



Description of *Sphaleroptera orientana meridionalis* subs. n. (Lepidoptera: Tortricidae: Cnephasiini) from the Pale di San Martino Mountain plateau (Dolomites, NE Italy)

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Abstract

On the base of newly collected material, a new subspecies of the alpine endemic moth *Sphaleroptera orientana* Whitebread, 2006 (Lepidoptera: Tortricidae) from the Pale di San Martino Group (Dolomites, Northern Italy) is described. Morphological characters of the adults and DNA barcode suggests the presence of allopatric populations of *S. orientana* in the South-eastern Alps, attributable to two distinct subspecies: *S. o. suborientana* Whitebread, 2006 in the Catinaccio, the Sella, and the Fanes group, and Julian Alps, and *S. o. meridionalis* **subs. nov.** known from the Pale of San Martino group. The main biogeographic barrier is constituted by the complex of the Fiemme valleys—Val di Fassa—by the Pordoi Pass and by the Val Cordevole which from west to north to east separate the distribution area of *S. o. suborientana* from *S. o. meridionalis*.

Key words: alpine environment, alps, biodiversity, Dolomiti, moth

Introduction

Sphaleroptera Guenée 1845 (Tortricidae: Cnephasiini) is a moth genus inhabiting the high mountain environments of the Austrian, French, German, Italian, and Swiss Alps as well as the Pyrenees (Whitebread 2006). The *Sphaleroptera alpicolana*-species complex (*sensu* Whitebread, 2006) includes five species and two subspecies: *Sphaleroptera adamelloi* Whitebread, 2006, *Sphaleroptera alpicolana alpicolana* (Frölich, 1830), *Sphaleroptera alpicolana buseri* Whitebread, 2006, *Sphaleroptera dentana* Whitebread, 2006, *Sphaleroptera occidentana* Whitebread, 2006, *Sphaleroptera orientana orientana* Whitebread, 2006 and *Sphaleroptera orientana suborientana* Whitebread, 2006.

All *Sphaleroptera* are day-flying moths, despite their activity is usually restricted to early morning and sunshine (Whitebread 2006). Males possess developed wings and are able to fly while females are brachypterous, with very limited dispersal capabilities.

As for other alpine micromoths, very little is known about biology and host plants of *Sphaleroptera* species; furthermore, with the exception of *S. alpicolana* that may be locally common, most of the species remain known for a limited number of specimens, most of which are part of the type series (Whitebread 2006).

Sphaleroptera orientana is a species inhabiting high alpine environments between 1800 and 2300 m a.s.l. The nominotypical subspecies is distributed in central Austrian Alps and Alpi Carniche (Italy), while *Sphaleroptera orientana suborientana* presents a more oriental distribution, ranging from south Tyrol up to Julian Alps (Italy).

During faunistic research aimed at investigating micromoths biodiversity in the Parco di Paneveggio–Pale di San Martino Natural Park (Dolomites, Northern Italy) a new moth taxon was found. This new subspecies, as the southernmost population of *S. orientana*, is described here below as *Sphaleroptera orientana meridionalis* **n. ssp.**

Material and methods

The specimen cited in this paper is currently deposited in the private collection of Timossi Giovanni, Preganziol (Treviso), Italy.

Abbreviations

RCTG: Research Collection Giovanni Timossi (Preganziol, Italy).

Genitalia preparation

Genitalia dissection and microscopic slide preparation of the abdomen was performed using the methodology described in Robinson (1976), Timossi & Ruzzier (2014), and Scaccini *et al.* (2021).

Photographs

All-in-focus images were produced after stacks of several photos taken with Canon 760D camera. Photos of the habitus and the labial palps were taken with a Canon MP-E 65 mm f/2.8 1-5x macro lens provided by Yongnuo YN-14EX flash macro ring. The specimens were mounted on a macro focusing rail and lightly moved at every shot obtaining different focusing layers. A number of 15-45 shots were taken for each photo session. Stacking and editing processes were finally performed using Photoshop CC 2019 software. For imaging of the slide-mounted genitalia, a Nikon Eclipse E100 microscope was used, equipped with a Sony Colour CCD 5.1 Mp TP 5100 microcamera with X-Entry software.

DNA barcoding

The DNA was extracted from the holotype (dry legs) using the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. A region of mitochondrial DNA, corresponding to a fragment of the COI gene, was amplified using the universal primers LCO-1490/HCO-2198 (Folmer *et al.* 1994). DNA extraction, purification, and amplification followed the methodology described in Ruzzier *et al.* (2020). PCR products were purified using Exonuclease and Antarctic Phosphatase (GE Healthcare) and sequenced at the BMR Genomics Service (Padova, Italy). The sequences were edited using MEGA 11 (Tamura *et al.* 2021) and subsequently translated with Transeq (EMBOSS) to exclude the presence of stop codons in the coding region. An analysis of the sequences obtained was run through the integrated bioinformatics platform Barcode of Life Data (BOLD) System database to assess the identity of the species.

Phylogenetic reconstruction

The COX1 sequences of *S. orientana orientana* (GenBank codes: PHLAE433-11, PHLAE434-11, PHLAB269-10, LEATB893-13, PHLAG519-12, PHLAG520-12) and *S. orientana suborientana* (GenBank codes: PHLAB266-10, PHLAB267-10, PHLAE435-11, PHLAE436-11) were used in the phylogenetic reconstruction. *Sphaleroptera dentana* was included in the analysis as outgroup (GenBank codes: PHLAB270-10, PHLAE439-11). Evolutionary analysis was conducted in MEGA 11 and the phylogeny was inferred by using the Maximum Likelihood (ML) method and T92 = Tamura 3-parameter model, with 1000 bootstrap replications. The pairwise genetic distances among sequences were calculated using MEGA 11, under default settings.

Results

Sphaleroptera orientana meridionalis ssp. nov.

Type material. Holotype: adult ♂, Italy, Trento, PPPSM, Rifugio Pedrotti 46.2679 11.8409, 2581 m; 20.VIII.2019: legit Timossi G.; genitalia slide number 1730. Day fly.

Paratype: adult ♀, Italy, Trento, PPPSM, Rifugio Pedrotti, 46.2679 N, 11.8409 E, 2581 m; 20.VIII.2019: legit Timossi G.; genitalia slide number 1950. Attracted to UV lights.

Description. Adult ♂ (Fig. 1–2). Head: frons and palpi dark grey with few scales with light grey ends, black antennae with little white innellation; legs black with few light grey scales, tarsi with white rings, spurs light grey. Abdomen grey. Forewing: wingspan 16.8 mm, ground colour grey with few light grey, black and yellow scales. Forewing pattern (see Razowski 2002, page 9, fig. 6, 8): basal blotch and median fascia slate gray edged in black, subapical blotch and apical area confluent slate grey, fringe grey. Hindwing grey.

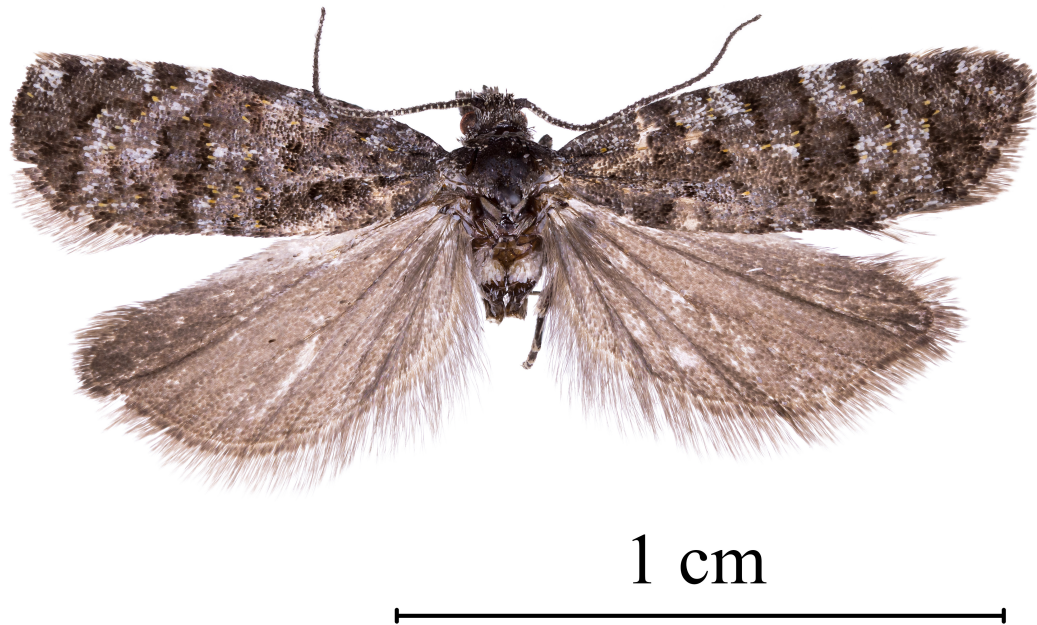


FIGURE 1. *Sphaleroptera orientana meridionalis* sp. nov. holotype, male, dorsal habitus.



FIGURE 2. *Sphaleroptera orientana meridionalis* sp. nov. holotype, labial palpus.

Male genitalia: elongated uncus (Fig. 3A), broad and elongated socius, slightly sclerotic gnathos, narrow tegumen, elongated pedunculus. Valva very broad basally, with a short terminal part (Fig. 3B); sacculus bilobed. Vinculum large; juxta pentagonal, with one incision at the posterior margin. Aedeagus sclerotized, gun-shaped (Fig. 3C), with protruding apical process bent at the distal end and passing through the stem: aedeagus is 116.604 μm : the length is taken following the indications of Whitebread (2006).

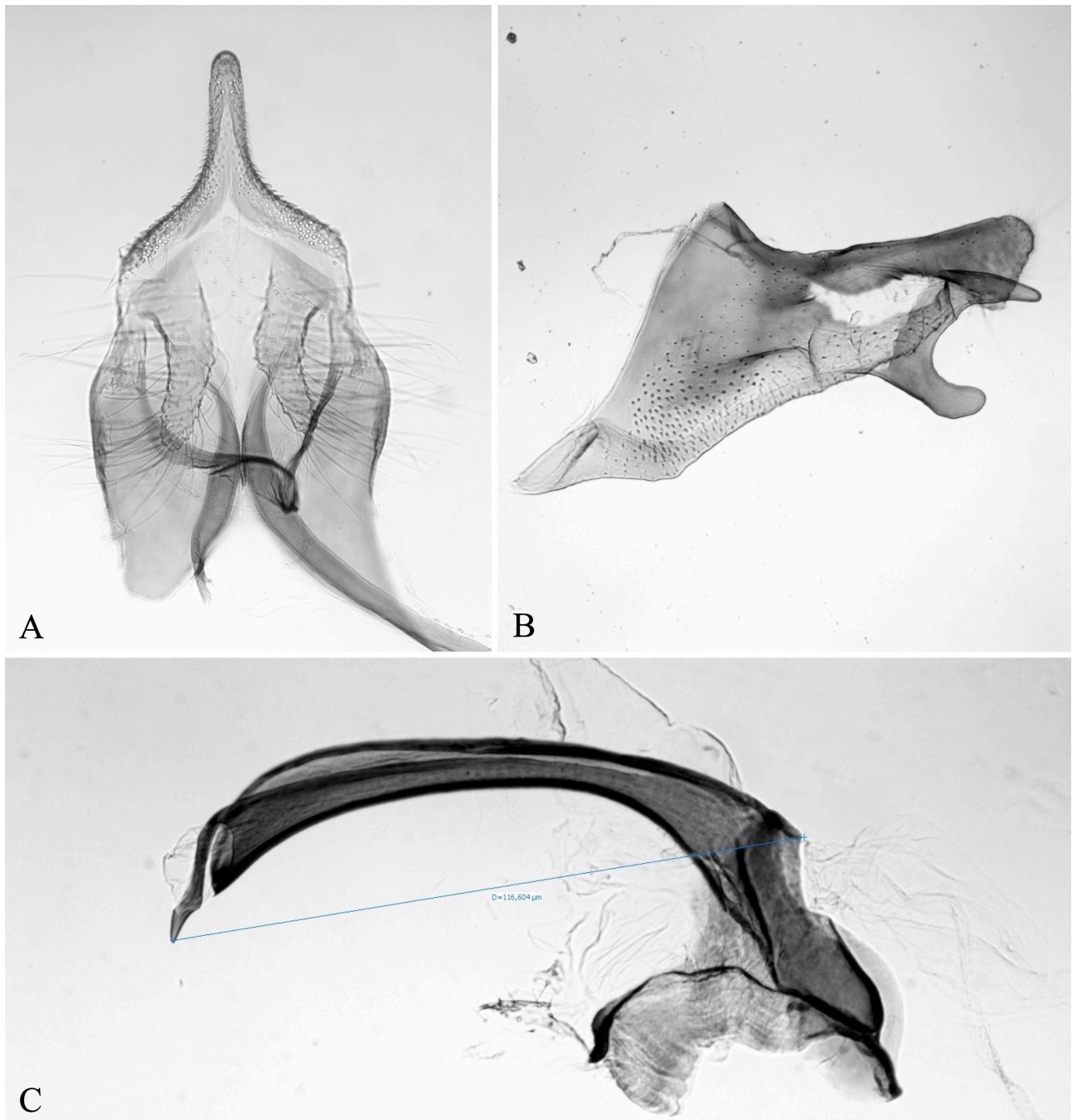


FIGURE 3. *Sphaleroptera orientana meridionalis* sp. nov. holotype, male, genitalia slide number 1730. A) uncus; B) valva; C) aedeagus in lateral view; length 116.6 μm (blue line).

Adult ♀ (Fig. 4–5). Head: frons light grey, second segment of the palps dark grey externally for half length, third segment slate grey, black antennae with white bands; prothoracic and mesothoracic legs with tarsus and tibia light grey, tarsi black with white rings, metathoracic legs light grey. Abdomen grey with light bands. Forewing: wingspan 14.4 mm, ground colour light grey, post basal fascia and median fascia dark grey, fringe dark and light grey. Hindwing white.

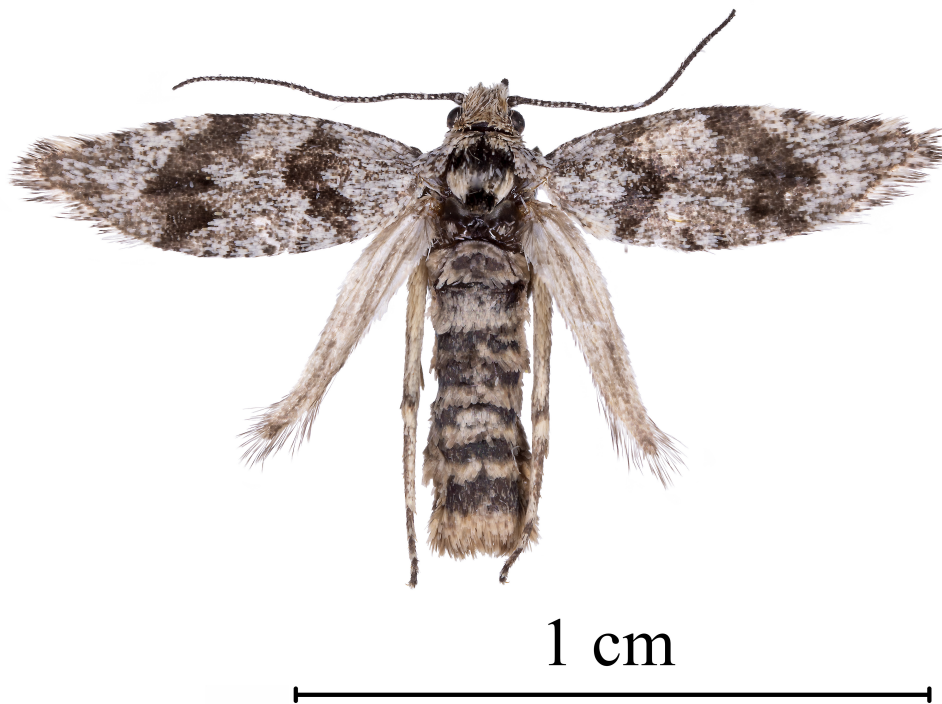


FIGURE 4. *Sphaleroptera orientana meridionalis* sp. nov. paratype, female, dorsal view.



FIGURE 5. *Sphaleroptera orientana meridionalis* sp. nov. paratype, labial palpus.

Female genitalia: (Fig. 6) ovipositor telescopic, short. Papillae anales flat, hairy; posterior apophyses about twice the anterior; Strongly sclerotic antrum, asymmetrical V-shaped, tubular, and slightly flared at the end, incised and with a cap-shaped protrusion; ductus burse short, ductus seminalis very close to the extremity of the antrum. Bursa about as long as the antrum without signum.



FIGURE 6. *Sphaleroptera orientana meridionalis* sp. nov. paratype, female genitalia (slide number 1952).

DNA barcoding and phylogenetic analysis. The DNA barcoding resulted in a sequence of 630 bp deposited in GenBank as *Sphaleroptera* sp. (ON721387).

The phylogenetic reconstruction, inclusive of *Sphaleroptera dentana* as outgroup, resulted in the consensus tree shown in Fig. 7. The topology of the whole tree is highly supported with *Sphaleroptera orientana meridionalis* forming a separate lineage in the respect of the *orientana*-*suborientana* cluster (bootstrap = 71%).

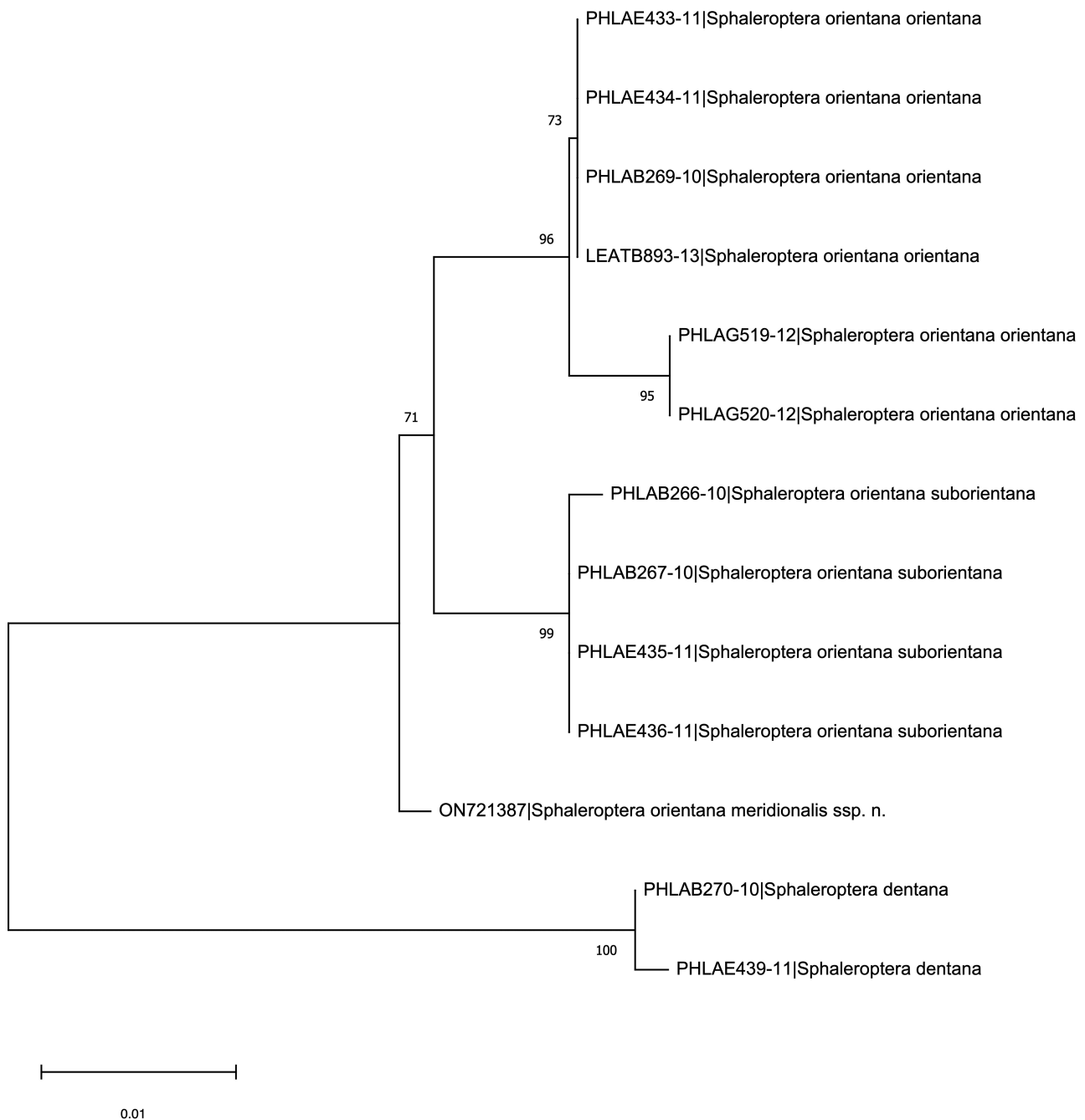


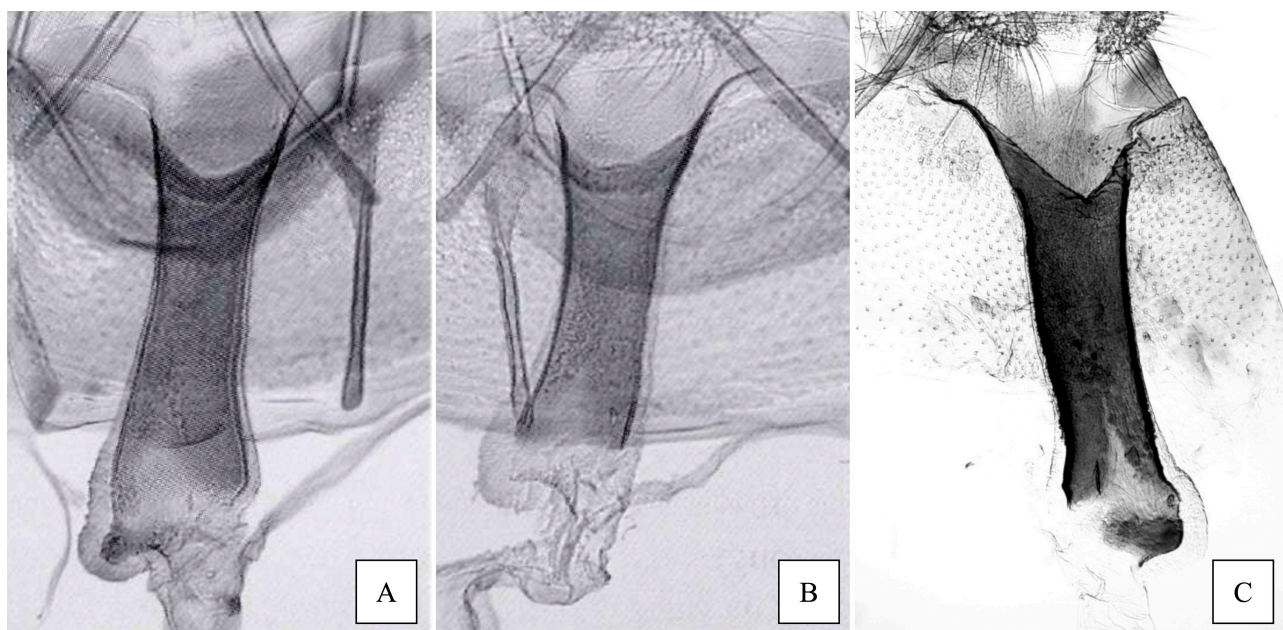
FIGURE 7. Maximum Likelihood tree of *Sphaleroptera orientana*.

Both *S. orientana orientana* and *S. orientana meridionalis*, both with specimens from two different localities, show very low intraspecific variability with a genetic distance of 0.2% and 0.1% respectively. *Sphaleroptera orientana meridionalis* is equally divergent from the previously mentioned taxa, with a genetic distance of ~1.1%. Genetic distances among the three subspecies are summarised in Table 1.

TABLE 1. Triangular matrix showing the genetic distances among *Sphaleroptera orientana* subspecies.

	<i>Sphaleroptera orientana orientana</i>	<i>Sphaleroptera orientana suborientana</i>	<i>Sphaleroptera orientana meridionalis</i>
<i>Sphaleroptera orientana orientana</i>	-		
<i>Sphaleroptera orientana suborientana</i>	0.0145	-	
<i>Sphaleroptera orientana meridionalis</i>	0.0109	0.0105	-

Diagnosis. The new ssp. resembles *S. orientana suborientana* both in external appearance as well as in the male genitalia. *Sphaleroptera orientana meridionalis* in comparison with *Sphaleroptera orientana suborientana* is distinguished by the darker grey background colour of forewing, which bear a few grey–white scales, the darker palps, head, femurs and tarsi. In the male of *S. orientana meridionalis* the uncus is more elongated and the phallus is longer than that of *S. o. orientana* and *S. o. suborientana* (see Whitebread 2006 for comparison). The female of *S. o. meridionalis* is similar in the general appearance to that of *S. o. orientana*, but it can be clearly separated by the latter on the base of genitalia morphology: in *Sphaleroptera orientana orientana* the antrum is rounded, U-shaped (Fig. 8A–B) while in *S. orientana meridionalis* is markedly asymmetrical V-shaped (Fig. 6, 8C). Moreover, the antrum of new subspecies is narrowed and incised at its proximal end, a unique feature when compared to the other *Sphaleroptera* species (see Whitebread 2006, figure 8, pag. 191). The female of *S. o. suborientana* remains unknown.

**FIGURE 8.** Comparison table of *Sphaleroptera orientana* female genitalia: A–B. *Sphaleroptera orientana orientana* from Austria (Whitebread, 2006; modified); C. *Sphaleroptera orientana meridionalis* **sp. nov.**

Etymology. The new subspecies has the southernmost distribution among those known, hence the name *meridionalis*.

Biology. Unknown. One generation observed. In the available literature (Razowski 2002, Whitebread 2006) *Sphaleroptera* are described as diurnal, but during the research the female of *S. o. meridionalis* was collected at night in the proximity of the light trap.

Habitat. Habitats are rock formations on a karst limestone plateau with sparse vegetation at elevations of about 2500 m cod. 8120: Calcareous and calcschist screes of the montane to alpine levels (*Thlaspietea rotundifolii*, cod. 8210: Calcareous rocky slopes with chasmophytic vegetation) (Fig. 9).

Conservation notes. The *locus typicus* of *Sphaleroptera orientana meridionalis* is in the proximity of the Pedrotti refuge and close to the arrival station of the cable car connecting the area with San Martino di Castrozza

(Trento Province, Trentino Alto Adige). Especially during summer, the whole area is subject to a significant influx of tourists, often associated with cultural and sporting events. Fortunately, the disturbance caused by tourists is still quite limited and localised above all thanks to the important extension of the plateau and the total lack of structures, with the exception of the refuge, which limit the usability of the environment. However, it cannot be excluded that an increase in the tourist load, combined with a reckless use of the area for recreational purposes, could threaten the survival of *S. orientana meridionalis* and other species present in the area, many of which are endemic. On the other hand, the global rise in temperatures may constitute a serious threat for this taxon which has a very low dispersal capacity due to the brachypterous females.



FIGURE 9. Pale di San Martino plateau, *locus typicus* of *Sphaleroptera orientana meridionalis* sp. nov.

Conclusion and comment

After Whitebread's contribution (2006), no further research has been published on the *Sphaleroptera alpicolana*-species complex. The few faunistic reports existing on *Sphaleroptera* testify to the rarity of the species and the general difficulty in finding fresh material to be studied. Since 2014, the Pale di San Martino has been the subject of an intense research aimed to investigate biodiversity of microlepidoptera in the area; consequently, the discovery of *Sphaleroptera orientana meridionalis* was totally unexpected.

The distribution of the subspecies of *S. orientana* reflects the orographic subdivision of the Alps called SOIUSA (Marazzi, 2005), at the level of subsections of the Dolomites section (SOIUSA Cod. II / C—31). *Sphaleroptera orientana orientana* is present in the Austrian Alps of southern Tyrol (SOIUSA cod. II / C—23, 17) while *S. o. suborientana* is present in the Dolomites section of the Sella, Catinaccio and Fanes groups (SOIUSA cod. II / C—31.III, 31.I); *Sphaleroptera o. meridionalis* is known exclusively from the Pale di San Martino (SOIUSA cod. II / C—31.IV, fig. 12).

The reconstruction of the landscape during the last glacial maximum, dated about 20,000 years, illustrates how

the surface of the Pale di San Martino plateau was occupied by a large glacier cap that flowed out with several glacial tongues (Giordano 2021). With the deglaciation, portions of soil that can be colonised by plant and animal organisms were freed from the ice. Due to their southern position close to the southern plain, the Pale di San Martino have a rainy regime with rainfall higher than the innermost Dolomites and this has allowed the presence of glaciers and frigidophilous fauna until today. Paleogeographic and paleoclimatic factors therefore determined the peculiar, and in most cases endemic fauna of Lepidoptera of the Pale di San Martino Plateau.

A similar pattern of populations isolation and allopatric speciation was already observed for multiple moth taxa, such as *Sattleria* Povolný, 1965 (Gelechiidae), *Sciadia* Hübner, 1822, and *Colostygia* Hübner, 1825 (Geometridae), *Kessleria* Nowicki, 1864 (Yponomeutidae) and the *Dichrorampha bugnionana* complex (Tortricidae) (Huemer 1993; Huemer & Karsholt 2010, Huemer & Hebert 2011, Huemer & Timossi 2014, Huemer & Hausmann 2009, Huemer & Mayr 2015, Huemer & Mutanen 2015, Timossi & Huemer 2022).

The presence of *Sattleria sophia* Timossi, 2014, *Sphaleroptera orientana meridionalis* sp. n., *Dhalica* sp. (in description) of the same locality, and other Dolomite endemics such as *Sciadia dolomitica* Huemer & Hausmann, 2009 and *Udea carniolica* Huemer & Tarmann, 1989, *Dichrorampha bugnionana dolomitana* Huemer, 1993 (Timossi 2018) indicate the high biodiversity value of Pale di San Martino plateau and its role as refugia during glaciations. However, one wonders if within the Alps, the Dolomites may represent an area in which the biodiversity of Lepidoptera is underestimated due to the lack of adequate studies.

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