133–138, 2005
ISSN 1210-5759

Molecular phylogeny of the Aphidiinae (Hymenoptera: Braconidae) based on DNA sequences of 16S rRNA, 18S rDNA and ATPase 6 genes

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Key words. Hymenoptera, Braconidae, Aphidiinae, 16S rRNA, 18S rDNA, ATPase 6, phylogeny

Abstract. Phylogenetic relationships among 16 genera of the subfamily Aphidiinae (Hymenoptera: Braconidae) were investigated using sequence data from three genes: the mitochondrial large ribosomal subunit (16S), 18S ribosomal DNA and mitochondrial ATPase 6. All sequences were downloaded from the GenBank database. A total of 2775 base pairs of aligned sequence were obtained per species from these three genes. The results support the existence of three-tribes: Ephedrini, Praini and Aphidiini, with the Ephedrini occupying the basal position; Aphidiini could be further subdivided into three subtribes: Monoctonina, Trioxina and Aphidiina. The genus *Aphidius* is a paraphyletic group. The taxonomic status of the subfamily Aphidiinae within the Braconidae is probably closer to the non-cyclostome than the cyclostome subfamilies.

INTRODUCTION

Aphidiinae is one of the subfamilies of the family Braconidae (Insecta: Hymenoptera) with approximately 50 genera and 400 species (Mackauer & Starý, 1967; Starý, 1988). They are exclusively solitary endoparasitoids of aphids. Several species have been used successfully in biological control programs throughout the world (Carver, 1989). Because of their importance as biological control agents, many aspects of their biology have been studied (Starý, 1970).

Aphidiines have often been treated as a separate family, the Aphidiidae, because of their specialization on aphids, the presence of a flexible suture between the second and third mesosomal tergites and reduced wing venation. However, recent phylogenetic studies have shown aphidiines to be a lineage within the Braconidae (Quicke & van Achterberg, 1990, 1992; Wharton et al., 1992), but it still remains unclear that to which of the many braconid subfamilies the aphidiines are most closely related.

Although the Aphidiinae is a coherent group defined by a number of synapomorphies, significant differences exist in morphology, biology and behaviour among tribes, genera and species, and the phylogenetic relationships within this subfamily remain to be resolved. Several phylogenies, based on adult and larval morphology, embryology and DNA sequences, have been proposed for Aphidiinae (Mackauer, 1961; Tremblay, 1967; Tremblay & Calvert, 1971; Chou, 1984; Gärdenfors, 1986; Quicke & van Achterberg, 1990, 1992; Whitfield, 1992; Belshaw & Quicke, 1997; Dowton et al., 1998; Smith et al., 1999; Kambhampati et al., 2000; Sanchis et al., 2000). The most widely accepted classification scheme for Aphidiinae is that of Mackauer (1961) who divided the subfamily into four tribes: Aclitrini, Aphidiini, Ephedrini and Praini. The Aphidiini is the largest of the four tribes, includes the majority of genera and species, and is further subdivided into two subtribes, Aphidiina and Trioxina. Because the Aclitini is poorly represented and hardly available (Kmabhampati et al., 2000 are the only authors to have included them in a molecular analysis) most authors accept the existence of four natural groups: Ephedrini, Praini, Trioxini and Aphidiini. Trioxini and Aphidiini are treated as independent tribes, forming a four-tribe hypothesis (Ephedrini + (Praini + (Trioxini + Aphidiini))) (Belshaw & Quicke, 1997) or they are placed in the same tribe, resulting in a three-tribes hypothesis: Ephedrini, Praini and Aphidiini (Smith et al., 1999; Sanchis et al., 2000). However, Sanchis et al. (2000) claimed that their results favour either the three-tribes system or a new classification of at least five tribes (Ephedrini, Praini, Monoctonini, Trioxini and Aphidiini).

One of the main phylogenetic controversies concerns the basal lineage among extant aphidiines. Each of the four tribes mentioned above have been suggested as being basal. Ephedrini, based on adult morphology (Mackauer, 1961; Gärdenfors, 1986) and DNA sequences (Belshaw & Quicke, 1997; Sanchis et al., 2000); Praini, based on pupation habit and venom apparatus (Tobias, 1967; Edson & Vinson, 1979) and DNA sequences (Dowton et al., 1998; Smith et al., 1999); Aclitini, based on morphology and behaviour (Chou, 1984) and DNA sequences (Kambhampati et al., 2000); and Trioxina (=Aphidiini), based on final instar larval morphology (Finlayson, 1990).

Therefore, the aim of our study was to determine which tribe might be basal within the Aphidiinae. This was done using three different molecular markers, the mitochondrial ATPase 6, the ribosomal 18S rDNA and the mitochondrial 16S rRNA genes, whose sequences for the taxa studied are already available in the GenBank database. In

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addition, whether there are three or four main clades within this subfamily was tested and the phylogenetic trees inferred here and those based on other characters compared.

MATERIAL AND METHODS

Sampling of taxa

Twenty three species belonging to 16 genera were examined in this study. The species are listed in Table 1 and the arrangement of the tribes is based on morphological and biological characters. DNA sequences of the three genes used in this study were downloaded from the GenBank database with accession numbers listed in Table 1.

Outgroup selection

Three outgroups were selected for the phylogenetic analysis: the genera *Jarra* (Doryctinae) and *Mesostoa* (Mesostoinae) of the cyclostome lineage and genus *Schizoprymnus* (Helconinae) of the non-cyclostome lineage. Helconinae is widely recognized as a sister group of the Aphidiinae, and the Doryctinae and Mesostoinae are postulated to occupy a relatively basal position within Braconidae (Quicke & van Achterberg, 1990).

Sequence alignments

Sequences were aligned using CLUSTAL X version 1.81 (Thompsom et al., 1997) with default parameters. The manual alignment was followed to remove some regions with high variation. The lengths of the resulting alignments of 18S rDNA ranged between 1752 to 1820 bp, of 16S rRNA between 394 to 486 bp and of ATPase 6 between 618 to 624 bp.

Phylogenetic analysis

Following alignment, three different methods of phylogenetic analyses were performed using PAUP* 4.0 (beta 10 version) (Swofford, 2001). First, maximum parsimony (MP) was used to find the most parsimonious tree(s), and heuristic parsimony search (Hillis et al., 1996) were performed using 100 replicates

TABLE 1. Aphidiine species included in the study.

Taxa	Aphid host	Accession Number		
		16S rRNA	18S rDNA	ATPase 6
Tribe Ephedrini				
Ephedrus niger Gaut., Bon. & Gau., 1939	*Macrosiphoniella* sp.	—	AJ009328[2]	AJ400617[5]
Ephedrus persicae Froggatt, 1904	*Brachyungis tamaricis*	AF174348[1]	AJ009329[2]	AJ400618[5]
Tribe Praini				
Praon dorsale (Haliday, 1833)	*Uroleucon* sp.	—	AJ009341[2]	AJ400616[5]
Dyscritulus planiceps (Marshall, 1896)	*Drepanosiphum oregonensis*	AF174350[1]	AJ009340[2]	AJ400615[5]
Tribe Troxini				
Trioxys brevicornis (Haliday, 1833)	*Hyadaphis phoeniculi*	—	AJ009350[2]	AJ400610[5]
Trioxys pallidus (Haliday, 1833)	*Hoplocallis picta*	AF174336[1]	AJ009351[2]	AJ400613[5]
Monoctonia vesicarii Tremblay, 1991	*Pemphigus spirotecae*	AF174341[1]	AJ009337[2]	AJ400618[5]
Lipolexis gracilis Förster, 1862	*Aphis ruborum*	AF174338[1]	AJ009334[2]	AJ400609[5]
Tribe Aphidiini				
Aphidius colemani Viereck, 1912	*Hyalopterus pruni*	AF289145[8]	AJ009318[2]	AJ400586[5]
Aphidius matricariae Haliday, 1834	*Myzus cerasi*	AF289148[8]	AJ009324[2]	AJ400590[5]
Aphidius rosae Haliday, 1834	*Macrosiphum rosae*	AF003478[3]	AJ009325[2]	AJ400519[5]
Diaeretiella rapae (M'Intosch, 1855)	*Xerophyllaphis suaedae*	AF174315[1]	AJ009323[2]	AJ400592[5]
Diaeretus leucopterus (Haliday, 1834)	*Eulachnus rileyi*	AF174332[1]	AJ009323[2]	AJ400606[5]
Lysaphidus santolinae Michelena & Sanchis, 1997	*Coloradoa* sp.	—	AJ009333[2]	AJ400593[5]
Pauesia pini (Haliday, 1834)	*Cinara* sp.	AF174325[1]	AJ009344[2]	AJ400602[5]
Pauesia sylvestris (Starý, 1960)	*Cinara* sp.	AF174327[1]	AJ009342[2]	AJ400603[5]
Protaphidius wissmannii Ratzenburg, 1848	*Stomaphis* sp.	AF174317[1]	AJ009348[2]	AJ400605[5]
Pseudopauesia prunicola Halme, 1986	*Myzus cerasi*	AF174318[1]	AJ009346[2]	AJ400599[5]
Adialytus salicaphis (Fitch, 1855)	*Chaitophorus leucomelas*	AF174329[1]	AJ009319[2]	AJ400596[5]
Lysiphlebus cardui (Starý)	*Aphis fabae*	AF174319[1]	AJ009330[2]	AJ400597[5]
Lysiphlebus fabarum (Marshall, 1896)	*Aphis urticata*	AF174321[1]	AJ009332[2]	AJ400594[5]
Lysiphlebus testaceipes (Cresson, 1880)	*Aphis gossypii*	AF174323[1]	AJ009335[2]	AJ400595[5]
Xenostigmus bifasciatus (Ashmead, 1891)	*Cinara* sp.	—	AJ009353[2]	AJ400607 [5]
Outgroup				
Jarra maculipennis Marsh & Austin, 1994		AF003485[6]	AJ307459[4]	—
Mesostoa kerri Austin & Wharton		AF003490[3]	AJ307460[4]	—
Schizoprymnus sp.		AF176060[7]	AJ307463[4]	—

[1]Sequences from Kambhampati et al., 2000; [2]Sequences from Sanchis et al., 2000; [3]Sequences from Dowton et al., 1998; [4]Sequences from Belshaw & Quicke, 2002; [5]Sequences from Sanchis et al., in prep.; [6]Sequences from Whitfield, 2002; [7]Sequences from Belshaw et al., 2000; [8]Sequences from Chen et al., 2002; " — " means sequence data not available.

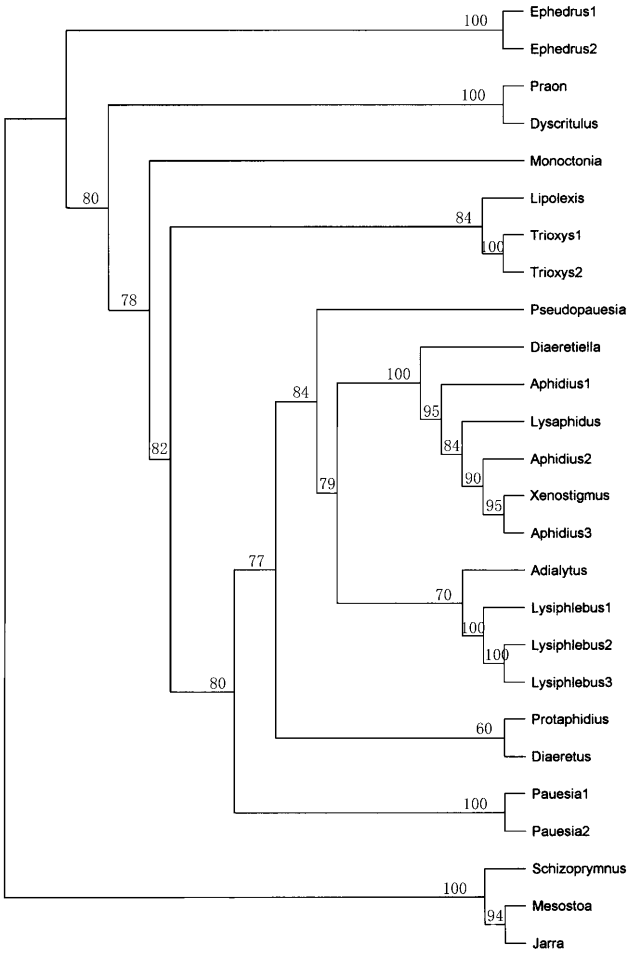

Fig. 1. Phylogeny of the Aphidiinae based on 3 genes using the NJ method (PAUP*). *Jarra*, *Mesostoa* and *Schizoprymnus* were used as outgroups. Numbers at nodes are bootstrap values (%).

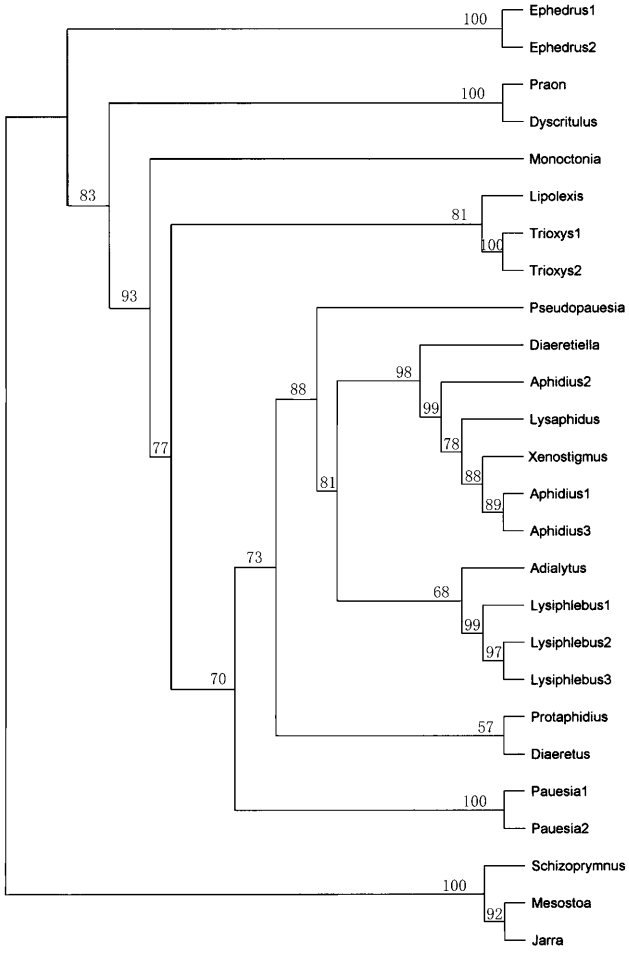

Fig. 2. Phylogeny of the Aphidiinae based on 3 genes using the MP method (PAUP*). *Jarra*, *Mesostoa* and *Schizoprymnus* were used as outgroups. Numbers at nodes are bootstrap values (%).

of random addition sequences and TBR option for branch swapping followed by additional rounds of branch swapping on the resulting trees with restriction on the number of trees to one. Each base was treated as an unordered character of equal weight, with gaps treated as missing data. Where more than one most parsimonious tree was found, a strict consensus tree was calculated. Downweighting transitions or treating gaps as a fifth base did not markedly affect the results. Statistical support for each node was evaluated by bootstrap analysis (Felsemstein, 1985) with 1000 replications. Second, a distance-based method based on the neighbor-joining algorithm (NJ) with Tamura-Nei correction (Saitou & Nei, 1987; Tamura & Nei, 1993) was used for obtaining a minimum-evolution tree and bootstrapping evaluation of each node was performed as above. Third, maximum likelihood (ML) trees were generated under the HKY85 model, using base frequencies estimated by PAUP, default number of substitution type (2, HKY85 variant) and transition/transversion ratio (2). Heuristic search were used with 100 replicates of random addition sequence and TBR branch swapping. Bootstrap analysis was performed with 100 replicates. The Bayesian approach to phylogenetic reconstruction (Yang & Rannala, 1997; Huelsenbeck et al., 2001) was implemented using MRBAYES 3.0B4 (Huelsenbeck & Ronquist, 2001). Each run was performed using default staring parameters and comprised 5 000 000 generations. Bayesian posterior probabilities (P_{bay}) were calculated from majority-rule consensus of trees sampled every 100 generations once the Markov chain reached stationary (determined by empirical checking of likelihood values).

RESULTS AND DISCUSSION

We tested alignment using the Clustal X program with different gap opening and gap extension values, and resulted in different length of aligned sequences. This result is identical with that of Morrison & Ellis (1997). They conclude that the multiple alignments, using different procedures, vary greatly in length and those produced using the Clustal W program with different gap weights are at least as different from each other as those produced by different alignment algorithms (Morrison & Ellis, 1997). Because the default parameters in version 1.81 (gap opening 15, gap extension 6.66) were optimized using the balibase multiple alignment in the 142 alignment test in balibase (J. Thompson, pers. comm.), we used the alignments with default parameters for the analysis presented here.

Because using several genes generally improves phylogenetic accuracy (Remsen & DeSalle, 1998), we com-

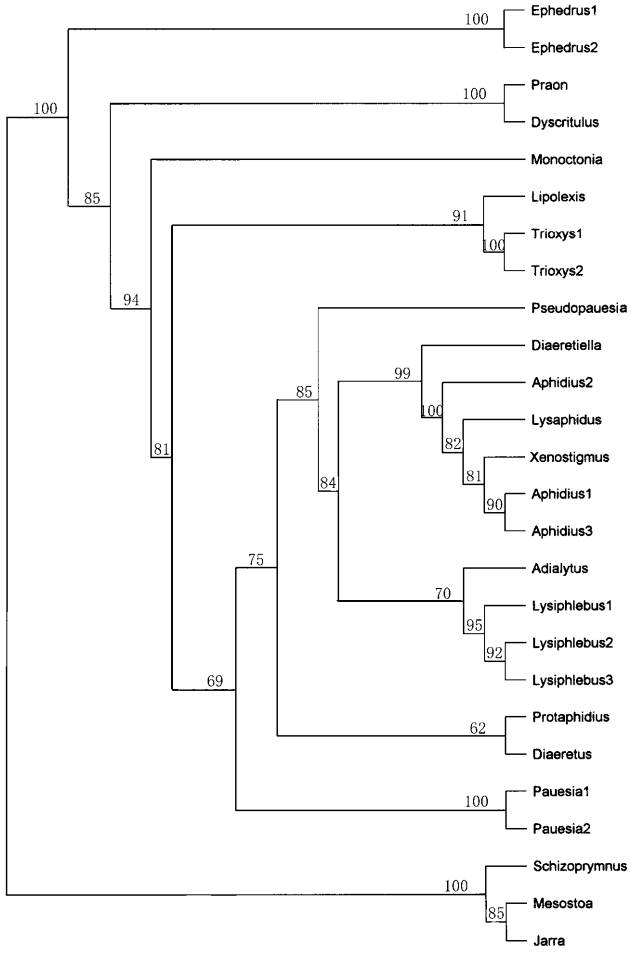

Fig. 3. Phylogeny of the Aphidiinae based on 3 genes using the ML method (PAUP*). *Jarra*, *Mesostoa* and *Schizoprymnus* were used as outgroups. Numbers at nodes are bootstrap values (%).

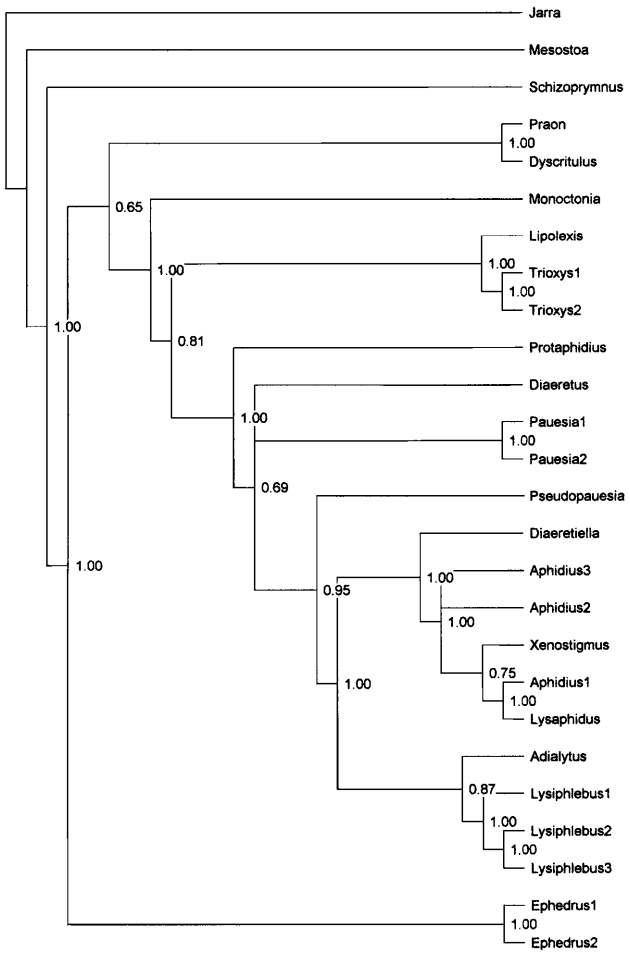

Fig. 4. Phylogeny of the Aphidiinae based on 3 genes using MrBayes. *Jarra*, *Mesostoa* and *Schizoprymnus* were used as outgroups. Numbers at nodes are Bayesian posterior probabilities.

bined sequence data of the 18S rDNA, 16S rRNA and ATPase 6 genes, giving 2775 characters in total, including gaps. Of these 2775 characters, 929 (33.5%) were variable and 422 (15.2%) were parsimony informative. Regarding the base composition, the overall GC content of 16S rRNA is 17.65%, ranging from 16.63% to 18.78%; that of 18S rDNA is 47.59%, ranging from 45.77% to 50.11%; and of ATPase 6 is 17.08%, ranging from 14.10% to 23.56%.

The trees resulting from PAUP* and MrBayes analyses are presented in Figs 1–4. We also show the bootstrap values and Bayesian posterior probabilities obtained from the identical analysis.

All the trees generated from the molecular data using different analyses and three taxa as outgroups support the monophyletic nature of the Aphidiinae and indicate that the subfamily Aphidiinae is a natural group as suggested by previous studies (Mackauer, 1961; Mackauer & Starý, 1967; Tremblay, 1967; Tremblay & Clavert, 1971; Chou, 1984; Gärdenfors, 1986; Quiche & van Achterberg, 1990, 1992; Whitfield, 1992; Belshaw & Quicke, 1997; Smith et al., 1999; Kambhampati et al., 2000). Although only three different genera were used as outgroups, it might indicate from Fig. 4 that the taxonomic status of Aphidiinae within Braconidae is probably closer to non-cyclostome (Helconid-complex) than cyclestome subfamilies.

The topology of all trees inferred from molecular data using different methods was similar. They confirmed the existence of two of the four traditionally accepted tribes, Ephedrini and Praini, but questioned the existence of the Trioxini and Aphidiini s. str. Our analyses support the three-tribe hypothesis: ((Ephedini + Praini) + Aphidiini s. lat.), as do the results of Smith et al. (1999) and Sanchis et al. (2000). Because our analyses support the monophyletic nature of the tribe Aphidiini s. lat. (tribal defintion of three-tribe system) we do not accept the classification system of five tribes proposed by Sanchis et al. (2000) and merge the two tribes, Trioxini and Aphidiini s. str., into one tribe – Aphidiini s. lat.

As shown in the figures the clade Praini seems to be the sister group of the Aphidiini, with the Ephedrini occupying the basal position, which is supported by the results of Belshaw & Quicke (1997) and Sanchis et al. (2000) based on molecular data, and Mackauer (1961) and Gärdenfors (1986) based on adult morphology, but not by

those of Dowton et al. (1998), Smith et al. (1999) and Kambhampati et al. (2000). Belshaw & Quicke (1997), using sequence data from three genes (elongation factor-1α, cytochrome b and the second expansion segment of the 28S rRNA), suggested the following tribal relationships: (Ephedrini + (Praini + (Aphidiini + Trioxini))), while Sanchis et al. (2000), using only the 18S rDNA gene, established the basal position of the tribe Ephedrini within the subfamily.

Kambhampati et al. (2000) considered that the basal lineage of Aphidiinae was *Aclitus* (Aclitini) and that the Praini was basal relative to Ephedrini based on the sequence data of the 16S rRNA gene alone while similar topologies were inferred from a combined analysis that included DNA sequences of 16S rRNA, NADH1 dehydrogenase and 28S rRNA (*Aclitus* was not included), resulting in that Praini was basal. At the same time, they also pointed out that *Aclitus* possesses both presumed synapomorphic and plesiomorphic characters with Aphidiina and Trioxina on the one hand, and its phylogenetic position is further complicated by the presence of characters that are related to its parasitization of root-feeding aphids on the other hand (Takada & Shiga, 1974). Therefore, the proposed basal position of *Aclitus* requires further study (Kambhampati et al., 2000).

The tribe Aphidiini s. lat., can be further subdivided into three subtribes, Monoctonina (containing the genus *Monoctonia*), Trioxina and Aphidiina, with the genus *Monoctonia* occupying a basal clade within the tribe, as proposed by Sanchis et al. (2000). Within the tribe *Xenostigmus* has a close relationship with *Aphidius* (Figs 1–4) while it was closer to *Protaphidius* in the trees produced by Sanchis et al. (2000), suggesting that *Xenostigmus* might be a member of the subtribe Aphidiina, and not the Protaphidina as defined by Sanchis et al. (2000). Our results also indicate that *Aphidius* is a paraphyletic group, as suggested by Smith et al. (1999) and Sanchis et al. (2000), but the nature of this genus needs to be validated.

ACKNOWLEDGMENTS. We sincerely thank J.B. Whitfield (Urbana, USA) and C. van Achterberg (Leiden, the Netherlands) for reviewing an early draft of the manuscript and valuable comments. We also thank three anonymous referees for their invaluable comments. The project was partly supported by National Natural Science Foundation of China (NSFC, No. 30170120) and an award by the Teaching and Research Award Program for Outstanding Young Teachers in Higher Education Institutions of MOE, China (TRAPOYT) to the second author.

REFERENCES

Belshaw R. & Quicke D.L.J. 1997: A molecular phylogeny of Aphidiinae (Hymenoptera: Braconidae). *Mol. Phylogenet. Evol.* **5**: 423–428.

Belshaw R. & Quicke D.L.J. 2002: Robustness of ancestral state estimates: evolution of life history strategy in ichneumonid parasitoids. *Syst. Biol.* **51**: 450–477.

Belshaw R., Dowton M., Quicke D.L.J. & Austin A.D. 2000: Estimating ancestral geographical distributions: a Gondwanan origin for Aphid parasitoids? *Proc. R. Soc. Lond. (B)* **267**: 491–496.

Carver M. 1989: Biological control of Aphids. In Minks A.K. & Harrewijn P. (eds): *Aphids, their Biology, Natural Enemies and Control*. *Vol. 2B.* Elsevier, Amsterdam, pp. 171–184.

Chen Y., Giles K.L. & Greenstone M.H. 2002: Molecular Evidence for a species complex in the genus Aphelinus (Hymenoptera: Aphelinidae), with additional data on Aphidiine phylogeny (Hymenoptera: Braconidae). *Ann. Entomol. Soc. Am.* **95**: 29–34.

Chou L.-Y. 1984: The phylogeny of Aphidiidae (Hymenoptera). *J. Agric. Res. (China)* **33**: 97–177.

Dowton M., Austin A.D. & Antolin M.F. 1998: Evolutionary relationships among the Braconidae (Hymenoptera: Ichneumonoidea) inferred from partial 16S rDNA gene sequences. *Insect Mol. Biol.* **7**: 129–150.

Edson K.M. & Vinson S.B. 1979: A comparative morphology of the vemon apparatus of the female braconids (Hymenoptera: Braconidae). *Can. Entomol*. **111**: 1013–1024.

Felsemstein J. 1985: Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783–791.

Finlayson T. 1990: The systematics and taxonomy of final-instar larvae of the family Aphidiinae (Hymenoptera). *Mem. Entomol. Soc. Can.* **152**: 3–74.

Gärdenfors U. 1986: Taxonomic and biological revision of palearctic Ephedrus Haliday (Hymenoptera: Braconidae, Ahpidiinae). *Entomol. Scand. (Suppl.)* **27**: 1–95.

Hillis D.M., Moritz C. & Mable B.K. 1996: *Molecular Systematic. 2nd ed.* Sinauer, Sunderland, MA.

Huelsenbeck J.P. & Ronquist F. 2001: MR BAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.

Huelsenbeck J.P., Ronquist F., Nielsen R. & Bollback J.P. 2001: Bayesian inference of phylogeny and its impact on evolutionary biology. *Science* **294**: 2310–2314.

Kambhampati S., Voelkl W. & Mackauer M. 2000: Phylogenetic relationships among genera of Aphidiinae (Hymenoptera: Braconidae) based on DNA sequence of the mitochondrial 16S rRNA gene. *Syst. Entomol*. **25**: 437–445.

Mackauer M. 1961: Die Gattungen der Familie Aphidiidae und ihre verwandtschaftliche Zuordnung (Hymenoptera: Ichneumonoidea). *Beitr. Entomol.* **11**: 792–803.

Mackauer M. & Starý P. 1967: World Aphidiidae (Hymenoptera: Ichneumonoidea). Le Francois, Paris, 195 pp.

Morrison D.A. & Ellis J.T. 1997: Effects of nucleotide sequence alignment on phylogeny estimation: a case study of 18S rDNA of apicomplexa. *Mol. Boil. Evol.* **14**: 428–441.

Quicke D.L.J. & Van Achterberg C. 1990: Phylogeny of the subfamilies of the family Braconidae (Hymenoptera: Ichneumonoidea). *Zool. Verhand.* **258**: 1–95.

Quicke D.L.J. & Van Achterberg C. 1992: Phylogeny of the subfamilies of the family Braconidae: a reassessed. *Cladistics* **8**: 237–264.

Remsen J.R. & DeSalle R. 1998: Character congruence of multiple data partitions and the origin of Hawaiian Drosophilidae. *Mol. Phylogenet. Evol.* **9**: 225–235.

Saitou N. & Nei M. 1987: The neighbor-joining method: a new method for reconstructing phylogenetic tree. *Mol. Phylogenet. Evol.* **4**: 406–425

Sanchis A., Latorre A., Gonzalez-Candelas F. & Michelena J. M. 2000: An 18S rDNA-based molecular phylogeny of Aphidiinae (Hymenoptera: Braconidae). *Mol. Phylogenet. Evol.* **2**: 180–194.

Smith P., Kambhampati S., Völkl W. & Mackauer M. 1999: A phylogeny of aphid parasitoids (Hymenoptera: Braconidae: Aphidiinae) inferred from mitochondrial NADH 1 dehydrogenase gene sequence. *Mol. Phylogenet. Evol.* **11**: 236–245.

Starý P. 1970: *Biology of Aphid Parasites (Hymenoptera: Aphidiidae) with Respect to Integrated Control. Series Entomologica 6.* Dr. W. Junk, The Hague, 643 pp.

Starý P. 1988: Aphidiidae. In Minks A.K. & Harrewijn P. (eds): *Aphids, their Biology, Natural Enemies and Control. Vol. 2B.* Elsevier, Amsterdam, pp. 171–184.

Swofford D.L. 2001: PAUP*: Phylogenetic Anallysis Using Parsimony (* and Other Methods). Sinauer Associates, Sunderland, MA.

Tamura K. & Nei M. 1993: Estimation of the number of nucleotide substitution in the control region of mitochrondrial DNA in humans and chimpanzees. *Mol. Phylogenet. Evol.* **10**: 512–526.

Takada H. & Shiga M. 1974: Description of a new species and notes on the systematic position of the genus Aclitus (Hymenoptera: Aphidiindae). *Kontyu* **42**: 283–292.

Thompson J.D., Gibson T.J., Plewniak F., Jeanmougin F. & Higgins D.G. 1997: The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucl. Acids Res.* **24**: 4876–4882.

Tobias V.I. 1967: A review of the classification, phylogeny and evolution of the family Braconidae (Hymenoptera). *Entomol. Rev.* **46**: 387–399.

Tremblay E. 1967: Sulla posizione sistematica degli Aphidiinae (Hymenoptera: Braconidae). *Boll. Lab. Entomol. Agr. Filippo Silvestri* **25**: 93–108.

Tremblay E. & Calvert D. 1971: Embryosystematics in the Aphidiinae (Hymenoptera: Braconidae). *Boll. Lab. Entomol. Agr. Filippo Silvestri* **29**: 223–249.

Wharton R.A., Shaw S.R., Sharkey M.J., Wahl D.B., Woolley J.B, Whitfield J.B., Marsh P.M. & Johnson J.W. 1992: Phylogeny of the subfamilies of the family Braconidae (Hymenoptera: Ichneumonoidea): a reassessment. *Cladistics* **8**: 199–235.

Whitfield J.B. 1992: The polyphyletic origin of endoparasitism in the cyclostome lineages of Braconidae (Hymenoptera). *Syst. Entomol.* **17**: 273–286.

Whitfield J.B. 2002: Estimating the age of the plydnavirus/Braconid wasp symbiosis. *Proc. Nat. Acad. Sci.* **99**: 7508–7513.

Yang Z. & Rannala B. 1997: Bayesian phylogenetic inference using DNA sequence: a Markov chain Monte Carlo method. *Mol. Biol. Evol.* **14**: 717–724.

Received October 22, 2004; revised and accepted December 20, 2004