

***Agonopterix issikii* (Lepidoptera: Depressariidae), New to Korea**

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ABSTRACT

A depressariid moth, *Agonopterix issikii* Clarke, 1962, feeding on *Orixa japonica* was newly recorded from Korea. *Agonopterix issikii* can be characterized by the brownish forewing mottled dark brown and black scales that show a dentate line at the base and three minute blackish dots in triangular at the median, and the basally pale gray hindwing. *Agonopterix issikii* is similar to *A. ocellana* Fabricius in external appearance but can be distinguished by the presence of brownish forewing with three blackish triangular dots at the median. The male genitalia of *Agonopterix issikii* can be characterized by the elliptic gnathos, the triangular socius, the rectangular valva with rather straight cuiller, and the stout phallus with a short basal process and the absence of cornutus. The female genitalia can be characterized by the funnel-shaped ostium bursae, the long ducuts bursae, and the ovate corpus bursae with a rounded patch of signa. To date, 12 species of the genus *Agonopterix* have been recorded in Korea.

Keywords: Lepidoptera, Depressariidae, *Agonopterix*, *Orixa japonica*, Korea

INTRODUCTION

The family Depressariidae Meyrick, 1883, comprises over 550 species in 35 genera worldwide, but the high species diversity occurs in the forest zone of the Holarctic region (Lvovsky, 2006). The monophyly of the Depressariidae was defined based on the male genitalia and pupal structures: a spinose, lobe like gnathos in the male genitalia and the presence of lateral condyles on the abdominal segments and a paired spur group, often in swellings that may be developed to pupal legs on the 9th abdominal segment (Heikkilä et al., 2014). In Korea, the family has been insufficiently known until recently, namely, only 32 species in 12 genera were listed (NIBR, 2021).

The genus *Agonopterix* Hübner, 1825 consists of about 230 species in Depressariidae (Heikkilä et al., 2014; Becaloni et al., 2017). The monophyly of the genus was defined using the species in North America: the extra cutting teeth on the larval mandible and the quadrate signum of corpus bursae (Berenbaum and Passoa, 1999). In Korea, 11 species of *Agonopterix* were recorded (Byun et al., 2009; Park et al., 2016). This paper reports the occurrence of

Agonopterix issikii feeding on *Orixa japonica* (Rutaceae) for the first time in Korea.

MATERIALS AND METHODS

Larvae were collected in the field and reared in captivity. The newly hatched moths were preserved in a freezer and mounted for examination. For slide preparation of male and female genitalia, each specimen was prepared by boiling the abdomen in 10% KOH for approximately 20 min. The scales and tissues were removed, stained with Chlorazol black, and mounted on slides in a Euparal solution. For wingspan measurements, the distance from the tip of the left forewing to the tip of the right forewing was used.

Genomic DNA was extracted from the legs of an adult moth using the DNeasy Blood and Tissue Extraction Kit (Qiagen, UK), according to the manufacturer's instructions. The obtained gDNA, the 658-bp 5' end region of the COI (cytochrome c oxidase I) gene was amplified, using a polymerase chain reaction (PCR) method. For amplification, we used a previously described standard barcoding primer pair:

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Fig. 1. Adult of *Agonopterix issikii* in Korea. A, Male; B, Female. Scale bars: A, B= 10 mm.



Fig. 2. Head of *Agonopterix issikii* in Korea. Scale bars= 1 mm.

LCO1490, and HCO2198 (Folmer et al., 1994). The PCR was conducted using AccuPower PCR kits (Bioneer, Korea) under the following conditions: initial denaturation for 3 min at 94°C, followed by 35 cycles of 94°C for 1 min, 50°C for 30 s, and 72°C for 1 min, with a subsequent final 3 min extension at 72°C. Electrophoresis was carried out using 1 × TAE buffer on 1% agarose gel with Top Green Nucleic Acid Gel Stain (LED; Genomic Base, Korea) for 15 min at 135 V to confirm successful DNA amplification. The obtained PCR products were purified with a PCR purification kit (iNtRON, Korea) and were sequenced with forward and reverse primers (Genotech Korea, Korea). Sequences were edited manually to check ambiguous bases by applying forward and reverse primer sequences using MEGA version 10.1.7 (Kumar et al., 2018).

The DNA barcoding sequence was compared with publicly available sequence databases, such as Biotechnology In-

formation (NCBI) and GenBank, and through BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and the Barcode of Life Data (BOLD) system (<http://boldsystems.org>) (Ratnasingham and Hebert, 2007). Taxonomic data of sequence taxa, sample ID, collection information, GenBank accession, are shown in Appendix 1.

General Time Reversible model using an assumption with a discrete gamma distribution (+G) and a certain fraction of sites are evolutionarily invariable (+I) was suggested as the best model for the data and it was used during the calculations of genetic distances and phylogenetic trees. Maximum likelihood computation method was used including 500 bootstrap replications for each tree.

The terminology of the adult, including the female genitalia, refers to Buchner (2015). The specimen is now deposited in the Collection of National Institute of Biological Resources (NIBR), Incheon, and Mokpo National University, Muan.

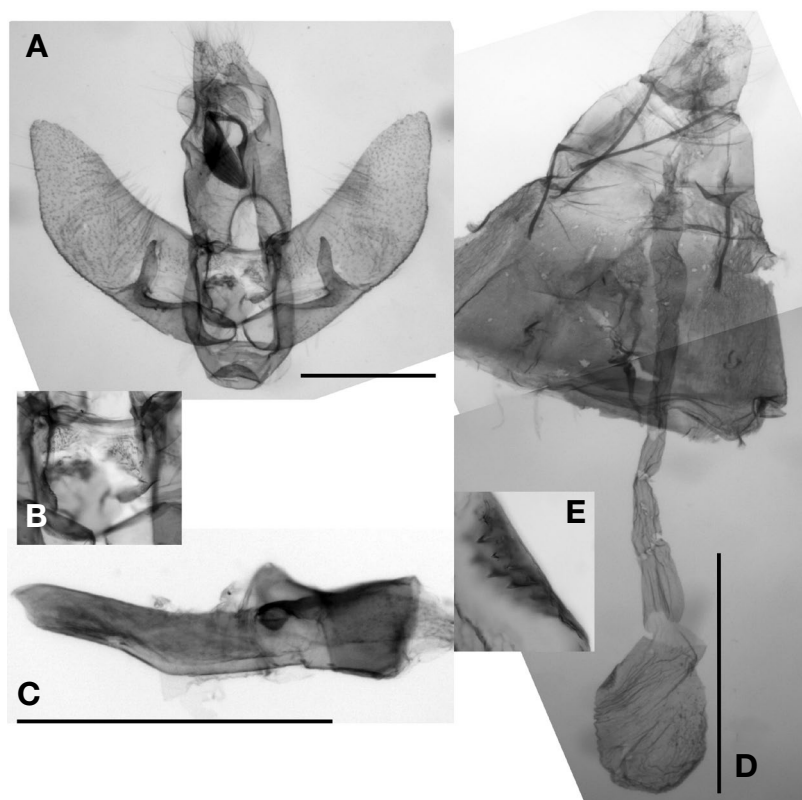


Fig. 3. Male and female genitalia of *Agonopterix issikii* in Korea. A, Male genital capsule; B, Juxta; C, Aedeagus; D, Female genitalia; E, Signum. Scale bars: A-C=0.5 mm, D=1 mm.

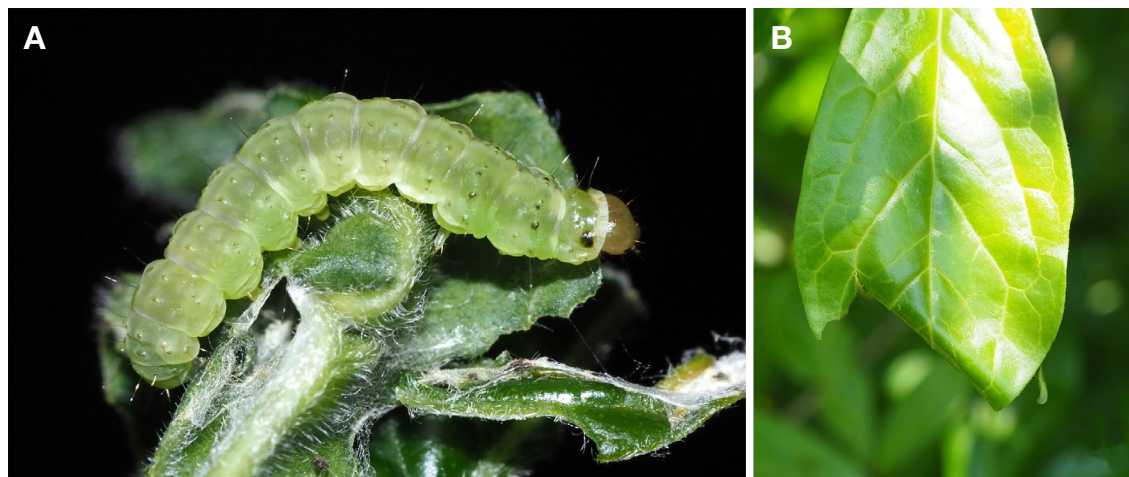


Fig. 4. Larva (A) of *Agonopterix issikii* and pupa (B) in a curled leaf.

SYSTEMATIC ACCOUNTS

Order Lepidoptera Linnaeus, 1758
Superfamily Gelechioidea Fracker, 1915
Family Depressariidae Meyrick, 1883

Subfamily Depressariinae Meyrick, 1883
Tribe Depressariini Meyrick, 1883

Genus *Agonopterix* Hübner, 1825
Type species: *Tinea signella* Hübner, 1796.

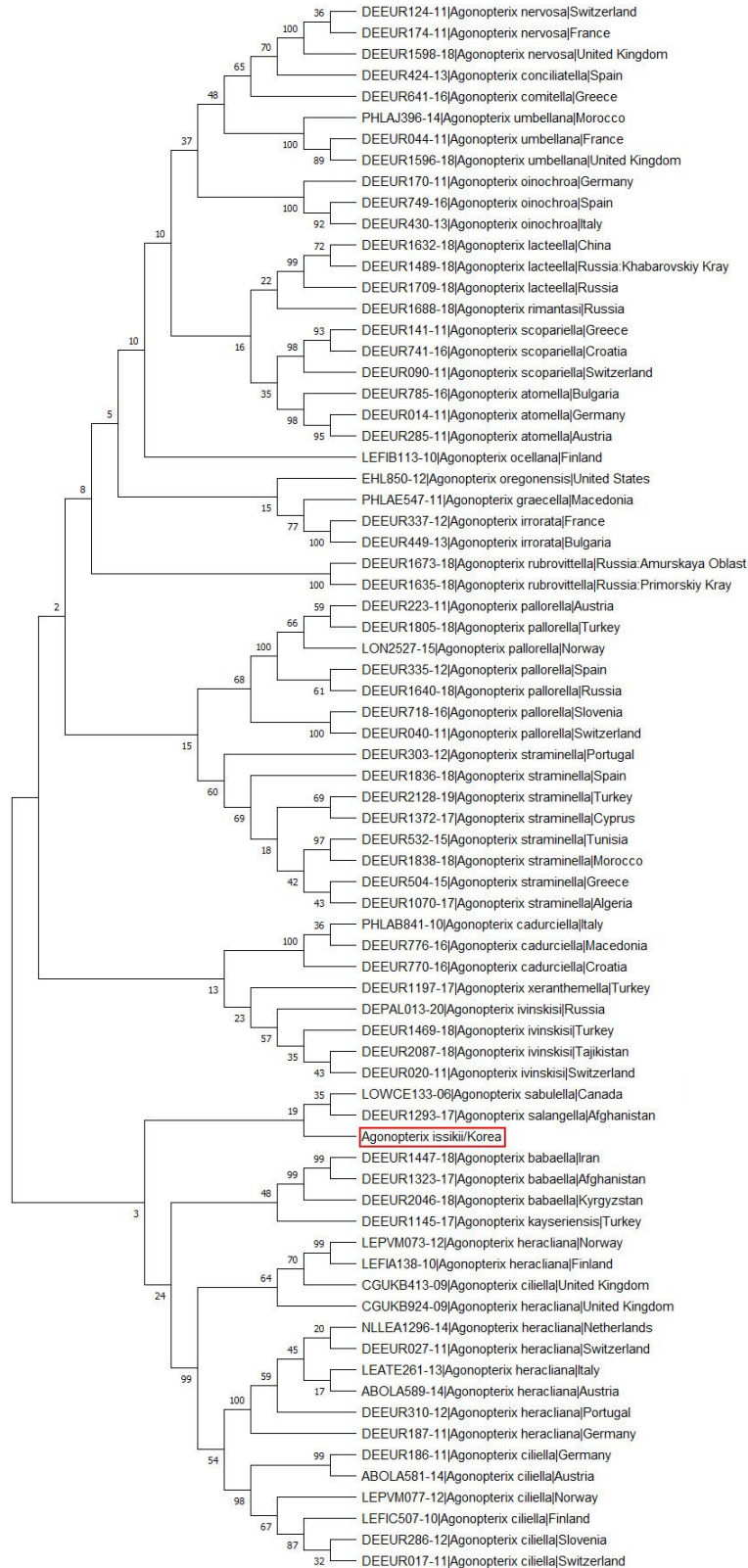


Fig. 5. A consensus tree from Maximum likelihood analysis using General Time Reversible model with a discrete gamma distribution (+G) and a certain fraction of sites are evolutionarily invariable (+I). Maximum likelihood computation method was used including 500 bootstrap replications for each tree.

¹**Agonopterix issikii* Clarke, 1962

Agonopterix issikii Clarke, 1962: 96. Type Locality: [Japan] Honshu.

Material examined. 4 males, 3 females, Korea: Gwangju, Yongyeon-dong, Youngchu fall, 35°6'20"N, 126°58'0"E, 19 Apr 2020 on larvae from *Orixa japonica* Thunb. (SY Ahn). KTSN 120000475578 (NIBR).

Diagnosis. *Agonopterix issikii* can be characterized by the brownish forewings interspersed with scattered dark brown scales that do not form a distinct pattern, except for the dentate basal line and three triangular dark brown or blackish central dots. *Agonopterix issikii* is similar to *A. ocellana* Fabricius but can be distinguished from the latter by the brownish forewing ground color with three blackish triangular dots at the median.

Description. Adults (Fig. 1). Wingspan 17–19 mm. Head brown mixed with dark brown scales, antenna dark brown, flagellum of male antenna thickened at the base and middle. Labial palpi brownish, second segment covered with long dark reddish-brown scales, third segment naked, light brownish (Fig. 2). Thorax brownish, mixed with dark brown scales; tegulae with dark reddish brown scales on outer margin. Forewings rather broad, termen slightly concave: ground color brown, intermixed with dark brown scales; dentate dark brown basal line; three blackish central dots in a triangular shape; distinct dark brown dots on termen, distinct from distal half of costa to bottom of termen. Hindwing ground color basally light gray, distally dark grayish, veins darker and visible; cilia grayish. Male genitalia (Fig. 3). Gnathos elliptic (length/width ratio about 1.5), not exceeding socii; socius almost triangular in shape, apex weakly pointed, uncus minute. Anellus lobe slender, with broad elliptic appendages on its upper margin; transtilla even width. Valva rectangular in shape with even width, apex pointed; cuiller rather straight, medially bent outward, apex pointed, not reaching the costa. Phallus rather stout (length/width ratio in lateral view about 4.4), basal process short in length, medially bent, distal process minute. Female abdomen and genitalia (Fig. 3). VIII segment width-length ration 1 : 2.6, craniad edge of sternite VIII straight. Ostium located in the middle of the sternite VIII, flanked by triangular, funnel-shaped processes placed at the connection between ostium and ductus bursae. Ductus bursae without distinct structure, thin, gradually widening close to corpus bursae. Corpus bursae ovate, diameter about 1/3 of the length of ductus bursae; a rounded patch of signa with spicules.

Immatures. Larvae were collected on 19 Apr 2020 from the leaves of *Orixa japonica* Thunb. in Gwangju. They were

reared with *Orixa japonica* and emerged from mid-May to early June 2020. Larvae can be distinguished by the grayish-green and unmarked head, and the pale green elongate body with a yellowish-white subdorsal stripe (Fig. 4). Pupa hides in a spun leaf.

DNA taxonomy. Pairwise distance analysis using 74 sequences from 26 species of *Agonopterix* indicates that *Agonopterix ivinskisii* was the nearest neighbor with *A. issikii* in 6.8% p-distance while the average distance among the total sequences was 5.13% p-distance (Fig. 5).

Distribution. Korea, Japan.

Remarks. Many species of *Agonopterix* are associated with Apiaceae, Asteraceae, and Fabaceae (Berenbaum and Passoa, 1999; Buchner, 2015; Buchner and Stănescu, 2019), while *Agonopterix issikii* feeds on *Orixa japonica*, a plant species of Rutaceae in Korea and Japan (Sakamaki, 2013).

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CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGMENTS

This work was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR 202102204).

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Korean name: ¹*삼각점큰원뿔나방 (신칭)

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Received October 2, 2021
Revised October 18, 2021
Accepted October 18, 2021

Appendix 1. Taxonomic details for sequenced data

Species	Sample ID	Sequence ID	Collector site and year	Collector(s)	GeneBank accession
<i>Agonopterix atomella</i>	BC TLMF Lep 19310	DEEUR785-16	Bulgaria, 2009	Nupponen K.	KY754269
<i>Agonopterix atomella</i>	TLMF Lep 06310	DEEUR285-11	Austria, 2010	Stark W.	KP976160
<i>Agonopterix atomella</i>	TLMF Lep 04804	DEEUR014-11	Germany, 2010	Sonderegger P.	KP976143
<i>Agonopterix babaella</i>	TLMF Lep 25946	DEEUR2046-18	Kyrgyzstan, 2015	Korb S.K.	MW126470
<i>Agonopterix babaella</i>	MFN-29197-B10	DEEUR1447-18	Iran, 1977	Thomas W.	MW126468
<i>Agonopterix babaella</i>	MFN-29134-H04	DEEUR1323-17	Afghanistan, 1975	Thomas W.	MT551921
<i>Agonopterix cadurciella</i>	BC TLMF Lep 19301	DEEUR776-16	Macedonia, 2014	Junnilainen J.	MN413883
<i>Agonopterix cadurciella</i>	TLMF Lep 01641	PHLAB841-10	Italy, 2010	Huemer P.	HQ968845
<i>Agonopterix cadurciella</i>	BC TLMF Lep 19295	DEEUR770-16	Croatia, 2014	Junnilainen J.	MN413888
<i>Agonopterix ciliella</i>	TLMF Lep 06965	DEEUR286-12	Slovenia, 2007	Pekarsky O.	
<i>Agonopterix ciliella</i>	NorBOL LepVM77	LEPVM077-12	Norway, 1958	Unknown	
<i>Agonopterix ciliella</i>	TLMF Lep 04976	DEEUR186-11	Germany, 2011	Deuring H.P.	KX042812
<i>Agonopterix ciliella</i>	MM04215	LEFIC507-10	Finland, 2006	Mutanen, T., Mutanen, M.	HM872332
<i>Agonopterix ciliella</i>	UKLB16A02	CGUKB413-09	United Kingdom, 2007	SMP 532	
<i>Agonopterix ciliella</i>	TLMF Lep 04807	DEEUR017-11	Switzerland, 2006	Sonderegger P.	KX042322
<i>Agonopterix ciliella</i>	TLMF Lep 16268	ABOLA581-14	Austria, 2014	Huemer P.	
<i>Agonopterix comitella</i>	TLMF Lep 19071	DEEUR641-16	Greece, 2000	Fibiger M.	MK566760
<i>Agonopterix conciliatella</i>	TLMF Lep 07103	DEEUR424-13	Spain, 2006	Galovic D.	MK566765
<i>Agonopterix graecella</i>	TLMF Lep 05052	PHLAE547-11	Macedonia, 2011	Huemer P. & Tarmann G. M.	KX042308
<i>Agonopterix heracliana</i>	TLMF Lep 06989	DEEUR310-12	Portugal, 2005	Corley M.	
<i>Agonopterix heracliana</i>	NorBOL LepVM73	LEPVM073-12	Norway, 1973	Unknown	
<i>Agonopterix heracliana</i>	RMNH.INS.538640	NLLEA1296-14	Netherlands, 2013	Unknown	
<i>Agonopterix heracliana</i>	TLMF Lep 11673	LEATE261-13	Italy, 2013	Huemer P.	
<i>Agonopterix heracliana</i>	TLMF Lep 04977	DEEUR187-11	Germany, 2011	Deuring H.P.	KX042743
<i>Agonopterix heracliana</i>	MM01086	LEFIA138-10	Finland, year unknown	Mutanen, M., Vaelimaeki, P.	HM396484
<i>Agonopterix heracliana</i>	UKLB21D09	CGUKB924-09	United Kingdom, 2007	NRL 0168	
<i>Agonopterix heracliana</i>	TLMF Lep 04817	DEEUR027-11	Switzerland, 2007	Sonderegger P.	KX042640
<i>Agonopterix heracliana</i>	TLMF Lep 16276	ABOLA589-14	Austria, 2014	Huemer P.	
<i>Agonopterix irrorata</i>	TLMF Lep 07016	DEEUR337-12	France, 2001	Peslier	KP976157
<i>Agonopterix irrorata</i>	TLMF Lep 07128	DEEUR449-13	Bulgaria, 2011	Ignac, R.	KP976136
<i>Agonopterix ivinskisi</i>	TLMF Lep 25987	DEEUR2087-18	Tajikistan, 1986	Ivinskis P.	MW201278
<i>Agonopterix ivinskisi</i>	NGS-29197-D08	DEEUR1469-18	Turkey, 1992	Szaboky Cs.	MW201274
<i>Agonopterix ivinskisi</i>	TLMF Lep 04810	DEEUR020-11	Switzerland, 2007	Sonderegger P.	MW201277
<i>Agonopterix ivinskisi</i>	TLMF Lep 26004	DEPAL013-20	Russia, 1926	Rjabov M.A.	
<i>Agonopterix kayseriensis</i>	TLMF Lep 21939	DEEUR1145-17	Turkey, 1999	Junnilainen J.	MT551917
<i>Agonopterix lacteella</i>	MNINGA-29197-F04	DEEUR1489-18	Russia:Khabarovskiy Kray, 1907	Korb, S.K.	MK566762
<i>Agonopterix lacteella</i>	TLMF Lep 23547	DEEUR1709-18	Russia, 2009	Dubatolov V.	MK566761
<i>Agonopterix lacteella</i>	TLMF Lep 23470	DEEUR1632-18	China, 2000	Kullberg J.	MK566763
<i>Agonopterix nervosa</i>	TLMF Lep 23341	DEEUR1598-18	United Kingdom, 2015	Buchner	MK566770
<i>Agonopterix nervosa</i>	TLMF Lep 04914	DEEUR124-11	Switzerland, 2005	Sonderegger P.	KX042206
<i>Agonopterix nervosa</i>	TLMF Lep 04964	DEEUR174-11	France, 1994	Nel J.	KX042264
<i>Agonopterix ocellana</i>	MM00461	LEFIB113-10	Finland, year unknown	Mutanen, M., Vaelimaeki, P.	HM871020
<i>Agonopterix oinochroa</i>	TLMF Lep 19179	DEEUR749-16	Spain, 2015	Palm E.	MK566769
<i>Agonopterix oinochroa</i>	TLMF Lep 07109	DEEUR430-13	Italy, 2012	Deutsch H.	KY754250
<i>Agonopterix oinochroa</i>	TLMF Lep 04960	DEEUR170-11	Germany, 2010	Sonderegger P.	KX042649
<i>Agonopterix oregonensis</i>	1209626B16Apr1999	EHL850-12	United States, 1999	Lagasa, E.	
<i>Agonopterix pallorella</i>	TLMF Lep 23453	DEEUR1805-18	Turkey, 1997	Nupponen K.	MT406265

Appendix 1. Continued

Species	Sample ID	Sequence ID	Collector site and year	Collector(s)	GeneBank accession
<i>Agonopterix pallorella</i>	TLMF Lep 07014	DEEUR335-12	Spain, 1991	Vives A.	KY754263
<i>Agonopterix pallorella</i>	TLMF Lep 19148	DEEUR718-16	Slovenia, 2012	Morandini C.	KY754236
<i>Agonopterix pallorella</i>	TLMF Lep 23478	DEEUR1640-18	Russia, 1998	Kullberg J.	MN968469
<i>Agonopterix pallorella</i>	NHMO-DAR-6238	LON2527-15	Norway, 2006	Voith, R.	
<i>Agonopterix pallorella</i>	TLMF Lep 06248	DEEUR223-11	Austria, 2010	Stark W.	MK585824
<i>Agonopterix pallorella</i>	TLMF Lep 04830	DEEUR040-11	Switzerland, 2005	Sonderegger P.	KX042642
<i>Agonopterix rimantasi</i>	TLMF Lep 23526	DEEUR1688-18	Russia, 2012	Strelzov A.N.	MK566768
<i>Agonopterix rubrovittella</i>	TLMF Lep 23473	DEEUR1635-18	Russia:Primorskiy Kray, 1998	Kullberg J.	
<i>Agonopterix rubrovittella</i>	TLMF Lep 23511	DEEUR1673-18	Russia:Amurskaya Oblast, 2014	Dubatolov V.	
<i>Agonopterix sabulella</i>	CGWC-3893	LOWCE133-06	Canada, 1994	Guppy, C.S.	MG364602
<i>Agonopterix salangella</i>	SMNK-29134-E10	DEEUR1293-17	Afghanistan, 1966	Amsel H.G.	MT551916
<i>Agonopterix scopariella</i>	TLMF Lep 19171	DEEUR741-16	Croatia, 2005	Sumpich J.	MK566767
<i>Agonopterix scopariella</i>	TLMF Lep 04880	DEEUR090-11	Switzerland, 2005	Sonderegger P.	KP976168
<i>Agonopterix scopariella</i>	TLMF Lep 04931	DEEUR141-11	Greece, 2009	Sonderegger P.	KP976151
<i>Agonopterix straminella</i>	TLMF Lep 26028	DEEUR2128-19	Turkey, 1998	Dvorak M.	MT406261
<i>Agonopterix straminella</i>	TLMF Lep 17727	DEEUR532-15	Tunisia, 2008	Lehmann L.	KY754243
<i>Agonopterix straminella</i>	TLMF Lep 06982	DEEUR303-12	Portugal, 2011	Corley M.	KP976162
<i>Agonopterix straminella</i>	TLMF Lep 17699	DEEUR504-15	Greece, 2007	Fibiger M.	KY754276
<i>Agonopterix straminella</i>	TLMF Lep 26306	DEEUR1836-18	Spain, 2016	Jeppesen G.	MN968404
<i>Agonopterix straminella</i>	TLMF Lep 26308	DEEUR1838-18	Morocco, 2016	Hviid C.	MW201265
<i>Agonopterix straminella</i>	TLMF Lep 23210	DEEUR1372-17	Cyprus, 1997	Mueller B.	MT406263
<i>Agonopterix straminella</i>	NHMW2.Lep. 0025	DEEUR1070-17	Algeria, 1929	Zerny	MT406262
<i>Agonopterix umbellana</i>	TLMF Lep 16451	PHLAJ396-14	Morocco, 2012	Meyer M.	MK566764
<i>Agonopterix umbellana</i>	TLMF Lep 23339	DEEUR1596-18	United Kingdom, 2016	Buchner	MW201280
<i>Agonopterix umbellana</i>	TLMF Lep 04834	DEEUR044-11	France, 2011	Sonderegger P.	KX042486
<i>Agonopterix xeranthemella</i>	TLMF Lep 21991	DEEUR1197-17	Turkey, 1998	Knud, L.	MK308402
<i>Agonopterix issikii</i>		MNUIG0001	South Korea, 2020	Ahn, S.	OK501165