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Ypsolopha rhinolophi sp. nov. (Lepidoptera: Ypsolophidae), a new species from Portugal and France unveiled by bats

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Abstract

A new species *Ypsolopha rhinolophi* Corley is described from northern Portugal and south-east France. It resembles *Y. alpella* (Denis & Schiffermüller, 1775) and *Y. lucella* (Fabricius, 1775) but shows clear differences from both species in DNA barcode and in male and female genitalia. Male genitalia of *Y. lucella* are illustrated for the first time. The new species has been collected at light, reared from larvae on *Quercus pyrenaica* Willd. and recognised from DNA barcode fragments obtained from droppings of horseshoe bats.

Key words: Yponomeutoidea, DNA barcoding, horseshoe bats, *Ypsolopha lucella* male genitalia

Introduction

Ypsolopha Latreille, 1796 is a large genus with more than 160 described species (Jin *et al.* 2013) found all over the Palaearctic and Nearctic regions, with greatest diversity in the eastern Palaearctic. Thirty-seven species are known in Europe (de Jong *et al.* 2014). They are medium-sized Microlepidoptera, often with distinctive wing shape and markings. In the Iberian Peninsula 21 species are known to occur (Vives 2014), none is endemic, and many of them have a wide distribution in the middle latitudes of Europe, extending into northern parts of Spain, but not reaching Portugal. Currently, only five species are known to occur in Portugal (Corley 2015): *Ypsolopha alpella* (Denis & Schiffermüller, 1775), *Ypsolopha lucella* (Fabricius, 1775), *Ypsolopha persicella* (Fabricius, 1787), *Ypsolopha scabrella* (Linnaeus, 1761) and *Ypsolopha ustella* (Clerck, 1759). *Ypsolopha* species are not very commonly attracted to light, so they are potentially under recorded.

The larvae of *Ypsolopha* species feed on the leaves of woody plants, especially trees. The majority feed on a single genus of plants (monophagous) or a few closely related genera (oligophagous). Of the species known in the Iberian Peninsula, the food-plants of three species appear to be unknown (Robinson *et al.* 2010), only *Y. parenthesella* (Linnaeus, 1761) is polyphagous, feeding on *Quercus*, *Corylus*, *Carpinus*, *Betula*, *Alnus* and probably other trees. Four species are oligophagous on Rosaceous trees including *Prunus*, *Crataegus*, *Malus* and *Sorbus*. The remaining species are monophagous: four species on *Quercus* (*Y. lucella*, *Y. alpella*, *Y. sylvella* (Linnaeus, 1767), *Y. ustella* (Clerck, 1759)), one species on each of *Fagus*, *Acer*, *Ulmus*, *Euonymus* and two on *Lonicera*. There are also three species feeding on *Ephedra*, a strange plant belonging to the small class Gnetopsida, which is distantly related to Gymnosperms. It is an unusual food-plant for Lepidoptera, but in arid areas of the central Palaearctic region it hosts a large diversity of *Ypsolopha* species.

Since 2015, the authors have been collecting specimens of moth species throughout continental Portugal within the frame of the InBIO Barcoding Initiative. Currently four of the five *Ypsolopha* species are represented in the reference collection by a total of eight samples, including one *Ypsolopha* specimen identified as *Y. alpella* collected at Constantim (north-east Portugal) in 2016.

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In a parallel study that aims to characterise the diet of bat communities in north-east Portugal using a metabarcoding approach, one of the authors (V.M.) found DNA sequences of *Ypsolopha* specimens in the bat diet samples (unpublished data). Droppings from different individual bats of the genus *Rhinolophus* Lacépède, produced two different DNA barcodes, both supposedly matching the same species, *Ypsolopha alpella.* The "Constantim" DNA barcode sequence was found in bat droppings samples she was processing from two individuals of *Rhinolophus ferrumequinum* (Schreber) and one of *R. euryale* Blasius. Although the DNA barcode of the Constantim specimen shows clear differences from both *Y. lucella* and *Y. alpella* DNA barcodes, this information had not yet been fully processed within InBIO Barcoding Initiative project at the time. This paradox caught the attention of V.M. who then asked M.C. to confirm the species identity of the specimen collected at Constantim. M.C. dissected a male from Constantim and it was immediately apparent that this was a distinct species with very clear differences from *Y. alpella*. This is described in this paper as a new species of *Ypsolopha* based on morphological and genetic data, present in Portugal and France.

Abbreviations

NHMUK—Natural History Museum, London, UK
USNM—National Museum of Natural History, Smithsonian Institution, Washington, DC, USA

Material and methods

Moths were collected at night using 125 w mercury vapour lights over a white sheet, netted by day and reared from larvae found by searching in daylight. Genitalia were dissected using standard techniques (Robinson 1976), with preparations mounted in Euparal.

Bat droppings were collected for a community level trophic study. Bats were captured at various locations in north-east Portugal using mist nets. Captured bats were placed inside clean cotton bags and droppings were collected from these bags.

DNA extraction and sequencing. 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 DNA barcodes were sequenced in an Illumina Miseq platform, following the approach described by Shokralla *et al.* (2015).

TABLE 1. Specimens of *Ypsolopha* sequenced. [Code = InBIO Barcoding Initiative sample code; Date = date of collection; Locality = collecting locality; Lat = latitude; Long = longitude; Genbank = GenBank code for cytochrome c oxidase I (COI).

Taxa	Code	Date	Locality	Lat	Long	GenBank
Ypsolopha alpella	INV01867	03/10/2015	França	41.905	-6.776	MK300076
Ypsolopha alpella	INV05455	21/07/2017	2 km East of Ansião	39.917	-8.413	MK300077
Ypsolopha persicella	INV02647	15/07/2017	Freixiel	41.313	-7.222	MK300080
Ypsolopha rhinolophi	INV00598	04/07/2016	Constantim	41.631	-6.274	MK300075
Ypsolopha scabrella	INV03636	31/08/2016	França	41.905	-6.776	MK300081
Ypsolopha scabrella	INV05376	19/07/2017	Dine	41.909	-6.933	MK300082
Ypsolopha ustella	INV01030	18/06/2015	Freixiosa	41.425	-6.308	MK300078
Ypsolopha ustella	INV03672	02/09/2016	Dine	41.909	-6.933	MK300079

We used OBITools (https://git.metabarcoding.org/obitools/obitools) for general sequence processing. Geneious v.6.1.5 (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).

DNA was extracted from the bat droppings using the E.Z.N.A. Tissue DNA Kit (Omega Bio-Tek, USA). Target fragments of DNA were amplified using two different COI primer-sets: fwhF2/fwhR2n (Vamos *et al.* 2017) and ZBJ-ArtF1c/ZBJ-ArtR2c (Zeale *et al.* 2011). Both PCR reactions had 10 µL of final volume, containing 5 µL of Multiplex PCR Master Mix (QIAGEN, Hilden, Germany), 0.3µM of each primer, and 1µL 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; 35 cycles at 95ºC for 30 s, 45ºC for ZBJ and 50ºC for fwh2 for 30 s, 72ºC for 30 s; and a final elongation step at 60ºC for 10 min. PCR fragments were sequenced in a HiSeq Illumina Platform following the manufacturer's protocol. Bioinformatic procedures and DNA barcode identification followed Mata *et al.* (2019).

Results

Molecular results: All samples amplified the partial COI gene sequence. The specimen from Constantim exhibited a distinct haplotype with *Ypsolopha lucella* being the closest related species (uncorrected p-distance 3.8%) and all other 40 *Ypsolopha* species including *Y. alpella* with sequences available with over 6.5% divergence (SM Table 1).

Taxonomic account

***Ypsolopha rhinolophi* Corley, sp. nov.**
(Figs 1A–D, 2A–B, 3A–B)

Material examined: Holotype: ♂, 'PORTUGAL │Constantim │ Trás-os-Montes │ 4.vii.[20]16 ex l[arva] 3.vi.[20]16 │ M.F.V. Corley │ *Quercus pyrenaica*' '5491 │M. Corley │ gen. prep.' Barcoded (INV00598). To be deposited in Natural History Museum, London (registration number NHMUK010219609) (Figs. 1A, 2A).

Paratypes: ♂, same locality, emerged 5.vii.2016, coll. Corley. (Fig. 1B); ♂, Portugal, Trás-os-Montes, Montalegre, Serra do Larouco, 23.ix.2003, leg. M. Corley, Corley gen. prep. 5495, coll. Corley; ♀, Portugal, Trás-os-Montes, Montalegre, Serra do Larouco, 21.ix.2005, leg. M. Corley, Corley gen. prep. 2517, coll. Corley (Fig. 3A); ♂, Portugal, Beira Litoral, Miranda do Corvo, Vila Nova, Parque Eólico, 30.vii.2015, leg. J. Rosete, Corley gen. prep. 5510, coll. Rosete; ♂, Portugal, Beira Alta, Penedono, Barragem da Dama, 25.vii.2017, leg. J. Rosete, coll. Rosete; ♀, Portugal, Beira Alta, Penedono, Barragem da Dama, 25.vii.2017, leg. J. Rosete, Corley gen. prep. 5511, coll. Rosete (Fig. 3B); ♂, France, Basses Alpes, Digne, Vallée Miraux, 15.vii.1969, leg. E. Jäckh, [gen. prep.] 5441, (USNMENT 01480147) (USNM) (Figs 1D, 2B).

The new species has also been identified from a photograph taken at Portugal, Minho, Melgaço, Assureira, Ponte Nova, 8.viii.2015, photo by J. Nunes (Fig. 1C). The specimen was not collected but can clearly be identified from the photo.

Description. (Figs 1A–D). Wingspan 17–18.5 mm. Head with erect hair-like scales, pale ochreous; labial palp pale buff, segment 2 with tuft of long, forward-projecting, hair-like scales, longer than segment, segment 3 with appressed scales, slender, pointed; antennal scape buff, flagellum white, ringed black. Thorax ochreous to ochreous-cinnamon. Forewing broad, faintly hooked at apex, termen slightly concave, ground colour ochreous, sometimes overlaid cinnamon except costal area to one-third wing length and with less cinnamon in an indistinct band across wing from two-thirds to seven-eighths, terminal area darker cinnamon, at least ochreous costal area crossed by ochreous-brown strigulae, often such strigulae present over most of wing surface, combining with slightly darker lines on veins to form a reticulate pattern; spots on dorsum at one-quarter and one-half light brown to blackish, additional black scales, spots or dashes present in some specimens particularly in fold and on dorsal margin of cell; fringe with two darker cinnamon lines. Hindwings light grey, towards margins darker grey; fringe light grey-buff with dark grey basal line. Abdomen light grey-buff.

Variation. The French specimen (Fig. 1D) has forewing coloration less ochreous, more uniformly reticulate, without black markings and with the two dorsal spots faint; hindwing fringe whitish with distinct light grey-brown fringe line.

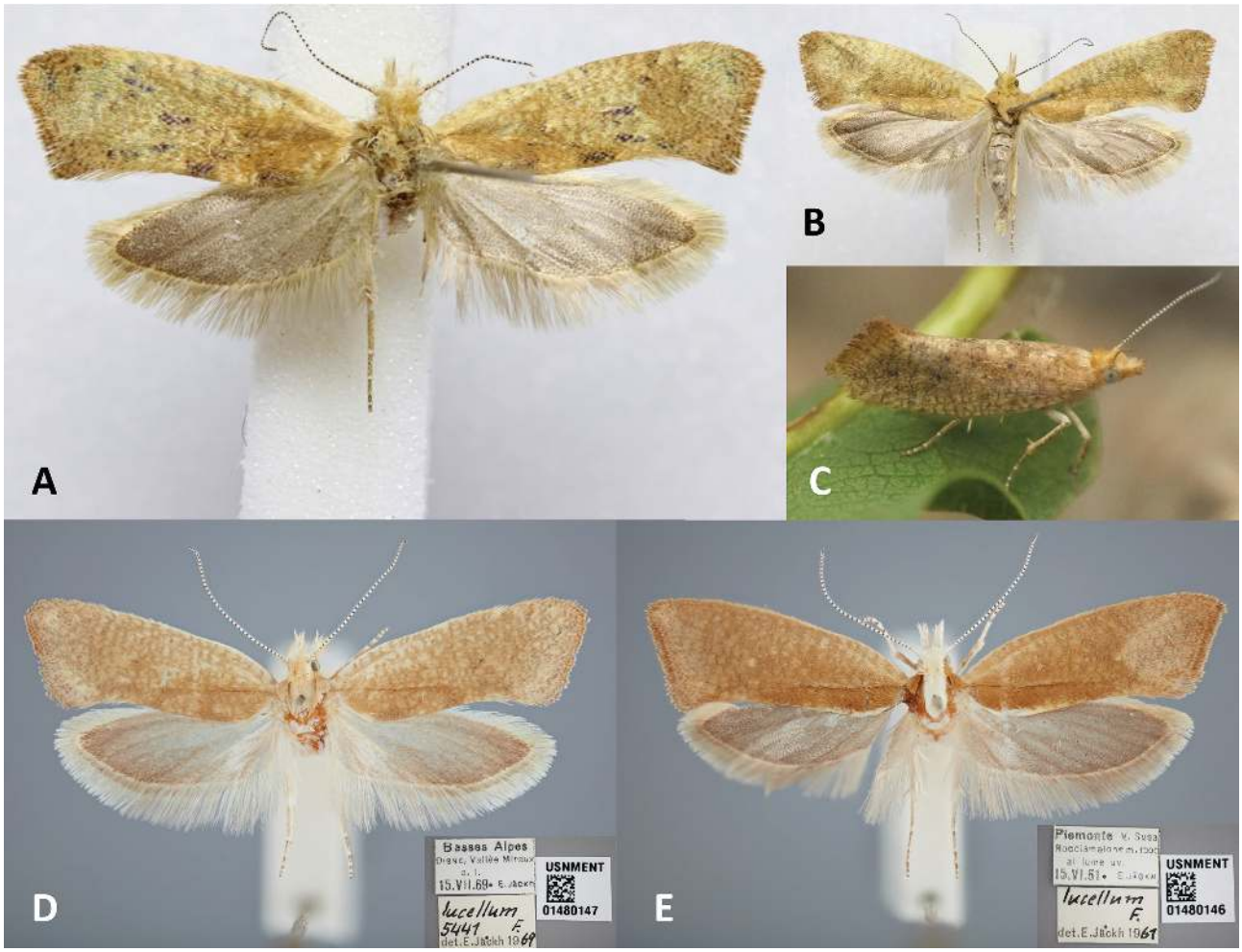

FIGURE 1. A. *Ypsolopha rhinolophi* Corley, **sp. nov.**, holotype male. Portugal, Trás-os-Montes, Miranda do Douro, Constantim, 4.vii.2016, ex larva on *Quercus pyrenaica*, leg. M. Corley. **B**. *Y. rhinolophi*, paratype male, same locality. **C**. *Y. rhinolophi*, Portugal, Minho, Melgaço, Assureira, Ponte Nova, 8.viii.2015 (J. Nunes). **D**. *Y. rhinolophi*, paratype, France, Basses Alpes, Digne, Vallée Miraux, 15.vii.1969, leg. E. Jäckh, (USNM). **E**. *Ypsolopha lucella* (Fabricius, 1775), Italy, Piemonte, V. Susa, Rocciamelone, 1000m, 15.vi.[19]61, leg. E. Jäckh (USNMENT01480146) (USNM).

Male genitalia: (Figs. 2A–B). Uncus not developed, socii widely separated, narrow, pointed spikes, gnathos arms long, medial plate hardly widened, chanelled; valva obovate, narrow at base, costal margin straight, thickened, ventral margin thickened, with slight bend beyond middle, apex with tighter curve to costal margin than to ventral margin; saccus slender, parallel-sided, 0.4 times length of valva; anellus tube densely covered with minute thorns; aedeagus 1.25 times length of valva, ductus ejaculatorius attached at middle of aedeagus, basal half (caecum) straight, slender, but twice width of apical half, apical half slightly curved, with pair of elongate cornuti, apex acute, apical part surrounded by a mantle covered with minute thorns.

Female genitalia: (Fig. 3A–B). Papilla analis rounded at apex; apophysis posterioris long, more than three times as long as apophysis anterioris, as measured from base of its posterior branch to apex; segment VIII weakly sclerotised; ostium circular, antrum small, ovoid, ductus bursae slender, expanding gradually towards corpus bursae, slightly longer than apophysis anterioris; corpus bursae large, ovoid, signum large, not well defined, consisting of two rhombic plates joined to form an hourglass shape, the posterior rhombus slightly smaller, both halves with a conspicuous transverse fold at their widest point.

Diagnosis. *Y. rhinolophi* is immediately separable from *Y. alpella* by the black-ringed white antenna, and differs from the most closely related species *Y. lucella* (Fig. 1E) in the absence of white scales on head and thorax. Further differences externally and in genitalia from both of these species are presented in Table 2 and Figs 1–3. Both

Y. lucella and *Y. alpella* have larvae feeding on *Quercus*. There are other European *Ypsolopha* species feeding on *Quercus*: *Y. sylvella* (Linnaeus, 1767) is a close relative of *Y. alpella*, but is easily distinguished from the new species by two dark lines obliquely crossing most of the forewing. *Y. parenthesella* (Linnaeus, 1761) and *Y. ustella* (Clerck, 1759) also feed on *Quercus*, but are not similar in appearance to *Y. rhinolophi*.

Larva. The larvae found at Constantim were of a light green colour, but were not recognised as of particular interest at the time of collection and were not photographed or described.

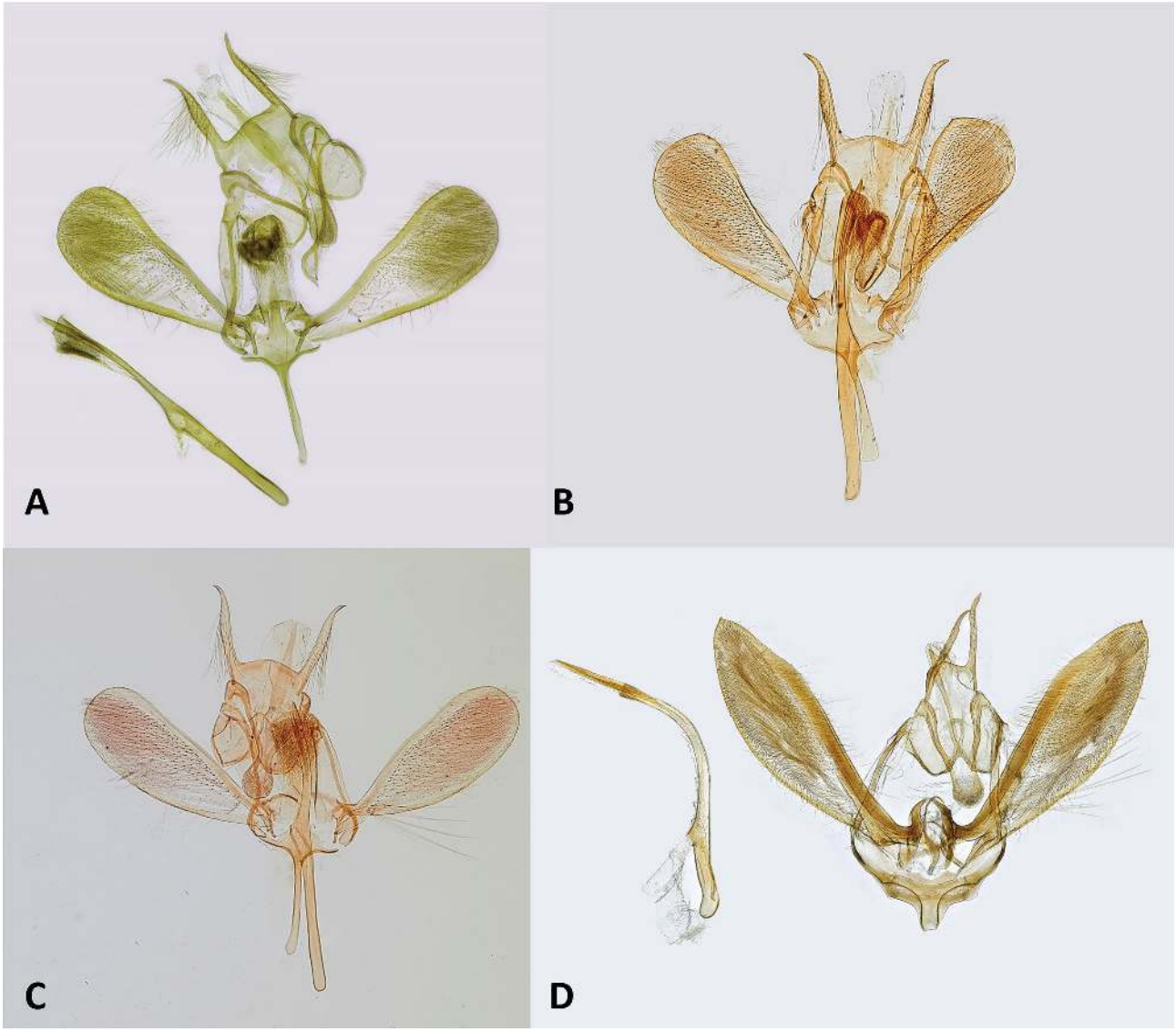

FIGURE 2. Male genitalia. **A.** *Y. rhinolophi* Corley, **sp. nov.**, holotype, Corley gen. prep. 5491. **B**. *Y. rhinolophi* Corley **sp. nov.**, paratype, France, Basses Alpes, Digne, Vallée Miraux, 15.vii.1969, leg. E. Jäckh, [gen. prep.] 5441, (USNM). **C**. *Ypsolopha lucella* (Fabricius, 1775), Italy, Piemonte, V. Susa, Rocciamelone, 1000m, 15.vi.[19]61, leg. E. Jäckh (USNMENT01480146) (ESNM). **D**. *Ypsolopha alpella* (Denis & Schiffermüller, 1775), Portugal, Beira Alta, Manteigas, 8.ix.2001, leg. M. Corley, Corley gen. prep. 5492.

Biology. Larvae were found at the beginning of June feeding on *Quercus pyrenaica* between adjacent leaves spun together. Moths emerged in early July. In the field, moths have been taken at light in the middle and end of July, in August and also on 21 and 23 September in different years. The site at Constantim is a low hill rising from the plateau at 840 m altitude, covered with *Q. pyrenaica* of moderate size. On Serra do Larouco the site was *Q. pyrenaica* scrub on a mountainside at 1370 m. The sites at Penedono and Miranda do Corvo were at 850 m and 800 m respectively, and both have *Quercus pyrenaica* present (J. Rosete pers. comm.). One additional site in Minho lies at 780m and also has *Q. pyrenaica* (Portugal, Minho, Melgaço, Castro Laboreiro, Assureira, Ponte Nova, 8.viii.2015,

J. Nunes, E. Marabuto and A. Gonçalves). *Quercus pyrenaica* is not present in south-east France where another *Quercus* species is likely to be the food-plant.

Distribution. In Portugal *Ypsolopha rhinolophi* is known from direct collection of larvae or adults from two localities in Trás-os-Montes in the north-east Portugal and from single localities in Beira Alta and Beira Litoral, and from a photographed moth in Minho in the north-west of the country which clearly shows the coloration, wing shape and black and white antennae of the new species (Fig. 1C). In addition, DNA metabarcode samples obtained from bat droppings indicate its presence in two additional localities in Trás-os-Montes (see Fig. 4). In France it is known from a single locality in Basses Alpes in the south-east of the country. Suitable habitat exists for *Y. rhinolophi* in Spain, so the species is likely to occur in the country.

Etymology. The species name *rhinolophi* recognises the part played in the discovery of the new species by the horseshoe bats *Rhinolophus ferrumequinum* and *Rhinolophus euryale*. The name is a noun in genitive case.

FIGURE 3. Female genitalia. **A**. *Ypsolopha rhinolophi* Corley **sp. nov.**, paratype, with ovipositor contracted, Portugal, Trás-os-Montes. Montalegre, Serra do Larouco, 21.ix.2005, leg. M. Corley, Corley gen. prep. 2517. **B**. *Ypsolopha rhinolophi* Corley **sp. nov.**, paratype, with ovipositor extended, Portugal, Beira Alta, Penedono, Barragem da Dama, 25.vii.2017, leg. J. Rosete, Corley gen. prep. 5511. **C**. *Y. lucella* (Fabricius, 1775), England, Lincolnshire, Broadholme, 23.viii.2013, leg. M.J. Gray, gen. prep. B. Goodey. **D**. *Y. alpella* (Denis & Schiffermüller, 1775), England, Essex, Colchester, Layer-de-la-Haye, 15.viii.2004, leg. and gen. prep. B. Goodey.

Discussion

The described morphological and genetic characteristics demonstrate the distinctiveness of *Ypsolopha rhinolophi* from other species of *Ypsolopha*. Nevertheless, the specimens from Serra do Larouco and those from Constantim were originally identified by M.C. as *Y. alpella*, including the female from Serra do Larouco, which was dissected in 2006. The long overlooked presence of *Y. rhinolophi* in Portugal resulted in some confusion in the identification of Portuguese *Ypsolopha* species, particularly *Y. alpella* (the morphologically most similar species), but also *Y. lucella* (the genetically closest species). The French specimen was identified by Jäckh as *Y. lucella.* Externally it shows some differences from Portuguese material (cf Figs 1D and 1E), but no difference in male genitalia (cf Figs 2A and 2B).

TABLE 2. Summary of the main morphological characteristics that differentiate *Ypsolopha rhinolophi* from the two morphologically most similar species.

	Y. rhinolophi	*Y. lucella*	*Y. alpella*
Head	Pale ochreous	White	Pale ochreous
Antenna	White ringed black	White ringed black	Creamy ochreous ringed grey-brown
Thorax	Ochreous-cinnamon	White centrally	Ochreous
Forewing apex	Weakly falcate	Weakly falcate	Distinctly falcate
Darker spots on dorsum	Present	Absent	Present
Male genitalia			
Valva shape	Obovate, narrower near base	Obovate, broader near base	Oblanceolate
Valva apex	Broadly rounded, more abruptly on costal side	Broadly and evenly rounded	Apiculate
Gnathos	Channelled	Broadly spatulate	Rounded, not channelled
Saccus	7–10 times as long as wide	About 8 times as long as wide	2–4 times as long as wide
Aedeagus shape	Nearly straight	Nearly straight	Strongly curved
Width of caecum relative to remainder of aedeagus	Caecum twice as wide as posterior part	Caecum 1.5 times as wide as posterior part	Caecum 1.5 times as wide as posterior part
Ductus ejaculatorius	Near middle	Near middle	One-quarter from base
Female genitalia			
Antrum	One-eighth length of apophysis anterioris	One-seventh length of apophysis anterioris	One-half length of apophysis anterioris
Ductus bursae	Shorter than corpus bursae	About equalling corpus bursae	Longer than corpus bursae
Corpus bursae	Large, ovoid	Smaller, elliptical	Smaller, elliptical
Signum	Posterior end smaller than anterior end	Posterior end smaller than anterior end	Two ends nearly equal

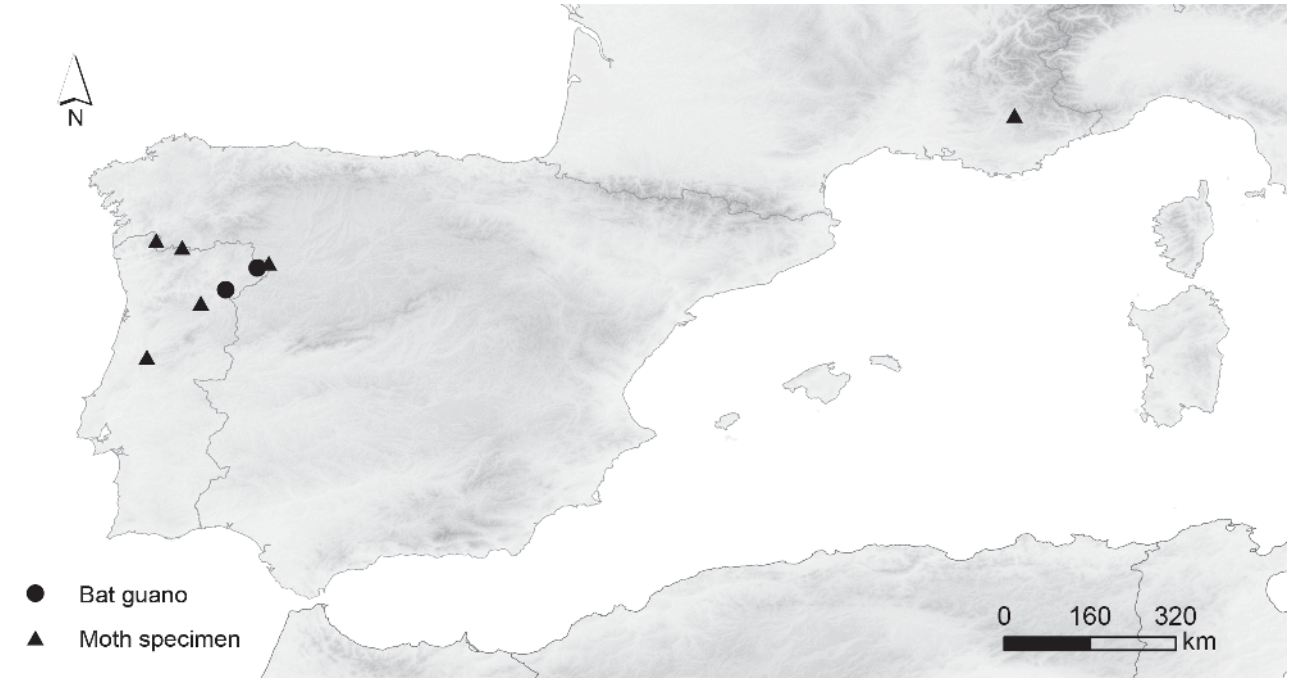

FIGURE 4. *Ypsolopha rhinolophi* Corley **sp. nov.**, known distribution.

The only illustration of *Y. lucella* male genitalia we were able to find is in Zagulajev (1981) which is indistinguishable from his drawing of male genitalia of *Y. alpella* and surely represents that species, not *Y. lucella.* No specimens have been collected by the authors and no male was found among about 30 specimens in NHMUK (David

Lees pers. comm.). According to Suomalainen (1978) the species is largely parthenogenetic over most of Europe, although he found a report of males found by E. Jäckh in north-west Italy and south-east France. Jäckh's collection is in USNM. Mark Metz kindly sent photographs of two males named *Y. lucella* by Jäckh together with photos of their genitalia. One specimen (USNMENT 01480146) (Fig. 1E) is indeed *Y. lucella* and male genitalia are illustrated here for the first time (Fig. 2C). The other specimen (Figs 1D and 2B) proved to be *Y. rhinolophi sp. n*. which greatly extends the known range of this species to south-east France.

The distribution of *Y. alpella* and *Y. lucella* in Portugal as given in Corley (2015) needs to be reviewed, but there is a shortage of collection material available and further efforts should be made in future fieldwork.

Horseshoe bats forage mostly in deciduous woodlands (Goiti *et al.* 2008). Such woodland in north-east Portugal is usually dominated by *Quercus pyrenaica*, the known food-plant of *Y. rhinolophi*. Horseshoe bats do not forage at any great distance from their roosts (Flanders & Jones, 2009), so the moth is likely to be present in the areas where the dropping samples were obtained. We include these localities on the map presented (Fig. 3). Diet analysis of bats might prove to be a relevant source of information in cases of moth species that are not commonly attracted to light such as *Ypsolopha* species.

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References

Clerck, C.A. (1759) *Icones insectorum rariorum cum nominibus eorum trivialibus locisque e C. Linnaei Systemae Naturae allegatis,* Stockholm, 21 pp., 55 pls.

Corley, M.F.V. (2015) *Lepidoptera of Continental Portugal. A fully revised list*. Martin Corley, Faringdon, 288 pp.

de Jong, Y., Verbeek, M., Michelsen, V., Bjørn, P., Los, W., Steeman, F., Bailly, N., Basire, C., Chylarecki, P., Stloukal, E., Hagedorn, G., Wetzel, F., Glöckler, F., Kroupa, A., Korb, G., Hoffmann, A., Häuser, C., Kohlbecker, A., Müller, A., Güntsch, A., Stoev, P. & Penev, L. (2014) Fauna Europaea—all European animal species on the web. *Biodiversity Data Journal*, 2, e4034. (accessed 5 November 2018) https://doi.org/10.3897/BDJ.2.e4034

Denis, M. & Schiffermüller, I. (1775) *Ankündung eines systematischen Werkes von den Schmetterlingen der Wienergegend*. Vienna, 324 pp.

Fabricius, J.C. (1775) *Systema Entomologicae, sistens Insectorum classes, ordines, genera, species, adjectis, synonymis, locis, descriptionibus, observationibus.* Flensburg, Lipsia, 832 pp. https://doi.org/10.5962/bhl.title.36510

Fabricius, J.C. (1787) *Mantissa Insectorum sistens eorum species, nuper detectas adjectis characteribus generis, differentiis specificis emendationibus, observationibus*. *Vol. 2.* Impensis Christ. Gottl. Proft, Hafniae, 382 pp. https://doi.org/10.5962/bhl.title.11657

Flanders, J. & Jones, G. (2009) Roost use, ranging behaviour and diet of Greater Horseshoe Bats (*Rhinolophus ferrumequinum*) using a transitional roost. *Journal of Mammalogy*, 90, 888–896. https://doi.org/10.1644/08-MAMM-A-352.1

Goiti, U., Garin, I., Almenar, D., Salsamendi, E. & Aihartza, J. (2008) Foraging by Mediterranean Horsehoe Bats (*Rhinolophus*

euryale) in relation to prey distribution and edge habitat. *Journal of Mammalogy*, 89, 493–502. https://doi.org/1644/07-MAMM-A-054R2.1

Hajibabaei, M., Janzen, D.H., Burns, J.M., Hallwachs, W. & Hebert, P.D.N. (2006) DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 968–971. https://doi.org/10.1073/pnas.0510466103

Hebert, P.D.N., Penton, E.H., Burns, J.M., Janzen, D.H. & Hallwachs, W. (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 14812–14817. https://doi.org/10.1073/pnas.0406166101

Jin, Q., Wang, S.X. & Li, H.H. (2013) Review of the genus *Ypsolopha* Latreille, 1796 from China. *Zootaxa*, 3705 (1), 1–91. https://doi.org/10.11646/zootaxa.3705.1.1

Linnaeus, C. (1761) *Fauna Svecica. 2nd Edition*. Laurentii Salvii, Stockholm, 578 pp.

Linnaeus, C. (1767) *Systema Naturae per regna tria naturae secundum classes, ordines, genera, species, cum characteribus differentiis, synonymis, locis*. *Editio duodecima, reformata. 1 (2)*. Impensis Direct. Laurentii Salvii, Holmiae, 533–1327 + 248 pp. Holmiae. https://doi.org/10.5962/bhl.title.156765

Mata, V.A., Rebelo, H., Amorim, F., Mccracken, G.F., Jarman, S. & Beja, P. (2019) How much is enough? Effects of technical and biological replication on metabarcoding dietary analysis. *Molecular Ecology*, 28, 165–175. https://doi.org/10.1111/mec.14779

Robinson, G.S., (1976) The preparation of slides of Lepidoptera genitalia with special reference to the Microlepidoptera. *Entomologist's Gazette*, 27, 127–132. Available from: http://itp.lucidcentral.org/id/lep/lbam/Robinson_1976.pdf (accessed 29 April 2019)

Robinson, G.S., Ackery, P.R., Kitching, I.J., Beccaloni, G.W. & Hernández, L.M. (2010) *HOSTS—A Database of the World's Lepidopteran Hostplants*. Natural History Museum, London. http://www.nhm.ac.uk/hosts (accessed 27 November 2018)

Shokralla, S., Porter, T.M., Gibson, J.F., Dobosz, R., Janzen, D.H., Hallwachs, W., Golding, G.B. & Hajibabaei, M. (2015) Massively parallel multiplex DNA sequencing for specimen identification using an Illumina MiSeq platform. *Scientific Reports*, 5, 9687. https://doi.org/10.1038/srep09687 pmid:25884109

Suomalainen, E. (1978) Two new cases of parthenogenesis in moths. *Nota lepidopterologica*, 1, 65–68.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution*, 28 (10), 2731–2739. https://doi.org/10.1093/molbev/msr121

Vamos, E.E., Elbrecht, V. & Leese, F. (2017) Short COI markers for freshwater macroinvertebrate metabarcoding. *Metabarcoding and Metagenomics*, 1, e14625. https://doi.org/10.3897/mbmg.1.14625

Vives Moreno, A. (2014) Catálogo sistemático y sinonímico de los Lepidoptera de la Península Ibérica, de Ceuta, de Melilla y de las Islas Azores, Baleares, Canarias, Madeira y Salvajes. (Insecta: Lepidoptera.). *SHILAP Revista de Lepidopterología*, Supplement, 1–1184.

Zagulajev, A.K. (1981) Plutellidae. *In*: Medvedev, G.S. (Ed.), *Keys to the insects of the European part of the USSR*, 4 (2), pp. 359–397.

Zeale, M.R.K., Butlin, R.K., Barker, G. & Gareth, J. (2011) Taxon-specific PCR for DNA barcoding arthropod prey in bat faeces. *Molecular Ecology Resources*, 11 (2), 236–244. https://doi.org/10.1111/j.1755-0998.2010.02920.x