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Short Communication

Genetic divergence of a newly documented population of the cecidogenous micromoth *Eugnosta azapaensis* Vargas & Moreira (Lepidoptera: Tortricidae) in the Atacama Desert of northern Chile



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ABSTRACT

*Eugnosta azapaensis* Vargas and Moreira, 2015 (Lepidoptera: Tortricidae) is a micromoth native to the Atacama Desert whose larvae induce fusiform galls in shoots of *Baccharis salicifolia* (Ruiz & Pav.) Pers. (Asteraceae). The presence of this cecidogenous tortricid was previously recorded only from the type locality, the Azapa Valley, Arica Province, northern Chile. However, fusiform galls on shoots of *B. salicifolia* were recently found in Chaca, another coastal valley of the Atacama Desert. The adults obtained from these galls were preliminarily identified as *E. azapaensis* based on morphology. Subsequently, to assess an additional source of evidence for the taxonomic identification of *E. azapaensis* in this new locality, sequences of the DNA barcode fragment of the cytochrome oxidase subunit I mitochondrial gene from the two localities were analyzed. Four haplotypes were detected, two restricted to Azapa and two restricted to Chaca. The genetic divergence (K2P) between haplotypes of each locality was 0.2–0.8%, while it was 1.1–1.4% between haplotypes of different localities, and 8.7–13.5% between the Chilean haplotypes and other species of *Eugnosta* Hübner, 1825. In addition, all the sequences of Azapa and Chaca were clustered in a well-supported group in a Maximum Likelihood (ML) analysis. Accordingly, divergence and ML analyses support the morphological identification of *E. azapaensis* in the Chaca Valley. Furthermore, although preliminary, the analyses suggest that the genetic variation of the populations of this insect could be geographically structured, a pattern that must be assessed in further studies.

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Tortricidae is one of the more diverse families of micromoths with more than 10,000 species described worldwide, with the highest level of species diversity in the Neotropical region (Regier et al., 2012). In a recent revision, Razowski and Pelz (2010) recorded more than 80 species from Chile, mostly based on the study of specimens from the central and south-central areas of this country. In contrast, the species from the arid environments of northern Chile have been scarcely collected and studied (Clarke, 1987; Bobadilla and Vargas, 2015). Larval feeding habits are variable in this family of micromoths. The larvae of many species are leaf-tiers and leaf-rollers; others bore into twigs, feed on reproductive organs (seeds, flower parts or fruits), or are gall inducers (Haghani et al., 2014; Razowski and Giliomee, 2014; Brown and Nishida, 2003). The gall-inducing habit has been described for several genera, mostly belonging to the

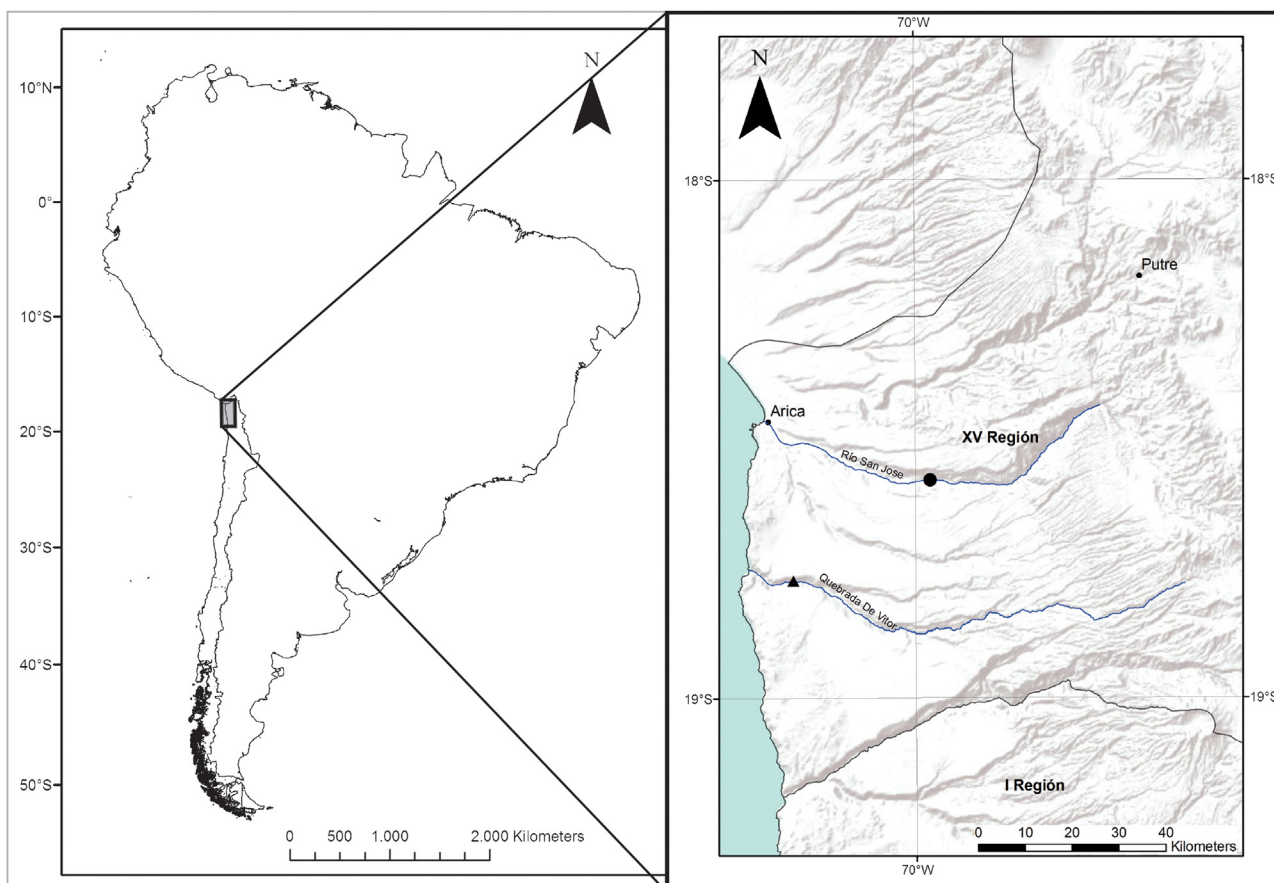
tribes Cochylini, Grapholitini and Eucosmini (Brown and Nishida, 2007).

*Eugnosta* Hübner, 1825 (Tortricinae: Cochylini) is a worldwide genus with more than 90 species described, about 40 of which are from the New World (Gilligan et al., 2014). Natural history has been described for a few species of *Eugnosta*, and in the New World all are gall inducers in Asteraceae (Comstock, 1939, 1940; Goeden and Ricker, 1981; Vargas et al., 2015). *Eugnosta azapaensis* Vargas and Moreira, 2015 is the only representative of the genus currently recorded in Chile. Its larvae induce fusiform galls on shoots of the shrub *Baccharis salicifolia* (Asteraceae) in the Atacama Desert (Vargas et al., 2015). This cecidogenous micromoth was previously known only from the type locality, the Azapa Valley, Arica Province. However, fusiform galls were recently detected on shoots of *B. salicifolia* in the Chaca Valley, about 30 km south the type locality (Fig. 1). The adults obtained from these galls were identified as *E. azapaensis* based on morphology of the male and female genitalia.

The Atacama Desert is the most arid desert in the world (Clarke, 2006), where the coastal valleys of the northernmost part of Chile

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**Fig. 1.** The study area in South America (left) and the sampling sites (right) of *Eugnosta azapaensis* in the Atacama Desert of northern Chile. The type locality Azapa Valley (black circle) and the newly documented locality Chaca Valley (black triangle).

are recognized as valuable reservoirs of biodiversity for plants (Luebert and Plissock, 2006), birds (Estades et al., 2007), mammals (Ossa et al., 2016) and insects (Vargas and Parra, 2009; Méndez-Abarca et al., 2012). Accordingly, it is important to record the taxonomic composition accurately at a local scale, especially in little studied groups such as micromoths.

The analysis of different sources of evidence is important to reach sound taxonomic identifications (Dayrat, 2005; Schlick-Steiner et al., 2010). This approach is especially important when morphologically similar species are involved (Taft et al., 2016; Warren and Grishin, 2017), as is the case in many groups of micromoths (Landry and Hebert, 2013; Huemer et al., 2014; Kirichenko et al., 2015; Luz et al., 2015; Pereira et al., 2017). It has been shown that the analysis of the DNA barcode fragment (sensu Hebert et al., 2003) of the cytochrome oxidase subunit I is a useful complement to morphology for identifying small organisms, including species of the family Tortricidae (Jaeger et al., 2013; Brown et al., 2014; Gilligan et al., 2016; Corley and Ferreira, 2017; Razowski et al., 2017). Accordingly, DNA barcode sequences were analyzed to assess the taxonomic status of the newly discovered population of *Eugnosta* in the Chaca Valley.

### Sampling

Fusiform galls on shoots of *B. salicifolia* were collected in the Azapa ( $18^{\circ}31'12''\text{S}$ ,  $70^{\circ}10'41''\text{W}$ ) and Chaca ( $18^{\circ}48'56''\text{S}$ ,  $70^{\circ}09'07''\text{W}$ ) valleys between May and August 2016. The collected galls were placed in plastic bags and brought to the laboratory and dissected to extract the cecidogenous larvae, which were kept in 95% ethanol at  $-20^{\circ}\text{C}$  for DNA extraction.

### DNA extraction and sequencing

Genomic DNA was extracted from larvae following the procedures described in Huanca-Mamani et al. (2015) and subsequently was sent to Macrogen Inc. (South Korea) for amplification by polymerase chain reaction (PCR) and sequencing with the primers LCO-1490 and HCO-2198 (Folmer et al., 1994). The amplification program was: 5 min at  $94^{\circ}\text{C}$ , 35 cycles of 30 s at  $94^{\circ}\text{C}$ , 30 s at  $47^{\circ}\text{C}$ , 1 min at  $72^{\circ}\text{C}$ , and a final elongation step of 10 min at  $72^{\circ}\text{C}$ .

### Sequence analysis

According to the procedures described by Hall (2013), the software MEGA6 (Tamura et al., 2013) was used to perform the sequence alignment by the ClustalW method, to estimate the sequence divergence by the Kimura 2-Parameters (K2P) method and to perform a Maximum Likelihood (ML) analysis. Besides the Chilean sequences reported here, the following DNA barcode sequences (658 bp) of *Eugnosta* with species identification were downloaded from BOLD (Ratnasingham and Hebert, 2007) for the ML analysis: *E. beevorana* (Comstock, 1940) (LNAUU4001-15), *E. brownana* Metzler and Forbes, 2012 (LNAUU4004-15), *E. busckana* (Comstock, 1939) (LOCB682-06), *E. erigeronana* (Riley, 1881) (LILLA210-11), *Eugnosta* sp. (LNAUU3996-15), *E. percnopitila* (Meyrick, 1933) (AFTOR133-12), *E. sartana* (Hübner, 1833) (LOFLA761-06). In addition, *Aethes argenteimitana* (Robinson, 1869) (BBLPA769-10) was included in the analysis, as *Aethes* Billberg, 1820 is close to *Eugnosta* (Regier et al., 2012). Previous to the ML analysis, the GTR+G was selected as the best model to describe the substitution pattern following the Bayesian information criterion.

**Table 1**  
Nucleotide variation among haplotypes of the DNA barcode fragment (658 bp) of the cytochrome c oxidase subunit I (COI) gene of *Eugnosta azapaensis* collected in the Azapa and Chaca valleys, Arica Province, northern Chile.

	Variable sites <sup>a</sup>											n	Locality
	92	139	274	286	302	316	364	370	374	473	625		
Substitution <sup>b</sup>	Ts	Tv	Ts	Tv	Ts	Ts	Ts	Tv	Ts	Ts	Ts		
Haplotype													
H1	C	T	C	A	A	G	C	T	A	G	A	2	Azapa
H2	T	–	–	T	G	A	–	–	G	–	–	2	Azapa
H3	–	A	T	–	G	A	T	A	–	A	–	6	Chaca
H4	–	A	T	–	G	A	T	A	–	A	G	1	Chaca

<sup>a</sup> “–” indicates nucleotide identity to the H1 haplotype.

<sup>b</sup> Ts, transition; Tv, transversion.

The statistical support of the nodes was assessed by 1000 bootstrap replicates. The genealogy of the haplotypes was inferred by the Median-Joining (MJ) method (Bandelt et al., 1999) in the software Network 5.0.0.1 (Fluxus Technology Ltd.).

Eleven DNA barcode sequences (658 bp) were obtained; four from Azapa and seven from Chaca (GenBank accessions MF000408–MF000418), all of which were characterized by 30.6% (A), 39.1% (T), 15.4% (C), 14.8% (G).

### Genetic divergence

Eleven variable sites were found in the alignment of all the sequences of Azapa and Chaca valleys, with eight transitions and three transversions (Table 1). When the sequences of the different localities were aligned separately five variable sites were found in Azapa, while just one variable site was found in Chaca. The mutations determined the presence of four haplotypes, two (H1, H2) restricted to Azapa, and two (H3, H4) found only in Chaca. H1 and H2 were represented by two individuals each, H3 by six individuals, and H4 by one (Table 1). The divergence (K2P) was 0.8% between haplotypes of Azapa and 0.2% between those of Chaca. The divergence between haplotypes of different localities was 1.1–1.4%. Accordingly, the haplotype network shows a lower number of mutations between haplotypes of the same (1–5) than of different (7–9) localities (Fig. 2). The divergence between haplotypes of

Chaca and Azapa with other species of *Eugnosta* included in the ML analysis was 8.7–13.5%.

DNA barcode divergence above 2% (K2P) is generally suggestive of different species of Lepidoptera (Hausmann et al., 2011). However, cases of higher intraspecific divergence have been reported, mostly involving widely distributed species (Wiemers and Fiedler, 2007; Hausmann and Huemer, 2011). In addition, cases of interspecific DNA barcode distances less than 2% also have been described, mostly dealing with morphologically close species (Kirichenko et al., 2015). Regardless of these exceptional cases, the divergence found between haplotypes of the Azapa and Chaca valleys (1.1–1.4%) appears to be at the intraspecific level. This suggestion is reinforced by the fact that the lowest divergence found between the other species of *Eugnosta* analyzed was 2.6% (*E. busckana* and *E. beevorana*).

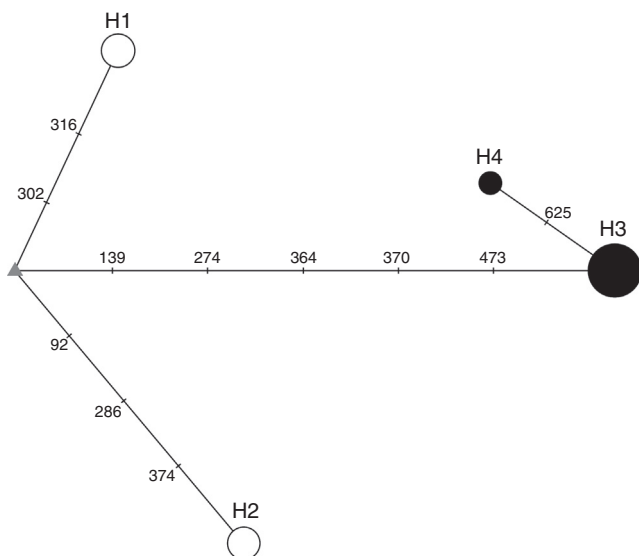
### ML analysis

The alignment for the ML analysis included nineteen DNA barcode sequences of 658 bp length with 166 variable sites, 108 of which were parsimony informative. The eleven Atacama Desert sequences (Azapa and Chaca) were clustered with high support (Fig. 3), suggesting that all these belong to the same species (*E. azapaensis*). In addition, the sequences of Azapa were clustered with high support as a subgroup into the *E. azapaensis* clade (Fig. 3), suggesting that the genetic variation is associated with geographic distribution, although a similar scenario was not found for the sequences of Chaca.

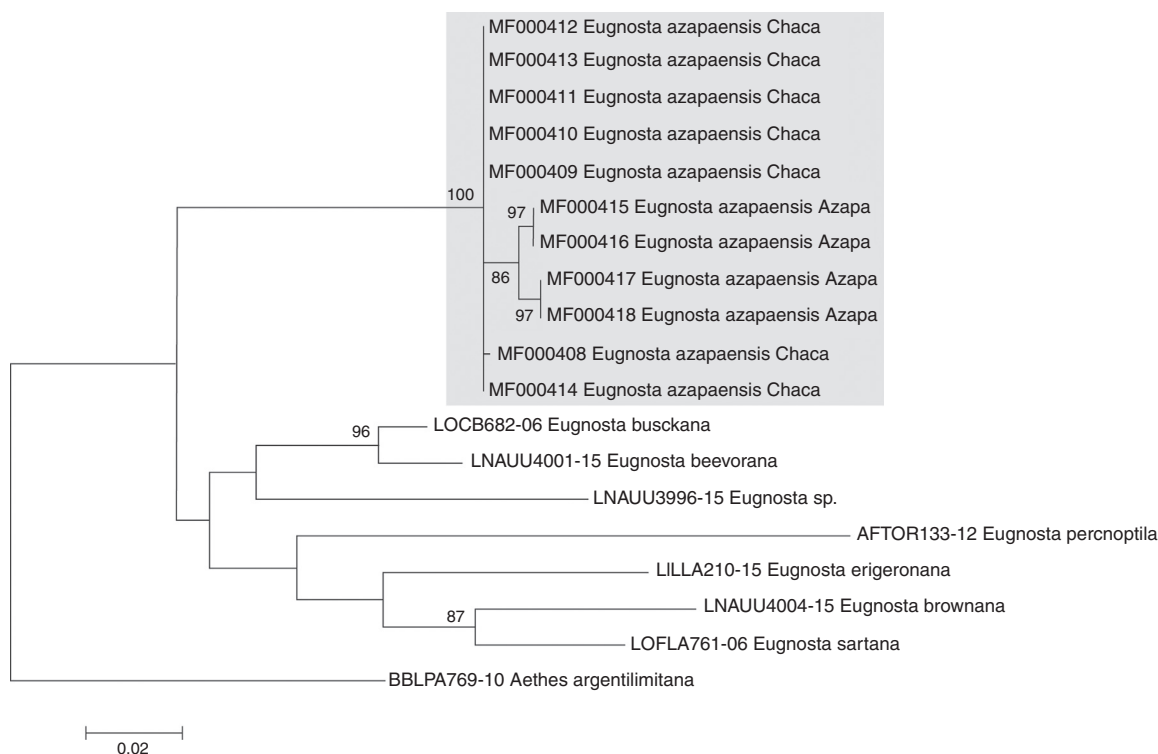
The relationships of *E. azapaensis* with congeners were not resolved in the analysis. However, this was expected since only a few species of *Eugnosta* are represented in BOLD, most of which are Nearctic. The Neotropical *E. argentinae* (Razowski, 1967) described from Argentina and *E. ochrolemma* (Razowski, 1986) from Mexico are morphologically similar to *E. azapaensis* (Vargas et al., 2015), but DNA barcode sequences of these species are unknown. In addition, the gall shape of *E. azapaensis* is similar to that of the Nearctic *E. busckana* and *E. beevorana*. Interestingly, these two Nearctic species were clustered with high support in the ML tree, although they were not found to be close to *E. azapaensis*. Evidently, additional morphological (adult and immature stages) and molecular (mitochondrial and nuclear) data of other Neotropical species are necessary to understand the evolutionary relationships of *E. azapaensis*.

### Further remarks

This is the first report of *E. azapaensis* outside the type locality. Galls of *E. azapaensis* were searched for on shoots of the only other species of *Baccharis* represented in the study area (*B. scandens*) without success. In addition, surveys for galls on *B. salicifolia* were carried out on the western slopes of the Andes of



**Fig. 2.** Median joining network of the haplotypes of the DNA barcode fragment (658 bp) of the cytochrome c oxidase subunit I (COI) gene of *Eugnosta azapaensis* from Azapa (white) and Chaca (black) valleys, Atacama Desert of northern Chile. H1, H2, H3, H4 haplotypes; circles proportional to the frequency of the respective haplotype; numbers between circles indicates variable sites; gray triangle a median vector.



**Fig. 3.** Maximum likelihood tree of the sequences of the DNA barcode fragment (658 bp) of the cytochrome c oxidase subunit I (COI) gene of *Eugnosta azapaensis* (gray area) from Azapa and Chaca valleys, Atacama Desert of northern Chile. Bootstrap supports higher than 70% are shown above branches.

the Parinacota Province, at about 3000 m elevation, also without success. Thus *E. azapaensis* appears to be a host-specialist with a geographic range restricted to the low elevation environments of the coastal valleys of the Atacama Desert. This geographic pattern has been described for different insect groups (Porter, 1985; Howden, 2008). Additional coastal valleys in which the host plant is present must be surveyed in order to better understand the geographic range of *E. azapaensis* along this extensive desert area.

The only DNA barcode sequence previously available for *E. azapaensis* was the analyzed in the original description of the species (GenBank accession KM023733.1; 622 bp) which matches 100% with one of the haplotypes of Azapa (H2), while H1, H3 and H4 are here reported for the first time. The variation found in this study suggests that the DNA barcode fragment is a valuable tool to assess the taxonomic status of eventual new populations of *Eugnosta* of the Atacama Desert. Analysis of sequences of mitochondrial DNA is useful to explore geographic patterns of genetic variation, at least at a preliminary stage (Harper et al., 2008; Maita-Maita et al., 2015; Velasco-Cuervo et al., 2016; Maia et al., 2016). In this regard, an interesting finding is that each haplotype is restricted to a specific locality. This spatial segregation of the genetic variation suggests that the arid conditions of the area separating the valleys could be an effective barrier for the dispersal of adults of *E. azapaensis*, enhancing the genetic differentiation of the local populations of each valley. However, due to the low density of galls in the field, only a few specimens were collected and analyzed in this study. Thus further sampling and analyses of additional molecular markers (Valade et al., 2009; Seraphim et al., 2016) would be needed to verify this pattern of geographic structure of the genetic variation in the driest desert of the world.

#### Conflicts of interest

The authors declare no conflicts of interest.

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