A new species of *Brachmia* Hübner (Lepidoptera, Gelechiidae) from South Europe

KAI BERGGREN, LEIF AARVIK, OLE KARSHOLT, PER KRISTIAN SLAGSVOLD & GUNNHILD MARTHINSEN

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Brachmia cretica **sp. n.** from South Europe is described new to science. The new species is characteristic both in the first part of the COI gene (the standard DNA barcode region) and the morphology of the genitalia. It is compared with the related species, *B. blandella* (Fabricius, 1798). Remarks on the two species' biology and distribution are given.

Key words: Lepidoptera, Gelechiidae, Brachmia, Brachmia cretica, new species, Crete, giant reed.

Kai Berggren, Bråvann terrasse 21, NO-4624 Kristiansand, Norway. E-mail: kberggr@online.no

Leif Aarvik, Natural History Museum, University of Oslo, P.O. Box 1172 Blindern, NO-0318 Oslo Norway. E-mail: leif.aarvik@nhm.uio.no

Ole Karsholt, Zoological Museum, Natural History Museum of Denmark, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen Ø, Denmark. E-mail: okarsholt@snm.ku.dk

Per Kristian Slagsvold, Natural History Museum, University of Oslo, P.O. Box 1172 Blindern, NO-0318 Oslo, Norway. E-mail: p.k.slagsvold@nhm.uio.no

Gunnhild Marthinsen, Natural History Museum, University of Oslo, P.O. Box 1172 Blindern, NO-0318 Oslo, Norway. E-mail: g.m.marthinsen@nhm.uio.no

Introduction

Recently collected material of the genus *Brachmia* Hübner, 1825 (Lepidoptera: Gelechiidae) from the large Greek island, Crete, deviate from known European species of the genus both in the genitalia and in the first part of the COI gene (the standard DNA barcode region). Following the discovery from Crete, additional specimens from the Greek mainland and other South European countries were examined and found to agree with the Cretan taxon. The genus *Brachmia* Hübner, 1825, as currently understood, is a relatively small genus with distribution in the Old World. Many species have been described in *Brachmia*, especially by Edward Meyrick who (Meyrick 1925) listed 93 species in that genus. Most of these have since been transferred to *Helcystogramma* Zeller, 1877, or have even been shown to belong to the families Autostichidae or Lecithoceridae. The global distribution of *Brachmia* is uncertain, but the genus occurs at least in the Palaearctic region and has four European species (Huemer & Karsholt 2020).

Material and methods

Most adults from Crete were collected by night using a LepiLED lamp. The three specimens from 2017 were netted after being spotted by means of a headlamp. Dissections of the genitalia followed Robinson (1976). Photos of the genitalia were taken through a microscope using a Canon digital camera. DNA barcodes refer to a 658 base-pair long fragment of the mitochondrial cytochrome c oxidase subunit 1 (CO1). Legs from three specimens were prepared according to the prescribed standards and processed at the Canadian Centre for DNA Barcoding (CCDB, Biodiversity Institute of Ontario, University of Guelph) to obtain DNA barcodes. A Neighbour joining dendrogram was constructed in MEGA7 (Kumar et al. 2016) based on COI sequences from 14 species additional to the one described here, mined from BOLD (Ratnasingham & Hebert 2007). Hypatima rhomboidella (Linnaeus, 1758) was chosen as outgroup. The Kimura 2-parameter model (Kimura 1980) of nucleotide substitution was used, and 1000 bootstrap replications were performed. The terminology of forewing pattern elements and the genitalia follows Gregersen & Karsholt (2022).

Depository of examined material. KBE (Collection of Kai Berggren, Kristiansand, Norway); NHMO (Natural History Museum, University of Oslo, Norway); ZMUC (Zoological Museum, Natural History Museum of Denmark, Copenhagen, Denmark).

Brachmia cretica Berggren, Aarvik, Karsholt & Slagsvold sp. n. Figures 1–3

Type material. Holotype \mathcal{J} , GREECE: Crete, Chania Province: Kolymbari, 35.536201°N 23.801236°E, 5 m., 22–29.IX.2018, K. Berggren leg., genitalia on slide KBE 13446, coll. NHMO. Paratypes, $3\mathcal{J}\mathcal{J}$, $11\mathcal{Q}\mathcal{Q}$, same data as holotype, in coll. KBE; $1\mathcal{J}$, $1\mathcal{Q}$, Kolymbari, 35.539080°N 23.789793°E, 3 m.a.s.l., 2–9.IX.2017, K. Berggren leg. & coll.; $1\mathcal{J}$, $8\mathcal{Q}\mathcal{Q}$, Kolymbari, 35.536688°N 23.775811°E, 29 m.a.s.l., 11.IX.2019, K. Berggren leg., genitalia slide (\bigcirc) 13444 and 13445 (O), coll. KBE; 1O, $2\bigcirc\bigcirc$, Chania Province: Agia, 35.47482°N 23.93231°E, 44 m.a.s.l., 6.V.2022, K. Berggren & L. Aarvik leg., 1^{\uparrow} and 1° coll. KBE, 1° coll. NHMO; 1° , Skines, 35°25'44"N 23°55'22"E, 75 m.a.s.l., 5-11.VI.2004, C. Hviid, B. Skule & E. Vesterhede leg., coll. ZMUC; 233, 299, Kato Stalos, 19-27.IV.1995, J.P. Baungaard leg., genitalia slide OK 5406, coll. ZMUC; GREECE: Corfu, 12 \bigcirc , 19 \bigcirc , Agios Georgios, 19.IX.2001, H. Hendriksen leg., genitalia slide OK 5415, coll. ZMUC; GREECE: Kos, 19, 16.IX.1992, R. Johansson leg., genitalia slide OK 5415, coll. ZMUC; GREECE (mainland): 1^Q, C Makedonia, Stavros, 11.VII.1987, E. Baraniak leg., genitalia slide OK 5407, coll. ZMUC; 13° , 19° , prov. Aetolia-Akamania, Amvrakikos Bay, Katafourka, 2 m.a.s.l., 6.IX.2008, P. Skou leg., genitalia in vial, coll. ZMUC; BULGARIA: 2♂♂, 1♀, Dobrich region, Kaverna Beach, 4-8.VIII.2012, B.S. Larsen leg., genitalia slide OK 5408, coll. ZMUC; CROATIA: $2 \stackrel{\circ}{\downarrow} \stackrel{\circ}{\downarrow}$, Zadar County: Vir, 44.282802°N 15.127520°E, 18.VI.2019, L. Aarvik & K. Berggren leg., genitalia slide NHMO 4232, coll. NHMO; MALTA: 1♀, Mellieha, 4.VII.2002, H. Hendriksen leg., genitalia slide OK 5412, coll. ZMUC; ROMANIA: 1♀, Donau Delta, Caraoman, 14-17.V.1992, L. Rakosy leg., genitalia slide OK 5411, coll. ZMUC.

Diagnosis. Brachmia cretica sp. n. (Figure 1) is very similar to B. blandella (Fabricius, 1798), and the two species cannot be separated with certainty externally. However, B. blandella is on average larger (wingspan 9.0-13.5 mm) and has darker labial palps and darker, less contrasting forewings. In the male genitalia the two species differ as follows; the phallic process is thicker and longer in *B. cretica* sp. n. and without apical hook; the uncus is slenderer in B. blandella, the gnathos is distinctly longer, the valva slightly longer, and the distal hooked-shaped process of the juxta is slenderer in B. cretica sp. n. In the female genitalia B. cretica sp. n. differs from the other European Brachmia species by the presence of a conspicuous longitudinally ribbed, V-shaped sclerite in the antrum.

Description. Male. Wingspan 7.5–10.0 mm. *Head and thorax* pale ochreous yellow, thorax



FIGURE 1. Adult of Brachmia cretica sp. n. Photo: K. Sund.



FIGURE 2. Male genitalia of Brachmia cretica sp. n. Genitalia slide KBE 13446. Photo: P.K. Slagsvold.



FIGURE 3. Female genitalia of *Brachmia cretica* sp. n. Genitalia slide KBE 13444. Photo: P.K. Slagsvold.

with slight brown mottling; palp four times diameter of eye, third segment shorter than second, concolorous with head, second segment with few dark scales externally, third segment with dark ring before tip; scape of antenna blackish on upper side, pale ochreous yellow on underside, flagellum of antenna ringed blackish and pale yellowish, lighter at underside. Antenna filiform. Legs pale ochreous yellow, with some dark scales which on tarsi form dark spots. Forewing slightly widening towards termen, costa slightly concave in apical half, apex produced, termen concave; ochreous yellow, suffused with brown and blackish brown particularly along costa to 2/3, at dorsum at 1/4, and in terminal area, slight grey suffusion on costa, discal spots 1 & 2 and plical spot 2 distinct, black; a yellow costal spot before apex; cilia cream, grey at apex and tornus, with dark cilia line. Hindwing light grey, cilia pale grey, becoming whitish towards alar angle, darker grey at apex, with indistinct cilia line. Abdomen grey on upper side, underside and rear tuft cream. Female. On the average slightly larger than male, wingspan 7.5-11.5 mm

Variation. The examined specimens show slight variation in the forewing colour, and especially in the distinctness of the blackish brown area before the margin of the forewing.

Male genitalia. (Figure 2) Uncus relatively broad, apically triangular, tip pointed; gnathos broad, parallel-sided, with small apical process; valvae slightly curved, of nearly even width; vinculum broad, rounded; juxta lobes strong, hooked; phallus with broad, rod-like process, vesica with numerous denticles.

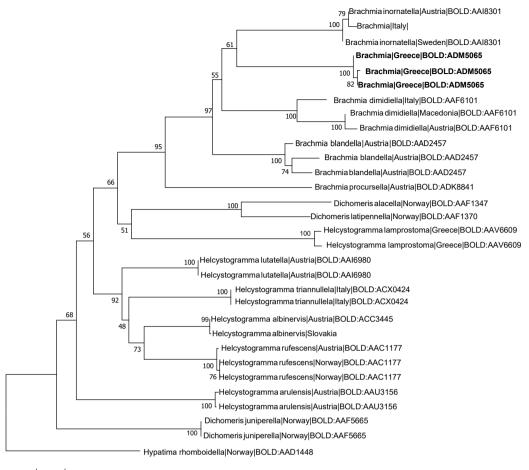
Female genitalia. (Figure 3) Ovipositor broad; segment 8 short; apophyses anteriores circa 3.2 times the length of apophyses posteriores; antrum heart-shaped, with conspicuous longitudinally ribbed, V-shaped sclerite; ductus bursae very short; corpus bursae oval, with numerous denticles posteriorly and medially; tube of accessory bursae inserted in middle of corpus bursae.

Molecular diagnosis. Sequences of *B. cretica* sp. n. forms a well-defined cluster which received the Barcode Index Number (BIN-code): ADM5065. The DNA barcode region of *B. cretica* sp. n. shows no intraspecific divergence and a minimum divergence of 7.28 % to its closest relative *B. inornatella* (Douglas 1850) (Figure 4). The distance to its morphologically similar congener, *B. blandella* is slightly larger, 7.43 %.

Etymology. Named after the island Crete where most of the type material was collected.

Distribution. Known from Greece, the mainland as well as the islands Crete, Corfu and Kos; Bulgaria; Croatia; Malta and Romania (Figure 5). A very small specimen (wingspan 7.5 mm) without abdomen from South Italy: Calabria resembles most *B. cretica* sp. n., but is excluded from the type series because its genitalia could not be examined. Karsholt & Huemer (2017) recorded *Brachmia blandella* new to Crete. We consider that all these specimens belong to *B. cretica* sp. n., and that *B. blandella* should be deleted from the list of Cretan Gelechiidae.

Biology. In Crete, all specimens were collected among or close to giant reed (*Arundo donax* L.) (Poaceae). At one of the Kolymbary (Figure 6) sites, the lamp was attached directly on the reeds. Our hypothesis is that *Arundo donax* is the foodplant. The larva of *Brachmia inornatella* feeds



0,0100

FIGURE 4. Neighbour joining dendrogram with *Brachmia cretica* sp. n. (in bold letters) and relatives. The number depicted for each sample (BOLD:XXXXXX) gives the specimen's BIN assignment (Ratnasingham & Hebert 2013). The numbers placed at each node in the tree report bootstrap support for the group (1000 replications). *Hypatima rhomboidella* (Linnaeus, 1758) is an outgroup.

on the related *Phragmites australis* (Cav.) Steud., whereas the larva of *B. blandella* feeds on several non-Poaceae plants (Gregersen & Karsholt 2022: 171). The species has been collected from April to September and probably has at least two generations. Specimens from the autumn are on average smaller.

Discussion

The main diagnostic character separating *Brachmia cretica* sp. n. from *B. blandella* is the

paired longitudinally ribbed, V-shaped sclerite in the antrum of the female. According to Hodges (1986: 124) «females of *Helcystogramma* have paired, often pointed sclerites in the dorsal wall of the antrum. These are lacking in *Brachmia*». We are not aware of other *Brachmia* species with females having such paired sclerites, but we are convinced that *B. cretica* sp. n. belongs to *Brachmia*. Another character separating *Brachmia* and *Helecystogramma* is, according to Hodges (*op cit.*), the absence of a juxta in males of *Helcystogramma*. However, Gregersen & Karsholt (2022: 174) pointed out that the lateral lobes of the



FIGURE 5. Map showing the known distribution of Brachmia cretica sp. n. in Europe.



FIGURE 6. Kolymbari, Crete. Locality for *Brachmia cretica* sp. n. with giant reed (*Arundo donax*). Photo: K. Berggren.

vinculum in *Helcystogramma* should be regarded as juxta. Ponomarenko (1997: 313; 2009: 165– 166) excluded *Brachmia* from Dichomeridinae and moved it to an independent tribe, Brachmiini, in Anacampsinae. This was based primarily upon the muscle protractors of the phallus, m5, which form a single bunch in *Brachmia* (divided in two bunches in *Helcystogramma* and other Dichomeridinae). This was not followed by most subsequent authors (e.g., Karsholt *et al.* 2013: 343) who, based on molecular studies, found *Brachmia* nested deeply within the Dichomeridinae, next to *Helcystogramma*.

Brachmia blandella was described as Tinea blandella from an unstated number of specimens from Germany, Kiel. A lectotype was designated by Karsholt & Nielsen (1976: 247), and its synonym Gelechia gerronella was described from a series of specimens collected in Poland, Zielona Góra, Głogów («Glogau»), and a few specimens from Hungary and Italy (Zeller 1850: 156). It is distributed in most of Europe, apart from the northern parts of Great Britain and Fennoscandia. Eastwards it is reported from South-West Siberia (Ponomarenko 2019: 109), and towards the south it is recorded from Iran (Wieser et al. 2002: 66). The authors have examined a number of specimens identified as B. blandella from various South European countries. We can confirm the presence of the species in Armenia, France (including Corsica), Croatia, Georgia, Greece (including the islands of Corfu and Lefkada), southern Italy and Spain.

Elsner *et al.* (1999) illustrated the male genitalia of a *B. blandella* specimen from Greece: Litochoro. The illustration has much in common with the genitalia of *B. cretica* sp. n., *viz.* the shape of the uncus and the process of the phallus. This indicates that it is a misidentification of the latter.

The discovery of *B. cretica* sp. n. is yet another example among European Lepidoptera of the usefulness of DNA barcoding to discover cryptic species. The suspicion that *B. blandella* was a «double» species arose after the DNA barcoding of specimens from Crete. Subsequent examination of the genitalia showed that this suspicion was indeed correct. Much taxonomic work is needed to clarify already known cases of suspected cryptic diversity (e.g., Huemer *et al.* 2020, Lopez-Vaamonde *et al.* 2021), and continued DNA barcoding is likely to reveal additional cases.

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References

- Elsner, G., Huemer, P. & Tokár, Z. 1999. Die Palpenmotten (Lepidoptera, Gelechiidae) Mitteleuropas. Bestimmung – Verbreitung – Flugstandort – Lebensweise der Raupen. František Slamka, Bratislava. 208 pp.
- Gregersen, K. & Karsholt, O. 2022. *The Gelechiidae* of North-West Europe. Norwegian Entomological Society, Oslo. 939 pp.
- Hodges, R.W. 1986. *Gelechioidea: Gelechiidae (in part): Dichomeridinae. In:* R. B. Dominick et al.: The Moths of America North of Mexico 7 (1), 1–195, vii–xiii.
- Huemer, P. & Karsholt, O. 2020. Commented checklist of European Gelechiidae (Lepidoptera). *ZooKeys* 921, 65–140.
- Huemer, P., Karsholt, O., Aarvik, L., Berggren, K., Bidzilya, O., Junnilainen, J., Landry, J.-F., Mutanen, M., Nupponen, K., Segerer, A., Šumpich, J., Wieser, C., Wiesmair, B. & Hebert, P.D.N. 2020. DNA barcode library for European Gelechiidae (Lepidoptera) suggests greatly underestimated species diversity. *ZooKeys* 921, 141–157.
- Karsholt, O. & Huemer, P. 2017. Review of Gelechiidae (Lepidoptera) from Crete. *Linzer biologische Beiträge* 49, 159–190.
- Karsholt, O. & Nielsen, E.S. 1976. Notes on some Lepidoptera described by Linnaeus, Fabricius and Ström. *Entomologica scandinavica* 7, 241–251.
- Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences.

Journal of Molecular Evolution 16, 111–120. https://doi.org/10.1007/BF01731581

- Kumar, S., Stecher, G. & Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology* and Evolution 33(7), 1870–1874. <u>https://doi.org/10.1093/molbev/</u>msw054
- Lopez-Vaamonde, C., Kirichenko, N., Cama, A., Doorenweerd, C., Godfray, H.C.J., Guiguet, A., Gomboc, S., Huemer, P., Landry, J.-.F., Laštůvka, A., Laštůvka, Z., Min Lee, K., Lees, D.C., Mutanen, M., Nieukerken, E.J. von, Segerer, A.H., Triberti, P., Wieser, C. & Rougerie, R. 2021. Evaluating DNA barcoding for species identification and discovery in European gracillariid moths. *Frontiers in Ecology* and Evolution 9, 1–16.
- Meyrick, E. 1925. Lepidoptera Heterocera. Fam. Gelechiadae. *Genera Insectorum* 184, 1–290, pls 1–5.
- Ponomarenko, M.G. 1997. Phylogeny and taxonomy of the subfamily Dichomeridinae (Lepidoptera: Gelechiidae). *Zoosystematica rossica* 6, 305–314.
- Ponomarenko, M.G. 2009. The Gelechiid moths of the subfamily Dichomeridinae (Lepidoptera: Gelechiidae) of the world. 389 pp., 4 pls. Vladivostok [in Russian with English summary]
- Ponomarenko, M.G. 2019. Gelechiidae. Pp. 91– 113 & 375–376. In: S.Yu. Sinev (ed.): Katalog Cheshuekrylykh (Lepidoptera) Rossii. Catalogue of the Lepidoptera of Russia. Edition 2. 448 pp. St. Petersburg [in Russian].
- Ratnasingham, S. & Hebert, P.D.N. 2007. BOLD: The Barcode of Life Data System (www.barcodinglife. org). *Molecular Ecology Notes* 7, 355–364. <u>https://doi.org/10.1111/j.1471-8286.2007.01678.x</u>
- Robinson, G.S. 1976. The preparation of slides of Lepidoptera genitalia with special reference to the Microlepidoptera. *Entomologist's Gazette* 27, 127–132.
- Wieser, C., Huemer, P. & Stangelmaier, G. 2002. Schmetterlinge (Lepidoptera). Pp. 52–81. *In*: Gutleb,
 B. & Wieser, C.: Ergebnisse einer zoologischen Exkursion in den Nordiran, 2001. *Carinthia* 11, 192/112, 33–140.
- Zeller, P.C. 1849-50. Verzeichniss der von Herrn Jos. Mann beobachteten Toscanischen Microlepidoptera. *Stettiner entomologische Zeitung* 10 (1849): 200 –223, 231–256, 275–287, 312–317; 11 (1850): 59– 64, 134–136, 139–162, 195–212.

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