Zootaxa 4706 (2): 201–230
https://www.mapress.com/j/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)
https://doi.org/10.11646/zootaxa.4706.2.1
http://zoobank.org/urn:lsid:zoobank.org:pub:A398B735-215D-4C11-8486-A8F78ACC5BB7

Taxonomic notes on the *Ectoedemia suberis* and *angulifasciella* species groups in Japan (Lepidoptera: Nepticulidae)

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Abstract

Diagnoses and notes on biology and distribution of Japanese species of the *suberis* and *angulifasciella* groups of the genus *Ectoedemia* Busck, 1907 are given. Two species, *E. chasanella* Puplesis, 1984 and *E. ortiva* Rocienė & Stonis, 2013, are reported for the first time from Japan and their morphology is redescribed. In addition, we provide molecular identification of the two species and their relatives based on COI barcode sequences and the nuclear gene (EF1-α) sequences. The female of *E. chasanella* is described for the first time. We confirm that *E. chasanella* utilizes *Quercus* species (Fagaceae) as its hostplant: *Q. dentata*, *Q. serrata*, *Q. crispula*, and *Q. acutissima*. *Ectoedemia ortiva* was formerly placed in the *suberis* group, and its host plant was unknown. We move this species to the *angulifasciella* group and provide evidence that it utilizes *Ulmus* sp. (Ulmaceae) as its host plant.

Key words: *Ectoedemia chasanella*, *Ectoedemia ortiva*, female genitalia, *Quercus*, taxonomy

Introduction

Nepticulidae include some of the smallest moths in the world, with over 900 species described to date. *Ectoedemia* Busck, 1907 is the third largest genus in Nepticulidae and consists of about 95 named species (van Nieukerken *et al*. 2016; Stonis *et al*. 2016; Stonis & Remeikis 2018). This genus is mainly distributed in the temperate zone, with 76 species described from the Palearctic region. In *Ectoedemia*, Fagaceae form the most important host plant family and are also the most likely ancestral hosts for three species groups: the *suberis*, *ornatella*, and *subbimaculella* groups (Doorenweerd *et al*. 2015). The *suberis* group is composed of ten species, three of which are known from the East Palearctic, i.e., *E. chasanella* Puplesis, 1984, *E. ortiva* Rocienė & Stonis, 2013 and *E. paraortiva* Rocienė & Stonis, 2014.

There are no host records for the *suberis* group in the East Palearctic, although *E. chasanella* was expected to utilize *Q. dentata* Thunb. and *Q. mongolica* Fisch. ex Ledeb (Puplesis 1994). Until now, the *suberis* group has not been recorded from Japan, although the larvae can be easily distinguished from other species groups, because they feed only on *Quercus* species and are green in color, whereas larvae in other groups are usually yellow or pale green (van Nieukerken 1985; van Nieukerken *et al.* 2010).

The *Ectoedemia angulifasciella* group is the most diverse group globally and is composed of 26 named species (van Nieukerken *et al*. 2016; Stonis *et al*. 2016). The *angulifasciella* group consists mainly of Rosaceae-feeders, but with some species known to utilize Ulmaceae, Betulaceae, Nyssaceae (sometimes included in Cornaceae), or Staphyleaceae (Wilkinson & Newton 1981; van Nieukerken 1985; Doorenweerd *et al*. 2015). In Japan, four species, i.e., *E. pilosae*, *E. picturata*, *E. occultella*, and *E. jacutica* (Puplesis 1994; van Nieukerken *et al*. 2010, 2012, 2016; Hirano 2013, 2014) and at least three unnamed species are recorded (Oku 2003; Owada *et al*. 2006; Hirano 2009).

In this paper, we document two newly recorded species for Japan, *E. chasanella* and *E. ortiva*, both recognized as members of the *suberis* group, and both previously known only from their type localities in far eastern Russia.

Accepted by J.W. Brown: 1 Nov. 2019; published: 9 Dec. 2019

The description of the female genitalia and biology of *E. chasanella* is provided for the first time. We also show the relationships of the species based on sequence data from COI barcodes and EF1-α regions. Moreover, the Japanese *angulifasciella* group is also introduced with photos including male genitalia. We illustrate Japanese specimens of *E. picturata*, *E. occultella*, and *E. jacutica* for the first time.

Material and methods

Specimens were collected by the authors or borrowed from the collections of Osaka Prefecture University (OPU), Hokkaido University Museum (SEHU), and the personal collections of Mr. H. Kogi and Mr. H. Shimizu. Adults were collected by light traps using a mercury vapor lamp, throughout Japan. Rearing methods differed between each collector, and one of the authors (Yagi) followed the methods of Ohshima (2013). We modified the latter rearing method that was developed for Gracillariidae. First, in the rearing container, a layer of Japanese traditional toilet paper (Yawatahama Paper Industry, 123057) was used as substrate for pupation. To overwinter, the rearing containers were preserved at 5°C in the incubators. Around late March, the temperature was increased to 20°C or specimens were taken out of the incubator and kept at room temperature. Before changing the temperature, we decreased the temperature in the incubators by 2°C for one day to emphasize the temperature rise.

After emergence or collection by light trap, adults were killed by ammonia in a killing bottle. Then, wings of specimens were spread using a spreading board, and then dried in a specimen room for three weeks or using a drying oven (DO-450, Iuchi) at 45°C for a week. For preparation of the genitalia, the abdomen was boiled in 10% KOH for about 5 minutes to macerate, or soaked in ATL buffer with Proteinase K overnight at 56°C in order to extract DNA at the same time. Softened abdomens were stained with Chlorazol Black E. The genitalia were then dehydrated in a series of 70–100% ethanol solutions and mounted in Euparal on a glass slide.

DNA extraction was performed with the Qiagen DNeasy Blood and Tissue kit. Almost all samples were extracted from the abdomen of adult specimens in a non-destructive method, except for one sample of *E. chasanella* (SaY218 was extracted from the larva using destructive method). Abdominal pelts were preserved in 70% ethanol temporarily to observe the genitalia. PCR was carried out according to our laboratory's protocols; however, annealing temperatures followed Doorenweerd *et al*. (2015). PCR products were purified with the ExoSAP-IT kit (Amersham Biosciences). A 665 bp fragment of the partial mitochondrial COI barcoding region was amplified using the primers LepF1 and LepR1 (Herbert *et al*. 2003). When DNA was too degraded, we used internal primers mLepF1 and mLepR1 (Hajibabaei *et al*. 2006). A 482 bp fragment of the nuclear EF1-α gene was also amplified using EF-NepF and EF-NepR (van Nieukerken *et al*. 2012). The COI sequences of six Japanese species that were treated in this paper were searched using BLASTn v 2.9.0 (Zhang *et al*. 2000; Morgulis *et al*. 2008). Some sequence data were utilized from previous studies (refer to Table 1). Sequence alignment and pairwise sequence distances were generated using Mega 7.0.26 (Kumar *et al*. 2016). To identify similar species, pairwise sequence distances were calculated based on the Kimura-two parameter (K2P) model. For our analysis, we also used the data of the species of *Ectoedemia* that are registered to BOLD SYSTEMS and Genbank, except for some samples that had short sequences and/or undescribed species.

Phylogenetic trees were construct using Maximum Likelihood methods and Bayesian methods; model selection was calculated based on the Akaike Information Criterion (AIC) (Akaike 1974) using Kakusan 4 (Tanabe, 2011) and GTR+G model were selected in both analyses. To construct phylogenetic trees using the two markers, we used almost all named species of *Ectoedemia* and one sample (*Ectoedemia UlmusKorea*), the latter of which is most similar to *E. ortiva*. The sequence data of *E. aegilopidella* (RMNH.INS.23875) wasn't included in the analysis because the length was too short, and Doorenweerd *et al*. (2015) showed negatively influencing support values. Maximum Likelihood trees were created using RAxML 8.2.9 (Stamatakis, 2014) and bootstrap values were calculated with 1,000 replicates. Bayesian analyses were performed using MrBayes v3.2.6 (Ronquist *et al*. 2012).

Morphological terminology follows Johansson *et al.* (1990) and Puplesis (1994), and the scientific names follow van Nieukerken *et al.* (2016). The scientific names of plants follow Yonekura & Kajita (2003).

The names of localities in Nagano Prefecture as used here are from before the merger of smaller municipalities into larger ones in the early 2000s. That is, the following localities on labels, Azumi-mura and Azusagawa-mura have been merged into Matsumoto-shi. Akashina-machi and Toyoshina-machi have been merged into Azumino-shi. Unless otherwise noted, the specimens are deposited in the Entomological Laboratory of Kyushu University (ELKU).

TABLE 1. Sample information used in this study for DNA analysis.

Species name	Sample ID	Process ID	BIN ID	GenBank accession number (COI)	GenBank accession number (EF1-α)	References
***commiphorella* group**						
Ectoedemia expeditionis	RMNH.INS.23987	NEPT283-10	BOLD:AAK9936	JN201855	JN208856	Nieukerken *et al*. (2012)
Ectoedemia tersiusi	RMNH.INS.23988	NEPT284-10	BOLD:AAK9963	JN201854	JN208855	Nieukerken *et al*. (2012)
***terebinthivora* group**						
Ectoedemia terebinthivora	RMNH.INS.17747	NEPT139-10	BOLD:AAI9353	JN201853	JN208854	Nieukerken *et al*. (2012)
***populella* group**						
Ectoedemia intimella	RMNH.INS.11373	NEPT032-10	BOLD:AAD0467	JN201755	JN208769	Nieukerken *et al*. (2012)
Ectoedemia insularis	RMNH.INS.23890	NEPT259-10	BOLD:AAD0468	JN201756	JN208770	Nieukerken *et al*. (2012)
Ectoedemia populella	RMNH.INS.23886	NEPT255-10	BOLD:AAB9666	JN201794	JN208802	Nieukerken *et al*. (2012)
Ectoedemia hannoverella	RMNH.INS.11738	NEPT051-10	BOLD:AAE8153	JN201712	JN208733	Nieukerken *et al*. (2012)
Ectoedemia canutus	RMNH.INS.23727	NEPT212-10	BOLD:AAF6428	JN208625	JN201604	Nieukerken *et al*. (2012)
Ectoedemia turbidella	RMNH.INS.11320	NEPT024-10	BOLD:AAD4374	JN201859	JN208860	Nieukerken *et al*. (2012)
Ectoedemia klimeschi	RMNH.INS.17827	NEPT156-10	BOLD:ABX4998	JN201760	JN208774	Mutanen et al. (2016)
Ectoedemia argyropeza	RMNH.INS.11689	NEPT048-10	BOLD:AAC1036	JN201679	JN208698	Nieukerken *et al*. (2012)
***subbimaculella* group -satellite taxa**						
Ectoedemia arisi	RMNH.INS.29358	NEPTA176-13	BOLD:ACG9190	KM077681	KM077780	Doorenweerd *et al*. (2017)
Ectoedemia christopheri	RMNH.INS.23897	NEPT266-10	BOLD:ADF5874	–	JN208716	Doorenweerd *et al*. (2015)
Ectoedemia trinotata	RMNH.INS.18297	NEPTA238-13	BOLD:AAH4504	KM077719	KM077800	Doorenweerd *et al*. (2015)
Ectoedemia preisseckeri	RMNH.INS.23870	NEPT239-10	BOLD:AAI9363	JN201797	JN208804	Doorenweerd *et al*. (2015)
Ectoedemia quadrinotata	RMNH.INS.23900	NEPT269-10	BOLD:AAI9358	JN201806	JN208811	Nieukerken *et al*. (2012)
***subbimaculella* group**						
Ectoedemia givipennella	RMNH.INS.23840	NEPT231-10	BOLD:AAI9359	JN201708	JN208728	Nieukerken *et al*. (2012)
Ectoedemia quinquella	RMNH.INS.17672	NEPT137-10	BOLD:AAF620	JN201808	JN208813	Nieukerken *et al*. (2012)
Ectoedemia coscoja	RMNH.INS.23845	NEPT236-10	BOLD:AAX9882	JN201701	JN208720	Nieukerken *et al*. (2012)
Ectoedemia algeriensis	RMNH.INS.23842	NEPT233-10	BOLD:AAI9361	JN201640	JN208660	Nieukerken *et al*. (2012)
Ectoedemia leucothorax	RMNH.INS.23834	NEPT225-10	BOLD:AAF6505	JN201762	JN208776	Nieukerken *et al*. (2012)
Ectoedemia haraldi	RMNH.INS.23662	NEPT199-10	BOLD:AAE8152	JN201714	JN208735	Nieukerken *et al*. (2012)
Ectoedemia pseudoilicis	RMNH.INS.23831	NEPT223-10	BOLD:AAF6511	JN201799	JN208807	Nieukerken *et al*. (2012)
Ectoedemia ilicis	RMNH.INS.23841	NEPT232-10	BOLD:AAE8134	JN201747	JN208761	Nieukerken *et al*. (2012)
Ectoedemia heringella	RMNH.INS.23674	NEPT203-10	BOLD:AAD0479	JN201722	JN208743	Nieukerken *et al*. (2012)
Ectoedemia alnifoliae	RMNH.INS.23883	NEPT252-10	BOLD:AAF6393	JN201641	JN208661	Nieukerken *et al*. (2012)

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TABLE 1. (Continued)

Species name	Sample ID	Process ID	BIN ID	GenBank accession number (COI)	GenBank accession number (EF1-α)	References
Ectoedemia cerviparadisicola	RMNH.INS.17902	NEPT180-10	BOLD:AAI6174	JN201607	JN208628	Nieukerken *et al.* (2012)
Ectoedemia rufifrontella	RMNH.INS.23629	NEPT197-10	BOLD:AAE8131	JN201823	JN208827	Nieukerken *et al.* (2012)
Ectoedemia pubescivora	RMNH.INS.12900	NEPT115-10	BOLD:ACE4977	JN201804	JN208809	Nieukerken *et al.* (2012)
Ectoedemia albifasciella	RMNH.INS.12644	NEPT078-10	BOLD:ABZ3177	JN201635	JN208654	Nieukerken *et al.* (2012)
Ectoedemia contorta	RMNH.INS.12948	NEPT123-10	BOLD:AAH6393	JN201700	JN208719	Nieukerken *et al.* (2012)
Ectoedemia cerris	RMNH.INS.23836	NEPT227-10	BOLD:ABZ3179	JN201695	JN208714	Nieukerken *et al.* (2012)
Ectoedemia subbimaculella	RMNH.INS.11383	NEPT033-10	BOLD:ACE7752	JN201847	JN208849	Nieukerken *et al.* (2012)
Ectoedemia phyllotomella	RMNH.INS.11416	NEPT039-10	BOLD:ACF1459	JN201788	JN208798	Nieukerken *et al.* (2012)
Ectoedemia heringi	RMNH.INS.11353	NEPT030-10	BOLD:AAB7903	JN201741	JN208757	Nieukerken *et al.* (2012)
Ectoedemia liechtensteini	RMNH.INS.11418	NEPT041-10	BOLD:ACF1459	JN201766	JN208780	Nieukerken *et al.* (2012)
***platanella* group**						
Ectoedemia similella	RMNH.INS.18373	NEPTA220-13	BOLD:AAH5602	KM077657	KM077766	Nieukerken *et al.* (2012)
Ectoedemia platanella	RMNH.INS.18358	NEPTA172-13	BOLD:AAH4492	KM077592	KM077739	Doorenweerd *et al.* (2015)
Ectoedemia clemensella	RMNH.INS.18282	NEPTA084-13	BOLD:ACC8118	KM077729	KM077804	Doorenweerd *et al.* (2015)
Ectoedemia virgulae	RMNH.INS.18265	NEPTA079-13	BOLD:ACG9109	KM077702	KM077793	Doorenweerd *et al.* (2015)
***ornatella* group**						
Ectoedemia ivinskisi	RMNH.INS.23899	NEPT268-10	BOLD:AAX9873	JN201758	JN208771	Nieukerken *et al.* (2012)
Ectoedemia olvina	RMNH.INS.29235	NEPTA165-13	BOLD:ACG9261	KM077673	KM077774	Doorenweerd *et al.* (2015)
Ectoedemia ornatella	RMNH.INS.23913	NEPT282-10	BOLD:AAI9365	JN201783	JN208794	Nieukerken *et al.* (2012)
***suberis* group**						
Ectoedemia chasanella	SaY50	NEPSY001-19	–	LC474631	–	This study
Ectoedemia chasanella	SaY35	NEPSY002-19	BOLD:AAX9883	LC467975	LC468033	This study
Ectoedemia chasanella	SaY85	NEPSY003-19	BOLD:AAX9883	LC467974	LC500258	This study
Ectoedemia chasanella	SaY218	NEPSY004-19	BOLD:AAX9883	LC474630	LC500259	This study
Ectoedemia chasanella	RMNH.INS.23912	NEPT281-10	BOLD:AAX9883	JN201697	JN208715	Nieukerken *et al.* (2012)
Ectoedemia andalusiae	RMNH.INS.11633	NEPT044-10	BOLD:AAE8168	JN201649	JN208668	Nieukerken *et al.* (2012)
Ectoedemia caradjai	RMNH.INS.12862	NEPT110-10	BOLD:AAF6427	JN201693	JN208712	Nieukerken *et al.* (2012)
Ectoedemia caradjai	RMNH.INS.23839	NEPT230-10	BOLD:AAF6427	JN201692	JN208711	Nieukerken *et al.* (2012)
Ectoedemia caradjai	RMNH.INS.23872	NEPT241-10	BOLD:AAF6427	JN201691	JN208710	Nieukerken *et al.* (2012)
Ectoedemia heckfordi	RMNH.INS.23730	NEPT213-10	BOLD:AAF6423	JN201719	JN208740	Nieukerken *et al.* (2012)
Ectoedemia hendrikseni	RMNH.INS.23720	NEPT210-10	BOLD:AAI9362	JN201721	JN208742	Nieukerken *et al.* (2012)

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TABLE 1. (Continued)

Species name	Sample ID	Process ID	BIN ID	GenBank accession number (COI)	GenBank accession number (EF1-α)	References
Ectoedemia phaeolepis	RMNH.INS.23829	NEPT221-10	BOLD:AAI9364	JN201787	JN208797	Nieukerken *et al*. (2012)
Ectoedemia suberis	RMNH.INS.11407	NEPT036-10	BOLD:AAF6517	JN201851	JN208852	Nieukerken *et al*. (2012)
Ectoedemia aegilopidella	RMNH.INS.23875	NEPT244-10	–	JN201614	JN208634	Nieukerken *et al*. (2012)
***angulifasciella* group**						
Ectoedemia ortiva	SaY51	NEPSY005-19	BOLD: ADX0813	LC467976	–	This study
Ectoedemia ortiva	SaY144	NEPSY006-19	BOLD: ADX0813	LC467977	LC468034	This study
Ectoedemia hexapetalae	RMNH.INS.31242	NEPTA2181-18	BOLD:ADQ7803	–	–	–
Ectoedemia rosae	RMNH.INS.12587	NEPT072-10	BOLD:AAI6173	JN201609	JN208629	Nieukerken *et al*. (2012)
Ectoedemia marmaropa	RMNH.INS.24570	NEPTA1049-15	BOLD:ACG7148	–	–	–
Ectoedemia spiraeae	RMNH.INS.18490	NEPTA226-13	BOLD:AAI9355	KM077682	KM077782	Doorenweerd *et al*. (2015)
Ectoedemia jacutica	SaY181	NEPSY010-19	BOLD:AAI9354	LC467970	LC468029	This study
Ectoedemia agrimoniae	RMNH.INS.17805	NEPT153-10	BOLD:AAE0677	JN201615	JN208635.1	Nieukerken *et al*. (2012)
Ectoedemia nyssaefoliella	RMNH.INS.24214	NEPTA160-13	BOLD:AAH0032	KM077602	KM77743	Doorenweerd *et al*. (2015)
Ectoedemia pilosae	SaY151	NEPSY007-19	BOLD:AAP1383	LC467968	LC468027	This study
Ectoedemia picturata	SaY12	NEPSY008-19	BOLD:ACU6987	LC467969	LC468028	This study
Ectoedemia minimella	RMNH.INS.12778	NEPT094-10	BOLD:AAE0820	JN201777	JN208788	Nieukerken et al. (2012)
Ectoedemia occultella	SaY150	NEPSY009-19	BOLD:ADX7742	LC467971	LC468030	This study
Ectoedemia angulifasciella	RMNH.INS.11768	NEPT058-10	BOLD:AAB6754	JN201666	JN208685	Nieukerken *et al*. (2012)
Ectoedemia arcuatella	RMNH.INS.12593	NEPT074-10	BOLD:AAB9664	JN201671	JN208689	Nieukerken *et al*. (2012)
Ectoedemia atricollis	RMNH.INS.12021	NEPT069-10	BOLD:AAB9664	JN201687	JN208706.1	Nieukerken *et al*. (2012)
Ectoedemia rubivora	RMNH.INS.11325	NEPT025-10	BOLD:AAB9664	JN201819	JN208823	Nieukerken *et al*. (2012)
Ectoedemia spinosella	RMNH.INS.12581	NEPT070-10	BOLD:AAE0786	JN201829	JN208832	Nieukerken *et al*. (2012)
Ectoedemia mahalebella	RMNH.INS.11674	NEPT046-10	BOLD:AAD8581	JN201774	JN208785	Nieukerken *et al*. (2012)
Ectoedemia erythrogenella	RMNH.INS.11534	NEPT043-10	BOLD:AAE0809	JN201706	JN208726	Nieukerken *et al*. (2012)
Ectoedemia rubifoliella	RMNH.INS.18327	NEPTA204-13	BOLD:ABX4311	KM077786	KM077690	Doorenweerd *et al*. (2015)
Ectoedemia ulmella	RMNH.INS.18286	NEPTA973-14	BOLD:AAI6172	MH917753	KX821286	Nieukerken *et al*. (2018)
"*Ectoedemia UlmusKorea*"	RMNH.INS.29226	NEPTA243-13	BOLD:ACG8518	KM077642	KM077758	Doorenweerd *et al*. (2015)
"*Ectoedemia RosaTaiwan*"	RMNH.INS.29404	NEPTA199-13	BOLD:ACG9450	KM077678	–	Doorenweerd *et al*. (2015)
"*Ectoedemia StephanandraKorea*"	RMNH.INS.30118	NAPTA1112-15	BOLD:ACU7394	KX281268	–	Doorenweerd *et al*. (2015)
Etainia parva	SaY196	NEPSY013-19	BOLD:ADW7453	LC467978	LC468035	This study

Results and discussion

DNA analysis. Five specimens of *E. chasanella* (3 ♂, 1 ♀, 1 larva) and two specimens of *E. ortiva* (2 ♂) were used for DNA analysis. One specimen of *E. chasanella* (SaY50, NEPSY001-19) was in poor condition and only part of the barcode region (321 bp) was obtained. Based on this sequence, SaY50 showed only 1 bp difference (Table 2, K2P distance, 0.31%) from two specimens of Japanese *E. chasanella* (SaY35, NEPSY002-19 and SaY85, NEPSY003-19). The intraspecific variation of *E. chasanella* (665 bp) showed 0.15–0.94% pairwise distances in the COI region. The intraspecific variation of *E. chasanella* was 0.21% in the EF1-α region.

According to the interspecific pairwise K2P distances in the COI region, *E. chasanella* showed 4.17–6.15% pairwise distances from the most similar species, *E. caradjai* (Table 2), which is consistent with the results of Doorenweerd *et al*. (2015). *Ectoedemia chasanella* also showed 5.36–5.70% pairwise distances from *E. aegilopidella*. In the EF1-α region, the interspecific pairwise distances of *E. chasanella* is similar to *E. caradjai* (3.19–3.85%). On the other hand, the sequence of *E.aegilopidella* isn't similar to *E. caradjai* (5.39–5.82) compared to other *suberis* group species (Table 3). *Ectoedemia heckfordi* is the most similar species based on the EF1-α region of *E. aegilopidella* (4.11%). To identify the closely related species of *E. chasanella*, we need to get reliable sequcences of *E. aegilopidella* because the sequence data for *E. aegilopidella* is only one sample in each gene and half length.

Based on the pairwise distances, two samples of *E. ortiva* were very similar to that of "*Ectoedemia UlmusKorea*" (Doorenweerd *et al*. 2015), which belongs to the *angulifasciella* group (Table 4, 2.82%). The results of pairwise distances of *E. ortiva* in the EF1-α region are also similar to that of "*Ectoedemia UlmusKorea*" (Table 5, 3 bp, 0.62%). In Lepidoptera, COI-based divergence values between species are usually larger than 3% (Herbert *et al*. 2003). In *Ectoedemia*, the maximum intraspecific distance of COI was as high as 3.5% while EF1-α values remain below 2.0% (van Nieukerken *et al*. 2012). In this case, the pairwise distances of two genetic regions fall below these thresholds. Therefore, "*Ectoedemia Ulmus Korea*" most likely is the same species as *E. ortiva*, unless it is an exceptional case of barcode sharing, as in the European *E. rubivora* complex in which COI fails to distinguish species, and interspecific pairwise distances of the EF1-α region fall below 1% (van Nieukerken *et al*. 2012). Based on male genitalia, *E. ortiva* and *E. paraortiva* are very similar like *E. rubivora* complex, and we thus cannot exclude the possibility that "*Ectoedemia Ulmus Korea*" is in fact *E. paraortiva*.

Both Maximum Likelihood and Bayesian trees showed the same topology (Fig. 37). Doorenweerd *et al*. (2015) showed the monophyly or paraphyly of the species group using six gene regions, and our results also seems to support their results. *Ectoedemia chasanella* was supported as the member of the *suberis* group, while *Ectoedemia ortiva* was shown to be a member of the *angulifasciella* group.

Taxonomy

Check list of the *Ectoedemia suberis* and *angulifasciella* groups in Japan

suberis **group**
1. ***Ectoedemia chasanella*** Puplesis, 1984

angulifasciella **group**
2. ***Ectoedemia ortiva*** Rocienė & Stonis, 2013
3. ***Ectoedemia pilosae*** Puplesis, 1984
4. ***Ectoedemia picturata*** Puplesis, 1985
5. ***Ectoedemia occultella*** (Linnaeus, 1767)
6. ***Ectoedemia jacutica*** Puplesis, 1988

Ectoedemia suberis group

Species of this group mine leaves of *Quercus* species. The shape of the mines is a blotch, and the color of larvae is green. According to van Nieukerken (1985), the male genitalia of this species group have a large curved valva, one pair of single carinae, and a simple gnathos. Female genitalia are characterized by weak development of the vaginal sclerite and spiculate pouch, a globular bursa covered with pectinations, and wide, similar, oval signa.

This species group is distributed only in the Palearctic region. All species in the West Palearctic and the East Asiatic *E. chasanella* form monophyletic clusters with high bootstrap support (van Nieukerken *et al.* 2012; Doorenweerd *et al*. 2015), while the phylogenetic position of other members of the *suberis* group (*E. ortiva* and *E. paraortiva*) is unknown.

1. *Ectoedemia chasanella* Puplesis, 1984
(Figs. 1–8, 13–18, 24–34, 58)

Ectoedemia chasanella Puplesis, 1984: 124.
Type locality: Primorskiy Kray, 15 km SW Slavyanka, Ryazanovka (Russia).

Material examined. JAPAN. Hokkaido: 1 ♂, Tomikawa, Monbetsu, 28.vii.2002, H. Kogi, genitalia slide no. 6267; 1 ♂, Shiratukari, Ishikari, 18.viii.2011; 1 ♂, 17.vii.2013, H. Kogi; 1 ♀, Sibi, Ishikari, 23.vii.2009, genitalia slide no. SY354; 3 ♂, 4 ♀, 3.iv.2010, Host (No. 09-127): "Kashiwa" [=*Q. dentata*], genitalia slide no. 7493(♂); 2 ♂, 1 ♀, 15.iv.2010, Host (No. 09-127F): "Kashiwa" [=*Q. dentata*], genitalia slide no. 7492(♂), 7494(♂); 1 ♀, 19.vii.2013; 1 ♂, 15.iii.2014, Host (No. 13-122): "Kashiwa" [=*Q. dentata*], genitalia slide no. 472; 1 ♂, 1.v.2014, Host (No. 13-122B): "Kashiwa" [=*Q. dentata*], genitalia slide no. SY350, DNA extraction no. SaY50; 1 ♂, 6.v.2014, Host (No. 13-157): "Kashiwa" [=*Q. dentata*]; 1 ♀, 4.vi.2014, Host (No. 13-122): "Kashiwa" [=*Q. dentata*]; 1 ♀, 11.vii.2014, H. Kogi; 1 ♀, Oyafuru, Ishikari, 21.vii.2004, H. Kogi; 1 ♂, Sinkoh, Ishikari, 18.viii.2002, H. Kogi, genitalia slide no. 6269; 1 ♀, Kitaurimaku, Sikaoi, 23.vii.2012, H. Kogi; 1 ♂, Shikaribetu, Sikaoi, 13.vii.2014, H. Kogi; 1 ♀, Asari, Otaru, 15.vii.2014, H. Kogi; 1 ♂, Sizukawa, Tomakomai, 9.vii.2012, H. Kogi. **Honshu**: [Nagano Pref.] 1 ♂, Ikezawa, Ikusaka-mura, 29.vii.1995, N. Hirano; 1 ♀, Higashikawate, Akashina-machi, 12.viii.1969, N. Hirano; 1 ♀, Komuro, Azusagawa-mura, 25.vi.1994, N. Hirano; 1 ♀, Kamanosawa, Azusagawa-mura, 30.vii.1978; 1 ♀, 22.vii.1979; 1 ♀, 2.viii.1980; 1 ♂, 10.vii.1982, N. Hirano; 1 ♀, Ohkuchizawa, Toyoshina-machi, 7.viii.1980; 1♂, 25.vii.1981; 1 ♀, 12.viii.1982; 2 ♀, 2.vii.1994; 1 ♂, 1 ♀, 8.vii.1994; 2 ♂, 10 ♀, 15.vii.1994; 1 ♂, 7 ♀, 25.vii.1995; 1 ♀, 5.viii.1995; 1 ♀, 26.viii.1995; 1 ♂, 3 ♀, 12.vii.1996, genitalia slide no. ♂NH-1081, N. Hirano; 1 ♀, Tazawa, Toyoshina, Azumino-shi, 16.ix.2017 larva on *Quercus crispula* (No. M1407), 17.v.2018 em., Sadahisa Yagi, genitalia slide no. 471; 2 ♀, Shimashimadani, Azumi-mura, 26.vii.1995; 1 ♂, 1 ♀, 2.viii.1995; 1 ♀, 19.viii.1995; 1 ♀, 26.viii.1995, genitalia slide no. NH-1082; 4 ♀, 19.vii.1996; 2 ♀, 20.vii.1997; 1 ♂, 4.vii.1998; 1 ♀, 29.vii.1998, N. Hirano; 1 ♀, Tomikusa, Anan-cho, 24–25.vii.2004; 1 ♀, 21.viii.2004, N. Hirano; 3 ♀, Kojiro, Tenryu-mura, 19–20.vii.2008; 1 ♀, 19.vi.2009, N. Hirano; [Osaka Pref.] 1 ♀, Ikoma, Osaka, 30.vi.1995, S. Koshino (OPU); 1 ♀, Osaka, Yao-shi, Kodachi, Jyusan Toge, Fumin no mori, 25.vi.2011, H. Shimizu, genitalia slide no. SY493; 1 ♂, Kyosi, Osaka, 8.vi.1997, S. Koshino (OPU). **Tsushima Is.**: [Nagasaki Pref.] 1ex., Mt. Asaji, Kofunakoshi, Mitsushima Tsushima-shi, 13.x.2018, larva on *Quercus serrata*, S. Yagi. **Kyushu**: [Fukuoka Pref.] 1 ♀, Mt. Hiko, Soeda, Tagawa, 13.viii.2016, LT, S. Yagi, N: 33.4755, E: 130.8957, 450 m, genitalia slide no. SY330, DNA extraction No. SaY35; [Oita Pref.] 1 ♂, Jizobaru, Machida, Kokonoe, Kusu-gun, 2.x.2017 larva on *Quercus acutissima* (No. M1662), ex. pupa 14.vii.2018, genitalia slide no. SY476, DNA extraction No. SaY85; 1 ♂, larva on *Quercus acutissima* (No. M1662), ex. pupa 23.vii.2018; 1 ♂, larva on *Quercus crispula* (No. M1661), ex. pupa 14.vii.2018, S. Yagi.

Diagnosis. *Ectoedemia chasanella* is similar to *E. aegilopidella* (Klimesch, 1978); the two share special scales on the underside of forewing in the male and similar female genitalia. However, *E. chasanella* can be identified by the flatten pseuduncus (ventral globular in *E. aegilopidella*), the central element of gnathos (broad in *E. aegilopidella*), and the pectinations in spiculate pouch (very few small spines, without pectinations in *E. aegilopidella*). This species is also similar to *E. caradjai*, but can be identified by the absence of a hair-pencil (white in *E. caradjai*) and the presence of distinct yellow special scales under costal fold on the underside of the forewing (absent in *E. caradjai*) in the male, and the smaller number of antennal segments (43–51 segments in the male of *E. caradjai* and 30–32 segments in the female of *E. caradjai* (van Nieukerken *et al*. 2010)). This species also can be distinguished by the distinct anterior emargination and broad central element of gnathos in the male genitalia and spiculate pouch with a slightly sclerotized area anteriorly in the female genitalia.

Male (Figs. 1, 2, 4, 5, 7, 8). Forewing length 1.8–2.8 mm (n = 10), wingspan 4.4–5.9 mm (n=10), antennae with 39–40 segments (n = 5). Head with frontal tuft yellowish orange; collar yellow cream; scape and pedicel cream; flagellum light grayish yellow. Thorax and tegula yellow cream posteriorly with fuscous scales. Forewing with basal half fuscous; medial fascia cream, posteriorly wide; distal to fascia with fuscous coarse scales, ochreous ground coloration appearing near the edge, scales of these areas long and basally transparent; cilia line interrupted; fringe cream, posteriorly brownish gray; underside light grayish brown; basal half with yellow cream scales, at costal margin with long hair-like scales (Figs. 5, 7 left wing), under hair-like scales with large, cream and oval lamellar androconial scales (Figs. 5, 8 right wing) covered with transparent large scales (Fig. 8a). Hindwing brownish gray, basal half covered with yellow cream scales; costal margin basally weakly expanded; frenulum yellowish orange; hair-pencil and androconial scales absent; fringe brownish gray; underside basally ochreous, other parts brownish gray. Legs and abdomen dark brownish gray, abdomen dark brownish gray, posteroventrally cream; anal tuft dark cream.

Female (Figs. 3, 6). Forewing length 2.2–2.6 mm (n = 10), wingspan 5.0–6.0 mm (n =10), antennae with 24–27 segments (n = 5). Essentially as in male. Underside of forewing basally without long hair-like scales and androconial scales. Hindwing basally slender with costal bristles; frenulum absent. Fascia of forewing tending to be wider than that of male.

Male genitalia (Figs. 13–18). Capsule length ca. 290 μm (n = 6). Valva ca. 180 μm (n = 6). Phallus 330–370 μm (n = 6). Pseuduncus developed; tip roundish. Lateral arm of gnathos basally thick and tapering toward the tip; central element tapering but not sharp. Valva strongly curved inwardly; distal process tapering and pointed; inner margin basally projected and concaved at middle; outer margin roundish. Transverse bar of transtilla moderately; lateral arm thick; sublateral process moderate in length. Vinculum with anterior emargination; lateral lobe developed and apically sharp. Phallus cylindrical; median carinae thick and short tip roundish; vesica with many minute spinelike cornuti.

Female genitalia (Figs. 24–28). Corpus bursae 530–580 μm (n = 4). Signa 320–380 μm (n = 4). T8 with two lateral patches of ca. 27 setae on each side. T8 posteriorly slightly concave. Anal papillae with 7–11 setae on each side. Anterior apophyses short, posterior apophyses slender, longer than anterior apophyses. Vaginal sclerite oval; anteriorly broadly projected anteriorly; posteriorly thickened. Spiculate pouch with a group of pectinations; basally weakly sclerotized. Corpus bursae with many minute pectinations and a pair of large, oval, reticular signa. Wide ductus spermathecae with ca. 3 convolution; vesicle large.

Distribution (Fig. 58). Japan (new record): Hokkaido, Honshu, Tsushima Is., Kyushu; Russia: Far East.

Host plants. *Quercus dentata* Thunb., *Q. crispula* Blume, *Q. serrata* Murray and *Q. acutissima* Carruth.

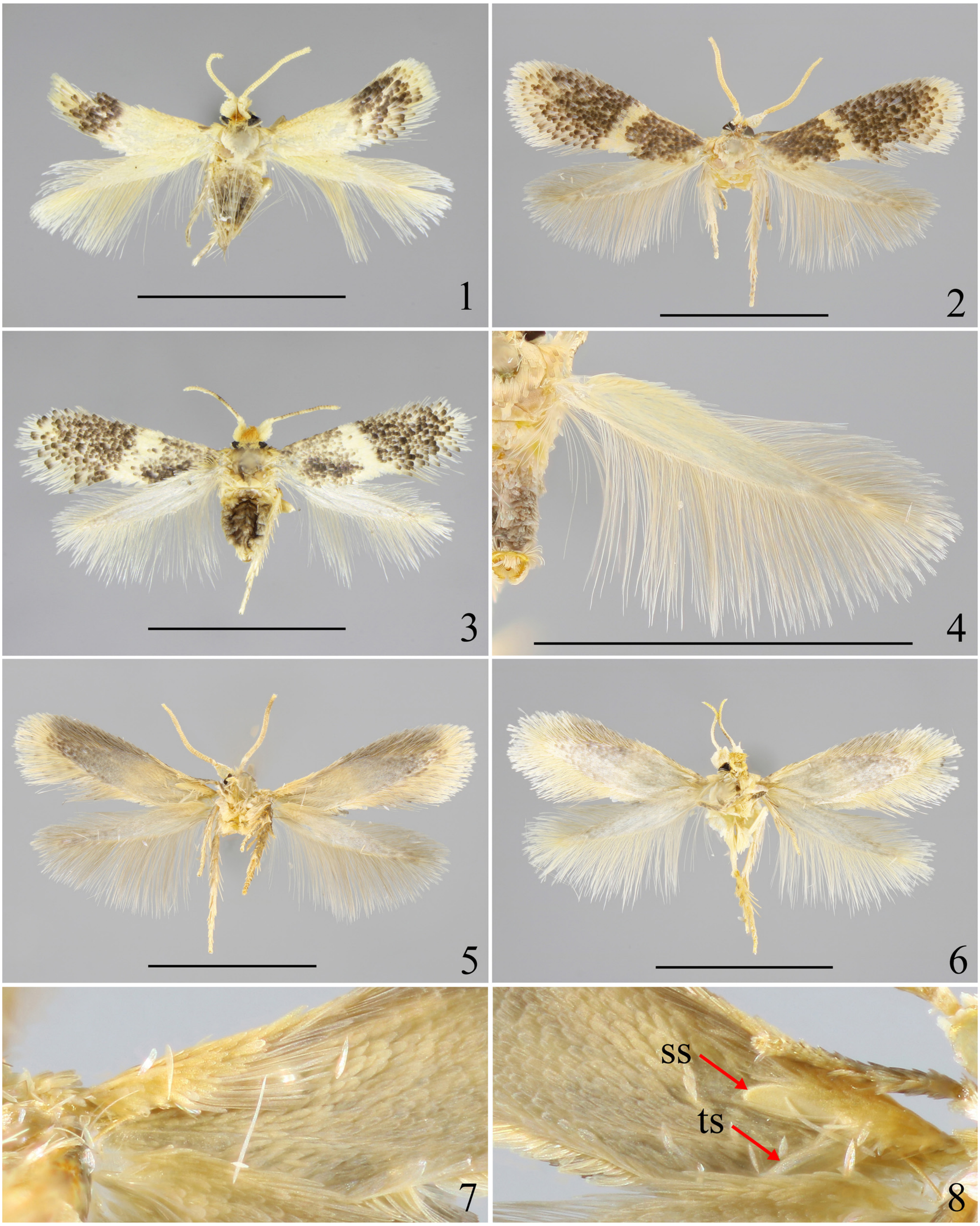
Puplesis (1994) predicted the host plants of *E. chasanella* to be *Q. dentata* and *Q. mongolica* and our result confirmed this prediction. *Quercus dentata*, *Q. crispula* (the latter sometimes regarded as a subspecies of *Q. mongolica* (Okaura *et al*. 2007)), and *Q. serrata* belong to the section *Quercus*, whereas *Q. acutissima* belongs to the section *Cerris* (Satake *et al*. 1989). According to this wide host range over the section, *E. chasanella* seems to be able to utilize other *Quercus* species. In Japan other deciduous oaks also occur: *Q. variabilis* Blume and *Q. aliena* Blume. However, *E. chasanella* may not occur in these forests, because these plants grow mainly in the warm temperate zone, whereas *E. chasanella* was collected in the cool temperate zone in Japan.

Biology (Figs. 29–36). Eggs are deposited on the upper side of the leaf, apparently in a random manner. The early mine is a linear gallery filled with dense frass. The later mine becomes suddenly a blotch, with dense frass, but not filling the entire blotch. The cocoon is light brown. Larvae are green, collected in October. The adults emerged from May to July of the following year, which indicates univoltinism.

Remarks. Two reared males from Hokkaido (Fig. 1) have basal wing markings that differ from those of other specimens (Fig. 2). This unique intraspecific difference may be an aberration or a desquamation of surface black scales. Otherwise, morphological characters such as male genitalia (Fig. 16) showed no difference, and the molecular data showed these specimens to be conspecific; i.e., 1/321 bp (0.31%) different from Japanese (Kyushu) specimens and 3/321 bp (0.94%) different from Russian specimens. Paler basal wing marking owing to desquamation of scales is also observed in *Ectoedemia olvina* Puplesis, 1984.

Ectoedemia angulifasciella group

Many species in the *angulifasciella* group utilize Rosaceae, and almost all the described Japanese species, except *E. occultella* and *E. ortiva*, are known to utilize Rosaceae. The adults usually have a shining metallic fascia, and males have a hair-pencil, which in some species is lost secondarily (van Nieukerken 1985). In the male genitalia, the phallus has one pair of carinae, often with additional spines, and the valva has a more-or-less straight inner margin (van Nieukerken 1985).



FIGURES 1–8. Adults of *Ectoedemia chasanella*. 1. Male collected in Ishikari-shi, Hokkaido (Genitalia slide No. SY350). 2. Male collected in Oita Pref., Kyushu (Genitalia No. SY476). 3. Female, collected in Fukuoka Pref., Kyushu (genitalia no. SY330). 4. Hindwing of male (frenulum removed). 5. Underside of male. 6. Underside of female. 7. Underside of male left forewing, basal part of costal margin with long hair scales. 8. Yellow special scales (ss) on underside of male right forewing at the base (long hair-like scales are removed) and a transparent large scale (ts) is shifted. Scale bars: 2.0 mm.

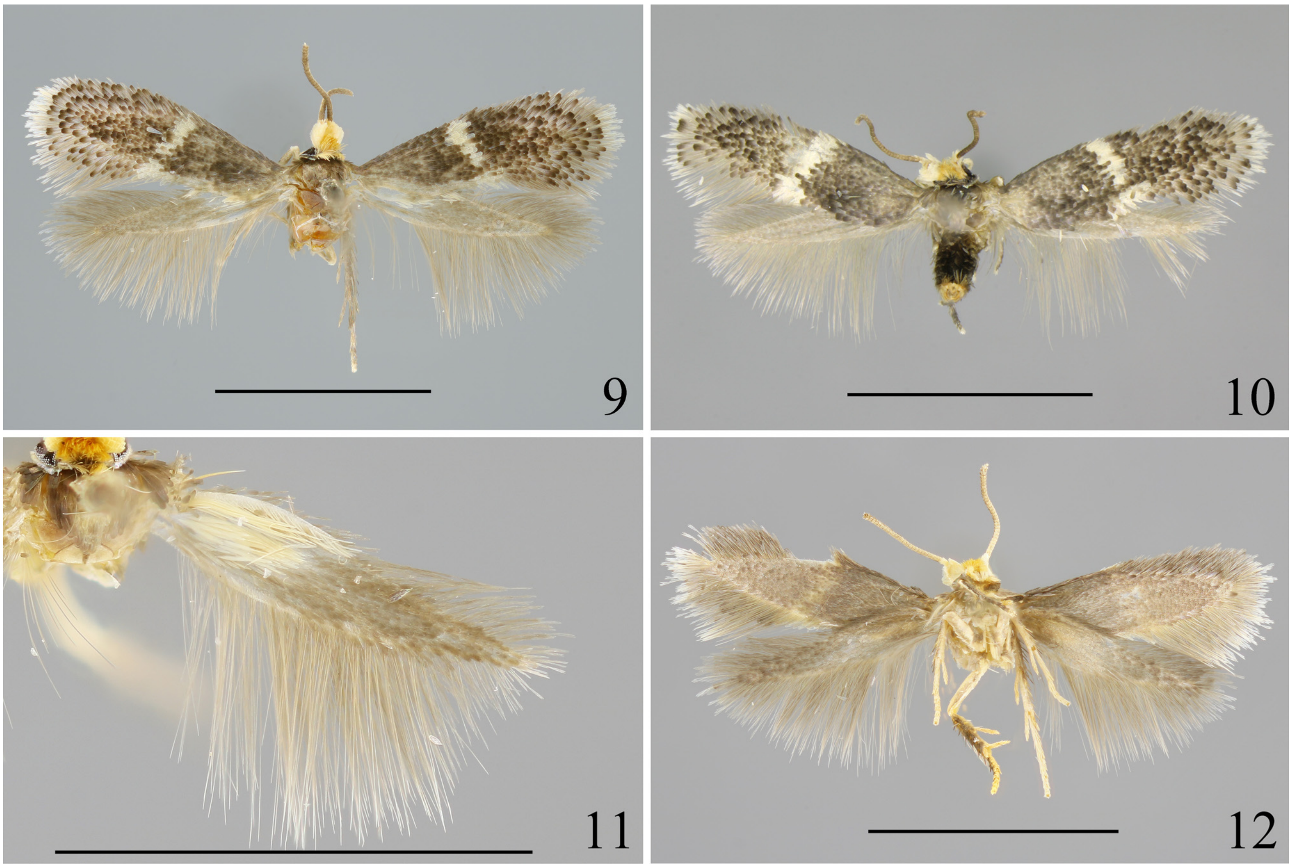
2. *Ectoedemia ortiva* Rocienė & Stonis, 2013
(Figs. 9–12, 19–23, 59)

Ectoedemia ortiva Rocienė & Stonis, 2013: 76.
Type locality: The Russian Far East, Primorskiy Kray, 20 km E Ussuriysk, Gornotayezhnoe.

Material examined. JAPAN. Hokkaido: 2 ♂, Sibi, Ishikari, Hokkaido, 15.vii.2012, H. Kogi, genitalia slide nos. 351, 568; 1 ♂, Tomamu, Shimukappu, 28.vi.2006, H. Kogi; 1 ♂, Katsuranosawa, Atuta, 15.vii.2002, H. Kogi, genitalia slide no. 6299.

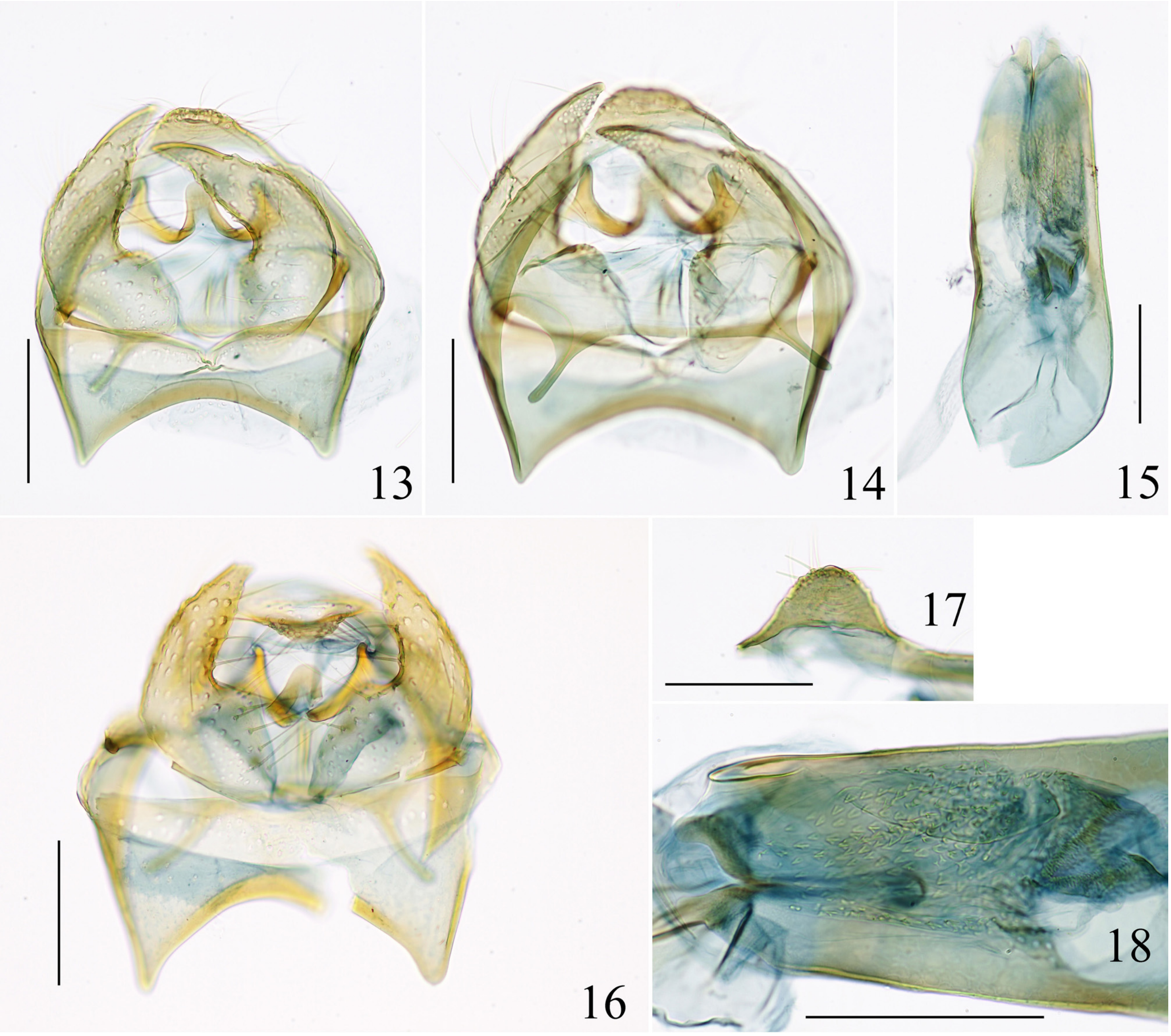
Diagnosis. This species is very similar to *E. paraortiva* Rocienė & Stonis, 2014 based on the male genitalia. It can be identified by the fascia of the forewing (absent in *E. paraortiva*) and the androconial scales surrounding the hair-pencil (absent in *E. paraortiva*). These extensive androconial scales are absent in other East Palearctic species in the *angulifasciella* group. This species and *E. paraortiva* also can be distinguished from other *Ectoedemia* by their short and thick phallus (whereas other species have a slenderer phallus).

Male (Figs. 9–12). Forewing length 2.2–2.5 mm (n = 4), wingspan 4.9–5.6 mm (n = 4), antennae with 31–32 segments (n = 4). Head with frontal tuft yellowish orange; collar yellow cream; scape and pedicel cream; flagellum grayish yellow. Thorax and tegula fuscous. Forewing fuscous; medial fascia cream; cilia line interrupted; fringe outer margin cream, posterior brownish gray; underside grayish brown. Hindwing dark brownish gray; costal margin basally expanded; frenulum yellow cream; hair-pencil yellowish cream, ca. 0.3 length of hindwing; androconial scale surrounding hair-pencil slender and yellowish cream; fringe of hindwing dark brownish gray. Legs and abdomen dark brownish gray; anal tuft brownish yellow.



FIGURES 9–12. Adults of *Ectoedemia ortiva*. 9. Male (genitalia slide no. SY351). 10. Male (Genitalia slide No. SY568). 11. Hindwing. 12. Underside of male. Scale bars: 2.0 mm.

Male genitalia (Figs. 18–22). Capsule length ca. 250 µm (n = 2). Valva ca. 160 µm (n = 2). Phallus 290–310 µm (n = 2). Pseuduncus weakly projected. Lateral arm of gnathos basally slender and subapically curved inwardly; central element of gnathos with small sclerite apically. Valva trapezoid; basally not projected; distal process tapering and sharp; subapically with a small projection inwardly near outer margin; inner margin more-or-less straight, slightly curved inwardly, subapically slightly roundish; outer margin roundish. Transverse bar of transtilla moderate; lateral arm thick; sublateral process of transtilla moderate length. Vinculum with weak anterior emargination; lateral lobe developed, apically weakly roundish. Phallus short and ovate; median carinae slender, usually with additional spine; vesica with of many spinelike cornuti, which are larger and sharp near carina with smaller spines near cathrema.



FIGURES 13–18. Male genitalia of *Ectoedemia chasanella*. 13, 14. Genital capsule with phallus removed (Genitalia slide No. SY419). 15. Phallus (Genitalia slide No. SY419). 16. Genital capsule with phallus removed (Genitalia slide No. SY350). 17. Pseuduncus (Genitalia slide No. SY476). 18. Cornuti (Genitalia slide No. SY419). Scale bars: 100 μm (13–16), 100 μm (17, 18).

Female. Unknown.

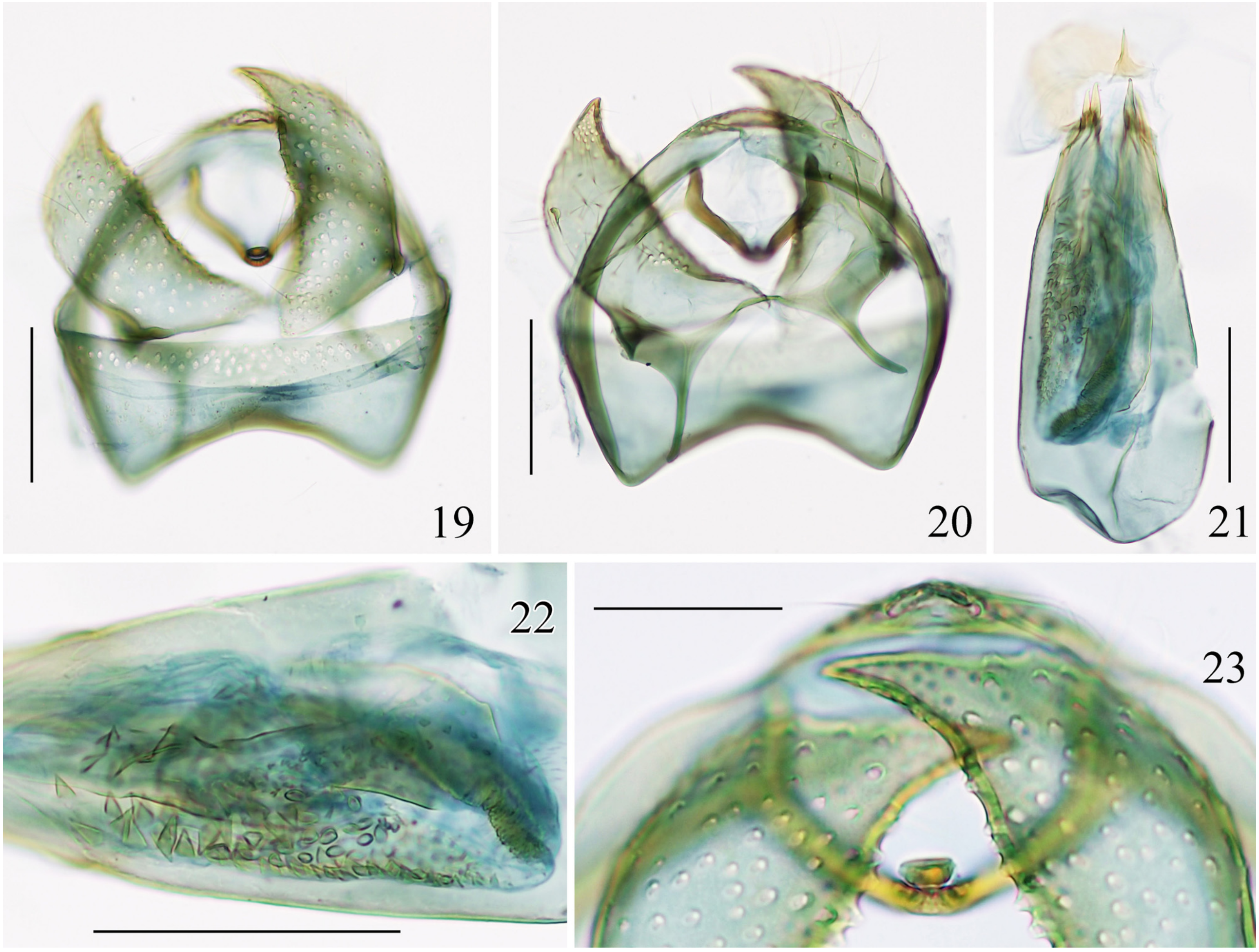
Distribution (Fig. 59). Japan (new record): Hokkaido; Russia: Far East.

Host plant. Unknown. Probably *Ulmus* sp. (Ulmaceae) because similar species utilize *Ulmus* as larval hosts.

Biology. Adults were collected in June and July.

Remarks. This species is herein recognized as a member of the *angulifasciella* group. In male genitalia, species of the *suberis* group have a large curved valva, while *E. ortiva* has a more-or-less straight inner margin that is recognized as a diagnostic character of the *angulifasciella* group. Moreover, in the *angulifasciella* group, the carinae of male genitalia often have additional spines, and *E. ortiva* also shares this trait. However, *E. ortiva* has remarkable androconial scales surrounding the hair-pencil. This is not observed in other East Asian species in the *angulifasciella* group, while modest androconial scales are observed in some species such as *Ectoedemia pilosae*. Moreover, European *E. spinosella* (de Joannis, 1908) and American *E. ulmella* (Braun, 1912) and *E. nyssaefoliella* (Chambers, 1880) have distinct androconial scales (van Nieukerken 1985; Johansson *et al*. 1990; van Nieukerken *et al*. 2018; van Nieukerken pers. comm.). Thus, the presence of a hair-pencil and androconial scales on hindwing are not the best characters for assigning a species to a species group, although these traits are very useful for identification at the species level.

Based on the similarity of the male genitalia, it is suggested that *E. paraortiva* also belongs to the *angulifasciella* group.



FIGURES 19–23. Male genitalia of *Ectoedemia ortiva*. 19, 20. Genital capsule with phallus removed (Genitalia slide No. SY351). 21. Phallus. 22. Cornuti. 23. Pseuduncus and gnathos (Genitalia slide No. SY568). Scale bars: 100 µm (19–22), 50 µm (23).

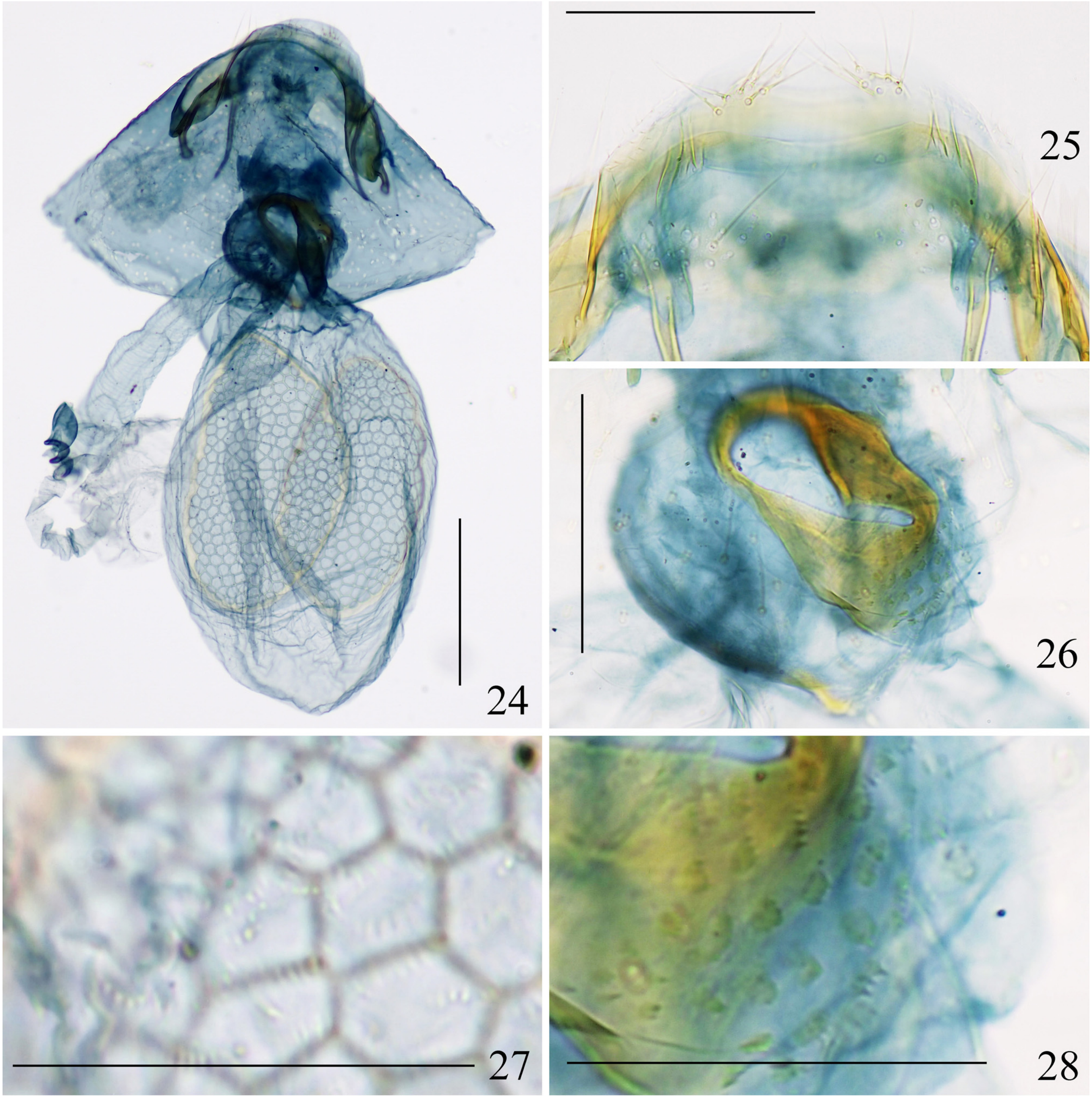
3. *Ectoedemia pilosae* Puplesis, 1984
(Figs. 38, 42–44, 54, 60)

Ectoedemia pilosae Puplesis, 1984: 123.
Type locality: Primorskiy Kray, 20 km E Ussuriysk, Gornotayezhnoe (Russia).

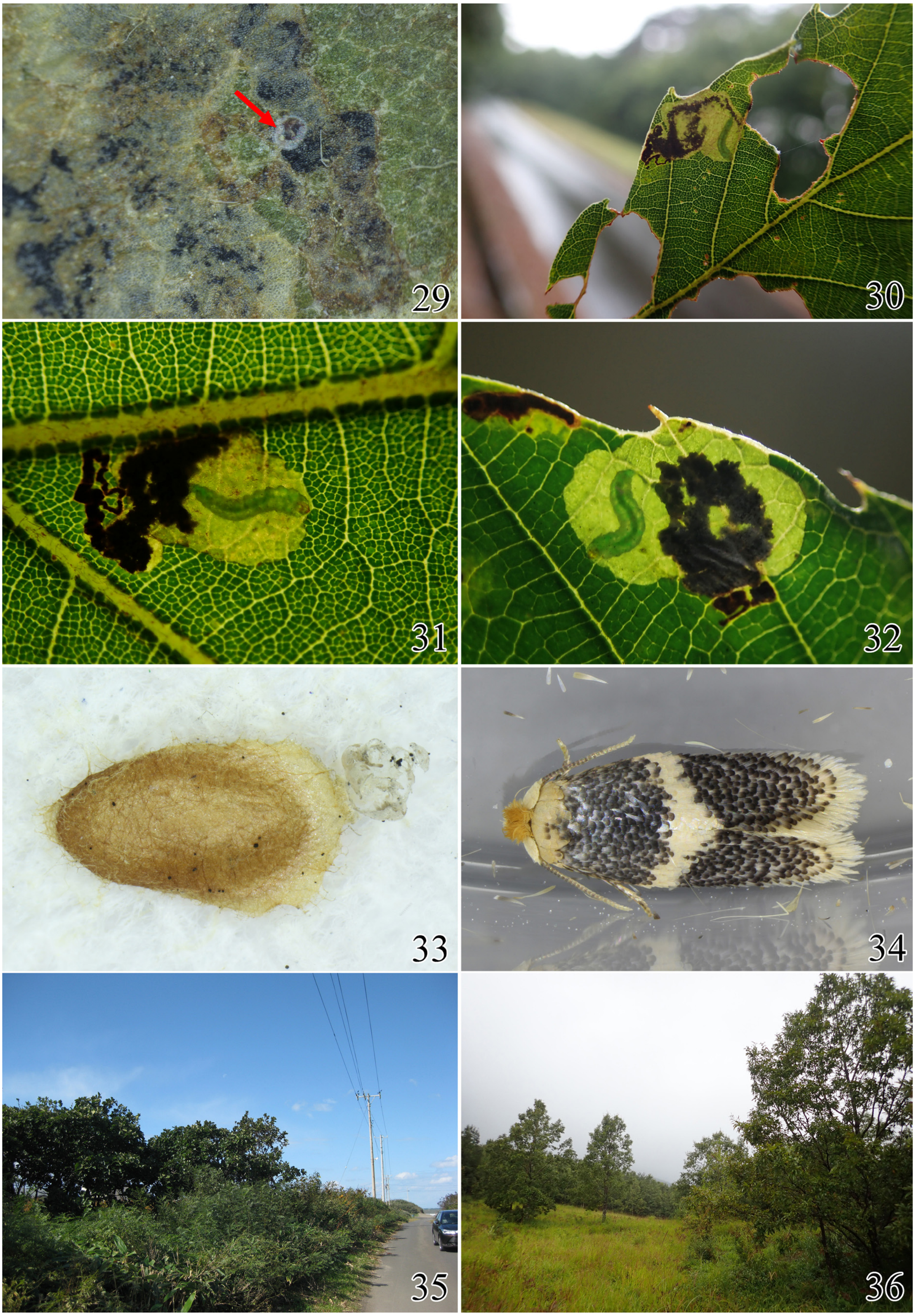
Material examined. JAPAN. Hokkaido: 1 ♂, Makinouti, Nemuro, 29.vii.2001, H. Kogi, genitalia slide no. 6295; 7 ♂, 5 ♀, Sapporo, 24.iv–18.v.1959, T. Kumata (SEHU); 1 ♂, Kitaurimaku Sikaoi, 23.vii.2012, H. Kogi, genitalia slide no. 7520; 3 ♂, 3 ♀, Kannonzawa, Minami-ku, Sapporo, 2.x.2015 larvae on *Agrimonia pilosa*, ex. pupa 30.v–15.vi.2016, S. Yagi; 1 ♂, Komasato, Chitose, 11.iv.2014, host: "Kinmizuhiki" [=*Agrimonia pilosa*], H. Kogi; 1 ♀, Sizukawa, Tomakomai, 26.iii.2013, host: "Kinmizuhiki" [=*Agrimonia pilosa*]; 1 ♂, 26.iii.2013, H. Kogi; 2 ♂, Kashiwabara, Tomakomai-shi, 1.x.2015 larvae on *Agrimonia pilosa*, ex. pupa 29.v–3.vi.2016, S. Yagi, genitalia slide no. SY224; 2 ♂, 1 ♀, Uenae, Tomakomai-shi, 5.x.2017, larvae on *Agrimonia pilosa*, ex. pupa 21–31.v.2018, S.

Yagi; 1 ♀, Futamata, Osyamanbe, 19.vii.2012, H. Kogi; 8 ♂, 11 ♀, Omine, Oshamanbe, Yamakoshi-gun, 6.x.2017, larvae on *Agrimonia pilosa*, ex. pupa 9–13.v.2018, S. Yagi. **Honshu**: [Iwate Pref.] 4 ♂, 1 ♀, Matsuoyoriki, Hachimantai-shi, 16.x.2016, larvae on *Agrimonia pilosa*, ex. pupa 14–20.v.2017, S. Yagi; [Akita Pref.] 2 ♂, Chokai, Toshi, Nikaho-shi, 23.ix.2017, larvae on *Agrimonia pilosa*, ex. pupa 31.v–3.vi.2018, S. Yagi; [Nagano Pref.] 10 ♂, 7 ♀, Hirao Yamanouchi, Shimotakai-gun, 17.ix.2017, larvae on *Agrimonia pilosae*, ex. pupa 10.v–22.vi.2017, S. Yagi, DNA extraction No. SaY151; 1 ♀, Togakushi, 15.x.1986, host: "Kinmizuhiki" [= *Agrimonia pilosa*], ex. pupa 25.v.1987, H. Kuroko (OPU); 1 ♀, Osirakawa, Nagawamura, 11.vii.2004, B. W. Lee (OPU); 2 ♂, 1 ♀, Oshirakawa, Matsumoto-shi, 8.x.2015, larvae on *Agrimonia pilosa*, ex. pupa 5.vi.2016, S. Yagi; 2 ♂, 2 ♀, Tateshina-kogen, 2.x.1969, host: *Agrimonia pilosa*, ex. pupa 21.iv.1970, H. Kuroko, genitalia slide VU no. 2465 (OPU); 1 ♂, Minamijo, Anan, Shimoina, 26.x.2016, larva on *Agrimonia pilosa*, ex. pupa 29.v.2017, S. Yagi.

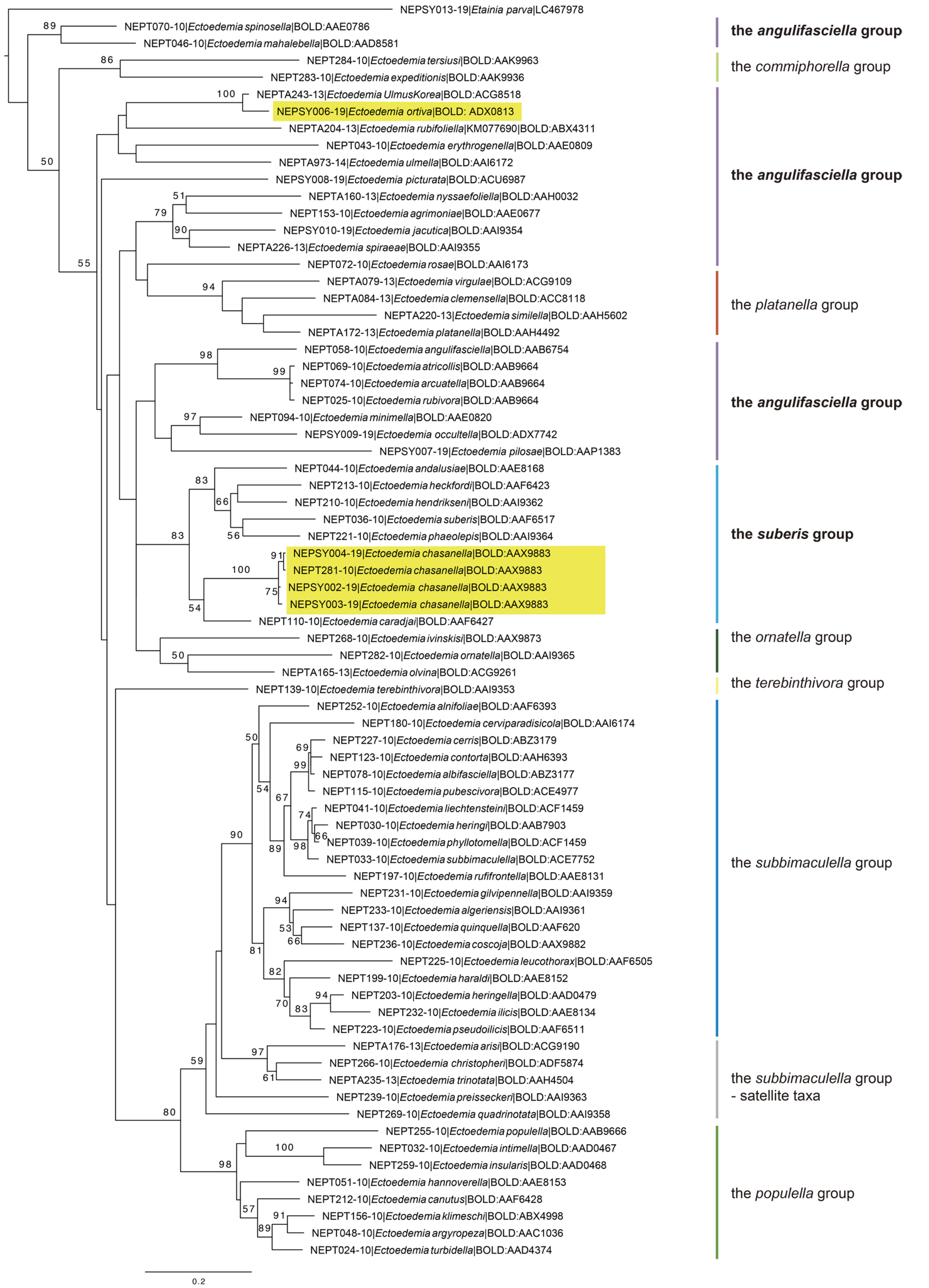
Male. Forewing length 1.8–2.2 mm (n = 20), wingspan 3.9–5.2 mm (n = 20). Female. Forewing length 2.0–2.4 mm (n = 20), wingspan 4.5–5.3 mm (n = 20).



FIGURES 24–28. Female genitalia of *Ectoedemia chasanella*. 24. Whole genitalia (Genitalia slide No. SY471). 25. T8+T9 (Genitalia slide No. SY330). 26. Vaginal sclerite with spiculate pouch. 27. Pectinations on corpus bursae (Genitalia slide No. SY471). 28. Spiculate pouch with a group of pectinations (Genitalia slide No. SY330). Scale bars: 200 µm (24), 100 µm (25, 26), 50 µm (27, 28).



FIGURES 29–36. Biology and habitat of *Ectoedemia chasanella*. 29. Egg scale on the upper side of the leaf of *Quercus crispula*. 30. Mine on *Q. crispula*. 31. Larva in the leaf of *Q. crispula*. 32. Larva in the leaf of *Q. acutissima*.33. cocoon with exuviae. 34. Freshly emerged adult, male. 35. Habitat at Ishikari-shi, with *Q. dentata* trees. 36. Habitat at Oita Pref., *Q. acutissima* and *Q. crispula* are present.



FIGURES 37. Phylogenetic tree based on ML method using two-gene region. Numbers associated with branches indicate bootstrap values higher than 50%. Species names are followed by each specimen's ID within sample names.

TABLE 2. Intraspecific and interspecific pairwise K2P distances in the COI barcoding region among the *Ectoedemia suberis* group.

	Species name (Process ID)	1	2	3	4	5	6	7	8	9	10	11	12			
1	*E. chasanella* (NEPSY001-19)	–														
2	*E. chasanella* (NEPSY002-19)	0.0031	–													
3	*E. chasanella* (NEPSY003-19)	0.0031	0.0015	–												
4	*E. chasanella* (NEPSY004-19)	0.0063	0.0045	0.0061	–											
5	*E. chasanella* (NEPT281-10)	0.0094	0.0060	0.0076	0.0015	–										
6	*E. aegilopidella* (NEPT244-10)	0.0536	0.0536	0.0536	0.0536	0.0570	–									
7	*E. andalusiae* (NEPT044-10)	0.0549	0.0693	0.0709	0.0693	0.0709	0.0536	–								
8	*E. caradjai* (NEPT110-10)	0.0417	0.0449	0.0465	0.0465	0.0465	0.0571	0.0594	–							
9	*E. caradjai* (NEPT230-10)	0.0449	0.0497	0.0513	0.0513	0.0513	0.0640	0.0562	0.0137	–						
10	*E. caradjai* (NEPT241-10)	0.0451	0.0482	0.0498	0.0498	0.0498	0.0608	0.0595	0.0030	0.0137	–					
11	*E. caradjai* (NEPTA074-13)	0.0579	0.0579	0.0579	0.0615	0.0615	0.0797	0.0723	0.0267	0.0301	0.0234	–				
12	*E. caradjai* (NEPTA075-13)	0.0456	0.0473	0.0490	0.0490	0.0490	0.0614	0.0588	0.0015	0.0123	0.0015	0.0234	–			
13	*E. heckfordi* (NEPT213-10)	0.0654	0.0743	0.0760	0.0760	0.0777	0.0750	0.0776	0.0694	0.0677	0.0728	0.0836	0.0723	–		
14	*E. hendrikseni* (NEPT210-10)	0.0518	0.0677	0.0693	0.0693	0.0710	0.0573	0.0498	0.0562	0.0530	0.0579	0.0687	0.0555	0.0595	–	
15	*E. phaeolepis* (NEPT221-10)	0.0550	0.0627	0.0644	0.0644	0.0660	0.0676	0.0627	0.0579	0.0562	0.0612	0.0796	0.0605	0.0778	0.0514	–
16	*E. suberis* (NEPT036-10)	0.0686	0.0759	0.0776	0.0776	0.0793	0.0856	0.0628	0.0611	0.0546	0.0612	0.0908	0.0605	0.0878	0.0728	0.0727

TABLE 3. Intraspecific and interspecific pairwise K2P distances in the EF1-α region among the *Ectoedemia suberis* group.

	Species name (Process ID)	1	2	3	4	5	6	7	8	9	10
1	*E. chasanella* (NEPSY002-19)	–									
2	*E. chasanella* (NEPT281-10)	0.0021	–								
3	*E. aegilopidella* (NEPT044-10)	0.0582	0.0539	–							
4	*E. andalusiae* (NEPT044-10)	0.0451	0.0429	0.0541	–						
5	*E. caradjai* (NEPT110-10)	0.0385	0.0363	0.0539	0.0564	–					
6	*E. caradjai* (NEPT230-10)	0.0341	0.0319	0.0496	0.0519	0.0042	–				
7	*E. caradjai* (NEPT241-10)	0.0385	0.0363	0.0539	0.0564	0.0000	0.0042	–			
8	*E. heckfordi* (NEPT213-10)	0.0453	0.0431	0.0411	0.0407	0.0408	0.0364	0.0408	–		
9	*E. hendrikseni* (NEPT210-10)	0.0565	0.0542	0.0541	0.0518	0.0474	0.0430	0.0474	0.0298	–	
10	*E. phaeolepis* (NEPT221-10)	0.0496	0.0474	0.0539	0.0518	0.0518	0.0474	0.0518	0.0276	0.0362	–
11	*E. suberis* (NEPT036-10)	0.0587	0.0564	0.0581	0.0563	0.0564	0.0519	0.0564	0.0319	0.0407	0.0297

TABLE 4. Intraspecific and interspecific pairwise K2P distances in the COI barcoding region among the *Ectoedemia angulifasciella* group.

	Species name (Process ID)	1	2	3	4	5	6	7	8	9	10	11	12
1	*E. ortiva* (NEPSY005-19)	–											
2	*E. ortiva* (NEPSY006-19)	0.0000	–										
3	*E. hexapetalae* (NEPTA2181-18)	0.1239	0.1237	–									
4	*E. rosae* (NEPT072-10)	0.1155	0.1153	0.0922	–								
5	*E. marmaropa* (NEPT1049-15)	0.1188	0.1186	0.0770	0.0738	–							
6	*E. spiraeae* (NEPTA226-13)	0.0999	0.0997	0.0807	0.0824	0.0860	–						
7	*E. jacutica* (NEPSY010-19)	0.1193	0.1191	0.1045	0.1051	0.1172	0.0695	–					
8	*E. agrimoniae* (NEPT153-10)	0.1015	0.1013	0.0938	0.0777	0.0888	0.0572	0.0761	–				
9	*E. nyssaefoliella* (NEPTA160-13)	0.1293	0.1290	0.0887	0.1060	0.1080	0.0756	0.0856	0.0784	–			
10	*E. pilosae* (NEPSY007-19)	0.0895	0.0893	0.0990	0.0945	0.1132	0.0841	0.0877	0.0742	0.0955	–		
11	*E. picturata* (NEPSY008-19)	0.1102	0.1100	0.0921	0.1032	0.1115	0.0808	0.0930	0.0809	0.1060	0.1047	–	
12	*E. minimella* (NEPT094-10)	0.0948	0.0947	0.1009	0.0982	0.0959	0.0740	0.0968	0.0878	0.1043	0.0980	0.0963	–
13	*E. occultella* (NEPSY009-19)	0.0929	0.0928	0.0700	0.0843	0.0888	0.0824	0.0928	0.0809	0.0921	0.0759	0.0927	0.0580
14	*E. angulifasciella* (NEPT058-10)	0.1103	0.1101	0.1044	0.1103	0.0975	0.0858	0.1265	0.0962	0.1095	0.1032	0.1206	0.0880
15	*E. arcuatella* (NEPT074-10)	0.0946	0.0944	0.0785	0.0860	0.0940	0.0824	0.1050	0.0792	0.0938	0.0759	0.0910	0.0744
16	*E. atricollis* (NEPT069-10)	0.0895	0.0893	0.0768	0.0860	0.0940	0.0824	0.1050	0.0792	0.0938	0.0742	0.0910	0.0761
17	*E. rubivora* (NEPT025-10)	0.0912	0.0910	0.0785	0.0826	0.0905	0.0790	0.1015	0.0759	0.0903	0.0759	0.0876	0.0744
18	*E. spinosella* (NEPT070-10)	0.1156	0.1154	0.0956	0.0962	0.0956	0.0739	0.1087	0.0843	0.1007	0.0860	0.0963	0.0947
19	*E. mahalebella* (NEPT046-10)	0.0964	0.0962	0.0921	0.1082	0.1043	0.0705	0.0930	0.0792	0.1007	0.0793	0.0979	0.0911
20	*E. erythrogenella* (NEPT043-10)	0.1154	0.1152	0.0904	0.1118	0.1114	0.0996	0.1313	0.0996	0.1183	0.1082	0.1014	0.1101
21	*E. rubifoliella* (NEPTA204-13)	0.1140	0.1138	0.1067	0.1282	0.1175	0.0893	0.1139	0.0909	0.1137	0.1155	0.1066	0.0980
22	*E. ulmella* (NEPTA973-14)	0.1080	0.1078	0.0972	0.1095	0.0991	0.0909	0.1238	0.1042	0.1077	0.1147	0.1148	0.1077
23	*E. UlmusKorea* (NEPTA243-13)	0.0282	0.0281	0.1094	0.1095	0.1060	0.0892	0.1150	0.0938	0.1183	0.0852	0.1024	0.0870
24	*E. Rosa Taiwan* (NEPTA199-13)	0.1061	0.1059	0.0835	0.0904	0.0991	0.0739	0.0922	0.0684	0.0852	0.0618	0.0955	0.0888
25	*E. StephanandraKorea* (NAPTA1112-15)	0.0924	0.0922	0.0870	0.0820	0.0926	0.0756	0.0871	0.0819	0.0972	0.0802	0.0871	0.0540

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TABLE 4. (Continued)

	Species name (Process ID)	13	14	15	16	17	18	19	20	21	22	23	24
1	*E. ortiva* (NEPSY005-19)												
2	*E. ortiva* (NEPSY006-19)												
3	*E. hexapetalae* (NEPTA2181-18)												
4	*E. rosae* (NEPT072-10)												
5	*E. marmaropa* (NEPT1049-15)												
6	*E. spiraeae* (NEPTA226-13)												
7	*E. jacutica* (NEPSY010-19)												
8	*E. agrimoniae* (NEPT153-10)												
9	*E. nyssaefoliella* (NEPTA160-13)												
10	*E. pilosae* (NEPSY007-19)												
11	*E. picturata* (NEPSY008-19)												
12	*E. minimella* (NEPT094-10)												
13	*E. occultella* (NEPSY009-19)	–											
14	*E. angulifasciella* (NEPT058-10)	0.0929	–										
15	*E. arcuatella* (NEPT074-10)	0.0628	0.0778	–									
16	*E. atricollis* (NEPT069-10)	0.0611	0.0778	0.0045	–								
17	*E. rubivora* (NEPT025-10)	0.0628	0.0777	0.0030	0.0045	–							
18	*E. spinosella* (NEPT070-10)	0.0843	0.1065	0.0894	0.0894	0.0860	–						
19	*E. mahalebella* (NEPT046-10)	0.0810	0.0980	0.0876	0.0876	0.0876	0.0712	–					
20	*E. erythrogenella* (NEPT043-10)	0.0894	0.1259	0.0893	0.0876	0.0893	0.0962	0.0996	–				
21	*E. rubifoliella* (NEPTA204-13)	0.0927	0.1247	0.1049	0.1049	0.1014	0.1120	0.0961	0.1173	–			
22	*E. ulmella* (NEPTA973-14)	0.0972	0.1078	0.0990	0.0990	0.0955	0.0974	0.0835	0.1025	0.1121	–		
23	*E. UlmusKorea* (NEPTA243-13)	0.0886	0.1078	0.0903	0.0886	0.0869	0.1042	0.0938	0.1130	0.1120	0.1007	–	
24	*E. Rosa Taiwan* (NEPTA199-13)	0.0701	0.0921	0.0869	0.0869	0.0835	0.0819	0.0700	0.1059	0.1014	0.0972	0.1007	–
25	*E. StephanandraKorea* (NAPTA1112-15)	0.0489	0.0890	0.0669	0.0652	0.0635	0.0889	0.0836	0.0921	0.0892	0.1025	0.0836	0.0836

TABLE 5. Intraspecific and interspecific pairwise K2P distances in the EF1-α region among the *Ectoedemia angulifasciella* group.

	Species name (Process ID)	1	2	3	4	5	6	7	8	9	10
1	*E. ortiva* (NEPSY006-19)	–									
2	*E. rosae* (NEPT072-10)	0.0930	–								
3	*E. spiraeae* (NEPTA226-13)	0.0923	0.0826	–							
4	*E. jacutica* (NEPSY010-19)	0.0954	0.0955	0.0412	–						
5	*E. agrimoniae* (NEPT0153-10)	0.0957	0.0791	0.0458	0.0609	–					
6	*E. nyssaefoliella* (NEPTA160-13)	0.0772	0.0842	0.0505	0.0542	0.0477	–				
7	*E. pilosae* (NEPSY007-19)	0.0952	0.1012	0.1046	0.1126	0.1079	0.1209	–			
8	*E. picturata* (NEPSY008-19)	0.0815	0.0957	0.0851	0.1008	0.0961	0.0964	0.0956	–		
9	*E. minimella* (NEPT094-10)	0.0860	0.0767	0.0897	0.1002	0.0909	0.0914	0.0887	0.0887	–	
10	*E. occultella* (NEPSY009-19)	0.1122	0.0953	0.1064	0.1121	0.1026	0.1082	0.1100	0.1051	0.0562	–
11	*E. angulifasciella* (NEPT058-10)	0.1002	0.1025	0.0778	0.0906	0.0860	0.0984	0.1099	0.0838	0.0788	0.0998
12	*E. arcuatella* (NEPT074-10)	0.1031	0.1103	0.0781	0.0791	0.0864	0.0867	0.1054	0.0771	0.0861	0.1098
13	*E. atricollis* (NEPT069-10)	0.1031	0.1103	0.0781	0.0791	0.0816	0.0915	0.1054	0.0771	0.0861	0.1049
14	*E. rubivora* (NEPT025-10)	0.1005	0.1126	0.0804	0.0766	0.0887	0.0890	0.1078	0.0793	0.0884	0.1122
15	*E. spinosella* (NEPT070-10)	0.1004	0.1004	0.1021	0.0956	0.1107	0.0962	0.1177	0.0792	0.0933	0.1025
16	*E. mahalebella* (NEPT046-10)	0.1078	0.1004	0.1097	0.1129	0.1183	0.1036	0.1227	0.0961	0.1006	0.1025
17	*E. erythrogenella* (NEPT043-10)	0.0744	0.1100	0.1024	0.1030	0.1032	0.1063	0.1124	0.0983	0.0933	0.1026
18	*E. rubifoliella* (NEPTA204-10)	0.0656	0.0984	0.0929	0.0934	0.0866	0.0778	0.1152	0.1038	0.1109	0.1128
19	*E. ulmella* (NEPTA973-14)	0.0747	0.0910	0.0900	0.0935	0.0867	0.0751	0.1178	0.0793	0.0886	0.1030
20	*E. UlmusKorea* (NEPTA243-13)	0.0063	0.0860	0.0852	0.0883	0.0887	0.0703	0.0882	0.0746	0.0814	0.1050

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TABLE 5. (Continued)

	Species name (Process ID)	11	12	13	14	15	16	17	18	19
1	*E. ortiva* (NEPSY006-19)									
2	*E. rosae* (NEPT072-10)									
3	*E. spiraeae* (NEPTA226-13)									
4	*E. jacutica* (NEPSY010-19)									
5	*E. agrimoniae* (NEPT0153-10)									
6	*E. nyssaefoliella* (NEPTA160-13)									
7	*E. pilosae* (NEPSY007-19)									
8	*E. picturata* (NEPSY008-19)									
9	*E. minimella* (NEPT094-10)									
10	*E. occultella* (NEPSY009-19)									
11	*E. angulifasciella* (NEPT058-10)	–								
12	*E. arcuatella* (NEPT074-10)	0.0429	–							
13	*E. atricollis* (NEPT069-10)	0.0429	0.0042	–						
14	*E. rubivora* (NEPT025-10)	0.0451	0.0063	0.0063	–					
15	*E. spinosella* (NEPT070-10)	0.1099	0.0981	0.0981	0.1004	–				
16	*E. mahalebella* (NEPT046-10)	0.1225	0.1104	0.1054	0.1128	0.0298	–			
17	*E. erythrogenella* (NEPT043-10)	0.0953	0.1030	0.1030	0.1053	0.1003	0.1077	–		
18	*E. rubifoliella* (NEPTA204-10)	0.1054	0.1110	0.1161	0.1134	0.1032	0.1057	0.0818	–	
19	*E. ulmella* (NEPTA973-14)	0.0958	0.0962	0.0962	0.0985	0.0886	0.1031	0.0984	0.0872	–
20	*E. UlmusKorea* (NEPTA243-13)	0.0931	0.0960	0.0960	0.0934	0.0933	0.1006	0.0676	0.0588	0.0678

FIGURES 38–41. Adults of the Japanese *Ectoedemia angulifasciella* group, male 38. *E. pilosae* collected in Shimotakai-machi, Nagano Pref. 39. *E. picturata* collected in Ishikari-shi, Hokkaido Pref. 40. *E. occultella* collected in Kutchan-cho, Hokkaido Pref. 41. *E. jacutica* collected in Tomakomai-shi, Hokkaido Pref.

Diagnosis. This species can be distinguished from other described species of the *angulifasciella* group species by the pale color of the scape, the long cream-white hair-pencil (ca. 0.45 times as long as hindwing), and the forewing with strong silver reflections at the base. The thin triangular pseuduncus, slender sublateral process of transtilla, and slender median carina in the male genitalia are also useful in distinguishing it from other members of the *angulifasciella* group.

Barcode data. The DNA barcode of one specimen (BOLD: AAP1383) was generated and deposited in GenBank with accession number LC467968. The DNA barcode of this species is closest to that of "*Ectoedemia Rosa-Taiwan*" based on BLAST in GenBank, and the similarity between them is 94.07%.

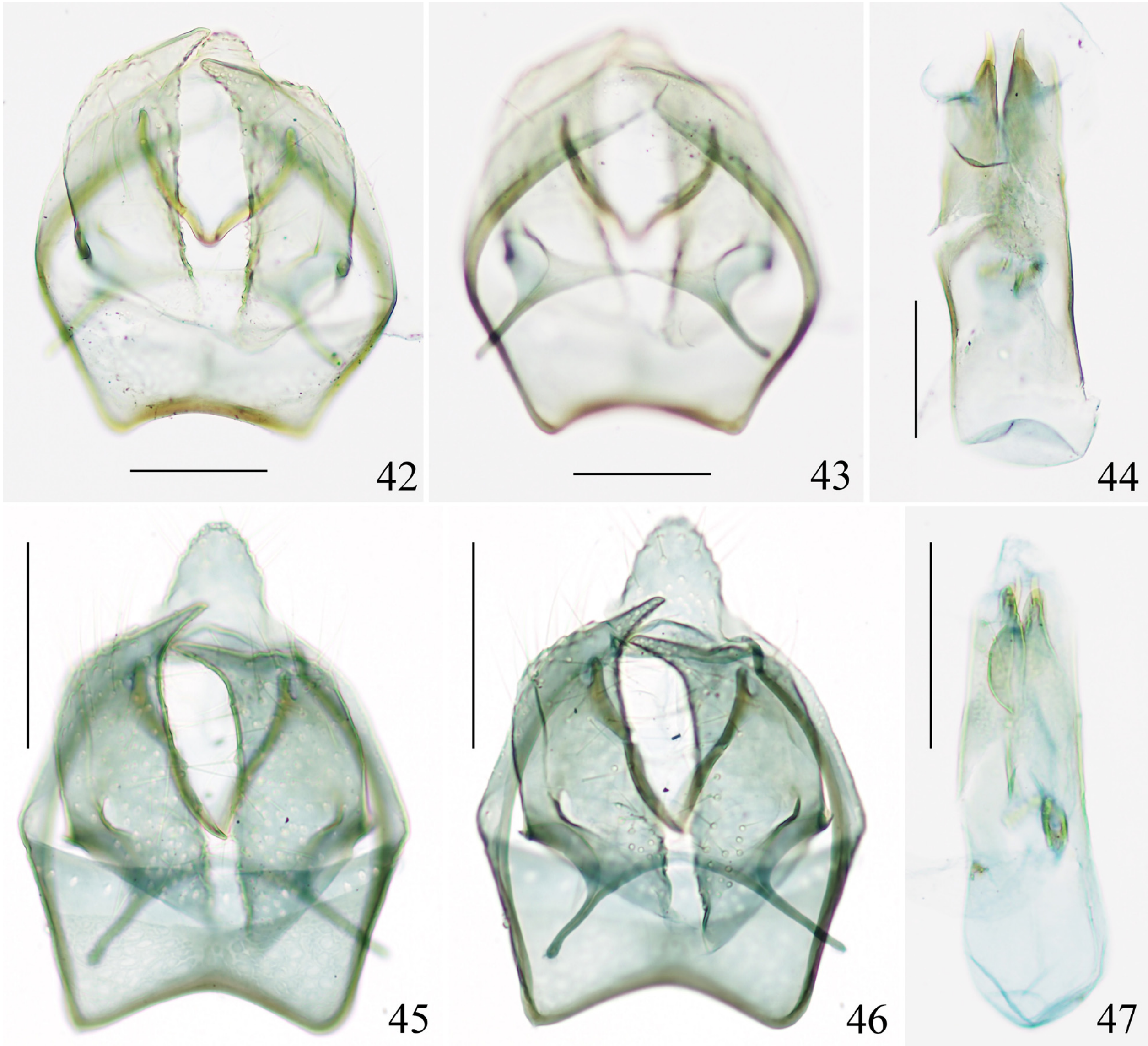
Biology. The Japanese population utilizes *Agrimonia pilosa* Ledeb. var. *japonica* (Miq.) Nakai as the host plant. Eggs are depostied on the underside of the leaf. The leafmine starts as a contorted gallery with blackish frass, and later becomes a broad serpentine gallery with dense, narrow, blackish frass. Larvae light yellow, found between September and October, which indicates univoltinism.

Distribution (Fig. 60). Japan: Hokkaido, Honshu, Kyushu; Russia: Far East; South Korea: Gangwon-do.

Remarks. *Stigmella alikurokoi* Kemperman & Wilkinson, 1985, recorded as *Stigmella* sp. 2 in Owada *et al*. (2006), also utilizes *Agrimonia pilosa* in Japan. However, the two can be easily distinguished because *E. pilosae* makes a contorted mine whereas *S. alikurokoi* makes a linear mine. Moreover, *E. pilosae* is distributed in the cool temperate zone, whereas *S. alikurokoi* is distributed in the warm temperate zone. Therefore, these species do not occur sympatrically.

4. *Ectoedemia picturata* Puplesis, 1985
(Figs. 39, 45–47, 55, 61)

Ectoedemia picturata Puplesis, 1985: 65.
Type locality: Primorskiy Kray, 20 km E Ussuriysk, Gornotayezhnoe (Russia).



FIGURES 42–47. Male genitalia of the Japanese *Ectoedemia angulifasciella* group. 42, 43. *E. pilosae*, genital capsule with phallus removed (genitalia slide no. SY224). 44. Ditto, phallus (Genitalia slide No. SY224). 45, 46. *E. picturata*, genital capsule with phallus removed (Genitalia slide No. SY183). 47. Ditto, phallus (Genitalia slide No. SY287).

Material examined. JAPAN. Hokkaido: 3 ♂, 6 ♀, Bansei, Taiki-cho, Hiroo-gun, 6.x.2017, larvae on *Rosa rugosa*, ex. pupa 6.v–8.vi.2018, S. Yagi; 1 ♀, Shinkoh, Isikari, 29.v.2002; 1 ♀, 3.vi.2004, H. Kogi; 1 ♂, Sibi, Ishikari, 15.vii.2012; 2 ♂, 4 ♀, 23.ii–28.v.2014, host: "Hamanasu [= *Rosa rugosa*]", H. Kogi; 2 ♂, 3 ♀, Ishikarihama, Ishikari-shi, 3.x.2015, larvae on *Rosa rugosa*, ex. pupa 14–16.vi.2016, genitalia no. SY183; 5 ♂, 3 ♀, 4.x.2015, larvae on *Rosa rugosa*, ex. pupa 14.v–14.vi.2016, S. Yagi; 85 ♂, 101 ♀, Hama-machi, Ishikari-shi, 7.x.2017, larvae on *Rosa rugosa*, ex. pupa 14.iv–22.vi.2018, S. Yagi; 4 ♀, Oyafune, Oyafune-cho, Ishikari, 18.vii.2018, larvae on *Rosa rugosa*, ex. pupa 7–9.viii.2018, S. Yagi. **Honshu**: [Fukushima Pref.] 1 ♂, Akai, Minato-machi, Aizuwakamatsu-shi, 21.x.2016, larva on *Rosa multiflora*, ex. pupa 30.iv.2017, S. Yagi, DNA extraction no. SaY12; [Nagano Pref.] 1 ♂, Sugadaira-kogen, Ueda-shi, 17.ix.2017, larva on *Rosa dauvurica*, ex. pupa 30.iv.2018, S. Yagi; 1 ♂, Kitayama, Chino-shi, 19.ix.2017, larva on *Rosa multiflora*, ex. pupa 25.v.2018, S. Yagi; 1 ♂, Yatsugatake-nojo, Tamagawa, Chino-shi, 15.ix.2017, larva on *Rosa multiflora*, ex. pupa 12.v.2018, S. Yagi; [Yamanashi Pref.] 9 ♂, 9 ♀, Mt. Mikuni, Hirano, Yamanakako-mura, 24.x.2016, larvae on *Rosa hirtula*, ex. pupa 6.v–21.vi.2017, S. Yagi, genitalia no. SY287; [Shizuoka Pref.] 9 ♂, 10 ♀, Otome-toge, Fukasawa, Gotenba, 27.x.2016, larva on *Rosa hirtula*, ex. pupa 11.v–29.vi.2017, S. Yagi; [Aichi Pref.] 1 ♂, Dando, Damine, Shitara, Kitashitara, 11.ix.2018, larva on *Rosa multiflora*, ex. pupa 30.ix.2018, S. Yagi; [Mie Pref.] 2 ♀, Nishi-6, Yurigaoka, Nabari, 30.vii.2011, larvae on *Rosa multiflora*, ex. pupa 10.viii.2011, S. Kobayashi (OPU). **Kyushu**: [Fukuoka Pref.] 1 ♂, Hikosan (Buzen), 29.ix.1954, "Noibara no miner" [=miner of *Rosa multiflora*] (OPU); 1 ♀, 2.x.1954, genitalia slide VU no. 2488 (OPU); 1 ♀, 19.vi.1957, H. Kuroko (OPU); [Kagoshima Pref.] 1 ♂, 1 ♀, Hanazato-cho, Kanoya-shi, 8.v.2018, larvae on *Rosa multiflora*, ex. pupa 1–3.vi.2018, S. Yagi.

Male. Forewing length 1.6–2.0 mm (n = 30), wingspan 3.4–4.5 mm (n = 30). Female. Forewing length 1.8–2.1 mm (n = 30), wingspan 3.7–4.7 mm (n = 30).

Diagnosis. Worldwide, five *Rosa*-feeding species are recognized, i.e., *E. angulifasciella* (Stainton, 1849), *E. marmaropa* (Braun, 1925), *E. rosiphila* Puplesis, 1992, *E. rosae* van Nieukerken & Berggren, 2011, and *E. picturata*. *Ectoedemia picturata* can be easily distinguished from *E. rosae*, *E. marmaropa*, and *E. rosiphila* by the yellowish orange frontal tuft, forewing with a metallic fascia, and white-cream hair-pencil (ca. 0.30 times as long as hindwing, and absent in three species). It also can be distinguished from *E. angulifasciella* by the long sublateral process of the transtilla and the straight inner margin of the valva. The male genitalia are similar to those of *E. rubivora* complex, but they can be distinguished by the wider ventral plate of the vinculum; geographic distribution and host plant also serve to distinguish it.

Barcode data. The DNA barcode of one specimen (BOLD: ACU6987) was generated and deposited in GenBank with accession number LC467969. The barcode of this species is closest to that of *Ectoedemia spiraeae* based on BLAST in GenBank, and the similarity between them is 92.40%.

Biology. This species seems to utilize only *Rosa* spp.; the Japanese population utilizes *Rosa rugosa* Thunb., *R. multiflora* Thunb., *R. hirtula* (Regel) Nakai and *R. amblyotis* C.A. Mey. Eggs are deposited on the upper side of the leaf. The leafmine starts as a linear gallery, filled with blackish frass, sometimes more or less contorted. The later mine becomes a broad serpentine or blotch gallery with dense blackish frass, sometimes more or less dispersed. Larvae are light yellow, found between May and October, which indicates bivoltinism or more.

Distribution (Fig. 61). Japan: Hokkaido, Honshu, Kyushu; Russia: Far East; China: Heilongjiang Sheng.

Remarks. This species exhibits some variation in the median carina of male genitalia (Stonis & Rocienė, 2013; Rocienė & Stonis, 2014), and the holotype (Stonis & Rocienė, 2013) has short median carinae while other specimens collected in the same locality as the holotype (Rocienė & Stonis, 2014) have slender and sharp median carinae. This variation is also observed in Japan where the median carinae are short in specimens collected in the northern part of the country while they are slender in specimens collected in the southern part. However, no other differences in external morphology or genitalia were observed, so we consider this as intraspecific variation.

5. *Ectoedemia occultella* (Linnaeus, 1767)

(Figs. 40, 48–50, 56, 62)

For synonymy see van Nieukerken *et al*. 2016.
Type locality: Hammerby (Sweden).

Material examined. JAPAN. Hokkaido: 3 ♂, 1♀, Hanazono, Kutchan-cho, 6.x.2017, larvae on *Betula ermanii*, ex. pupa 20.v.2018, S. Yagi, genitalia slide no. SY578, DNA extraction no. SaY150. **Honshu**: [Nagano Pref.] 1 ♂, Tateshina-kogen, 2.x.1969, larva on *Betula platyphylla*, 9.v.1970, H. Kuroko (OPU).

Male. Forewing length 2.2–2.3 mm, wingspan 5.0–5.3 mm (n = 4). Female. Forewing length 2.7 mm, wingspan 6.2 mm (n = 1).

Diagnosis. This species and *E. minimella* (Zetterstedt,1839) can be easily distinguished from others of the *angulifasciella* group by the absence of a cilia line and a long pseuduncus. *Ectoedemia occultella* is similar to *E. minimella*, and it can be distinguished by the frontal tuft in female (male with blackish tuft in *E. minimella*) and vesica without elongated cornuti.

In East Asia a morphologically very similar *Sorbus*-feeding *Ectoedemia* occurs sympatrically with *E. occultella* (Hirano 2013; van Nieukerken *et al*. 2016: Supplementary material 2). However, *E. occultella* can be separated from it by the fuscous forewing (fuscous forewing with stronger purple sheen in the *Sorbus*-feeding *Ectoedemia*), sharper pseuduncus (a little shorter and not so sharp in the *Sorbus*-feeding *Ectoedemia*), and slender sublateral process of transtilla in male genitalia (basally thick in the *Sorbus*-feeding *Ectoedemia*). These two species are very difficult to distinguish and their host plants occur sympatrically. Therefore, records that are not based on reared specimens need to be confirmed.

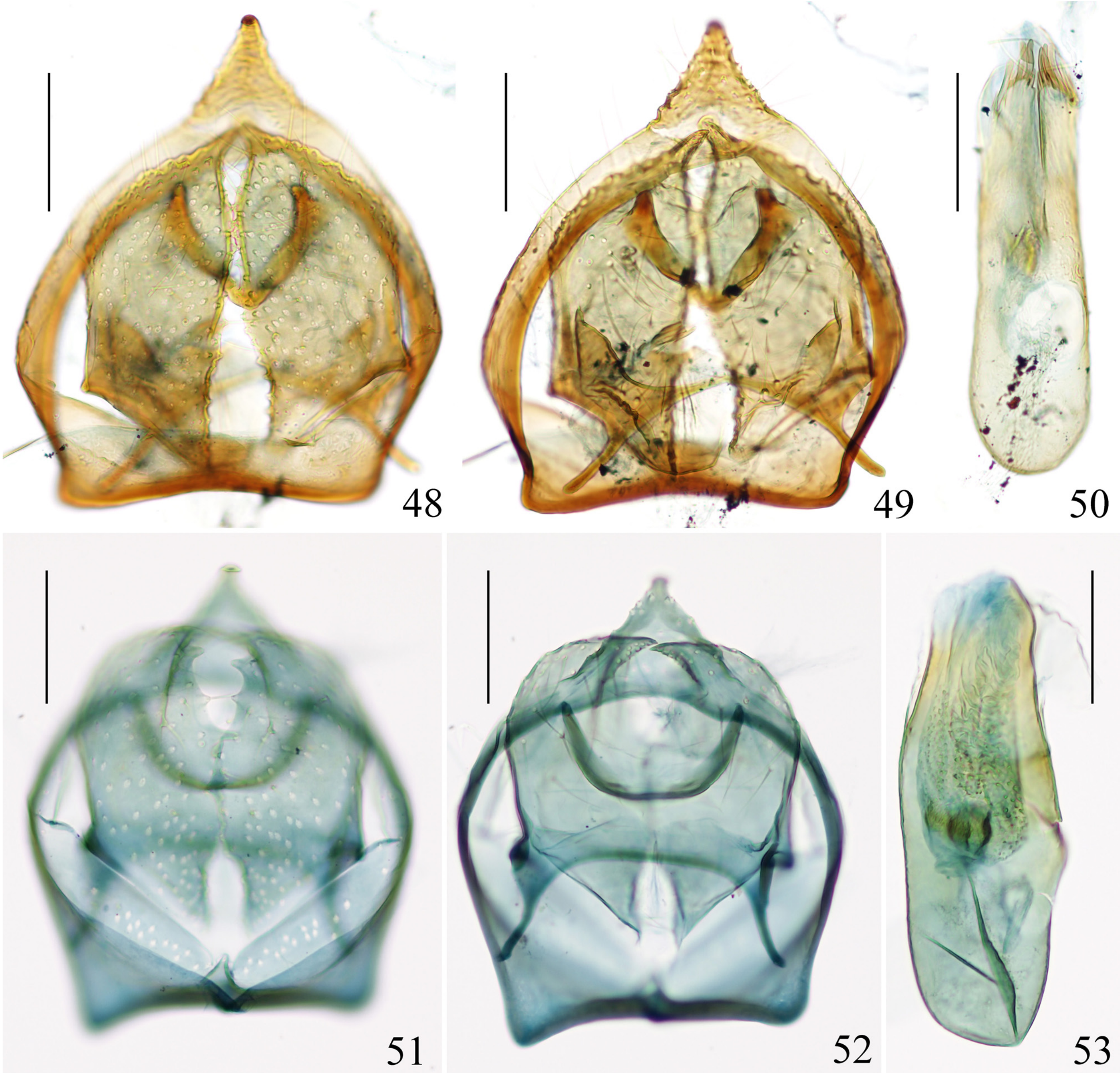
Barcode data. A DNA barcode of one specimen (BOLD: ADX7742) was generated and deposited in the Gen-

Bank with accession number LC467971. The barcode of this species is closest to that of *"Ectoedemia StephanandraKorea"* based on BLAST in GenBank, and the similarity between them is 95.29%.

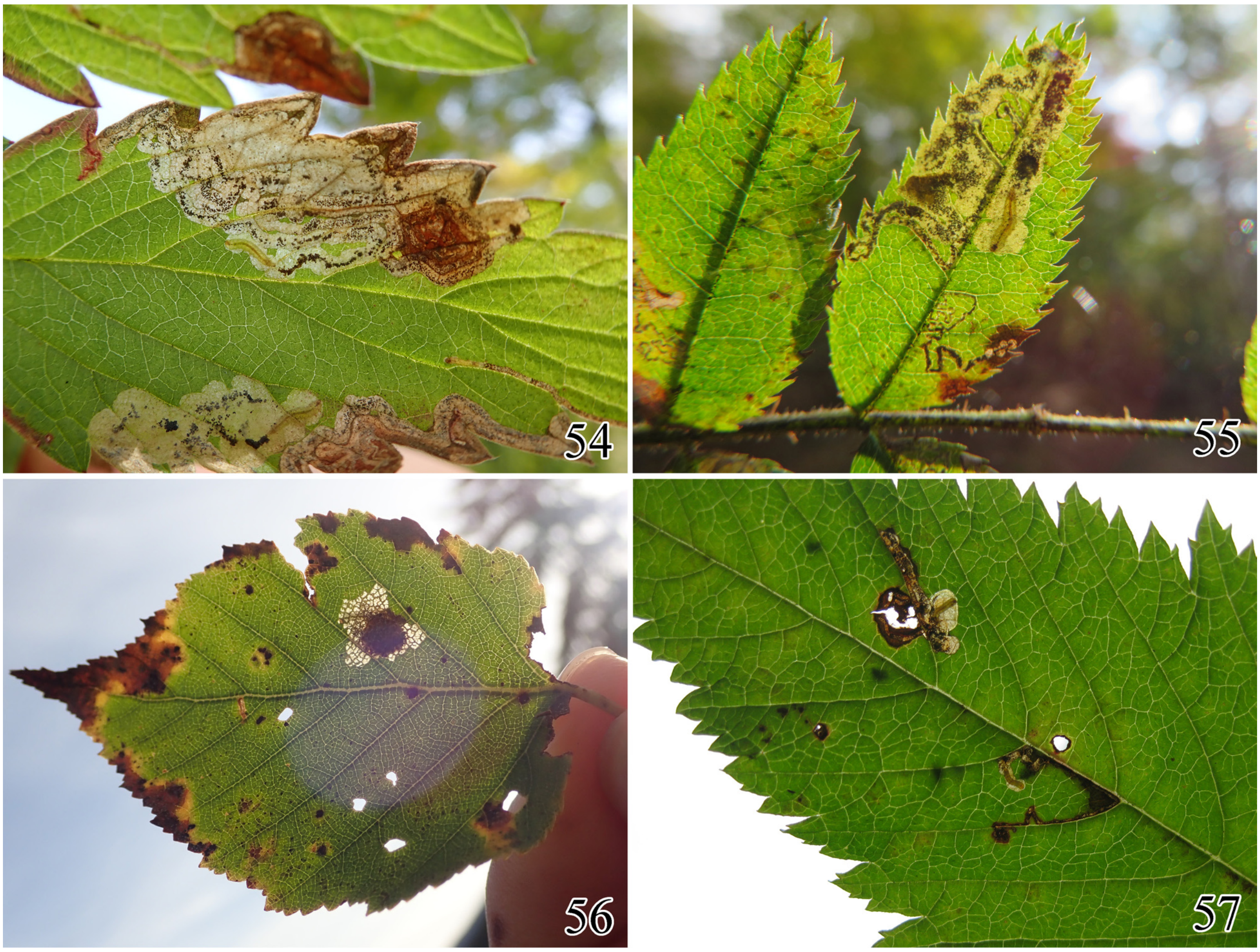
Biology. The Japanese population utilizes *Betula platyphylla* Sukaczev var. *japonica* (Miq.) H. Hara and *B. ermanii* Cham. Similar mines also were found on *B. maximowicziana* Regel and *B. corylifolia* Regel et Maxim. Eggs are deposited on the underside of the leaf. The leafmine starts as a blotch, filled with blackish frass, and later becomes a larger blotch and the blackish dense frass is glued to the upper surface at the center of the mine, forming a dark spot under which the larva can hide. Larvae are pale greenish cream; found in October, which indicates univoltinism.

Distribution (Fig. 62). Northern Holarctic: from westernmost Europe to Japan and throughout northern North America (Puplesis 1994).

Remarks. A similar shaped mine and larvae are found on *Stephanandra* spp. in Korea and Japan (Doorenweerd *et al*. 2015; Yagi unpubl.).



FIGURES 48–53. Male genitalia of the Japanese *Ectoedemia angulifasciella* group. 48, 49. *E. occultella*, genital capsule with phallus removed (Genitalia slide No. SY578). 50. Ditto, phallus (Genitalia slide No. SY578). 51, 52. *E. jacutica*, genital capsule with phallus removed (Genitalia slide No. SY189). 53. Ditto, phallus (Genitalia slide No. SY172).



FIGURES 54–57. Leafmines of the Japanese species of the *Ectoedemia angulifasciella* group. 54. *E. pilosae* on *Agrimonia pilosa*. 55. *E. picturata* on *Rosa hirtula*. 56. *E. occultella* on *Betula platyphylla*. 57. *E. jacutica* on *Aruncus dioicus*.

6. *Ectoedemia jacutica* Puplesis, 1988
(Figs. 41, 51–53, 57, 63)

Ectoedemia jacutica Puplesis, 1988: 26; Stonis *et al*. 2015: 117.
Ectoedemia species 219: Rocienė & Stonis 2013: 77.
Type locality: Yakutia, Yakutsk (Russia).

Material examined. JAPAN. Hokkaido: 1 ♂, Manzi, Iwamizawa, 15.vii.2012, H. Kogi; 8 ♂, 6 ♀, Tomakomai, 1–15.vi.1970, host: *Spiraea salicifolia*, T. Kumata, genitalia slide no. SY172 (SEHU); 5 ♂, 3 ♀, Tomakomai, 1–13.vi.1970, host: *Spiraea salicifolia*, T. Kumata. (OPU); 17 ♂, 17 ♀, Kashiwabara, Tomakomai-shi, 1.x.2015, larvae on *Spiraea salicifolia*, ex. pupa 27.v–17.vi. 2016, genitalia slide no. SY189; 4 ♂, 3 ♀, 8.x.2017, larvae on *Spiraea salicifolia*, ex. pupa 20.v–22.vi.2018, S. Yagi. **Honshu**: [Akita Pref.] 5 ♂, 6 ♀, Kaginotaki, Ani, Akita-shi, 24.ix.2017, larvae on *Aruncus dioicus*, ex. pupa 13.v–1.vi.2018, S. Yagi, DNA extraction no. SaY181; 1 ♀, Babatoshiyama Nikaho-shi, 24.ix.2017, larva on *Aruncus dioicus*, ex. pupa 19.v.2018, S. Yagi; [Iwate Pref.] 8 ♂, 6 ♀, Takinoue, 30.ix.1975, larvae on "Shoma"[=*Aruncus dioicus*], 13.v–2.vi.1976, H. Kuroko (OPU); 1 ♀, Matsuoyoriki, Hachimantai-shi, 16.x.2016, larva on *Aruncus dioicus*, ex. pupa 29.v.2017, S. Yagi; [Miyagi Pref.] 8 ♂, 3 ♀, Okura, Aoba-ku, Sendai-shi, 14.x.2017, larvae on *Aruncus dioicus*, ex. pupa 30.iv–9.v.2018, S. Yagi; 2 ♂, 1 ♀, Kumazawa-Rindo, Okura, Aoba-ku, Sendai-shi, 14.x.2017, larvae on *Aruncus dioicus*, ex. pupa 6–16.v.2018, S. Yagi; [Fukushima Pref.] 5 ♂, 3 ♀, Yaheishiro, Ine, Nishiaizu-machi, 21.ix.2017, larvae on *Aruncus dioicus*, ex. pupa 13.v–18.vi.2018, S. Yagi; [Nagano Pref.] 1 ♂, Shibu-onsen, Shiga-kogen, 18.x.1986, larva on *Aruncus dioicus*, H. Kuroko (OPU); 1 ♀, Shiga-kogen, 30.v.1964, Host: *Aruncus dioicus*, H. Kuroko leg. (OPU); 9 ♂, 12 ♀, Hirao, Yamanouchi-machi, Shimotakai, 17.ix.2017, larvae on *Aruncus dioicus*, ex. pupa 5–20.v.2018, S. Yagi; 6 ♂, 7 ♀, Hijiri-kogen, Omi-mura, 20.x.2015, larvae on *Spiraea salicifolia*, ex. pupa 20.v–3.vii.2016, S. Yagi; 3 ♂, Mineno-tyaya Karuizawa-machi Nagano-ken, 9.x.1989, larvae on *Spiraea chamaedryfolia*, ex. pupa 28.v–6.vi.1990, N. Hirano leg., genitalia slide NH-393, host no.728; 2 ♂, Minodo, 9.v.1970, host: "Shimotsuke" [=*Spiraea japonica*], H. Kuroko leg. (OPU); 4 ♂, 2 ♀, Minodo, Tamagawa, Chino-shi, 15.ix.2017, larvae on *Spiraea chamaedryfolia*, ex. pupa 1.vi.2018, S. Yagi; 7 ♂, 7 ♀, Ichirino, (Hakusan), Ishikawa Pref., 9.x.1982, larvae on *Aruncus dioicus*, H. Kuroko (OPU). **Kyushu**: [Fukuoka Pref.] 8 ♂, 6 ♀, Hikosan, Fukuoka Pref., 20.vi.1956, host: "Shimotsuke" [=*Spiraea japonica*], H. Kuroko (OPU).

Male. Forewing length 1.6–2.5 mm (n = 30), wingspan 3.8–5.5 mm (n = 30). Female. Forewing length 1.9–2.6 mm (n = 30), wingspan 4.2–5.8 mm (n = 30).

Diagnosis. This species is similar to *E. spiraeae*, but can be identified by the dark brown hair-pencil (yellowish white in *E. spiraeae*), and the shorter and thicker sublateral process of the transtilla (long and thin in *E. spiraeae*).

Barcode data. A DNA barcode of one specimen (BOLD: AAI9354) was generated and deposited in the GenBank with accession number LC467970. The barcode of this species is closest to that of *E spiraeae* based on BLAST in GenBank, and the similarity between them is 93.55%.

Biology. The Japanese population utilizes *Spiraea salicifolia* L., *S. chamaedryfolia* L. var. *pilosa* (Nakai) H. Hara, *S. japonica* L.f., and *Aruncus dioicus* (Walter) Fernald var. *kamtschaticus* (Maxim.) H. Hara. Eggs are deposited on the underside of the leaf. The leafmine starts as a linear gallery with blackish frass, later becoming a blotch with dense, narrow, blackish frass or dispersed frass. Larvae are pale greenish cream; found only in September and October, which indicates univoltinism. Adults emerged from May to July and a male was collected by light trap in July.

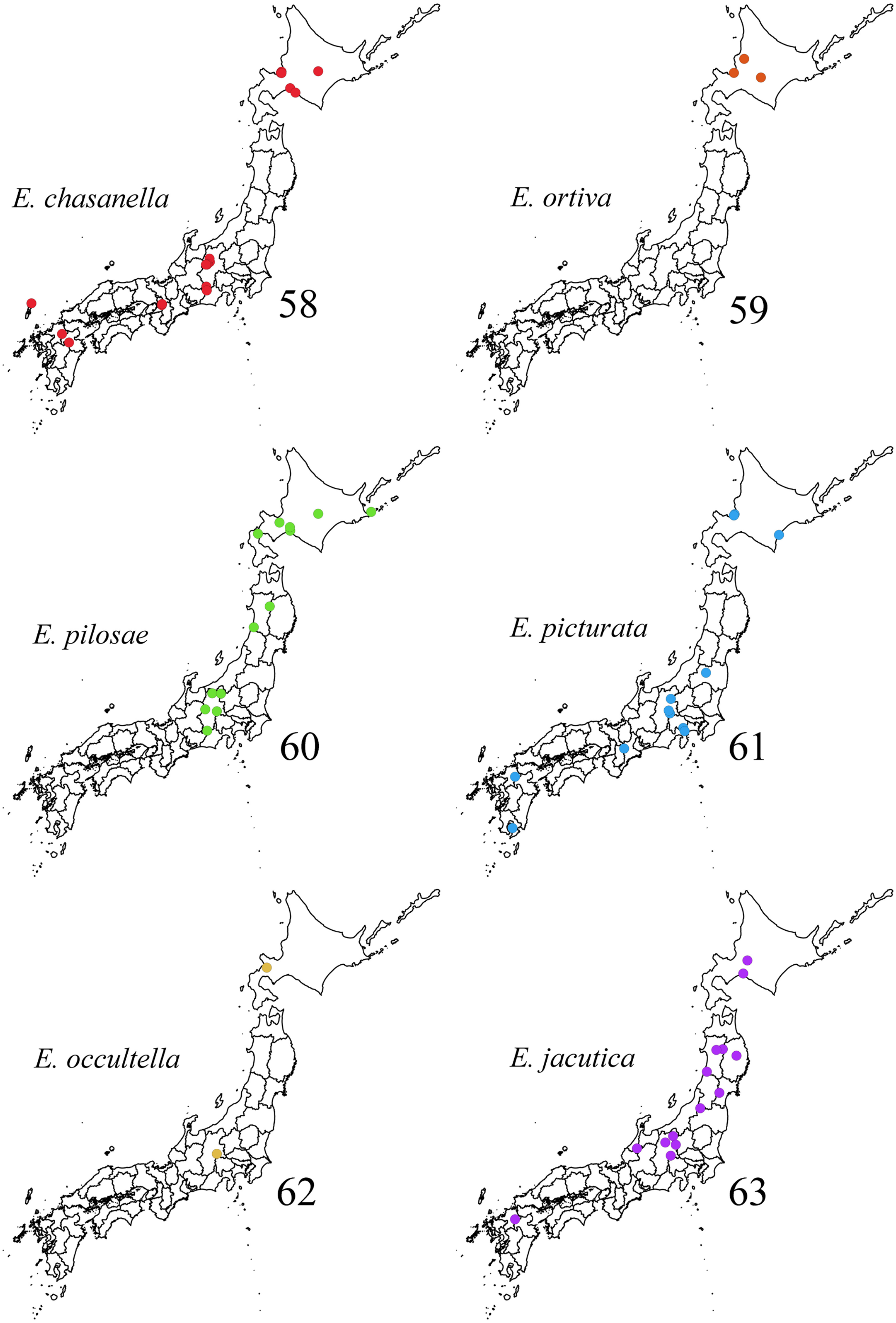
Distribution (Fig. 63). Japan: Hokkaido, Honshu, Kyushu; Russia: Yakutsk, West Altai; China: Heilongjiang Sheng.

Remarks. This species was recorded from Japan in 2010 for the first time as *E. spiraeae*, then it was reported as *E. jacutica* in 2016 (van Nieukerken *et al*. 2010; van Nieukerken *et al*. 2016). Japanese populations show some differences from the type specimen (Stonis *et al*. 2015), i.e., the color of fascia of forewing is cream white (slightly shining in holotype) and the hair-pencil is conspicuous (inconspicuous in the holotype), whereas the male genitalia are very similar and difficult to distinguish. There are no sequence data for *E. jacutica* from the type locality or the area close by. Therefore, the identification of the Japanese population is tentative. For a more accurate identification, additional sampling in the type locality is necessary.

The morphology of Japanese *E. jacutica* is similar to that of "*Ectoedemia* species 219", which is treated as a member of the *subbimaculella* group (Rocienė & Stonis 2013). However, this specimen is similar to some species of the *angulifasciella* group, e.g., *E. agrimoniae*, *E. spiraeae*, and *E. jacutica*, in the absence of median carinae and the developed pseuduncus.

Acknowledgements

We greatly appreciate the generous guidance of Erik J. van Nieukerken (Naturalis Biodiversity Center, Netherlands) and Camiel Doorenweerd (University of Hawai'i) on the taxonomy of the family Nepticulidae and for providing us with DNA sequences of *Ectoedemia* worldwide. We are grateful to H. Kogi (Sapporo-shi) and H. Shimizu (Fujiidera-shi) for the gift of specimens of *Ectoedemia*. We also thank M. Ishii (OPU), N. Hirai (OPU), S. Kobayashi (OPU), M. Ohara (SEHU), and T. Kumata (Ebetsu-shi) for allowing us to examine the collection of OPU and SEHU. We thank Editage (www.editage.jp) for English language editing. This study was supported by a Grant-in-Aid for JSPS Research Fellow (JAG7J07644). This is a contribution from the Entomological Laboratory, Kyushu University, Fukuoka (Ser. 7, No. 67).



FIGURES 58–63. Japanese distribution maps of Japanese species of the *Ectoedmeia suberis* and *angulifasciella* groups. 58. *E. chasanella*. 59. *E. ortiva*. 60. *E. pilosae*. 61. *E. picturata*. 62. *E. occultellla*. 63. *E. jacutica*.

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