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SCIENCE DIRECT
Biological Control 37 (2006) 131–140
Biological Control
www.elsevier.com/locate/ybcon

Phylogenetic study of *Diadegma* species (Hymenoptera: Ichneumonidae) inferred from analysis of mitochondrial and nuclear DNA sequences

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Received 9 March 2005; accepted 12 January 2006
Available online 3 March 2006

Abstract

The genus *Diadegma* consists of a large group of parasitoid wasps with a confusing taxonomy based on morphological characters. No phylogenetic studies on this genus or any of its species have been published so far. We examined DNA variations in a 498 bp segment of the *cytochrome oxidase* I gene (COI) of mitochondrial DNA (mtDNA) and in the internal transcribed spacer 2 (ITS2) of ribosomal DNA (rDNA) and compared nine different *Diadegma* species (i.e., *D. semiclausum*, *D. insulare*, *D. fenestrale*, *D. mollipla*, *D. rapi.*, *D. leontiniae*, *D. chrysostictos*, *D. armillata*, *D. blackburni*) from various geographical origins. Interspecific variation ranged from 3.9 to 16.8% in the COI region and from 5.4 to 32.5% in the ITS2. Phylogenetic relationships were constructed on maximum parsimony and maximum likelihood trees for each marker. Likelihood ratio tests were used to find the best evolutionary model fitting each of the data sets. The cladistic analysis of both data sets yielded different tree topologies. The sequences of the COI gene provided very little resolution of relationships among species whereas the nuclear DNA sequence data (ITS2 region) seems to be phylogenetically more valuable.

Keywords: *Diadegma*; Parasitoid populations; Biological control; *Cytochrome oxidase* I; Mitochondrial DNA; Internal transcribed spacer 2; Ribosomal DNA; Phylogeny

1. Introduction

The genus *Diadegma* (Hymenoptera: Ichneumonidae, Campopleginae) represents a large group of parasitoid wasps with 201 species known to occur worldwide (Yu and Horstmann, 1997). *Diadegma* species are present in all major biogeographic regions with the majority (131 species) having a palearctic and 33 species a nearctic distribution. Twelve *Diadegma* species are known to occur in more than one geographical region. At least one of the twelve *Diadegma* species (*D. semiclausum* (Hellén)) was introduced by man as biological control agent into other geographical areas (Talekar and Shelton, 1993) whereas *D. blackburni* Cameron might have been introduced accidentally to Hawaii (Henneman and Memmott, 2001).

Adult *Diadegma* females parasitize larvae of various lepidopteran species. The host range can be restricted to a few species such as in *D. semiclausum* that is known to parasitize Lepidoptera of the family Plutellidae (*Plutella xylostella* Linnaeus, *Prays oleae* Bernard and *Prays citri* Millière). However, host range can also be as wide as in *D. blackburni*, where several species from eight different families (Crambidae, Gelechiidae, Geometridae, Oecophoridae, Pterophoridae, Pyralidae, Scythrididae, Tortricidae) and the superfamily Tineoidea are known as suitable hosts (Banko et al., 2002; Perkins, 1913; Zimmerman, 1978). Among the potential hosts of *Diadegma* species, the diamondback moth, *P. xylostella*, is regarded as one of the most destructive pests of cruciferous crops. Accordingly, some parasitoid species, in particular *D. insulare* and *D. semiclausum* have gained economic importance as biological control agents of *P. xylostella* and are therefore the best known and well-examined species of the genus *Diadegma*. The importance of *Diadegma* species as biological control

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doi:10.1016/j.biocontrol.2006.01.004

agents has increased interest in their taxonomy and phylogeny. The current understanding of the taxonomic relationships in this genus is based on morphological characters (i.e., dorsal view of the head, propodeum), a technique that requires expertise and time. Furthermore, diagnostic characters are very variable in the subfamily of the Campopleginae. Thus, many misidentifications have been perpetuated in the literature of this genus (Azidah et al., 2000; Fitton and Walker, 1992). To provide an example for the unclear taxonomy, in the catalogue of Yu and Horstmann (1997) *D. niponica* Kusigemati (paleartic distribution), *D. varuna* Gupta (oriental distribution), and *D. fenestrale* Holmgren (paleartic, oriental, and oceanic distribution), are listed as three different species, whereas Azidah et al. (2000) considered all three species to be *D. fenestrale*. *Diadegma niponica* Kusigemati syn. nov. and *D. varuna* Gupta syn. nov. are now regarded as synonyms.

In addition, crossbreeding experiments are often applied to determine the status of a species. *Diadegma fenestrale* and *D. semiclausum* are occurring in the same geographical region and parasitize the same host. Hardy (1938) reported mating studies of both species with the F1 generation showing characters of both species. Therefore, additional studies are needed to confirm the existence of separate species.

Molecular studies of insects are becoming increasingly important in resolving taxonomic relationships (for review, see, Caterino et al., 2000). The most commonly analyzed regions for resolving genetic relationships within the species-rich insect group of the Hymenoptera have been the *cytochrome oxidase* subunits (COI and COII), the 16S ribosomal DNA, the cytochrome *b* from the mitochondrial DNA (mtDNA), as well as the internal transcribed spacers (ITS1 and ITS2) and the 28S region of the nuclear ribosomal DNA (rDNA) (e.g., Cameron et al., 1992; Campbell et al., 1993; Dowton and Austin, 2001; Mardulyn and Whitfield, 1999; Rokas et al., 2002; Whitfield and Cameron, 1998).

In the present study, we analyzed phylogenetic relationships within the genus *Diadegma* on the basis of sequence information of a fragment of the mitochondrial COI gene and the ITS2 of rDNA. These genes were chosen since they appear to have a different mode of evolution and transmission: the COI gene consists of highly conserved and variable regions (Lunt et al., 1996; Zhang and Hewitt, 1996), while the ITS2 of the rDNA is a noncoding and therefore rapidly evolving region. This region is easy to amplify in PCR because of being present in multiple copies. Each copy of an rDNA array evolves very similar to the other copies within individuals or species and low variation among rDNA arrays within individuals and/or species indicates that the multiple copies are homogenized, a process of concerted evolution (Dover, 1982). Nonetheless, the utility of ITS2 sequences as phylogenetic markers may be restricted due to high intraspecific variation (Harris and Crandall, 2000; Rich et al., 1997) or low interspecific variation (Kuperus and Chapco, 1994). However, this region has been successfully used in many genetic and phylogenetic studies of insect populations (e.g., Alvarez and Hoy, 2002; Clark et al., 2001; Gallego and Galián, 2001; Hackett et al., 2000).

2. Materials and methods

2.1. Insects and DNA extraction

Individuals from nine different species of the genus *Diadegma* were collected from various localities (Table 1). Prior to DNA extraction, specimens from East and Southern Africa were identified to species level using the morphological key provided by Azidah et al. (2000). Hosts for rearing *Diadegma* wasps were either *P. xylostella* (for *D. semiclausum*, *D. mollipla*, *D. fenestrale*, *D. insulare*, *D. leontiniae*, and *D. rapi*), *Cydia* spp. (Lepidoptera: Tortricidae) (for *D. blackburni*), *Yponomeuta cagnagellus* Hübner (Lepidoptera: Yponomeutidae) (for *D. armillata*) or *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) (for *D. chrysostictos*) (Table 1). The emerging *Diadegma* wasps were killed in 70–99.6% ethanol and sent to the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya, where they were stored at 4 °C for subsequent analysis. *Meloboris* sp. (Hymenoptera: Ichneumonidae, Campopleginae) from Ethiopia served as outgroup. Voucher specimens were deposited at ICIPE with exemption of *D. rapi*, where only one specimen was available for the present study.

Total genomic DNA was extracted using single *Diadegma* and *Meloboris* sp. specimens, applying a slightly modified protocol from Baruffi et al. (1995). Isolated DNA was resuspended in 20–30 μl TE buffer (10 mM Tris–HCl, 1 mM EDTA (pH 8.0)) and stored at −20 °C until further use. The specimens of *D. chrysostictos* were shipped as extracted DNA in TE buffer.

2.2. PCR amplification and sequencing

Two target DNA fragments were amplified with polymerase chain reaction (PCR), a fragment of the COI gene and the complete ribosomal ITS2.

Amplification of the COI gene fragment was performed in a 25 μl reaction mixture containing 10 mM Tris–HCl (pH 8.8), 50 mM KCl, 0.08% Nonidet P40, 1.5 mM $MgCl_2$, 15 pmol of each primer, 1.25 nmol of each dNTP, 1 μg of bovine serum albumin (BSA), 1 U of *Taq*-Polymerase (MBI Fermentas, St. Leon, Germany), and 1 μl of template DNA. The DNA fragments were amplified with the following primer combinations: (i) C1-J-1718 (5′-GGA GGA TTT GGA AAT TGA TTA GTT CC-3′) and C1-N-2329 (5′-ACT GTA AAT ATA TGA TGT GCT CA-3′) (Simon et al., 1994) or (ii) C1-J-1718 and P8 (5′-GCC AAT GGT TAA TAT TGC A-3′). For amplification of the COI fragment of *D. leontiniae* (*D. leontiniae*-B1) and *D. chrysostictos* (*D. chrysostictos*-UK13), a third primer combination was employed: (iii) C1-J-1718 and C1-N-2735 (5′-AAA AAT GTT GAG GGA AAA ATG TTA-3′) (Simon et al., 1994). Primer P8 was designed for *Diadegma* species and anneals to nucleotide 2612–2630 of the honeybee mitochondrial