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Short Communication

First record of the sedge feeder *Bactra verutana* Zeller (Lepidoptera: Tortricidae) in Chile based on morphology and DNA barcodes

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ABSTRACT

The sedge-feeding moth *Bactra verutana* Zeller, 1875 (Lepidoptera: Tortricidae: Olethreutinae: Bactrini), described from Dallas, Texas, USA, is widespread, recorded throughout much North America, Central and South America, including the Caribbean, and Africa. The species is recorded for the first time from Chile based on specimens collected in the coastal valleys of the Atacama Desert, where its larvae feed on *Cyperus corymbosus* Rottb. var. *subnodosus* (Nees & Meyen) Kük. (Cyperaceae). A single DNA barcode haplotype, which is widespread in USA, was found in two Chilean specimens sequenced.

Bactra Stephens, 1834 (Olethreutinae: Bactrini) is a widespread genus of Tortricidae (Lepidoptera) with 106 described species worldwide (Gilligan et al., 2014), 13 of which are recorded in the Neotropical Region (Razowski and Becker, 2010). Host plant records, available for 21 species, are mainly restricted to representatives of Cyperaceae, Juncaceae and Poacae, upon which the larvae feed on stems (Horak, 2006; Brown et al., 2008). In contrast to the general habit of feeding on monocotyledenous hosts, the Palearctic *Bactra bactrana* (Kennel, 1901) was recently recorded as a pest of sweet pepper *Capsicum annuum* L. (Solanaceae) (Roditakis et al., 2016).

Bactra verutana Zeller, 1875 is one of the species in the genus whose biology has been best studied. It has been proposed as a biological control agent of weed sedges, because its larvae are voracious stem borers on these plants (Keeley et al., 1970; Frick and Garcia, 1975; Frick and Wilson, 1978). Originally described from Dallas, Texas, USA, it is currently widespread in the New World, with records from much of North America and several localities in Central and South America, the Caribbean Islands and Africa (Diakonoff, 1964; Powell, 1997; Razowski and Becker, 2010).

We provide the first record of *B. verutana* from Chile based on sampling conducted in the northernmost part of this country. Because the genitalia of some representatives of *Bactra* are morphologically similar (e.g.: Diakonoff, 1963, 1964), DNA barcode sequences (sensu Hebert et al., 2003) were used to assess the relationships of the Chilean specimens.

Sampling. Adults were collected at light in the Azapa Valley, Atacama Desert of northern Chile, between July 2015 and January 2018. Larvae were collected on the sedge *Cyperus corymbosus* Rottb. var. *subnodosus* (Nees & Meyen) Kük. (Cyperaceae) in the same locality. Stems bored by larvae were placed in plastic vials with paper towel at the bottom; additional pieces of stems were provided when needed. Plastic vials were periodically checked for adult emergence. Two larvae were placed in 95% ethanol at −20 °C until DNA extraction. The abdomens of adults were dissected using hot 10% KOH for a few minutes; the genitalia were stained with Chlorazol Black or Eosin Y and slide mounted with Euparal. Vouchers are deposited in IDEA (Colección Entomológica, Universidad de Tarapacá, Arica, Chile).

DNA extraction and analysis. DNA extraction followed the procedures described in Huanca-Mamani et al. (2015). Genomic DNA was sent to Macrogen (South Korea) for amplification, purification and sequencing of the barcode region using the primers LCO-1490 and HCO-2198 (Folmer et al., 1994) following the amplification program described in Escobar-Suárez et al. (2017). For phylogenetic analysis, additional DNA barcode sequences (658 bp; Table 1) were downloaded from BOLD (Ratnasingham and Hebert, 2007): one of each haplotype of *B. verutana* and one of each other species of *Bactra*, except for *B. blepharopis* Meyrick, 1911, in which the two available haplotypes were included, as this species showed the lowest K2P distance with *B. verutana*. A sequence of *Endothenia* Stephens, 1852 (Endotheniini) was also included in the analysis

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Table 1
Sequences used in the Bayesian analysis.

Species	BOLD accession	GenBank accession	Country
Bactra ablabes Turner, 1946	ANICV1650-11	KF404098.1	Australia
Bactra blepharopis Meyrick, 1911	ANICC250-09		Australia
Bactra blepharopis Meyrick, 1911	MAMOT3760-13	KX862772.1	Pakistan
Bactra furfurana (Haworth, 1811)	BBLOD1417-11		United States
Bactra lacteana Caradja, 1916	DEEUR370-12		Germany
Bactra maiorina Heinrich, 1923	LOCB575-06		United States
Bactra optanias Meyrick, 1911	ANICB519-06		Australia
Bactra passercula Turner, 1916	ANICV1671-11	KF402375	Australia
Bactra priapeia Heinrich, 1923	LMEMB250-09		United States
Bactra robustana Christoph, 1872	CGUKC306-09		United Kingdom
Bactra scalopias Meyrick, 1911	ANICV1656-11	KF404800.1	Australia
Bactra suedana Bengtsson, 1989	LEEUA224-11		Denmark
Bactra venosana (Zeller, 1847)	ANICB611-06		Australia
Bactra verutana Zeller, 1875	BBLOE331-11		United States
Bactra verutana Zeller, 1875	BBLOE1159-12		United States
Bactra verutana Zeller, 1875	BBLOB1839-11		United States
"*Bactra verutana* Zeller, 1875"	BLPDG174-09		Costa Rica
"*Bactra verutana* Zeller, 1875"	BLPDH685-09		Costa Rica
Bactra verutana Zeller, 1875	LMEMB244-09		United States
"*Bactra verutana* Zeller, 1875"	MIMAD026-15		Madagascar
"*Bactra verutana* Zeller, 1875"	MIMAD662-15		Madagascar
Bactra verutana Zeller, 1875		MH938331	Chile
Endothenia gentianaeana (Hubner, [1796–1799])	CGUKC916-09		United Kingdom

as a representative of the sister tribe of Bactrini (Regier et al., 2012). The sequences were aligned by the ClustalW method, and sequence divergence was estimated by the Kimura 2-parameter model (K2P) in the software MEGA6 (Tamura et al., 2013) using 1000 bootstrap replications. Coalescent trees were inferred using the Bayesian method through BEAST 1.8.4 (Drummond et al., 2012) based on a Yule species tree prior. The nucleotide substitution model JC was the most appropriate according to the Bayesian information criterion obtained in JModelTest 2.1 (Darriba et al., 2012). A Markov Chain Monte Carlo (MCMC) process was run for 10 million generations, sampling every 1000 generations. We used MCMC sampling with priors above 200 to ensure effective sample sizes, which were assessed in Tracer 1.6 (Rambaut et al., 2014). Ten percent of the samples were removed as "burn-in" in TreeAnnotator 1.8.4 (http://beast.community/treeannotator). Finally, the consensus tree obtained was viewed and edited in FigTree 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree).

Morphology. Eight adults (four females, four males), two of which were reared from larvae collected on *C. corymbosus*, were dissected and identified as *B. verutana* based on genitalia morphology (Diakonoff, 1964; Powell, 1997) (Fig. 1).

DNA barcodes and phylogenetic analysis. Two DNA barcode sequences (658 bp), representing a single haplotype (GenBank accession MH938331), were obtained from the borer larvae collected on stems of *C. corymbosus*. A search in BOLD revealed that this haplotype is widespread in the USA (Alabama, Arizona, Arkansas, Florida, Illinois, Maryland, Massachusetts, Mississippi, Oklahoma, Tennessee and Texas). Pairwise distances (K2P) of the Chilean haplotype were 0–0.3%, 2.0–2.2% and 6.0–6.2% with those of *B. verutana* from USA, Madagascar and Costa Rica, respectively. Agreeing with this deep divergence, the phylogenetic analysis showed that the *B. verutana* haplotypes are not monophyletic; the Chilean haplotype clustered with high support only with those from the USA (Fig. 2). This USA-Chile group was sister to *B. blepharopis*. The haplotypes from Costa Rica and Madagascar were clustered with high support according to geographic origin in two distantly related groups. Those from Costa Rica were clustered with *Bactra ablabes* Turner, 1946 and those from Madagascar were sister to the group (*B. verutana* USA-Chile + *B. blepharopis*). Pairwise distances of the Chilean haplotype with other representatives of *Bactra* ranged from 1.7 (*B. beplarophis*) to 8.6% (*B. scalopias* Meyrick, 1911).

Remarks. The Tortricidae of Chile were revised by Razowski and Pelz (2010), providing a valuable base for a wide range of studies, including taxonomic ones. Recent field surveys have revealed previously unknown endemic representatives of this micromoth family in this country (e.g.: Cepeda, 2017; Urra, 2017), suggesting that the diversity of this fauna remains insufficiently described. Based on the available data, the Tortricidae of South and Central Chile are characterized by a high level of endemism (Razowski and Pelz, 2010; Cepeda, 2017; Urra, 2017), whereas less endemism is found in the arid environments of the northernmost part of the country (Brito and Vargas, 2018; Vargas-Ortiz and Vargas, 2018). Finding *B. verutana* in the coastal valleys of the Atacama Desert provides an additional record of a widespread, non-endemic tortricid moth for northern Chile.

The habit of feeding on sedges as a stem borer has been well described previously for *B. verutana* (Keeley et al., 1970; Frick and Garcia, 1975; Frick and Wilson, 1978), with at least three species of *Cyperus* recorded as its hosts, besides unidentified representatives of *Scirpus* (Cyperaceae) and *Juncus* (Juncaceae) (Brown et al., 2008). Thus, the record of *C. corymbosus* here reported for the Chilean specimens agrees with the main association of *B. verutana* with Cyperaceae. Obviously, the presence of such a suitable host was indispensable for the colonization of the coastal valleys of the Atacama Desert by this specialized sedge-feeding micromoth.

In accordance with the patterns described for other Lepidoptera families (Hebert et al., 2003), intraspecific DNA barcode divergences are in general lower than 2% in Tortricidae (Hulcr et al., 2007; Gilligan et al., 2016; Corley and Ferreira, 2017; Vargas-Ortiz et al., 2017), although cases of greater divergence have been described for a few widespread species (Gilligan et al., 2016). The deep divergence of the BOLD sequences of *B. verutana* and the results of the phylogenetic analysis, in which the haplotypes from Madagascar and Costa Rica were not clustered with those from USA, strongly suggest that more than one species is present under this name in this database. It is supposed that the haplotypes from North America represent the true *B. verutana*, as this group includes sequences sampled close to the type locality (Texas, USA). The divergence between the North American haplotypes of *B. verutana* and the two haplotypes of *B. blepharopis* (1.7–2.0%) is close to the highest values of intraspecific divergence reported for a few widespread tortricids (Gilligan et al., 2016); however, the two are currently considered valid species based on morphology (Horak, 2006; Gilligan et al., 2014). In addition, the reciprocal monophyly found in the phylogenetic analysis between the North American haplotypes of *B. verutana* and those of *B. blepharopis* reinforces their heterospecific status.

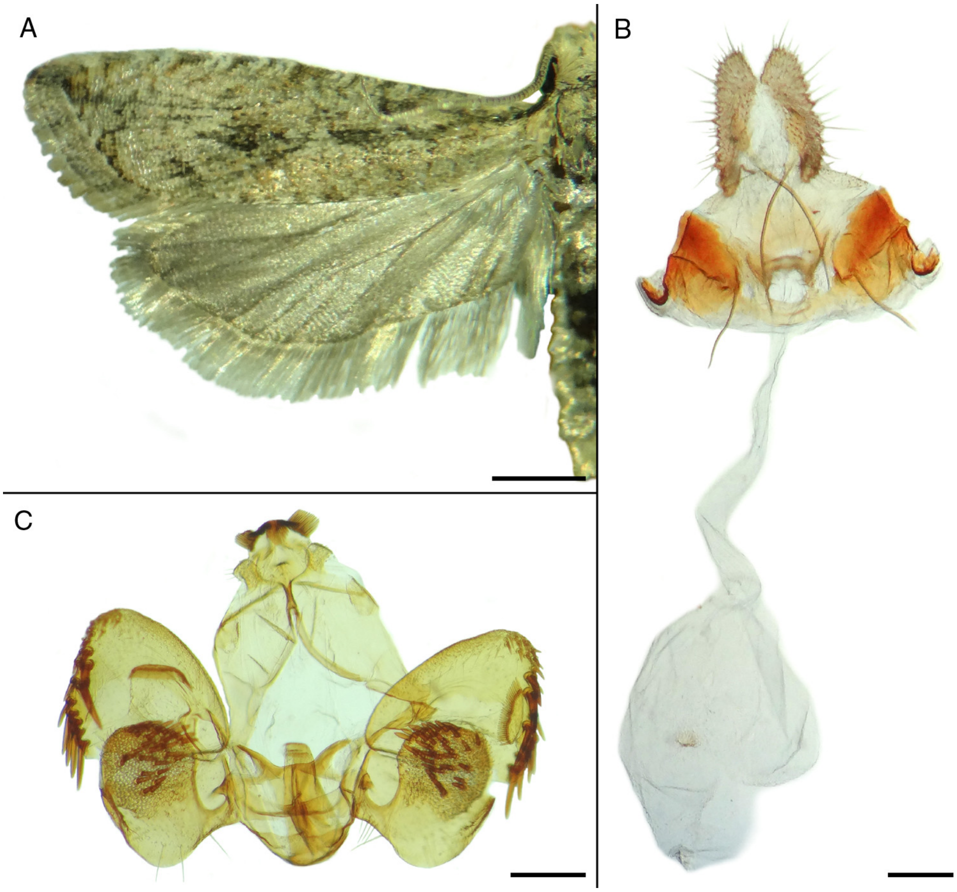

Fig. 1. Adult *Bactra verutana* collected in the Azapa Valley, northern Chile. (A) Male in dorsal view. (B) Female genitalia in ventral view. (C) Male genitalia in ventral view. Scale bars 1, 0.2 and 2.2 mm, respectively.

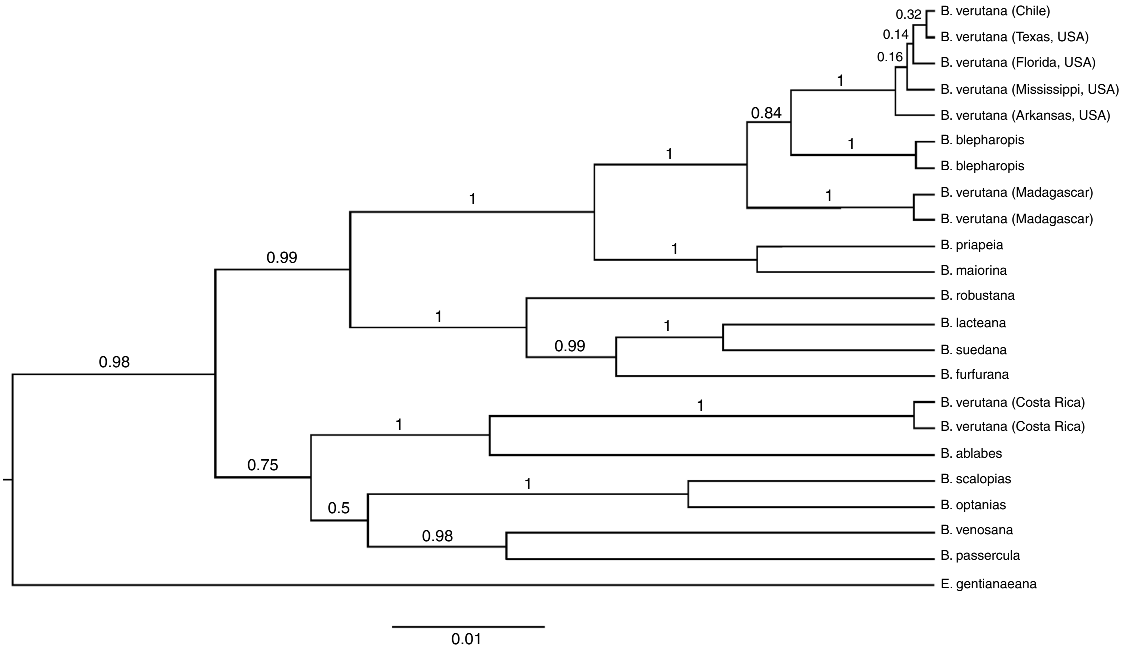

Fig. 2. Bayesian tree of *Bactra verutana* and congenerics based on sequences of the DNA barcode fragment (658 bp) of the cytochrome c oxidase subunit I (COI) gene. Node supports (posterior probability) indicated above branches.

The low divergence and the grouping of the only Chilean haplotype with the North American ones in the phylogenetic analysis provides additional support for the morphological identification of *B. verutana* in Chile. These results also suggest a recent arrival of this species to the Atacama Desert; however, understanding the phylogeographic patterns throughout its remarkably wide New World range deserves further molecular studies. Meanwhile, the taxonomic status of the populations from Costa Rica and Madagascar should be assessed using integrative approaches.

Conflicts of interest

The authors declare no conflicts of interest.

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References

Brito, R., Vargas, H.A., 2018. A new leaf-tying *Episimus* Walsingham (Lepidoptera: Tortricidae) feeding on the vulnerable tree *Haplorhus peruviana* (Anacardiaceae) in the Atacama Desert of northern Chile. Stud. Neotrop. Fauna E 53, 113–119.

Brown, J.W., Robinson, G., Powell, J.A., 2008. Food plant database of the leafrollers of the world (Lepidoptera: Tortricidae) (Version 1.0), http://www.tortricid.net/foodplants.asp (accessed 25.07.17).

Cepeda, D.E., 2017. Una nueva especie del género *Eugnosta* Hübner, de Chile (Lepidoptera: Tortricidae) Insecta Mundi 0565, 1–6.

Corley, M.F.V., Ferreira, S., 2017. DNA Barcoding reveals sexual dimorphism in *Isotrias penedana* Trematerra 2013 (Lepidoptera: Tortricidae, Chlidanotinae). Zootaxa 4221, 594–600.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9, 772.

Diakonoff, A., 1963. African species of the genus *Bactra* Stephens (Lepidoptera Tortricidae). Tijdschr. Entomol. 106, 285–356.

Diakonoff, A., 1964. Further records and descriptions of the species of *Bactra* Stephens (Lepidoptera Tortricidae). Zool. Verh. 70, 1–81.

Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7 Mol. Biol. Evol. 29, 1969–1973.

Escobar-Suárez, S., Huanca-Mamani, W., Vargas, H.A., 2017. Genetic divergence of a newly documented population of the cecidogenous micromoth Eugnosta azapaensis Vargas & Moreira (Lepidoptera: Tortricidae) in the Atacama Desert. Rev. Bras. Entomol. 61, 266–270.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 294–299.

Frick, K.E., Garcia, C., 1975. *Bactra verutana* as a biological control agent for purple nutsedge. Ann. Entomol. Soc. Am. 68, 7–14.

Frick, K.E., Wilson, R.F., 1978. Larval biology of *Bactra verutana*, an indigenous biological control agent of the weedy nutsedges. Ann. Entomol. Soc. Am. 71, 680–684.

Gilligan, T.M., Baixeras, J., Brown, J.W., Tuck, K.R., 2014. T@RTS: online world catalogue of the Tortricidae (Ver. 3.0), Available from: http://www.tortricid.net/catalogue.asp (accessed 25.07.18).

Gilligan, T., Huemer, P., Wiesmair, B., 2016. Different continents, same species? Resolving the taxonomy of some Holarctic *Ancylis* Hübner (Lepidoptera: Tortricidae). Zootaxa 4178, 347–370.

Hebert, P.D.N., Cywinska, A., Ball, S.L., deWaard, J.R., 2003. Biological identification through DNA barcode. Proc. R. Soc. B 270, 313–321.

Horak, M., 2006. Olethreutine Moths of Australia (Lepidoptera: Tortricidae) Collingwood. CSIRO, Australia.

Huanca-Mamani, W., Rivera-Cabello, D., Maita-Maita, J., 2015. A simple, fast, and inexpensive CTAB-PVP-Silica based method for genomic DNA isolation from single, small insect larvae and pupae. Gen. Mol. Res. 14, 7990–8000.

Hulcr, J., Miller, S.E., Setliff, G.P., Darrow, K., Mueller, N.D., Hebert, P.D.N., Weiblen, G.D., 2007. DNA barcoding confirms polyphagy in a generalist moth *Homona mermerodes* (Lepidoptera: Tortricidae). Mol. Ecol. Notes 7, 549–557.

Keeley, P.E., Thullen, R.J., Miller, J.H., 1970. Biological control studies on yellow nutsedge with *Bactra verutana* Zeller. Weed Sci. 18, 393–395.

Powell, J.A., 1997. Three additional *Bactra* in California, one native but overlooked, one probably introduced, one new species (Tortricidae). J. Lepid. Soc. 51, 128–134.

Rambaut, A., Suchard, M.A., Xie, D., Drummond, A.J., 2014. Tracer v1.6, Available from http://tree.bio.ed.ac.uk/software/tracer/.

Ratnasingham, S., Hebert, P.D.N., 2007. BOLD: the barcode of life data system (www.barcodinglife.org). Mol. Ecol. Notes 7, 355–367.

Razowski, J., Becker, V.O., 2010. Systematic and faunistic data on Neotropical Bactrini (Lepidoptera: Tortricidae). Pol. J. Entomol. 79, 187–193.

Razowski, J., Pelz, V., 2010. Tortricidae from Chile (Lepidoptera: Tortricidae). SHILAP Rev. Lepidopt. 38, 5–55.

Regier, J.C., Brown, J.W., Mitter, C., Baixeras, J., Cho, S., Cummings, M.P., Zwick, A., 2012. A molecular phylogeny for the leafroller moths (Lepidoptera: Tortricidae) and its implications for classification and life history evolution. PLoS ONE 7 (4), e35574.

Roditakis, E., Morin, S., Baixeras, J., 2016. Is *Bactra bactrana* (Kennel, 1901) a novel pest of sweet peppers? Bull. Entomol. Res. 106, 161–167.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729.

Urra, F., 2017. *Foikeulia razowskii*, nuevo género y nueva especie de tortrícido (Lepidoptera: Tortricidae) asociada a *Drimys winteri* (Winteraceae). Rev. Chil. Entomol. 43, 47–54.

Vargas-Ortiz, M., Vargas, H.A., 2018. A new species of *Strepsicrates* Meyrick (Lepidoptera: Tortricidae) from the Atacama Desert of northern Chile previously misidentified as *S. smithiana* Walsingham. Zootaxa 4370, 569–579.

Vargas-Ortiz, M., Bobadilla, D., Vargas, H.A., Huanca-Mamani, W., Gilligan, T.M., Brown, J.W., 2017. Exploring DNA barcodes of Neotropical and Afrotropical species of *Eccopsis* Zeller (Lepidoptera: Tortricidae). J. Lepid. Soc. 71, 211–217.