



Systematics, Morphology and Biogeography

A morphological reappraisal of the immature stages and life history of *Elachista synethes* Meyrick (Lepidoptera, Elachistidae), an Australian leaf miner alien to Chile



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ABSTRACT

Elachista synethes was recently recognized as an alien species in northern Chile, where its larvae mine the rescue grass *Bromus catharticus* (Poaceae). In order to provide the necessary information to allow field detection of *E. synethes* during early ontogeny, we conducted a morphological reappraisal of the immature stages of this leaf-miner moth, based on light and scanning electron microscopy, including the first descriptions of the egg and the first-instar larva. This is the first report of the existence of an apodal early larva for a species of *Elachista* Treitschke. The legs and prolegs are absent in the first two instars, but are well developed in the last two. Additional observations on the life history are also provided, including a description of the mine.

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Introduction

Elachistidae is a family of Gelechioidea moths, with 830 recognized species in three subfamilies, Parametriotinae, Agonoxeninae and Elachistinae (Heikkilä et al., 2014). The cryptic life mode of many of these species is among the reasons that large numbers of elachistids are still waiting to be discovered and described, even from geographic areas where micro-moth faunas have been the subject of intensive taxonomic studies (Kaila and Sippola, 2010). They are collected relatively seldom in South America (Kaila, 1999b, 2000).

Elachista Treitschke, 1833 is the most diverse genus within the Elachistinae, including more than 600 species (Kaila and Ståhls, 2006; Kaila and Sippola, 2010; Mutanen et al., 2013). In general, their larvae are leaf miners on species of Poaceae, Cyperaceae or Juncaceae (Braun, 1948; Kaila, 1999a; Baran, 2002, 2009; Sruoga, 2010). Species-level identification can be difficult, as in many cases the external appearance of the adults is extremely uniform and differences in genital morphology are subtle, resulting in the formation of apparent species complexes (Kaila and Varalda, 2004; Kaila and Sippola, 2010; Kaila, 2011a,b; Kaila and Sruoga, 2014). The genus occurs worldwide; the northern hemisphere is better known,

especially in Europe where detailed taxonomic studies have been carried out.

Kaila (2000) recognized seven species of *Elachista* in South America, five of which he described as new, and listed three others as *Incertae sedis*. Subsequently, Sruoga (2010) described five species from Ecuador, including one not named, and also reported the first Ecuadorian record of *Elachista saccharella* (Busck, 1934). Kaila (2000) mentioned two species of Elachistinae from Chile, describing both from the southern part of this country: *Elachista beltira* Kaila, 2000, also known from southern Argentina; and *Perittia morgana* Kaila, 2000. In addition, *Elachista synethes* Meyrick, 1897, with its type locality in Sydney, Australia, and also present in New Zealand and Tasmania (Kaila, 2011c), was recently recorded as an alien species in the coastal valleys of the northern Chilean Atacama Desert, mining the leaves of the rescue grass *Bromus catharticus* Vahl (Poaceae) (Gonçalves et al., 2015).

Although native to South America, *B. catharticus* has been introduced into many localities in the world as forage (Rosso et al., 2009). Its use as a host plant by *E. synethes* was previously reported from Australia, where it is an exotic plant (Common, 1990). The host plants of *E. synethes* in Australia, Tasmania and New Zealand include a wide spectrum of grasses, mostly with broad soft leaves, but the rescue grass is the most important (Kaila, 2011c). The recent discovery of *E. synethes* in hyperarid northern Chile suggests that the primary factor underlying the successful colonization was the presence of *B. catharticus* in the area (Gonçalves et al., 2015). Currently

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there are no records of pest status for *E. synethes*; however, its ability to mine leaves of rescue grass could be a threat in areas where this grass is cultivated.

Thus, accurate description of *E. synethes* is important, not only to follow its eventual population expansion in Chile, but also to detect new occurrences of this leaf-miner moth at other localities, if any. Detailed morphological characterizations of the adult stage of *E. synethes* are already available, particularly for the male and female genitalia (Common, 1990; Kaila, 2011c; Gonçalves et al., 2015). Gross morphologies of the pupa, last-instar larva and the mature mine in *Holcus mollis* L., 1759 (Poaceae) were provided by Kaila (2011c). Here we provide a reappraisal of the external morphology of the immature stages of *E. synethes*, based on light and scanning electron microscopy, including the first descriptions of the egg and early larval instars. Information on the life history is also provided, together with a detailed description of the leaf mine.

Material and methods

All the specimens used in this study were collected as either leaf-miner larvae or attached eggs and pupae, on *B. catharticus* in the Azapa Valley (18°31'S; 070°10'W) between June 2012 and August 2013. The rearing was conducted in plastic vials at room temperature, in the Departamento de Recursos Ambientales, Facultad de Ciencias Agronómicas, Universidad de Tarapacá, Arica, Chile.

For observations of the gross morphology, the specimens were cleared in 10% KOH, dissected, and slide-mounted in either glycerin jelly or Canada balsam; Chlorazol Black was used to stain membranous structures. Observations were performed with the aid of a Leica M125 stereomicroscope, where structures selected to be drawn were photographed with an attached Sony® DSC-H10 digital camera; or using an Olympus BX51 microscope, where the selected structures were photographed with a QImaging™ MicroPublisher™ 3.3 RTV-digital camera. Vectorized line drawings were then made with the software CorelDraw® X4, using the corresponding digital images as a guide. Measurements were made with

an attached ocular micrometer; unless noted, values are presented as mean ± standard deviation.

For scanning electron microscope examination, the specimens were dehydrated in a Bal-tec™ CPD 030 critical-point dryer, mounted with double-sided tape on metal stubs, and coated with gold in a Bal-tec SCD050 sputter coater. They were then examined and photographed in a JEOL JSM-5800 scanning electron microscope at the Centro de Microscopia Eletrônica (CME) of the Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, state of Rio Grande do Sul, Brazil.

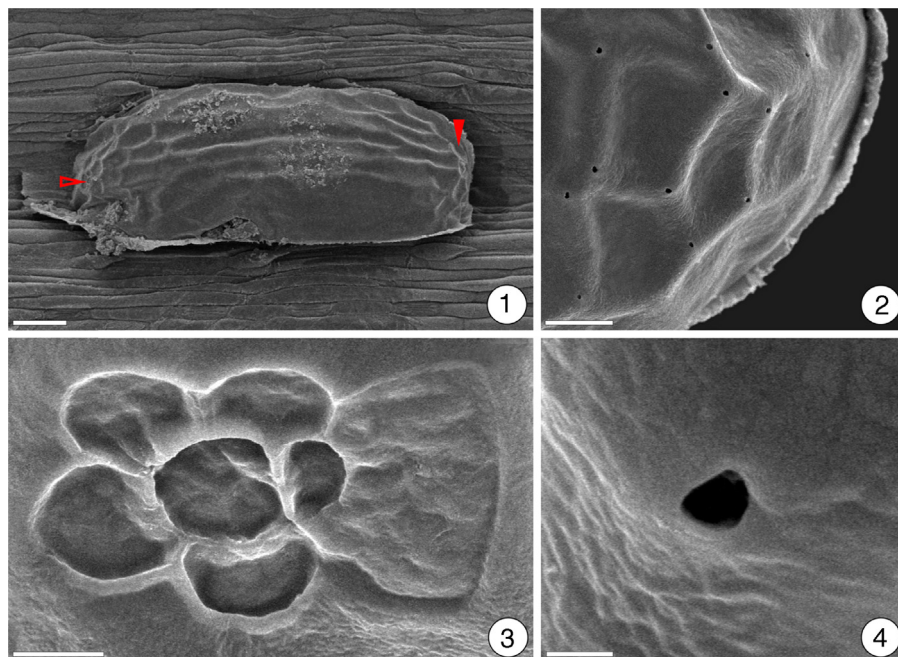
Descriptions of plant anatomy were based on diaphanized, field-collected leaf mines ($n=5$) from leaves of *B. catharticus* that were fixed in FAA (37% formaldehyde, glacial acetic acid, and 50% ethanol, 1:1:18, v/v), stained with toluidine blue (aqueous solution: 0.05 g/100 ml) and mounted either whole or in freehand section in glycerin on slides, following a procedure described in detail by Brito et al. (2012).

In addition to those vouchers with their respective collection data and accession numbers listed by Gonçalves et al. (2015), the following slide preparations should be added as material examined in the present study [all with the same data, deposited in the insect collection of the Laboratório de Morfologia e Comportamento de Insetos (LMCI), of the Departamento de Zoologia, UFRGS]: five larvae mounted in glycerin jelly (LMCI 191-25A-E), and five mature leaf mines, mounted in glycerin (LMCI 191-25F-J).

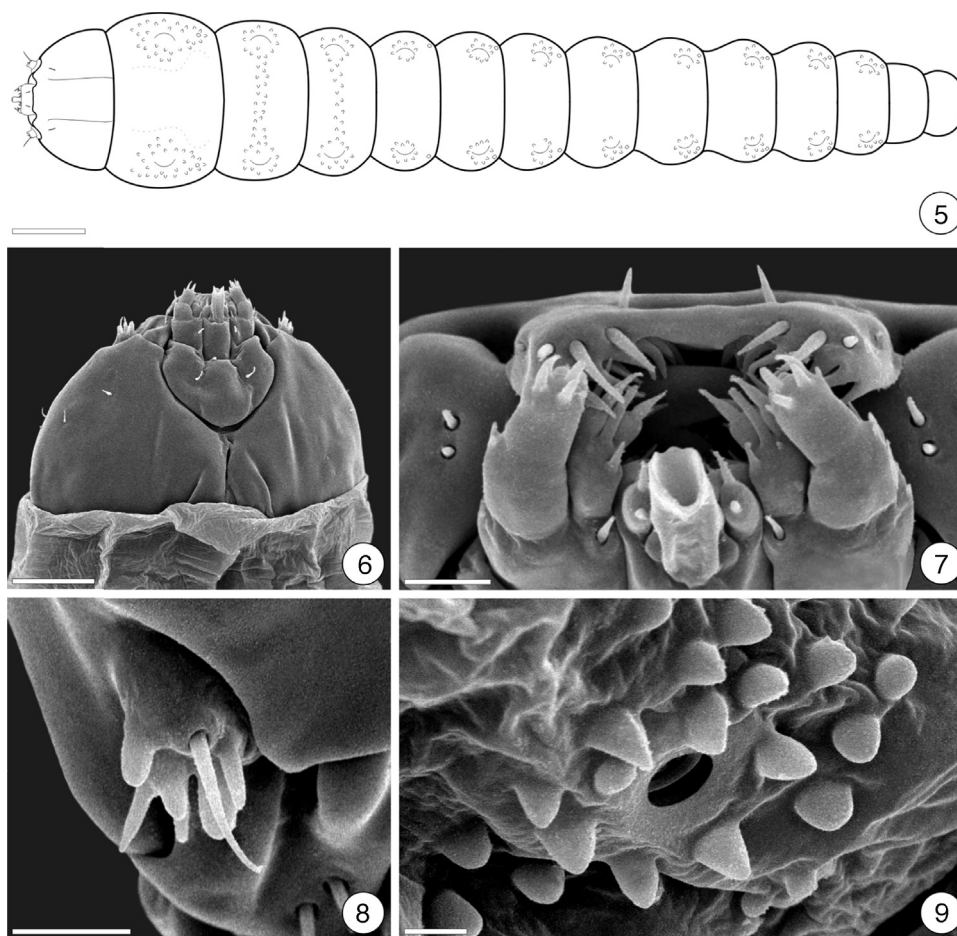
Results

Egg (Figs. 1–4)

Dimensions; $n=4$: length = 0.42 ± 0.02 mm; width = 0.19 ± 0.01 mm. Flat and elliptical, deposited with micropylar axis parallel to longitudinal leaf veins (Fig. 1); chorion translucent; surface sculptured with slightly differentiated, broad ridges, forming elongated and poorly defined cells that are longitudinally arranged (Fig. 2); circular aeropyles at intersections of ridges (Figs. 2 and 4); micropylar area with 4–5 subcircular cells (Fig. 3).



Figs. 1–4. *Elachista synethes* Meyrick, 1897. Egg: 1, dorsolateral view; 2, chorionic cells showing location of aeropyles (indicated by closed arrow in Fig. 1); 3, micropylar region (indicated by open arrow in Fig. 1); 4, aeropyle in detail. Scale bars = 50, 10, 5 and 1 μ m, respectively.



Figs. 5–9. *Elachista synethes* Meyrick, 1897. First larval instar: 5, general, dorsal view; 6, head, ventral; 7, mouth parts, ventral; 8, antenna, laterodorsal; 9, prothoracic spiracle, anterolateral. Scale bars = 100, 25, 5, 5 and 2 μm , respectively.

Larva

With two clearly different morphs: first and second instars without legs and prolegs; third and fourth instars with a more typical eruciform appearance, with well-developed legs and prolegs.

First instar (Figs. 5–9)

Dimensions ($n=4$): length = 1.03 ± 0.04 mm; head-capsule width = 0.10 ± 0.006 mm.

Head (Figs. 5–8): Prognathous, slightly flattened (Figs. 5 and 6), brown; without stemmata; with few short hair-like setae; frontoclypeus subrectangular, abruptly narrowing posteriorly. Antennae (Fig. 8) greatly reduced, bisegmented, with five sensilla on broad basal segment, two sensilla on narrow distal segment. Mouthparts of chewing type (Fig. 7); labrum bilobed, distal margin with spiniform serrations and median notch, external face with ten short hairlike setae; mandibles with two short hairlike setae on outer face, three cusps on distal margin; maxillae with galea and palpi well developed and provided with many sensilla; labium with slightly developed palpi, spinneret cylindrical with orifice at tip.

Thorax (Figs. 5 and 9): Cream-white, setae extremely reduced or absent; legs absent. Prothorax: spiracle circular, without pronounced peritreme; dorsal shield as narrow brownish band; ventral surface with pair of brown circular blotches; integument sculptured with broadly rounded coniform processes, mostly around spiracles, and forming broad transverse band on ventral surface near posterior margin; one pair of callus-like

structures laterodorsally and another pair of similar structures lateroventrally. Meso- and metathorax: without spiracles; coniform processes similar to those on prothorax, forming dorsal and ventral transverse bands; one pair of callus-like structures laterodorsally and another ventrolaterally.

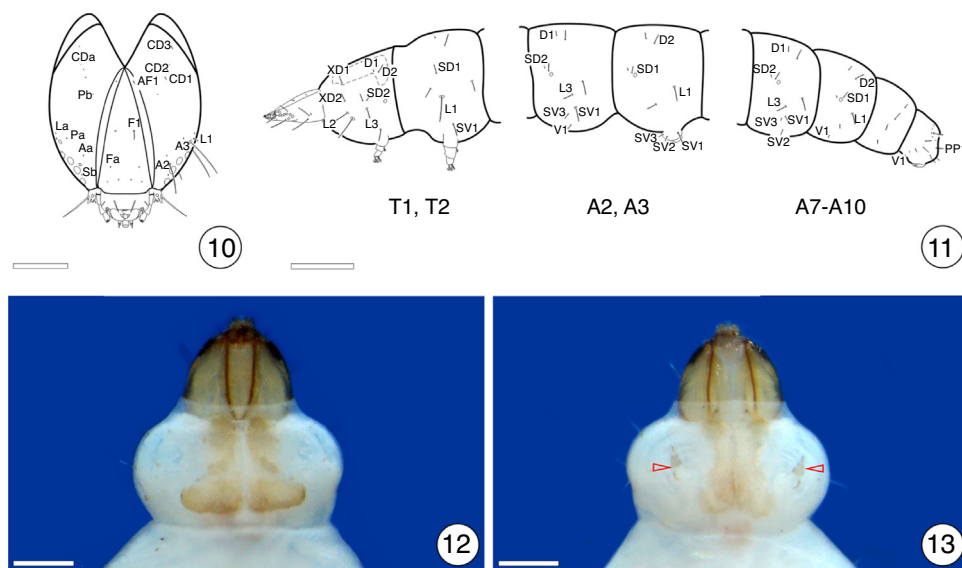
Abdomen (Fig. 5): Cream-white, setae extremely reduced or absent; prolegs absent; circular spiracles with slightly developed peritreme, laterally on A1–8; ornamentation of integument similar to that on thorax but widely scattered, except near spiracles.

Last instar (Figs. 10–25)

Dimensions ($n=6$): length = 4.81 ± 0.53 mm; head-capsule width = 0.34 ± 0.02 mm.

Head (Figs. 12–19): Prognathous, brown, slightly depressed; frontoclypeus subtriangular, with lateral sides ca. three times length of anterior side; six subcircular stemmata near lateral margin (Fig. 15); stemma 6 ventrally between setae S2 and S3, stemma 5 hidden by antenna in dorsal view, stemmata 1–4 between seta A3 and antenna. Antenna (Fig. 16) greatly reduced, bisegmented; five sensilla on broad basal segment, two sensilla on narrow distal segment. Mouthparts of chewing type (Figs. 18 and 19); labrum bilobed with ten short hairlike setae; mandibles with three cusps.

Thorax (Figs. 12–13, 17, 20–23): Cream-white, integument slightly rough, provided with hairlike setae; prothoracic dorsal shield composed of two light yellowish-brown semi-triangular plates, divided postero-longitudinally by indistinctly marked, unpigmented area (Fig. 12); one ellipsoid callus-like structure (Fig. 21) between dorsal shield and SD group; one pair of circular



Figs. 10–13. *Elachista synthes* Meyrick, 1897. Last larval instar: 10, head chaetotaxy, frontal view; 11, thoracic and abdominal chaetotaxy, lateral view; 12–13, head and prothorax, dorsal and ventral views, respectively (open arrows indicate prothoracic legs). Scale bars = 150, 300, 200 and 200 μ m, respectively.

spiracles laterally on T1; prothoracic sternum with elongated, sub-rectangular shield (Fig. 20), with lateral margins broadly excavated, and anterior margin slightly broader than posterior margin; pair of well-developed legs on each segment, each bearing one pair of flattened setae and one curved claw at apex (Fig. 23); one callus-like structure anterior to each leg.

Abdomen (Figs. 24 and 25): Cream-white, integument slightly rough, provided with hairlike setae, and anal shield slightly differentiated; one pair of circular spiracles laterally on A1–8; one pair of prolegs on segments A3–6 and A10, each with 4–6 hooklike crochets arranged in transverse bars.

Chaetotaxy of the last-instar larva (Figs. 10 and 11)

Head: F group unisetose; F1 ca. equidistant between epicranial notch and distal margin of frontoclypeus; Fa pore between F1 and distal margin of frontoclypeus; C group replaced by small pores; AF group unisetose, AF1 greatly reduced, near epicranial notch; CD group trisetose, CD1, CD2 and CD3 almost in straight line, CDa pore between CD2 and CD3; A group bisetose, A1 absent, A2 between stemmata 2 and 3 and frontoclypeus, Aa pore posteromedial to A2, A3 proximal to stemma 1; P not represented by setae, Pa pore medial to A3, Pb distomedial to CD1; L group unisetose, L1 a small seta posterolateral to A3, La pore posterior to L1; S group bisetose, S2 posterolateral to stemma 6, a pore between S2 and stemma 6, S3 between stemmata 5 and 6, Sb pore between stemmata 3 and 4; Mg group bisetose, both setae greatly reduced, near posterior margin of gena, MGa pore between MG setae.

Prothorax: D group bisetose, with D1 and D2 posterolaterally on dorsal shield; XD group bisetose, with XD1 anterolaterally on dorsal shield, XD2 ventral to XD1, outside of dorsal shield; SD group bisetose, SD1 and SD2 ca. equidistant between XD2 and spiracle; L group trisetose, L2 ventral to XD2, L3 ca. equidistant between L2 and spiracle, L1 posteroventral to L2; SV group unisetose, SV1 posteroventral to L3; V group unisetose, V1 between coxa and ventral shield; MV group bisetose, MV2 and MV3 on ventral shield, near lateral margin.

Meso- and Metathorax: D group bisetose, D2 posteroventral to D1; SD group unisetose, SD1 anteroventral to D2; L group trisetose, L2 anteroventral to SD1, L1 posteroventral to L2, L3 posterodorsal to L1; SV group unisetose, SV1 between L3 and coxa; V group unisetose, V1 medial to coxa.

Abdominal segment 1 (A1): D group bisetose, D1 greatly reduced, D2 posteroventral to D1; SD group bisetose, SD1 dorsal to spiracle, SD2 greatly reduced, anteroventral to SD1; L group bisetose, L1 posteroventral to spiracle, L3 anteroventral to L1; SV group bisetose, between L3 and V1, SV3 anterior to SV1; V group unisetose.

Abdominal segments 2, 7 (A2, 7): Similar to A1, except SD1 anterodorsal to spiracle, SD2 anteroventral to SD1, and SV3 anteroventral to SV1.

Abdominal segments 3–6 (A3–6): Similar to A2, except SV group trisetose, inserted anterolaterally on proleg, V1 on medial surface of proleg.

Abdominal segment 8 (A8): Similar to A7, except L and SV groups unisetose.

Abdominal segment 9 (A9): D, L, SV and V groups bisetose, with D1, L1, SV1 and V1 almost in straight line.

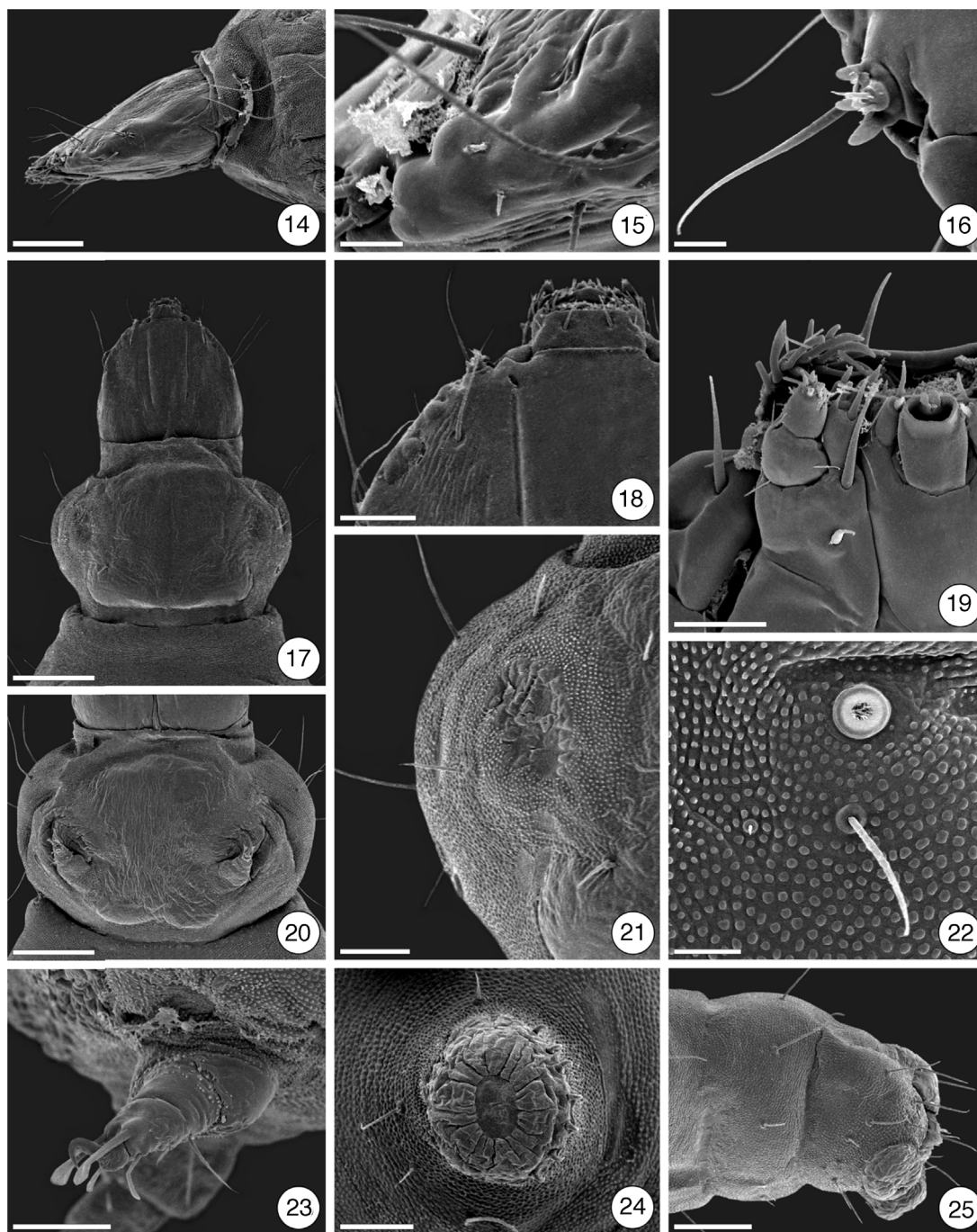
Abdominal segment 10 (A10): Provided with 10 pairs of setae with uncertain identities; one pair on anal shield, remaining nine pairs outside of dorsal shield.

Pupa (Figs. 26–40)

Dimensions ($n = 5$): length = 3.24 ± 0.23 mm. Overall dark yellowish brown (Fig. 28), occurring within a cocoon that is generally constructed on a leaf blade of *B. catharticus*.

Head (Figs. 26–31): Integument with abundant tiny punctures, almost evenly distributed; frons and eyes not separated by sutures, forming subrectangular area, anterior margin forming broad carina interrupted at middle, posterior margin slightly projected between galeae, tentorial pits near posterior margin; vertex triangular, with two tubercle-like projections anteriorly, posterior margin with conspicuous carina. Antennae narrow, elongated, reaching posterior margin of A5. Proboscis on ventral surface broad anteriorly, narrowing distally, reaching middle of A2.

Thorax (Figs. 26–29, 31–32): Integument ornamented as on head. Prothorax a narrow band between head and mesothorax. Mesothorax mostly evident by forewings, pair of broad longitudinal carinae dorsally, and dorsolateral group of longitudinally arranged tubercle-like process; tip of forewings reaching middle of A6; dorsal margin strongly sinuous between meso- and metathorax; broad longitudinal carina along middle of metathorax; hindwings mostly covered by forewings. Forelegs lateral to proboscis, not reaching



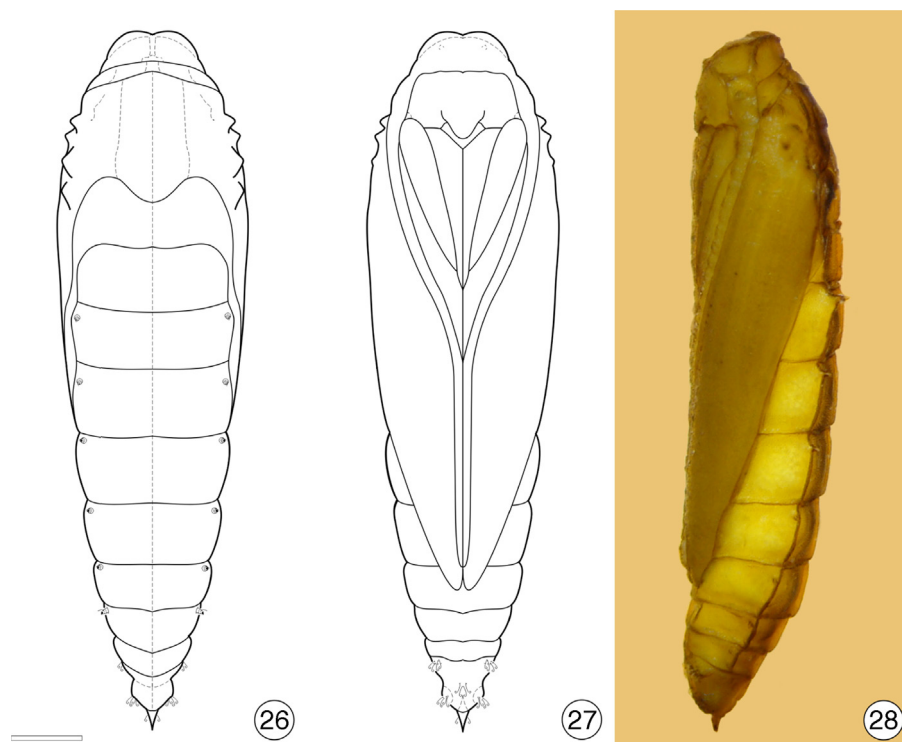
Figs. 14–25. *Elachista synthes* Meyrick, 1897. Scanning electron micrographs of last larval instar: 14, head, lateral view; 15, stemmata, lateral; 16, antenna, lateral; 17, head and prothorax, dorsal; 18, labrum and dorsal stemmata in detail, dorsal; 19, maxilla and labium, ventral; 20, prothorax, ventral; 21, detail of prothorax left portion, dorsal; 22, spiracle of abdominal segment A1, lateral; 23, prothoracic leg, posterolateral; 24, proleg of abdominal segment A4, ventral; 25, last abdominal segments, lateral. Scale bars = 100, 15, 10, 200, 50, 20, 150, 50, 20, 50, 50 and 100 μm , respectively.

proboscis tip; middle legs between forelegs and antennae, almost reaching posterior margin of A2.

Abdomen (Figs. 26–28, 33–40): Integument ornamented as on head and thorax; each segment with one dorsal longitudinal carina, one pair of dorsolateral carinae (Fig. 28), and two transverse carinae (Figs. 33 and 34), one on anterior margin, another on posterior margin; spiracles of A1 not visible, spiracles A2–7 at tip of coniform projection (Figs. 35 and 36); spiracle of A8 greatly reduced; group of elongated, hooklike setae (Figs. 37–40) ventrolaterally on broadly rounded outgrowths on A9–10, an additional group of similar setae ventrally at middle on A10; posterior half of A10 coniform (Figs. 37–39), with broadly rounded tip.

Life history (Figs. 41–47)

Eggs are deposited mostly on the adaxial surface of the leaf (Figs. 41–43). The first-instar larva bores into the leaf mesophyll through the chorionic surface adhered to the epidermis; the tip of the abdomen remains in the egg, while the cephalic portion of the larva progressively penetrates into the leaf tissues. As the larval feeding progresses, some feces are deposited into the empty sub-chorionic space (Fig. 43). Initially, the mine is narrow and straight, elongated and parallel to the leaf veins (Fig. 41), and remains restricted to the upper mesophyll portion (Fig. 46). Later on, starting with the third instar, it changes to a blotch type, as large



Figs. 26–28. *Elachista synethes* Meyrick, 1897. 26, pupa in dorsal, 27, ventral and 28, lateral views, respectively. Scale bar = 300 μ m.

numbers of cells are eaten by the larva; almost all the mesophyll is consumed, the epidermal cells and the main vascular bundles remaining mostly intact (Fig. 47). When it finishes feeding, the last-instar larva exits the mine and builds the silk cocoon on the leaf surface (Figs. 44 and 45), in which the pupa is concealed. The cocoon is white, made of flimsy silk threads that are arranged diagonally, crossing from one side of the pupa to the other; generally constructed on the upper surface of a leaf blade of *B. catharticus*.

The pupa has no silken girdle, remaining attached to the substrate by the hooklike setae of abdominal segments A9–10.

Discussion

Morphological studies dealing with immature stages of *Elachista* and other Elachistinae regularly include detailed descriptions and figures of the last-instar larva and/or pupa only (e.g., Baran, 2002, 2009, 2010; Parenti, 2005; Baran and Buszko, 2005, 2010; Kaila, 2011c). Apparently, the present contribution is the first in which the egg and the first-instar larva of a member of this lineage are examined in detail. Egg morphology has been little studied in Elachistinae, which prevents comparison of the details provided here, especially those obtained by using scanning electron microscopy. The overall shape of the egg of *E. synethes* closely fits the general pattern described for *Elachista* by Braun (1948), based on eggshells collected from the beginning of mines. However, the egg of *E. synethes* clearly differs from those of *Stephensia* Stainton, which are cone-shaped (Braun, 1948; Sruoga and Diškus, 2001).

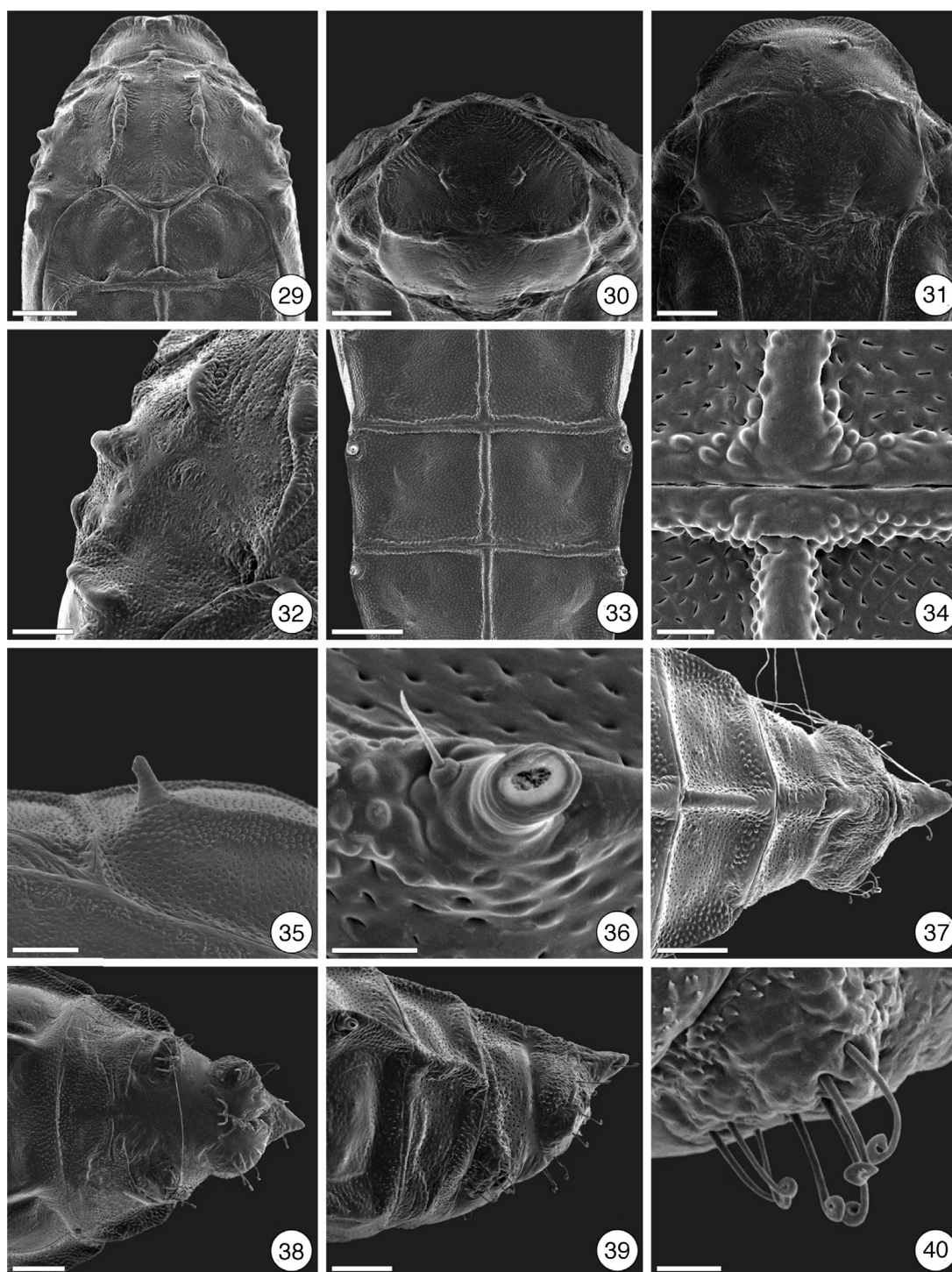
The new information presented here, in particular that related to the first instar, highlights the importance of performing morphological studies with the early life stages of elachistid moths, for comparative purposes. A remarkable characteristic in *E. synethes* is the absence of legs and prolegs in the two early larval instars, contrasting with the presence of these structures in the two later instars. As far as we know, this pattern has not been reported

elsewhere for the family. Reduction of thoracic legs has been mentioned for some elachistid species; and the absence of prothoracic legs was reported for the last instar of some Australian members of *Elachista* (Wagner, 1987; Common, 1990).

The pattern illustrated in the present study resembles the hypermetamorphic development described for some species of Bucculatricidae (e.g., Davis et al., 2002; Vargas and Moreira, 2012) and in the gracillariid *Spinivalva gaucha* Moreira and Vargas (Brito et al., 2013) whose early apodal, tissue-feeder instars are followed by later larval instars with well-developed thoracic legs and abdominal prolegs. This ontogenetic transformation in the larval stage of *E. synethes* is presumably associated with progressive changes in the mine appearance; that is, from a narrow elongated type at the beginning, to a blotch type at the end. These two larval morphotypes may represent morphological adaptations to locomotion under these different conditions within the mine, which should be further explored.

Although legs and prolegs are absent in the early larva of *E. synethes*, the thoracic and abdominal segments are provided with a pair of callus-like structures on both the terga and sterna. Also, similar structures were found between the dorsal shield and the SD group of setae on the prothorax, and anteriorly to each leg on each thoracic segment of the later instars. These have been mentioned as probable tonofibrillary platelets or other structures that facilitate movement (Kaila, 2011c). The function of these structures in the early larval instars of *E. synethes* is very likely associated with lateral anchorage to the narrow, elongated mine gallery, and deserves further attention. Additional studies in this regard should also involve comparisons with other species of *Elachista* and allied genera.

As far as we know, this is the first description of the chaetotaxy for a species of the *synethes* complex, and the information presented here may be useful in future comparative studies of this species complex. Many setae are absent on the cephalic head capsule of *E. synethes* larvae compared with those of *Stephensia*, another genus of



Figs. 29–40. *Elachista synethes* Meyrick, 1897. Scanning electron micrographs of pupa: 29, head and thorax, dorsal view; 30, head, anterior; 31, head, ventral; 32, mesothoracic lateral tubercles in detail, dorsal; 33, terga of abdominal segments A2–4, dorsal view; 34, tergal ridges in detail, dorsal; 35, spiracle of abdominal segment A2, lateral; 36, spiracle of abdominal segment A6, dorsal; 37–39, last abdominal segments, in dorsal, ventral and lateral views, respectively; 40, cremaster setae on ninth abdominal segment, lateral. Scale bars = 200, 150, 150, 100, 200, 25, 250, 20, 100, 100, 100 and 20 μm , respectively.

Elachistinae for which the chaetotaxy has been described (Baran, 2010). For instance, all setae from the C-group were replaced by pores in *E. synethes*, but C1 is present in *Stephensia*; also, setae of the P-group are absent in *E. synethes*, whereas this group is bisetose in *Stephensia*.

In relation to the thoracic and abdominal chaetotaxy of *E. synethes*, the closest pattern is found in *Elachista baltica* Hering, 1891, as described by Baran and Buszko (2005). This is interesting,

as *E. baltica* belongs to the *freyerella* species group (Baran and Buszko, 2005), in which the *synethes* complex is included (Kaila, 2011c). Apparently the D-group is absent in A10 of the two species; the D-group may be unisetose or bisetose in species belonging to other groups of *Elachista* (Baran, 2002, 2009; Baran and Buszko, 2010). However, the homology of the A10 setae should be carefully examined before any conclusion is attempted in this regard (Baran and Buszko, 2005).

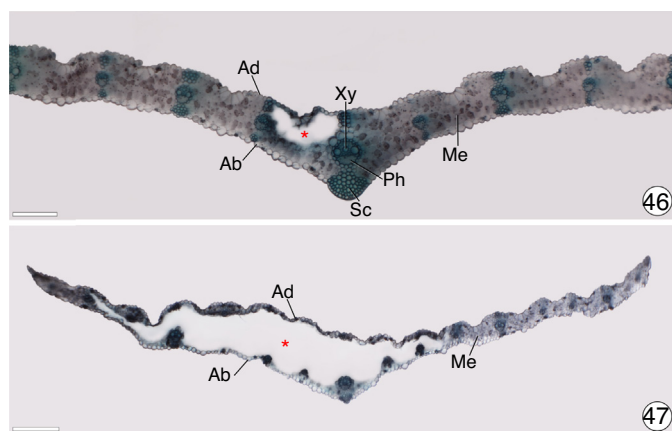


Figs. 41–45. *Elachista synethes* Meyrick, 1897. Life history: 41, mine on leaf of *Bromus catharticus*, Azapa Valley, Arica municipality, Chile (open arrows indicate empty chorion and beginning of linear section of the mine; closed arrow indicates last-instar larva visible through transparent blotch section of the mine); 42, egg on leaf upper surface; 43, young mine in detail (open and closed arrows indicate respectively the empty chorion and first-instar larva seen by transparency); 44, last-instar larva weaving the cocoon; 45, pupa seen by transparency within cocoon. Scale bars = 2, 0.5, 0.5, and 1 mm, respectively.

Although the microsetae are greatly reduced on the thorax and the abdomen of *E. synethes*, the bisetose MV group is well represented on the ventral shield of the prothorax. A similar situation for MV2 and MV3 was described for *E. baltica* by Baran and Buszko (2005) and for *Elachista irenae* Buszko, 1989 of the *bifasciella* group by Baran and Buszko (2010). According to Baran and Buszko (2010), this feature could be an apomorphy indicating a close relationship between the *freyerella* and *bifasciella* species groups. Despite the great similarity between the chaetotaxy patterns of *E. synethes* and

E. baltica, some clear differences were found. For example the XD1, D1 and D2 setae of the prothorax are located on the dorsal shield in *E. synethes*, but outside this structure in *E. baltica*; the L group of A9 is unisetose in *E. synethes* but bisetose in *E. baltica*; one pair of setae (probably SD1) is present on the anal shield of *E. synethes*, but no seta is present on that of *E. baltica*.

Similarly to many of the pupae described for other species of *Elachista* (e.g. Braun, 1948; Sugisima and Kaila, 2005), the integument is highly ornamented in *E. synethes*, mostly with tubercles



Figs. 46–47. Transverse histological sections of leaf of *Bromus catharticus*, showing the organization levels of mine of *Elachista synthes* Meyrick, 1897 in relation to larval ontogeny: 46, first instar, initial, linear section of mine; 47, last instar, final, blotch section of mine. Asterisks indicate leaf mines. **Ab** abaxial surface of epidermis; **Ad** adaxial surface of epidermis; **Me** mesophyll; **Ph** phloem; **Sc** sclerenchyma; **Xy** xylem. Scale bars = 150 and 400 μm , respectively.

and carinae. In general, the pupal morphology of *E. synthes* fits the pattern described by Patočka (1999) for the *freyerella* species group, especially regarding that of *Elachista freyerella* (Hübner, 1825). However, the pupae of these two species can be separated by some morphological features, especially those of the terminalia; for instance, hook-like setae are restricted to the A10 in *E. freyerella*, but are present not only on this abdominal segment but also on A9 in *E. synthes*; furthermore, the posterior projection of A10 is little developed in *E. freyerella*, but reaches to about midlength of the segment and has a distinctive cone-like appearance in *E. synthes*.

Conflicts of interest

The authors declare no conflicts of interest.

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References

Baran, T., 2002. *Elachista nolkeni* Šulcs, 1992: morphology and bionomics of immature stages (Gelechioidea: Elachistidae). *Nota Lepidopterol.* 25, 97–107.

- Baran, T., 2009. The immature stages of *Elachista zonulae* (Sruoga, 1992) (Lepidoptera: Elachistidae). *Entomol. Fenn.* 20, 239–244.
- Baran, T., 2010. Larval morphology of Central European species of genus *Stephensia* Stainton, 1858 (Lepidoptera, Elachistidae). *Deutsche Entomol. Z.* 57, 149–158.
- Baran, T., Buszko, J., 2005. *Elachista baltica* Hering 1891 sp. rev. – a valid species of Elachistidae from the Baltic shore (Lepidoptera: Gelechioidea). *Entomol. Fenn.* 16, 9–18.
- Baran, T., Buszko, J., 2010. Preimaginal stages and life history of *Elachista irenae* Buszko, 1989 (Insecta: Lepidoptera: Elachistidae) – a local montane moth from Central Europe. *Ital. J. Zool.* 77, 323–330.
- Braun, A.F., 1948. Elachistidae of North America (Microlepidoptera). *Mem. Am. Entomol. Soc.* 13, 1–110.
- Brito, R., Gonçalves, G.L., Vargas, H.A., Moreira, G.R.P., 2012. A new species of *Phyllocnistis* Zeller (Lepidoptera: Gracillariidae) from southern Brazil, with life-history description and genetic comparison to congeneric species. *Zootaxa* 3582, 1–16.
- Brito, R., Gonçalves, G.L., Vargas, H.A., Moreira, G.R.P., 2013. A new Brazilian *Pasiflora* leafminer: *Spinivalva gaucha* gen. n., sp. n. (Lepidoptera, Gracillariidae, Gracillariinae), the first gracillariid without a sap-feeding instar. *ZooKeys* 291, 1–26.
- Common, I.F.B., 1990. *Moths of Australia*. Melbourne University Press, Melbourne.
- Davis, D.R., Landry, B., Roque-Albelo, L., 2002. Two new neotropical species of *Bucculatrix* leaf miners (Lepidoptera: Bucculatricidae) reared from *Cordia* (Boraginaceae). *Rev. Suisse Zool.* 109, 277–294.
- Gonçalves, G.L., Moreira, G.R.P., Brito, R., Vargas, H.A., 2015. Stranger in a known land: Bayesian analysis confirms the presence of an Australian leaf miner in the Chilean Atacama Desert. *Bioinvasions Rec.* 4, 67–73.
- Heikkilä, M., Mutanen, M., Kekkonen, M., Kaila, L., 2014. Morphology reinforces proposed molecular phylogenetic affinities: a revised classification for Gelechioidea (Lepidoptera). *Cladistics* 30, 563–589.
- Kaila, L., 1999a. A revision of the Nearctic species of *Elachista* s. l. III. The *bifasciella*, *praelineata*, *saccharella* and *freyerella* groups (Lepidoptera Elachistidae). *Acta Zool. Fenn.* 211, 1–235.
- Kaila, L., 1999b. Phylogeny and classification of the Elachistidae s.s. (Lepidoptera: Gelechioidea). *Syst. Entomol.* 24, 139–169.
- Kaila, L., 2000. A review of the South American Elachistidae s. str. (Lepidoptera Gelechioidea), with descriptions of 15 new species. *Steenstrupia* 25, 159–193.
- Kaila, L., 2011a. A review of species related to *Elachista catalana* Parenti (Lepidoptera, Elachistidae: Elachistinae), with descriptions of two new species. *Entomol. Fenn.* 22, 85–96.
- Kaila, L., 2011b. On species related to *Elachista pollutella* Duponchel (Lepidoptera, Elachistidae), with descriptions of four new Palearctic species. *Entomol. Fenn.* 22, 129–139.
- Kaila, L., 2011c. Elachistine moths of Australia (Lepidoptera: Gelechioidea: Elachistinae). *Monographs on Australian Lepidoptera Series 11*. CSIRO Publishing.
- Kaila, L., Valada, P.G., 2004. The *Elachista juliensis* complex revisited (Elachistidae). *Nota Lepidopterol.* 27, 217–237.
- Kaila, L., Ståhls, G., 2006. DNA barcodes: evaluating the potential of COI to differentiate closely related species of *Elachista* (Lepidoptera: Gelechioidea: Elachistidae) from Australia. *Zootaxa* 1170, 1–26.
- Kaila, L., Sippola, L., 2010. *Elachista saarelai* sp. n. (Lepidoptera Elachistidae: Elachistinae), a new species from southern Finland. *Entomol. Fenn.* 21, 129–138.
- Kaila, L., Sruoga, V., 2014. Definition of the *Elachista pupulesis* Sruoga complex (Lepidoptera, Gelechioidea, Elachistidae), with description of a new species. *Zootaxa* 3821, 583–589.
- Mutanen, M., Kaila, L., Tabell, J., 2013. Wide-ranging barcoding aids discovery of one-third increase of species richness in presumably well-investigated moths. *Sci. Rep.* 3, 2901.
- Parenti, U., 2005. Elachistid moths of the collection Antonio Curó and the pre-imaginal stages of *Elachista adscitella* Stainton and *E. pollinariella* Zeller. *Riv. Mus. civ. Sc. Nat. E. Caffi.* 24, 3–9.
- Patočka, J., 1999. Die Puppen der mitteleuropäischen Elachistidae (Lepidoptera Gelechioidea). *Bonn. Zool. Beitr.* 48, 283–312.
- Rosso, B., Pagano, E., Rimieri, P., Ríos, R., 2009. Characteristics of *Bromus catharticus* Vahl (Poaceae) natural populations collected in the central area of Argentina. *Sci. Agric.* 66, 276–279.
- Sruoga, V., 2010. The Elachistinae (Lepidoptera: Gelechioidea: Elachistidae) of Ecuador with descriptions of five new species. *Zootaxa* 2524, 33–50.
- Sruoga, V., Diškus, A., 2001. *Stephensia brunnicella* (Lepidoptera: Elachistidae) new species for Lithuania. *Acta Zool. Lit.* 11, 73–77.
- Sugisima, K., Kaila, L., 2005. Japanese *Elachista* mining on the leaf of woody Poaceae (Lepidoptera: Elachistidae s. str.). *Entomol. Fenn.* 16, 83–102.
- Vargas, H.A., Moreira, G.R.P., 2012. A new species of *Bucculatrix* Zeller (Lepidoptera: Bucculatricidae) associated with *Baccharis salicifolia* (Asteraceae) in northern Chile. *Zootaxa* 3300, 20–33.
- Wagner, D., 1987. Elachistidae (Gelechioidea). In: Stehr, F.W. (Ed.), *Immature Insects*, vol. I. Kendall/Hunt Publishing Company, Dubuque, pp. 383–385.