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DNA barcoding species inventory of Microgastrinae wasps (Hymenoptera, Braconidae) from a Mexican tropical dry forest

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Abstract

The cosmopolitan Microgastrinae is probably the most diverse braconid subfamily of parasitoid wasps, yet its species diversity is far from being known. As part of a global initiative for DNA barcoding Microgastrinae species, here we show the results of a study that assessed the species richness of this subfamily in a Mexican tropical dry forest located in the Chamela region, near the Pacific coast of Jalisco. Barcoding sequences of a total of 551 microgastrine specimens were generated, corresponding to 238 haplotypes. Performance of two species delineation approaches, a 2% corrected pairwise distance criterion and the general mixed Yule-coalescent (GMYC) method, yielded 100 and 112 putative species, respectively, which belong to 13 genera. The species delimited by the above two approaches were mostly congruent with our morphospecies identification. Ten molecular operational taxonomic units (MOTUs) were split into twenty-two species by the GMYC approach. We found morphological differences between the GMYC species corresponding to three of these MOTUs. Thus, a total of 103 microgastrine species were confirmed for the region of study. Thirty-three species were only represented by males, and therefore, their generic assignment is only tentatively proposed. A new record for the country is provided for the *Diolcogaster-basimacula* species group. Based on a comparison of nearly 20 000 barcoding sequences released for Microgastrinae from 75 countries, only five microgastrine species from Chamela were found to occur in other countries, four in Costa Rica and one in Canada and the United States.

Keywords: COI, GYMC model, Mexico, Microgastrinae, parasitoid

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Introduction

Hymenopteran parasitoid wasps represent approximately 20% of all insect species on the planet, with Braconidae the second most diverse family behind Ichenumonidae (Quicke 1997). Microgastrinae is perhaps the most diverse braconid subfamily, having approximately 2000 species described worldwide (Yu *et al.* 2012), but with an estimated of 8–20 times this number (Rodriguez *et al.* 2012). Microgastrines are endoparasitoids of Lepidoptera larvae, generally being host specialists (Smith *et al.* 2008), and therefore playing an essential role in all the food chains where they occur. Moreover, because of their relatively narrow host specificity (i.e. usually one or few species of lepidopterans are parasitized by one wasp species), several microgastrine species have been successfully employed in biological control programmes against agricultural and forestry pests (Austin & Dangerfield 1992; Whitfield 1997). Despite this, the enormous diversity and extremely conservative morphology found in this subfamily makes its taxonomic study considerably difficult and hence restricted to few specialists (Smith *et al.* 2008).

In an effort to overcome the considerable taxonomic impediment in this megadiverse, taxonomically neglected insect taxon, a group of taxonomists from various countries have been collaborating with the generation and releasing in the Barcode of Life Data System (BOLD) of DNA barcodes of microgastrine species from various geographical regions. Since 2004 to date, this group has released more than 20 000 barcode sequences belonging

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to 50 microgastrine genera and more than 1 700 species from 75 countries (Smith *et al.* 2013). To date, however, only two barcoding studies devoted to microgastrine faunas from particular geographical regions have been published, one in Costa Rica (Smith *et al.* 2008) and the other one in Canada and Alaska (Fernández-Triana 2010). In particular, in one of these studies, 313 provisional species assigned to six microgastrine genera were discovered for the Área de Conservación Guanacaste (ACG) in north-western Costa Rica using DNA barcoding, morphological and natural history data (Smith *et al.* 2008). This pioneer study not only revealed the presence of a largely overlooked species richness of Microgastrinae for the above region, but also the likely existence of an extraordinary species richness of this subfamily along the Neotropics.

Here, we show the results of an intensive barcoding species inventory of microgastrine fauna from Chamela, a region located in the northernmost Neotropics, near the Pacific coast of Jalisco, Mexico, which comprises one of the most-well preserved tropical dry forests in the country (Noguera *et al.* 2002). This ecosystem in particular has received little attention in taxonomic studies of arthropods compared to other tropical ecosystems. Our aim was to assess the species richness of the subfamily in the above region, as well as to compare it with the results derived from the two barcoding studies performed for this group both in the Neotropical (Costa Rica) and Nearctic (USA/Canada) regions. A preliminary barcoding study carried out in Chamela for the braconid subfamily Doryctinae found a considerably high but largely unknown species richness, with various new generic records for the country (Zaldívar-Riverón *et al.* 2010). Similar to the latter work, we also report an extraordinary, mostly neglected species richness in Microgastrinae, thus highlighting the importance to carry out biodiversity inventories of this kind, which may help to unveil the species richness of highly diverse invertebrate taxa in a short period of time.

Materials and methods

Taxon sampling and laboratory procedure

A total of eight field trips were carried out from June 2009 to May 2011 to the Chamela Biological Station owned by the Instituto de Biología, Universidad Nacional Autónoma de México (UNAM). The station is located within the Chamela-Cuixmala biosphere reserve, near the Pacific coast of Jalisco, Mexico (Noguera *et al.* 2002). The Chamela region has two well-defined seasons, one rainy and one dry. It is mainly composed of tropical dry forest, though other types of vegetation are also present in the region, including patches of tropical subdeciduous forest (Noguera *et al.* 2002). A total of ten collecting sites were established within the station during both seasons, covering the above two types of vegetation and using Malaise, light and yellow pan traps, as well as sweeping nets. The collected material was preserved in absolute or 96% ethanol and kept at −20°C. Microgastrinae specimens were sorted out, identified to genus level using the relevant literature (Mason 1981; Whitfield 1997) and subsequently segregated to morphospecies.

A single hind leg was removed for each examined specimen and placed in a 96-well plate. Plates were subsequently sent to the Canadian Centre for DNA Barcoding, University of Guelph, Ontario, Canada, for DNA extraction, amplification and sequencing (see laboratory protocols in Smith *et al.* 2008). A 615–658-bp fragment belonging to the barcoding locus (cytochrome oxidase subunit I of the mitochondrial DNA gene; Hebert *et al.* 2003) was amplified using both the LepF1/LepR1 (Hebert *et al.* 2004) or the LCO1490/HCO2198 (Folmer *et al.* 1994) primers. Samples were sequenced in both directions and contigs were edited with Sequencher version 4.0.5 (Gene Codes) and aligned manually. Most of the sequences generated in this work have been already released and published elsewhere (Smith *et al.* 2013; GenBank accession nos JN281535-986). Newly generated sequences have the following GenBank accession nos: KC755270-365. All these sequences and their specimen information are available in the project file 'Microgastrinae from Chamela, Mexico' (MXBMC project) in the projects section of the Barcode of Life Data Systems (www.barcodinglife.org).

Species delineation analyses

Our delimitation of microgastrine species based on the barcoding locus was explored with two different approaches, the general mixed Yule-coalescent (GMYC) model (Pons *et al.* 2006; Fontaneto *et al.* 2007) and a 2% corrected pairwise distance criterion, which is widely employed for determination of molecular operational taxonomic units (MOTUs; Jones *et al.* 2011). The latter approach has proved to be a rapid, generally reliable tool for identification of species in most animal groups (Hebert *et al.* 2003; Hajibabaei *et al.* 2007). Its use, however, has been criticized due to its assumption that COI substitution rates vary uniformly over time in all animal taxa (Papadopoulou *et al.* 2010). The GMYC model on the other hand is an analytical, more rigorous approach that discriminates species as the most likely point of transition from coalescence to speciation branching patterns in an ultrametric phylogenetic tree (Pons *et al.* 2006; Fontaneto *et al.* 2007). This model has been recently employed with success to delimit species in highly diverse, poorly known insect taxa (Monaghan *et al.* 2009;

Ceccarelli *et al.* 2012). Nevertheless, the exclusive use of COI sequences has been shown to be problematic for the above two approaches in the case of recently diverging species (Burns *et al.* 2008; Ceccarelli *et al.* 2012).

Species delineation with the GMYC model was obtained generating an ultrametric phylogenetic tree with branch lengths scaled to time with BEAST version 1.7.4 (Drummond *et al.* 2012), using a relaxed lognormal clock, a coalescent prior, the GTR + Γ + I model of evolution (Lanave *et al.* 1984), and running the analysis for 10 million generations. Duplicated haplotypes were excised from the analyses with Collapse version 1.2 (Posada 2004) and one sequence belonging to other noncyclostome braconid subfamily (BOLD accession nos. MXBMC-0475) was employed to root the tree. We discarded the first 5000 sampled trees and employed the remaining trees to build a maximum clade credibility tree with posterior probability of clades with TreeAnnotator version 1.7.4 (part of the BEAST 1.7.4 package). The reconstructed tree was employed to delimit species using the GMYC model implemented in the SPLITS package (available from http://r-forge.r-project.org/projects/splits/) with the program R version 2.10.1 (R core Development Team 2009). Sequence divergences were obtained with the K2P distance model (Kimura 1980) and a neighbour-joining (NJ) tree was reconstructed using BOLD (www.boldsystems.org).

Results and discussion

Performance of species delineation approaches

We generated COI sequences belonging to 557 of 569 microgastrine specimens obtained after three years of collecting effort in the Chamela Biological Station. Of these sequences, three belong to the genus *Mirax* of Miracinae, which had previously been placed in Microgastrinae, and the remaining three to other braconid noncyclostome subfamilies whose specimens were originally misidentified as microgastrines. The 551 sequences assigned to Microgastrinae specimens corresponded to 238 haplotypes. A list with the identified genera and their number of species delimited both by the 2% corrected pairwise distance criterion and the GMYC method is given in Table 1. The ultrametric tree and phenogram reconstructed by the two methods showing their delimited species is shown in Appendix S1.

All the species delimited by the two DNA sequence-based approaches were congruent with our identified morphospecies. The GMYC approach yielded a total of 112 putative species, whereas 100 MOTUs were delimited using the 2% corrected pairwise distance criterion. This difference was due to 10 of the recovered MOTUs were split into twenty-two species by the GMYC approach. These cases involved seven microgastrine genera, including two MOTUs assigned to *Dolichogenidea* Viereck and three to *Apanteles* Foester, which appeared split into four and six 'GMYC' species, respectively. Moreover, one MOTU assigned to *Cotesia* Cameron, *Diolcogaster* Ashmead, *Microplitis* Foester and *Pseudapanteles* Ashmead and one MOTU assigned to *Glyptapanteles* Ashmead were split into two and four 'GMYC' species, respectively. A detailed external morphological examination of the taxa involved found consistent differences between the specimens belonging to GMYC species of *Cotesia*, *Pseudapanteles* and *Apanteles* that were merged in three of the above 10 MOTUs (*Cotesia* sp. Sff3a and Sff3b; *Apanteles* sp. Sff7a and Sff7b; *Pseudapanteles* sp. Sff4a and Sff4b; MXBMC project, BOLD). Therefore, 103 microgastrine species belonging to 13 genera are confirmed for the Chamela region based on both morphological and DNA sequence data. However, in the seven remaining cases of incongruence found between GMYC species and MOTUs, we did not observe external morphological differences that supported the species delineation recovered by the GMYC method.

The available keys to microgastrine genera (Mason 1981; Whitfield 1997) are mainly based on female features. Thirty-three of the species delimited by the two approaches were only represented by males, and therefore, their generic assignment could not be confirmed. However, we tentatively assigned a genus to these spe-

Table 1 List with the identified Microgastinae genera and their number of species delimited by the 2% corrected pairwise distance criterion (2% CPD) and the GMYC method

Genus	2% CPD	GYMC species
Apanteles Foerster	26	27
Cotesia Cameron	3	4
Diolcogaster Ashmead	5	6
Distatrix Mason	2	2
Dolichogenidea Viereck	1	1
Fornicia Brullé	1	1
Glyptapanteles Ashmead	2	5
Microplitis Foerster	3	4
Parapanteles Ashmead	1	1
Pholetesor Mason	1	1
Promicrogaster Brues & Richardson	2	2
Pseudapanteles Ashmead	6	6
Unconfirmed generic assignations		
Apanteles Foerster	16	18
Cotesia Cameron	1	1
Dolichogenidea Viereck	9	11
Glyptapanteles Ashmead	15	15
Pseudapanteles Ashmead	4	5
Promicrogaster Brues & Richardson	1	1
Rhygoplitis Mason	1	1
Total	100	112

cies based on the above keys, using comparative material and running BLAST searches to corroborate these identifications. The tentative generic assignations for these species are listed in Table 1.

This study provides the first record in the Mexican territory for the *Diolcogaster-basimacula* species group with an undescribed species. This species group had only previously been recorded for the Oriental and Afrotropical regions, although collection records show that is widespread in the Neotropics.

A comparison between the sequences examined here and the remaining ones released from Mexico and other 74 countries (nearly 20 000 sequences in total) revealed that only five MOTUs occurring in Chamela are shared with other countries. Four of these MOTUs also occur in Costa Rica (named as *Apanteles* Rodriguez142, *Apanteles* Rodriguez200, *Apanteles* Rodriguez24 and *Glyptapanteles* Whitfield111 in Smith *et al*.'s 2008 study) and one in Canada and the United States (*A. samarshalli* Fernández-Triana; Fernández-Triana 2010). The above four species found in Costa Rica were all collected in tropical dry forest, although the latter two were also collected in tropical rain forest. To date, records of Microgastrinae in the Mexican territory are considerably scarce, limited to only 41 described species (Coronado-Blanco 2011). Our results therefore not only substantially increased the taxonomic knowledge of this group in the country, but also have potential applications for further biological control and host–parasitoid association studies.

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J.L.F.T., A.Z.R. and S.F.F. conceived and designed the experiments. S.F.F., A.Z.R., J.L.F.T. and J.J.M. performed the experiments.

Data Accessibility

DNA Sequences: GenBank accession nos JN281535-986, KC755270-365. All sequences generated in this study are also available in the project file 'Microgastrinae from Chamela, Mexico' (MXBMC project), Barcode of Life Data System (www.barcodinglife.org).

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Trees derived from the two species delineation approaches based on the DNA barcoding sequences generated in this study. A) Neighbour-joining tree reconstructed with BOLD and 552 COI sequences using the K2P model of evolution. B) Ultrametric tree reconstructed with BEAST and 242 haplotype sequences, which was employed for performing the GMYC approach. 'GMYC' species are represented both by sequence clusters (in red) and singletons (black terminal branches).