

Prey identification in nests of the potter wasp *Hypodynerus andeus* (Packard) (Hymenoptera, Vespidae, Eumeninae) using DNA barcodes

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ABSTRACT. Prey identification in nests of the potter wasp *Hypodynerus andeus* (Packard) (Hymenoptera, Vespidae, Eumeninae) using DNA barcodes. Geometrid larvae are the only prey known for larvae of the Neotropical potter wasp *Hypodynerus andeus* (Packard, 1869) (Hymenoptera, Vespidae, Eumeninae) in the coastal valleys of the northern Chilean Atacama Desert. A fragment of the mitochondrial gene cytochrome oxidase c subunit 1 was amplified from geometrid larvae collected from cells of *H. andeus* in the Azapa Valley, Arica Province, and used to provide taxonomic identifications. Two species, *Iridopsis hausmanni* Vargas, 2007 and *Macaria mirthae* Vargas, Parra & Hausmann, 2005 were identified, while three others could be identified only at higher taxonomic levels, because the barcode reference library of geometrid moths is still incomplete for northern Chile.

KEYWORDS. Boarmiini; Cyclophorini; Geometridae; Insecta; Neotropical.

The animal DNA barcode is a short, standardized region of the cytochrome c oxidase subunit 1 (COI) gene proposed as the core of a DNA-based identification system (Hebert *et al.* 2003a). DNA barcoding provides a very useful tool for species identification and species delimitation (Hebert *et al.* 2003b, 2010; Dincă *et al.* 2011; Goldstein & DeSalle 2011; Teletchea 2010; Hausmann *et al.* 2011, 2013; Puillandre *et al.* 2011; Strutzenberger *et al.* 2011; Mutanen *et al.* 2012; Laiho & Ståhls 2013; Nagy *et al.* 2013), and is especially valuable for associating extreme forms, sexes and different stages of the life cycle of organisms (Miller *et al.* 2005; Hausmann & Parra 2009; Silva-Brandão *et al.* 2009; Jinbo *et al.* 2011; Li *et al.* 2011). DNA barcodes may be potentially useful for studying trophic links in complex field scenarios (Valentini *et al.* 2009), as in the case of webs involving host-parasitoid interactions, where many times host identification must be performed using immature stages (Hrcek *et al.* 2011).

Potter wasps (Hymenoptera, Vespidae, Eumeninae) are primarily solitary hunters, with some species having primitively social behavior (West-Eberhard 2005). Although nesting habits are variable among potter wasps, and various types of substrata may be used for nest construction, mud is broadly employed. Each nest is composed of one or more cells, into which the female lays one egg and a number of prey, which are eaten by the wasp larva after eclosion (*e.g.*, Matthews & González 2004; Matthews & Matthews 2009). Thus, a detailed knowledge of the prey spectrum used by the potter wasps is important to understand their field biology (Chiappa & Rojas 1991; Sears *et al.* 2001; Budriene 2003). This information may be useful in order to design ecological studies or to plan the conservation of these species.

The Neotropical potter wasp genus *Hypodynerus* de Saussure, 1855 is mostly associated with the Andes from Colombia to southern Chile (Willink 1970; Carpenter & Garcete-Barrett 2002; Garcete-Barrett & Hermes 2013). Although this genus is represented by thirty species in Chile (Barrera-Medina 2011), their nesting biology is poorly documented, and accurate prey identification at species level is not available (Claude-Joseph 1930; Pérez-D'Angello 1968, 1974, 1991; Méndez-Abarca *et al.* 2012).

Hypodynerus andeus (Packard, 1869) is known from Ecuador, Peru and Chile (Willink 1970). Larvae of the moth family Geometridae have been mentioned as the only prey for this potter wasp in the coastal valleys of the northern Chilean Atacama Desert (Méndez-Abarca *et al.* 2012). However, morphological identification of immature stages is rarely successful within Neotropical Lepidoptera, because their external morphology is still poorly known and often does not offer unambiguous differential features. Thus, the objective of this contribution is to identify prey larvae collected from nests of *H. andeus* using DNA barcodes.

MATERIAL AND METHODS

Specimen collection. Larvae were collected from three cells of *H. andeus* in January 2013 in the Azapa valley (18°13'14"S, 70°10'42"W), Arica Province, northern Chile. Each cell was taken from a different nest, with maximum distance among nests of about 30 m. The cells were not completely filled, as shown by the presence of a funnel-like structure surrounding the entrance orifice. This structure is generally removed when the cell is fully provisioned

(Méndez-Abarca *et al.* 2012). All the prey larvae were taken from the respective cell and preliminarily identified as morphotypes (M1 to M6) based on combinations of external characters, color and relative size (Table I). Additionally, two male adults of Geometridae were collected at the light on the same date and site, which were identified as *Iridopsis hausmanni* Vargas, 2007 (voucher: IDEA-002-03-20; GenBank accession number: KF663057; 616 bp) and *Macaria mirthae* Vargas, Parra & Hausmann, 2005 (voucher: IDEA-002-03-19; GenBank accession number: KF663056; 596 bp), based on morphological characters (Vargas 2007; Vargas *et al.* 2005).

Table I. Geometrid larvae collected and barcoded from cells of three nests of the Neotropical potter wasp *Hypodynerus andeus* (Packard) in the Azapa valley, northern Chilean Atacama Desert.

Morphotype	n	Nest	Voucher	Sequence length(bp)	GenBank Accession
M1	3	1	IDEA-002-03-2	658	KF663044
		1	IDEA-002-03-3	639	KF663045
		2	IDEA-002-03-14	658	KF663053
M2	5	1	IDEA-002-03-6	658	KF663046
		1	IDEA-002-03-7	581	KF663047
		1	IDEA-002-03-8	581	KF663048
		2	IDEA-002-03-11	658	KF663051
		2	IDEA-002-03-12	578	KF663052
M3	1	1	IDEA-002-03-9	658	KF663049
M4	1	1	IDEA-002-03-10	658	KF663050
M5	1	3	IDEA-002-03-15	658	KF663054
M6	1	3	IDEA-002-03-16	658	KF663055

DNA extraction and sequencing. The two adults and fourteen larvae were used for DNA extraction with the Insect DNA Kit (Omega bio-tek) according to the manufacturer's instructions. Subsequently, a fragment of the COI gene was amplified by polymerase chain reaction (PCR) with the primers LCO 1490 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO 2198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3', developed by Folmer *et al.* (1994). PCR reactions were performed in a final volume of 20 µl. Each reaction contained 1 µl of DNA extract, 10 pmoles of each primer, 2,5 mM of each dNTP, 2 mM MgCl₂, 1X PCR buffer (KCl), 1 unit of Taq DNA polymerase (Thermo Scientific) and sterile distilled water. Cycling conditions were: 5 min at 94°C; 35 cycles of 30 sec at 94°C; 30 sec at 55°C; 30 sec at 72°C and final elongation step of 2 min at 72°C. PCR blank reactions controls were incorporated. Three µl of each PCR product was visualized on 1.5% agarose gel stained with gel-red (Biotium). Reactions containing fragments of the expected size were directly sequenced by a commercial facility (Macrogen, South Korea).

Data analysis. Sequences were edited and aligned using MEGA 5.05 (Tamura *et al.* 2011). Following Hebert *et al.* (2003a), sequence divergences were calculated using the Kimura-2-parameter method and a neighbor-joining tree was constructed with MEGA 5.05 (Tamura *et al.* 2011) based on these values. A search with the BOLD v.3 Identification System (Ratnasingham & Hebert 2007) was performed for each

sequence in order to identify larvae that did not match adults sequenced in this study. Five sequences of the genera *Physocleora* Warren, 1897, *Pherotesia* Schaus, 1901, *Neodora* Warren, 1897, *Pantherodes* Guenée, [1858] and *Cyclophora* Hübner, 1822, which resulted as nearest 'neighbors' when blasting the larval sequences are deposited in the public data project GZPUV on BOLD database where they can easily be accessed and downloaded (specimen ID's BC ZSM Lep 03021, 18780, 57455, 59014, 59120, respectively).

RESULTS

Overall, 578–658 base pairs (bp) of COI fragments were sequenced from fourteen individuals, including two adults and twelve larvae (Table I). Genetic distance among individuals was 0–15.6%, suggesting that more than one species of geometrid was involved as prey of *H. andeus* among the samples. Six larval morphotypes (M1 to M6) were identified, among which only five DNA barcodes were recognized.

Six larvae (IDEA-002-03-6, IDEA-002-03-7, IDEA-002-03-8, IDEA-002-03-10, IDEA-002-03-11 and IDEA-002-03-12) shared their DNA barcode with the adult of *M. mirthae* (Fig. 1), at a genetic distance of 0.2–0.5%. Five of these larvae were previously assigned to M2, which was characterized by a light green coloration with a white lateral stripe, while the remaining one was the only representative of M4, characterized by a pale grey coloration without a lateral stripe. One larva (IDEA-002-03-15), which was the only representative of M5, matched exactly the barcode of *I. hausmanni* (Fig. 1; aligned fragment length 616 bp).

Three other unidentified geometrid species were detected in the sample (Fig. 1). One was represented by three larvae (IDEA-002-03-2, IDEA-002-03-3 and IDEA-002-03-14); all of these were previously assigned to M1, with no variation along a fragment of 639 bp length. Blasting that sequence on the BOLD database (with >20,000 sequences of Neotropical geometrids, belonging to >5,000 species covering almost all described genera) suggests it to be a member of the subfamily Ennominae. The nearest neighbor genera are from the tribe Boarmiini: *Physocleora* at 7.8% minimum distance (Kimura 2 parameter) and *Pherotesia* at 8.5% distance. Two other species were represented by one larva each: one (M6: IDEA-002-03-16) likely also belongs to Ennominae, nearest neighbor genera *Neodora* at a genetic distance of 4.7% and *Pantherodes* at a distance of 5.1%; the other one (M3: IDEA-002-03-9) to the Sterrhinae, tribe Cyclophorini, nearest neighbor *Cyclophora nanaria* (Walker, 1861) at a distance of 4.7%.

DISCUSSION

The only previous report of DNA barcodes of Chilean Geometridae was performed for 87 species from the southern zone of this country, in which host plant relationships were revealed for some species based on barcodes of larvae and adults (Hausmann & Parra 2009). This study reports the first DNA barcodes for the geometrid fauna from the north-

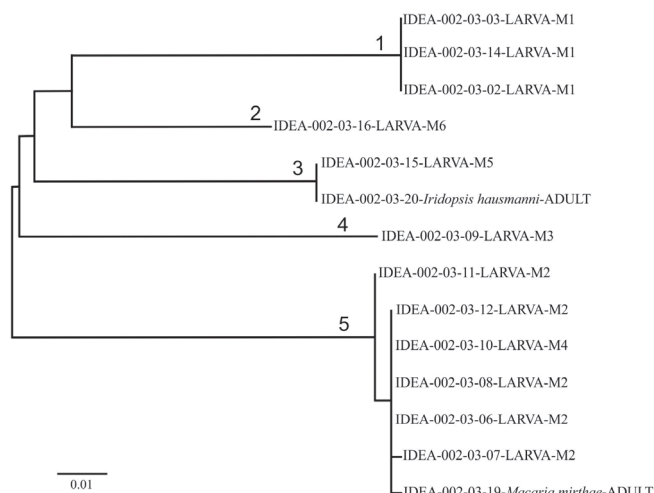


Fig. 1. Neighbor Joining tree of geometrid larvae collected in cells of *Hypodynerus andeus* wasps in the Azapa valley, Arica Province, northern Chile, and adults of *Iridopsis hausmanni* and *Macaria mirthae* from the same locality, based on 578 bp of the barcoding region (cytochrome oxidase c subunit I gene (5'), COI). Numbers above branches indicate species: (1) Ennominae, Boarmiini, nearest neighbor genus *Physocleora*; (2) Sterrhinae, Cyclophorini, nearest neighbor *Cyclophora nanaria*; (3) *I. hausmanni*; (4) Ennominae, nearest neighbor genus *Neodora*; (5) *M. mirthae*.

ern Chilean Atacama Desert. Specifically, DNA barcodes were employed here to explore the prey spectrum of a potter wasp.

The number of prey species detected in this study was five (Fig. 1), all Geometridae. This fact confirms the specialization of *H. andeus* females in hunting geometrid larvae, as previously reported for the same locality based on morphological evidence (Méndez-Abarca *et al.* 2012). Interestingly, a high proportion (50%) of the barcoded larvae matched with *M. mirthae*; however, four other species belonging to multiple genera were also detected in this study, suggesting that females of *H. andeus* are not prey specialist at species level. Even more, the capacity of the females of *H. andeus* for hunting more than one prey species could be an important factor underlying the presence of this potter wasp in many different environments in northern Chile, because the taxonomic composition and abundance levels of available prey must be different among places. Moreover, a significant variation in abundance levels of prey is also expected among seasons in some sites. Thus, identity and relative importance of each prey species in *H. andeus* nests should vary at geographic and temporal levels.

Although *M. mirthae* was already mentioned as a probable prey of *H. andeus* in this locality based on morphology (Méndez-Abarca *et al.* 2012), this is the first confirmation through DNA barcodes. The presence of *M. mirthae* in the cells may be related to the presence of some Fabaceae trees in the study site, which are used as hosts by the larvae of this species (Vargas *et al.* 2005). Similarly, one of the Anacardiaceae host plants used by *I. hausmanni* in the coastal valleys of the northern Chilean Atacama Desert was also present in the study site (Vargas 2014).

Macaria mirthae was represented by two morphotypes in the sample, which were clustered based on barcodes (Fig. 1); thus overestimation of the number of prey species was avoided by using barcodes in this study. Similarly, in other occasions geometrid larvae with the same two color patterns here reported for *M. mirthae* have been collected on *Acacia macracantha* Willd. in the same study site. The subsequent rearing of these larvae to adult stage has always revealed *M. mirthae* based on genitalia morphology (HAV, unpublished). Even more, it is known that identifications of geometrid larvae based only on external morphology may be unsatisfactory for two reasons: more than one larval morph may be present for each species (Bocaz *et al.* 2003; Vargas & Parra 2013), or external morphological attributes may not be variable among different species (Grehan *et al.* 1994).

The results here reported on the prey of *H. andeus* constitute one more example highlighting the usefulness of DNA barcoding as an effective tool to be used in field ecology studies. A more detailed DNA barcode study of northern Chilean Geometridae, together with a better documentation of their host plant relationships, is necessary in order to clarify the trophic ecology of this potter wasp in this geographic area.

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