

Rapid Communication

Stranger in a known land: Bayesian analysis confirms the presence of an Australian leaf miner in the Chilean Atacama Desert

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Abstract

Several species of leaf-mining moths have become invasive in many parts of the world. Their success seems to be related, at least in part, to their small body size, short life cycles, high dispersal capabilities and relatively plastic host-plant associations. Here, we provide the first report of established populations of the elachistid moth, *Elachista synethes*, in South America (specifically the Azapa Valley, northern Chilean Atacama Desert). *E. synethes* is a widespread polyphagous species native to Australia, Tasmania and New Zealand. The immatures in the Azapa Valley were found associated with the South American native rescue grass, *Bromus catharticus*. Interestingly *E. synethes* is known to use *B. catharticus* as a host plant in the Australian region (i.e. where the plant is introduced but the moth native). In addition to comparing the morphology of immature and adult moths, we sequenced a partial region of the cytochrome oxidase subunit I gene from putative specimens from the Azapa Valley, and reconstructed a Bayesian phylogeny using representative species from different groups of *Elachista* as terminals. The specimens from Chile fell within the lineage of *E. synethes*, with 0% of genetic divergence. Thus, we hypothesize that this leaf-miner species was accidentally introduced into northern Chile, and has established there due to the existence of suitable host plants in that area.

Key words: alien species, COI, DNA barcoding, Elachistidae, elachistid moths, Neotropical region

Introduction

Lepidoptera is among the most diverse insect orders and one of the largest phytophagous groups. In several parts of the world lepidopteran diversity has been historically affected by human activity, in particular due to the transport of invasive species through the trade in plants and stored plant products. Most data in this regard come from Europe, where about 9,428 native species (83 families) and 97 alien species (20 families) of Lepidoptera have been recorded (Lopez-Vaamonde et al. 2010). Data on invasive insects in the neotropics are more scattered in the literature. A total of 13 insect alien species have been reported in Chile, none within the Lepidoptera (GISD 2015). As far as we know,

there has previously been no confirmed case of introduction of any elachistid species worldwide, nor any successful invasion of an Australian lepidopteran in South America.

In a recent survey for leaf-miner moths in the Atacama Desert, Arica, northern Chile, we found in abundance a species of *Elachista* Treitschke (Lepidoptera: Elachistidae) associated with the rescue grass *Bromus catharticus* Vahl (Poaceae). This plant grows spontaneously in open fields in the more humid parts of the region, including artificial habitats in suburban areas, such as in the Azapa, Lluta and Codpa valleys, where it occurs occasionally. Although native to Peru (Pinto-Escobar 1976, 1985), rescue grass has been introduced as winter forage in many warm-temperate and (sub-) tropical regions of the

world, including South and North America, Africa, Asia, Europe and Oceania (Weintraub 1953; Verloove 2012). A preliminary comparison at the gross-morphology level revealed that adults of this moth did not match the description of any elachistid species reported for the Neotropical region (for a review, see Kaila 2000 and Sruoga 2010), but did conform to those of the *Elachista freyerella* group s.l. This group is not known from the Neotropics, and members of the *synthes* complex are known only from the Australian region (Kaila 2011). Additional observations of the genital structure indicated a close match with *Elachista synthes* Meyrick. Even though Bradley (1956) established the synonymy of *E. archaeonoma* Meyrick and *E. synthes*, he did not examine the holotypes. In fact, *E. archaeonoma* does not belong strictly to the same species complex. Therefore *E. synthes* is the valid name for the species treated here (L. Kaila, personal communication).

Some leaf-miner moths, for example in the gracillariid subfamily Lithocolletinae, are invasive in many parts of the world (De Prins et al. 2013; Lees et al. 2011). This is due in part to their small size, short life cycles, high dispersal capabilities and relatively plastic use of host plants. This seems to be case for *E. synthes*, which is widespread in Australia, Tasmania and New Zealand. It accepts several grass species as host plants, including introduced grasses such as *B. catharticus* (Kaila 2011). Therefore, we decided to examine whether this *Elachista* species is present in Chile. We used a molecular approach to compare specimens from the Azapa Valley to representative species from the major lineages (=subgenera: *Atachia*, *Apheloseitia*, *Elachista*, Kaila [2011]) of *Elachista* from online databases (Bold and GenBank). A Bayesian tree was constructed based on sequences of the standard “barcoding” region (cytochrome oxidase I gene), and the results are presented here.

Materials and methods

Leaf mines of *B. catharticus* containing either larvae or pupae of *Elachista* were collected in the Azapa Valley, Arica, Chile. Some specimens were reared to the adult stage in small plastic pots under laboratory conditions, and others were stored in 95% ethanol for morphological studies and for DNA extraction. For observations of the gross morphology, adults were cleared in 10% KOH, dissected and slide-mounted using either glycerin jelly or Canada balsam; and stained

with Chlorazol Black E to reveal membranous structures. Observations were performed with the aid of a Leica M125 stereomicroscope; genitalia selected for illustration were photographed with an attached Sony® DSC-H10 digital camera. Vectorized line drawings were then made with the software CorelDraw® X4, using the corresponding digital images as a guide. Additional observations were performed using an Olympus BX51 microscope, where the selected genital structures were photographed with a Micropublisher 3.3 RTV-QImaging digital camera.

Total genomic DNA was isolated using an Invitrogen PureLink Kit. A fragment of 658 base pairs (bp) of the cytochrome oxidase I gene was amplified through a polymerase chain reaction (PCR) using primers and conditions described by Folmer et al. (1994). This region was chosen based not only on its efficiency (see Hebert et al. 2003), but mainly due to its previous successful use in discovering cryptic species within *Elachista*, as demonstrated by Mutanen et al. (2013) using the *E. bifasciella* group. Sequences from 43 specimens, representative of major lineages of *Elachista*, particularly those from the *freyerella* group within the subgenus *Elachista* (focused based on previous morphological evidence; Vargas H.A. unpub. data) were incorporated from the BOLD and GenBank databases (Table 1). We selected specimens that would maximize representative diversity within each lineage (based on Kaila 2011). A total of 30 species were surveyed. Coalescent species trees of *Elachista* were inferred using the Bayesian method through BEAST 2.01 (Bouckaert et al. 2014), based on a Yule species tree prior and the GTR+ Γ model of nucleotide substitution with four rate categories. The Markov Chain Monte Carlo (MCMC) was run for 50 million generations and repeated 10 times to test for MCMC convergence, and priors exceeded 200 to ensure effective sample sizes, which were assessed in Tracer 1.5. TreeAnnotator was used for all analyses, with 10% of the samples removed for burn-in to generate a tree that was then visualized and edited in FigTree 1.3.1 (<http://tree.bio.ed.ac.uk/software/figtree>). Genetic divergence was calculated using a pair of sequences (from Chile) based on Kimura 2-parameters (Kimura 1980), using 1000 bootstrap replications.

Dried and pinned adult vouchers were deposited as follows: 3 males, 3 females, from Azapa valley, Arica, Chile, September 2013, H.A. Vargas coll., reared from leaf-miner larvae on *Bromus catharticus* leaves, collected August 2013, deposited

Table 1. Specimens used to confirm the identity of specimens of *Elachista* from the Atacama Desert, collected in *Bromus catharticus*, based on phylogenetic reconstruction using cytochrome oxidase I (standard “barcoding”) sequences.

Subgenus	Genus	Species	Voucher	Database accession number		Reference
				GenBank	BOLD	
<i>Elachista (Atachia)</i>						
	<i>Elachista</i>	<i>catagma</i>	10ANIC-04708	HQ948479	ANICH711-10	Hebert et al. (2013)
		<i>catarata</i>	10ANIC-04502	HQ948336	ANICH505-10	Hebert et al. (2013)
		<i>deusta</i>	10ANIC-04483	HQ948317	ANICH486-10	Hebert et al. (2013)
		<i>evexa</i>	10ANIC-04822	HQ948580	ANICH825-10	Hebert et al. (2013)
		<i>gerasmia</i>	10ANIC-04672	HQ948444	ANICH675-10	Hebert et al. (2013)
		<i>melanthes</i>	10ANIC-04755	HQ948522	ANICH758-10	Hebert et al. (2013)
<i>Elachista (Elachista)</i>						
	<i>Elachista</i>	<i>abiskoella</i>	MM18594	KF809277	LEFIII167-10	Mutanen et al. (2013)
		<i>aepsera</i>	10ANIC-04727	HQ948495	ANICH730-10	Hebert et al. (2013)
		<i>synthes</i>	10ANIC-04801	HQ948561	ANICH804-10	Hebert et al. (2013)
			10ANIC-04802	HQ948562	ANICH805-10	Hebert et al. (2013)
			10ANIC-04804	HQ948564	ANICH807-10	Hebert et al. (2013)
			10ANIC-04805	JF840434	ANICH808-10	Hebert et al. (2013)
			10ANIC-04836	HQ948591	ANICH839-10	Hebert et al. (2013)
			LMCI 191-2-1	KM023733	-	This study
			LMCI 191-2-2	KM023734	-	This study
		<i>cycotis</i>	10ANIC-04711	HQ948481	ANICH714-10	Hebert et al. (2013)
		<i>commoncomme</i>	10ANIC-04946	HQ948648	ANICH949-10	Hebert et al. (2013)
		<i>linae</i>				
		<i>elaphria</i>	10ANIC-04551	HQ948365	ANICH554-10	Hebert et al. (2013)
		<i>ensifera</i>	10ANIC-04958	HQ948658	ANICH961-10	Hebert et al. (2013)
		<i>delira</i>	10ANIC-04656	HQ948429	ANICH659-10	Hebert et al. (2013)
			10ANIC-04657	HQ948430	ANICH660-10	Hebert et al. (2013)
			10ANIC-04658	HQ948431	ANICH661-10	Hebert et al. (2013)
		<i>diligens</i>	10ANIC-04731	HQ948499	ANICH734-10	Hebert et al. (2013)
		<i>dieropa</i>	10ANIC-04548	HQ948362	ANICH551-10	Hebert et al. (2013)
		<i>fucosa</i>	10ANIC-04843	HQ948594	ANICH846-10	Hebert et al. (2013)
		<i>ignicolor</i>	10ANIC-04944	HQ948646	ANICH947-10	Hebert et al. (2013)
		<i>maculicerusella</i>	MM16980	KF809375	ELACA1217-11	Mutanen et al. (2013)
		<i>ravella</i>	10ANIC-04540	HQ948360	ANICH543-10	Hebert et al. (2013)
		<i>seductilis</i>	10ANIC-04788	HQ948549	ANICH791-10	Hebert et al. (2013)
		<i>strenua</i>	10ANIC-04795	HQ948555	ANICH798-10	Hebert et al. (2013)
			10ANIC-04796	HQ948556	ANICH799-10	Hebert et al. (2013)
			10ANIC-04797	HQ948556	ANICH800-10	Hebert et al. (2013)
			10ANIC-04798	HQ948557	ANICH801-10	Hebert et al. (2013)
			10ANIC-04799	HQ948558	ANICH802-10	Hebert et al. (2013)
			10ANIC-04800	HQ948559	ANICH803-10	Hebert et al. (2013)
		<i>subnigrella</i>	MM16245	KF809219	ELACA330-10	Mutanen et al. (2013)
		<i>talgarella</i>	MM21429	KF809329	ELACA1366-12	Mutanen et al. (2013)
		<i>tanaella</i>	MM16154	JF847513	ELACA421-10	Mutanen et al. (2013)
		<i>velox</i>	10ANIC-04556	HQ948368	ANICH559-10	Hebert et al. (2013)
		<i>zophosema</i>	10ANIC-04554	HQ948367	ANICH557-10	Hebert et al. (2013)
<i>Elachista (Aphelosetia)</i>						
	<i>Elachista</i>	<i>nuraghella</i>	MM16963	JQ952638	ELACA1200-11	Mutanen et al. (2013)
		<i>subalbidella</i>	MM18154	JF854167	AAC7905	Mutanen et al. (2012)
-	<i>Stephensia</i>	<i>brunnichella</i>	MM06437	JF818793	-	Kaila et al. (2011)

at Museo Nacional de Historia Natural de Santiago (MNNC), Santiago, Chile; 2 males, 9 females, same data, deposited at Colección Entomológica de la Universidad de Tarapacá (IDEA), Arica, Chile; 4 males, 4 females, same data, July 2012, collected June 2012 (IDEA). Immature stages,

with the same collection data, were deposited in the insect collection of the Laboratório de Morfologia e Comportamento de Insetos (LMCI), of the Departamento de Zoologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil, under the following accession numbers: 9 larvae,

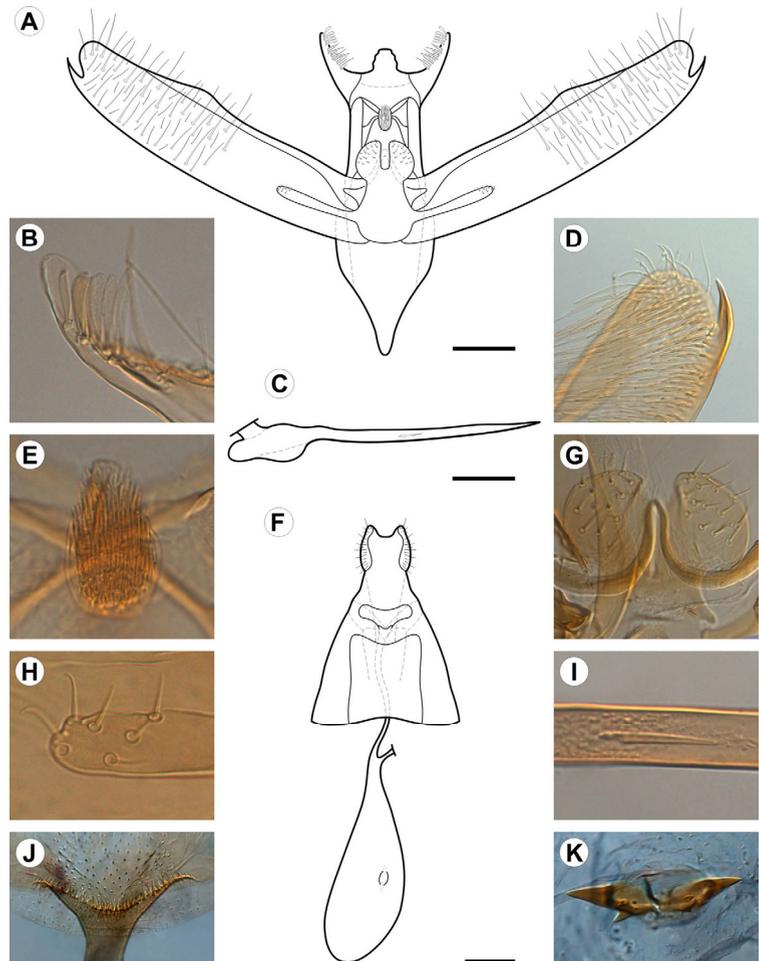


Figure 1. *Elachista synethes* genital morphology under light microscopy: (A) male genitalia, posterior view; (B) distal portion of socius, posterior view; (C) aedeagus, lateral view; (D) distal portion of valve, mesal view; (E) gnathus, posterior view; (F) female genitalia, ventral view; (G) juxta lobes, posterior view; (H) digitate process of juxta, posterior view; (I) cornutus, lateral view; (J) antrum, ventral view; (K) signum, internal view. Scale bars = 100, 100, and 200 μm , respectively.

preserved in 100% ethanol, in -20°C (LMCI 191-2); 14 larvae, fixed with Dietrich's fluid, preserved in 70% ethanol (LMCI 191-25, 26); 12 pupae, fixed in Dietrich's fluid and preserved in 75% ethanol (LMCI 191-23); 18 intact mines, fixed and preserved in 75% ethanol (LMCI 191-18). Host-plant vouchers were deposited in the herbarium of Universidad de Concepción, under accession number CONC 179889.

Results

The adults of *E. synethes* are micromoths with a maximum forewing length of 3.7 mm. The wing pattern (Figure 2B) enables easy recognition among other micromoth species of northern Chile, for both male and female, as no other member of *Elachista* is known to occur in that geographic area. However, examination of the genitalia (Figure 1) is required to reliably differentiate

this species from other members of the *synethes* complex, and also from members of the *freyerella* species group (Kaila 1999) occurring elsewhere. In contrast to other species in this group, in the male genitalia of *E. synethes* the median plate of the juxta has dorsally directed lateral pockets (Figure 1G), while in the female genitalia the antrum is typically bell-shaped (Figure 1J). A histological description of the mine and integumentary morphology of the immature stages, under both light and scanning electron microscopy, was carried out in complement and will be published elsewhere.

The Bayesian analysis of a broad section of *Elachista* spp. placed the specimens from Chile in the *freyerella* group (Figure 2A). A refined tree using only taxa from the *freyerella* group, but with more individuals, reinforced our finding that specimens from Chile belong to the same species as those from Australia: *E. synethes*

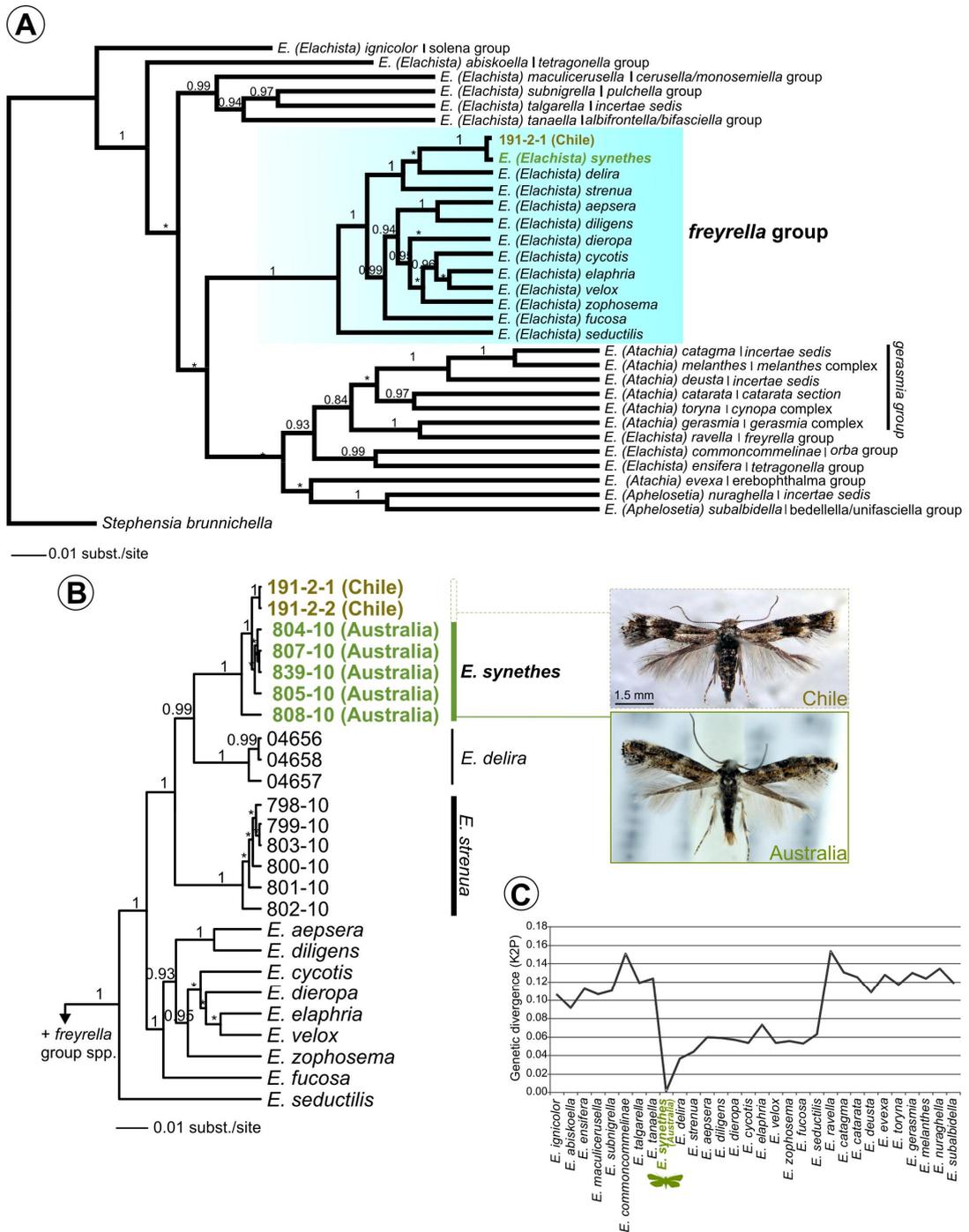


Figure 2. Evolutionary relationships of an alien species of *Elachista* in Chile. **A**, Bayesian consensus phylogeny based on 658 base pairs (bp) of the mitochondrial gene cytochrome oxidase I, including a wide range of specimens from major lineages (=subgenera *Atachia*, *Apheloseitia* and *Elachista*, see Kaila [2011]) in order to establish the position of specimens from the Atacama Desert. Blue square indicates that a Chilean specimen collected in *B. catharticus* is closely related to the *freyrella* group. **B**, unrooted Bayesian tree reconstructed with only one species from the *freyrella* group in order to characterize in detail relationships within this closely related lineage, using more individuals. Numbers above branch indicate node support (posterior probability). Asterisks represent values < 0.8. **C**, Graph depicting mean genetic divergence based on individuals of *Elachista* from Chile vs. other lineages, using the K2P method. The distance in relation to *E. synethes* from Australia is 0%.

(Figure 2B). An analysis of genetic divergence showed variation \pm standard deviation roughly from 4 ± 1 to $15\pm 1\%$ between *E. synethes* from Chile and all the other specimens, except those from Australia (Figure 2C). The pairwise distance between specimens from Chile and Australia was 0%.

Discussion

The results presented here clearly show that the elachistid specimens sequenced from the Azapa Valley are co-specific to those of *E. synethes* that have been sequenced from the Australian region. We hypothesized that by unknown means and at an unknown time, this species has been accidentally introduced to the area. We argue that because of their small size and narrow width, *E. synethes* mines excavated by early instars cannot be easily detected. The blotch-type mines of later instars are usually located on the distal portion of the leaf lamina: after the larva has exited, they resemble wilt areas and can be confused with physiological damage. Thus, their immature stages may pass unnoted by nonspecialists when they are present, for example, in potted specimens of *B. catharticus* or any other grass used as a host. In Europe, cereals coming from different sources, such as those from birdseed wastes, are recognized as imported vectors for *B. catharticus* introductions, including those associated with potted palm plants that are transported to garden centers (Verlove 2012). The existence of native populations of *B. catharticus* in Chile would facilitate prompt establishment of *E. synethes* after invasion. Importantly, more than one larva of this elachistid moth can be found in a given *B. catharticus* leaf. Also, several leaves can be attacked per plant, and many plants, even the smallest ones in a stand, can harbor their eggs, larvae and pupae at the same time. Therefore, relatively few infected plants are needed to support a substantial invasion by this species.

As mentioned above, the *Elachista freyerella* group/*synethes* complex, to which *E. synethes* belongs, is known to be endemic to the Australian region, from which the founder stock of the Chilean population likely came. However, precise location of the original invasion remains uncertain, and should be further explored by using comparatively specific gene markers at the population level (e.g. Valade et al. 2009, and references therein). The species was described from material collected in Australia. Type localities, however, do not attest to the locale of evolutionary origin of a given species. Subsequently to its description,

E. synethes has been recorded in several locations in New Zealand, Australia and Tasmania (Kaila 2011). Elachistids are small cryptic moths that are sampled relatively seldom, particularly in South America (Kaila 1999, 2000). Thus, the possibility that the Chilean stock reported here came from another, still-undetected alien population of *E. synethes* established in another part of the world cannot be ruled out.

It is also unclear whether this species will develop into a major problem in Chile, and this possibility should be examined further. *E. synethes* is a multivoltine species, with both immature and adult stages present throughout the year. This seems also to be the case for the population reported here, where up to now larvae have been found feeding only on *B. catharticus*. In the Australian region, however, it has been reared from several native and exotic grasses that are either spontaneously grown or cultivated, and also on wheat (Kaila 2011).

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