Annotationes zoologicae et botanicae, 230: 1–9 (2022)
https://doi.org/10.54719/AMVZ5625

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First record of *Mordellistena semiferruginea* (Coleoptera: Mordellidae) in Italy and analysis of intraspecific variation in the COI gene

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Recieved: 15 November 2022
Accepted: 30 November 2022
Published: 9 December 2022
Subject editor: Milan Kozánek

Abstract: *Mordellistena semiferruginea* Reitter, 1911, despite its wide distributional range that spans Europe and Central Asia, is a rarely encountered member of the family Mordellidae. In this paper, we present the first record of this species in Italy based on 15 individuals captured in Malaise traps. We have compared these individuals with type specimens and provided a differential diagnosis of the species together with high-resolution photographs of the habitus and male genitalia. Additionally, we generated 14 sequences of the standard DNA barcoding fragment of the mitochondrial gene coding for cytochrome c oxidase subunit I (COI). The analyses of COI sequences showed a relatively high intraspecific divergence reaching 3.65% (*p*-distance), which is the highest within-species genetic variation documented so far in Mordellidae. Of the total number of 30 nucleotide polymorphisms, only one led to a divergence in the amino acid sequences. The predicted 3D structure of the encoded proteins showed that the variation occurred in Loop 1–2, far from the active site of the protein.

Key words: beetles, distribution, DNA barcoding, Malaise trap, variability.

Introduction

Although the family Mordellidae Latreille, 1802 has been continuously documented in Italy since the 19th century (e.g., Costa 1854; Emery 1876; Baudi 1878; Bertolini 1899; Luigioni 1929; Porta 1934; Ragusa 1904), the number of species recorded in this country has continued to increase in recent years. In the checklist of the Italian fauna, Franciscolo (1995) listed 89 species. Ruzzier (2013) summarized the published data and added several new records in a comprehensive catalogue that listed 90 species. Since then, four more species have been added to the list by Selnekovič and Ruzzier (2019) and Selnekovič and Kodada (2022). Considering the published distributional data, as well as the taxonomic changes that have taken place in recent years (Selnekovič and Improta 2020, Selnekovič et al. 2021), the Italian fauna of the family Mordellidae currently consists of 95 species. Here, we increase the number by reporting the occurrence of *Mordellistena semiferruginea* Reitter, 1911 in Italy for the first time.

Mordellistena semiferruginea was described in 1911 on the basis of two specimens collected in the vicinity of the town of Trenčín in western Slovakia. The species is very rare and since the original description, only 27 specimens have been reported in 12 publications. Currently, *M. semiferruginea* is known in 12 countries in Europe and Central Asia (Fig. 1). As the records show, adults have been found on flowers in open woodland and grassland communities (Reitter 1911; Roubal 1936). No further information on the bionomy and development of this species is known. In 2021, the senior author conducted a faunistic survey focusing on insects near the village of Gaville in central Italy (Tuscany). Fifteen specimens of *M. semiferruginea* were captured in two Malaise traps together with approximately 500 other individuals of Mordellidae. The fixation methods used during sampling allowed subsequent DNA isolation and analysis, enabling us to generate the first publicly available sequences of standard DNA barcoding fragment coding for cytochrome c oxidase subunit I (COI) of *M. semiferruginea*, and to explore the intraspecific variation in the COI gene as well as the predicted 3D structure of the encoded protein.

Material and methods

Material acquisition and deposition
A total of 20 individuals of *M. semiferruginea* were exam-

https://virnat.sk/journals/ | ISSN (print): 1336-1546
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ined during the preparation of the present paper. Fifteen specimens were collected in 2021 in the vicinity of the village of Gaville in central Italy (Tuscany). The specimens were captured from June to August 2021 using two Malaise traps, which were approximately 200 m apart. One trap was situated between an olive grove and a mixed forest, mainly with *Pinus nigra* J.F. Arnold, *Arbutus unedo* L., and *Quercus pubescens* Willd. (43.571342°N, 11.421425°E). The other trap was situated in a sparse oak forest with *Q. pubescens* (43.570000°N, 11.420556°E). Seventy percent ethanol was used as a fixation fluid and the samples were removed from the traps every ten days. The specimens mentioned above were compared with the lectotype and a paralectotype of *M. semiferruginea*, a holotype of *M. taurica* Csiki, 1949, and three additional specimens of *M. semiferruginea* from Hungary and Slovakia. The specimens examined are deposited in the following collections: Dávid Selnekovič collection, Bratislava, Slovakia (DSBS); Hungarian Natural History Museum (Termeszettudomanyi Muzeum), Budapest, Hungary (HNHM); Slovak National Museum, Natural History Museum, Bratislava, Slovakia (SNM). DNA samples are deposited at the Department of Zoology, Comenius University in Bratislava Faculty of Natural Sciences, Bratislava, Slovakia (ZCUB).

DNA isolation and amplification

Fifteen individuals of *M. semiferruginea* and one individual of *Stenalia testcea* (Fabricius, 1775) were used for DNA extraction (Tab. 1). Genomic DNA was extracted from whole specimens using the E.Z.N.A.® Tissue DNA Kit (Omega Bio-tek, Inc., Norcross, GA, USA) according to the manufacturer's protocol. Before DNA extraction, the cuticle of each specimen was penetrated at the hind coxae, using a sterile insect pin. The forward and reverse strands of the COI gene fragment were amplified using standardised primes LCO1940 and HCO2198 (Folmer 1994). Each individual polymerase chain reaction (PCR) mixture contained 5 µl of extracted DNA, 10 µl of GoTaq® Green Master Mix (Promega, Madison, WI, USA), 0.52 µl of each primer (10 pmol/l), and 3.96 µl of nuclease-free water. The PCR reaction was set as follows: initial denaturation at 94 °C for 2 min followed by 40 cycles of 94 °C for 40 s, 52 °C for 40 s, 72 °C for 60 s, and a final extension at 72 °C for 10 min. PCR products were visualised in 1% agarose gel using a GoodView™ stain (SBS Genetech Co. Ltd., Beijing, China). PCR products were purified using the EPPiC fast kit (A&A Biotechnology, Gdansk, Poland) and sequenced in Macrogen Europe (Amsterdam, The Netherlands).

DNA analyses

Nucleotide sequences were trimmed and assembled into contigs using Unipro UGENE 44.0 (Okonechnikov et al. 2012). The final alignment was performed in Unipro UGENE 44.0 using the MUSCLE algorithm. *Stenalia testacea* was selected as an outgroup. The uncorrected pairwise distances were calculated in the MEGA11 software (Tamura et al. 2021). Haplotypes were detected and a TCS haplotype network was calculated using PopART 1.7 (http://popart.otago.ac.nz). Amino acid sequences were generated from aligned nucleotide sequences in MESQUITE 3.70 (Maddison and Maddison 2021). Maximum likelihood analysis (ML) was performed on the IQ-TREE web server (http://iqtree.cibiv.univie.ac.at/) using the best substitution model (HKY+F+I) identified by the built-in ModelFinder. Node support values were obtained from 1,000 ultrafast bootstrap replicates (Hoang et al. 2017) and tested by the SH-aLRT branch test (Guindon et al. 2010). The resulting tree was visualized and edited in FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) and Adobe Illustrator CC (https://www.adobe.com/uk/products/illustrator.html). The predicted protein 3D structures were modelled using I-TASSER (Yang et al. 2015), and visualised and aligned using UCSF Chimera 1.16 (Pettersen et al. 2004).

Table 1. Specimens of *Mordellistena semiferruginea* Reitter, 1911 and *Stenalia testacea* (Fabrisius, 1775) used for DNA extraction and analyses.

Locality	Sample #	GenBank #	BOLD #	Haplotype
Mordellistena semiferruginea Reitter, 1911				
Italy, Cinipetta	DSBS_246	OP320356	BTFB155-22	H1
Italy, Cinipetta	DSBS_247	OP320350	BTFB156-22	H5
Italy, Cinipetta	DSBS_256	OP320357	BTFB157-22	H1
Italy, Cinipetta	DSBS_331	OP320358	BTFB158-22	H3
Italy, Cinipetta	DSBS_332	OP320352	BTFB159-22	H2
Italy, Cinipetta	DSBS_333	OP320359	BTFB160-22	H1
Italy, Cinipetta	DSBS_353	OP320360	BTFB161-22	H1
Italy, Cinipetta	DSBS_354	OP320362	BTFB162-22	H4
Italy, Cinipetta	DSBS_355	OP320353	BTFB163-22	H2
Italy, Cinipetta	DSBS_356	OP320354	BTFB164-22	H1
Italy, Cinipetta	DSBS_357	OP320355	BTFB165-22	H2
Italy, Cinipetta	DSBS_358	OP320361	BTFB166-22	H1
Italy, Cinipetta	DSBS_359	OP320363	BTFB167-22	H4
Italy, Cinipetta	DSBS_360	OP320351	BTFB168-22	H5
Stenalia testacea (Fabricius, 1775) (outgroup)				
Italy, Cinipetta	DSBS_253	OP320364		

Morphological observation and documentation

After DNA extraction, the individuals were soaked in distilled water, the genitalia were dissected, and the individuals were mounted on cardboard labels using dimethyl hydantoin formaldehyde (DMHF; Entomopraxis Scp, Barcelona, Spain). Leica M205 C (Leica Microsystems, Wetzlar, Germany) with magnification up to 160×, equipped with diffused LED lighting (6400 K) was used for morphological observations. Measurements were taken using eye-piece micrometer. Body length (BL) is given as a combination of head, pronotal, and elytral lengths. The elytral length was measured from the scutellar apex to the elytral apices along the midline and the elytral width as a maximum width of the elytra. Genitalia were cleared in lactic acid for several days, then dehydrated in ethanol and temporarily mounted on slides using Euparal (Paradox Company, Cracow, Poland). Photographs of habitus were taken with an EOS 5D mark II camera (Canon, Tokyo, Japan) attached to an Axio Zoom V16 stereoscope (Zeiss, Oberkochen, Germany); photographs of genitalia were taken with an Axio Imager 2

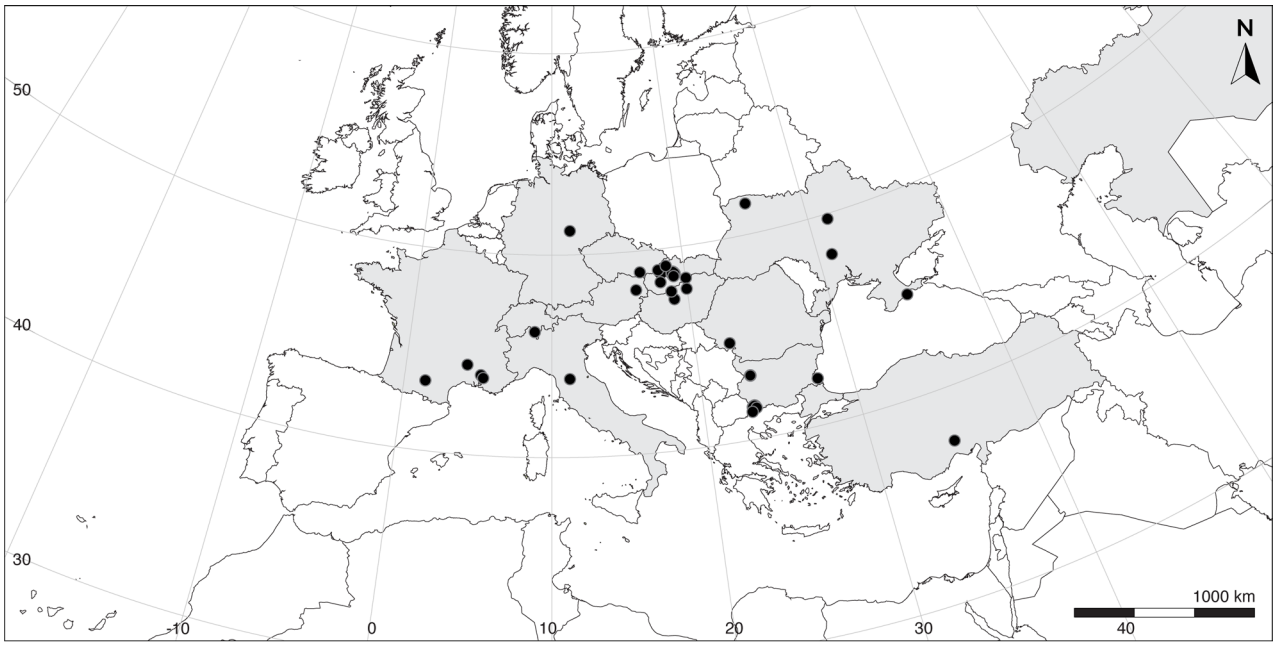

Figure 1. Distribution of *Mordellistena semiferruginea* Reitter, 1911. Countries of known occurrence are highlighted in grey; individual localities are shown with dots.

(Zeiss, Oberkochen, Germany). The images were stacked in Zerene Stacker 1.4 software (https://zerenesystems.com/cms/stacker) and edited in Adobe Photoshop CC (https://www.adobe.com/products/photoshop.html). The final photographs are equipped with the browsing software Krpano version 1.20.8 (https://krpano.com/home/), which enables the observation of detailed structures in the original resolution.

Data availability

The sequences and alignment used in this paper were uploaded to The Barcode of Life Data System (BOLD) and can be accessed in dataset DS-AZB2022 (dx.doi.org/10.5883/DS-AZB2022). The sequences are also available on GenBank with the accession numbers listed in Tab. 1.

Results

***Mordellistena* (s. str.) *semiferruginea* Reitter, 1911**

(Figs 1–4)

Mordellistena (*Tolida*) *semiferruginea* Reitter, 1911: 376, original description, type locality: "Trencsen", Slovakia—Csiki (1915: 50), catalogue—Schaufuss (1916: 766), catalogue—Winkler (1928: 886), catalogue—Roubal (1936: 284), localities.

Mordellistena (*Mordellochroa*) *taurica* Csiki, 1949: 45–46, original description, type locality: "Krim", Ukraine—Batten (1977: 117–120), synonymy.

Mordellistena balazuci Ermisch, 1966: 37–39, original description, type locality: "Bois de Paiolive", France—Batten (1976: 168), description of male, figures—Batten (1977: 117–120), synonymy.

Mordellistena (*Mordellochroa*) *semiferruginea*: Roubal (1931: 184), localities, description of form *nigroangulata*.

Mordellistena (*Mordellistena*) *semiferruginea*: Csiki (1949: 45), first record from Hungary—Ermisch (1956: 310), localities—Ermisch (1963: 34), first record from Romania—Ermisch (1969: 186), identification key—Batten (1977: 117–120), localities, first record from Bulgaria, figures—Ermisch (1977: 172), identification key—Kaszab (1979: 36), identification key, figures—Odnosum (1993: 27), identification key—Odnosum (2005: 110), localities—Horák (2008: 101), catalogue, first records from Austria, Switzerland, and Turkey—Odnosum (2010: 234–235), identification key, description, figures, localities, first record from Kazakhstan—Leblanc (2014), catalogue—Valladares et al. (2016: 72), localities—Horák (2020: 98), catalogue, first records from Czechia and Germany.

Material examined. *Lectotype* of *M. semiferruginea*, female, (Fig. 2C): "♀ / Hung. Trencsén Nagy-Sziklás [Omšenie] Kocsi / Zsihlavnik [Žihlavník] / semiferruginea m. / Holotypus 1911 Mordellistena semiferruginea Reitter / Lectotype Mordellistena semiferruginea Reitter, 1911 Batten des. (Ent. Ber., 37: 117)" (HNHM).

Paralectotype of *M. semiferruginea*, male, (Fig. 3A, C): "♂ / Hung. Trencsén Nagy-Sziklás [Omšenie] Kocsi / Zsihlavnik [Žihlavník] / semiferruginea m. / Paratypus 1911 Mordellistena semiferruginea Reitter / Paralectotype Mordellistena semiferruginea Reitter, 1911 Batten des. (Ent. Ber., 37: 117)" (HNHM).

Holotype of *M. taurica*, female: "*Топлиб. Мон.* [?, handwritten Cyrillic] Krim 24.7.08 W. Pliginski / Monotypus 1948 Mordellistena (Mordellochroa) taurica Csiki" (HNHM).

Additional material: HUNGARY: 1 male: Mátraháza, Mátra hegység, 28 July 1956, E. Hamori leg. (HNHM); 1 female: Budapest, Kamara erdő, 15 July 1919 (HNHM); ITALY: 13 males: Tuscany, Firenze, Gaville env. (Figline Valdarno), Cinipetta, 43.570000°N, 11.420556°E, 445 m a.s.l., 20–30 Jun 2021 (5 ex.), 1–10 Jul 2021 (4 ex.), 10–20

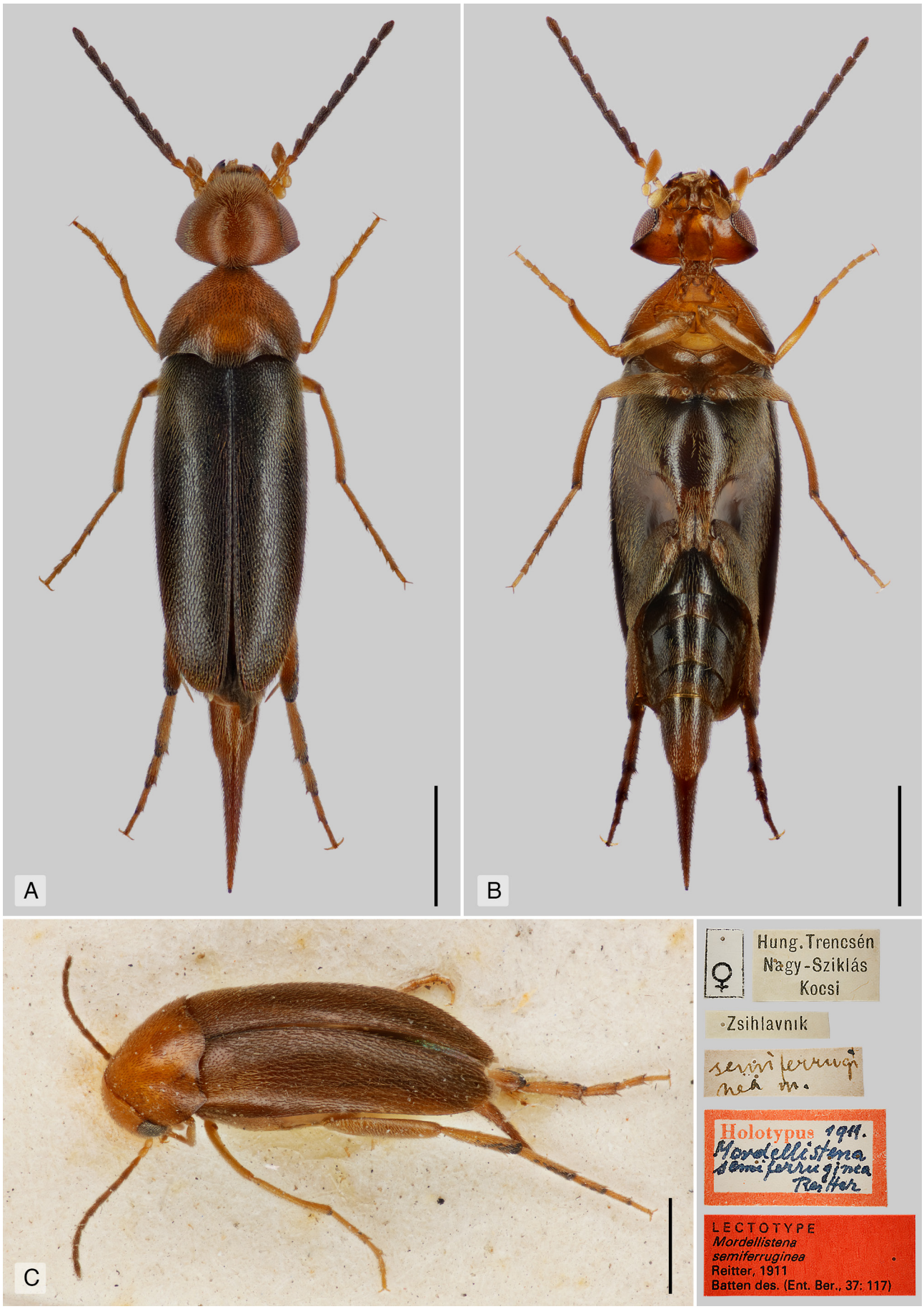

Figure 2. Habitus of *Mordellistena semiferruginea* Reitter, 1911. **A.** Male from Italy (DSBS 256), dorsal aspect (full-size image at https://virnat.sk/pc/Fig_2A/). **B.** Same, ventral aspect (full-size image at https://virnat.sk/pc/Fig_2B/). **C.** Lectotype (HNHM), female, with labels (full-size image at https://virnat.sk/pc/Fig_2C/). Scale = 1 mm.

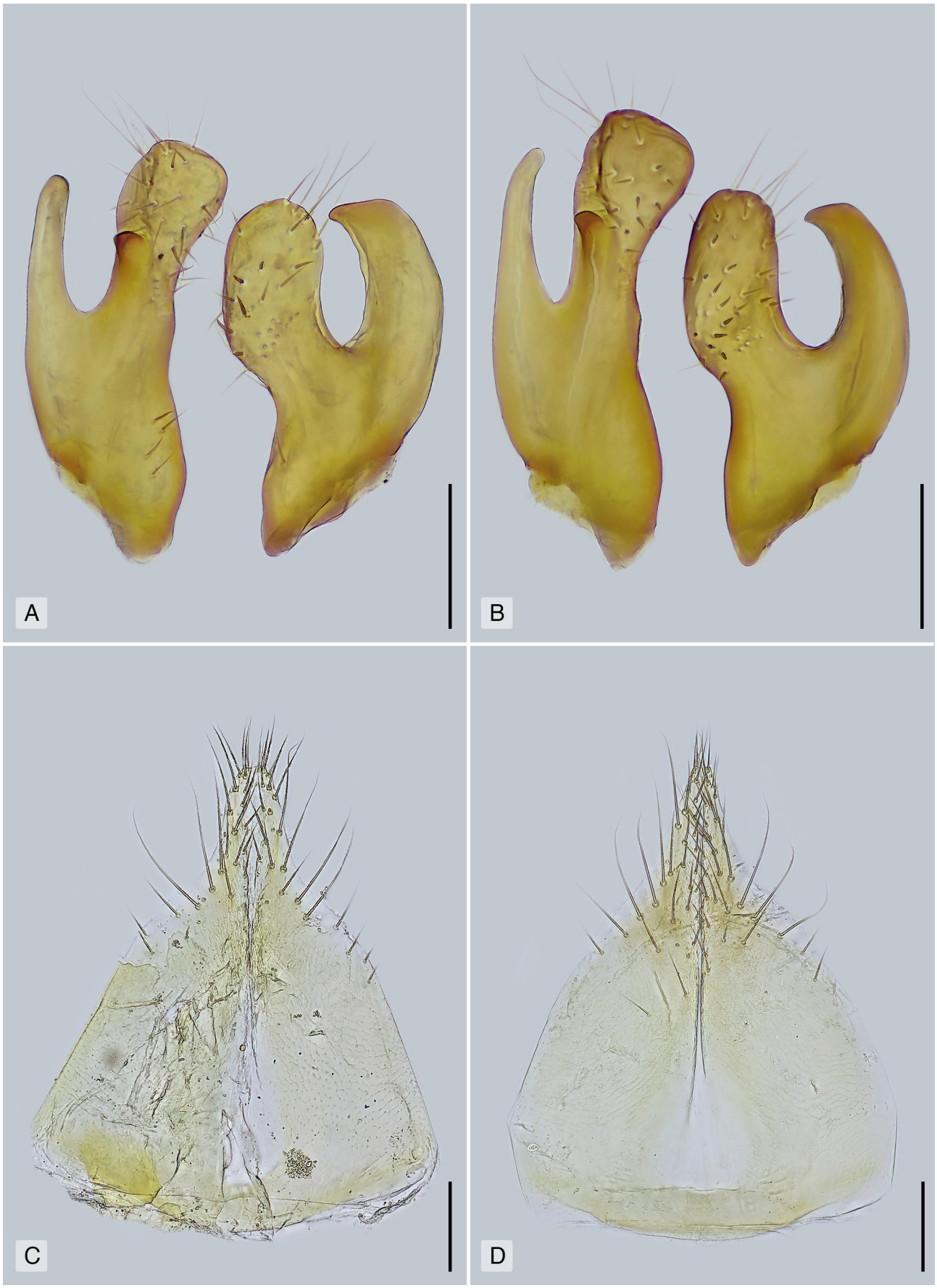

Figure 3. Parts of male terminalia of *Mordellistena semiferruginea* Reitter, 1911. **A.** Parameres, paralectotype (HNHM) (full-size image at https://virnat.sk/pc/Fig_3A/). **B.** Parameres, specimen from Italy (DSBS 256) (full-size image at https://virnat.sk/pc/Fig_3B/). **C.** Sternite VIII, paralectotype (HNHM) (full-size image at https://virnat.sk/pc/Fig_3C/). **D.** Sternite VIII, specimen from Italy (DSBS 256) (full-size image at https://virnat.sk/pc/Fig_3D/). Scale = 0.1 mm.

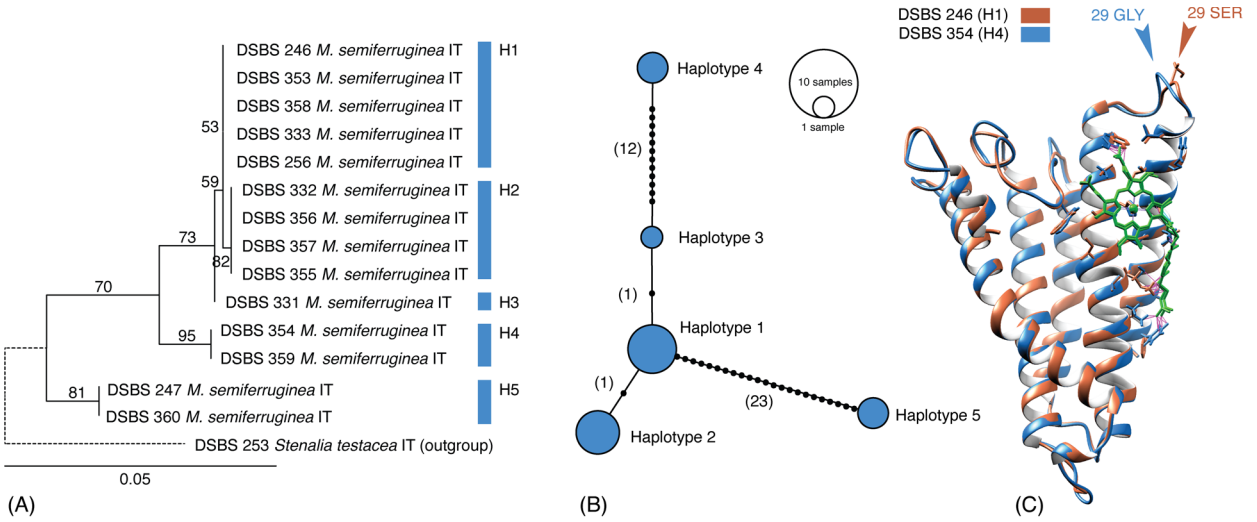

Figure 4. Results of analyses of a 658 bp fragment of the cytochrome c oxidase subunit I gene (COI) in *Mordellistena semiferruginea* Reitter, 1911 specimens from Italy. **A.** Maximum likelihood tree based on HKY+F+I substitution model. Statistic support values are obtained from 1,000 ultrafast bootstrap replicates. **B.** TCS haplotype network with the numbers of nucleotide substitutions between haplotypes shown in parentheses. **C.** Aligned 3D structures of the encoded proteins in Haplotype 1 (orange) and Haplotype 4 (blue). Difference in one amino acid at position 29 is shown by arrows.

Jul 2021 (2 ex.), 20–30 Jul 2021 (1 ex.), 1–10 Aug 2021 (1 ex.), forest, Malaise trap, L. Bartolozzi leg., vouchers DSBS 246, DSBS 247, DSBS 256, DSBS 333, DSBS 353 to DSBS 360 (DSBS, ZCUB); 2 males: same data but 43.571342°N, 11.421425°E, 420 m a.s.l., 20–30 Jun 2021 (1 ex.), 1–10 Jul 2021 (1 ex.), vouchers DSBS 331, DSBS 332 (DSBS, ZCUB); SLOVAKIA: 1 ex., sex unknown: Nitra, Zobor hill, 24 Jul 1962, I. Okáli leg. (SNM).

Differential diagnosis. Body elongated, wedge-shaped (Fig. 2), BL: ♂♂ 4.01–4.89 mm (4.41 ± 0.26, n = 14). Head reddish-brown, mouth parts and antennomeres 1–3 yellowish to reddish brown, following antennomeres dark brown to black; pronotum reddish brown, postero-lateral angles often darkened to various extent; elytra black; prosternum and mesoventrite reddish brown; metaventrite and abdominal ventrites reddish brown to dark brown; pygidium reddish brown at base, darkened posteriad. Vestiture on most of body surface yellowish; elytra with yellowish pubescence in humeral portions and brown pubescence in remaining portions (Fig. 2A). First three antennomeres narrower and shorter than following ones; antennomeres 4–10 ca. 2× as long as wide (Fig. 2A–B). Second maxillary palpomere strongly expanded; terminal palpomere scalene, with inner angle located in distal quarter of the segment (Fig. 2A–B). Lateral pronotal sides straight in lateral aspect; postero-lateral angles obtuse. Elytral length/width ratio: ♂♂ 2.00–2.23 (2.14 ± 0.06, n = 14). Protrochanter and profemur each with one conspicuous erect black seta on anterior edge; penultimate segment of protarsus subtruncate at apex. Metatibia with short subapical ctenidium and three oblique lateral ctenidia that nearly reach middle of tibial width. Metatarsomere 1 with three ctenidia; metatarsomere 2 with two ctenidia. Parameres and male sternite VIII as in Fig. 3.

Mordellistena semiferruginea differs from most of the western Palaearctic congeners in having the antennomeres 1–3 shorter than the following ones. In general appearance and colouration, it resembles *M. signicollis* Schilsky, 1894, *M. humeropicta* Ermisch, 1963, *Natirrica humeralis* (Linnaeus, 1758), and *N. variegata* (Fabricius, 1798). It is easily recognisable based on body colouration shape of the male genitalia. The head capsule is entirely reddish brown in *M. semiferruginea*, compared to yellowish in the anterior portions and black in the posterior portions in *N. humeralis* and *N. variegata*. The pronotum is reddish brown, sometimes with darkened posterior angles in *M. semiferruginea*, while in *M. signicollis* and *N. humeralis*, it is usually yellowish in the lateral portions and black in the medial portions, or entirely yellowish, or black. In *N. variegata*, the pronotum is dark brown to black, with two yellowish areas in the lateral portions. Furthermore, *M. humeropicta* and *N. variegata* differ from *M. semiferruginea* in having the antenna completely yellowish. Elytra are entirely black in *M. semiferruginea*, while they are at least partially yellowish in the latter three species. *Mordellistena cypria* Ermisch, 1963, *M. oranensis* Pic, 1900, *M. goetzi* Ermisch, 1969, and *Natirrica neuwaldeggiana* (Panzer, 1796) differs from *M. semiferruginea* in having the body entirely yellowish brown.

DNA sequences and variation. Overall, 14 sequences of 658 bp COI gene fragment of *M. semiferruginea* were generated and submitted to GenBank and BOLD (Tab. 1). The dataset contained 628 constant and 30 parsimony-informative sites. Seventy percent of the substitution sites occurred at the third codon position, while the remaining 30% occurred at the first codon position. The sequences fell into five haplotypes (H1–H5). The most diverged haplotype was H5, separated from H1 by 23 substitutions in the nucleotide sequence (*p*-distance 3.50%), and from H2, H3, and H4 by 24 substitutions (*p*-distance 3.65%) (Fig. 4B, Tab. 2). The smallest divergence was between haplotypes H1 and H2, and H1 and H3, which were separated by one substitution

in nucleotide sequence (*p*-distance, 0.15%). The ML tree shows separate clade for each haplotype (Fig. 4A). Only one substitution in the nucleotide sequence between adenosine (A) and guanine (G) at position 86 resulted in a variation in the amino acid sequences. This is the first position of the AGA codon (nucleotide sequence positions 86–88) coding for polarly uncharged serine (Ser) in haplotype H4 and the GGA codon coding for nonpolar aliphatic glycine (Gly) in all other haplotypes (amino acid sequence position 29). All other substitutions in the nucleotide sequences were synonymous. The model of the predicted 3D structure of the encoded protein revealed, that the amino acid divergence occurred in the Loop 1–2, far from the active site of the protein (Fig. 4C). The alignment of the predicted 3D structures did not reveal any structural differences in the active sites of the proteins between H1 and H4 (Fig. 4C). The amino acid at the position 29 does not interact with ligand. There is no apparent morphological variation in the examined specimens that would correspond to the genetic divergence.

Distribution. *Mordellistena semiferruginea* has so far been recorded from Austria (Horák 2008; pers. comm.), Bulgaria (Batten 1977), Czechia (Horák 2020; pers. comm.), France (Ermisch 1966; Batten 1976; Valladares et al. 2016), Germany (Horák 2020; pers. comm.), Hungary (Csiki 1949), Kazakhstan (Odnosum 2009), Italy (new record), Romania (Ermisch 1963), Slovakia (Reitter 1911; Roubal 1931, 1936; Kaszab 1979), Switzerland (Horák 2008; pers. comm.), Ukraine (Csiki 1949; Odnosum 1993, 2005, 2009), and Turkey (Horák 2008; pers. comm.) (Fig. 1). Here, we present the first record of the species from Italy.

Discussion

Mordellistena semiferruginea is, despite its wide distributional range (Fig. 1), a species that is very rarely encountered. Previous records show that adult specimens have been found in small numbers (up to three specimens per collecting event) on flowers. In this paper, we present the sampling of 15 individuals that were captured between June and August 2021 using two Malaise traps. Thirteen individuals were captured by a trap placed in a sparse oak forest with *Quercus pubescens*, and the remaining two individuals were captured by a trap placed between an olive orchard and a mixed forest with *Pinus nigra*, *Arbutus unedo*, and *Quercus pubescens*. The two traps captured approximately 500 individuals of Mordellidae during one season (2021). In the literature, we can find another example where an interesting finding was made with the help of Malaise traps. This is the discovery of 14 individuals of the very rare species *Conalia baudii* Mulsant & Rey, 1956 in France (Calmont 2019). Malaise traps therefore prove to be useful for sampling Mordellidae since they are able to capture rare species or those that are difficult to collect by conventional sampling methods, such as individual collecting or sweeping from vegetation.

Table 2. Uncorrected *p*-distances between haplotypes H1–H5 in *Mordellistena semiferruginea* Reitter, 1911 calculated in Mega X software.

Haplotype	H1	H2	H3	H4
H2	0.0015			
H3	0.0015	0.0030		
H4	0.0198	0.0213	0.0182	
H5	0.0350	0.0365	0.0365	0.0365

Analysis of the COI gene fragment in 14 individuals of *M. semiferruginea* revealed a relatively high intraspecific divergence, reaching 3.65% among individuals captured at a single site. This genetic variation does not correspond to any apparent morphological variation. This is the highest intraspecific divergence recorded so far in Mordellidae. The only study to date that has provided data on intraspecific divergence for six species (altogether 30 specimens) in the genus *Mordellistena* recorded the highest value of 1.9% between two distant populations of *M. hirtipes* (Selnekovič et al. 2021). Similarly, the first author's unpublished data on intraspecific variation of species from the *M. pumila* and *M. confinis* species groups show values that do not exceed 1.5%. In the case of *M. semiferruginea*, out of a total of 30 substitutions in the 658 bp fragment of the COI gene, only one was non-synonymous, causing a difference in one amino acid between the protein sequences in haplotype H4 versus all other haplotypes. Analysis of the predicted 3D structure of the encoded proteins showed that the variation between the two amino acids occurred in Loop 1–2 (Fig. 4C), far from the functional site of the protein and therefore unlikely to affect its functioning (Pentinsaari et al. 2016). Research utilizing genetic data is still in its infancy in the family Mordellidae, and we do not yet have sufficient data to assess the extent to which such high intraspecific divergence in mitochondrial DNA as we found in *M. semiferruginea* is present in the genus *Mordellistena* or within the entire family.

Acknowledgements

The authors thank Milan Kozánek (Bratislava, Slovakia) for providing the Malaise traps. We thank Janka Poláková and Alica Christophoryová (ZCUB) for their help with DNA isolations and editing of the photographs. We thank Győző Szél (HNHM) for loaning the type material. We thank Jan Horák (Prague, Czechia) for sharing the distributional data. We also thank two anonymous reviewers for their valuable comments and suggestions. The present paper was supported by the Slovak Research and Development Agency under project no. APVV-19-0076.

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