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Ypsolopha milfontensis Corley & Ferreira, a new species from the Portuguese coast (Lepidoptera: Ypsolophidae)

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Abstract

Ypsolopha milfontensis Corley & Ferreira, sp. n. is described from the coast of south-west Portugal where its hostplant is *Ephedra fragilis* Desf. It is closely related to *Ypsolopha instabilella* (Mann, 1866), but with some differences externally, in male and female genitalia and a significant difference in DNA barcode.
KEY WORDS: Lepidoptera, Ypsolophidae, new species, *Ypsolopha*, *Ephedra*, Portugal.

Ypsolopha milfontensis **Corley & Ferreira, uma espécie nova da costa portuguesa (Lepidoptera: Ypsolophidae)**

Resumo

Ypsolopha milfontensis Corley & Ferreira, sp. n. é descrita da costa sudoeste de Portugal, onde a planta hospedeira é *Ephedra fragilis* Desf. É uma espécie próxima de *Ypsolopha instabilella* (Mann, 1866), apresentando diferenças na morfologia externa, na genitália do macho e da fêmea e no código de barras de ADN.
PALABRAS CHAVE: Lepidoptera, Ypsolophidae, espécie nova, *Ypsolopha*, *Ephedra*, Portugal.

Ypsolopha milfontensis **Corley & Ferreira, una nueva especie de la costa portuguesa (Lepidoptera: Ypsolophidae)**

Resumen

Ypsolopha milfontensis Corley & Ferreira, sp. n. se describe de la costa sudoeste portuguesa, donde la planta huésped es *Ephedra fragilis* Desf. Es una especie próxima de *Ypsolopha instabilella* (Mann, 1866), presentando diferencias en la morfología externa, en la genitalia del macho y de la hembra y en el código de barras genético ADN.
PALABRAS CLAVE: Lepidoptera, Ypsolophidae, nueva especie, *Ypsolopha*, *Ephedra*, Portugal.

Introduction

The Portuguese Lepidoptera fauna has approximately half as many species as that of Spain. While this might be expected due to smaller area and more restricted altitude and latitude together with correspondingly less diverse climate and habitats, there are still gaps in the knowledge of the Portuguese fauna due to a lack of fieldwork in some areas and habitats. We recognised that one such gap was the fauna associated with the shrub *Ephedra* Tourn. ex L. *Ephedra* is a member of the

Gnetopsida, a small class within the Gymnospermae (more familiarly including trees and shrubs such as *Pinus* and *Juniperus*). In Spain there are at least six species of Lepidoptera which feed exclusively on species of *Ephedra* (ROBINSON *et al*., 2010). As far as we were aware, no-one had ever examined *Ephedra* in Portugal for Lepidoptera, and no *Ephedra*-feeding species had been recorded.

On a field trip to south-west Portugal in May 2019 we made a special effort to find *Ephedra fragilis* Desf., the only Portuguese species of the genus, which we understood was locally abundant along the coast between Sines and Odeceixe. On 27-V-2019 we visited a number of sites along the coast between Vila Nova de Milfontes and Cabo Sardão without finding any *Ephedra*. Night time field work was further inland, but we had left a single UV light trap near our base at Vila Nova de Milfontes between sand dunes and a car park. On returning to base in the small hours we checked this light and found a single *Ypsolopha* which we could not identify.

The genus *Ypsolopha* Latreille, 1796 shows great diversity of forewing shape, coloration, and markings, often allowing easy identification in the field where the fauna is well-known. There are a number of species feeding on *Ephedra* in southern Europe, North Africa, the Middle East and on into Central Asia as well as in North America. As the specimen collected was not recognised, MC concluded it was likely to be one of the *Ephedra*-feeding species with which he was not familiar. In expectation that there must be *Ephedra* nearby, MC went searching for it the following morning and soon found one stand of the plant about 200 metres from the UV light. That night, we left a UV light by the *Ephedra* plants. On our return to the light late that night we found four more *Ypsolopha* on the plant near the trap, but none in the trap. SF noticed that there were small brown larvae feeding on the *Ephedra* stems, and we collected nine of these. In spite of further searching northwards towards and beyond Sines on 28 May we found no more *Ephedra*.

The larvae collected from *Ephedra* continued to feed and all pupated. Nine adults emerged between 20 June and 3 July 2019. From their external appearance they were provisionally identified as *Y. instabilella* (Mann, 1866), a species already known to be present in Spain (VIVES MORENO, 2014) which has a wide distribution from southern Spain, south France, Switzerland, Croatia (type locality), Ukraine, Turkey, Israel (GERSHENSON *et al*., 2002) and on into central Asia, where it is recorded from Uzbekistan, Tajikistan, and Kyrgyzstan.

Legs were taken from the specimens captured in Portugal and placed in alcohol for subsequent DNA sequencing. Genitalia dissection of one male showed slight differences from male genitalia of a Spanish specimen illustrated on the Lepiforum website, but at this early stage it was not clear if these were significant.

When barcodes were obtained, these showed over 4% difference from barcodes of *Y. instabilella* on BOLD from Spain and Croatia. This led to the supposition that the species was not *Y. instabilella*, but an undescribed species. A further male and two females were dissected and compared closely with the male and female genitalia photographs of *Y. instabilella* on Lepiforum e. V. (2020). We conclude that the differences in genitalia are sufficient to justify the description of a new species, supported by differences in DNA barcode and external morphology.

Abbreviations

GP	Genitalia preparation
INV*****	InBIO Barcoding Initiative sample code
NHMUK	Natural History Museum, London, England
P*****	Martin Corley collection number of specimens
SU18/***	Jan Šumpich collection number of specimens

Materials

Ypsolopha instabilella. All material examined as photos. SPAIN, Almería, Sierra de Alhamilla, 37º 00’ 02” N, 02º 09’ 88” W; Nijar env. 560 m, 1 ♂, 30-IV-2008, J. Šumpich leg. 18/308; Mojacar, 4-

V-2008, J. Šumpich leg. 18/309; Sierra de Alhamilla, Huebro, 1 ♀, 700-800 m, 29-IV-2008, J. Šumpich leg. 18/310; GRANADA, Valle de Lecrín, Padul, 941 m, 2 ♀♀, 09-VI-2018, GP Friedmar Graf; TERUEL, Albarracín env. 1100 m, 1 ♀, 7-VIII-2010, J. Šumpich leg. 18/311. CROATIA, PIROVAC, env. 33 m, Tijesno-Ivinj, 43º 47' 27" N, 15º 37' 31" E, 6-12-VIII-2007, J. Šumpich leg. 18/307; Manušiči, 35 km S. E. of Split, 250 m, 43º 24' 16" N, 16º 50' 26" E, 1 ♀, 3-7-VIII-2008, J. Šumpich leg. 18/339. SWITZERLAND, VALAIS, Venthône, 1 ♂, 6-IV-2011, leg. Hermann Gerber, GP Wolfgang Wittland (photo Rudolf Bryner).

The specimens of *Ypsolopha milfontensis* Corley & Ferreira, sp. n. are listed under that species.

Methods

Morphological examination: genitalia preparations were made following standard techniques (ROBINSON, 1976).

Genomic DNA was extracted from leg tissue (Table 1) using EasySpin Genomic DNA Tissue Kit (Citomed, Lisboa, Portugal) following manufacturer's protocol, except for the lysis period which was extended to enhance extraction success. The cytochrome c oxidase I (COI) barcoding fragment was amplified as two overlapping fragments using two sets of primers. For the first fragment, primers LepF (HEBERT *et al*., 2004) and MlepR (HAJIBABAEI *et al*., 2006) were used, while primers LepR (HEBERT *et al*., 2004) and MlepF (HAJIBABAEI *et al*., 2006) were used to amplify the second fragment. Both PCR reactions had 10 µL of final volume, containing 5 µL of Multiplex PCR Master Mix (QIAGEN, Hilden, Germany), 0.4µM of each primer, and 1-2µL of DNA. PCR amplification was carried out on a T100 Thermal Cycler (BioRad, Hercules, CA, USA) using the following conditions: initial denaturation at 95ºC for 15 min; 5 cycles at 95ºC for 30 s, 47ºC for 45 s, 72ºC for 45 s; then 40 cycles at 95ºC for 30 s, 51ºC for 45 s, 72ºC for 45 s; and a final elongation step at 60ºC for 10 min. The barcodes were sequenced in an Illumina Miseq platform, following the approach described by SHOKRALLA *et al*. (2015).

Table 1.– Specimens of *Ypsolopha* sequenced in the present work. [Code = InBIO Barcoding Initiative sample code; Date = date of collection; Locality = collecting locality; Lat = latitude; Long = longitude; BOLD = BOLD Process ID for cytochrome c oxidase I (COI) DNA barcodes access. (http://www.boldsystems.org).

Taxa	Code	Date	Locality	Lat	Long	BOLD
Ypsolopha milfontensis	INV08683	7/05/2019	Vila Nova de Milfontes	37.7235	-8.7890	IBILP1700-20
Ypsolopha milfontensis	INV08836	28/05/2019	Vila Nova de Milfontes	37.7235	-8.7890	IBILP1732-20
Ypsolopha milfontensis	INV08837	28/05/2019	Vila Nova de Milfontes	37.7235	-8.7890	IBILP1733-20
Ypsolopha milfontensis	INV08838	28/05/2019	Vila Nova de Milfontes	37.7235	-8.7890	IBILP1734-20
Ypsolopha milfontensis	INV08839	28/05/2019	Vila Nova de Milfontes	37.7235	-8.7890	IBILP1735-20
Ypsolopha alpella	INV09730	15/09/2019	Serra do Larouco	41.8816	-7.7286	IBILP2897-20
Ypsolopha alpella	INV09768	17/09/2019	Gondesende	41.8464	-6.8794	IBILP2907-20
Ypsolopha rhinolophi	INV09890	20/09/2019	Guadramil	41.9212	-6.5747	IBILP2951-20

We used OBITools (available from https://git.metabarcoding.org/obitools/obitools) for general sequence processing. Geneious v.6.1.5 (available from http://www.geneious.com/) was used for final sequence assembly. The sequence obtained was blasted against GenBank and BOLD databases. The average divergence (uncorrected p-distance) between the sequence of Portuguese specimens and sequences available in GenBank and BOLD was calculated in MEGA v.5.2.1 (TAMURA *et al*., 2011).

Results

The molecular results of the specimens from Vila Nova de Milfontes exhibited distinct

haplotypes of the partial COI gene sequence with *Y. instabilella* being the closest related species (uncorrected p-distance 4.7%) followed by *Y. alpella* ([Denis & Schiffermüller], 1775) and *Y. scabrella* (Linnaeus, 1758) with sequences available with over 8.7% and 9.0% divergence, respectively (Table 1).

Ypsolopha milfontensis Corley & Ferreira, sp. n.

Material examined: Holotype ♀ (Fig. 1), "P11795 | PORTUGAL | Vila Nova de Milfontes | Baixo Alentejo | 27-V-2019 | M. Corley & S. Ferreira" "Holotype ♀ | *Ypsolopha milfontensis* | Corley & Ferreira" "GP ♀ | CORLEY | 5770" "INV08683". The specimen will be placed in NHMUK. Paratypes: P11801 Portugal, Baixo Alentejo, Vila Nova de Milfontes, 1 ♀, 28-V-2019, M. Corley & S. Ferreira, INV08837; P11802, INV08839, 1 ♂, same data; P11887, 1 ♂, 1 ♀, same locality, ex. *Ephedra fragilis*, 20-VI-2019, M. Corley & S. Ferreira; P11888, same data but 21-VI-2019, GP MD02820; P11893, P11894, same data 2 ♂♂, but 25-VI-2019; P11895, same data 1 ♀, but 26-VI-2019; P11896 same data and date, GP MD02818, P11897, not dissected; P11903 same data 2 ♂♂, but 3-VII-2019 GP MD02819, P11904, same data and date, not dissected.

Two further male specimens in poor condition were collected at the type locality on 28-V-2019. Barcodes were obtained from these (INV08836, INV08838) and they have been retained by MC, but they have not been set and mounted as museum specimens and they are not included in the paratype series.

Description (Figs 1-4): Males and females similar. Wingspan 15-20.5 mm. Head and labial palp pale grey-buff, scales paler-tipped, labial palp segment 2 with long tuft of forward projecting scales, segment 3 short, slender, just projecting from upper side at about one-third of tuft length, often invisible. Antenna grey-buff. Thorax grey-buff. Forewing elongate, apex falcate, acute; light brownish buff with scattered individual brown scales, particularly towards dorsum; basic markings consist of a few blackish or dark brown dots close to costa in basal one-third, a dark grey spot at two-fifths which makes a small bump on the costal edge and a few brown dots on costa towards apex; a black dot at end of cell and another in fold at one-third, the latter preceded by an oblique whitish streak crossing fold, edged chestnut-brown proximally; terminal fringe short beneath apex, becoming longer to tornus, with at least two darker fringe lines. Additional forewing markings often present, see below under Variation. Hindwing grey, darker towards costa; fringe grey at base, light brownish buff distally. Abdomen light grey.

Variation. Sometimes one or more of the basic elements of the wing pattern may be missing, most notably the dark spot and bump on costa at one-third, but also the blackish dots at end of cell and in fold. However, variation mainly consists of additional elements of pattern which are highly individual. Weak lighter or darker longitudinal streaks may be present, the brown edging to whitish fold mark can be extended across wing, sometimes forming a complete fascia extending to blackish spot on costa at one-third; dorsal margin may have a row of brown spots, dorsal area beneath fold can be more or less infilled blackish; one specimen has a black streak along costa from base to one-fifth extending beneath costa to beyond half with additional black lines from middle of base, two overlapping lines below cell in mid-wing and another from beyond end of cell to termen beneath apex; fringe lines more or less suffused blackish, additional lines sometimes present.

Male genitalia (Figs 9-10): Uncus a small rounded protuberance between narrow elongate pointed socii, separated by less than half their length; gnathos rounded, scobinate, on long arms; valva twice as long as wide, costal margin straight and thickened to beyond middle, slightly concave at two-thirds to three-quarters length, apex very broadly rounded, ventral margin hardly thickened, with slight nearly constant curvature, inner surface of valva with a large field of elongate scales; vinculum broad, rounded, saccus one-quarter length of valva; aedeagus 0.85 times length of valva, ductus ejaculatorius at one-quarter of its length, caecum more slender than distal part which is slightly curved, a single long cornutus present.

Female genitalia (Fig. 12): Papillae analis elongate, pointed; posterior apophysis twice as long

as free part of anterior apophysis; segment VIII weakly sclerotised; ostium with short lateral margins, antrum sclerotised, cylindrical, twice as long as wide; ductus bursae with a curve in anterior half, slightly expanded and sclerotised towards corpus bursae, but not ringed, about twice as long as free part of anterior apophysis; corpus bursae elongate ovoid, signum large, flask-shaped, slightly widened posteriorly, with a deep transverse fold across widest part and another just posterior to narrowest part.

Diagnosis: Externally most similar to *Y. instabilella* (Mann, 1866) (figs 5-8). *Y. instabilella* is on average slightly larger with wingspan 18-22.5 mm compared with 15-20.5 mm in *Y. milfontensis*. The majority of specimens of the new species were reared from larvae which were obliged to feed on *Ephedra* in increasingly suboptimal condition, which may have led to specimens being smaller, but the wild caught specimens were also smaller than the majority of *Y. instabilella*. Both species show considerable variation in wing markings, but there is a tendency for the ground colour of both fore and hindwings to be darker and greyer in *Y. instabilella*, however the differences are rather less than appears when comparing figures 1-4 with figures 5-8, as the photos were not taken under identical conditions. Most specimens of *Y. instabilella* have forewing veins clearly delineated black. In *Y. milfontensis* the veins are not well-marked or occasionally marked brown. The dark spot and bump on costa at one-third which is present in some *Y. milfontensis* has not been seen in *Y. instabilella*. There are clear differences in male and female genitalia. In male genitalia (fig. 11) *Y. instabilella* differs in shape of valva, which has very slight concavity on costal margin immediately beyond middle, less broad apex, curvature of ventral margin most marked at one-third, field of scales on ventral surface absent; vinculum narrower, not rounded, saccus longer, two-fifths length of valva; aedeagus straight. In female genitalia (figs 13-14) *Y. instabilella* differs mainly in length of ductus bursae which is 3.5 times as long as free part of anterior apophysis and has a series of papillose rings close to corpus bursae; corpus bursae more rounded; signum flask-shaped, but with narrower neck than in *Y. milfontensis* and without a fold in the neck.

Early stages: Larvae were found at night lightly attached to the stems with silk while feeding on the epidermis of the stems. They were not visible by day, so presumably descend into the interior of the *Ephedra* bushes by day. The plants form a very dense mass of stems. Full grown larva about 10 mm long, tapering to both ends, slightly contracted between segments, without prolegs on segment 12; head dull fuscous with small black dots, body dull fuscous, with a dull creamy white dorsal line, sometimes interrupted and a dull pale lateral line; each segment with dorsal line slightly expanded in middle, a pair of obliquely placed black dots near dorsal line, three lateral dots forming an equilateral triangle above lateral line and 2 to 4 dots below lateral line; underside and legs pale coloured; dark brown V-shaped (angle at front) marks on dorsal surface of segments 6-10; head and body have a wrinkled appearance due to a network of fine purplish-fuscous lines; head and body with dark hairs, shortest on head.

Bionomics: Adult moths were collected at end of May in varying condition. At the same time larvae were found that were no more than 5 mm long and others that were almost full grown (≈10 mm). This suggests the possibility that the species is continuous brooded, but with only one sampling this is speculative. The hostplant, *Ephedra fragilis* is evergreen and the local climate without severe winter cold would certainly allow continuous development.

Distribution: At present known only from a single site at Vila Nova de Milfontes, Beja, Portugal, in contrast with *Y. instabilella* which has a wide distribution from southern Spain, into central Asia.

Etymology: The species name *milfontensis* is a latinised adjective in genitive case derived from the type locality Vila Nova de Milfontes.

Discussion: With the present work the number of Portuguese *Ypsolopha* species is raised to seven. Five species were known to occur in Portugal in 2015: *Y. alpella* ([Denis & Schiffermüller], 1775), *Y. lucella* (Fabricius, 1775), *Y. persicella* (Fabricius, 1787), *Y. scabrella* (Linnaeus, 1761) and *Y. ustella* (Clerck, 1759) (CORLEY, 2015). The existence of a sixth species in the country was unveiled by bats and described in 2019: *Ypsolopha rhinolophi* Corley, 2019 (CORLEY *et al*., 2019).

CORLEY (2015) listed 20 Lepidoptera species endemic to Portugal. Since then, three of those species have been found in Spain: *Depressaria cinderella* Corley, 2002 (BUCHNER & ŠUMPICH, 2018), *Agnoea nonscriptella* Corley, 2014 (LAŠTŮVKA & LAŠTŮVKA, 2020) and *Isotrias penedana* Trematerra, 2013 (CORLEY & FERREIRA, 2017); *Elachista occidentella* Traugott-Olsen 1992 has been relegated to synonymy with *Elachista hispanica* Traugott-Olsen, 1992 (KAILA, 2015). Six newly described endemic species have been added: *Ekboarmia miniaria* Skou, Stüning & Sihvonen, 2016, *Chrysoclista soniae* Corley, 2017, *Megacraspedus occidentellus* Huemer & Karsholt, 2018, *Afriberina salemae* Skou & Sihvonen, 2019, *Mondeguina atlanticella* Corley & Rosete, 2020 and *Heterogynis cynetis* de Freina, Monasterio, Escobés, Hinojosa & Vila, 2020 (SKOU *et al*., 2016; CORLEY, 2017; HUEMER & KARSHOLT, 2018; MÜLLER *et al*., 2019; CORLEY *et al*., 2020; FREINA *et al*., 2020). With the new *Ypsolopha* described in this paper the number of Portuguese endemic Lepidoptera species increases to 23.

The Iberian Peninsula is considered to have been a refugium for warmth-loving species during the Pleistocene glaciations, but within this area there would have been local areas that were particularly sheltered from colder conditions (WEISS & FERRAND, 2007). If sufficiently isolated these had the potential to evolve into new species which in turn could remain as local endemics if for one reason or another, they did not greatly expand their range during the ensuing interglacial period. In Portugal, one such area is the south-west coast, including part of Alentejo and the western Algarve. The Geometridae *Ekboarmia miniaria* and *Afriberina salemae* occupy this area, as does *Ypsolopha milfontensis* Corley & Ferreira, sp. n. (SKOU *et al*., 2016; SKOU & SIHVONEN, 2019).

If we are correct in our impression that *Ephedra fragilis* has suffered a major reduction in population in recent years, then the continued existence of *Ypsolopha milfontensis* is threatened by the disappearance of its hostplant. The plant is listed as Vulnerable in the Portuguese Red List (CARAPETO *et al*., 2020). The species is nowadays particularly scarce and localised, existing in small areas, some with less than 10 individuals (Paula Canha, pers. comm., 2018) and is currently threatened by habitat destruction due to urban pressure (CARAPETO *et al*., 2020). Further investigation of the current distribution of *Ephedra fragilis* and whether *Y. milfontensis* occurs anywhere else is urgently needed.

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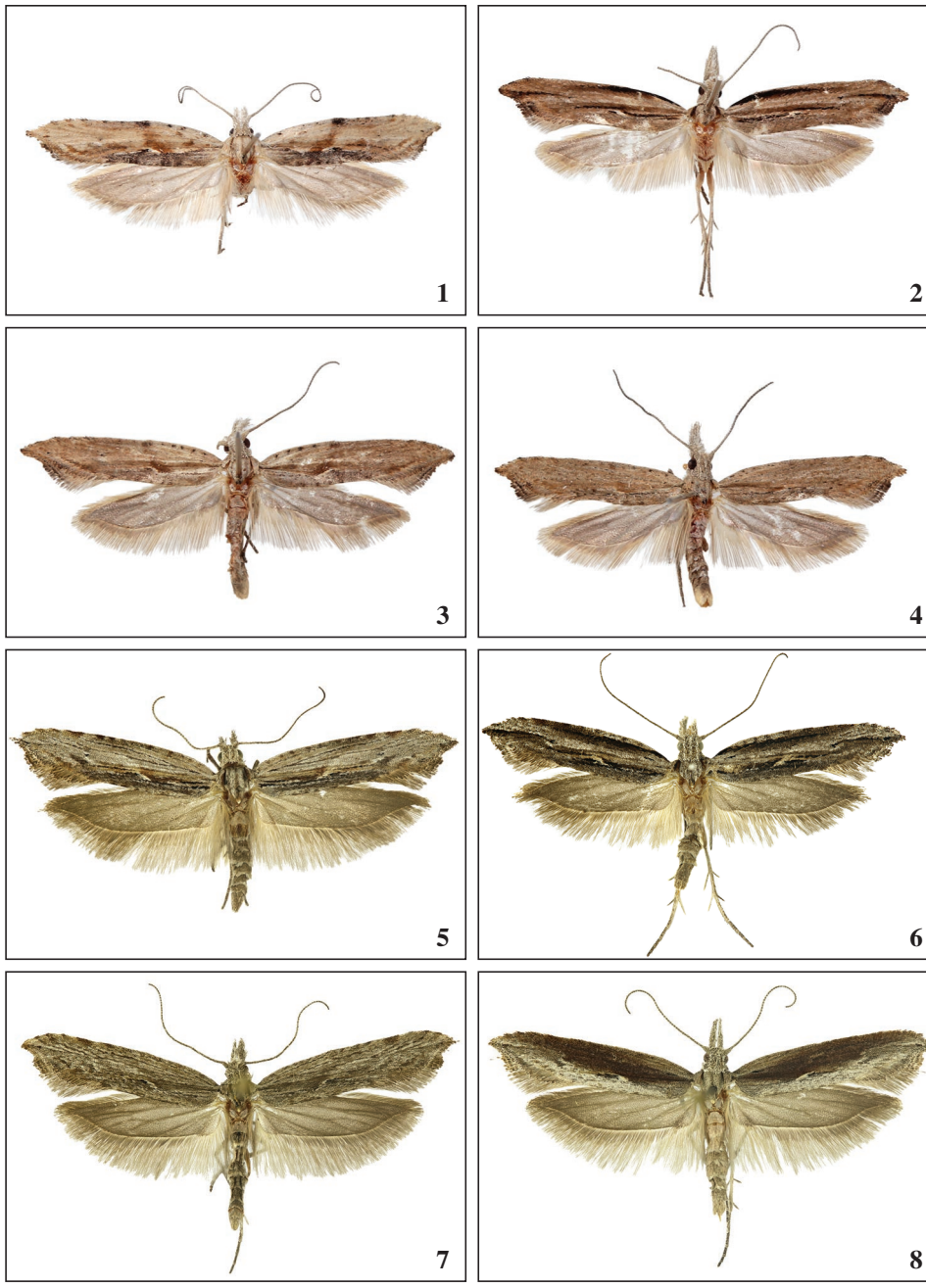
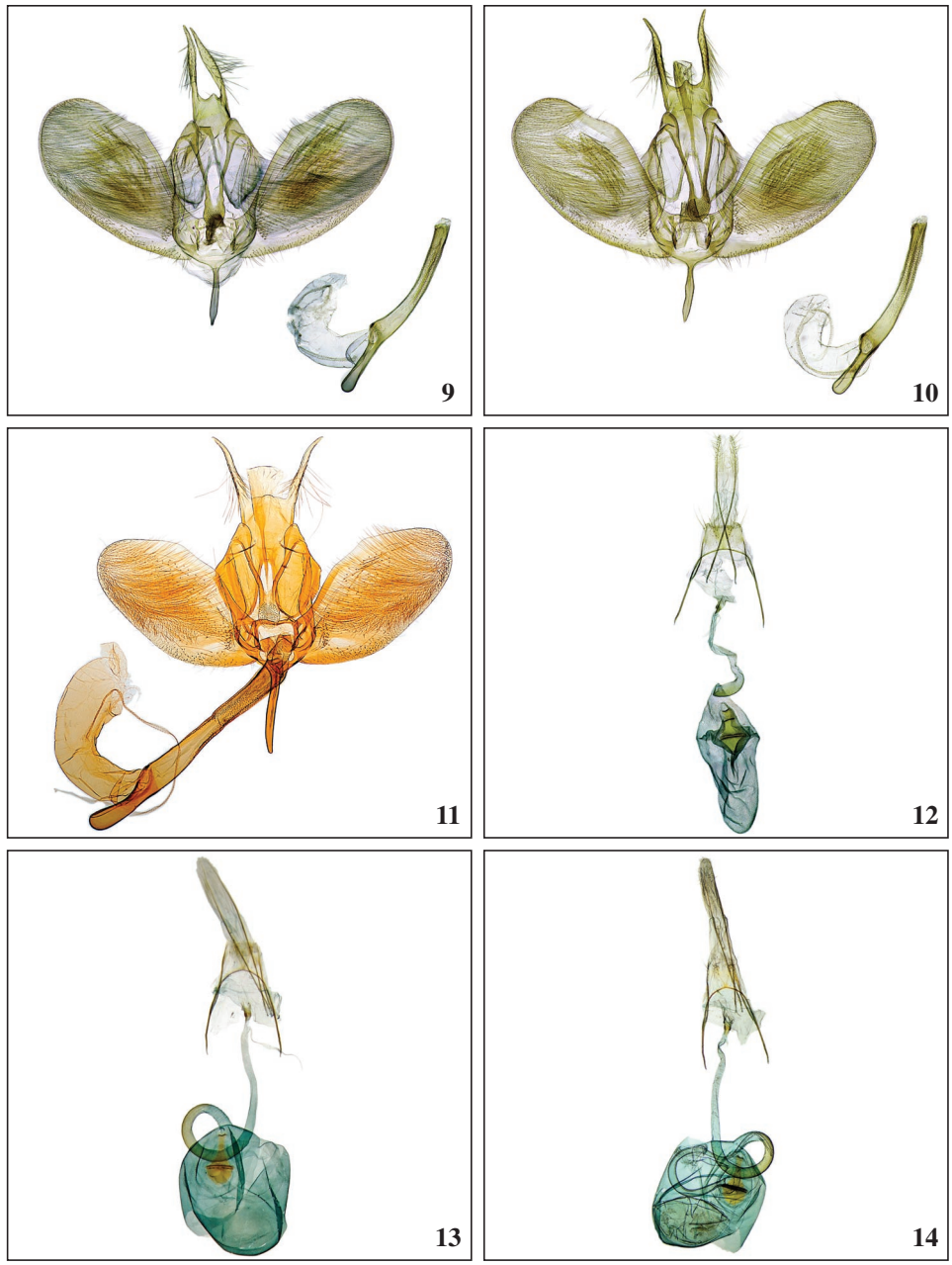


Figs 1-8.–1-4. Adults *Ypsolopha milfontensis* Corley & Ferreira, sp. n. **5-8.** *Ypsolopha instabilella* (Mann).



Figs 9-14.– 9-10. Male genitalia *Ypsolopha milfontensis* Corley & Ferreira, sp. n. **11.** Male genitalia *Ypsolopha instabilella* (Mann). **12-13.** Female genitalia *Ypsolopha milfontensis* Corley & Ferreira sp. n., **14.** *Ypsolopha instabilella* (Mann).