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A new species of *Anthophila* Haworth, 1811 with variable male genitalia from the Canary Islands (Spain) (Lepidoptera: Choreutidae)

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Abstract

We describe and illustrate *Anthophila variabilis* Falck, Karsholt & Rota, sp. n. (Choreutidae) from Tenerife (Canary Islands, Spain). The new species is outstanding due to the variability of its male genitalia. It is closely related to *A. fabriciana* (Linnaeus, 1767), and more distantly related to *Anthophila threnodes* (Walsingham, 1910), which is endemic to Madeira. Based on the DNA barcode, the new species is molecularly very distinct from its closest relative, *A. fabriciana*, with a pairwise K2P distance of more than 6.5%. The previous record of *A. fabriciana* from the Canary Islands is based on misidentification, and the species should be removed from the list of Lepidoptera found in the Canary Islands.
KEY WORDS: Lepidoptera, Choreutidae, *Anthophila*, new species, Canary Islands, Spain.

Una nueva especie de *Anthophila* Haworth, 1811 con la variable genitalia del macho de las Islas Canarias (España) (Lepidoptera: Choreutidae)

Resumen

Describimos e ilustramos *Anthophila variabilis* Falck, Karsholt & Rota, sp. n. (Choreutidae) de Tenerife (Islas Canarias, España). La nueva especie se destaca por la variabilidad de la genitalia del macho. Es relativamente próxima a *A. fabriciana* (Linnaeus, 1767) y relativamente más distante de *Anthophila threnodes* (Walsingham, 1910), la cual es endémica de Madeira. Basándose sobre el AND código de barras genético, la nueva especie es, a nivel molecular, muy distinta de su pariente más próximo, *A. fabriciana*, con dos parámetros K2P y con una distancia mayor del 6.5%. Los registros previos de *A. fabriciana* de las Islas Canarias están basados sobre malas identificaciones y la especie debería se retirada de la lista de Lepidoptera encontradas en las Islas Canarias.
PALABRAS CLAVE: Lepidoptera, Choreutidae, *Anthophila*, nueva especie, Islas Canarias, España.

Introduction

Choreutidae are a small family of usually diurnal and often brightly coloured moths, with 414 described species in 20 genera (ROTA, unpublished). Most choreutids are found in the tropics, and a comparatively large number occur on oceanic islands (ROTA *et al*., 2016). So far five species of Choreutidae have been recorded from the Canary Islands (Spain): *Anthophila fabriciana* (Linnaeus, 1767), *Choreutis nemorana* (Hübner, 1799), *C. pariana* (Clerck, 1759), *Tebenna micalis* (Mann, 1857)

and *T. bjerkandrella* (Thunberg, 1784) (BÁEZ & MARTÍN, 2001: 237; VIVES MORENO, 2014: 201-203). However, records of *T. bjerkandrella* are due to misidentification of *T. micalis*, and the species has already been removed from the list of Lepidoptera found in the Canary Islands (VIVES MORENO, 2014: 202; ROTA *et al*., 2014: 100), and also *A. fabriciana* should be removed from the list (see Discussion below). While Madeira, the closest archipelago to the Canary Islands, shares some of the species with the Canary Islands (*C. nemorana*, and *T. micalis*), it also has an endemic species of *Anthophila* - *A. threnodes* (Walsingham, 1910).

During field work in Tenerife in 2016 the first author collected a few specimens of an *Anthophila* species, believed to belong to *A. fabriciana* (L.). The specimens flew in numbers around *Urtica morifolia* Poir. (MUER *et al*., 2016: 247). Dissection of the genitalia revealed a possible new species, and during 2017-2019 more larvae and adult specimens were collected for studying. Because of considerable variation in the adult habitus, and especially in the male genitalia, at some point it was assumed that there might be two separate species. The results from DNA barcoding showed no genetic difference between the two forms, and therefore this hypothesis was rejected.

In this study we describe the new species, we carry out a phylogenetic analysis including all of the sequenced species of *Anthophila* to try to infer the placement of the new species within the genus and especially its sister lineage, and we comment on the possible historical biogeography.

Material and methods

A part of the material was subjected to DNA barcoding (sequencing of the 658 bp fragment of the mitochondrial COI gene; HEBERT *et al*., 2003) for detection of genetically distinct taxa and for obtaining molecular data for the new species. DNA barcodes for some of the specimens were sequenced at the University of Guelph, Canada; some of the sequences were downloaded from online databases such as GenBank and BOLD systems (RATNASINGHAM & HEBERT, 2013); while laboratory work for other specimens was done at Lund University (Table 1). The laboratory protocols followed WAHLBERG & WHEAT (2008).

In addition to sequencing the DNA barcode region (first half of the COI gene, what we refer to as COI-begin), we also sequenced the second half of the mitochondrial COI gene (COI-end), as well as seven nuclear genetic loci (WAHLBERG & WHEAT, 2008) (Table 1). The sequenced nuclear loci are the following: CAD, EF1alpha, GAPDH, IDH, MDH, RpS5, and wingless. The list of specimens with sequences, including their basic geographical data and collection year where known, is provided in Table 1. The molecular data were analyzed with maximum likelihood implemented in RAxML (STAMATAKIS *et al*., 2014) with GTR+G model as a concatenated and unpartitioned dataset. The final dataset had 6404 base pairs and 30 taxa, two of which were outgroups (Chroeutidae: Brenthinae: *Brenthia hexaselena* and Choreutidae: Choreutinae: *Choreutis pariana*). Branch support was calculated using bootstrapping (100 replicates); values below 70 are omitted from branches as they signify lack of statistical support. The K2P divergences between the examined taxa were calculated using analytic tools in BOLD systems.

The photographs of specimens were taken with Canon EOS700D camera. Those of the genitalia by using a Soptop CX40T Trinocular microscope and a Toup Tek P10500A-E3 / E3ISPM05000KPA-E3 / 5.0MP USB3 camera.

Abbreviations used

GP	Genitalia preparation
PF	Collection of Per Falck, Neksø, Denmark
MNCM	Collection A. Vives, Museo Nacional de Ciencias Naturales, Madrid, Spain

MZLU Entomology Section, Biological Museum, Department of Biology, Lund University, Lund, Sweden
ZMUC Zoological Museum, Natural History Museum of Denmark, Copenhagen, Denmark

Results

Eleven specimens of the newly discovered species were successfully sequenced. The new species is genetically distinct (BIN BOLD:ADZ8341; RATNASINGHAM & HEBERT, 2013) from morphologically similar *Anthophila* species. The average corrected pairwise distance between the specimens of the new species is 0.17% and the average distance between the new species and its nearest neighbor (*A. fabriciana*, BIN BOLD:AAC8582) is 6.58%. In the DNA barcode tree (Fig. 1), the new species is sister to *A. fabriciana*, but in the tree based on the expanded molecular dataset including the nuclear gene fragments (Fig. 2), it is sister to a clade consisting of *A. alpinella*, a North American species, and *A. fabriciana*. However, bootstrap values for these branches are very low: a bootstrap of 50 in the DNA barcode tree and only 38 in the full dataset.

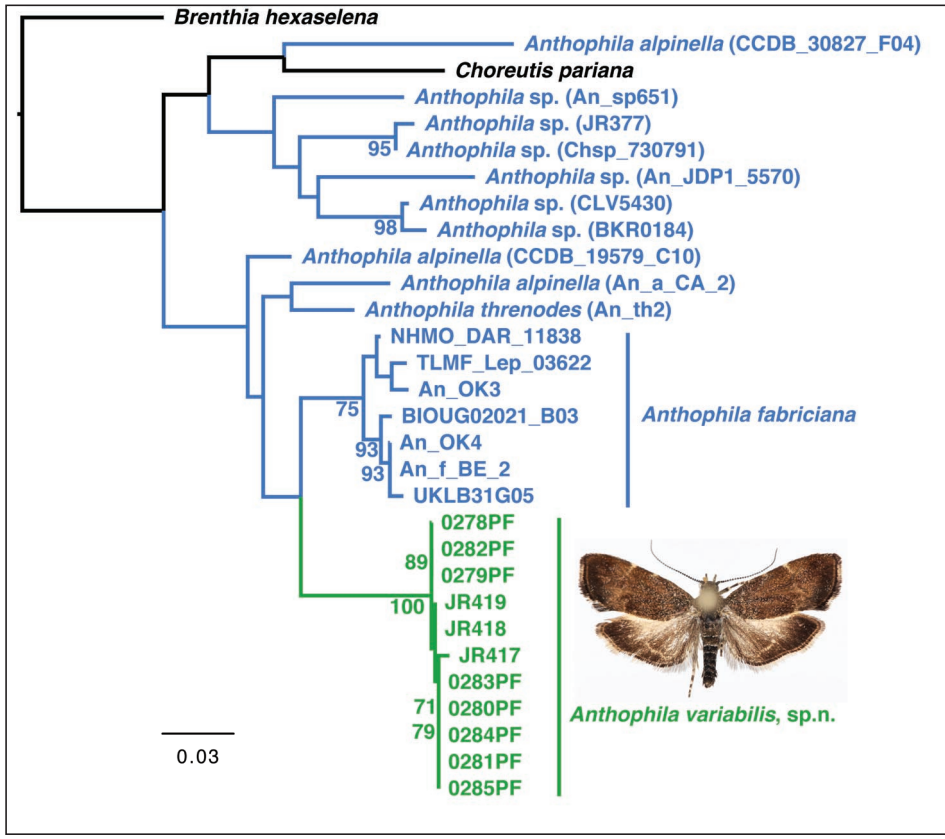

Figure 1.– A maximum likelihood tree of *Anthophila* based on the DNA barcode sequences. The numbers on branches are bootstrap values.

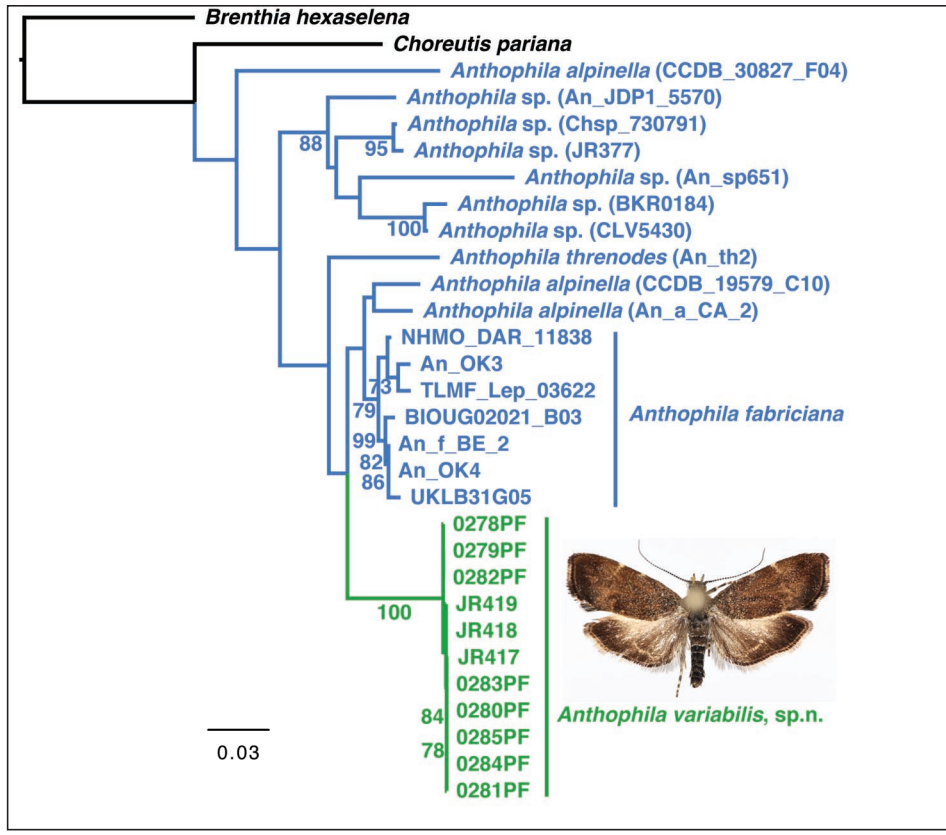

Figure 2.– A maximum likelihood tree of *Anthophila* based on seven nuclear genes as well as the entire COI gene. The numbers on branches are bootstrap values.

***Anthophila variabilis* Falck, Karsholt & Rota, sp. n.** (Figs 3-6)

Holotype ♂: SPAIN, Tenerife, Aguamansa, 1050 m, 21-V-3-VI-2019, leg. P. Falck, genitalia slide 3109PF, DNA sample Lepid Phyl 0285PF (ZMUC).

Paratypes: SPAIN, Tenerife, Aguamansa, 1050 m, 4 ♂♂, 3 ♀♀, 8-22-XI-2016, leg. P. Falck; 4 ♂♂, 1 ♀, ibidem, 1-20-III-2017, leg. P. Falck; 7 ♂♂, 3 ♀♀, ibidem, ex. larvae in spun shots (*Urtica morifolia* Poir), 6-III-2017, leg. P. Falck; 2 ♂♂, 1 ♀, ibidem, ex. larvae in spun shots (*Urtica morifolia* Poir), 18-XI-8-XII-2018, leg. P. Falck; 7 ♂♂, 5 ♀♀, ibidem, 21-V-3-VI-2019, leg. P. Falck; 6 ♂♂, 4 ♀♀, ibidem, 13-26-VIII, leg. P. Falck; genitalia slides 2484PF, 2501PF, 2502PF, 2503PF, 2504PF, 2505PF, 2506PF, 2526PF, 2527PF, 2528PF, 3281PF, 3282PF, 3283PF,3284PF, DNA samples Lepid Phyl 0278PF, 0279PF, 0280PF, 0281PF, 0282PF, 0283PF, 0284PF, JR417, JR418, JR419 (PF, MNCN, MZLU).

Description male: Wingspan 11-13 mm. Head and neck blackish brown with creamy white-tipped scales; labial palpus creamy white, second segment with scattered blackish brown scales, third segment ringed dark basally and with dark tip. Antenna dark grey ringed white, scapula with a few creamy white scales. Thorax blackish brown, with scattered creamy white scales. Forewing blackish brown, scattered with white-tipped scales, especially in basal part and apically to transverse fascia; costa with a creamy

white diffuse mark just beyond 1/3, and a creamy white comma-shaped mark before 2/3, continued by a diffuse creamy white zigzag line across the wing to dorsum well before tornus; fringe-line black, fringes black and creamy white. Hindwing blackish brown, a white irregular streak from above tornus to about middle of termen. Abdomen blackish brown.

Description female: Wingspan 12-14.5 mm. Wing-pattern and coloration as in male, but mixture with white-tipped scales in basal part and distally to transverse fascia more pronounced, giving these areas a dusty appearance; zigzag line more distinct.

Variation: The mixture of white-tipped scales on the forewing can be very pronounced giving adult a speckled appearance, other times the white-tipped scales are nearly absent giving the specimen a more uniform appearance. Hindwing sometimes becoming gradually paler towards costa.

Genitalia ♂ (Figs 9-13): Tegumen triangular, uncus small and rounded. Papillae anales elongate, somewhat elliptical patches with long hairs. Gnathos well developed, curved, hook-like with pointed apex. Vinculum ventrally rounded with a small triangular saccus. Valva broad, somewhat oval, with a pointed costal process and an unsclerotized rounded distal extension; distally and ventrally covered with hairs. Harpe rounded, apically covered with spines. Juxta a hood-shaped plate. Phallus shorter than valva, slightly sigmoidal, with a small, sometimes rounded spine at one-third from apex.

Variation: The pointed costal process varies from well developed (Fig. 9) to totally absent (Fig. 11). The spine on the phallus varies from a small, sharp spine (Fig. 12) to a nearly absent, rounded process (Fig. 13).

Genitalia ♀ (Fig. 18): Posterior apophysis slender, slightly broader at base, about 1.5 times as long as anterior apophysis. Anterior apophysis greatly enlarged from 1/3, tapering slightly towards apex, being about twice as thick in distal 2/3 as in basal 1/3. Ostium on segment VIII. Ductus bursae slightly widening into corpus bursae, heavily twisted with about eight revolutions. Corpus bursae rounded, small; signum as small patch of dentations.

Molecular variability: Among the DNA barcode sequences from 11 specimens there are four haplotypes, which differ from one another by between one and four bases. One haplotype is shared by specimens 0278PF, 0279PF, and 0282PF; one haplotype is shared by JR418 and JR419; one haplotype is shared by 0280PF, 0281PF, 0283PF, 0284PF, and 0285PF; and the fourth haplotype is found in JR417.

Differential diagnosis: *Anthophila variabilis* Falck, Karsholt & Rota, sp. n., resembles *A. fabriciana* (Fig. 7) and *A. threnodes* (Fig. 8). It is characterized by its blackish brown wings, but the adult cannot be distinguished from *A. fabriciana* with certainty. From *A. threnodes* it differs by the distinct white streak on the hindwing. In the male genitalia it differs from *A. fabriciana* (Figs 14, 15) by having a much shorter spine on the phallus. From *A. threnodes* (Figs 16, 17) it differs by the shorter and less pointed spine on the phallus. In the female genitalia it differs from *A. fabriciana* (Fig. 19) by the thick distal part of the anterior apophyses, and the shorter ductus bursae with fewer revolutions (about twelve revolutions in *A. fabriciana*). From *A. threnodes* (Fig. 20) it differs by the apparent spiralization of ductus bursae and the rounded corpus bursae.

Biology: The larva is off-white with dark brown spots and brownish head. It lives under a spun web on or around the young leaves on which it feeds. The hostplant is *Urticae morifolia* Poir. The larvae have been found in March and late November. The adults have been collected from March to late November, probably in several broods, flying actively especially in late afternoon sunshine. The type locality is situated at the north-facing slopes of Tenerife at an altitude of 1050 m a.s.l.

Distribution: Known only from the type-locality Canary Islands (Spain): Tenerife, Aguamansa. The species is most likely an endemic species.

Etymology: The species is named after the variable male genitalia.

Discussion

The corrected pairwise genetic distances within *Anthophila variabilis* Falck, Karsholt & Rota, sp. n. and between *A. variabilis* and its nearest neighbor *A. fabriciana* display a typical barcoding gap (MEYER & PAULAY, 2005), demonstrating that this species is genetically a well-separated lineage

from the other known species of *Anthophila*. With our molecular dataset we cannot answer the question of which species is the sister group to *A. variabilis* with great certainty due to low branch support, but it does appear, as suggested also by the morphological similarity, that *A. variabilis* is the sister species of *A. fabriciana*. We were somewhat surprised that *A. variabilis* is not so closely related to *A. threnodes*, the Madeiran endemic.

Although *A. fabriciana* is a common species in mainland Europe, its occurrence in the Canary Islands is "based on a single specimen ("61978"), taken in April 1884 [in Tenerife], by the late Mr. J. H. Leech" (WALSINGHAM, 1908: 989). The Lepidoptera fauna of the Canary Islands (and especially Tenerife) is relatively well studied, and it is surprising that *A. fabriciana* has not been found again in the islands. REBEL (1911: 349) suggested that the single specimen might have resulted from an accidental importation.

We have not been able to examine the above mentioned specimen but considering that *A. fabriciana* externally is hardly separable from *A. variabilis* we find it most likely that the specimen collected by Leech belonged to the latter. As the record of *A. fabriciana* in the Canary Islands is based on that specimen it should be removed from the list of Canary Island Lepidoptera and replaced by *A. variabilis*.

Interestingly J. H. Leech also collected a specimen of *Anthophila* in Madeira on which WALSINGHAM (1910: 257) based the description of the endemic *A. threnodes* (ROTA *et al*., 2014: 93).

Based on our phylogenetic hypothesis, it appears that the colonization of Madeira by the ancestor of *A. threnodes* happened earlier than the colonization of the Canary Islands by the ancestor of *A. variabilis*. *Anthophila* larvae are Urticaceae specialists and therefore one can imagine that the establishment of a species of *Anthophila* on these islands is not too difficult once gravid females arrive. It is a little less clear how exactly such small moths that are relatively poor fliers can cross hundreds of kilometers from mainland to oceanic islands, but choreutids are apparently good at that given that a number of oceanic island endemics are known in this family (ROTA *et al*., 2016 and references therein). One possibility is that storm systems facilitate such dispersal. One should also consider the possibility that more volcanic islands may have existed in this part of the Atlantic oceans during the last millions of years, and as "stepping stones" thereby having facilitated distribution of the biota from the continent to the Macaronesian Islands.

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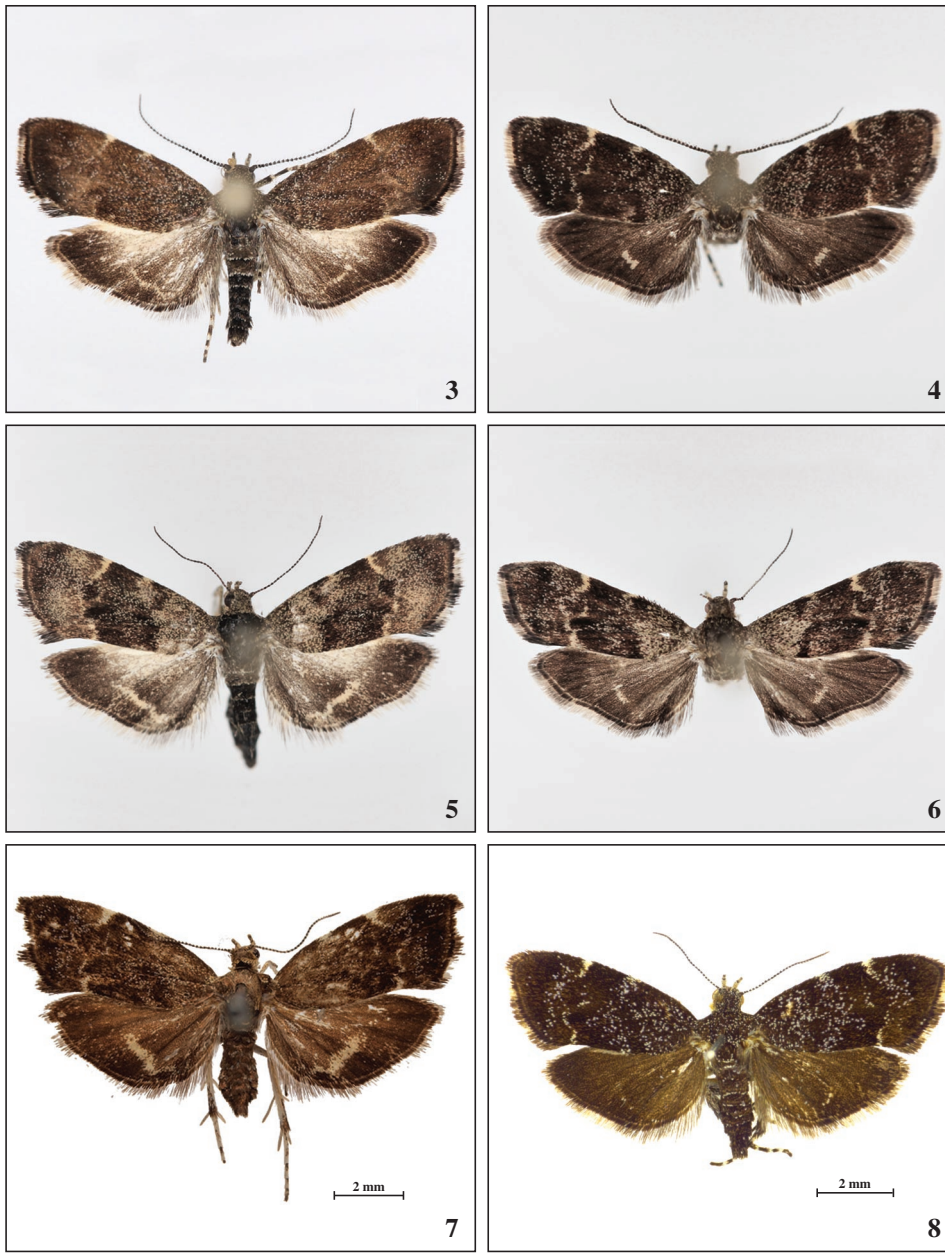
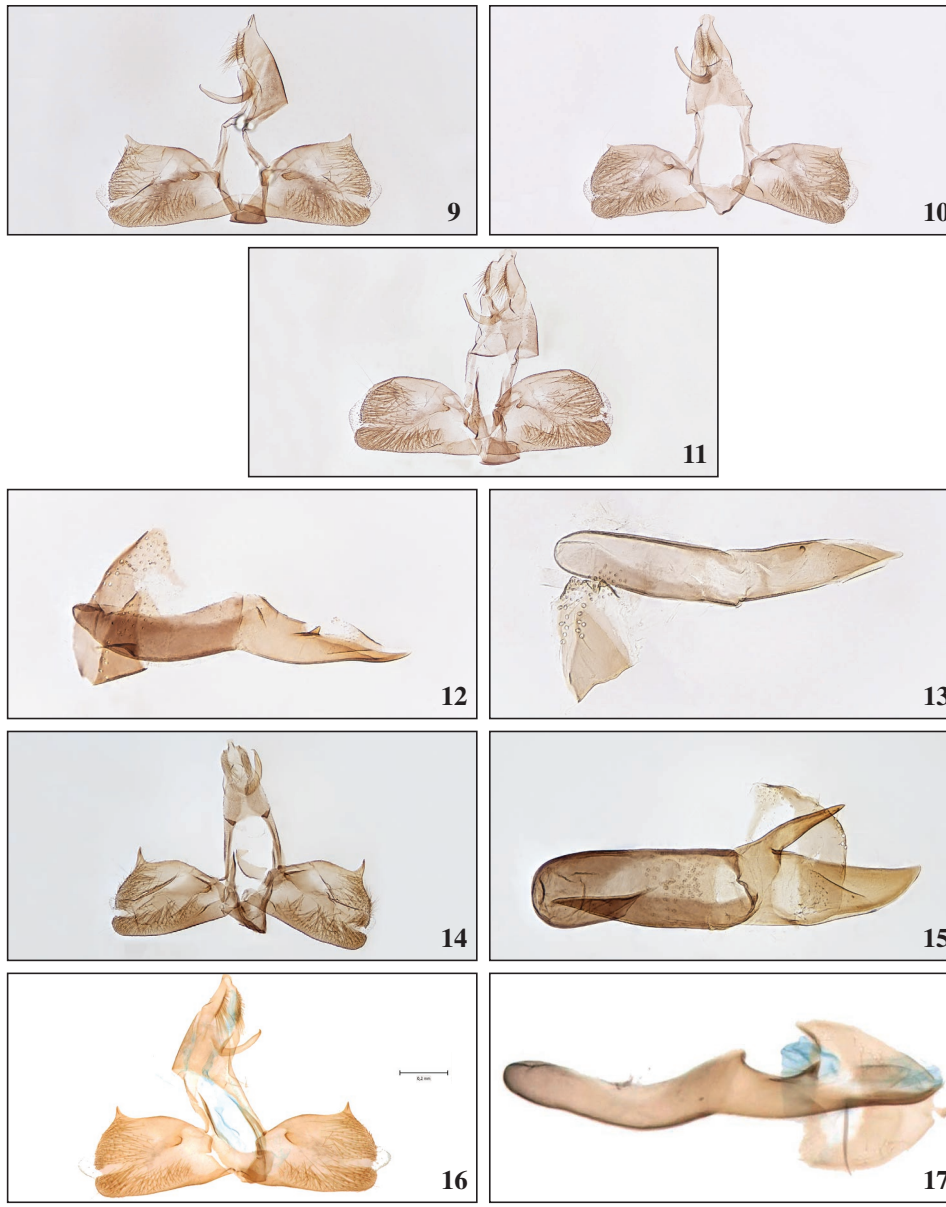
Table 1.– List of specimens sequenced, their voucher codes, collection country, gene fragments sequenced, and GenBank accession numbers.

Voucher code	Genus	Species	Country	Coll. year	CAD	COI-begin	COI-end	EF1a	GAPDH	IDH	MDH	RpS5	Wgl400	Sequnce source
Brh	Brenthia	hexaselena	Costa Rica	2003	KT956454	JQ958512	HQ533063	HQ541460	-	-	KT956661	JQ958481	HQ541541	GenBank
Chp	Choreutis	pariana	USA	2003	JQ958409	JQ958519	HQ533076	HQ541473	JQ958438	JQ958461	JQ958552	JQ958487	HQ541553	GenBank
0278PF	Anthophila	variabilis, sp.n.	Spain, Canary Islands	2017	-	X	-	-	-	-	-	-	-	CILEP BOLD
0279PF	Anthophila	variabilis, sp.n.	Spain, Canary Islands	2017	-	X	-	-	-	-	-	-	-	CILEP BOLD
0280PF	Anthophila	variabilis, sp.n.	Spain, Canary Islands	2017	-	X	-	-	-	-	-	-	-	CILEP BOLD
0281PF	Anthophila	variabilis, sp.n.	Spain, Canary Islands	2019	-	X	-	-	-	-	-	-	-	CILEP BOLD
0282PF	Anthophila	variabilis, sp.n.	Spain, Canary Islands	2017	-	X	-	-	-	-	-	-	-	CILEP BOLD
0283PF	Anthophila	variabilis, sp.n.	Spain, Canary Islands	2016	-	X	-	-	-	-	-	-	-	CILEP BOLD
0284PF	Anthophila	variabilis, sp.n.	Spain, Canary Islands	2019	-	X	-	-	-	-	-	-	-	CILEP BOLD
0285PF	Anthophila	variabilis, sp.n.	Spain, Canary Islands	2019	-	X	-	-	-	-	-	-	-	CILEP BOLD
An_a_CA_2	Anthophila	alpinella	USA	2006	JQ958398	KJ844049	HQ533053	HQ541450	JQ958430	JQ958454	JQ958542	JQ958476	HQ541531	GenBank
An_f_BE_2	Anthophila	fabriciana	Belgium	2006	JQ958399	JQ958507	HQ533054	HQ541451	JQ958431	KJ844055	JQ958543	JQ958477	HQ541532	GenBank
An_JDP1_5570	Anthophila	sp.	Rwanda		KJ844057	KJ844051	KJ844051	KJ844058	KJ844054	KJ844056	-	KJ844060	KJ844061	GenBank
An_OK3	Anthophila	fabriciana	Armenia		-	X	-	-	-	-	-	-	X	LU
An_OK4	Anthophila	fabriciana	Greece		-	X	-	-	-	-	-	-	-	LU
An_sp651	Anthophila	sp.	Peru		-	KJ844050	-	-	KJ844053	-	-	-	-	GenBank
An_th2	Anthophila	threnodes	Madeira		-	KJ844048	-	X	KJ844052	-	-	-	-	GenBank
BIOUG02021_B03	Anthophila	fabriciana	Canada	2010	-	KF808606	-	-	-	-	-	-	-	BOLD
BKR0184	Anthophila	sp	Madagascar	2011	-	MH416295	-	-	-	-	-	-	-	BOLD
CCDB_19579_C10	Anthophila	alpinella	USA	1962	-	X	-	-	-	-	-	-	-	BOLD
CCDB_30827_F04	Anthophila	alpinella	USA	1933	-	X	-	-	-	-	-	-	-	BOLD
Chsp_730791	Anthophila	sp.	Kenya		-	X	-	-	-	-	-	-	-	LU
CLV5430	Anthophila	sp.	Madagascar	2011	-	MH415902	-	-	-	-	-	-	-	BOLD
JR377	Anthophila	sp.	Kenya	2013	-	X	X	X	X	-	X	X	X	LU
JR417	Anthophila	variabilis, sp.n.	Spain, Canary Islands	2017	-	X	X	X	X	-	X	X	-	LU
JR418	Anthophila	variabilis, sp.n.	Spain, Canary Islands	2017	-	X	X	X	X	-	-	-	-	LU
JR419	Anthophila	variabilis, sp.n.	Spain, Canary Islands	2017	-	X	X	X	-	X	-	-	-	LU
NHMO_DAR_11838	Anthophila	fabriciana	Norway	2016	-	X	-	-	-	-	-	-	-	BOLD
TLMF_Lep_03622	Anthophila	fabriciana	Austria	2010	-	JN265169	-	-	-	-	-	-	-	BOLD
UKLB31G05	Anthophila	fabriciana	United Kingdom	2007	-	KF808852	-	-	-	-	-	-	-	BOLD



Figures 3-8.– 3. *Anthophila variabilis* Falck, Karsholt & Rota, sp. n., ♂, Tenerife, 13.5 mm. **4.** *A. variabilis* Falck, Karsholt & Rota, sp. n., ♂, Tenerife, 11 mm. **5.** *A. variabilis* Falck, Karsholt & Rota, sp. n., ♀, Tenerife, 14.5 mm. **6.** *A. variabilis* Falck, Karsholt & Rota, sp. n., ♀, Tenerife, 13 mm. **7.** *A. fabriciana* (Linnaeus, 1767), ♀ Finland, 14 mm. **8.** *A. threnodes* (Walsingham, 1910), ♀, Madeira (Portugal), 12 mm.



Figures 9-17.– 9. *Anthophila variabilis* Falck, Karsholt & Rota, sp. n., ♂, Tenerife, GP 2484PF. **10.** *A. variabilis* Falck, Karsholt & Rota, sp. n., ♂, Tenerife, GP 2503PF. **11.** *A. variabilis* Falck, Karsholt & Rota, sp. n., ♂, Tenerife, GP 2501PF. **12.** *A. variabilis* Falck, Karsholt & Rota, sp. n., phallus, Tenerife, GP 2502PF. **13.** *A. variabilis* Falck, Karsholt & Rota, sp. n., phallus, Tenerife, GP 2503PF. **14.** *A. fabriciana* (Linnaeus, 1767), ♂, Denmark, GP2500PF. **15.** *A. fabriciana* (Linnaeus, 1767), phallus, Denmark, GP2500PF. **16.** *A. threnodes* (Walsingham, 1910), ♂, Madeira (Portugal), GP5236OK. **17.** *A. threnodes* (Walsingham, 1910), phallus, Madeira (Portugal), GP5236OK.

Figures 18-20.– 18. *Anthophila variabilis* Falck, Karsholt & Rota, sp. n., ♀, Tenerife, GP 3284PF. **19.** *A. fabriciana* (Linnaeus, 1767), ♀, Denmark, GP3280PF. **20.** *A. threnodes* (Walsingham, 1910), ♀, Madeira (Portugal), GP5240HH.