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A new species of *Meligethes* (Coleoptera: Nitidulidae: Meligethinae) of the *M. lugubris* complex from Sardinia*

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Abstract

A combined morphological and bionomical analysis was performed to clarify the position of a problematic species of the *Meligethes lugubris* group from Sardinia and Corsica (Coleoptera, Nitidulidae, Meligethinae). This species-group is represented by a dozen anthophagous species associated with Lamiaceae, and distributed from North Africa to Japan. The analysis was mainly focused on the specific distinction and formal description of a new species, *M. foddaii* Audisio, De Biase & Trizzino **sp. nov.**, from Sardinia and Corsica. The species is morphologically scarcely distinguishable from the allopatric *M. lugubris* Sturm and *M. gagathinus* Erichson (both widespread in southern Europe). An identification key to Euro-Mediterranean members of the *M. lugubris* complex is provided. Combined morphological, ecological, phenological, and preliminary molecular data are presented to support the distinction of the new species. The palaeogeographical scenario explaining the likely Plio-Pleistocene differentiation of the three species, which are all associated with the related Lamiaceae genera *Mentha* and *Thymus*, is also briefly discussed.

Key words: Pollen beetles, *Meligethes*, new species, Corso-Sardinia, palaeogeography

Introduction

Meligethes Stephens, 1830 is the largest genus of Nitidulidae, and includes, worldwide, some 600 pollen-feeding species (Audisio 1993). They are to be found in association with flowers of a huge number of species of several botanical families. Most of the known species are distributed in the Palaearctic and Afrotropical Regions, with more than 200 and probably more than 300 species, respectively (Audisio 1993). Recent molecular data (Trizzino *et al*. 2008) strongly support the delimitation of *Meligethes* into a monophyletic clade including only the [M. atratus (Olivier) + M. denticulatus (Heer)] group (~35 described and undescribed species, mostly from the East Palaearctic; associated with Rosaceae during larval development), close to the Meligethes aeneus (Fabricius) group (~40 Palaearctic and Nearctic species; associated with Brassicaceae during larval development). The type species of *Meligethes* is, in fact, the European *M. atratus*. One of the natural assemblages within the present-day *Meligethes* is the *M. umbrosus* Sturm + *M. obscurus* Erichson + *M. lugubris* Sturm + *M. exilis* Sturm species-groups (globally including some 50 Palaearctic species, all strictly associated with Lamiaceae during larval development), probably representing a monophyletic lineage needing description as a pair of separate genera (Audisio et at 2009).

Members of the *Meligethes lugubris* group, including a dozen species distributed from North Africa to China and Japan (Easton 1954; Kirejtshuk 1992; Audisio 1993; Jelínek & Audisio 2007) are mostly

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associated with flowers of *Mentha* (Lamiaceae), and live in wet habitats. Two exceptions are the rare and isolated N and NE European species *Meligethes norvegicus* Easton, and the closely related *M. abiens* Kirejtshuk, both associated with *Dracocephalum* (Lamiaceae) in moderately xeric meadows (Audisio 1993, and unpublished data). The only other known exception is represented by *Meligethes lugubris*, an uncommon Sibero-European species widespread from NE Spain to eastern Russia, which is associated with moderately xeric meadows, and developing exclusively on *Thymus* (Lamiaceae) (Audisio 1993). As first discussed by Audisio (1993), the Sardinian populations of *M. lugubris* are morphologically intermediate between the true *Meligethes lugubris* and the closely related *M. gagathinus* Erichson. Indeed, these populations combine a small, narrow and parallel-sided body shape, and male genitalia similar to those of the true *M. lugubris*, with a markedly larger and more heavily pigmented tegmen. These populations also possess a much shinier dorsal surface, as in *M. gagathinus* (absent in Sardinia) and the related *M. submetallicus* Sainte-Claire Deville (common in Sardinia). In addition, Sardinian populations are present exclusively in wet places where they develop on *Mentha* spp. (Lamiaceae), especially on the subendemic *M. insularis* Requien. The taxonomic status and specific identification of these populations of "*M. lugubris/gagathinus*" from Sardinia and Corsica has so far remained unresolved. Herein, we analyse the status of these populations based also on preliminary data from molecular markers, to definitively ascertain the true distribution of *M. lugubris* and its allies (i.e., members of the *M. lugubris* complex within the larger *M. lugubris* species-group) in the Corso-Sardinian plate. We sequenced a fragment of the mitochondrial DNA (mtDNA) cytochrome oxidase I (COI). The latter has been successfully used both for insect population studies and for species distinction (Simon *et al.* 1994).

Molecular methods and material

Our preliminary molecular analyses were focused on two specimens of *M. gagathinus* from peninsular Italy (Liguria, Savona province, Quiliano, 16 m, 11.VI.2008; northern Latium, Rieti province, Ripa Sottile Lake, 410 m, 8.VIII.2008) and four specimens of *M. foddaii* sp. nov. (SW Sardinia, Medio Campidano province, Siuru Lake near Domusnovas, 390 m, 19.VI.2008).

After DNA isolation, using the polymerase chain reaction (PCR), a fragment of the mitochondrial cytochrome c oxidase subunit I (COI) was amplified and sequenced (see De Biase *et al.* 2003 and references therein for experimental procedures).

Sequences were aligned using Staden Package v. 1.6.0 (Bonfield *et al.* 2005) and analyzed using MEGA v. 3.1 (Kumar *et al.* 2004).

Abbreviations

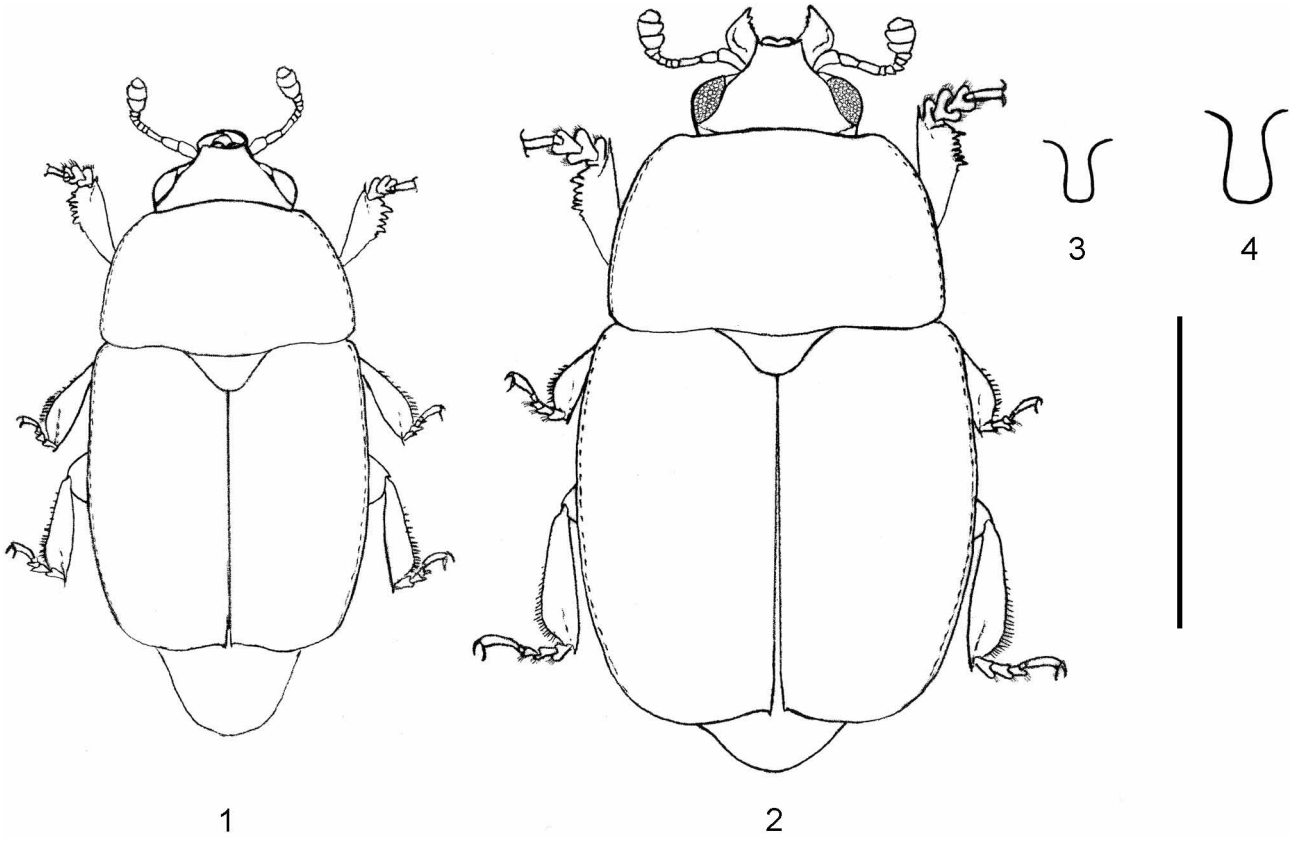
CAR	P. Audisio collection, "La Sapienza" University, Rome, Italy.
CNBFVR	Centro Nazionale per lo Studio e la Conservazione della Biodiversità Forestale "Bosco Fontana", Verona, Italy.
NMP	National Museum, Prague, Czech Republic.
ex	specimen/s

Meligethes foddaii Audisio, De Biase & Trizzino, sp. nov.

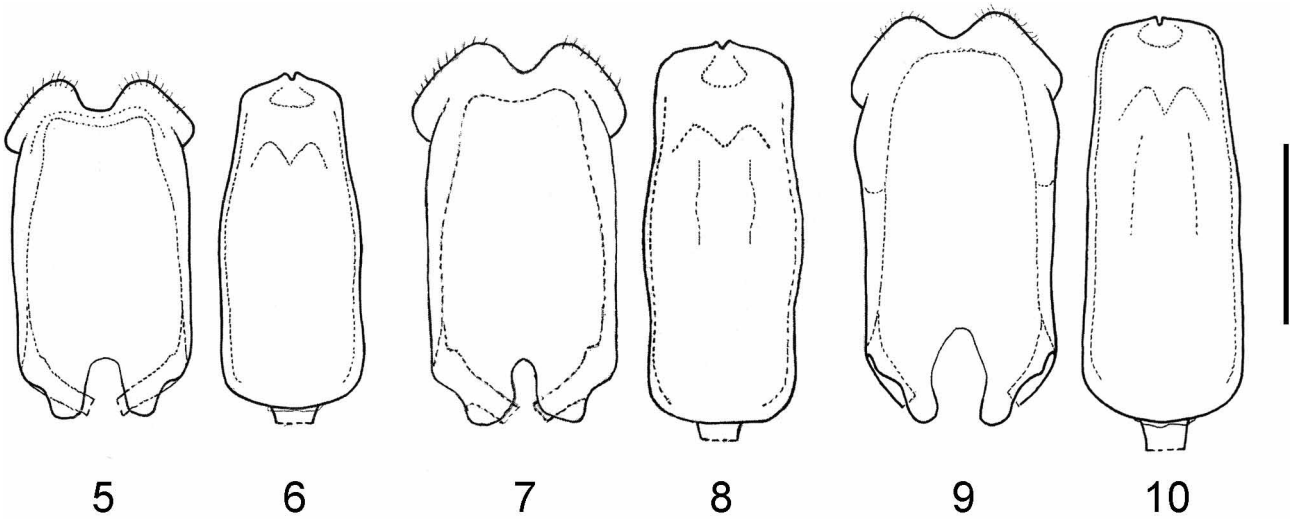
(Figs 1, 3, 7–8)

Diagnosis. Small-sized (length 1.78–2.02 mm), body shiny black, legs and antennae blackish to chestnut brown. Maximum pronotal width near posterior angles (Fig. 1). Similar to the widespread European species *M. lugubris* recently re-described by Audisio (1993), but with interspaces between dorsal punctures on

pronotum and most of elytra smooth and shining. Median lobe of aedeagus distinct, slightly more parallel-sided distad (Fig. 8), and tegmen darker, distinctly more pigmented than median lobe. Differentiated from the closely related *M. gagathinus* by smaller, more elongate, and more parallel-sided body (length 2.1–2.6 mm in *M. gagathinus*, 1.8–2.0 in *M. foddaii* sp. nov.), elytral sides less arcuate (Figs 1–2), more extruded paramere apices (Figs 7, 9), and more narrow prosternal process (Figs 3–4).



FIGURES 1–4. *Meligethes* spp. **1–2.** Male habitus. **1.** *Meligethes foddaii* sp. nov. (paratype, Sardinia, Lago Siuru). **2.** *Meligethes gagathinus* (Italy, Latium, Rieti province, Monteleone Sabino, 300 m). **3–4.** Outline of male prosternal process in ventral view. **3.** *Meligethes foddaii* sp. nov. (paratype, Sardinia, Lago Siuru). **4.** *Meligethes gagathinus* (Latium, Monteleone Sabino). Scale bar: 1 mm.



FIGURES 5–10. Aedeagus and tegmen of *Meligethes* spp. (dorsal view). **5–6.** *Meligethes lugubris* (Italy, Lombardy, Sondrio province, Lovero Valtellino, 530 m). **7–8.** *Meligethes foddaii* sp. nov (paratype, Sardinia, Lago Siuru). **9–10.** *Meligethes gagathinus* (Italy, Latium, Rieti province, Monteleone Sabino, 300 m). Scale bar: 0.2 mm.

Type material. Holotype ♂: Italy, Sardinia, Medio Campidano province, Domusnovas, Lago Siuru, 390 m, 39.22.12 N, 8.36.59 E, 19.V.2008, P. Audisio, M. Trizzino, G. Nardi & M. Bardiani leg., on apical (non-flowered) stems of *Mentha insularis* Requien (= *Mentha suaveolens* Ehrh. subsp. *insularis* (Req.) Greuter; Lamiaceae) (CAR).

Paratypes: same data as holotype, 4 ♂♂, 5 ♀♀ (CAR, NMP); Italy, Sardinia, Medio Campidano province, Villacidro, near Montimannu dam, 256 m, 39.24.02 N, 8.41.56 E, 6.IX.2006, G. Nardi leg., grassnetting on flowers of *Mentha insularis*, 2 ♀♀, 1 ♂ (CAR, CNBFVR).

Other material examined. Italy, Sardinia, Nuoro province, Oliena, near San Giovanni, 150 m, 6.VI.1995, F. Angelini leg., 1 ex (CAR); Italy, Sardinia, Nuoro province, Gennargentu Massif, Montarbu Forest, Mount Tonneri, 1300 m, 2.V.1983, P. Audisio leg., 2 ex (CAR); ibidem, 989 m, 16.V.2008, P. Audisio & M. Trizzino leg., 1 ex (CAR); Italy, Sardinia, Cagliari province, Geremeas, Geremeas river, 150 m, 18.VI.1976, C. Meloni leg., 2 ex (CAR); Italy, Sardinia, Cagliari province, Elmas near Cagliari, 10 m, 1.X.1973, P. Leo leg., 1 ex (CAR); France, Corsica, Casta, 270 m, 22.VII.1976, A. Sette leg., 5 ex (CAR).

Description. Male [measurements refer to holotype]. Length 1.90 mm; width (at elytral widest point) 1.00 mm. Body elongate, narrow, transversely convex and moderately parallel-sided (Fig. 1), black and shining, with short and fine silvery to golden-olivaceous pubescence. Legs and antennae blackish.

Head with dorsal punctures as large as or slightly smaller than an eye facet, moderately impressed, separated by nearly one diameter, surface smooth and shining; front margin of clypeus straight, completely bordered, with almost right angles. Fronto-genal grooves narrow and shallow, but distinct. Antennae small, of normal size for group (*cf.* Fig. 1), with third segment slender, longer than second; antennal club small.

Pronotum only moderately narrowed anteriorly, 1.65 times as wide as long, broadest close to posterior angles (*cf.* Fig. 1); sides narrowly bordered; posterior angles slightly obtuse but distinct. Posterior base faintly sinuate on either side of scutellum; pronotal punctures and surface smooth and shining, each puncture nearly as large as an eye facet and separated by one diameter or less.

Scutellum medium-sized, densely and uniformly punctate; surface exhibiting a faint trace of reticulate microsculpture. Elytra (*cf.* Fig. 1) nearly 1.17 times as long as wide, broadest in basal fifth, scarcely wider than pronotum (1.06) and nearly twice as long; feebly raised, humeral striae absent; elytral punctures in basal half as on head and pronotum, but slightly coarser and more elongate, exhibiting a feeble transverse rugosity and with a shiny surface between punctures. Elytral punctures becoming finer and shallower towards their hind end.

Ventral surface black, with sparse fine silvery pubescence. Prosternal antennal furrows strongly raised. Prosternal process rather long, moderately wide and parallel-sided (*cf.* Fig. 1), subtruncate at apex, as wide as posterior tibiae.

Metasternum moderately convex (punctures as on head and pronotum, surface shiny), with a barely distinct and posteriorly widened longitudinal impression on posterior two-thirds, and a well-raised elongate medial tubercle. Caudal marginal line of posterior coxal cavity closely following posterior edge, turning back just before outer end. Last visible abdominal sternite with a markedly raised obtuse projection, distally concave, as in males of *M. lugubris* and *M. gagathinus*.

Protibiae (*cf.* Fig. 1) with outer edges finely crenulate from basal third, with subapical group of 5–7 sharp teeth, the first and penultimate tooth markedly bigger than the rest; protarsi barely as wide as antennal club; metatibiae narrow, inner edges not sinuate (*cf.* Fig. 1); tarsal claws simple.

Genitalia. Tegmen as in Fig. 7, rather strongly sclerotized and dark, with a shallow, V-shaped median excision, and apex markedly projected distad and scarcely pubescent; median lobe of aedeagus elongate, narrow, nearly 2.4 times as long as wide (*cf.* Fig. 1), less sclerotized and paler than tegmen, widest in distal three-fifths, parallel-sided distad and proximad, apex subtruncate and minutely incised. Male genitalia exhibiting a rather unusual contrast between the rather strongly pigmented, blackish-brown tegmen, and the orange-yellowish and scarcely pigmented median lobe.

Female. Protarsi more narrow (~0.7 times as wide) than in male. Metasternum not impressed longitudinally. Ovipositor nearly indistinguishable from that of *M. lugubris* (fig. 160*l* in Audisio 1993),

yellowish, with apical suture of coxites slightly darker, apex acuminate, styli relatively short and inserted at a distance from apex equal to their length; outer subdivision of coxites moderately long and narrow; "central point" placed at distal three fifths, without ventral spicule; transverse suture nearly straight; external angles of basicoxites moderately sharp.

Variability. Length 1.78–2.04 mm. Dorsal punctures slightly variable in density, but usually conforming to the pattern described. Antennae, tibiae and tarsi frequently paler, at least partially nut-brown.

Comparative notes. *Meligethes foddaii* sp. nov. exhibits a dorsal habitus (Fig. 1) similar to that of small specimens of *M. lugubris* (widespread in Europe and peninsular Italy), but with a much shinier dorsal surface. The new species is also recognizable by the proportionally larger male genitalia, the more strongly pigmented and darkened tegmen, and by the more parallel-sided shape of the distal portion of the median lobe of the aedeagus (Figs 5–8). Easily distinguishable from the closely related *M. gagathinus* (widespread in Europe and peninsular Italy) by the smaller, narrower, and more parallel-sided body (Figs 1–2), by the parameres more protruding laterad to the distal portion of tegmen (Figs 7–10), by the narrower prosternal process (Figs 3–4), and by the slightly narrower male protarsal plate (Figs 1–2).

Geographic distribution. All known specimens are from the above-mentioned localities in Sardinia and Corsica. This species appears not to be particularly rare, and could be more widely distributed, in suitable habitats, throughout both islands.

Biological notes. The type and non-type specimens were all collected from apical stems of the common plant *Mentha insularis* Requien [= *Mentha suaveolens* Ehrh ssp. *insularis* (Req.) Greuter] (Lamiaceae), a subendemic W-Mediterranean species known to occur in the Corso-Sardinian plate and Balearic Islands, in wet places near lakes and ponds, and on the edges of river banks, from sea level up to 1300m (Gennargentu Massif). The adults are active on host-plants from early May to late November, two-three months before flowering time, but reproduction appears to be extremely tardive for a Mediterranean *Meligethes*, occurring mainly between late July and early October.

Etymology. This species is named after our Sardinian friend and colleague Dr. Sebastiano Foddai (Department of Vegetal Biology, Sapienza University of Rome), whose main scientific research is devoted to the biochemistry of secondary substances extracted from Sardinian endemic plant species.

Molecular results

Preliminary molecular data based on nucleotide sequence comparison (COI gene) show that genetic distances between *M. foddaii* sp. nov. (Sardinian specimens from the type locality) and its probably closest relative *M. gagathinus* (specimens from peninsular Italy) are ~2%, while distances between *M. foddaii* sp. nov. and the slightly less closely related *M. submetallicus* (Sardinian specimens from the same locality as *M. foddaii*) range around 4.2%.

Comparative COI sequences of *M. lugubris* are still pending, but on a morphological basis this species is likely phylogenetically less closely related to *M. foddaii* sp. nov. than the examined *M. gagathinus* (Audisio, 1993).

Results of more detailed molecular analyses on *M. foddaii* sp. nov., *M. gagathinus*, and *M. lugubris* will be summarised in a companion paper on molecular systematics of the *M. lugubris* species-group (Audisio *et al.* in prep.).

Discussion

As reported above, the preliminary values of the genetic distances scored between the individuals of *M. gagathinus* from peninsular Italy (Liguria and Latium) and those of *M. foddaii* sp. nov. (SW Sardinia), show a 2.0% difference between their haplotypes. This finding is congruent with the outcomes of other studies on

COI gene regarding the divergence between closely related taxa at the species level (e.g., Funk 1999 and citations therein; De Biase *et al*. 2003; Hebert *et al*. 2003).

On the other hand, the few available morphological diagnostic characters discussed in the Introduction and reported below in the identification key to members of the *M. lugubris* complex, appear sufficiently supported by host-plant data and phenology. Combining the available information summarized in the Introduction, it is highly likely that the plesiotypic life-style of members of the *M. lugubris* group was represented by a larval association with *Mentha* spp., while the association of the single *M. lugubris* with *Thymus* spp. must be considered as the relatively recent result of a single event of an ecological shift on a related genus of Lamiaceae. This phenomenon probably occurred in southern Europe in conjunction with speciation of *M. lugubris* from the stem of a closely related *Meligethes* associated with *Mentha* spp., and involving a group of populations shifted on *Thymus* sp., likely during an interglacial period. In one of the main southern European peninsulae, the increase of mean annual temperatures pushed lowland plant communities associated with wet habitats towards higher altitudes and more xeric communities into local montane areas, allowing for the above hypothesized host-plant shift for the hypothetical ancestor of *M. lugubris*, followed by subsequent specific differentiation. In Sardinia and Corsica this same ancestor, in an isolated condition, likely retained its original life-style, as well as in other southern European areas, allowing for allopatric differentiation of *M. foddaii* sp. nov. and *M. gagathinus*, respectively.

Meligethes lugubris, *M. gagathinus* and *M. foddaii* sp. nov. represent a triplet of closely related species, *M. lugubris* and *M. gagathinus* likely being two relatively recent (post-Würmian) colonizers of central and northern Europe. As for *M. foddaii* sp. nov., the common ancestor of this complex undoubtedly did not remain isolated in the Corso-Sardinian microplate before its counter-clockwise rotation, which began some 27–30 mya from its original position close to the present-day Mediterranean coastal areas of SE Spain and S France, included in the ancient Iberian Plate (Alvarez 1972, 1974; Cherchi & Montadert 1982; La Greca 1990; Sbordoni *et al*. 1990; Boccaletti *et al*. 1990; Cimmaruta *et al*. 1998; Oliverio *et al*. 2000; Ketmaier *et al*. 2003). In fact, the relatively low level of both morphological and molecular differentiation of the new species suggests a biogeographic scenario involving a more recent penetration of *M. foddaii*'s ancestor into the Corso-Sardinian plate from peninsular Italy, probably during the Messinian salinity crisis (5.6–5.3 mya: Rouchy & Caruso 2006; Carranza *et al*. 2008) or later during a Plio-Pleistocene (2.0–0.5 mya) glacial peak (combined with a marked lowering of the sea level), followed by subsequent allopatric differentiation.

Identification key to Euro-Mediterranean members of the *M. lugubris* complex

1 (2) Male genitalia large and peculiar shaped (as in figs 144*i*-*l* in Audisio (1993), tegmen with parameres moderately protruding at sides distad, but separated by a deep and narrow distal excision. Ovipositor (fig. 160*i* in Audisio 1993) with bases of coxites inversely V-shaped, and "central point" nearly at distal two-fifths. Last visible abdominal sternite in males with distinct and shining but not strongly raised median gibbosity. Body small (length: 1.6–2.3 mm), narrow, parallel-sided (fig. 122*c* in Audisio 1993). Larval stages monophagous on *Mentha pulegium* L., in periodically inundated places. Distributed nearly throughout the whole southern portion of the Palaearctic, except North Africa and the Iberian Peninsula ..... ***submetallicus*** Sainte-Claire Deville, 1908

2 (1) Male genitalia not as above (Figs 5–10, and figs 144*g*-*h* in Audisio 1993), tegmen with parameres more or less strongly protruding at sides distad, but separated by a much shallower and wider distal excision. Ovipositor (figs 160*l* in Audisio 1993, and fig. 18 in Easton 1954) with bases of coxites almost straight, and "central point" nearly in median position. **3**

3 (4) Surface between dorsal punctures on posterior third of pronotum with dull microscopic reticulation. Male genitalia (Figs 5–6), tegmen with parameres strongly protruding at sides distad, and separated by a deep V-shaped distal excision. Ovipositor (as in fig. 160*l* in Audisio 1993). Last visible abdominal sternite in males with a strongly raised median tubercle. Body small (length: 1.6–2.2 mm), narrow, parallel-sided. Larval stages known from *Thymus serpyllum* L. and allied species, mostly in xeric mountain meadows. Distributed throughout most of the Palaearctic, except North Africa, the Corso-Sardinian plate, Sicily, the southern Iberian Peninsula, Japan, and the Middle East ..... ***lugubris*** Sturm, 1845

4 (3) Surface between dorsal punctures on posterior third of pronotum completely smooth and shining. Male genitalia

with parameres differently shaped (Figs 7–10, and figs 144*g-h* in Audisio 1993). Larval stages on *Mentha* spp., in low altitude wet places **5**

5 (6) Male genitalia as in figs 144*g-h* in Audisio (1993), tegmen with parameres less protruding at sides distad, and separated by a shallow but wider distal excision. Ovipositor (as in fig. 18 in Easton 1954), with bases of coxites slightly wider, nearly reaching sides of ovipositor. Body small (length: 1.7–2.3 mm), narrow, parallel-sided. Larval stages monophagous on *Mentha pulegium* L., in periodically inundated places. Distributed throughout western North Africa, the Iberian Peninsula, and SW France ..... ***normandi*** Easton, 1954

6 (5) Male genitalia (as in Figs 7–10), tegmen with parameres more protruding at sides distad, and separated by a deeper and narrower distal excision. Ovipositor shaped similar to *M. lugubris* (fig. 160*l* in Audisio 1993), with bases of coxites slightly narrower, usually not reaching sides of ovipositor. Larval stages oligophagous on several *Mentha* spp., in permanently wet habitats. **7**

7 (8) Small-sized (length 1.8–2.0 mm), body narrow and parallel-sided (Fig. 1), male genitalia with darker tegmen and parameres more protruding at sides distad (Figs 7–8). Prosternal process as wide as or slightly narrower than posterior tibiae (Figs 1, 3). Larval stages likely monophagous on *Mentha insularis* Req. Distributed throughout Sardinia and Corsica. Reproduction between July and late October..... ..... ***foddaii*** Audisio, De Biase & Trizzino, **sp. nov.**

8 (7) Medium-sized (length 2.1–2.6 mm), body wider, oval and more convex (Fig. 2), male genitalia with paler tegmen and parameres less protruding at sides distad (Figs 9–10). Prosternal process 1.3 times wider than posterior tibiae (Figs 2, 4). Larval stages mostly monophagous on *Mentha aquatica* L. and allied species. Distributed throughout most of the Palaearctic, except North Africa, the Corso-Sardinian plate, Sicily, the southern Iberian Peninsula, and the Near East. Reproduction between June and late August ..... ***gagathinus*** Erichson, 1845

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