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Endemic and cryptic: different biogeographic histories of three Italian blister beetles of the genus *Meloe* (Coleoptera: Meloidae: Meloinae: Meloini)

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The Italian geographic region is characterized by complex and diversified biogeographic patterns and is represented by a high number of endemic species. Endemic species characterized by a limited distribution range should be a primary concern in conservation. This article aimed to investigate the phylogenetic and biogeographic relationships of 2 Italian endemic species of the wingless blister beetle genus *Meloe* Linnaeus, 1758: *Meloe* (*Eurymeloe*) *apenninicus* and *Meloe* (*E.*) *baudii*. Our inferences, based on morphological characters, 2 mitochondrial (16S and COI) and 2 nuclear (CAD and 28S) markers and the use of 3 species delimitation analyses approaches, pointed out the presence of a new Italian endemic species (*M.* (*E.*) *digiuliorum* **sp. n.**), here described, and 3 different patterns of phylogenetic and biogeographic affinities. *M. digiuliorum* is close to the Spanish endemic *M. orobates* **comb. n.**, revealing a possible fragmentation of the ancestor range in the Pleistocene (ca. 0.84 Mya) followed by isolation in Italy and Spain. *M. apenninicus* is the sister species of the European-Anatolian *M. rugous* and *M.* cfr. *rugosus*, and this pattern originated around the Plio-Pleistocene boundary (ca. 2.83 Mya) likely influenced by the climatic fluctuations and the presence of the Alpine barrier. Finally, 2 subspecies were referred to *M. baudii*: the nominal one, endemic to Italy, and the Turanian-E European *M. b. glazunovi* **stat. n.**, disclosing a third more recent (ca. 0.64 Mya) pattern of biogeographic disjunction.

Key words: cryptic species, Mediterranean biogeography, taxonomy, new species, molecular analyses

Introduction

Estimating species diversity is crucial to comprehend evolutionary processes, ecosystem functioning and to direct conservation efforts toward aspects of priority concern (Struck et al. 2018). Nevertheless, assessing the true number of species is challenged by a plethora of uncertainties like a non-univocal definition of *species*, an unknown number of taxonomic inaccuracies (i.e., synonymies due to the assignment of different names to somehow distinct entities of the same species), and the occurrence of cryptic species ("species classified as a single nominal species because they are at least superficially morphologically indistinguishable" *sensu* Bickford et al. 2007) (Bickford et al. 2007, Vrijenhoek 2009, Adams et al. 2014, Struck et al. 2018, Li and Wiens 2023).

Italy is the European country with the highest animal species richness and the recently updated list for this country reached about 60,000 species (Bologna et al. 2022). Such species richness can be traced back to the Tertiary events, that molded the central Mediterranean (i.e., the drift of the western microplates during the Oligocene and the Messinian salinity crisis), and the Plio-Pleistocene climatic fluctuations, but is also related to the pivotal position in the center of the Mediterranean basin, and the astonishing mosaic of ecosystems of the Italian geographic region (Schmitt et al. 2021). Therefore, Italian biota is characterized by complex and diversified biogeographic patterns and is represented by a high number of species, many of which are endemic (La Greca 2002, Ruffo and Vigna Taglianti 2002, Schmitt et al. 2021). Understanding how many species are present in such a diverse geographic area (also unveiling the cryptic taxa), individuating the number of endemism, and disclosing the biogeographic patterns behind such diversity, is essential in conservation (Burlakova et al. 2011).

Meloidae, with 3 subfamilies (Eleticinae, Nemognathinae, and Meloinae) and 133 recognized genera (Pan & Ren 2020, Riccieri et al. 2022, 2023), are known for their hypermetamorphic larval development and the production of a toxic terpene (Cantharidin), but are also peculiar for the predaceous habits of their first-instar larva (triungulin) which can be predators of Hymenoptera Apoidea or Orthoptera Acridiidae (Bologna 1991, Bologna and Pinto 2001, 2002, Bologna et al. 2010, Riccieri et al. 2022).

The Italian fauna of Meloidae, commonly called blister beetles, includes around 64 species, (Bologna 1991, 2020a), some of which might be extinct (Bologna 2018). From a biogeographic point of view, this fauna is relatively composite, including several species with Central Asiatic-Europeo-Mediterranean or Central Asiatic-Mediterranean distribution, but also some Mediterranean or Siculo-Maghrebian elements (Vigna Taglianti et al. 1999). Among the Italian blister beetles, 4 species and 5 subspecies are endemic but their phylogenetic and biogeographic affinities were never investigated. Respectively: *Alosimus tyrrhenicus* Bologna, 1989; *Meloe apenninicus* Bologna, 1988, *Meloe baudii* Leoni, 1907; *Mylabris obsoleta* Novicki, 1874; and *Lydus trimaculatus italicus* Kaszab, 1952; *Meloe autumnalis heydeni* Escherich, 1889; *Meloe tuccia corrosus* Brandt and Erichson, 1832; *Mylabris pusilla latialis* Magistretti, 1943; and *Mylabris impressa stillata* Baudi di Selve, 1878. *Mylabris maculosopunctata mendizabali* De la Rosa, 2008, considered as an endemic subspecies, probably represents an erroneous record.

The tribe Meloini, in the subfamily Meloinae, includes the monotypic Iberian genus *Physomeloe* Reitter, 1911 and the Holarctic and Afrotropical *Meloe* Linnaeus, 1758. The latter is one of the most speciose and widely distributed genera of the family, with about 150 species shared in 16 subgenera mostly Palaearctic (Bologna 1991, 2020b, Pan and Bologna 2021). The taxonomy of these subgenera was recently revised (Sánchez-Vialas et al. 2021). Nonetheless, based on larval and adult morphology (Bologna 1988, Bologna et al. 1989, Bologna and Pinto 1992, 2001, Pan and Bologna 2021), as well as

on molecular evidences (Salvi et al. in prep.), we reject the elevation of the subgenera at genus level and the division of *Eurymeloe* in 3 subgenera (*Eurymeloe*, *Coelomeloe* Reitter, 1911, *Bolognaia* Ruiz, García-París, Sánchez-Vialas and Recuero, 2022) proposed by Sanchéz-Vialas et al. (2021, 2022).

Meloe species are characterized by having the oviposition in the soil and a phoretic triungulin, that climbs on flowers to cling to wild bees, to be at last transported to their nest, where it feeds on their larvae, eggs, and provisions and completes its complex larval cycle (Bologna 1991, Bologna and Pinto 2001, Bologna et al. 2010). This strategy compensates the lack of wings of the adults, contributing to the dispersion of the species. Its dependence on the host as well as the adult scarce dispersion capacity, makes the genus *Meloe* particularly worthy of being investigated from a biogeographic perspective.

A total of 25 species of *Meloe* were recorded from Italy, of which, as noted above, *M. apenninicus* and *M. baudii* are endemic or subendemic (Bologna 1988, 1991; Fig. 1). These 2 species are included in the speciose subgenus *Eurymeloe* Reitter, 1911 (53 spp., 12 in Italy; Bologna 2020a, 2020b, Sánchez-Vialas et al. 2021, 2022) and both belong to the group of *M. rugosus* Marsham, 1802 (*sensu* Bologna 1988, for synthesis on the ecology of these species, see Bologna 1991 and *Results*). Like those of the nominate subgenus (Pan and Bologna 2021), species of *Eurymeloe*, and in particular those included in the *M. rugosus* group, are very hard to readily distinguish (Bologna 1988): slight differences can be observed in the head and pronotum shape and punctuation, but specimens with intermediate features can be frequently observed (Bologna 1988). Indeed, until the end of the XIX century, the *rugosus* group (*sensu* Bologna, 1988) included the nominate species and few others. The splitting of *M. rugosus* in several species was encouraged by accurate observations of the morphology of antennae, pronotum, and more recently by male genitalia, the latter representing the main diagnostic characters, as they maintain a constant shape at intraspecific level (e.g., Leoni 1907, Pliginsky 1910, Müller 1925, Bologna 1988, Ruiz & García-París 2009, 2015) and also by molecular evidence
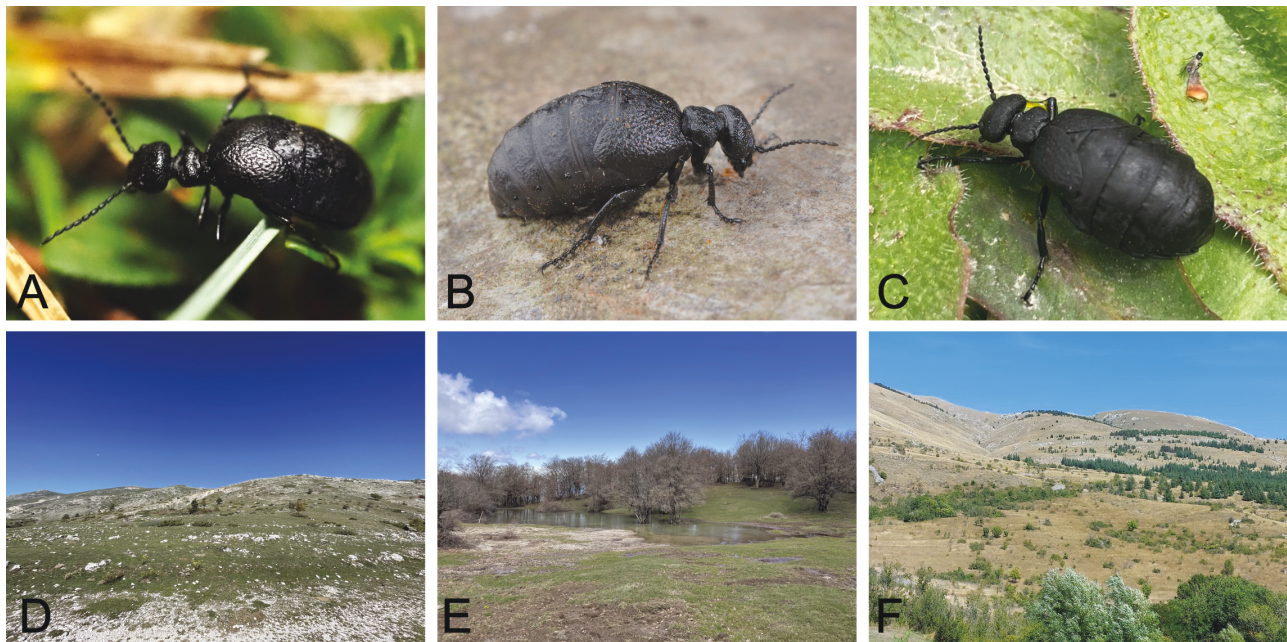
Fig. 1. Italian endemic taxa of *Meloe* (*Eurymeloe*) (upper row) and their type localities (lower row): (A) *M. digiuliorium* sp. nov.; (B) *M. apenninicus*; (C) *M. b. baudii*; (D) Abruzzo, L'Aquila prov., Ovindoli, Freddo Mt. western slope; (E) Sicily, Messina prov., Cesarò, nr. Portella di femmina morta; (F) Abruzzo, L'Aquila prov., Cerchio, basal western slope of the Sirente Mt. Photo's credits: A, L. Spagoni; B–E, A. Ricceri; F, M.A. Bologna (https://inkscape.org/it/).

(Sánchez-Vialas et al. 2021, 2022). At present, the *M. rugosus* group includes around 30 species (Bologna 2020b).

Based on morphological evidence, *M. apenninicus* seems close to *M. rugosus*, while *M. baudii* to *M. glazunovi* Pliginskji, 1910 (Bologna 1988, 1991, Di Giulio et al. 2013). However, none of these 2 species were included in the recent molecular revision of *Eurymeloe* by Sánchez-Vialas et al. (2022) and their phylogenetic position remains uncertain.

By investigating the phylogenetic affinities of these species with other closely related ones, our aim is to understand the biogeographic origin of these 2 Italian endemics. Moreover, considering the scarce morphological differentiation observed within the *M. rugosus* group, we aimed to investigate the possible presence of undescribed species among the taxa studied. Indeed, our molecular analyses led to the identification of a new cryptic species endemic to Italy (Fig. 1A), and we provided its description and included it in the discussion.

Material and Methods

Taxon Sampling, DNA Extraction, Amplification, and Sequencing

Samples of *Meloe apenninicus* and *M. baudii* were collected by hand in the field between 2010 and 2022 from different localities (Supplementary Table S1), and 24 and 5 samples, respectively, were used for molecular analyses. Other species of *Meloe* (*Eurymeloe*) were added to the dataset to define the phylogenetic placement of the 2 target species (Supplementary Table S1): 11 individuals of *M. rugosus*, 13 of *M. mediterraneus* Müller, 1925, 4 of *M. murinus* Brandt and Erichson 1832, 2 of *M. glazunovi* Pliginsky, 1910, and 16 of *M. ganglbaueri* Apfebleck, 1905. All samples were stored at 4 °C preserved in pure ethanol in the MAB alcohol' collection at University Roma Tre (MABCa) and were identified in lab by one of us (MAB) using an Olympus SZX12 Stereomicroscope (Bologna 1988, 1991).

DNA was extracted from 3 legs per specimen which were processed with the salting-out protocol (Sambrook et al. 1989) or with the Qiagen DNeasy Blood and Tissue kit following the manufacturer's protocol.

Two mitochondrial and 2 nuclear genes were amplified: (i) mitochondrial cytochrome c oxidase subunit I (COI; primer pairs: LCO1490/HCO2198, LCO1490/HCOoutout; Folmer et al. 1994, Prendini et al. 2005); (ii) mitochondrial 16S ribosomal DNA (16S rDNA; primer pairs: 16SK1/16SK2; Pitzalis 2007); (iii) nuclear carbamoylphosphate synthetase domain of the rudimentary gene (CAD; primer pairs: CD439F/CD688R; CD439F/CD668R; Wild and Maddison 2008); and (iv) nuclear 28S ribosomal RNA gene (28S rDNA; primer pairs: 28S01/28SR01; Kim et al. 2000).

PCR conditions, primer sequences, and thermal cycles used for each gene are reported in the supplementary material (Supplementary Table S2). Amplified products were purified and sequenced by Macrogen (Milan, Italy).

Sequences Editing and Alignment

The software Geneious Prime 2021.1.1 (https://www.geneious.com) was used to check and edit the sequences. Newly generated sequences were aligned with other downloaded from GenBank belonging to species of the subgenus *Eurymeloe* (see Supplementary Table S1) and to *M. (Meloe) proscarabaeus* Linnaeus, 1758 and *Physomeloe corallifer* (Reitter, 1911) which were used as outgroups. Sequences alignment was carried out with the online version of MAFFT v7 (Katoh and Standley 2013). The Q-INS-i algorithm was

used to align 16S and 28S rDNA, as it also considers the secondary structure of RNA, while the E-INS-i algorithm was used to align the coding genes. Specimen vouchers and GenBank accession numbers are provided in Supplementary Table S1.

Phylogenetic Analyses and Molecular Clock Calibration

Maximum likelihood (ML) and Bayesian inferences (BI) approaches were used on single genes and on a concatenated dataset to infer the phylogeny of the analyzed species.

ML was performed with the IQ-TREE web server which also implements the model selection through ModelFinder (Trifinopoulos et al. 2016, Kalyaanamoorthy et al. 2017). The concatenated dataset was partitioned by codon position for coding genes (COI, CAD) and by genes for non-coding genes (28S, 16S), and analyzed with the edge-linked model. The IQ-Tree stopping rule (-numstop) was set to 1000 both for single genes and for the concatenated dataset. To evaluate nodes support, 1000 replicates of ultrafast bootstrap (UFBboot2; Hoang et al. 2018) and Shimodaira–Hasegawa (SH)-like approximate ratio tests (SH-aLRT; Guindon et al. 2010) were applied.

BI was carried out with MrBayes v3.2.6 (Ronquist et al. 2012) and implemented on the webserver CIPRES (Miller et al. 2010). The best partitioning scheme and the substitution models were defined by ModelFinder on IQ-TREE web (Trifinopoulos et al. 2016). Two independent MCMC runs of 60 million generations (10 million for single genes analyses), with 4 chains each and a default 25% burn-in, implementing the reversible jump MCMC (Huelsenbeck et al. 2004) were used to perform Bayesian analyses. Trees were sampled every 6000 (1000 for single genes) generations and runs convergence was assessed with Tracer v1.7 (Rambaut et al. 2018). FigTree v1.3.1 (Rambaut and Drummond 2009) was used to visualize the trees.

We considered supported only clades with SH-aLRT values ≥ 80% (Guindon et al. 2010), UFBoot (UFB) values ≥ 95% (Minh et al. 2013), and posterior probability (PP) values ≥ 0.95 (Erixon et al. 2003).

Molecular clock analysis was performed in BEAST v2.6.6 (Bouckaert et al. 2019) using the combined dataset including only one sample per species. The following settings were implemented in BEAUTi to build the input file: unlinked substitution models (HKY + G), unlinked molecular clock model (uncorrelated relaxed lognormal; Drummond et al. 2006), linked tree model. To obtain a rough timescale of divergence of the 3 endemic species, molecular clock calibration followed the rates estimated by Papadopoulou et al. (2010) in Coleoptera Tenebrionidae (a family close to Meloidae, Cai et al. 2022) for the COI (lognormal prior, mean in real space = 0.0168; stdev = 0.075) and 16S (lognormal prior, mean in real space = 0.0054; stdev = 0.085). Uninformative prior on clock rates were used for all the other genes (uniform prior; lower = 0, upper = 0.25) and the Yule process was used as a tree prior. BEAST was run 2 times for 50 million generations, sampling every 5.000 generations. Runs convergence and ESS values higher than 200 were assessed with Tracer v1.7 (Rambaut et al. 2018). Log files and tree files were combined using Log Combiner v2.6.6, whereas Tree Annotator v2.6.6 was used to produce the maximum clade credibility tree.

Species Delimitation

Three different species delimitation methods were adopted: 2 distance-based approaches, Assemble Species by Automatic Partitioning (ASAP; Puillandre et al. 2021) and ad hoc nucleotide

distance threshold, and the tree-based approach multi-rate Poisson Tree Processes (mPTP; Kapli et al. 2017).

ASAP method is designed for single-locus data, does not require any a priori knowledge, and uses pairwise genetic distance to build a list of partitions ranked by a score that can be used to assess objectively the quality of species partitions (Puillandre et al. 2021). COI sequences alignment was analyzed using the ASAP web server (https://bioinfo.mnhn.fr/abi/public/asap/). Kimura (K80) ts/tv (Kimura 1980) was selected as nucleotide substitution model and all the other parameters were left as default.

For ad hoc nucleotide distance threshold, the R package *spider* v1.5.0 (Brown et al. 2012) was used and the threshold optimization analysis was estimated using the R function *localMinima*. COI nucleotide sequences were clustered with the ad hoc threshold using the function *tclust*.

As a tree-based approach, we used the mPTP, an improved method of PTP. The analysis was performed in mPTP web server (http://mptp.h-its.org; Kapli et al. 2017) using the default settings, the input for mPTP was a maximum likelihood tree based on COI sequences generated by IQ-TREE.

Morphological Study

For morphological descriptions, we used both specimens in ethanol (processed for molecular analyses and listed in Supplementary Table S1), and dried: 19 exx. *M. rugosus*; 32 exx. *M. digiuliorum*; 92 exx. *M. apenninicus*; 17 exx. *M. baudii*; 27 *M. glazunovi*; and 9 exx. *M. scabriusculus* Brandt & Erichson, 1832. All specimens are preserved in the M. A. Bologna collection at the University Roma Tre, Italy (MABC for dried and MABCa for EtOH ones); we considered only few specimens examined in other collections, which are indicated in the text. In addition, we studied for comparison several specimens of most of the described species of the group of *M. rugous*, including one damaged specimen possibly attributable to *M. orobates* Sánchez-Vialas, Ruiz, Recuero, Gutiérrez-Pérez & García-París, 2022 from S Spain (all MABC). Types of the new species (*M. digiuliorum*) are detailed in its description. Data from the examined specimens are listed in the taxonomy section of the results.

The study was carried out using an Olympus SZX 12 stereomicroscope. Photographs of dried specimens were taken with Visionary Digital LK Lab System (Visionary Digital, Palmyra, VA) equipped with a Canon EOS 6D mark II dSLR camera and an MP-E 65 mm *f*/2.8 1–5 × lens (Canon, Tokyo, Japan). This device allowed the automatic capture of stacks of images on different focal planes, which were combined with the Helicon Focus 7 software.

Results

Phylogenetic Results and Molecular Clock Calibration

Overall, we generated 69 sequences of COI (621 bp), 75 sequences of 16S (445 bp), 68 sequences of CAD (731 bp), and 70 sequences of 28S (794 bp). Our final tree (Fig. 2) consisted of 109 terminal taxa and a total length of 2591 bp. Bayesian inference (Supplementary Fig. S1) and maximum likelihood (Supplementary Fig. S2) supported similar topologies. Specimens attributed to *M. apenninicus* were split into 2 distinct clades: clade (a) including only specimens from the Velino Massif (Abruzzo) and the linked Duchessa Mts. (Latium); clade (c) including specimens from Sicily and Latium. The clade (a), which was close to the Spanish endemic species *M. orobates* [clade (b)], should be referred as a new endemic Italian species (see below). Specimens ascribed to *M. rugosus* from Piedmont and Lombardy

clustered within clade (c) and must be referred to *M. apenninicus* rather than to *M. rugosus*, while the other *M. rugosus* from the UK (topotypic), Germany [clade (d)], and from Turkey (Gümüşhane) formed 2 slightly distinct but related clades. Specimens of *M. baudii* [clade (o)], resulted closely related to *M. glazunovi* [clade (p)]. *M. murinus* appeared polyphyletic, with the specimens from Spain and Morocco downloaded from GenBank [clade (g)] close to *M. ganglbaueri* [clade (f)], and our specimens from Sicily (topotypic) and from Sardinia, well distinct in a separate clade (m). *M.* cfr. *glazunovi* from E Turkey (Bitlis) formed a distinct lineage [clade (l)] and likely represents a new species, which will be described separately. *M. corvinus* resulted included within *M. brevicollis* [clade (s)]. The remaining clades are monophyletic. Divergence times estimated in BEAST (Mya = Million years; Fig. 8, Supplementary Fig. S3) indicated a speciation event in the Pleistocene (late Calabrian age) ca. 0.84 Mya [95% highest posterior density (95%HPD): 0.37–1.37 Mya] leading to *M. orobates* and *M. digiuliorum*. The diversification between *M. apenninicus* and *M. rugosus* + *M.* cfr. *rugosus* occurred in the Plio-Pleistocene (between Piacentian and Gelasian ages) around 2.83 Mya (95%HPD: 1.94–3.69 Mya), while that between *M. baudii* and *M. glazunovi* is estimated in the Pleistocene (basal Ionian/Chibasian age) around 0.64 Mya (95%HPD: 0.28–1.05 Mya).

Species Delimitation Analysis

Results of species delimitation analyses based on COI mitochondrial marker revealed a different number of species. The best ASAP partition recovered 22 species groups (asap-score: 3; *p*-value: 2.19e-02; W: 4.31e-04) while in both ad hoc nucleotide distance threshold and mPTP methods 18 species were recognized (Fig. 3).

Best ASAP partition about the specimens of *M. apenninicus* was concurrent with phylogenetic results (Figs. 2 and 3). Indeed, all *M. apenninicus* seems to be divided into 2 species, one including *M. apenninicus* from the Velino Massif and Duchessa Mts. (Abruzzo and Latium), which represents a new Italian endemic species (see below). The other species corresponds to *M. apenninicus* from Sicily (topotypic) and central Latium (Fig. 3). *M. apenninicus* was split into 2 distinct species also considering the methods of ad hoc nucleotide distance threshold and mPTP, but in these scenarios, the new Central Apennine species resulted merged within *M. orobates*.

All 3 species delimitation analyses, split *M. rugosus* into 3 distinct species. Italian specimens of *M. rugosus* from Piedmont and Lombardy belong to *M. apenninicus* (Sicily and Latium), those from the UK (topotypic) and Germany represent the true *rugosus*, while that from Turkey correspond to a new species (Fig. 3). *M. baudii* and *M. glazunovi* were considered the same species, according to all the species delimitation analyses (Fig. 3).

Taxonomy

By integrating molecular phylogenetic results and morphological observations, and considering the distribution and the time of divergence of the examined taxa, we propose the description of a new endemic species from Central Apennines and a new subspecific status for *M. glazunovi*: *M. baudii glazunovi* stat. n.

More in detail, we highlight diagnostic characters of the new species, and improve the information on characters which permit to distinguish it and *M. rugosus*, *M. apenninicus*, and *M. orobates* by adding a synoptic comparison of these characters (Table 1) and by better defining the distribution and the ecology of these species. Similarly, we define diagnostic features of *M. baudii* and compare it with *M. b. glazunovi* and with *M. scabriusculus* (not available for
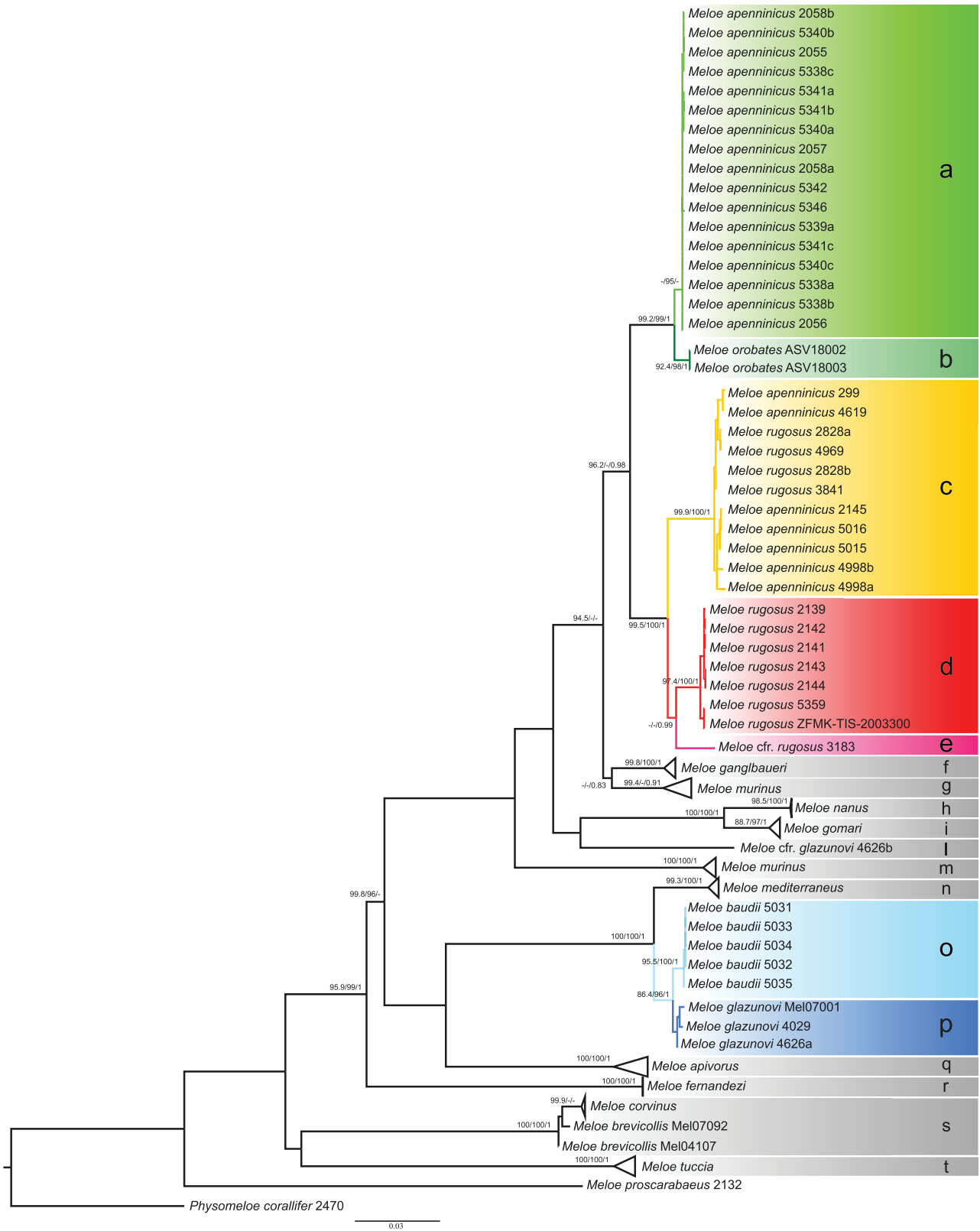
Fig. 2. Multilocus (16S, COI, CAD, and 28S) phylogenetic tree of *Meloe* (*Eurymeloe*). Topology corresponds to the maximum likelihood (ML) tree. Clades are indicated with letters (a–t). Only supported values of nodes (UFBootstrap, UFB ≥ 95 ands SH-aLRT ≥ 80%; posterior probability, PP ≥ 0.95) are reported (SH-LRT/UFB/PP). Dashes (–) indicate non-supported values. Clades of species that were not the main focus of the study are collapsed. For a non-collapsed version of the tree, see Supplementary Fig. S4 (https://inkscape.org/it/).

molecular analyses), as these taxa have been widely morphologically confused in the past (Table 2).

Description of the new Italian endemic species

Meloe (Eurymeloe) digiuliorum Bologna, Riccieri, Spagoni sp. n. urn:lsid:zoobank.org:act:A703C64C-FA93-4154-8624-BF350 3A7D165

(Figs. 4B and 5D–F)

Type material. HOLOTYPE ♂, Italy, Abruzzo, L'Aquila prov., Ovindoli, W slope Monte Freddo, 42°7'16''N 13°29'40''E, 1430–1500 m, 18.X.2003, A. Di Giulio leg. (MABC; male genitalia glued on the paper label). PARATYPES: 2 ♂♂ (one damaged), 2 ♀♀, Italy, Abruzzo, L'Aquila prov., Ovindoli, W slope M. Freddo, m 1430–1500 m, 4°7'6''E 13°29'0''E A. Di Giulio leg. (MABC); 1 ♂ and 1 ♀
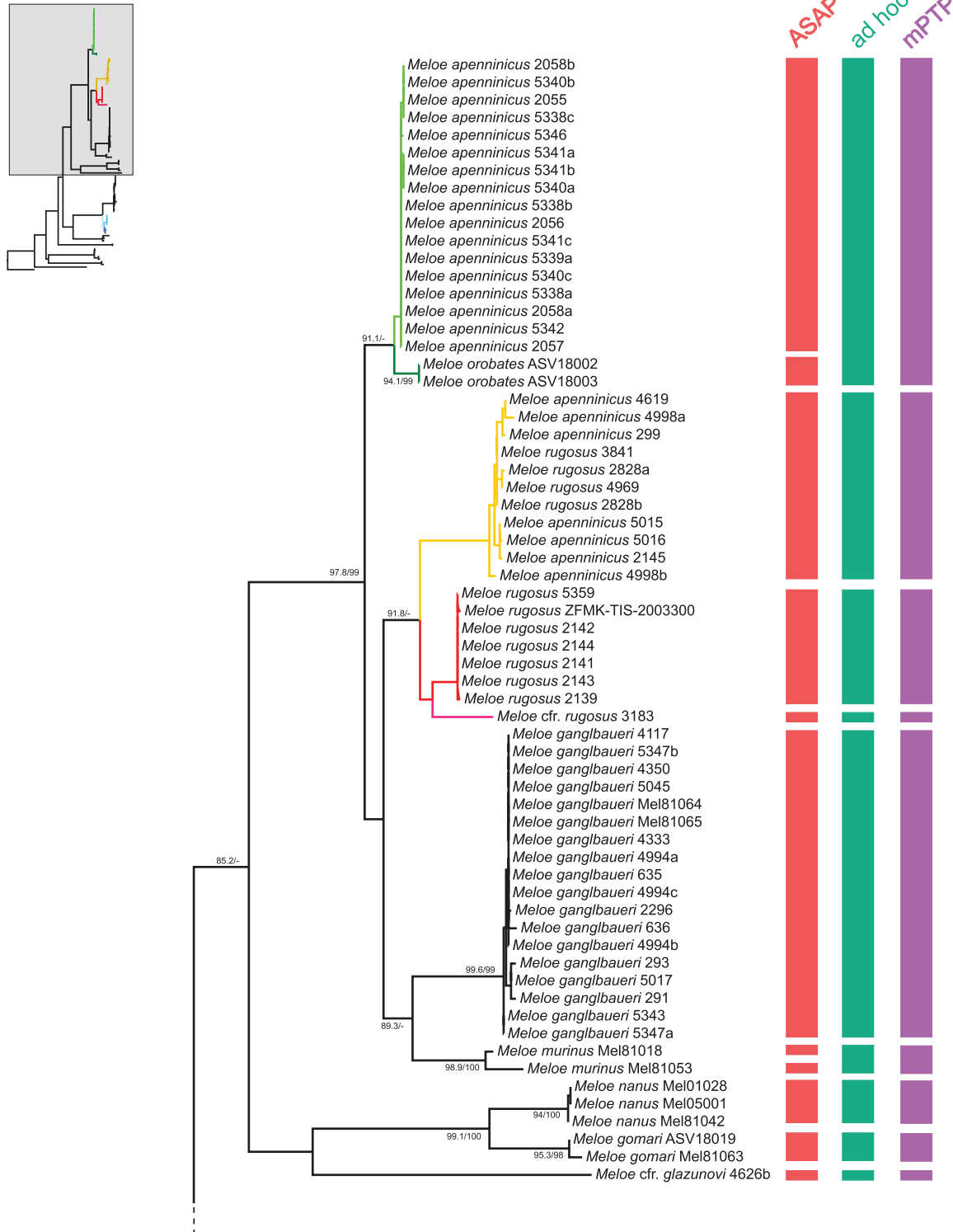
Fig. 3. Maximum likelihood tree based on COI sequences of analyzed specimens of *Meloe* (*Eurymeloe*). Only supported values of nodes (UFBootstrap, UFB ≥ 95 and SH-aLRT ≥ 80%) are reported (SH-LRT/UFB). Dashes (–) indicate non-supported values. Colored vertical bars on the right shown species delimitation analysis results (ASAP, ad hoc, and mPTP) (https://inkscape.org/it/).

Fig. 3. Continued

(MABC), and 5 ♂♂ and 9 ♀♀ (MABCa), same locality of Holotype, but 15.X.2022, M. Bologna, A. Riccieri, A. Di Giulio, L. Spagoni leg.; 3♂♂ and 2 ♀♀, Italy, Abruzzo, L'Aquila prov., Ovindoli, nr. Dolce vita parking, 1450–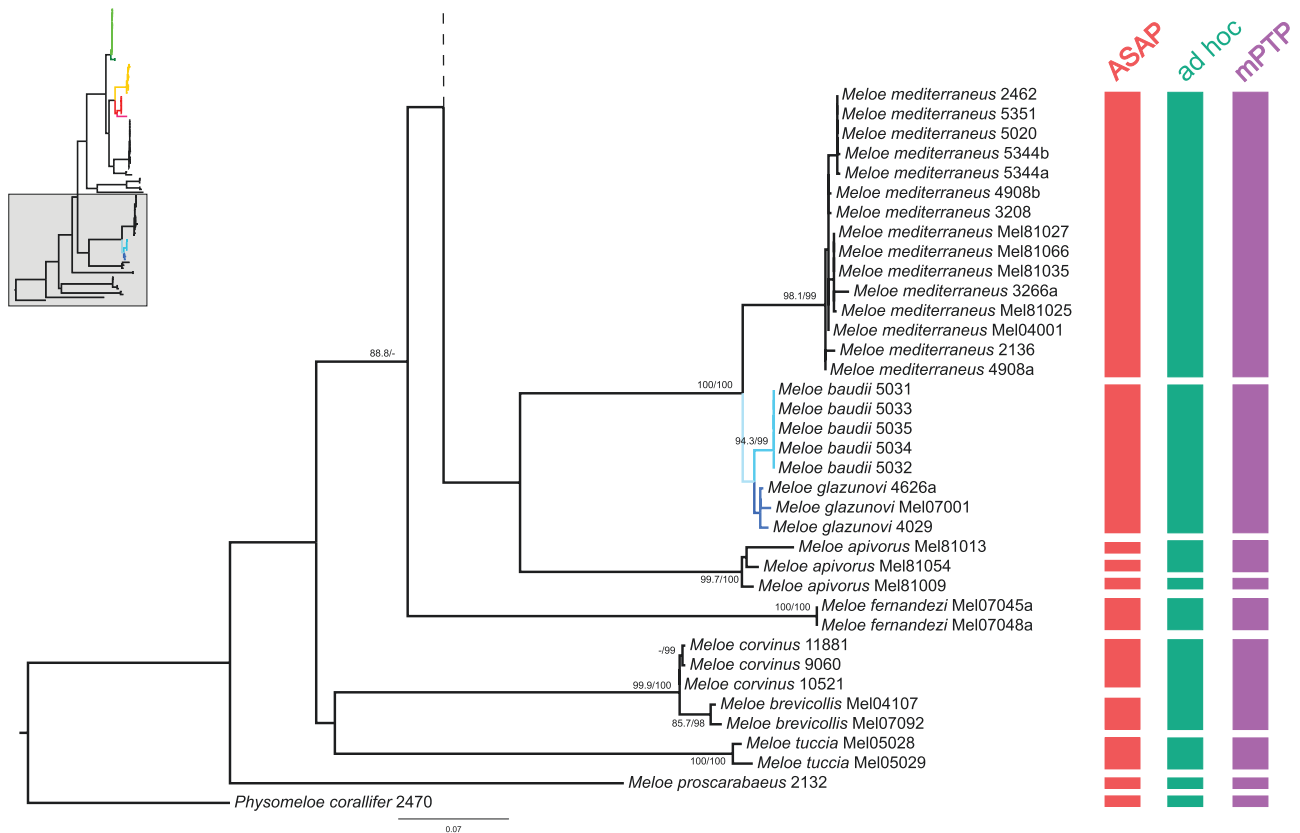1500 m, 1.XI.2010, A Di Giulio leg. (MABCa); 1 ♂ idem, 16.X.2010, A. Di Giulio leg. (MABCa); 1 ♀, idem, but 1.XI.2003, M. Bologna et al. leg. (MABC); 1 ♀, Italy, Latium, Rieti province, Duchessa Mts., 1800 m, 15.X.2022, E. Erbani, D. Lucente, M. Annessi leg. (MABCa); 1 ♂, Italy, Abruzzo, Campo Felice, La Chiesola, 1633 m, 22.X.1995, G. Pace leg. (MABC). Two additional and greatly damaged specimens (MABC) from both Monte Freddo and Dolce vita parking, were not considered as paratypes.

Diagnosis. The new species belongs to the group of *M. rugosus* (*sensu* Bologna 1988), and is very similar to 3 other species, namely, *M. apenninicus, M. rugosus,* and *M. orabates*, and it is phylogenetically very close to the last one. These 4 species, and especially *M. digiuliorum*, are distinguishable by the combination of some characters pointed out in Table 1 and Figs. 4 and 5. The main diagnostic characters of *M. digiuliorum* are: (i) dorsal setae of body black at base but light at apex, without reddish setae; (ii) male antennomere III subequal to IV, VI subequal to VII and VII subequal to VIII; (iii) pronotum pentagonal with rounded fore angles; (iv) male metatarsomere I distinctly curved ventrally, and II distinctly widened posteriorly; (v) apical lobes of male gonoforceps in dorsal view narrow and slightly convergent and intermediate space suboval and elongate rather than narrow; (vi) male gonoforceps in lateral view cylindrical until basal 1/2 rather than 2/3 and lobes progressively tapered and without lateral depression; (vii) gonocoxal plate very wide in both lateral and dorsal view; and (viii) aedeagal hooks elongate and robust, slightly distanced and proximal one distinctly inclined.

Male and female description. Body black, almost shiny; setae sparse, short, and erect, black but at apex light. Maximal length of body (from apex of labrum to apex of abdomen): 8–15.5 mm.

Head (Fig. 4B) with temples almost parallel and slightly widened posteriorly, where are widely rounded (holotype: max. width on temples 2.3 mm; max. length from frontal suture to occiput 2.l mm); eye ca. as wide as antennomere I length, convex; frontal suture only slightly obtusely incised in middle; punctures slightly deep and points scarcely confluent, intermediate surface shiny; middle longitudinal frontal furrow deep and extended from frontoclypeal suture to middle of the head or more posteriorly at bottom of a depression; clypeus subexagonal, transverse, smooth on the fore surface; labrum distinctly incised on fore margin; mandibles robust, extended over labrum. Antennae (Fig. 4B) slender (slighter in female), extended to fore third of elytra; antennomere I ca. twice as long as II, as subequal in length to III; III subequal to IV; VI subequal to VII; VII subequal to VIII; IX-X more cylindrical, narrower, and slightly longer than previous; and XI ca. 1.5 as long as X.

Pronotum (Fig. 4B) subpentagonal, as an inverted trapezoid, fore and posterior angles blunt (Holotype: max. width on fore angles 2.2 mm; max. length l.4 mm); middle depression slightly distinct, especially in fore middle; basal depression narrow and not extended on sides; fore-lateral depressions suboval, scarcely oblique; and punctuation similar than on head even if a little more confluent. Prosternum quite long and elongate posteriorly. Mesosternum with lateral portions quite inclined. Elytra (Fig. 4B) is only slightly convex dorsally, with humeral depressions evident and suboblique, rugosities scarcely raised and slightly shiny. Legs quite robust with setae short, robust; both protibial and mesotibial spurs pointed, inner metatibial spur slender and pointed, the outer

Table 1. Distinctive characters among 4 species of *Meloe* (*Eurymeloe*): *M. orobates*, *M. digiuliorum*, *M. apenninicus*, and *M. rugosus*. Characters that distinctly differ among the species are indicated in *Italic*.

Characters/Species	*M. orobates*	*M. digiuliorum*	*M. apenninicus*	*M. rugosus*
1. Dorsal setae of body	*Reddish, slightly tufted along the posterior margin of tergite*	*At base black, light at apex*	*Black, but in a few individuals mixed reddish*	*Black, but in a few individuals mixed reddish*
2. Frontal suture	Only few V-shaped	Only few V-shaped	Only few V-shaped	*Evidently V-shaped*
3. Head and pronotum punctures	Deep, distanced, slightly confluent	Slightly deep and slightly confluent	Slightly deep and slightly confluent	Deep and confluent
4. Length of male antennomere III	Subequal to IV	Subequal to IV	Subequal to IV	*Shorter than IV*
5. Length of male antennomere VI	Slightly shorter than VII	Subequal to VII	Subequal to VII	*Shorter than VII*
6. Length of male antennomere VII	Subequal to VIII	Subequal to VIII	Subequal to VIII	*Shorter than VIII*
7. Middle furrow of pronotum	Slightly distinct and only anteriorly	Slightly distinct and only anteriorly	Slightly distinct and only anteriorly	*Well distinct and extended until the base*
8. Basal depression of pronotum	Not extended on sides and narrow	Not extended on sides and narrow	Not extended on sides and narrow	*Extended also on sides and slightly wider*
9. Fore-lateral depressions of pronotum	*Almost absent in male, slightly distinct in female*	Suboval and scarcely oblique	Suboval and scarcely oblique	Oblique, less evident
10. Shape of pronotum and of their fore and posterior angles	*Transverse, rounded*	Pentagonal, rounded	Pentagonal, rounded	*Pentagonal, prominent*
11. Shape of prosternum	Shorter and scarcely elongated posteriorly	Longer and elongated posteriorly	Shorter and scarcely elongated posteriorly	Longer but not distinctly elongated posteriorly
12. Shape of mesosternum	*Lateral portions less inclined*	Lateral portions more inclined	*Lateral portions less inclined*	Lateral portions more inclined
13. Elytral rugosities	*Raised, slightly foveolate, shiny*	Few raised e slightly shiny	Few raised e slightly shiny	*Raised and shiny*
14. Male metatarsomere I in lateral view	Scarcely curved ventrally	*More curved ventrally*	Scarcely curved ventrally	Scarcely curved ventrally
15. Tergal plate	*Rugose*	Shagreened, scarcely rugose	Shagreened, scarcely rugose	*Rugose*
16. Male metatarsomeres II–III in dorsal view	Scarcely widened posteriorly?	*Widened posteriorly*	Scarcely widened posteriorly	Scarcely widened posteriorly
17. Gonoforceps in dorsal view	*Apical lobes very narrow and slender, not convergent apically, intermediate space drop-like at base*	*Apical lobes narrow and slightly convergent apically, intermediate space elongate suboval*	*Apical lobes relatively robust, and parallel, intermediate space narrow*	*Apical lobes relatively robust, vaguely convergent at apex, intermediate space distinctly narrow*
18. Gonoforceps and gonocoxal plate in lateral view	*Cylindrical until basal 2/3, apical lobes slightly robust, slightly curved, without lateral depression; gonocoxal plate narrow*	*Cylindrical until basal 1/2, apical lobes quite robust, progressively tapered, without lateral depression; gonocoxal plate very wide*	*Cylindrical until basal 2/3, apical lobes quite robust, more suddenly tapered, with a distinct and deep lateral depression; gonocoxal plate narrow*	*Cylindrical until basal 2/3, apical lobes quite robust and progressively tapered with a light lateral depression; gonocoxal plate slightly wide*
19. Gonocoxal plate in dorsal view	Not distinctly widened, suboval	*Wide, sides almost parallel in middle and posteriorly distinctly narrowed*	Not distinctly widened, suboval	Not distinctly widened, suboval
20. Aedeagal hooks	Short, distinctly distanced, similarly inclined	*Elongate and robust, slightly distanced, proximal one more inclined*	*Slightly elongate and robust, slightly distanced, proximal one more inclined and curved apically*	Short, distinctly distanced, proximal one slightly more inclined

Table 2. Distinctive characters among 2 species of *Meloe* (*Eurymeloe*): *M. b. baudii*, *M. b. glazunovi*, and *M. scabriusculus*. Characters that distinctly differ among the species are indicated in *Italic*.

Characters/Species	*M. b. baudii*	*M. b. glazunovi*	*M. scabriusculus*
1. Integument color	Piceous black	Piceous black	*Black-bluish or slate*
2. Head punctures	Usually slightly deep and not confluent	*Deep and usually confluent* (except in S Turkey populations)	Usually slightly deep and not confluent
3. Shape of temples	Widened and rounded	*Distinctly widened* (except in S turkey populations)	Widened and rounded
4. Length of antennomere III	Longer than IV (except few exceptions)	*As long as IV*	Longer than IV
5. Length of antennomeres IV–VI	Subcylindrical, longer than wide	Subcylindrical, longer than wide	*Pearliform, ca as wide as long*
6. Pronotum punctures	Usually slightly deep and not confluent	*Deep and usually confluent* (except in S Turkey populations)	Usually slightly deep and not confluent
7. Shape of pronotum	Subpentagonal but fore sides inclined	Subpentagonal but fore sides inclined	*Subpentagonal but fore sides more rounded; more convex on sides*
8. Middle longitudinal furrow of pronotum	Just outlined	*Usually deep and well outlined*	Just outlined
9. Elytral side	30° angulated along most of the side	30° angulated along most of the side	*Angulated only at basal third, posteriorly rounded*
10. Elytral surface	Slightly convex	Slightly convex	*Distinctly convex*
11. Elytral rugosities	*Moderately raised*	*Distinctly raised*	*Usually slightly raised*
12. Humeral depression	Oblique and well visible	Oblique and well visible	*Transverse and few visible*
12. Tergum surface	Slightly rugose	Slightly rugose	*Smooth*
13. Presence of tergal plate	*Rarely distinguishable*	*Distinguishable*	*Well visible on all*
14. Gonoforceps in dorsal view	*Inner margin of gonoforceps almost parallel, both in the basal third and pre-apically, apical lobes only quite slender, intermediate space long and narrow, with parallel margin*	*Inner margin of gonoforceps slightly converging in the basal third and before the apex, apical lobes quite robust, distinctly depressed on inner side, intermediate space long and narrow, converging pre-apically*	*Inner margin of gonoforceps relatively close in the basal third, and emarginate before the apex, apical lobes quite robust, distinctly depressed on inner side*
15. Gonoforceps in lateral view	*Subcylindrical until 2/3, very narrow, apical lobes almost straight and very narrow, with a distinct lateral and elongate depression extended to the middle*	*Subcylindrical until 2/3, relatively narrow, apical lobes tapered, with a distinct lateral and elongate depression extended to the basal third depression*	*Subcylindrical until 2/3, robust, apical lobes robust and conically narrowed, with a distinct lateral quite short depression, extended in the apical half*
16. Gonocoxal plate in dorsal view	Not widened, parallel on sides, elongate	Not widened, parallel on sides, elongate	*Widened, sides rounded*
17. Aedeagal hooks	Relatively distanced, robust, relatively similar in size, equally inclined	*Relatively distanced, robust, equally inclined but the proximal one more robust than the distal one, curved apically rather than straight*	Relatively distanced, robust, the proximal one almost 1.5 as long as the distal one, similar in shape, equally inclined

one spoon-like, but pointed at apex; male metasomeres II–III in dorsal view widened posteriorly; and male metatarsomere I in lateral view quite curved ventrally.

Abdominal sclerotized plate of tergite shagreened and scarcely rugose. Posterior margin of last ventrite is distinctly sinuate in males, and rounded in female. *Spiculum gastrale* elongate. Gonoforceps in dorsal view (Fig. 5D) narrow, divided into 2 lobes almost in the entire apical half, lobes apically tapered and slightly convergent; gonoforceps in lateral view (Fig. 5E) cylindrical until basal half, with a long laterodistal depression, apical lobes tapered in the apical half.

Aedeagus ([Fig. 5F](#)) with hooks quite long, distanced, proximal one more inclined.

Female differs because of the last ventrite, which is rounded rather than sinuate and the antennomeres are slightly slenderer.

Etymology. The new species is named after the late Giovanni Di Giulio, artist and entomologist, and his brother Andrea (Roma), a friend and colleague, who collected near Ovindoli (Abruzzo) the first specimens of the new species, and the uncommon endemic *M. baudii*, described from the close village of Cerchio, stimulating us to continue our research in that area.

Discussion. *M. digiuliorum* sp. n. until now was confused with *M. apenninicus* ([Bologna 2005](#); [Di Giulio et al. 2017](#)), which is also endemic to Italy and sympatric in Latium and Abruzzo, regions where it usually lives at lower altitudes (see below). Molecular analyses pointed out the differences of *M. digiuliorum* with *M. apenninicus*, as well as with *M. rugosus* and *Meloe* sp. cfr. *rugosus* from E
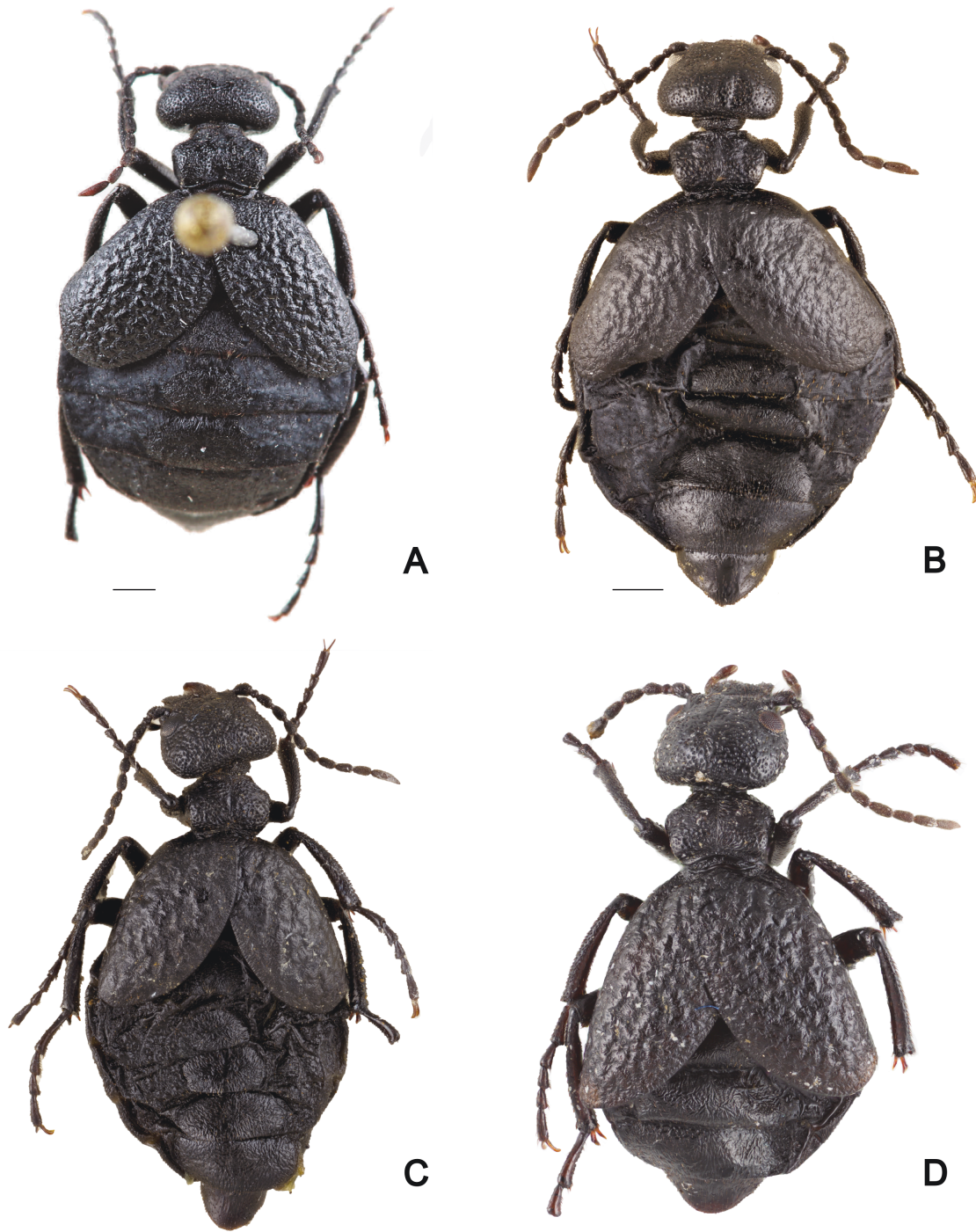

Fig. 4. Male habitus, dorsal view, of (A) *M. orobates* (from: [Sánchez-Vialas et al. 2022](#)), (B) *M. digiuliorum* sp. n., (C) *M. apenninicus*, (D) *M. rugosus*. Scale bar 1 mm (https://inkscape.org/it/).

Turkey (Malatya province, MABC, MABCa). At the same time, molecular phylogenetic results showed affinities with *M. orobates*, recently described from central Spain (Sánchez-Vialas et al. 2022). Morphological observation pointed out differences among the 4 European species especially considering male genitalia (Table 1; Figs. 4 and 5).

Biology. The new species is a middle mountain element of Central Apennines, where until now it was recorded only from 4 very close localities in the Velino–Duchessa range. It is related to mountain-derived pastures from Beech forests (*Fagus sylvatica* Linnaeus), between 1400 and 1800 m a.s.l., on both mesic or more thermophilic slopes (Fig. 1). Adults are active between October and November, but probably they live also in spring as other species of the group. Adults were collected both under stones and walking on the grass and feeding on leaves of Asteraceae; they are probably both nocturnal and diurnal as the remaining species of the group *rugosus*.

The single female collected at the Duchessa Mts., laid a mass of orange eggs 10 days after its collection (24.X.2022), and first-instar larvae hatched 18 days after. This development period is a little shorter than that one recorded by Di Giulio et al. (2013, as *M. apenninicus*: 25 days). These authors erroneously described the first-instar larvae of *M. digiuliorum* as *M. apenninicus*; they mixed these larvae with those of the true *M. apenninicus* (15 specimens) from Calabria (Sila Mts., Lorica) and Sicily (Nebrodi Mts., nr. Cesarò, type locality), found on bees or by netting flowers. Differences between larvae of these species need to be defined.

Taxonomy, distribution, and ecology of *M. apenninicus, M. rugosus,* and *M. orobates*

Meloe (Eurymeloe) apenninicus Bologna, 1988 (Figs. 4C and 5G–I)

Meloe (Eurymeloe) apenninicus Bologna, 1988: 20, Figs. 13, 26, 41, 53, 55, 70–73; Bologna, 1991: 315, Figs. 108g–l.
Eurymeloe (Bolognaia) apenninicus, Sánchez-Vialas et al. 2022: 28.

Material examined. (All from Italy) Holotype ♂ and 58 paratypes ♂♂ and ♀♀ housed in MABC (see Bologna 1988), and the following additional specimens (MABC and MABCa if not differently indicated): 4 exx., Piedmont, Turin prov., Torino Millerose; 1 ex., Piedmont, Turin prov., Ivrea; 1 ex., Lombardy, Sondrio prov., Berbenno, Mt. Caldenno; 1 ex., Lombardy, Sondrio prov., Mazzo; 1 ex., Emilia-Romagna, Forlì-Cesena prov., Ris. Nat, Sasso Fratino; 1 ex., Emilia-Romagna, Forlì-Cesena prov., Campigna.; 1 ex., Tuscany, Siena prov., Mt. Amiata, Arcidosso, Capenti; 1 ex., Abruzzo, Pescara prov., Capo Pescara; 1 ex., Abruzzo, L'Aquila prov., Castel di Sangro; 1 ex., Abruzzo, L'Aquila prov., Simbruini Mts., Camporotondo; 7 exx., Latium, Rome prov., Colli Albani, Vivaro-Doganella; 1 ex., Basilicata, Potenza prov., M. Volturino (Florence Museum); 1 ex., Basilicata, Potenza prov., Vaglio; 1 ex., Basilicata, Potenza prov., N. P. Pollino, Caramola Mt.; 1 ex., Calabria, Cosenza prov., Gariglione Mt.; 1 ex., Calabria, Reggio di Calabria prov., Piani di Aspromonte (Florence Museum); 5 exx., Sicily, Palermo prov., Madonie Mts., Piano Battaglia; 1 ex., Sicily, Palermo prov., btw. Geraci and Castelbuono; 2 exx., Sicily, Palermo prov., San Mauro Castelverde,
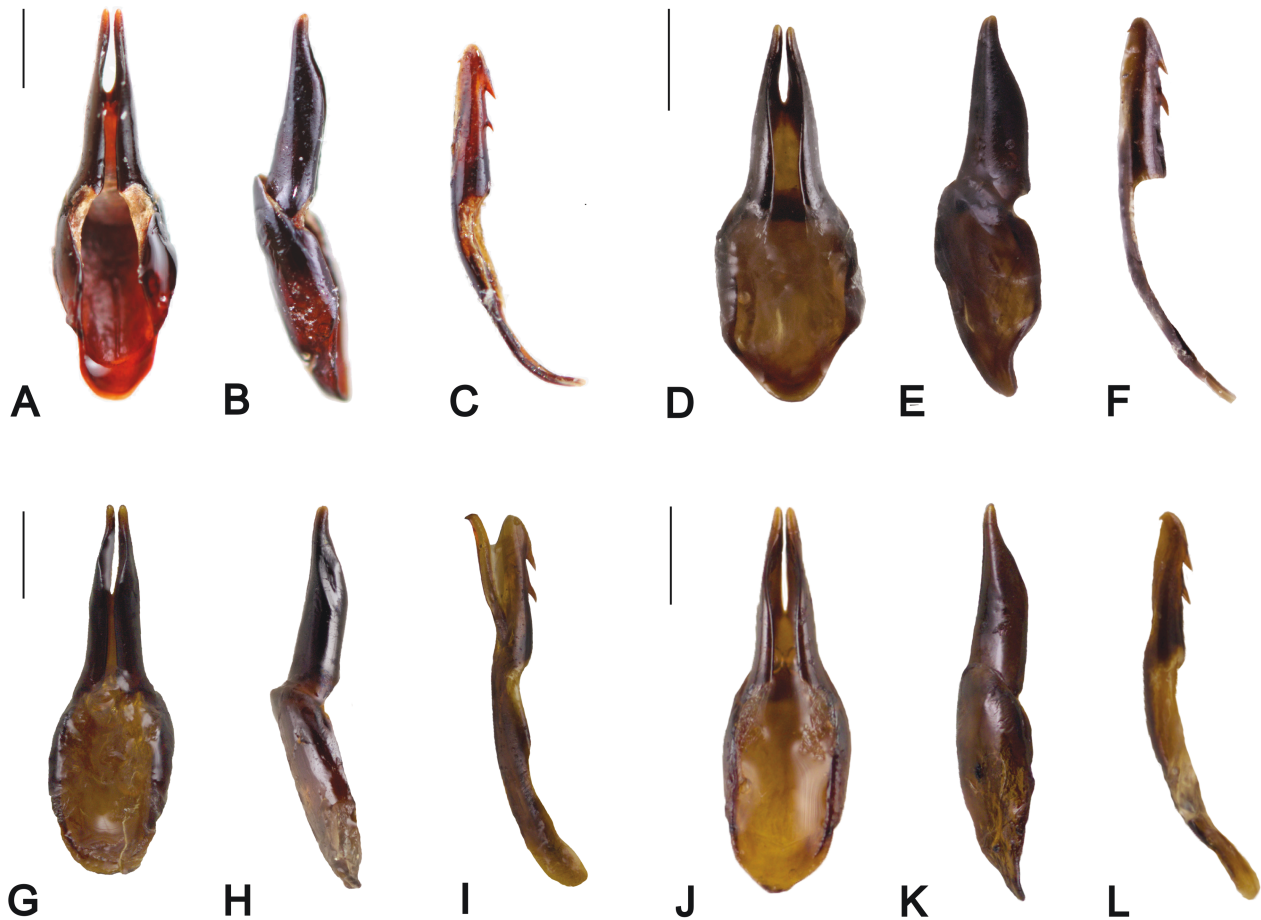

Fig. 5. Dorsal and lateral views of male genitalia: (A–C) *M. orobates* (from: Sánchez-Vialas et al. 2022), (D–F) *M. digiuliorum* sp. n., (G–I) *M. apenninicus*, (J–L) *M. rugosus*. Scale bar 0.5 mm (https://inkscape.org/it/).

Cernitello. Some of these new records better define the distribution of this endemism, especially in southern Italy.

Distribution. Species endemic to the Italian peninsula and Sicily. It seems distributed in NW Italy and along the Apennines as well as on the volcanic mountains of western peninsula (Amiata Mt., Colli Albani), at least from Emilia-Romagna south to Calabria, and in northern Sicily. Records from Piedmont and Lombardy, previously referred to *M. rugosus* (Bologna 2005, 2020a), are now referred to *M. apenninicus* after the present molecular and morphological studies. In Central Apennines, it is sympatric and probably syntopic with *M. digiuliorum*, an uncommon condition between close endemic species.

Biology. The ecology of this species was summarized by Bologna (1988, 1991). Distributed in NW Italy both at low (100–600 m a.s.l.) and high elevation (1700 m a.s.l.); in central Italy, it was recorded from 650 to 1500 m a.s.l., while in Sicily records are especially thickened between 1200 and 1800 m a.s.l.

Meloe (Eurymeloe) rugosus Marsham, 1802 (**Figs. 4D** and **5J–L**)
Meloe rugosa Marsham, 1802: 483.
Meloe rugosus, Müller, 1925: 22–24.
Meloe (Eurymeloe) rugosus, Bologna, 1988: 246, Figs. 12, 25, 40, 54, 67–69; Bologna, 1991: 313, Figs. 108a–f.
Eurymeloe (Bolognaia) rugosus, Sánchez-Vialas et al. 2022: 29.

Material examined. (MABC and MABCa if not differently indicated) 1 Neotype and 3 syntypes without locality but probably England (Natural History Museum, London) (see Bologna, 1988); 1 ex. (greatly damaged), the United Kingdom, England, Bristol, Ashton Court; 4 exx., Germany, Bensheim-Zell, Knodener-Hoehenweg; 1 ex., Germany, Bensheim-Auerbach, Fürstenlager; 1 ex., Austria, L. Bach, Neudörtal; 1 ex., Romania, Transilvania, Nucşoara; 1 ex., Greece, Karpenisi, Timphristos Mt., Sky center; 1 ex., Greece, Vitina; 1 ex., Greece, Viotia, Parnassos Mt.; 1 ex., Greece, Korinthias, Kyllini Mts., Kataphygion; 1 ex., Greece, Peloponnese, Taygetos Mt., Kataphygion; 1 ex., Turkey, Kastamonu, Masruf geç.; 1 ex., Turkey, Çorum, 9 km NE Çorum; 1 ex., Turkey, Çorum, pass N Iskilip; 1 ex., Turkey, Gümüshane, pass N Şiran; 1 ex., Adana, Otrukbeli geç.; 1 ex., Kaharamanmaraş, Baskonuş; 1 ex., Turkey, Adıyaman, Nemrut Dagı. Turkish specimens need to be checked with a molecular approach (see below).

Distribution. This species was recorded from England, Spain, and France, central and eastern Europe, and from Turkey to Central Asia. Records from Spain are restricted to northern and Madrid provinces (García-París et al. 2006). Possibly, records from the Turanian area refer to *M. glazunovi*, but in general, all records from Balkans, Turkey, and Caucasus, need a detailed revision using molecular techniques and new morphological characters.

Biology. Bologna (1988, 1991) summarized the information about the biology of this species. In central and eastern Europe, it is distributed in plain and hill regions, while in Greece and Turkey, it seems an orophilic element (1500–2100 m a.s.l.).

Meloe (Eurymeloe) orobates (Sánchez-Vialas, Ruiz, Recuero, Gutiérrez-Pérez & García-París, 2022) comb. n. (**Figs. 4A** and **5A–C** from Sánchez-Vialas et al. 2022)
Eurymeloe (Bolognaia) orobates Sánchez-Vialas et al. 2022: 33, Figs. 2I, 3A–D, 4A–E, 5A–C, 6B.

No specimens of this mountain species from Spain have been studied in the present work. However, we analyzed one specimen (MABC) from Malaga province (E Barranco de Madera) similar to *M. orobates* especially because of the pronotum shape but lacking the characteristic reddish setation. Therefore, its possible attribution to

M. orobates needs further confirmation. We refer to Sánchez-Vialas et al. (2022) for morphological and biological information.

Taxonomy and relationships of *M. (E.) baudii* and allied species

Before its description, *Meloe baudii* was repeatedly confused with *M. rugosus, M. glazunovi,* and *M. scabriusculus,* lacking in Italy (see Bologna 1988, 1991).

Our results point to identify *M. baudii* and *M. glazunovi* as the same species despite the presence of some morphological differences and the estimated 0.6 Mya of divergence. Consequently, the whole distribution of the species extends from Italy to Central Asia. Diagnostic characters between the 2 subspecies and with *M. scabriusculus* are listed in Table 2 and Figs. 6 and 7.

In particular, *M. b. baudii* differs from *M. b. glazunovi* because of: (i) head and pronotum punctures slightly deep and not confluent rather than deep and usually confluent; (ii) antennomere III longer than IV; (iii) middle longitudinal furrow of pronotum just outlined rather than deep and well visible; (iv) male gonoforceps in dorsal view with inner margin almost parallel, both in the basal third and pre-apically, apical lobes only quite slender, intermediate space between gonoforceps long and narrow, with parallel margin; apical lobes in lateral view almost straight and very narrow rather than tapered; (v) aedeagal hooks both robust, similar and straight, rather than proximal one more robust than the distal one, curved apically. *M. baudii* s.l. differs from *M. scabriusculus* because of the integument color black rather than dark bluish, the shape of antennomeres less moniliform, pronotum less rounded, and elytra.

Meloe (Eurymeloe) baudii baudii Leoni, 1907 (**Figs. 6A** and **7A–C**)
Meloe baudii Leoni, 1907: 353; Müller, 1925: 22–23.
Meloe (Eurymeloe) baudii, Bologna, 1988: 262, Figs. 16, 29, 44, 58, 80–82; Bologna, 1991: 309, Figs. 105f–k.
Eurymeloe (Bolognaia) baudii, Sánchez-Vialas et al. 2022: 33.

Material examined. (All from Italy; MABC and MABCa if not differently indicated) 1 Syntype ♂ and other 10 ♂♂ and ♀♀ syntypes, Abruzzo, Cerchio (Dipartimento di Scienze e Tecnologie Agro-Alimentari, Università Bologna); 1 ex., Piedmont, Turin; 3 exx., Piedmont, Alessandria prov., Novi Ligure (Museo civico di Storia naturale, Genova, MSNG); 1 ex., Lombardy, Brescia prov., Desenzano sul Garda; 1 ex., Lombardy, Cremona; 1 ex., Trentino-Alto Adige, Trento prov., Avio; 1 ex., Emilia-Romagna, Forli-Cesena prov., Massa (I. Gudenzi coll.); 1 ex., Latium, Rieti prov., Vallemare; 1 ex., Latium, Rome prov., Cerveteri; 1 ex., Latium, Latina prov., Agro Pontino (MSNG); 2 exx., Abruzzo, L'Aquila prov., Ovindoli; 6 exx., Calabria, Cosenza prov., Camigliatello silano; 1 ex., Sardinia, Sassari prov., Stintino capo Falcone; 1 ex., Olbia—Tempio Pausania prov., Aggius (D. Sechi coll.). Some of the unpublished records, better define the distribution of this endemism, especially in Romagna and Sardinia.

Distribution. *M. b. baudii* results endemic to Italy where it is distributed almost in all regions, including Sicily and Sardinia. Bologna (1988, 1991) reported generic records from Croatia (1 ex. housed at the Trieste Museum) and Slovenia (Bologna 2020b); both records are plausible because close to Italian localities of Venezia Giulia, but possibly they were confused with *M. b. glazunovi,* distributed also in E Austria, Czech Rep., and Balkans, and almost unknown at the time of previous studies. For this reason, we now leave them out.

Biology. The ecology of *M. b. baudii* was summarized by Bologna (1988, 1991). It is an uncommon even if euriecious element, distributed from the sea level to ca. 1500 m a.s.l. in the Apennines.

Meloe (Eurymeloe) baudii glazunovi Pliginsky, 1910 stat. n. (**Figs. 6B** and **7D–F**)

Meloe glazunovi Pliginsky, 1910: 170.
Meloe (*Eurymeloe*) *glazunovi*, Bologna, 1988: 243, Bologna & Di Giulio, 2013: 251.
Eurymeloe (*Bolognaia*) *glazunovi*, Sánchez-Vialas et al. 2022: 33.

Material examined. (all in MABC and MABCa if not differently indicated) No types were examined.1 ex., Austria, Neusiedl a.s.; 1 ex., Austria, Neusiedl a.s. prov., Neusfedl; 3 exx., Czech Rep., Brno prov., Ječmeniště (L. Cerny coll.; sympatric with *M. scabriusculus*); 1 ex., Greece, S Euboea, Karystos prov., nr. Vatisi; 1 ex., Greece, Korinthos prov., Sykion; 1 ex., Turkey, Bilecik; 1 ex., Turkey, Eskişehir prov., Karatepe; 1 ex., Turkey, Çorum prov., pass N Iskilip (sympatric with *M. rugosus*); 1 ex., Turkey, Çorum prov., Boğazkale; 1 ex., Turkey, Sivas prov., Beypinari; 1 ex., Turkey, Erzurum prov., 20 km W Erzurum; 2 exx., Turkey, Kars prov., nr. Susuz; 1 ex., Turkey, Bitlis, SE Yolalati, 38°14'44"N42°19'31"E;
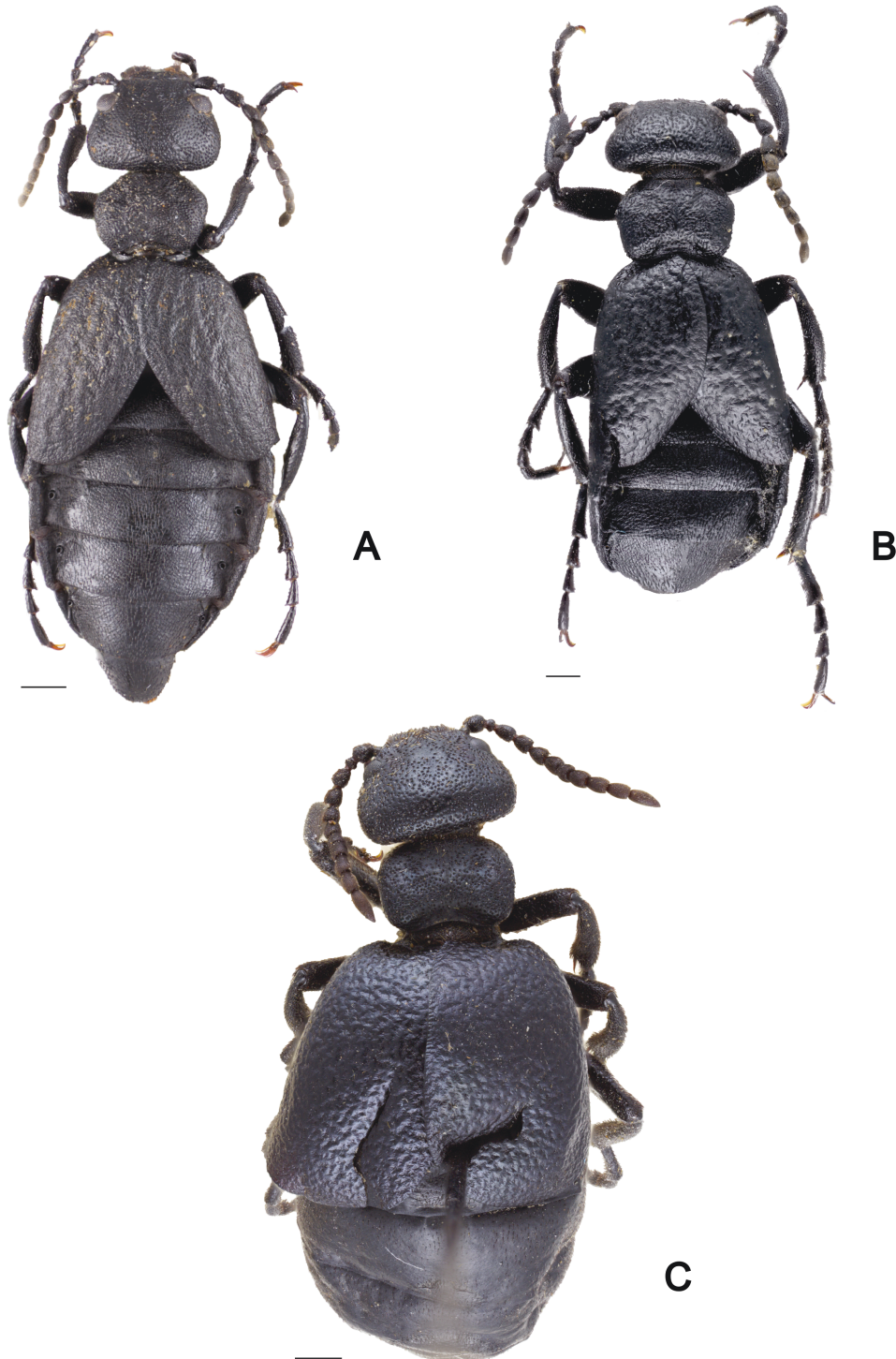
Fig. 6. Male habitus, dorsal view, of (A) *M. b. baudii*, (B) *M. b. glazunovi*, and (C) *M. scabriusculus*. Scale bar 1 mm (https://inkscape.org/it/).

2 exx., Turkey, Antalya prov., nr. Akseki; 2 exx., Turkey, Antalya prov., btw. Korkuteli and Kemer; 2 exx., Georgia, Shida Kartli prov., Gori distr.; 1 ex., Iran, Sultanabad; 2 exx., Tajikistan, Kuljab reg., Khozretisho Mt. (Florence Museum); 1 ex., Kazakhstan, SW Zailysky Mt., Keru vil; 1 ex., SE Kazakhstan, Chu-ili Ger.

Distribution. This subspecies was described (as species) from Crimea, Transcaucasia, Transcaspia, E Kazakhstan (Semirechye), and from Iran (var. *rufotarsalis*) (Pliginsky 1910). Our examined specimens from Georgia, Kazakhstan, and Iran are consequently representative of type localities. It is distributed from extreme eastern Austria (Neusiedl) to S Czeck Rep. (where it is sympatric with *M. scabriusculus*), Greece and Romania (but probably in most of the Balkan Peninsula), Crimea, S Russia, Turkey, Georgia, Armenia, N Iran, the whole Central Asia, east to Afghanistan and Kazakhstan (Bologna 2020b).

Biology. Larvae have been described by Di Giulio et al. (2013) on specimens from Turkey, Akseki (see above). Adults are active both in autumn (October–November) and in Spring (April–June). Records from eastern Europe and Georgia are at low elevations (130–700 m a.s.l.) but in northern Turkey and Kazakhstan, this species could reach more than 1800 m a.s.l..

Meloe (Eurymeloe) scabriusculus Brandt & Erichson, 1832 (Figs. 6C and 7G–I)
Meloe scabriusculus Brandt & Erichson, 1832: 125; Pliginsky, 1910: 172.

Meloe (Eurymeloe) scabriusculus, Bologna, 1988: 244; Bologna, 1991: 306, Figs. 105a–e.

Material examined. (all in MABC and MABCa if not differently indicated) No types were examined. 3 exx. (2 of which in L. Cerny coll.), Czech Rep., Brno prov., Ječmeniště (sympatric with *M. glazunovi*); 1 ex., Czech Rep., Bohemia, Klopusky; 1 ex., Czech Rep., Bohemia, Úhřetice; 1 ex., Hungaria (ill.); 1 ex., Hungaria, Budapest; 1 ex., Greece, Katerini, Olympus Mt.; 1 ex., Romania, Crudo (not detected locality); 1 ex., Turkey, Tekirdağ prov., Ballı.

Distribution. Widely distributed from E France through central and eastern Europe to Balkans, W Turkey, N Iran, S Russia, Kazakhstan, and Uzbekistan (Bologna 2020b). In eastern Europe and Balkans it is in sympatry with *M. baudii glazunovi*.

Biology. Information on this species have been published by Bologna (1991).

Discussion

The complex biogeographic history that characterizes the Italian geographic region is reflected in the composition of its biota and in the high percentage of endemic species and subspecies (more than 15%; Ruffo and Vigna Tagliani 2002, Bologna et al. 2022). This study represents an additional example of such complexity, though focused on a small group of species of insects. Indeed, we investigated 2 Italian endemic species in the wingless blister beetle genus *Meloe* of the subgenus

Fig. 7. Dorsal and lateral views of male genitalia: (A–C) *M. b. baudii*, (D–F) *M. b. glazunovi*, and (G–I) *M. scabriusculus*. Scale bar 0.5 mm (https://inkscape.org/it/).
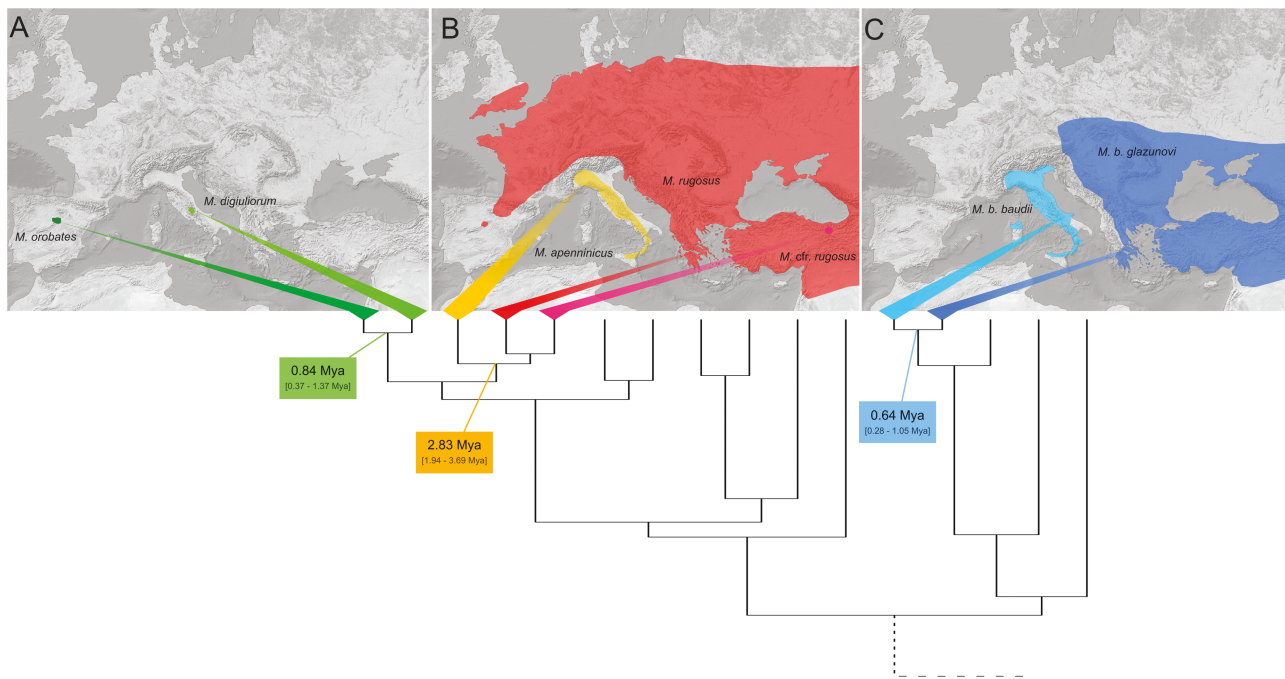
Fig. 8. Three different patterns of biogeographic disjunction and their relative molecular dating (with the 95%HPD in square brackets) observed in (A) *M. orobates* and *M. digiuliorum*; (B) *M. apenninicus* and *M. rugosus* + *M.* cfr. *rugosus*; and (C) *M. b. baudii* and *M. b. glazunovi* (https://inkscape.org/it/).

Eurymeloe, within the *M. rugosus* group (Bologna 1988) and pointed out (i) the presence of a new Italian endemic species, (ii) the conspecific status of *M. baudii* and *M. glazunovi*, and (iii) 3 different patterns of phylogenetic and biogeographic affinities. Moreover, we found 2 new lineages from Turkey and the polyphyly of *M. murinus*. These latter results will be further investigated, but highlight the presence of hidden and cryptic species that make very complex the achievement of a reliable taxonomic assessment of this group.

For a long time, several European species have been confused under the name *M. rugosus*, but were progressively distinguished as *M. ganglbaueri, M. baudii, M. glazunovi, M. mediterraneus, M. apenninicus,* and *M. orobates* (Bologna 1988, 1991, Sánchez-Vialas et al. 2022), and, after this study, as *M. digiuliorum*. In total, 2 of the 3 endemic species here analyzed, *M. digiuliorum* and *M. apenninicus*, were supported by morphological and molecular data. *M. baudii* was merged in the same partition with *M. glazunovi* in all species delimitation analyses, and the morphological differences here pointed out support the distinction of these taxa as 2 subspecies (see characters listed above; Table 2; Figs. 6 and 7).

The 3 endemic Italian taxa show distinct affinities (Fig. 2): *M. digiuliorum*, with a small range restricted to an apparently narrow area of Central Apennines, is the sister of *M. orobates*, endemic of a small mountain area of central Spain; *M. apenninicus*, distributed in the Italian peninsula and in Sicily, is close to the European-Anatolian *M. rugous* (and an undetermined Turkish specimen, here indicated as *M.* cfr. *rugosus*); *M. baudii* s.l., is represented by 2 distinct lineages, one distributed in Italy including Sicily and Sardinia, and one corresponding to the Turanian-E European *M. b. glazunovi*.

These distinct phylogenetic relationships open 3 different biogeographic scenarios, albeit in the same area and in the same group of species (Fig. 8).

The biogeographic relationships of *M. orobates* and *M. digiuliorum* reveal a possible Pleistocene (late Calabrian age, ca. 0.84 Mya; Fig. 8) vicariance event, likely during an interglacial stage,

after the fragmentation of the ancestor range and the isolation in refugia in the mountains of both Iberian and Italian peninsulas. This is an uncommon W Mediterranean distribution pattern which parallels, for example, that of the Pyrenean chamois (*Rupicapra pyrenaica* Bonaparte, 1845), a mountain mammal to which belong 3 genetically well distinct subspecies with disjunct range: *R. p. pyrenaica* and *R. p. parva* Cabrera, 1911 in Spain and *R. p. ornata* Neumann, 1899 in the Central Apennine (Pérez et al. 2002, 2022, Crestanello et al. 2009). Indeed, fossils of this species in intermediate areas support the evidence of a paleo-range more widely extended (Fioravanti et al. 2019).

The relationship between *M. apenninicus* and the complex of *M. rugosus* seems referable to a more common pattern of speciation which interested an ancestral species more widely distributed in Europe and afterward fragmented during the Plio-Pleistocene climatic fluctuations (between Piacentian and Gelasian ages, ca. 2.83 Mya; Fig. 8) in 2 subranges, trans-alpine and cis-alpine. This distribution pattern recalls that of the crested newts *Triturus cristatus* (Laurenti, 1768) and *T. carnifex* (Laurenti, 1768) (Canestrelli et al. 2012, Wielstra et al. 2013, 2019), and that of the slow worms *Anguis fragilis* Linnaeus, 1758 and *A. veronensis* Pollini, 1818 (Gvoždík et al. 2013, 2023, Jablonski et al. 2021, Dufresnes et al. 2023). Interestingly, the molecular results and the morphological revision (MABC, MABCa) carried out after the identification of additional diagnostic characters (Table 2), led us to refer to *M. apenninicus* the northern Italian populations (Piedmont and Lombardy), previously attributed to *M. rugosus*. Though the presence of *M. rugosus* especially in north-eastern Italy cannot be excluded, this result contributes to expanding the range of *M. apennincus*, who's previous northern limit was in Emilia-Romagna.

A third pattern of biogeographic disjunction is that relative to the 2 subspecies of *M. baudii* (Fig. 8). These taxa can be discriminated morphologically (see characters listed above; Table 2; Figs. 6 and 7), but molecular data denote few differences and a strict relationship

(Figs. 2 and 3). Indeed, the split between these 2 lineages seems recent, in the basal Ionian (Chibasian) age, around 0.64 Mya (Fig. 8), and occurred probably during a glaciation (MIS 16; Pope et al. 2023). The process of differentiation between *M. b. baudii* and *M. b. glazunovi* was, perhaps, slowed down by the geographic connection represented by the marine regression in the Adriatic during the glacial peaks. The observed pattern is typical of the steppe elements distributed from Central Asia to the Italian peninsula: among the numerous examples, it is worth mentioning the meadow viper, *Vipera ursinii* Bonaparte, 1835, as it shows several subspecies from the Apennines and the Balkans toward eastern areas implying an incomplete differentiation throughout its range (Ferchaud et al. 2012).

In conclusion, this article represents a contribution to improving the information on the Italian fauna of Meloidae and in particular on 3 endemic lineages. In addition, with our results we underline the relevance of integrative approaches to study biodiversity: indeed, even if in a small group, the combination of molecular and morphological traits allowed to identify new lineages that will be investigated with ongoing dedicated studies, to clarify the taxonomy of *M. baudii* and the former *M. glazunovi*, and to describe a new endemic species. To enlarge the knowledge on endemic evolutionary units as well as unveiling hidden and cryptic taxa must be a priority and represent an important step in applied conservation policies.

Supplementary material

Supplementary material is available at *Insect Systematics and Diversity* online.

Specimen Collection Statement

The authors attest that all legal and regulatory requirements, including export and import collection permits, have been followed for the collection of specimens from source populations at any international, national, regional, or other geographic level for all relevant field specimens collected as part of this study.

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Author contribution

Alessandra Riccieri (Conceptualization [equal], Data curation [equal], Formal analysis [equal], Investigation [equal], Methodology [equal], Writing—original draft [equal], Writing—review & editing [equal]), Lucrezia Spagoni (Data curation [equal], Formal analysis [equal], Methodology [equal], Writing—original draft [equal], Writing—review & editing [equal]), and Marco A. Bologna (Conceptualization [equal], Data curation [equal], Funding acquisition [lead], Methodology [equal], Writing—original draft [equal], Writing—review & editing [equal]).

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Data availability

Molecular data produced in this work are available in GenBank under the following accession numbers: *COI* OR635008-OR635077; *16S* OR635667-OR635742; *CAD* OR640161-OR640229; *28S* OR635744-OR635814. ZooBank registration for the new species *Meloe digiuliorum* is: urn:lsid:zoobank.org:act:A703C64C-FA93-4154-8624-BF3503A7D165. Input and output files and R script for species delimitation analysis are available on FigShare at https://doi.org/10.6084/m9.figshare.24235663.

Ethical statement

All samples used in this article were collected under appropriate collection permits and approved ethical guidelines.

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