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Phylogenetic relationships among the Braconidae (Hymenoptera: Ichneumonoidea) inferred from partial 16S rDNA, 28S rDNA D2, 18S rDNA gene sequences and morphological characters

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Abstract

Phylogenetic relationships among the Braconidae were examined using homologous 16S rDNA, 28S rDNA D2 region, and 18S rDNA gene sequences and morphological data using both PAUP* 4.0 and MRBAYES 3.0B4 from 88 in-group taxa representing 35 subfamilies. The monophyletic nature of almost all subfamilies, of which multiple representatives are present in this study, is well-supported except for two subfamilies, Cenocoelinae and Neoneurinae that should probably be treated as tribal rank taxa in the subfamily Euphorinae. The topology of the trees generated in the present study supported the existence of three large generally accepted lineage or groupings of subfamilies: two main entirely endoparasitic lineages of this family, referred to as the "helconoid complex" and the "microgastroid complex," and the third "the cyclostome." The Aphidiinae was recovered as a member of the non-cyclostomes, probably a sister group of Euphorinae or Euphorinae-complex. The basal position of the microgastroid complex among the non-cyclostomes has been found in all our analyses. The cyclostomes were resolved as a monophyletic group in all analyses if two putatively misplaced groups (*Mesostoa* and *Aspilodemon*) were excluded from them. Certain well-supported relationships evident in this family from the previous analyses were recovered, such as a sister-group relationships of Alysiinae + Opiinae, of Braconinae + Doryctinae, and a close relationship between Macrocentrinae, Xiphozelinae, Homolobinae, and Charmontinae. The relationships of "Ichneutinae + ((Adeliinae + Cheloninae) + (Miracinae + (Cardiochilinae + Microgastrinae)))" was confirmed within the microgastroid complex. The position of Acampsohelconinae, Blacinae, and Trachypetinae is problematic.

Keywords: Braconidae; Phylogenetic relationships; 28S rDNA; 16S rDNA; 18S rDNA; Morphology

1. Introduction

The Braconidae is a very large family of parasitic wasps with about 17,500 valid described species worldwide (TAXAPAD-database; data kindly supplied by Dr. D.S. Yu, Vancouver) and at least five times as many remain to be described. The species are currently classified into about 40+ subfamilies (van Achterberg, 1993). The precise number accepted by braconid workers has not yet stabilized but application of cladistic methodology in recent years has led to the creation of a number of additional subfamilies. Generally, the family shows significant specificity in host relationships at the subfamily level. For example, the Microgastrinae parasitize only lepidopteran larvae (with the exception of one species being a parasitoid of Trichoptera; van Achterberg, 2002), the Helconinae attack coleopteran larvae, the Alysiinae and Opiinae attack cyclorraphous dipteran larvae, whereas the Aphidiinae parasitize aphids. Thus, the Braconidae represent an important model system to examine the evolution of parasitoid lifestyles (Gauld, 1988; Whitfield, 1992).

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The relationships between the braconid subfamilies have been the subject of considerable discussion over past couple of decades (van Achterberg, 1976, 1984, 1993; Čapek, 1970; Fischer, 1972; Quicke and van Achterberg, 1990; Tobias, 1967), but few firm conclusions have been reached, though it has been generally accepted there are two major groupings of subfamilies, "cyclostomes" and relatives which are predominantly idiobiont ectoparasitoids and the remainder consisting of koinobiont endoparasitoids (van Achterberg, 1984; Askew and Shaw, 1986; Gauld, 1988). However, phylogenetic relationships within the family are controversial, with the most comprehensive morphological study (Quicke and van Achterberg, 1990) criticized by a number of workers (Wharton et al., 1992; but see van Achterberg and Quicke, 1992). Because morphology-based phylogenies often suffer from problems associated with reductional synapomorphies, it is difficult to determine whether lost structures are due to homologous or convergent events (van Achterberg, 1988; Gibson, 1985). So, molecular data have recently been employed to describe subfamily relationships within the family or generic relationships within subfamilies (Belshaw and Quicke, 1997; Chen et al., 2003; Gimeno et al., 1997; Li et al., 2003; Whitfield et al., 2002). Meanwhile, some other researchers think using a limited set of molecular data alone will not well reconstruct the phylogenetic relationship within Braconidae or other groups, therefore, they suggested that it is better to combine molecular data and morphological characters or other traits to analyze the phylogenetic relationships (Will and Rubinoff, 2004). Belshaw and Quicke (2002) used partial 28S rDNA (2–10 variable regions) and partial 18S rDNA gene sequences, combining lifestyle traits of different parasitic wasps, to estimate the phylogeny of Braconidae. Whitfield et al. (2002) combined 16S, COI, and 28S genes and morphology to reconstruct phylogenetic relationships among Microgastrinae. Dowton et al. (2002) investigated the phylogeny of the Braconidae, employing 16S and 28S rDNA gene fragments together with a suite of morphological characters, recovering the Aphidiinae as sister group to the cyclostomes and the Ichneutinae as sister group to the microgastroids. However, the phylogeny of the Braconidae is far from resolved. For a better understanding of the phylogenetic relationships among the Braconidae should be based on more analyses and of more genes. The purpose of the present study is to examine historical relationships among the Braconidae using both molecular and morphological data. Both PAUP* 4.0 and MRBAYES 3.0B4 were performed to generate phylogenetic trees. Three genes are chosen: mitochondrial 16S rDNA coding the large subunit of the mitochondrial ribosome, nuclear 28S rDNA D2 coding the second expansion segment of the nuclear ribosome subunit and ribosomal 18S rDNA partial gene sequence. These three genes have been extensively used in phylogenetic analysis within Hymenoptera, at both lower and higher taxonomic level. We combined 96 characters about adult external morphology and other traits of larval male and female reproductive systems.

2. Materials and methods

2.1. Sampling of taxa

We examined more than 100 species belonging to 88 genera in 35 subfamilies in this study. The species are listed in Table 1. The subfamily arrangement largely follows van Achterberg (1993) but a modified system is used based on morphological and biological characters. Both Acampsohelconinae and Hydragneocolinae are treated as independent subfamilies in this paper.

2.2. Laboratory protocols

We extracted DNA from single specimens preserved in 100% ethanol. Legs were removed from larger wasps (>5 mm long) and used for exaction whereas the abdomen and thorax were used for smaller wasps (<5 mm long). Ethanol was then removed by washing 3 times (15 min each) in 10 mM Tris–HCl (pH 8) containing 100 mM NaCl and 1 mM $MgCl_2$. Tissue was then ground in 400 μl of 10 mM Tris–HCl (pH 8), 10 mM EDTA, 1% SDS, and then added 100 μg (or 10 μl) proteinase K and incubated at 37 °C for 1 h. The homogenate was extracted with phenol/chloroform/isoamyl alcohol (25:24:1). DNA was resuspended in 60 μl TE buffer and stored at −20 °C. Double-strand PCR products were amplified in an Eppendorf Mastercycler gradient (Eppendorf AG, Hamburg), using 35 cycles [first denaturation, 4 min at 94 °C (denaturation, 1 min at 94 °C; annealing, 1.5 min at 55 °C; and elongation, 1.5 min at 72 °C) × 35; final elongation, 8 min at 72 °C]. The partial ribosomal 18S rDNA was amplified using the 18S up1 (5′-TGG TTG ATC CTG CCA GTA G-3′) and 18S 58-3 (5′-GAG TCT CGT TCG TTA TCG GA-3′) primers (Sanchis et al., 2000).

2.3. Sequence data and alignment

New sequences in this paper have been deposited in GenBank under accession numbers from AY920270 to AY920277. Most other sequences of three genes in this paper were retrieved from GenBank with accession numbers and references listed in Table 1. Two members of the Ichneumonidae (*Venturia canescens* and *Xorides praecatorius*) were included as outgroups. The Ichneumonidae is widely recognized as the sister group to the Braconidae (Belshaw and Quicke, 2002; Sharkey and Wahl, 1992). Before alignment some regions were removed manually because they were difficult to align.