



DNA barcoding and morphology support the division of *Elachista nuraghella* sensu auctorum (Lepidoptera: Elachistidae: Elachistinae) into two vicariant species

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Abstract

Elachista nuraghella sensu auct. (Lepidoptera, Elachistidae) is shown to display a striking division of haplotype groups in the DNA barcode sequence, one widely distributed in the western Mediterranean region, the other in the east: Bulgaria, Greece and Turkey. The haplotypes correspond with constant differences in both male and female genitalia, and generally also in outer appearance. *E. nuraghella* Amsel is thus considered to consist of two species displaying a vicariant distribution pattern. The eastern taxon is described as *Elachista grotenfelti* Kaila sp. nov.

Key words: barcodes, taxonomy, Elachistinae, *Elachista*, *Apheloseitia*, vicariant biogeography

Introduction

Elachista nuraghella Amsel, 1951 is a striking-looking elachistine species, being unicolorous, nearly white and, as an elachistine moth, very large-sized. It can only be confused with *E. argentella* (Clerck, 1759) with which it is not, however, known to co-exist, and *E. catalana* Parenti, 1978 which is usually a little smaller and more creamy white (see Kaila 2011a). In size it is also rivalled by *E. pollutella* Duponchel, 1843 which, unless worn, is scattered with grey scales on the forewings (cf. Kaila 2011b). *E. nuraghella* was originally described from Sardinia (Amsel, 1951), and has later been found to be widespread and often abundant in Mediterranean Europe and Turkey (Kaila 2009, and unpublished data). There are also hitherto unpublished records from Algeria and Tunisia in North Africa.

The author LK noted some variation in the genitalia of *E. nuraghella* from different areas, and samples from throughout its range were subjected to DNA barcoding. The resultant phylogram displays a deep divergence between specimens originating from the western half of Mediterranean up to Sicily and Malta in the east, and specimens originating from Bulgaria, Greece and Turkey. A closer scrutiny of male and female genitalia revealed constant differences between these populations, supporting the hypothesis that *E. nuraghella* actually consists of two species displaying a vicariant distribution pattern. The eastern taxon is here described as a new species, *Elachista grotenfelti* Kaila sp. nov.

Elachista nuraghella has been placed in the subgenus *Apheloseitia* of *Elachista* Treitschke, 1833 (Kaila 1999, Kaila & Sugisima 2011). The interrelationships within basal *Apheloseitia* are unclear; the original subdivision of *Elachista* by Traugott-Olsen & Nielsen (1977) was based on unreliable traits such as forewing colouration and wing venation (cf. Albrecht & Kaila 1997, Kaila 1997, 1999). Phenetically *E. nuraghella* is similar to *E. argentella*, as both are large species with unicolorous white forewings, and share a general similarity in the genital structures. This similarity is, however, at least in part symplesiomorphic based on the arrangements of Kaila (1999) and Kaila & Sugisima (2011): these species, as well as the *Elachista pollinariella* assemblage (Kaila, 2012) lack a y-shaped sclerotization ventrad of female papillae anales, and a dorsally projected funnel- or tongue-shaped appendix in the median plate of the male juxta (Kaila & Junnilainen 2002, Kaila 2007). A striking feature of *E. nuraghella* complex is the shape of the uncus which is very large and fused as a single lobe. The very broad valvae are also charac-

teristic and bear similarity to species of the subgenus *Dibrachia*. Members of *Dibrachia* are readily differentiated from *E. nuraghella* by their reduced uncus and by the peculiar-shaped gnathos (cf. Kaila 2005). The female ovipositor is an enlarged, sclerotized structure adapted for piercing, also reminiscent of *E. argentella* and species of *Dibrachia*. These characteristics distinguish *E. nuraghella* and *E. grotenfelti* sp. nov. also from the externally similar *E. catalana* and *E. pollutella* (cf. Kaila 2011a, b), and can be viewed by brushing the scales gently off from the tip of abdomen.

Material and methods

This paper is based on material obtained from the following collections:

MZH	Finnish Museum of Natural History, Zoology Unit, University of Helsinki, Finland (L. Kaila)
SMNK	Staatliches Museum für Naturkunde, Karlsruhe, Germany (R. Trusch)
ZMUC	Zoological Museum, University of Copenhagen, Denmark (O. Karsholt)

Specimens were also examined from the personal collections of Jari Junnilainen (Vantaa, Finland), Kari and Timo Nupponen (Espoo, Finland), Hartmut Roweck (Kiel, Germany) and Zdenko Tokár (Michalovce, Slovakia).

Terminology for anatomical structures follows Traugott-Olsen & Schmidt Nielsen (1977) and Kaila (1997, 1999, 2007). The specific epithet of the new species is a name in apposition.

A total of 39 specimens of the *E. nuraghella* complex from Turkey, Greece, Bulgaria, Italy, France, Malta and Spain were subjected to sequencing of DNA barcoding region of the mitochondrial COI gene. DNA barcodes of 33 specimens were successfully amplified and all except one of these yielded full-length barcodes (658 base pairs). Fourteen of these specimens represented *E. grotenfelti* and the remaining 19 specimens were *E. nuraghella*. DNA extraction and sequencing were performed at the Canadian Centre for DNA Barcoding largely following the protocol described in Ivanova et al. (2006). The protocol is accessible also under <http://www.dnabarcoding.ca/pa/ge/research/protocols>. Data for these samples and for one specimen of each of *E. argentella* and *E. pollinariella* Zeller, 1839 which were used as outgroups, are deposited in the BOLD database (www.boldsystems.org) in a public project “*Elachista nuraghella* complex”. Details of specimens, including Genbank accession numbers, are accessible in that project and the data is summarized in Table 1. Construction of a neighbor-joining tree and distance calculations were performed using BOLD analytical tools.

Results

The analysis of DNA barcodes of *E. nuraghella* revealed that the haplotypes are divided into two widely separate groups (Fig. 1). The minimum pairwise K2P distance between these groups is 6.63% and the maximum 7.99%. The groups follow strictly geographical division so that specimens from Turkey, Greece and Bulgaria (*E. grotenfelti*) were different from specimens from Italy, France, Spain, Malta and Tunisia (*E. nuraghella*). *E. grotenfelti* (n=14) does not show any intraspecific variation despite rather wide geographic coverage of specimens, while *E. nuraghella* (n=19) shows intraspecific variation of 0.925% a maximum. This variation follows geographic patterns. For example, the Tunisian haplotype is distinct from European haplotypes. Within the genus *Elachista*, our DNA barcode material of close to 200 Palearctic species of *Elachista* do not show any species showing less than 8.7% K2P distance to *E. nuraghella*, and we are unable to draw any conclusions of its affinities with other species of *Elachista* based on genetic data. The superficially rather similar *E. argentella* and *E. (Dibrachia) alicanta* Kaila show 10.42% and 14.53% minimum divergence to *E. nuraghella* complex, respectively, indicating that neither of these species are likely to be close relatives of the *E. nuraghella* complex.

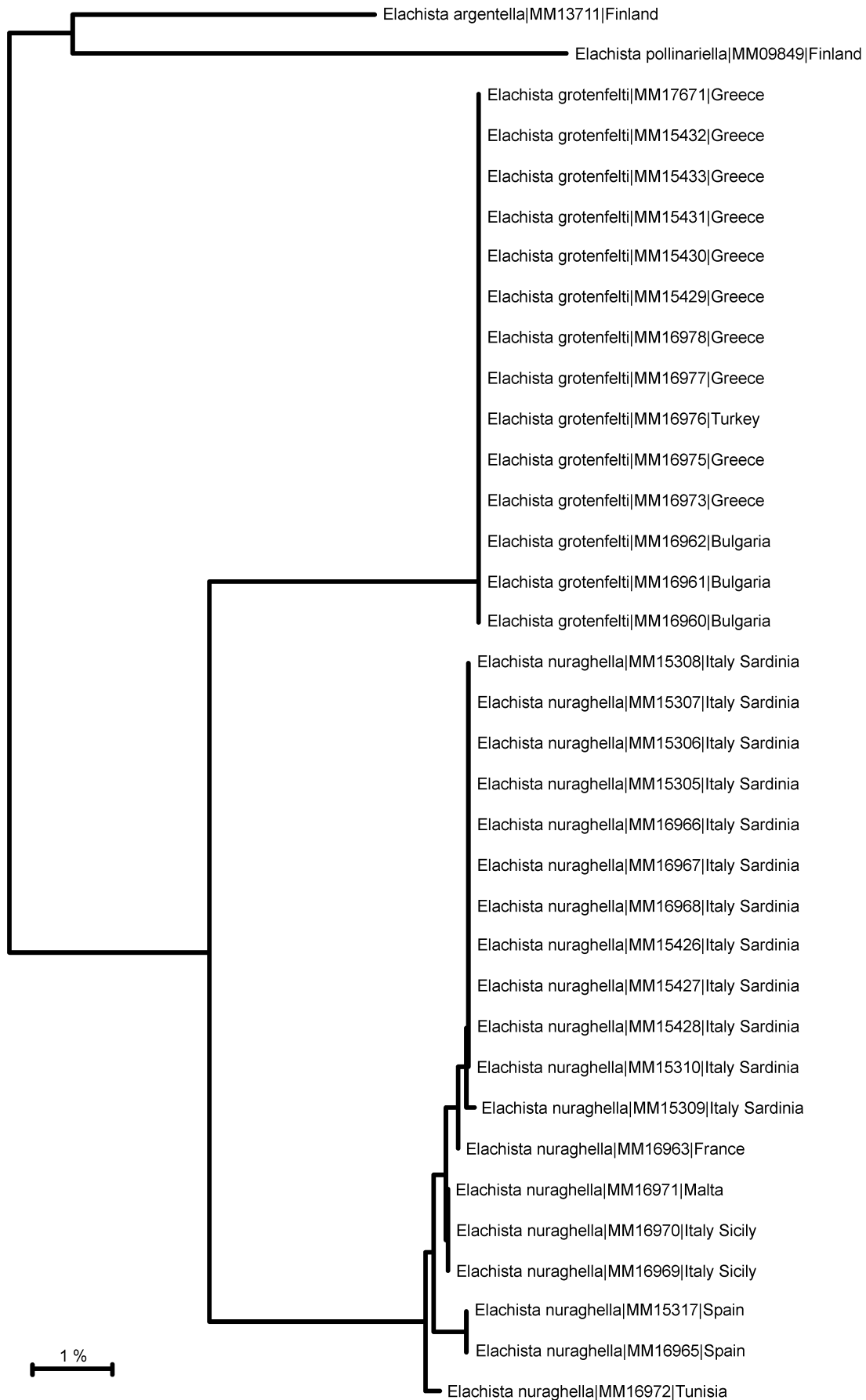


FIGURE 1. Neighbor-joining tree, implemented under the K2P model of nucleotide substitution, of studied taxa.

TABLE 1. Details of specimens used in molecular analysis of the DNA barcode region.

Sample ID	Species	Collectors	Date Collected	Country	Province	Exact Site	GenBank #
MM09849	<i>E. pollinariella</i>	Marko Mutanen	18-Jun-2008	Finland	Ka	Lappeenranta	HM874464
MM13711	<i>E. argentella</i>	M. Mutanen, P. Välimäki	2008	Finland	Ab	Dragsfjärd	HM875745
MM17671	<i>E. grotenfelti</i>	Jari Kaitila	21.-23.5.2003	Greece	Macedonia	Kozani 15 km W	JQ952624
MM15429	<i>E. grotenfelti</i>	L. Kaila, J. Kullberg	05-May-2007	Greece	Lesvos	Skala Kallonis, Malemi hotel	JF847714
MM15430	<i>E. grotenfelti</i>	L. Kaila, J. Kullberg	05-May-2007	Greece	Lesvos	Skala Kallonis, Malemi hotel	JF847715
MM15431	<i>E. grotenfelti</i>	L. Kaila, J. Kullberg	05-May-2007	Greece	Lesvos	Skala Kallonis, Malemi hotel	JF847716
MM15432	<i>E. grotenfelti</i>	L. Kaila, J. Kullberg	09-May-2007	Greece	Lesvos	Skala Kallonis, Malemi hotel	JF847717
MM15433	<i>E. grotenfelti</i>	L. Kaila, J. Kullberg	10-May-2006	Greece	Lesvos	Nr. Vadoussa	JF847718
MM16978	<i>E. grotenfelti</i>	O. Karsholt	23-May-1994	Greece	Ipiros	Konitsa area below Tymphi	JQ952625
MM16977	<i>E. grotenfelti</i>	G. Söderman	19-Mar-1994	Greece	Corfu	Ahravi	JQ952626
MM16975	<i>E. grotenfelti</i>	O. Karsholt	01-Jun-1994	Greece	Lakonia	Taygetos Mts.	JQ952628
MM16960	<i>E. grotenfelti</i>	J. Junnilainen	29-May-2006	Bulgaria		Pirin	JQ952641
MM16961	<i>E. grotenfelti</i>	J. Junnilainen	29-May-2006	Bulgaria		Pirin	JQ952641
MM16962	<i>E. grotenfelti</i>	J. Junnilainen	29-May-2006	Bulgaria		Pirin	JQ952639
MM16973	<i>E. grotenfelti</i>	J. Junnilainen	21-May-2005	Greece	Macedonia	Promahonas, 41 km NW Serres	JQ952629
MM16976	<i>E. grotenfelti</i>	T. Nupponen	08-Jun-2002	Turkey		Sultan Daglari, 30 km SW Aksehir	JQ952627
MM15305	<i>E. nuraghella</i>	K. Nupponen	24-May-2009	Italy	Sardinia	Alghero, Putifigari	JF847602
MM15306	<i>E. nuraghella</i>	K. Nupponen	26-May-2009	Italy	Sardinia	Alghero, Putifigari	JF847603
MM15307	<i>E. nuraghella</i>	K. Nupponen	26-May-2009	Italy	Sardinia	Alghero, Putifigari	JF847604
MM15308	<i>E. nuraghella</i>	K. Nupponen	26-May-2009	Italy	Sardinia	Alghero, Putifigari	JF847605
MM15309	<i>E. nuraghella</i>	K. Nupponen	26-May-2009	Italy	Sardinia	Alghero, Putifigari	JF847606
MM15310	<i>E. nuraghella</i>	K. Nupponen	26-May-2009	Italy	Sardinia	Alghero, Putifigari	JF847607
MM16966	<i>E. nuraghella</i>	K. Nupponen	21-May-2009	Italy	Sardinia	Gennargentu, Punta La Marmora	JQ952636
MM16967	<i>E. nuraghella</i>	K. Nupponen	21-May-2009	Italy	Sardinia	Gennargentu, Punta La Marmora	JQ952635
MM16968	<i>E. nuraghella</i>	K. Nupponen	21-May-2009	Italy	Sardinia	Gennargentu, Punta La Marmora	JQ952634
MM15426	<i>E. nuraghella</i>	L. Kaila, J. Kullberg	02-Jun-2008	Italy	Sardinia	Sardinia, Nuoro, Cantoni- era Cossatzu nr. Aritzo	JF847711
MM15427	<i>E. nuraghella</i>	L. Kaila, J. Kullberg	02-Jun-2008	Italy	Sardinia	Sardinia, Nuoro, Cantoni- era Cossatzu nr. Aritzo	JF847712
MM15428	<i>E. nuraghella</i>	L. Kaila, J. Kullberg	02-Jun-2008	Italy	Sardinia	Sardinia, Nuoro, Cantoni- era Cossatzu nr. Aritzo	JF847713
MM16969	<i>E. nuraghella</i>	M. Fibiger	01-May-1987	Italy	Sicily	Acate Vally, 18 km E Gela	JQ952633
MM16970	<i>E. nuraghella</i>	O. Karsholt	24-Apr-2006	Italy	Sicily	Pantano Longarini, 15 km W Portopalu	JQ952632
MM16963	<i>E. nuraghella</i>	C. Szaboky	04-May-2003	France	Korsika	25 km W. Ajaccio	JQ952638
MM16965	<i>E. nuraghella</i>	B. Skule	05-Apr-2009	Spain	Extremadura	1.3 km SW Canaweral	JQ952637
MM15317	<i>E. nuraghella</i>	T. Nupponen, K. Nupponen	24-Apr-2009	Spain	Huelva	Isla Bacuta	JF847614
MM16971	<i>E. nuraghella</i>	B. Skule	05-Apr-2004	Malta	Northern Part	Red Tower, Marfa Ridge	JQ952631
MM16972	<i>E. nuraghella</i>	ZMUC expedition	10-May-1988	Tunisia		25 km SE Ain Draham	JQ952630

Elachista nuraghella Amsel, 1951

Figs. 2, 3, 6, 7, 10, 12, 13

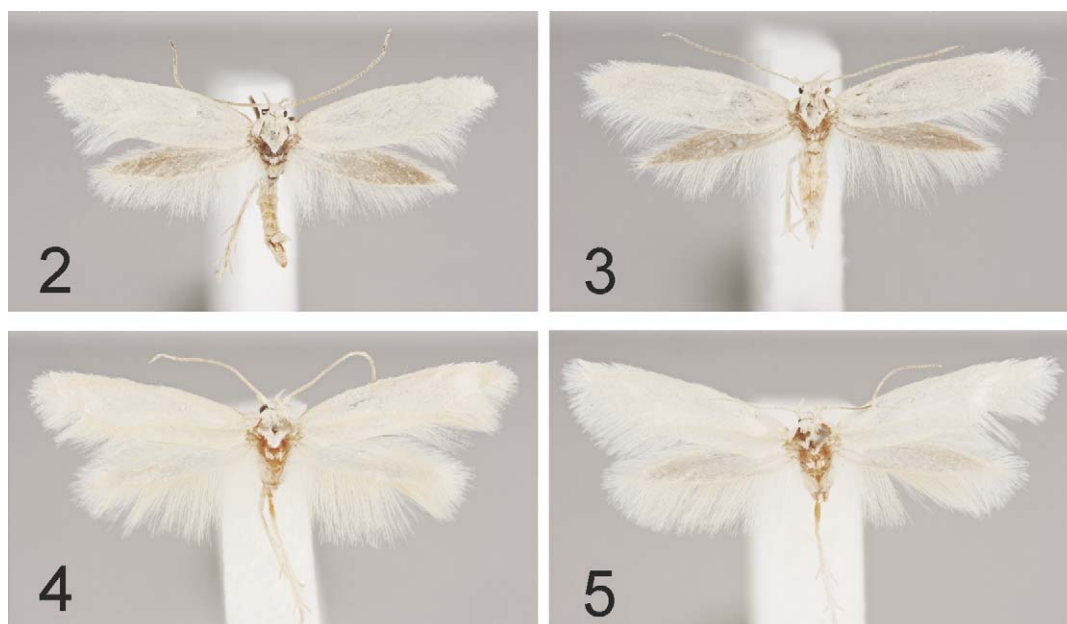
Elachista nuraghella Amsel, 1951: 140. Type locality: Italy: Sardinia, Tempio Pausania. Lectotype in LNK, designated by Parenti (1973).

Material studied. Type material. Lectotype ♂: Tempio Paus. Sardegna 15.V.1933 H. G. Amsel leg.; Prep. Nr. 5203 ♂; Paratypus leg. Amsel; Lectotypus *E. nuraghella* Amsel Teste U. Parenti 1973.; prep. genitale 273 ♂ U. Parenti 1973; ex Coll. H. G. Amsel (SMNK). Other material. **Algeria:** Prov. d'Oran "94 U. de B." 1 ♂, E. S. Nielsen prep. 1778 (Coll. ZMUC). **France:** Pyrenees orientales, Jujols, 650 m, 16.V.2004 1 ♂ J. Junnilainen leg., L. Kaila prep. 5313, DNA sample 16964 Lepid. Phyl. (Coll. Junnilainen); Corsica, 25 km W of Ajaccio, Camping les Vives 4.V.2003 Cs. Szabóky leg., DNA sample 16971 Lepid. Phyl. 16963 (Coll. Tokár). **Italy:** Italia merid. Lagi di Varano 20.V.1957 1 ♂ E. Jäckh leg. (Coll. ZMUC); Sicilia, Acate Valley, 18 km E. Cella, 50 m, 1.V.1987 1 ♂ M. Fibiger leg., L. Kaila prep. 5304 (Coll. ZMUC); Sicilia, Etna, Paterno 4.V.1987 1 ♂ M. Fibiger leg., E. Traugott-Olsen prep. B. 5.6.92 (Coll. ZMUC); Sicily, sea level, 15 km W Portopalu, Pantano Longarini 24.IV.2006 1 ♂ O. Karsholt leg., DNA sample 16970 Lepid. Phyl. (Coll. ZMUC); Sardinia, Sard. merid., Musei, 120 m, 25.IV.1972 1 ♂ Hartig leg. (Coll. ZMUC); Arizo, 1000 m, 26.–27.V.2007 2 ♂ J. Junnilainen leg. & Coll.; Prov. Nuoro 39°56'41"N 9°10'03"E Cantoniera Cossatzu, nr. Arizo 2.VI.2008 4 ♀ L. Kaila & J. Kullberg leg., L. Kaila prep. 55318, 5319, 5323, DNA samples 15426-8 Lepid. Phyl. (Coll. MZH); Sardinia 1 km S. Putifigari, 15 km E Alghero 20.V.2008 6 ♂ 1 ♀ T. Nupponen leg., L. Kaila prep. 5077 (Coll. Nupponen, 1 ♂ in MZH) 24.–26.V.2009 15 ♂ 3 ♀ K. Nupponen leg., L. Kaila prep. 5311, 5312, 5317, DNA samples 15305-9 Lepid. Phyl. (Coll. Nupponen, 1 ♂ in MZH), Sardinia, Gennargentu, 10 km E Punta la Marmora, 800 m, 21.V.2009 2 ♂ 1 ♀ K. Nupponen leg., L. Kaila prep. 5316, 5325, 5326, DNA samples 16966-8 Lepid. Phyl. (Coll. Nupponen). **Malta:** Mellieka 20.–20.V.1994 1 ♀ U. Seneca leg., L. Kaila prep 5324 (Coll. ZMUC); N. Malta 35°58'N 14°20'18"E at the Red Tower, 80 m, Marfa Ridge 5.–11.IV.2004 1 ♂ B. Skule leg., DNA sample 16971 Lepid. Phyl. (ZMUC). **Portugal:** Lisboa, Estremadura, 6.–10.V.1971 20 ♂ 1 ♀ P. Grotenfelt leg. (Coll. MZH); Algarve, pr. Bensafrim 13.V.1971 3 ♂ P. Grotenfelt & J. Kaisila leg., L. Kaila prep. 788, 789 (Coll. MZH); Algarve, pr. Lagos 13.V.1971 13 ♂ P. Grotenfelt leg. (Coll. MZH); Algarve, Praia Verde 27.V.1972 4 ♂ P. Grotenfelt leg. (Coll. MZH); Algarve, Carvoeiro 16.–17.IV.1997 5 ♂ K. Mikkola leg. (MZH); Viseu, Beira Alto 20.V.1971 1 ♂ P. Grotenfelt leg. (Coll. MZH). **Spain:** Extremadura 39°56'54"N 06°24'W 1.3 km SW Canaveral, 300 m, 5.IV. 2009 1 ♂ B. Skule leg., DNA sample 16965 Lepid. Phyl. (Coll. ZMUC); Cadiz, Castellar 26.V.1972 2 ♂ P. Grotenfelt leg. (Coll. MZH); Cadiz; Tarifa 23.V.1972 2 ♂ P. Grotenfelt leg. (Coll. MZH); Malaga, Fuengirola 19.V.1972 2 ♂ P. Grotenfelt leg. (Coll. MZH); Malaga 20 km NE Ronda 20.V.2004 8 ♂ 3 ♀ T. Nupponen leg., L. Kaila prep. 4264 (Coll. Nupponen, 1 ♀ in MZH); Huelva, Isla Bacuta 24.IV.2009 1 ♀ T. & K. Nupponen leg., L. Kaila prep. 5256, DNA sample 15317 Lepid. Phyl. (Coll. MZH); Malaga, 3 km N Calahonda 26.IV.2001 1 ♂ J. Junnilainen leg., L. Kaila prep. 3951 (Coll. Junnilainen). **Tunisia:** 25 km SE Ain Draham 10.–16.V.1988 1 ♂ exp. ZMUC leg., E. Traugott-Olsen prep. B.17.8.89, DNA sample 16964 Lepid. Phyl. 16972 (Coll. ZMUC).

Diagnosis. *Elachista nuraghella* is a large, unicolorous white or creamy white species with variably paler or darker grey hindwings (Figs 2, 3). It is externally closest to *E. argentella* and *E. catalana*, as well as to *E. grotenfelti*, described as new in this paper. Separation of *E. nuraghella* and *E. grotenfelti* is presented in the diagnosis of *E. grotenfelti*; the separation of *E. nuraghella* from other species as explained here is also applicable to identification of *E. grotenfelti* from the other species. *E. nuraghella* females are readily distinguishable from *E. catalana* by the membranous papillae anales of *E. catalana* (cf. Kaila 2011a); they are modified as strongly sclerotized and externally visible blade in *E. argentella* and *E. nuraghella*. The papillae anales of *E. argentella* are smaller than those of *E. nuraghella*, and they are distally rounded; in *E. nuraghella* the apex of papillae anales is pointed (Figs 12, 13). The ductus bursae is narrower and the corpus bursae smaller in *E. argentella* as compared to *E. nuraghella*. The male of *E. nuraghella* is usually identifiable from other species by the shape of the uncus lobes that can be examined without dissection, by carefully brushing off scales from uncus. The uncus lobes of *E. catalana* are well separate, nearly fused in *E. argentella* and entirely fused as pointed-looking apex *E. nuraghella*. The valva of *E. nuraghella* is very broad compared to other species of sg. *Apheloseitia*.

Biology. *E. nuraghella* has been reared from *Dasyphyrum villosum* (L.) P. Candargy (Poaceae) (Parenti & Varalda 1994). It appears to occupy a variety of habitats and has been observed at altitudes ranging from the sea level up to 800 m.

Distribution. France including Corsica, Italy including Sardinia and Sicilia, Malta, Portugal, Spain, Algeria, Tunisia. Records from Bulgaria, Greece and Turkey are attributable to *E. grotenfelti* sp. nov.

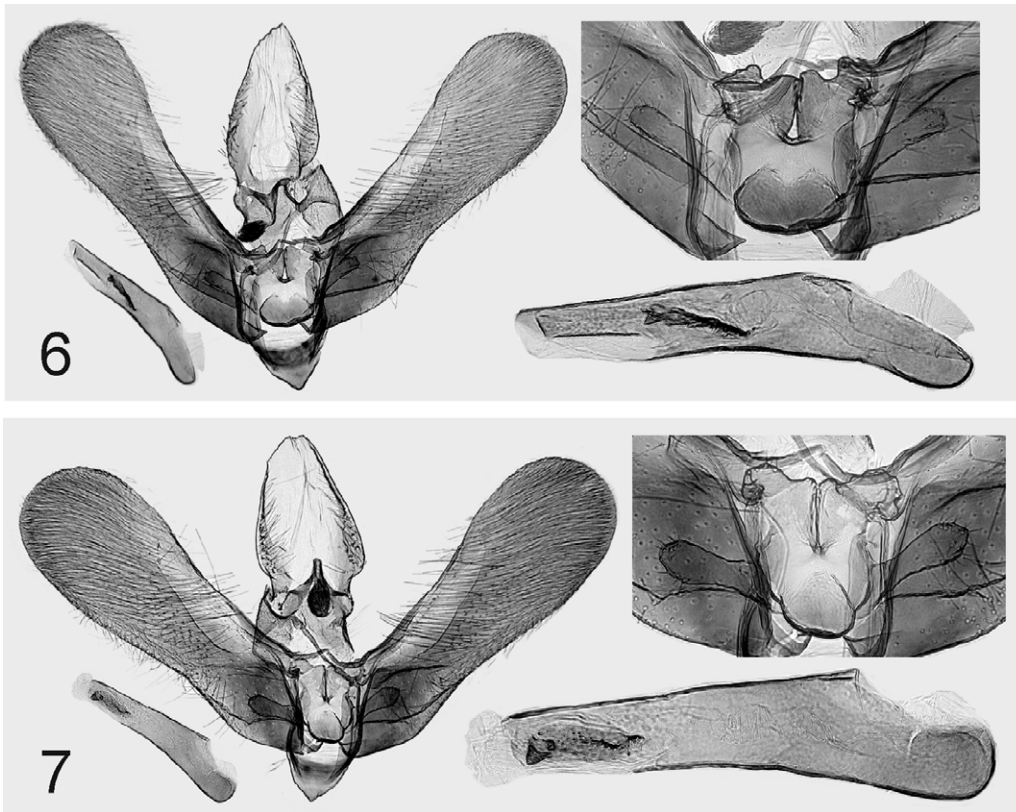


FIGURES 2–5. External appearance of *Elachista* sp. **2**, *E. nuraghella* Amsel, ♂ (Sardinia, Coll. MZH). **3**, *E. nuraghella*, ♀ (Sardinia, Coll. MZH). **4**, *E. grotenfelti* sp. nov., ♂ holotype (Greece, Lesvos, Coll. MZH). **5**, *E. grotenfelti* sp. nov., ♀ paratype (Greece, Lesvos, Coll. MZH).

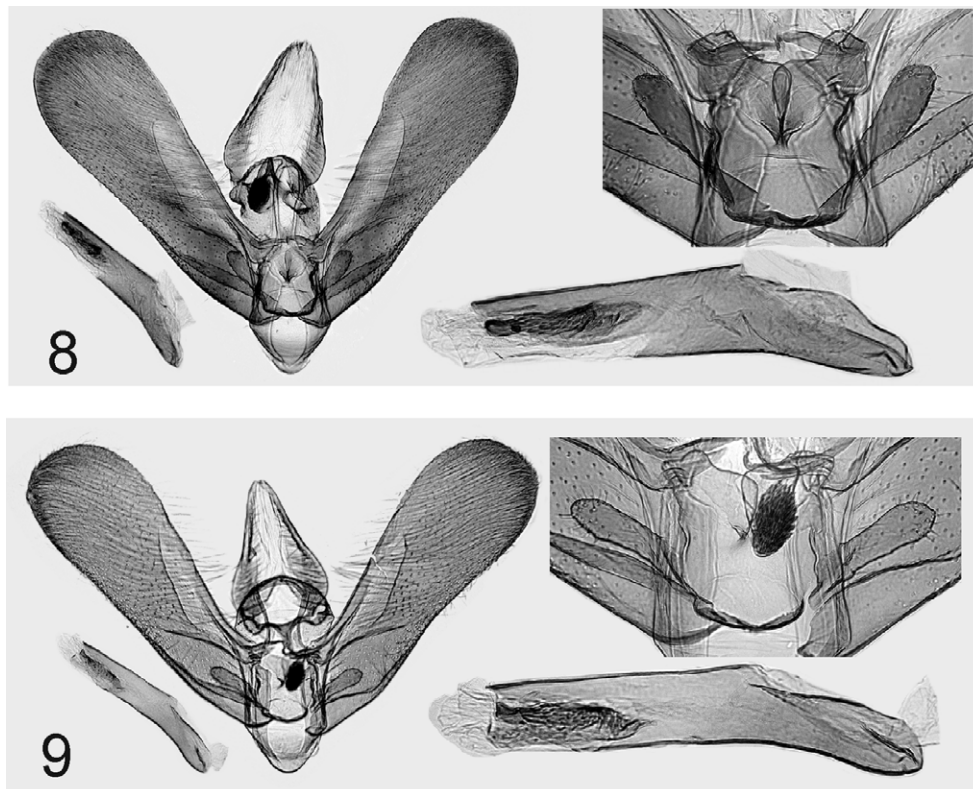
***Elachista grotenfelti* Kaila, sp. nov.**

Figs. 4, 5, 8, 9, 11, 14, 15

Type material. Holotype ♂: **Greece:** Lesvos, nr. Vadoussa, 39°15'55"N 26°04'57"E, 10.V.2006, exp. MZH, L. Kaila & J. Kullberg leg., L. Kaila prep. 5308, DNA sample 15433 Lepid. Phyl. (Coll. MZH). Paratypes (57 ♂ 19 ♀): 1 ♀ with the same collecting data as in the holotype (Coll. MZH); Lesvos, Skala Kallonis, Malemi Hotel, 39°12'3"N 26°12'13"E, 5.–9.V.2007 2 ♂ 6 ♀, L. Kaila prep. 5310, 5321, 5322, 5327, 5328, 5415, 5416, DNA samples 15429, 15430, 15432 Lepid. Phyl. (Coll. MZH); Lesvos, nr. Dafia, 39°14'37"N 26°11'11"E, 8.V.2007 1 ♂ exp. MZH, L. Kaila & J. Kullberg leg. L. Kaila prep. 5309 (Coll. MZH); Ipiros, Konitsa area below Tymphi 600–1000 m, 23.V.1994 2 ♂ 1 ♀ O. Karsholt leg. (ZMUC, 1 ♂ in MZH); Ipiros, Peristrei Mts. S. Metsovo 27.–28.V.1994 2 ♂ O. Karsholt leg. (ZMUC); Nafactus 5.VI.2010 6 ♂ J. Junnilainen leg. & Coll.; Kerkyra, Tabloni 18.V.1980 9 ♂ 1 ♀ P. Grotenfelt leg. (Coll. MZH); Kerkyra, Giannades 22.V.1980 5 ♂ P. Grotenfelt leg. (Coll. MZH); Kerkyra, Benitse 20.V.1980 1 ♂ P. Grotenfelt leg. (Coll. MZH); Kerkyra, Kinopiastes 23.V.1980 1 ♂ P. Grotenfelt leg. (Coll. MZH); Arkadia Vitina 24.VI.1981 2 ♂ P. Grotenfelt leg., L. Kaila prep. 4039, 5032, 5037 (Coll. MZH); Lakonia, Taygetos Mts. 1.VI.1994 1 ♂ 2 ♀ O. Karsholt leg., L. Kaila prep. 3592 (Coll. MZH, ZMUC); Lakonia, 5 km S. Monemvasia 16.–18.IV.1981 2 ♂ 2 ♀ B. Skule leg. (ZMUC); Prov. Makedonia, Kozani, nr. Xirolimni 3.VI.2005 1 ♂ 1 ♀ T. Nupponen leg. (Coll. Nupponen); Nr. Kozani 23.–24.V.2003 1 ♀ J. Junnilainen leg. DNA sample 16957 Lepid. Phyl. (Coll. Junnilainen); Makedonia, Mt. Olympos, 750 m, 15 km W Leptokaria 18.–21.V.2003 1 ♂ 1 ♀ J. Junnilainen leg., DNA sample 16958, 16959 Lepid. Phyl. (Coll. Junnilainen); Makedonia, Promahonas, 41 km NW Serres, 25.–26.V.2001 1 ♂ J. Junnilainen leg., L. Kaila prep. 4135, DNA sample 16973 Lepid. Phyl. (Coll. MZH); Makedonia, Xerolimni 11.VI.2010 1 ♂ J. Junnilainen leg. & Coll.; Peloponnisos, 13 km SE of Dhimitsana, 1200 m, 16.IV.1991 1 ♂ R. T. A. Schouten leg., DNA sample 16974 Lepid. Phyl. (MZH); Peloponnisos, Kalavrita 7.VI.2010 1 ♂ J. Junnilainen leg.; Corfu, Ahravi 1994 2 ♂ G. Söderman leg. DNA sample 16977 Lepid. Phyl. (MZH). **Bulgaria:** SW Bulgaria, Pirin, Sandanski, Ploski, 200–250 m, 17.–31.V.2010 2 ♂ N. Savenkov leg. (Coll. H. Roweck); Pirin, 1500 m, 29.V.2006 1 ♂ 2 ♀ J. Junnilainen leg., L. Kaila prep. 5087, 5088, DNA samples 16960-2 Lepid. Phyl. (Coll. Junnilainen). **Turkey:** Sultan Daglari, 30 km SW Aksehir, 1200 m, 8.VI.2002 12 ♂ T. Nupponen leg., L. Kaila prep. 4036, DNA sample 16974 Lepid. Phyl. 16976 (Coll. Nupponen, 1 ♂ in MZH).



FIGURES 6–7. Male genitalia of *Elachista nuraghella* Amsel. Left: general aspect of genitalia, phallus at same scale. Right top: juxta and digitate process. Right bottom: phallus as enlarged. **6**, Italy, Sardinia, L. Kaila prep. 5311. **7**, Italy, Sardinia, L. Kaila prep. 5312.



FIGURES 8–9. Male genitalia of *Elachista grotenfelti* Kaila sp. nov. Left: general aspect of genitalia, phallus at same scale. Right top: juxta and digitate process. Right bottom: phallus enlarged. **8**, holotype, L. Kaila prep. 5308. **9**, paratype, Greece: Lesvos, L. Kaila prep. 5310.

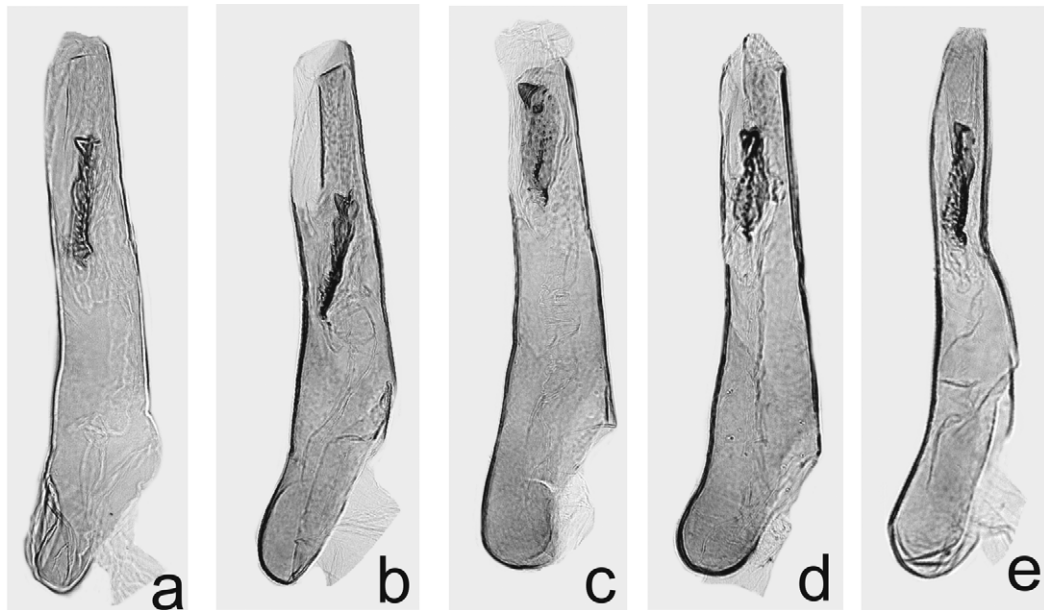


FIGURE 10. Male genitalia of *Elachista nuraghella* Amsel (phallus in lateral view). **a:** Algeria, E. Schmidt Nielsen prep. 1778; **b:** Italy: Sardinia, L. Kaila prep. 5311; **c:** Sardinia, L. Kaila prep. 5312; **d:** Italy, Sicilia, L. Kaila prep. 5304; **e:** Spain, Huelva, Isla Bacuta L. Kaila prep. 5256.

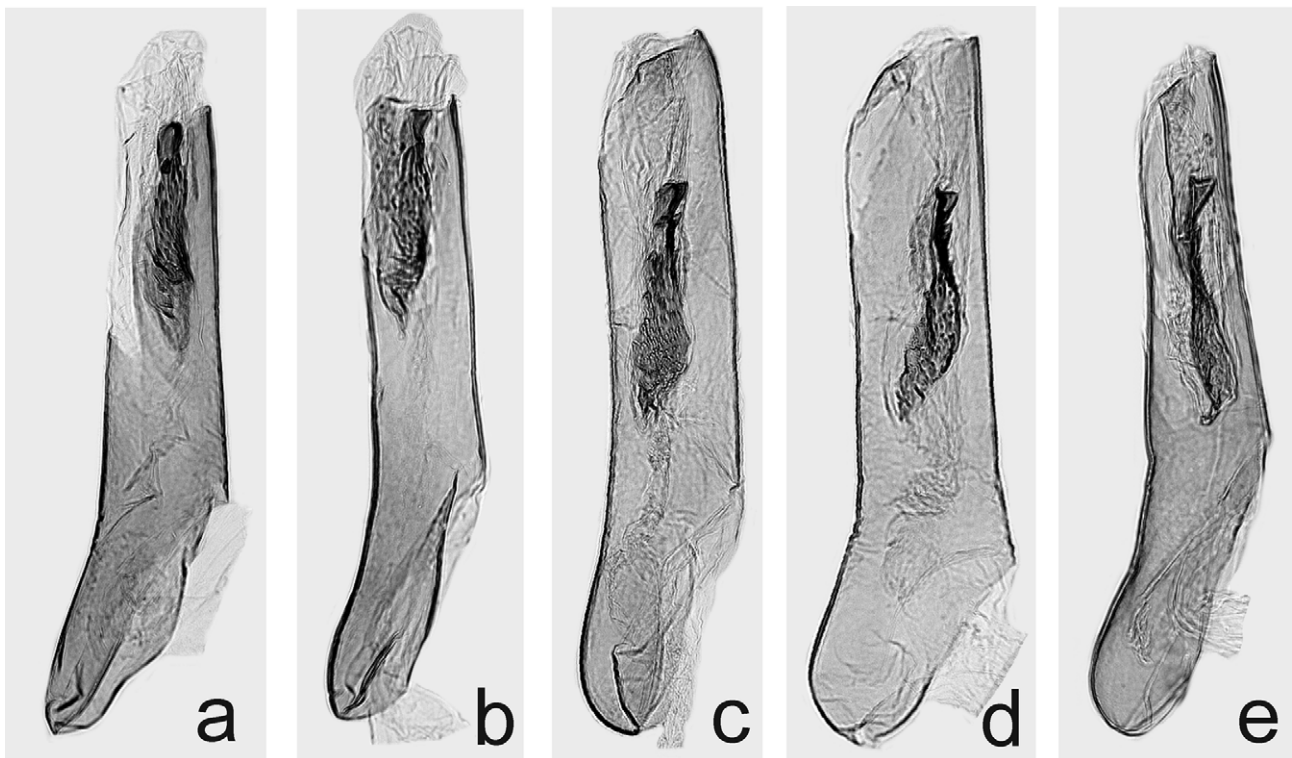
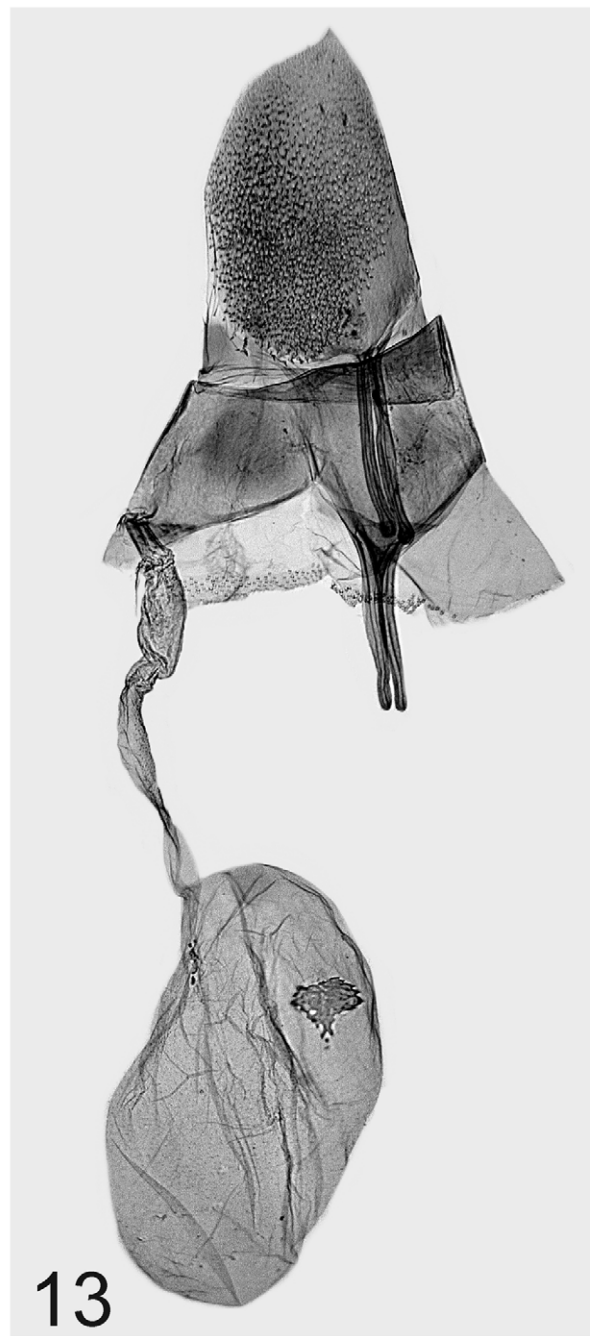


FIGURE 11. Male genitalia of *Elachista grotenfelti* Kaila sp. nov. (phallus in lateral view). **a:** holotype, Greece: Lesvos, L. Kaila prep. 5308; **b:** paratype, Greece: Lesvos, L. Kaila prep. 5310; **c:** paratype, Greece: Arkadia, Vitina, L. Kaila prep. 5032; **d:** paratype, Greece: Arkadia, Vitina, L. Kaila prep. 5037; **e:** paratype: Turkey, Sultan Daglari, L. Kaila prep. 4036.

Diagnosis. *Elachista grotenfelti* sp. n. is a close relative of *E. nuraghella*. For separation of these two species from other *Elachista*, see the diagnosis of *E. nuraghella* above. *E. grotenfelti* is externally similar to *E. nuraghella*, but it tends to be larger and usually yellowish and with paler hindwings than *E. nuraghella* (Figs 2–5). However, these characteristics vary a lot with occasional overlap between the species and therefore are not safe for species identification. These species differ in both male and female genitalia. In the male genitalia the size of the cornutus

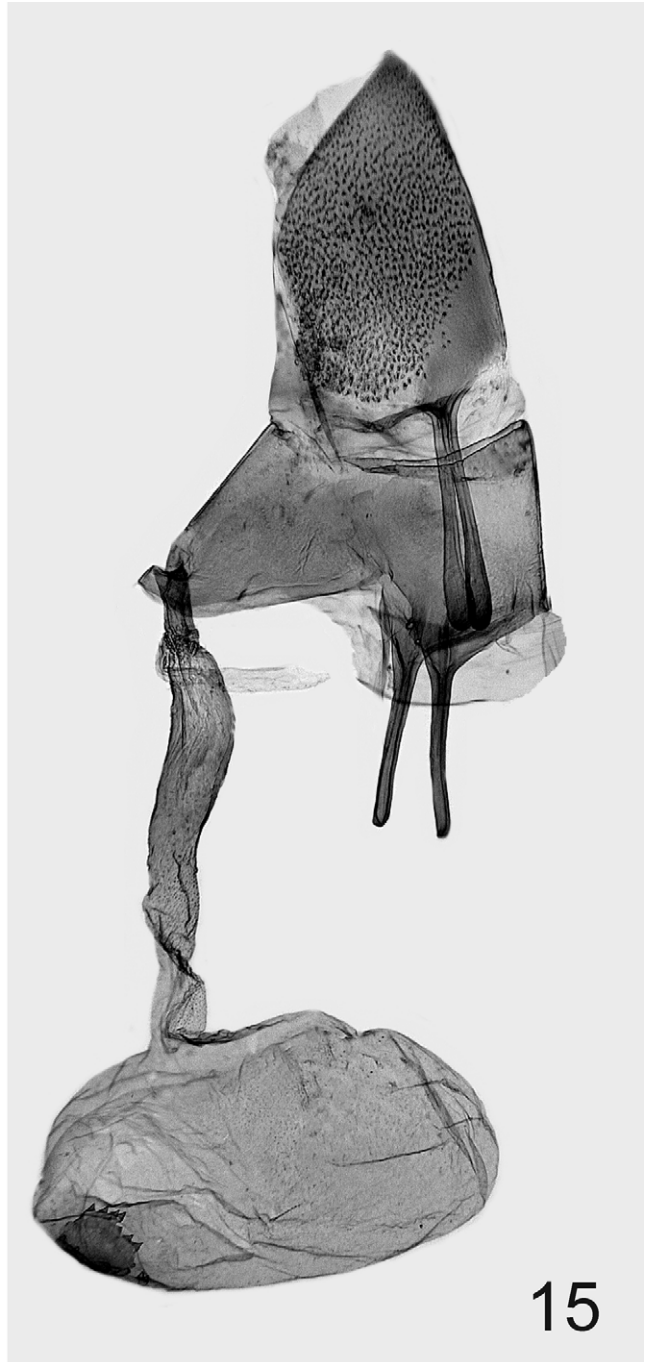
is diagnostic, being about $\frac{1}{4}$ of the length of the phallus in *E. nuraghella*, $\frac{1}{3}$ of the length of the phallus in *E. grottenfelti* (Figs 10, 11). In all dissections examined the distal horn of the cornutus is ventrally directed in *E. nuraghella*, more or less dorsally directed in *E. grottenfelti*. This trait is likely to be subject to artificial distortion and is to be used in caution. The juxta lobes are medially a little produced in *E. nuraghella*, not so in *E. grottenfelti*. While the ratio of the length of uncus and valva appears equal in these species, both these structures are somewhat more elongate in *E. nuraghella* than in *E. grottenfelti*. The female ductus bursae is thick and posteriorly dilated in *E. grottenfelti*, narrower and tubular in *E. nuraghella*. The shape of the corpus bursae also differs, being oval and its orientation transverse regarding the abdomen; the corpus bursae of *E. nuraghella* is rounded (note however, possible distortion due to preparation technique). The signum is in anterior half of the corpus bursae in *E. grottenfelti*, in posterior half in *E. nuraghella*.



FIGURES 12–13. Female genitalia of *Elachista nuraghella* Amsel, ventral view. **12**, from Spain, Malaga, L. Kaila prep. 4264. **13**, from Malta, L. Kaila prep. 5322.



14



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FIGURES 14–15. Female genitalia of *Elachista grotenfelti* Kaila sp. nov., ventral view. **14**, paratype from Greece: Lesvos, L. Kaila prep. 5328. **15**, paratype, Greece: Lesvos, L. Kaila prep. 5322.

Description. Wingspan 13–18.5 mm. Length of labial palpus 1.2 times diameter of head, creamy white. Head, neck tuft, scape and pedicel of antenna and thorax creamy white. Flagellum of antenna pale grey. Fore femur grey, legs otherwise pale ochreous. Forewing ground colour varying from snow white to yellowish white with concolorous fringe scales. Underside of forewing varying from yellowish white to grey, fringe white or yellowish white. Hindwing varying from yellowish white to grey both above and below, fringe scales white or yellowish white on both sides.

Male genitalia. Uncus lobes large, fused to form distally tapered and medially membranous single lobe that is densely setose laterobasally. Spinose knob of gnathos small and oval, attached to the mesially fused basal arms by narrow connection. Valva gradually broadened towards apex, three times as long as wide at its widest point near apex; costa straight, distally evenly bent and fused to termen without border; sacculus basally slightly bulbous,

somewhat concave in basal third, otherwise straight, distally bent and joins cucullus without border; distal margin of cucullus evenly rounded. Digitate process small, parallel-sided or distally slightly dilated, distally blunt and setose, extended to 1/9 length of valva. Mesial margin of juxta lobe straight, joining the medially concave distal margin in indistinct right angle, with a few setae, lateral margin concave; laterally joined to valval process. Median plate of juxta simple, concave, oval. Vinculum stout, broad and U-shaped. Phallus about 0.5 times as long as valva, straight, nearly parallel-sided; coecum elongate; distal opening extended to distal 2/5 of phallus; cornutus broad and elongate, dentate plate with a dorsally projected somewhat larger distal horn; length of cornutus 1/3 of the length of phallus.

Female genitalia. Papillae anales strongly sclerotized and covered by small but stout spines, 1.5 times as long as wide in lateral aspect, tapered into pointed apex. Apophyses posteriores very stout, distally widened, straight, as long as the length of sternum 8. Apophyses anteriores otherwise as apophyses posteriores, but not distally widened. Ostium bursae near anterior margin of tergum 8, small, rounded; no antrum present; colliculum sclerotized, short; ductus seminalis inserted near ostium bursae. Ductus bursae membranous, granulose, two to three times as long as apophyses, widened in caudal half; incepted in corpus bursae so that the oval-shaped corpus bursae appears in transverse position compared to the abdomen. Corpus bursae with minute internal granules, signum irregular-shaped, laterally dentate plate.

Biology. Immature stages are unknown. Adults have been collected by sweeping vegetation during night at sea level in Greece, Lesvos, and are attracted to artificial light during night. The species can be found in a variety of habitats, from sea level to the altitude of 1200 m.

Distribution. Bulgaria, Greece, Turkey.

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References

- Albrecht, A. & Kaila, L. (1997) Variation of wing venation in Elachistidae (Lepidoptera: Gelechioidea): methodology and implications to systematics. *Systematic Entomology*, 22, 185–198.
- Amsel, H. G. (1951) Lepidoptera Sardinica. Parte III. Descrizioni di specie nuove ed osservazioni sistematiche di carattere generale. *Fragmenta entomologica*, 1, 101–144.
- Clerck, C. (1759[–64]). *Icones Insectorum rariorum*. [xii] + [iii] pp., 55 pls. Holmiae.
- Duponchel, P.-A.-J. (1843) In: Godart, J.-B. 1842[–44]: *Histoire naturelle des Lépidoptères ou Papillons de France (Nocturnes, Supplément aux Tomes quatrième et suivants)*. Supplement 44, 534 pp., pls. 51–90. Paris.
- Ivanova, N. V., Dewaard, J. R., & Hebert, P. D. N. (2006) An inexpensive, automation-friendly protocol for recovering high-quality DNA. *Molecular Ecology Notes*, 6, 998–1002.
- Kaila, L. (1997) A revision of the Nearctic species of *Elachista* s. l. II. The *argentella* group (Lepidoptera, Elachistidae). *Acta Zoologica Fennica*, 206, 1–93.
- Kaila, L. (1999) Phylogeny and classification of the Elachistidae s.s. (Lepidoptera: Gelechioidea). *Systematic Entomology*, 24, 139–169.
- Kaila, L. (2005) A review of *Dibrachia* Sinev & Sruoga, 1992, a subgenus of *Elachista* (Elachistidae: Elachistinae). *Nota lepidopterologica*, 28, 139–155.
- Kaila, L. (2007) A taxonomic revision of the *Elachista bedellella* (Sircom) complex (Lepidoptera: Elachistidae: Elachistinae). *Zootaxa*, 1629, 1–25.
- Kaila, L. (2009) Fauna Europaea: Elachistidae. In Karsholt, O. & Nieukerken, E.J. van (eds.) (2009) Fauna Europaea: Lepidoptera, Moths. Fauna Europaea version 2.0, <http://www.faunaeur.org>; accessed 16th March 2012. Kaila, L. (2011a) A review of species related to *Elachista catalana* Parenti (Lepidoptera, Elachistidae: Elachistinae), with descriptions of two new species. *Entomologica Fennica*, 22, 85–96.

- Kaila, L. (2011a) A review of species related to *Elachista catalana* Parenti (Lepidoptera, Elachistidae: Elachistinae), with descriptions of two new species. *Entomologica Fennica*, 22, 85–96.
- Kaila, L. (2011b) On species related to *Elachista pollutella* Duponchel (Lepidoptera, Elachistidae), with descriptions of four new Palaearctic species. *Entomologica Fennica*, 22, 129–139.
- Kaila, L. (2012) On species related to *Elachista hedemanni* Rebel (Lepidoptera, Elachistidae), with descriptions of three new Palaearctic species. *Zootaxa*, 3316, 28–39.
- Kaila, L. & Junnilainen, J. (2002) Taxonomy and identification of *Elachista cingillella* (H.-S.) and its close relatives (Lepidoptera: Elachistidae), with descriptions of two new species. *Entomologica Fennica*, 13, 167–188.
- Kaila, L. & Sugisima, K. (2011) 1. Phylogeny, subfamily definition and generic classification. In: Kaila, L.: *Elachistine moths of Australia (Lepidoptera: Gelechioidea: Elachistidae)*. Monographs on Australian Lepidoptera, Vol 11. CSIRO Publishing, Melbourne, pp. 7–22.
- Parenti, U. (1973) Revisioni degli Elachistidi (Lepidoptera, Elachistidae) paleartici. III. Le specie di Elachistidi descritte da H. G. Amsel e F. Hartig. *Bollettino del Museo di Zoologia dell'Università di Torino*, 3, 41–58.
- Parenti, U. (1978) Nuove specie paleartiche del Genere *Elachista* Treitschke (Lepidoptera, Elachistidae). *Bollettino del Museo di Zoologia dell'Università di Torino*, 1978(4), 15–26.
- Parenti, U. & Varalda, P. G. (1994) Gli Elachistidi (Lepidoptera, Elachistidae) e loro piante ospiti. *Bollettino del Museo Regionale di Scienze Naturali – Torino*, 12, 73–136.
- Traugott-Olsen, E. & Nielsen, E. S. (1977) The Elachistidae (Lepidoptera) of Fennoscandia and Denmark. *Fauna Entomologica Scandinavica*, 6, 1–299. Klampenborg, Denmark.
- Treitschke, F. (1833) *Die Schmetterlinge von Europa*. 9 (2). 294 pp. Leipzig.
- Zeller, P. C. (1839) Versuch einer naturgemässen Eintheilung der Schaben. *Isis* (Leipzig), 1839, 167–220.