

First Remarks on Genetic Variation of the Little Known Leaf Miner *Angelabella tecomae* Vargas & Parra (Gracillariidae) in the Atacama Desert of Northern Chile

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FIRST REMARKS ON GENETIC VARIATION OF THE LITTLE KNOWN LEAF MINER *ANGELABELLA TECOMAE* VARGAS & PARRA (GRACILLARIIDAE) IN THE ATACAMA DESERT OF NORTHERN CHILE

Additional key words: gene flow, micromoth, mitochondrial DNA, Oecophyllembiinae, population structure

Knowledge of population genetic variation is important to achieve an adequate understanding of the evolutionary history of species and to plan conservation practices for endangered species (Méndez et al. 2006, Harper et al. 2008, Morales et al. 2011, Brito et al. 2013b, Cianferoni et al. 2013, Baranzelli et al. 2014, Carson et al. 2014, Wickson et al. 2014, Martins et al. 2015). A useful method to study population genetic diversity in animals is the analysis of mitochondrial DNA (mtDNA) polymorphisms (Harper et al. 2008, Gonçalves et al. 2009, Morales et al. 2011, Silva-Brandão et al. 2011, Siti-Balkhis et al. 2011, Brito et al. 2013b), since recent genetic divergence (base substitutions, insertions and deletions) accumulates at a mean rate about 5–10 times faster than in nuclear DNA (Brown et al. 1979). Furthermore, the different regions of the mitochondrial genome evolve at different rates, allowing specific options for each study (Avice 2000).

The Atacama Desert is one of the oldest regions under continuously arid conditions and the most arid desert of the world (Clarke 2006). Accordingly, its native biota lives in extremely fragile and isolated environments (Pinto et al. 2006, Vargas & Moreira 2012, Carevic et al. 2013). In some cases the Atacama Desert has imposed a significant barrier to gene flow among isolated populations, generating high levels of intraspecific divergence (Baranzelli et al. 2014). The coastal valleys of northern Chile have been largely recognized among the most important places for the biodiversity of the Atacama Desert; however, these valleys are currently impacted by heavy anthropic pressures mostly associated with intensive agricultural activities. Pristine habitats have been greatly reduced throughout these valleys, giving rise to many threats for all the native biota (Luebert & Plischoff 2006, Estades et al. 2007, Vargas & Parra 2009, Méndez-Abarca et al. 2012).

The Gracillariidae is a highly diverse plant-mining micromoth family, with 1,935 currently recognized species around the world (De Prins & De Prins 2014). Curiously, only a few more than 180 species have been described from the Neotropical Region until now (De Prins & De Prins 2014); however, this apparent low diversity is suggested to be an artifact arising from a poor sampling effort and a deficit of taxonomic descriptions for this fauna (Bruto et al. 2012, Lees et al. 2014). The

current knowledge of Chilean Gracillariidae is not an exception among the Neotropical representatives of this family, as only seven native species have been recorded (Davis 1994, Vargas & Landry 2005, Vargas & Parra 2005, De Prins & De Prins 2014, Mundaca et al. 2013a, b, Vargas et al. 2013).

Angelabella tecomae Vargas & Parra, 2005 is a leaf miner gracillariid micromoth native to the coastal valleys of the Atacama Desert of southern Peru and northern Chile (Vargas & Parra 2005, Vargas 2010). Its primary host plant is the native shrub *Tecoma fulva fulva* (Cav.) D. Don (Bignoniaceae) (Vargas & Parra 2005), whose geographic range is also restricted to a small area of the Atacama Desert, including some valleys of southern Peru and northern Chile (Wood 2008). Females of *A. tecomae* select new leaflets of the host plant for egg laying (Storey-Palma et al. 2014); hatching occurs through the surface of the egg adhered to the leaflet; the first instar larva enters into the leaflet, where it remains throughout the larval and pupal stages (Storey-Palma et al. 2012). Accordingly, the active dispersal of *A. tecomae* is only in the adult stage, as larvae and pupae are strictly endophytic.

The life history traits of *A. tecomae*, together with the arid and highly human-modified environment, suggest the possibility of high genetic divergence among isolated populations; however, genetic studies dealing with populations of *A. tecomae* have not been performed.

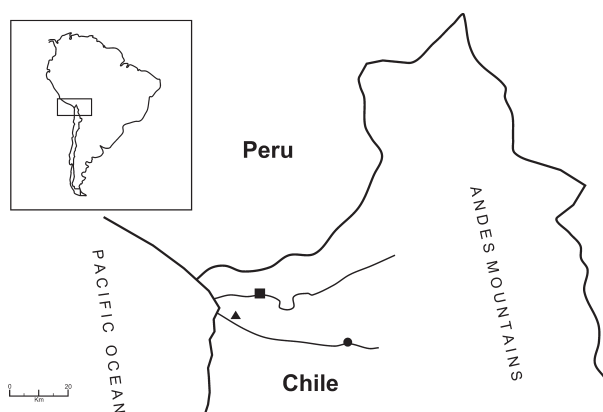


FIG. 1. Sampling sites of *Angelabella tecomae* in Lluta Valley (square), Arica City (triangle), Azapa Valley (circle) in the Arica Province, northern Chilean Atacama Desert.

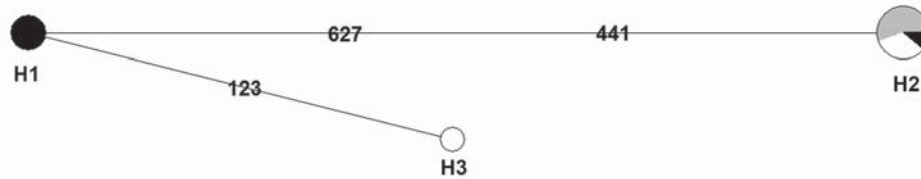


FIG. 2. Median joining network of DNA barcode (658 bp) haplotypes of *Angelabella tecomae* collected on *Tecoma fulva fulva*. H1, H2, H3: haplotypes; circle size proportional to number of individuals with the respective haplotype; numbers on the lines indicate DNA barcode position of nucleotide substitution. Nucleotide positions refer to the complete COI sequence of *Bombyx mori* (GenBank accession AY048187). Black: Azapa Valley; grey: Arica city; white: Lluta Valley.

Accordingly, the objective of this study is to provide an assessment of the genetic diversity of the northern Chilean populations of *A. tecomae* based on DNA barcode sequences as a first approach to the study of the population genetic structure of this little known leaf miner micromoth. We selected the animal DNA barcode fragment (Hebert et al. 2003) of the mitochondrial gene cytochrome oxidase c subunit I (COI) as a marker, as this has been successfully used in population studies of some species of gracillariid moths (Shapiro et al. 2008, Valade et al. 2009, Brito et al. 2013a, Davis et al. 2013).

Mined leaflets of *T. fulva fulva* were collected at three locations in the Arica Province between August and November 2013, covering all the northern Chilean range of *A. tecomae*: the city of Arica (18° 29' 24"S; 70° 17' 14"W), Azapa Valley (18° 34' 44"S; 69° 58' 03"W) and Lluta Valley (18° 24' 42"S; 70° 11' 48"W) (Fig. 1). The leaflets were brought to the laboratory and the mines were dissected in order to collect pupae of *A. tecomae* inside them, which were placed in ethanol (95%).

Genomic DNA was obtained from the pupae following the procedures described by Atashpaz et al. (2010) and Li et al. (2010). The DNA barcode fragment (658 bp) was amplified through PCR (Polymerase Chain Reaction) using the primers LEP-F1 (5'-ATTCAACCAATCATAAAGATAT-3') and LEP-R1 (5'-TAAACTTCTGGATGTCCAAAAA-3') developed by Hebert et al. (2004). PCR reactions were performed in a final volume of 20 µl. Each reaction contained 1 µl DNA extract, 10 pmoles each primer, 2.5 mM each dNTP, 2 mM MgCl₂, 1X PCR buffer (KCl), 1 unit 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 a

final elongation step of 2 min at 72 °C. PCR blank controls were incorporated. The PCR products were visualized on 1.5% agarose gels stained with gel-red (Biotium). Reactions containing fragments of the expected size were directly sequenced by a commercial facility (Macrogen, South Korea).

As a result, a fragment of 658 bp of the COI gene, comprising the complete animal DNA barcode (Hebert et al. 2003), was sequenced for 15 pupae of *A. tecomae* (GenBank accessions KM983591-KM983605). This is the third Chilean gracillariid species with DNA barcodes available, as these were previously reported for two other members of the family from the same geographic area (Vargas et al. 2013, Lees et al. 2014): *Acrocercops serrigera* Meyrick, 1915 and *Chileoptilia yaroella* Vargas & Landry, 2005.

The sequences were aligned with MUSCLE (Edgar 2004) as implemented in MEGA6 (Tamura et al. 2013). The mean nucleotide composition and the overall mean distance were calculated using the same software. Haplotype number, haplotype diversity and nucleotide diversity were calculated in DnaSP (Librado & Rozas 2009). The software Network 4.6.1.2 (Fluxus Technology Ltd) was used to infer the genealogical relationships of the haplotypes based on the median joining (MJ) method (Bandelt et al. 1999). The genetic differentiation among populations was estimated by the calculation of pairwise Φ_{st} in ARLEQUIN 3.5 (Excoffier & Lischer 2010) based on haplotype frequency.

The mean nucleotide composition of the *A. tecomae* sequences was 36.7% (T), 17.2% (C), 30.7% (A) and 15.4% (G). Three variable sites were detected among the sequences: 2 transversions and 1 transition (Table 1). The low value observed in the overall mean distance

TABLE 1. Nucleotide variation among haplotypes of the DNA barcode fragment (658 bp) of the cytochrome c oxidase subunit I (COI) gene of *Angelabella tecomae* (n = 15) collected in Azapa Valley, the city of Arica and Lluta Valley, Arica Province, northern Chile.

	Variable sites ^(a,b)			n	Locality
	123	441	627		
Substitution type ^(c)	Tv	Tv	Ts		
Haplotype					
H1	A	G	C	4	Azapa
H2	-	C	T	9	Azapa, Arica, Lluta
H3	C	-	-	2	Lluta

^(a) Nucleotide positions refer to the complete COI sequence of *Bombyx mori* (GenBank accession AY048187). ^(b) "-" indicates nucleotide identity to the H1 haplotype. ^(c) Tv: transversion, Ts: transition.

(0.002) indicates low variation among the DNA barcode sequences of *A. tecomae*. When the three populations are analyzed together (Table 2), the variation pattern is similar to that reported by Valade et al. (2009) for populations of the Palearctic species *Cameraria ohridella* Deschka & Dimić, 1986 from its native range using the same mitochondrial marker but with a much larger sample size. Contrarily, the genetic variation of *A. tecomae* is high compared to the recent colonization range of *C. ohridella* that includes a great part of Europe.

Only three haplotypes were detected in the sample (Table 1, Fig. 2), which differed by one (H1–H3), two (H1–H2) or three (H2–H3) nucleotide substitutions. Haplotype H1 was exclusive to the Azapa Valley (n = 4) and haplotype H3 was found only in the Lluta Valley (n = 2), while haplotype H2 was shared by all the three localities and also was the most abundant (n = 9) in the total sample. The urban population of the city of Arica was monomorphic, with only the most widespread haplotype (H2) represented in this sampling site. Accordingly, estimations of haplotype and nucleotide diversities were 0.00 for this population. Two haplotypes were represented in each of the two other populations of the Azapa (H1, H2) and Lluta (H2, H3) valleys, thus these reached higher values of haplotype and nucleotide diversities (Table 2).

As pairwise Φ_{st} values were not significant (Azapa-Arica: 0.750, $P = 0.072$; Azapa-Lluta = 0.432, $P = 0.081$; Arica-Lluta: 0.250, $P = 0.387$), there is no evidence that the genetic variation is geographically structured. However, it is interesting that Φ_{st} values are relatively high in numerical terms; similar Φ_{st} values are typical of populations with low gene flow (Morales et al. 2011). It is probable that the low genetic variation of the DNA barcode sequences of *A. tecomae* impedes the detection of biologically meaningful patterns. Accordingly, additional regions of the same and other genes should be explored, looking for sites with more variation. For instance, Brito et al. (2013a) reported 7% variable sites in a 1,583 bp mtDNA fragment, which also included the DNA barcode region, in populations of *Spinivalva* Moreira & Vargas, 2013 associated with different hosts and localities in southern Brazil.

Although in this study the genetic variation of *A. tecomae* was not found to be geographically structured, it is interesting that the two rural populations (Azapa and Lluta valleys) have exclusive haplotypes (Table 1, Fig. 2), which could indicate that environmental conditions of both localities allow harboring higher levels of genetic diversity, as both members of this system, the host plant *T. fulva fulva* and the leaf miner *A. tecomae*, reach larger population sizes in rural areas (Storey-Palma et al. 2014). Contrarily, the presence of only one haplotype in the

TABLE 2. Descriptive data of the genetic variation of the DNA barcode fragment (658 bp) of the cytochrome c oxidase subunit I (COI) gene of *Angelabella tecomae* collected in Azapa Valley, city of Arica and Lluta Valley, Arica Province, northern Chile. (n) sample size, (S) number of polymorphic sites, (Hn) haplotype number, (Hd) haplotype diversity, (π) nucleotide diversity; values following \pm indicate standard deviation.

	Azapa	Arica	Lluta	Total
n	5	5	5	15
S	2	0	3	3
Hn	2	1	2	3
Hd	0.400 \pm 0.237	0.00	0.600 \pm 0.175	0.590 \pm 0.106
π	0.00122 \pm 0.00072	0.00	0.00274 \pm 0.00080	0.00194 \pm 0.00034

population of Arica (Table 1, Fig. 2) could be associated with the low density of *T. fulva fulva* and the low population levels of *A. tecomae* regularly verified in the urban area.

Intraspecific genetic diversity has been little explored in Gracillariidae at the global level (Oshima 2008, Shapiro et al. 2008, Valade et al. 2009), and the Neotropical fauna is not an exception to this pattern (Brito et al. 2012, 2013a, Vargas et al. 2013, Lees et al. 2014). Although still at a preliminary level, the data here presented are the first dealing with the genetic diversity of the little known micromoth *A. tecomae* from its complete northern Chilean range, which is extremely narrow. Based on these results it appears that the Azapa and Lluta valleys, which are currently impacted by heavy anthropic pressures, deserve further attention in order to protect the genetic diversity of their leaf miner populations. However, additional efforts should be taken in the future to achieve a better characterization and understanding of the genetic variation of *A. tecomae* throughout its geographic range, also including southern Peruvian populations. Moreover, larger sampling sizes and additional molecular markers (Shapiro et al. 2008, Valade et al. 2009) should be used in these studies.

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