First description of the male and DNA barcode of *Euphyia vallantinaria* (Oberthür, 1890) from the Iberian Peninsula (Lepidoptera, Geometridae, Larentiinae)

Juan J. Guerrero[1], Axel Hausmann[2], Rosa M. Rubio[1], Manuel Garre[1], Antonio S. Ortiz[1]

1 *Department of Zoology and Physical Anthropology. University of Murcia. Campus de Espinardo, 30100 Murcia, Spain; juanjogf@um.es, rmrubio@um.es, manuel.garre@fripozo.com, aortiz@um.es*

2 *SNSB – Bavarian State Collection of Zoology, Münchhausenstr, 21, 81247 Munich, Germany; hausmann.a@snsb.de*

http://zoobank.org/EC231A77-580D-46DC-8328-3C41507E0527

Received 23 September 2021; accepted 13 December 2021; published: 6 January 2022
Subject Editor: Sven Erlacher.

Abstract. The male of *Euphyia vallantinaria* (Oberthür, 1890) has been recorded for the first time from the Iberian Peninsula and its genital morphology has been described and illustrated. DNA barcode data are presented and compared with the other European *Euphyia* species.

Introduction

The genus *Euphyia* Hübner, 1825 is distributed in the Holarctic and Neotropical regions and includes about 170 species (Scoble 1999). Presence of the genus in South America has been confirmed recently by Brehm et al. (2019). In Europe, six species have been cited of which only four fly on the Iberian Peninsula: *Euphyia biangulata* (Haworth, 1809), *E. vallantinaria* (Oberthür, 1890), *E. unangulata* (Haworth, 1809) and *E. frustata* (Treitschke, 1828) (Hausmann and Viidalepp 2012). All of them are silvicolous (except *E. frustata* which lives in almost any habitat including Mediterranean xeric scrub habitat). The larvae are oligophagous, preferring Caryophyllaceae (except the unknown larva of *E. vallantinaria*). *E. biangulata* and *E. unangulata* have Eurasiatic and Holarctic distribution, respectively, and are known from the northernmost part of the Iberian Peninsula while *E. frustata* is a Sub-Mediterranean species whose subspecies *fulvocinctata* Staudinger, 1871 flies in mountainous areas of the Iberian Peninsula and North Africa, from 700 up to 3,000 m above sea-level (Redondo et al. 2009; Hausmann and Viidalep 2012). Other European species are *Euphyia adumbraria* (Herrich-Schäffer, 1852) distributed in northern Italy and Austria to Greece and *E. mesembrina* (Rebel, 1927) distributed in the western Balkans from Croatia to Greece.

Euphyia vallantinaria was described initially as *Cidaria vallantinaria* from Bône in eastern Algeria. Prout (1938) combines this species with his subgenus *Euphyia*, but places it between the two species *basochesiata* (Duponchel, 1831) and *putridaria* (Herrich-Schäffer, 1832) which both belong to genus *Catarhoe* in current taxonomy. The species was definitely assigned to genus *Euphyia* by Hausmann (2011) and Hausmann and Viidalepp (2012) and confirme by Müller et al. (2019).

In the original description of *E. vallantinaria*, the sex differences among specimens were not indicated. Hausmann and Viidalepp (2012) published the female diagnosis and genitalia based on Iberian material and indicated that the male of this species had never been described properly. The female genitalia were described to be "without constant and significant differential features from *E. biangulata*" (Hausmann and Viidalepp 2012).

Previously, Hausmann (2011) studied two females from the southernmost part of the Iberian Peninsula and compared them with six females from Algeria in the Herbulot collection kept in Zoologische Staatssammlung München (SNSB-ZSM; Germany). He indicated that the dissected specimen earlier had been identified tentatively as *E. biangulata* because of the great similarity in female genitalia. Because of some differences in habitus and the distance between the southernmost distribution of *E. biangulata* and the new Andalusian populations (more than 600 km) these two female specimens were DNA barcoded and revealed large genetic distance from central European *E. biangulata* (5.6%), suggesting separate species rank. Hausmann (2011) reviewed and concluded that the specimens showed a great similarity in habitus with the North African species *E. vallantinaria*, which exactly corresponds also in female genitalia. Hausmann (2011) also noted that no significant and constant differential features in female genitalia between European *E. biangulata* and North African *E. vallantinaria* were found.

In this article, we provide a description of the male of *Euphyia vallantinaria*. In addition, DNA barcodes (COI) were used to confirm its occurrence in the southernmost part of the Iberian Peninsula and to assess genetic divergence between the *Euphyia* species.

Material and methods

Morphological study

The specimens were examined externally in order to evaluate possible differences in their colouration and wing shape, and they were dissected using standard procedures (Hausmann 2001) with minor modifications. The male adult image (Fig. 1A, B) was taken with a Nikon D70 digital camera and z-stacked using the software Zerene. Male morphology of genital structures (Fig. 1C–E) were studied using a Zeiss Stemi 508 stereomicroscope with a Zeiss Axiocam ICc5 digital camera. The specimen is deposited in the Research Collection of Animal Biology (RCBA-UMU) in the Department of Zoology and Physical Anthropology of Universidad de Murcia (Spain).

Molecular procedures

For DNA extraction, two legs were removed from two adult specimens (Table 1) in order to sequence the 658 base-pair long barcode segment of the mitochondrial COI gene (cytochrome c oxidase 1, 5' terminus). The tissue samples were submitted to the Canadian Centre for DNA

Table 1. Interspecific mean K2P (Kimura 2-Parameter) divergences (mean pairwise distances) based on the analysis of COI fragments (>600 bp) between European *Euphyia* species.

	biangulata	***frustata***	***mesembrina***	***unangulata***	***vallantinaria***
E. adumbraria	7.4%	6.2%	7.1%	4.9%	6.4%
E. biangulata		5.9%	6.7%	5.0%	5.3%
E. frustata			2.7%	5.1%	5.7%
E. mesembrina				6.1%	5.9%
E. unangulata					4.9%

Barcoding (CCDB, Biodiversity Institute of Ontario, University of Guelph) to obtain DNA barcodes using the high-throughput protocol described in deWaard et al. (2008) which can be accessed at www.dnabarcoding.ca/pa/ge/research/protocols. The DNA extracts are currently stored at the CCDB and the sequences are deposited in GenBank according to the iBOL data release policy (accession numbers: OK346331 and OK346332). Voucher data, images, sequences, and trace files are publicly available on the Barcode of Life Data System (BOLD) (Ratnasingham and Hebert 2007; Ratnasingham 2016).

Sequences were compared with a reference library of Lepidoptera DNA barcodes using the identification engine (BOLD-ID). The reference DNA barcode database for Geometridae used by BOLD-ID is continually validated by specialists to ensure accurate identifications and is particularly well parameterised due to a global campaign to DNA barcode the 24,000 species of the family.

Sequence divergences for the barcode region were calculated using the Kimura 2-parameter (K2P) model and the degrees of intra- and interspecific genetic variation were calculated using the analytical tools of BOLD. All the new and related species sequences were downloaded and aligned with the CLUSTAL algorithm of the MEGA6 software (Tamura et al. 2013). In order to assess the COI divergences between *E. vallantinaria* and the other five European *Euphyia* species, we included all sites with the pairwise deletion option. The sequences of *Euphyia adumbraria*, *E. biangulata*, *E. frustata*, *E. mesembrina* and *E. unangulata* were obtained from BOLD and *Epirrhoe sandosaria* (SampleID GWORO289-09) was used as outgroup. Neighbour Joining (NJ), Maximum Likelihood (ML) and Maximum Parsimony (MP) trees were calculated to visualise similarity among selected European *Euphyia* species. As all trees presented the same topology, only the NJ tree is presented here (Fig. 2). Since one gene is not providing enough information for warranting a reasonable phylogenetic analysis (Gatesy et al. 2007), the trees presented here do not reliably illustrate evolutionary relationships among the sequenced taxa.

Results

Euphyia vallantinaria (Oberthür, 1890)

Cidaria vallantinaria Oberthür, 1890: Études ent. 13: 31, pl. 7, fig. 49 (type locality: eastern Algeria: near Bône).

Type material. Syntype by type illustration in Oberthür (1890) allowing attribution to the genus *Euphyia*, confirmed by Claude Herbulot after study of the type material and by Prout (1938).

Specimens examined. Spain. 1♂, Andalusia, Tarifa, Llanos del Juncal (Cádiz), 770 m, (36.103, -5.541), 04.IX.2019, leg. JM Gaona, coll. RCBA-UMU, Barcode IBLAO1527-20 (Fig. 1A); 1♂, Andalusia, Tarifa, Llanos del Juncal (Cádiz), 770 m, (36.103, -5.541), 04.IX.2019; leg. JM Gaona, coll. RCBA-UMU, Barcode IBLAO1528-20 (Fig. 1B).

For comparison six females from El Tarf, Algeria (coll. Herbulot in ZSM), one male and three females from southern Andalusia in ZSM, all DNA barcoded (cf. Hausmann and Viidalepp 2012): GWORA2606-09, GWOSF937-10, GWOTL724-13, GWOTA860-13.

Description. Adult (Fig. 1A, B). Male wingspan (25.4 mm), smaller than female (28–32 mm). Forewing with basal area divided by the basal line which is brownish towards the base and with a greenish area between the basal line and the antemedial line. Costa with slight reddish tinge. Medial area dark olive green with dark brown on the inner margin of the antemedial and

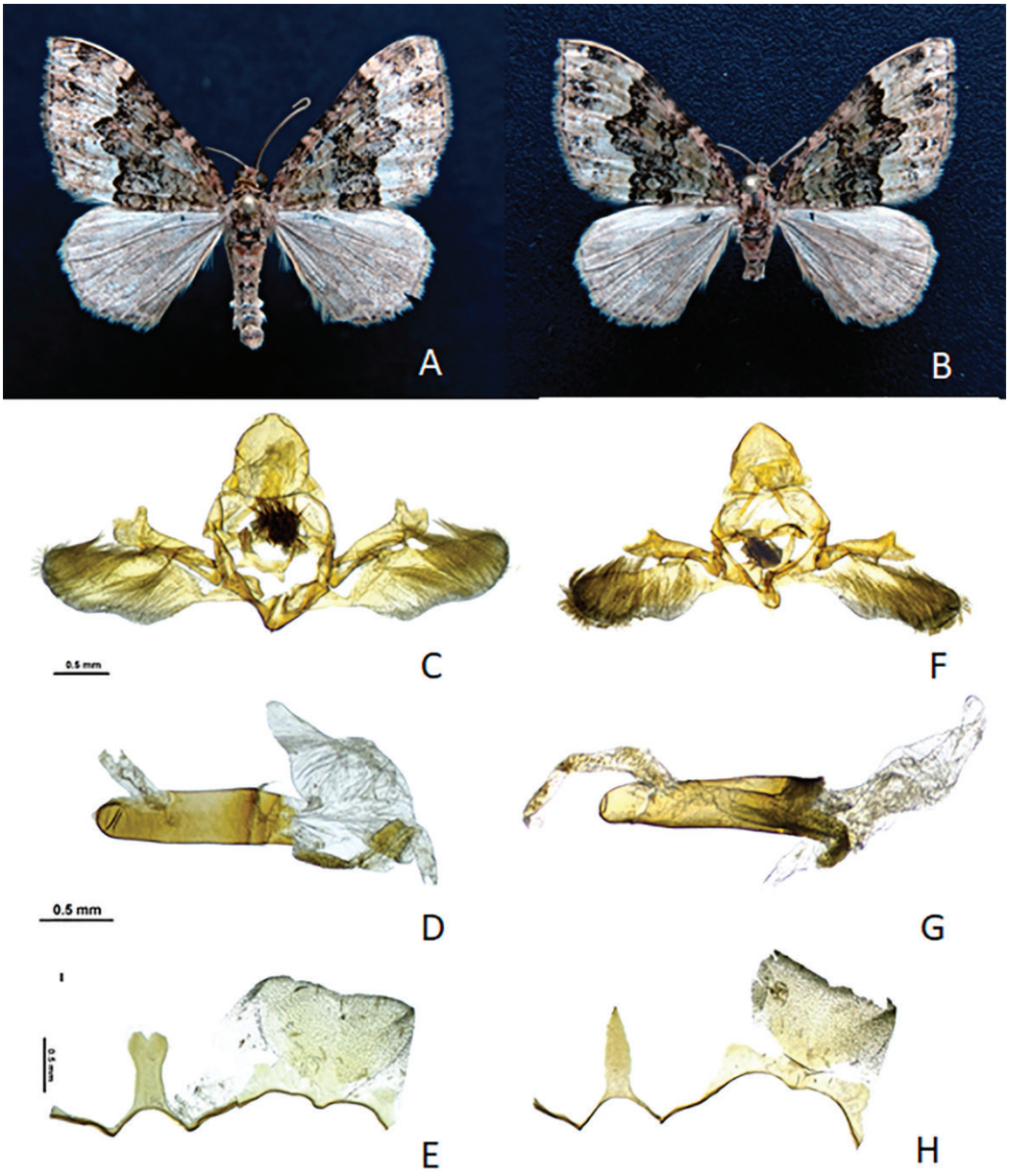

Figure 1. A. Male specimen of *Euphyia vallantinaria* (DNA barcoded: IBLAO1528-20); **B.** Male specimen of *Euphyia vallantinaria* with Barcode IBLAO1528-20; **C.** Andropygium; **D.** Aedeagus; **E.** Sclerotised tergum and sternum A8 detail. Male genitalia of *Euphyia biangulata* from Spain, Lugo, Robledo, 25.VI.2011: **F.** Andropygium; **G.** Aedeagus; **H.** Sclerotised tergum and sternum A8 detail.

postmedial lines, distally with bidentate projection towards the termen in the central zone, with some lobulation towards the anal margin and with an angled insertion towards the costa. Postmedial fascia, narrow, with shadows of a dividing line. Terminal area, less dark than in females, therefore it has a less marked wavy line and only slightly dark spots around this line in, and just

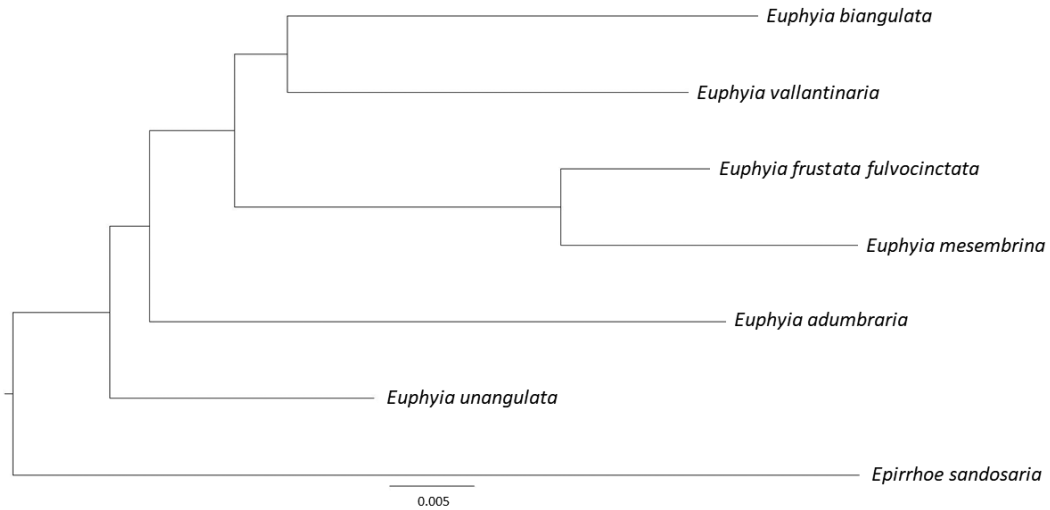

Figure 2. Neighbour Joining tree (K2P; constructed with MEGA6; COI 5' > 600 bp) including 65 sequences of selected *Euphyia* species, rooted with *Epirrhoe sandosaria* as outgroup. The depth of each branch shows divergence between lineages. The scale bar represents 0.005 genetic difference.

below, a subapical position and sometimes lower. Hindwing dark grey to brownish, with a barely contracted postmedial fascia.

Male genitalia (Fig. 1C) well matching genus characteristics with uncus, broad, rounded, dome-shaped. Sclerotised costa of valva with bifurcated projection dorsally with two projections with truncated tips, one narrower dorsally and one wider laterally, apex of the valva, rounded bent and with a slight dorso-subapical concavity. Bases of sacculi long. Saccus broad. Manica spinose on a plate located between juxta and distal part of the aedeagus, which during the dissection process may adhere to the aedeagus or remain adjacent to the juxta. Aedeagus length about 1.4 mm with a very small keel basally at coecum, vesica lobulated with a narrow elongated plate sclerotised with small spines basally (Fig. 1D). Tergum and sternum A8 sclerotised to a continuous ring with a sclerotised plate ventrally with bilobed and wider tip (Fig. 1E).

***Female genitalia*.** See Hausmann and Viidalepp (2012; p. 657, fig. 76).

Diagnosis. *Euphyia vallantinaria* is similar to *E. biangulata* differing in narrower postmedial fascia of forewing with shadows of a dividing line, females with terminal area darker, wavy line conspicuous, hindwing darker with out well-marked white postmedial fascia and a large genetic distance (5.6%) (Hausmann 2011). Can be easily confused with *Colostygia olivata* ([Denis & Schiffermüller], 1775), but the latter shows longer pectination of the male antennae, usually a wider medial area which is delimited by less straight lines. Diagnostic characters are found in the male genitalia which differ from *E. biangulata* in uncus sub-triangular and forked dorsal projections of costa, more pointed at tips, valvae straight dorsally, without dorsal cleft or concavity in subapical position and ventrally bilobous (Fig. 1F–H).

Phenology. Uni- or bivoltine: Scattered European data from early August to mid-September, in North Africa also recorded in April (Hausmann and Viidalepp 2012).

Biology. Larva unknown.

Habitat. Silvicolous in mountain areas. Deciduous and mixed forests of different types, forest fringes, scrub and scattered Mediterranean evergreen oak forest from 300 m up to 1,200 m above sea-level.

Similar species. *E. biangulata* (allopatric).

Distribution. West-Mediterranean with populations in the Iberian Peninsula (southern Andalusia) and in North Africa, only recorded from the type locality Annaba (formerly Bône) and El Tarf (coll. Herbulot/ZSM), both in Algeria.

Molecular analysis. Sequences of DNA barcode region were obtained from two male specimens and registered to Genbank (IBLAO1527-20: OK346332 and IBLAO1528-20: OK346331). No difference was found between the two fragments obtained from the males (658 bp) while a difference of two base pairs (0.3%) was found between male and earlier sequenced female from southern Spain (cf. Hausmann and Viiladepp 2012).

The species has a unique BIN BOLD:AAO2615 (n = 6; sequence length 658 bp in all six specimens). Mean intraspecific variation 0.26%. Maximum intraspecific distance 0.48%. The COI sequences indicated significant divergence with large mean distances to its nearest species in Europe: *E. unangulata* (n = 20; 4.9%), *E. biangulata* (n = 17; 5.3%), *E. frustata fulvocinctata* (n = 4; 5.7%), *E. mesembrina* (n = 6; 5.9%) and *E. adumbraria* (n = 12; 6.4%) (Table 1; Fig. 2).

Discussion

Alhough *E. vallantinaria* (Oberthür, 1890) was described many years ago, the male remained unknown to date. The male characters are described and illustrated here for the first time although the identification of the Andalusian populations needs further comparison through DNA barcoding of North African specimens.

The revision of the descriptions of all *Euphyia* species revealed similarities among them allowing for further grouping of the species within the genus. Among the six *Euphyia* species, *E. vallantinaria* is closely related and similar in female and male genitalia to *E. biangulata*, differing in narrower postmedial fascia of forewing, terminal area darker, wavy line conspicuous and hindwing darker. *E. frustata* is similar to *E. mesembrina* but it presents slightly narrower forewings, ground colour and pattern much paler, without vivid brown and yellow scales, transverse fasciae with dark costal spots while *E. adumbraria* differs from *E. mesembrina* in being larger, terminal area not paler in its tornal half, forewing underside with subapical white pattern. On the other hand, *E. unangulata* has a wing morphology that could be confused with *E. biangulata* and *E. vallantinaria*, although it looks much more like *Epirrhoe rivata* (Hübner, 1813) (Redondo et al. 2009). Female genitalia are very similar among all European *Euphyia* species while in male genitalia, the shape of sclerotised costa and juxta is a diagnostic character (Hausmann and Viidalepp 2012).

The results of the DNA barcoding data obtained in our study indicate the existence of two well-supported and reciprocally monophyletic groups in Europe (Fig. 2). One includes *E. biangulata* and *E. vallantinaria* differing from each other by 5.3% and the other clade consists of the Iberian subspecies *E. frustata fulvocinctata* and *E. mesembrina* differing from each other by 2.7% while *E. adumbraria* does not cluster with *E. unangulata* and differs from the latter by 4.9% (Table 1).

Acknowledgements

Thanks are due to José Manuel Gaona for collecting specimens while John Girdley and Claire Ward improved the manuscript linguistically. We are grateful to Paul D.N. Hebert and the entire team at the

Canadian Centre for DNA Barcoding (CCDB, Guelph, Canada) for carrying out the sequence analyses. This study has been supported by the Regional Excellence 19908-GERM-15 project of the Fundación Séneca (Regional Government of Murcia, Spain). Collecting permits were issued by Environmental Authority of Andalucia.

References

Brehm G, Murillo-Ramos L, Sihvonen P, Hausmann A, Schmidt BC, Õunap E, Moser A, Mörtter R, Bolt D, Bodner F, Lindt A, Parra LE Wahlberg N (2019) New World geometrid moths (Lepidoptera: Geometridae): Molecular phylogeny, biogeography, taxonomic updates and description of 11 new tribes. Arthropod Systematics & Phylogeny 77(3): 457–486. https://doi.org/10.26049/ASP77-3-2019-5

deWaard JR, Ivanova NV, Hajibabaei M, Hebert PDN (2008) Assembling DNA barcodes: Analytical Protocols. In: Martin C (Ed.) Methods in Molecular Biology: Environmental Genetics. Humana Press, Totowa, NJ, 275–293. https://doi.org/10.1007/978-1-59745-548-0_15

Gatesy J, DeSalle R, Wahlberg N (2007) Many genes ghould a systematist sample? Conflicting onsights from a phylogenomic matrix characterised by replicated incongruence. Systematic Biology 56: 355–363. https://doi.org/10.1080/10635150701294733

Hausmann A (2001) The Geometrid moths of Europe. Volume 1. Archiearinae, Orthostixinae, Desmobathrinae, Alsophilinae, Geometrinae. Apollo Books, Stenstrup, 282 pp.

Hausmann A (2011) An integrative taxonomic approach to resolving some difficult questions in the Larentiinae of the Mediterranean region (Lepidoptera, Geometridae). Mitteilungen der Münchner Entomologischen Gesellschaft 101: 73–97.

Hausmann A, Viidalepp J (2012) The Geometrid moths of Europe. Volume 3. Larentiinae I. Apollo Books, Stenstrup, 743 pp.

Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. Proceedings of the Royal Society B: Biological Sciences 270(1512): 313–321. https://doi.org/10.1098/rspb.2002.2218

Ivanova NV, DeWaard JR, Hebert PDN (2006) An inexpensive, automation-friendly protocol for recovering high-quality DNA. Molecular Ecology Notes 6: 998–1002. https://doi.org/10.1111/j.1471-8286.2006.01428.x

Oberthür C (1890). Faunes Entomologiques. Descriptions d'insectes nouveaux ou peu connus. Études d'Entomologie 13: 31. [pl. 7, fig. 49]

Prout LB (1934–1935a, additions 1938a) Brephinae, Oenochrominae, Hemitheinae, Sterrhinae, Larentiinae. In: Seitz A (Ed.) Die Groß-Schmetterlinge der Erde, Suppl. 4. Verlag A. Kernen, Stuttgart.

Ratnasingham S (2016) BOLD Barcode of Life Data System, version 3. http://www.boldsystems.org [Accessed on: 2017-7-01]

Ratnasingham S, Hebert PDN (2007) BOLD: The Barcode of Life Data System (http://www.barcodinglife.org). Molecular Ecology Notes 7: 355–364. https://doi.org/10.1111/j.1471-8286.2007.01678.x

Redondo VM, Gastón FJ, Gimeno R (2009) Geometridae Ibericae. Apollo Books, Stenstrup, 361 pp. https://doi.org/10.1163/9789004261013

Scoble MJ (1999) Geometrid Moths of the World: a catalogue (Lepidoptera, Geometridae). Vol. 1 and 2. CSIRO Publishing and Apollo Books, Stenstrup, 1016 pp. [+ 129 pp. of Index] https://doi.org/10.1071/9780643101050

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution 30: 2725–2729. https://doi.org/10.1093/molbev/mst197