DNA barcoding reveals a new cryptic species of *Denisia* Hübner, 1825 (Lepidoptera, Oecophoridae) from the south-western Alps

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Abstract

A new species of concealer moths (Oecophoridae), *Denisia cryptica* sp. nov., is described from specimens collected in the south-western Alps (Italy, France). It differs from similar congeneric taxa of the *Denisia stipella* species-group by a combined pattern of colour and markings of the forewing upperside, the colour of the head, particularly the yellow second segment of the labial palpi, and by highly divergent DNA barcode (cytochrome c-oxidase subunit 1) sequences. Male and female genitalia are like several other species, with only subtle diagnostic characters. Adult and partially genitalia of *D. cryptica* sp. nov. and the closest European species are shown for comparison. Finally, a complete checklist of European species of *Denisia* is added.

Key Words

Cottian Alps, cryptic morphology, endemism, France, Italy, Oecophorinae

Introduction

*Denisia* is one of the species-rich genera in Europe within the family Oecophoridae, which is represented here by 139 species (Lepiforum 2024). 23 species of *Denisia* have been recorded in Europe (Lepiforum 2024). Remarkably, four of these species were only discovered in the 21st century (Huemer 2001; Lvovsky 2007; Corley 2014; Nel and Varenne 2019), although one of them, *Denisia lutea* Varenne & Nel, 2019, has been incorrectly synonymized with the North African *Denisia curlettii* Lvovsky & Koster, 1996 (Leraut 2023; Nel and Varenne 2024). No additional congeneric species are currently known from Asia. However, recently studied molecular data suggest a significantly underestimated species diversity, especially among species with allopatric distribution patterns (Huemer and Mutanen 2015; Zlatkov and Huemer 2017; Huemer 2022; Huemer and Wieser 2023).

Only a few species like *Denisia stipella* (Linnaeus, 1758) and *Denisia similella* (Hübner, 1796) are widely distributed across the continent, especially in the temperate zones, while the vast majority have more localized ranges. Particularly, the various major mountain systems in Europe such as the Pyrenees, Alps, or Caucasus, as well as large Mediterranean islands, are partly inhabited by regional endemism. However, recently studied molecular data suggest a significantly underestimated species diversity, especially among species with allopatric distribution patterns (Huemer and Mutanen 2015; Zlatkov and Huemer 2017; Huemer 2022; Huemer and Wieser 2023).

The preimaginal life habits of *Denisia* species are insufficiently explored and often completely unknown. Tokár et al. (2005) mainly cite the bark of decaying or dead wood, rarely also lichens growing on trees, as larval substrates for the Central European fauna. However, there appear to be two trophic adaptation strategies: a large group of species, as far documented, lives on dead wood, while the other, as primarily demonstrated by Schmid (2019) for *D. rhaetica* (Frey, 1856), feeds on lichens growing on rocks. Typical megabiomes of *Denisia*
species thus encompass both forest habitats and rocky environments, extending into the alpine zone.

In the course of a comprehensive survey of Lepidoptera in the nature parks of the Cottian Alps (northwestern Italy) (Huemer and Wieser 2023), a new and previously unknown species of the genus *Denisia* has now been discovered, thanks to rigorous molecular identification controls using DNA barcoding.

In addition, a comprehensive checklist of European *Denisia* is provided in this paper.

Materials and methods

A total of 224 specimens of phenotypically similar *Denisia* have been examined morphologically: 4 *D. cryptica* sp. nov., 22 *D. nubilosella*, 133 *D. stipella*, 37 *D. similella*, 23 *D. fuscicapitella* (all Central Europe), and 1 *D. subaquilea* (Spain), 2 *D. obscurella* (Finland), 2 *D. luticiliella* (Russia). External and internal morphology of North American *D. haydenella* is based on Hodges (1974) and BOLD, with supplementing comments and unpublished figures from Landry (in litt.). Further externally very distinct species were only considered for genetic analysis. The material is preserved in the Research Collection of Tiroler Landesmuseum Ferdinandeum (Austria). Species identification is based on the phenotypic characteristics of the adults and partly confirmed by dissections and DNA barcoding.

Tissue samples (a single hind leg) from three *D. cryptica* sp. nov. were prepared according to prescribed standards to obtain DNA barcode sequences of an ideally 658 bp segment of the mitochondrial COI gene (cytochrome c oxidase subunit 1). The material was successfully processed at the Canadian Centre for DNA Barcoding (CCDB, Biodiversity Institute of Ontario, University of Guelph) using the standard high-throughput protocol described in deWaard et al. (2008). In addition, 155 public and private barcode sequences of 19 species of *Denisia* in the Barcode of Life Data Systems (BOLD; Ratnasingham and Hebert 2007; Ratnasingham 2018) were used for analysis. All barcode sequences, except for two shorter sequences of *D. pyrenaica*, range between 560 and 658 bp.

Further details including complete voucher data and images can be accessed in the public dataset DS-DENISIA1 “Denisia sp. n. - Alps” dx.doi.org/10.5883/DS-DENISIA1 in the Barcode of Life Data Systems BOLD (Ratnasingham and Hebert 2007).

All sequences were assigned to Barcode Index Numbers (BIN), algorithm-based operational taxonomic units that provide an accurate proxy for the true species. BINs were automatically calculated for records in BOLD that comply with the DNA Barcode standard (Ratnasingham and Hebert 2013).

Degrees of intra- and interspecific variation of DNA barcode fragments were calculated using the Kimura two-parameter model on the platform of BOLD systems v. 4.0. (https://boldsystems.org). A Neighbor-Joining tree was constructed using the Kimura two-parameter model in MEGA7 (Kumar et al. 2016).

Photographs of adults were taken with an Olympus OM-D Mark III camera and a 60 mm macro lens, genitalia photographs with a Zeiss Axiolab 5 microscope, mounted with an Olympus OM-D Mark III camera. 60 to 90 stacked photographs were edited using Helicon Focus 4.8 and Adobe Photoshop 6.0.

A linguistic review was conducted on ChatGPT.

Abbreviations. **TLMF** = Tiroler Landesmuseum Ferdinandeum, Innsbruck, Austria.

Results

Molecular analysis

Molecular analysis is based on 158 DNA barcodes sequences for 19 *Denisia* species including selected public sequences from BOLD. The interspecific distances to the nearest neighbor vary from 4.3% to 9.3% per species pair, except for a species pair with alleged barcode sharing. Intraspecific barcode variation is generally much lower, with a maximum of 1.5% in most species but exceeds 4% in three species (see discussion) (Table 1, Fig. 1). The sequences grouped into 23 distinct and strongly divergent clusters, each with different BINs (except for *Denisia pyrenaica* and *Denisia graminella* which currently have no BINs attached).

![Figure 1. Neighbor-Joining tree of Denisia spp. (Kimura 2-parameter, built with MEGA7, Kumar et al. 2016)](https://boldsystems.org)

Figure 1. Neighbor-Joining tree of *Denisia* spp. (Kimura 2-parameter, built with MEGA7, Kumar et al. 2016; Note: the scale bar only applies to internal branches between species. Width of triangles represent sample size, depth the genetic variation within the cluster. Source: DNA Barcode data from BOLD, DS-DENISIA1 (Barcode of Life Database; Ratnasingham 2018).
Table 1. Intraspecific mean K2P (Kimura 2 Parameter) divergences, maximum pairwise distances, Barcode Index Number (BIN), nearest species, distance to nearest neighbour (NN) (distances in %) of Denisia spp. Source: DNA Barcode data from BOLD, DS-DENISIA1 (Barcode of Life Database, cf. Ratnasingham 2018).

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean Intra-Sp</th>
<th>Max Intra-Sp</th>
<th>BIN</th>
<th>Nearest Species</th>
<th>Dist. NN</th>
</tr>
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<tbody>
<tr>
<td>Denisia albinaculea</td>
<td>2.23</td>
<td>5.51</td>
<td>BOLD:ACW8519; BOLD:ACE3228</td>
<td>Denisia augustella</td>
<td>7.13</td>
</tr>
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<td>0.36</td>
<td>BOLD:ACW5127</td>
<td>Denisia stipella</td>
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</tr>
<tr>
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<td>0</td>
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<td>8.34</td>
</tr>
<tr>
<td>Denisia cryptica</td>
<td>0</td>
<td>0</td>
<td>BOLD:AEF7084</td>
<td>Denisia haydenella</td>
<td>4.9</td>
</tr>
<tr>
<td>Denisia fuscicapitella</td>
<td>0.17</td>
<td>1.11</td>
<td>BOLD:AAU3742</td>
<td>Denisia obscurella</td>
<td>4.33</td>
</tr>
<tr>
<td>Denisia grassiinella</td>
<td>N/A</td>
<td>0</td>
<td>-</td>
<td>Denisia pyrenica</td>
<td>0.93</td>
</tr>
<tr>
<td>Denisia haydenella</td>
<td>0.63</td>
<td>2.5</td>
<td>BOLD:AAD4867</td>
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<tr>
<td>Denisia luctuosella</td>
<td>1.08</td>
<td>1.08</td>
<td>BOLD:AF9939</td>
<td>Denisia muellerrutzi</td>
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</tr>
<tr>
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<tr>
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<td>0</td>
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<tr>
<td>Denisia muellerrutzi</td>
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<td>4.58</td>
<td>BOLD:ACE1985; BOLD:ACE1986</td>
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<td>Denisia rhaetica</td>
<td>4.22</td>
<td>6.29</td>
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<td></td>
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<td>BOLD:AAU0847</td>
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<td>BOLD:AA1494</td>
<td>Denisia fuscicapitella</td>
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</tr>
<tr>
<td>Denisia subaqulae</td>
<td>N/A</td>
<td>0</td>
<td>BOLD:ACA9737</td>
<td>Denisia luctuosella</td>
<td>8.58</td>
</tr>
</tbody>
</table>

The three available D. cryptica sp. nov. sequences were assigned to one unique cluster. The mean intraspecific p-distance of D. cryptica sp. nov. is 0% (BIN:BOLD:AEF7084, n=3). The distance to the nearest neighbor D. haydenella from North America (BIN:BOLD:AAD4867) (n=92) is 4.9%.

Taxonomy

**Denisia cryptica** sp. nov.

https://zoobank.org/9D72F563-4785-4CF8-8CF0-B9C718962909

Figs 2, 3, 8, 9, 14, 16, 18

Material examined. **Holotype.** Italy • 1♀; Prov. Torino, Fenestrelle, Umg. Praetatinat, Forte delle Valli [type locality part of Orsiera-Rocciavrè Nature Park]; 1700–1720 m; 45°21’7"N, 7°4’14"E; 2 June 2022; leg. P. Huemer; DNA Barcode ID TLMF Lep 32850; TLMF.

**Paratypes.** Italy • 1♂; same collection data as holotype • 1♂, 1♀; Prov. Torino, PN Orsiera - Rocciavrè, Villaretto, Gran Faetto, Colletto; 1445 m; 45°00’28"N, 07°08’28"E; 19 June 2020; leg. P. Huemer; gen. slides GEL. 1305♀, 1306♀. P. Huemer; DNA Barcode IDs TLMF Lep 29494, TLMF Lep 30299; TLMF. France • 1♂, Hautes-Alpes, Brunissard, Arvieux; 1900 m; 5 July 1995; leg. J. Nel; gen. slide 3197♂ J. Nel; TLMF • 1♂, Alpes-de-Haute-Provence, La Fayèe de Lure; 1550 m; 4 July 1998; leg. J. Nel; gen. slide 7225♂ J. Nel; TLMF.

**Diagnosis.** Denisia cryptica sp. nov. differs from species with similar forewing patterns and coloration by the yellow-scaled head and the second segment of the labial palpus, which is at least partially yellow on the inner side. The overall similar species D. nubilosella (Figs 4, 5) and D. haydenella have a dark brownish-grey coloration of the head, which is sometimes mottled cream-white, particularly in females. However, the labial palps are grey-brown on the inner side with only few whitish scales on the second segment, lacking the extended yellow of the new species. Furthermore, the scapus is predominantly grey-brown and not cream-yellow in both relatives (Figs 10, 11). Additionally, the intense yellow speckling on the upper surface of the forewings of D. cryptica sp. nov. is absent in the mentioned species which at most exhibit some cream motting, particularly in females. A dark head and labial palps are also typical for D. fuscicapitella, D. obscurella and D. caucasiella which in addition differ by pattern of the forewing upperside. Denisia stipella and D. simililella (Figs 12, 13) also have a yellow head and extensive yellow coloration of the second segment of the labial palpus but both species differ immediately from D. cryptica sp. nov. by the striking yellow pattern of the upper surface of the forewings (Figs 6, 7). Significant differences in forewing patterns also apply to all other species of the genus. The male genitalia of D. cryptica sp. nov. are extremely like several species of Denisia, with at most subtle differences in the length and shape of the uncus and the valva (Figs 14, 15). Female genitalia are also very similar to other species, with potentially diagnostic features such as the shape of the signum (based on a single specimen) (Figs 16–19). However, except for one pair of species, all sequenced species (19 out of 24) exhibit significant divergences in DNA barcodes (Fig. 1).
Description. Adult (Figs 2, 3, 8, 9). Head brush cream-yellow, particularly scales above compound eye, medially and on frons brownish-yellow; scapus cream-yellow, with pecten, flagellum grey-brown with few whitish scales, pectinate in male, filiform in female; labial palpus recurved, second segment cream-yellow on inner side, outer surface and third segment grey-brown mottled with some cream-yellow. Thorax and tegulae grey-brown, mottled with cream-yellow, particularly in posterior part. Forewing length 7.4–7.6 mm (male), 6.8 mm (female); forewing ground color grey-brown with extended cream-yellow mottling (cream-withe in the only available female) except for costal base, medial dot-like area and apex; fringes dark grey-brown, lighter in tornal area with small cream-yellow spot at tornus; hindwing light grey-brown with concolorous fringes.
underside of wings unicolorous grey-brown. Legs grey-brown, mixed with cream at lower surface. Abdomen grey-brown.

**Male genitalia** (Fig. 14). Uncus broad and short, sub-triangular, apically pointed; gnathos massive, sub-triangular, distinctly longer than uncus, ventral part with pointed apex, dorsal part with dentate cushion like structure; valva broadly sub-oval, distal part rounded, sacculus broad but weakly demarcated, inner side largely covered with long setae; saccus broadly rounded; juxta lobe long and basally broad with recurved digitate distal part and upwards curved pointed apex, phallus short and slender, basally slightly inflated, without cornuti.

Female genitalia (Figs 16, 18). Posterior apophyses approximately two times length of segment VIII; anterior apophyses approximately reaching the length of segment VIII. Segment VIII without special processes or indentations; lateroventral area conspicuously sclerotized with some strong setae in posterior part, posterior edge rounded, anterior edge with advanced acute sclerotization that border the indistinctly separated membranous antrum laterally; ventromedial area continuously covered with numerous tiny microtrichia. Signum very small, irregularly bounded plate with several thorns on outer edge.

Biology. Adults have been collected in June at light (Fig. 20). Host-plant and early stages are unknown. However, like related species it possibly feeds under the bark of decaying or dead trees. From the habitat, Pinus sylvestris is the most likely candidate as host plant. Contrariwise, D. nubilosella seems to be restricted to Picea abies.

Distribution. Currently only known from two localities in the Cottian Alps (northern part) (Italy) and furthermore from two isolated spots in the French Alps but likely more widely distributed.

Etymology. The species name refers to the cryptic appearance of the new species and is derived from the latin adjective crypticus.

Remarks. Examined specimens from France were misidentified as D. nubilosella and the occurrence of that species in the south-western Alps requires confirmation.
Checklist of European *Denisia*

**Genus *Denisia* Hübner, 1825**

*Denisia* Hübner, 1825. Type species: *Phalaena* (*Tinea*) *stipella* Linnaeus, 1758

*Blepharocera* Chambers, 1877 nec Agassiz, 1847 (homonym)

*Chambersia* Riley, 1891

*Denisia stroemella* (Fabricius, 1779) (*Tinea*)
*Denisia obscurella* (Brandt, 1937) (*Borkhausenia*)
*Denisia rhaetica* (Frey, 1856) (*Oecophora*)
*Lampros engadinella* Herrich-Schaffer, 1856

*Denisia pyrenaica* Leraut, 1989
*Denisia graslinella* (Staudinger, 1870) (*Oecophora*)
*Denisia muellerrutzi* (Amsel, 1939) (*Borkhausenia*)

*Denisia luctuosella* (Duponchel, 1840) (*Lita*)
*Lita funestella* Duponchel, 1838
*Borkhausenia luctuosella sardiniella* Amsel, 1936

*Denisia ragonotella* (Constant, 1885) (*Oecophora*)
*Borkhausenia reducta* Walsingham, 1901
*Borkhausenia ragonotella* f. *bifasciella* Amsel, 1939 (intrasubsp.)

*Denisia augustella* (Hübner, 1796) (*Tinea*)
[(*Tinea*) *moestella* Geyer, 1832]
*Oecophora augustella* var. *latoniella* Milliere, 1875
*Lita tilicolella* Bruand, 1859

*Denisia piresi* Corley, 2014
*Denisia albimaculea* (Haworth, 1828) (*Tinea*)
*Oecophora albilabris* Zeller, 1850

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**Figure 20.** Type-locality of *Denisia cryptica* sp. nov. with light equipment used for collecting.
Oecophora schmidi Saalmüller, 1881
Schiffermuelleria staudeilha Mitterberger, 1914
Schiffermuelleria augustella var. coriscella Caradja, 1920
Borkhausenia albimaculae sardiniella Amsel, 1936

Denisia lutea Varenne & Nel, 2019
Denisia similisella (Hübner, 1796) (Tinea)
Denisia fuscicapitella Huemer, 2001
Denisia luticiliella (Erschoff, 1877) (Oecophora)
Oecophora irroratella Staudinger, 1880

Denisia caucasiella Lvovsky, 2007
Denisia stipella (Linnaeus, 1758) (Phalaena (Tinea))
Phalaena (Tinea) accessorla [Denis & Schiffermüller], 1775
Tinea sulphurella Hübner, 1796
Tinea tigrella Hübner, 1813
Lampros westermannella Zetterstedt, 1839

Denisia cryptica sp. nov.
Denisia nubilosella (Herrich-Schäffer, 1854) (Lampros)
Denisia subaqualea (Stainton, 1849) (Oecophora)
brittanicella Herrich-Schäffer, 1854 [uninominal]

Denisia fiduciella (Rebel, 1935) (Borkhausenia)
Denisia aragonella (Chréitén, 1903) (Oecophora)
Denisia coeruleopicta (Christoph, 1888) (Oecophora)

Remarks. Generic descriptions were published by Tokár et al. (2005) and are not repeated herein. The systematic arrangement of species follows Lepiforum (2024) and is mainly based on superficial morphological resemblance of wing pattern and genitalia structures. Original generic assignment in brackets.

Discussion

The discovery of a potentially local endemic species in the European Alps may come as a surprise at first glance. However, the Southern Alps exhibit a particularly high degree of endemism (Huemer 1998). The area of the Cottian Alps has long been recognized as a hotspot for endemic Lepidoptera, and even in the 21st century, several new species have been described from there (Baldizzone and Gaedike 2004; Gianti 2005; Huemer and Hebert 2011; Nel 2012; Huemer and Mutanen 2015; Huemer et al. 2020). The systematic application of molecular methods in identifying extensive newly collected material, however, suggests an astonishingly large number of additional, previously overlooked cryptic species (Huemer and Wieser 2023).

As a fundamental issue in the delineation of Denisia species, the widespread cryptic morphology of many representatives of the genus has proven to be a challenge. Similarities in external appearance occur in numerous species pairs or groups. However, even the delineation using genital morphological characteristics often reaches its limits in this genus, meaning that the specifically evaluated genital morphology is frequently based on subtle features. Here, a characterization of species using molecular data, especially DNA barcode sequences, appears to lead to more indisputable results. All analyzed species of Denisia exhibit significant interspecific divergences on the order of approximately 4–9%. This extent of genetic divergence appears to be highly representative for the genus due to the large number of sequenced taxa. Out of the globally described 24 species, barcode sequences are available for 19 species. Only the species pair D. pyrenaica and D. graminellina shows, based on the few known sequences, an overlap in the DNA barcode, which may, however, possibly be due to a previously overlooked synonymy of these two taxa. Conversely, striking intraspecific divergences have also been documented in the genus Denisia, exceeding 5% in D. rhaetica and D. albimaculea. However, initial analyses suggest further overlooked cryptic diversity, although taxonomic revisions in these species groups are still pending.

The newly described D. cryptica sp. nov. fits into the overall picture of a genetically strongly divergent species, which, however, is difficult to differentiate from other congeners both phenotypically and in genitalia structures. Most helpful and effective are especially complexes of features from color and pattern of the forewing upper surface combined with head coloration and colour of the labial palps and antennae.

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