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Three new cryptic species of the genus *Merodon* Meigen (Diptera: Syrphidae) from the island of Lesvos (Greece)

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Abstract

Descriptions are given of three new cryptic species of *Merodon* Meigen (Diptera: Syrphidae) from the island of Lesvos (Greece): *Merodon latifemoris* Radenković et Vujić **n. sp.** from the *nigritarsis* species group, *Merodon pulveris* Vujić et Radenković **n. sp.** from the *natans* species group and *Merodon puniceus* Vujić, Radenković et Pérez-Bañón **n. sp.** from the *aureus* species group. In addition to classical morphological characters, mitochondrial COI barcode sequences were generated for several specimens of each taxon.

Key words: hoverflies, *Merodon latifemoris*, *Merodon pulveris*, *Merodon puniceus*, *Merodon dobrogensis*, mitochondrial COI barcode sequences

Introduction

The genus *Merodon* Meigen (Diptera: Syrphidae: Eumerini) is the second largest genus of Syrphidae in Europe where it is represented by more than 50 species (Speight 2008). *Merodon* is one of the most widespread syrphid genera in the Mediterranean region (Dirickx 1994) and it is distributed in the Palaearctic and Ethiopian regions. The morphological diagnostic characters to separate *Merodon* spp. from all other hoverflies are the presence of a triangular projection beneath the distal part of the hind femora in combination with wing-vein R_{4+5} curving deeply into cell R_5. Several species of *Merodon* are known to have underground larval development and feed on bulbs or rhizomes of monocots such as Liliaceae, Hyacinthaceae and Amaryllidaceae (Hurkmans 1993; Rotheray 1993; Ricarte *et al.* 2008). Although the biology of the immature stages of many species of *Merodon* remains insufficiently studied or often completely unknown, it is likely that all species in this group have similar habits. The diversity of bulbous plants is very high in the Eastern Mediterranean area (Bazos 2005) which fits well with the known distribution and diversity of the genus *Merodon* in this region.

DNA barcoding (Hebert *et al.* 2003) is based on the observation that a short, standardized segment of the mitochondrial genome can enable species identification and facilitate species discrimination. Our results stress that barcode sequences of a single molecular marker cannot alone define species, but it can help in their discovery. We agree with the view of Sperling and Roe (2009) in that molecular taxonomy is not a parallel approach to insect taxonomy, but rather that molecular methods make an important part of a more holistic, integrative taxonomy. This is because molecular methods provide additional characters for addressing the classical problems of identifying specimens, discovering and delimiting species, as well as determining relationships (e.g. Will *et al.* 2005; Valdecasas *et al.* 2008). A recent barcoding study by Ståhls *et al.* (2009) on *Merodon* spp. of the Lesvos Island (Greece) revealed high taxonomic congruence between information obtained from the mtDNA barcode and that

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based on morphological characters, with only one morphologically distinct species pair sharing COI haplotypes. The study concluded that DNA characters proved to be a good source of additional data, facilitating identification of species and supporting the initial species delimitation conclusions made based on morphological characters. In addition, COI barcodes also highlighted three morphologically cryptic species, and two of these (*M. latifemoris* Radenković et Vujić **n. sp.** and *M. pulveris* Vujić et Radenković **n. sp.**) are described in the present paper. Third species belongs to *avidus* (Rossi) group and still has uncertain nomenclatural status. As a general rule, all cases of conflict between the DNA tree and morphological assignment deserve additional investigation. The third species described in this paper is *M. puniceus* Vujić, Radenković et Pérez-Bañón **n. sp.**, which is morphologically closely related to *M. dobrogensis* Bradescu, a species recently discovered in Greece (Petanidou *et al*. in prep.).

Research on cryptic species has increased exponentially over the past two decades (Bickford *et al.* 2007). Unexpected genetic diversity has proved that speciation is not always related with morphological changes, which is well exemplified as in the case of sympatric cryptic species complexes (Hebert *et al.* 2004; Stuart *et al.* 2006). In this paper we follow the distinction by Knowlton (1986) that cryptic species are difficult to recognize using traditional morphological methods (i.e. are superficially morphologically indistinguishable) whereas sibling species are those species which have been shown to be very closely related using molecular techniques (e.g. cryptic sister species; Bickford *et al.* 2007). There are many authors, however, that consider "sibling" and "cryptic" species to be synonymous.

Lesvos Island is the biggest of a series of islands located along the Aegean coast of Turkey in the zone where the biodiversities of two different regions merge: the Anatolian and the major west Mediterranean. Geophytes are outstandingly well represented on Lesvos, making 14.8% of the local flora, which is the second highest percentage found among 11 medium- to large-sized Aegean islands (range 10.7–15.2%) and six mountainous areas of the Greek mainland (range 9.0–13.9%) (Bazos 2005). Recent investigations have shown that the island of Lesvos is hosting more than 90 species of hoverflies from at least 40 genera (Vujić *et al.* 2007; Garcia-Gras 2008; Ståhls *et al.* 2009). Preliminary review of the data gathered on the syrphid fauna of Lesvos shows that at least 22 species of *Merodon* occur on the island (Garcia-Gras 2008; Ståhls *et al.* 2009), including two new species described from the island in 2007 (Vujić *et al.* 2007). This paper is the second in the series in which we update the results of our systematic survey of the Syrphidae of Lesvos. The paper presents the description of three *Merodon* species new to science, belonging to the *aureus*, *natans* and *nigritarsis* groups. A morphological key to these species was provided as supplementary data with Ståhls *et al.* (2009), where sp. nova 1 = *M. latifemoris*, sp. nova 3 = *M. pulveris* and sp. nova 4 = *M. puniceus*. Mitochondrial COI sequences of both 5'- and 3'-regions were generated for several specimens of each species, for future use in the study of the biodiversity of hoverflies of this region.

Material and methods

***Merodon* sampling.** The *Merodon* material was collected on the island of Lesvos during years 2001–2008 and identified using a morphological identification key to the *Merodon* species of Lesvos (Ståhls *et al.* 2009). Specimens were deposited in the insect collections of University of Alicante, Spain (CEUA), the Melissotheque of the Aegean, University of the Aegean, Mytilene, Greece (AEU), Faculty of Sciences, Department of Biology and Ecology, University of Novi Sad, Serbia (FSUNS), World Museum Liverpool, England (NML), Natural History Museum, London, England (NHML), Zoological Museum (MZH) of the Finnish Museum of Natural History, Helsinki, Finland, and Zoological Museum Amsterdam, Netherlands (ZMA). Number beside abbreviation AEU, MZH and FSUNS presents unique identifiers from specimen databases of these institutions.

Terminology follows McAlpine (1981) for non-genitalic morphology and Marcos-García *et al.* (2007) for male terminalia morphology.

Molecular analysis. DNA was extracted from two or three legs of the pinned adult fly. The protocols for DNA extraction, PCR amplification and sequencing were described in Ståhls *et al.* (2009). The Folmer fragment or "barcode fragment" of the 5' region of COI was amplified with forward primer LCO (5'-GCTCAACAAATCATAAAGATATTGG-3') and reverse primer HCO (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al.* 1994). The fragments were sequenced using the same primers and the Big Dye Terminator vs 3.1 cycle sequencing kit (Applied Biosystems). All COI barcodes sequences were submitted to EMBL Nucleotide Sequence Database, for accession numbers see text for each

species. Representatives of all taxa that are DNA voucher specimens were deposited in the Zoological Museum (MZH) of the Finnish Museum of Natural History, Helsinki, Finland, while types and additional material that were also used for DNA extraction were deposited in institutions indicated in respective species description. For comparisons of uncorrected pairwise divergences for the COI gene between the new species and their closest congeners in the Eastern Mediterranean region, see Discussion.

Results

Species descriptions

***Merodon latifemoris* Radenković et Vujić, n. sp.**
(Figs. 1–7, 10, 13, 16–20)

Type material. Holotype: ♂, GREECE: **Lesvos**: Agiassos, 10.06.2005; FSUNS_03566; (FSUNS).
Paratypes: GREECE: ♂, **Katara**, 13.07.1998, leg. S. Šimić, in Vujić *et al.*, 2000 as *Merodon femoratoides*; FSUNS_3561; **Lesvos:** ♂, 2.7 km SSE Agiassos, Alt. 700 m, 39° 3' 45" N; 26° 23' 30" E, chestnut, 21.06.2004, leg. M. Kapsali, *Trifolium* sp. (Fabaceae); AEU_0008878, FSUNS_03562; (AEU). ♂, Agiassos, 10.06.2005; FSUNS_03565; (AEU). ♂, Agiassos, 10.06.2005, stream valley; voucher genitalia MEP-5; DNA voucher specimen lab. No. MZH_S536; G. Ståhls, FMNH, FSUNS_03566; (MZH), EMBL accession number for mtDNA COI barcode is FM206514. 4 ♂, Agiassos, 8.06.2009, leg. A. Vujić; FSUNS_04433, FSUNS_04434, FSUNS_04435, FSUNS_04436; (AEU). ♀, Agiassos, 39° 04' 17" N, 26° 22' 22" E, 8.06.2009, leg. G. Ståhls; DNA voucher specimen lab. No. MZH_Y869; G. Ståhls, FMNH, FSUNS_04441; (MZH), EMBL accession number for mtDNA COI barcode fragment FR717821. ♀, Agiassos, 8.06.2009, leg. A. Vujić; DNA voucher specimen lab. No. MZH_A09 10; G. Ståhls, FMNH, (MZH), EMBL accession number for mtDNA COI barcode fragment is FR717822. ♀, Agiassos, 8.06.2009, A. Vujić; DNA voucher specimen lab. No. MZH_A09 11; G. Ståhls, FMNH, FSUNS_04438; (MZH), EMBL accession number for mtDNA COI barcode fragment is FR717822. ♀, Agiassos, 8.06.2009, A. Vujić; DNA voucher specimen lab. No. MZH_A09 12; G. Ståhls, FMNH, FSUNS_04439; (MZH), EMBL accession number for mtDNA COI barcode fragment is FR717823. ♀, Agiassos, 8.06.2009, A. Vujić; DNA voucher specimen lab. No. MZH_A09 14; G. Ståhls, FMNH, FSUNS_04440; (MZH), EMBL accession number for mtDNA COI barcode fragment is FR717824. ♂, Vafios, 5km E of Mithymna, Alt. 300–350m, 39° 21' 304" N, 26° 14' 450" E, 26.06.2001, leg. L. Sijstermans; AMA-05-26, FSUNS_03564; (ZMA). ♂, Vafios, 5km E of Mithymna, Alt. 300–350m, 39° 21' 304" N, 26° 14' 450" E, 26.06.2001, leg. L. Sijstermans; AMA-05-27, FSUNS_03563; (ZMA). ♂, Sikaminia, 24.07.–2.08.2001, ref VL_3; DNA voucher specimen lab. No. MZH_S522; G. Ståhls, FMNH, Helsinki, Finland; (MZH), EMBL accession number for COI barcode is FM206490. ♂, Parakila, 15.07.2007, leg. M. Hull, H-796, 39° 9' 35 N", 26° 9' 18" E; FSUNS_04383; (FSUNS). ♂, Parakila, 15.07.2007, leg. M. Hull, H-796, 39° 9' 35 N", 26° 9' 18" E; FSUNS_04384; (FSUNS). ♀, Parakila, 15.07.2007, leg. M. Hull, H-796, 39° 9' 35" N, 26° 9' 18" E; FSUNS_04393; (FSUNS). ♀, Parakila, 22.07.2007, leg. M. Hull, H-796, 39° 9' 35" N, 26° 9' 18" E; FSUNS_04394; (FSUNS). ♀, near Haramida beach, 7.06.2009, leg. A. Vujić; FSUNS_4437; (FSUNS).

Etymology. The word *latifemoris* is derives from Latin adjective *latus* meaning broad, wide; *femoris* is genitive of the noun *femur*, referring to the broad femur of the hind leg.

Diagnosis. Medium sized species (11–14mm); black mesoscutum with four white microtrichose longitudinal stripes (more distinct in females); tapering orange-black abdomen with white transversal microtrichose stripes on tergites 2–4 (figs. 16, 17); tarsi brown-black dorsally; metafemur wide and curved (fig. 7), without very long hairs posteroventrally (fig. 10).

M. latifemoris belongs to *nigritarsis* Rondani group which includes following species: *M. nigritarsis*, *M. femoratoides* Paramonov and *M. toscanus* Hurkmans. They are relatively large (11–17mm) species with white microtrichose longitudinal stripes on black mesoscutum and white microtrichose transverse stripes on orange-brown (in females orange-black) tergites (figs. 16, 17). Mesoscutum covered with erect, yellow hairs. Abdomen (figs. 16, 17) long, narrow and tapering. Posterior part of mesocoxa without long hairs. Tarsi dark brown-black

dorsally and orange ventrally. First flagellomere (figs. 5, 6) maximum two times longer than wide. Legs (figs. 7–15) without thorns and protuberances. Male genitalia (figs. 18–26): anterior surstyle lobe (fig. 19: a) more or less of rhomboid shape, covered with dense short hairs; posterior surstyle lobe (fig. 19: p) longer than anterior one; interior accessory lobe of posterior surstyle lobe narrow and long (in lateral view, fig. 19: i); cercus (fig. 19: c) rectangular, without prominences. Hypandrium (figs. 18, 20) narrow, elongated and sickle–shaped; posterior end of lateral sclerite of aedeagus (figs. 18, 20–24: la) tapering; hypandrium with a pair of lateral projections (figs. 18, 20–24: lt); lingula (figs. 18, 20–24: l) developed.

M. latifemoris **n. sp.** can be separated from the two closely related species, *M. nigritarsis* and *M. femoratoides*, by the diagnostic morphologhical characters presented in Table 1.

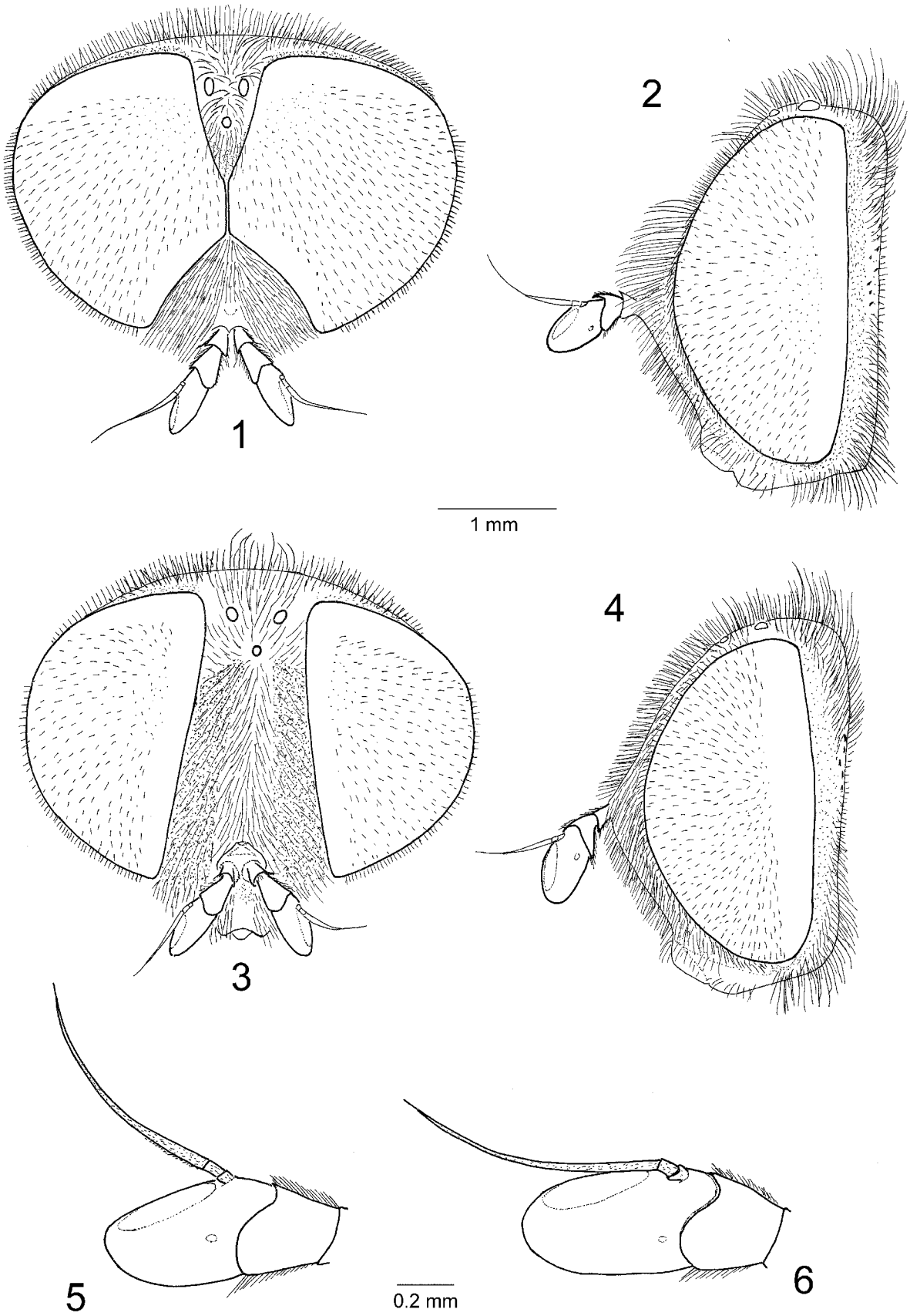
TABLE 1. Diagnostic characters for *Merodon latifemoris* **n. sp.** and two closely related species, *M. nigritarsis* and *M. femoratoides*.

character	*Merodon nigritarsis*	*Merodon latifemoris*	*Merodon femoratoides*
size of species	medium (13–14mm)	medium (11–14 mm)	larger (apx. 17 mm)
conspicuous short black setae on procoxa anteriorly	variable	present	absent
shape of metafemur	almost straight and narrower (in males 3–3.3 longer than deep, fig. 8; in females 3.3–3.5, fig. 14)	more curved and wider (2.7–2.9 times longer than deep, figs. 7, 13)	more curved and wider (2.7–2.9 times longer than deep, figs. 9, 15)
posteroventral hairs on metafemur	much shorter than width of tibia (figs. 8, 11, 14)	much shorter than width of tibia (figs. 7, 10, 13)	in males, longer than width of tibia (figs. 9, 12); in females, the longest hairs as long as width of tibia, or slightly shorter (fig. 15)
male genitalia: lingula of hypandrium	longer and not curved up (figs. 22, 24: l)	shorter and not curved up (figs. 18, 20: l)	longer and curved up (figs. 21, 23: l)
male genitalia: lateral projections of hypandrium	small (figs. 22, 24: lt)	medium and finger-shaped (figs. 18, 20: lt)	large and hook-shaped (figs. 21, 23: lt)
male genitalia: lateral sclerite of aedeagus	with elongated posterior end (figs. 22, 24: la)	with short posterior end (figs. 18, 20: la)	with elongated posterior end (figs. 21, 23: la)
male genitalia: posterior surstyle lobe	short and wide (2.2–2.4 times longer than wide; fig. 26)	long and not tapering (2.5–2.7 times longer than wide; fig. 19: p)	longer and tapering (2.9–3.1 times longer than wide; fig. 25)
two white microtrichose spots on tergite 2 in female	usually lacking	present (fig. 17)	present

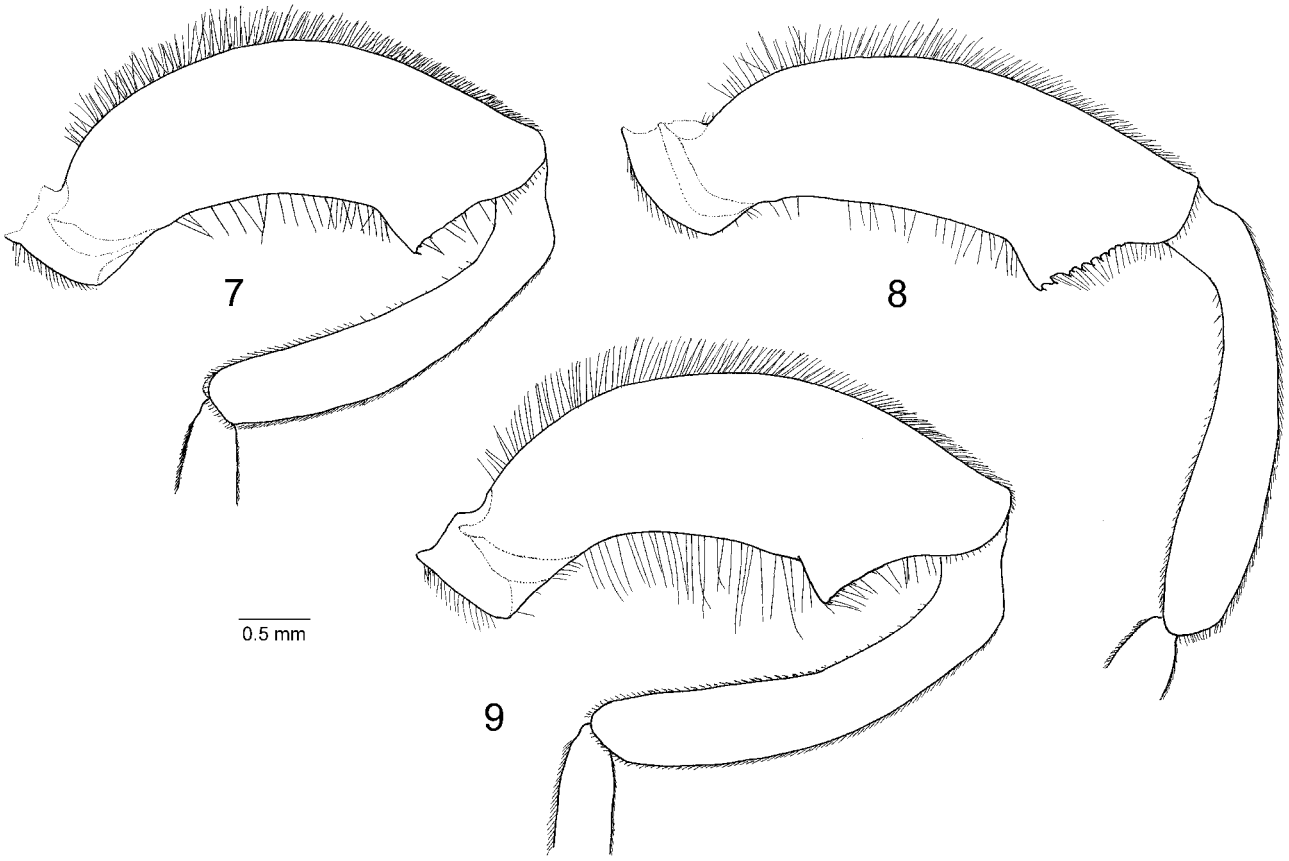
Description. Male.

Head (figs. 1, 2). Antenna (fig. 5) brown, first flagellomere 1.55–2 times as long as wide, 1.4 - 1.9 longer than pedicel, concave and darkened dorsally, apex acute; arista: second, third and basal part of fourth flagellomeres pale, fourth flagellomere dark brown and thickened basally, 1.3 times longer than first flagellomere; with short, dense microscopic hairs. Face and frons black, covered with long whitish-yellow hairs and silver, dense microtrichia. Oral margin shiny black. Vertical triangle isosceles, shiny black except in front of anterior ocellus with white microtrichia, covered with long whitish-yellow hairs except black ones in ocellar triangle. Ocellar triangle equilateral. Eye contiguity about 12 ommatidia long. Vertical triangle: eye contiguity: ocellar triangle = 2.2 : 1.2 : 1. Eye hairs long, dense, white. Occiput with whitish-yellow hairs, along the eye margin with dense white microtrichia and posteriorly with metallic bluish-greenish luster.

Thorax. Postpronotum with conspicuous tooth-like process posteriorly. Scutum and scutellum black with bronze luster, covered with relatively long, dense, erect yellow hairs. Scutum with two lateral and two submedian, longitudinal, white microtrichose stripes, anteriorly connected and posteriorly almost reaching the scutellum and postalar callus. Proepimeron, posterior anepisternum, antero-ventral and postero-dorsal part of katepisternum,



FIGURES 1–6. *Merodon latifemoris* **n. sp.** 1–4. head; 5, 6. antenna (first flagellomere and pedicel); 1, 2, 5. male; 3, 4, 6. female; 1, 3. dorsal view; 2, 4, 5, 6. lateral view.



FIGURES 7–9. Hind leg of male, lateral view. 7. *Merodon latifemoris* **n. sp.**; 8. *Merodon nigritarsis*; 9. *Merodon femoratoides.*

anepimeron, metasternum and katatergit with long yellow hairs and gray-green microtrichia. Wing hyaline, with dense microtrichia; veins dark brown except light brown C, Sc and R1. Calypter pale yellow. Halter with light brown pedicel and yellow capitulum. Legs dark brown-black, except following orange parts: apex of pro- and mesofemur, basal half of pro- and mesotibia (metatibia slightly paler basally and sometimes apically) and tarsi ventrally. Hairs on legs yellow, except some black hairs on procoxa, metatrochanter, basal and apical parts of metafemur. Metafemur (figs. 7, 10) thickened and curved, about 2.7–2.9 times as long as deep. Posteroventral hairs on metafemur shorter than anteroventral ones; the longest anteroventral hairs shorter than width of tibia and slightly longer or of equal size as hairs on dorsal surface.

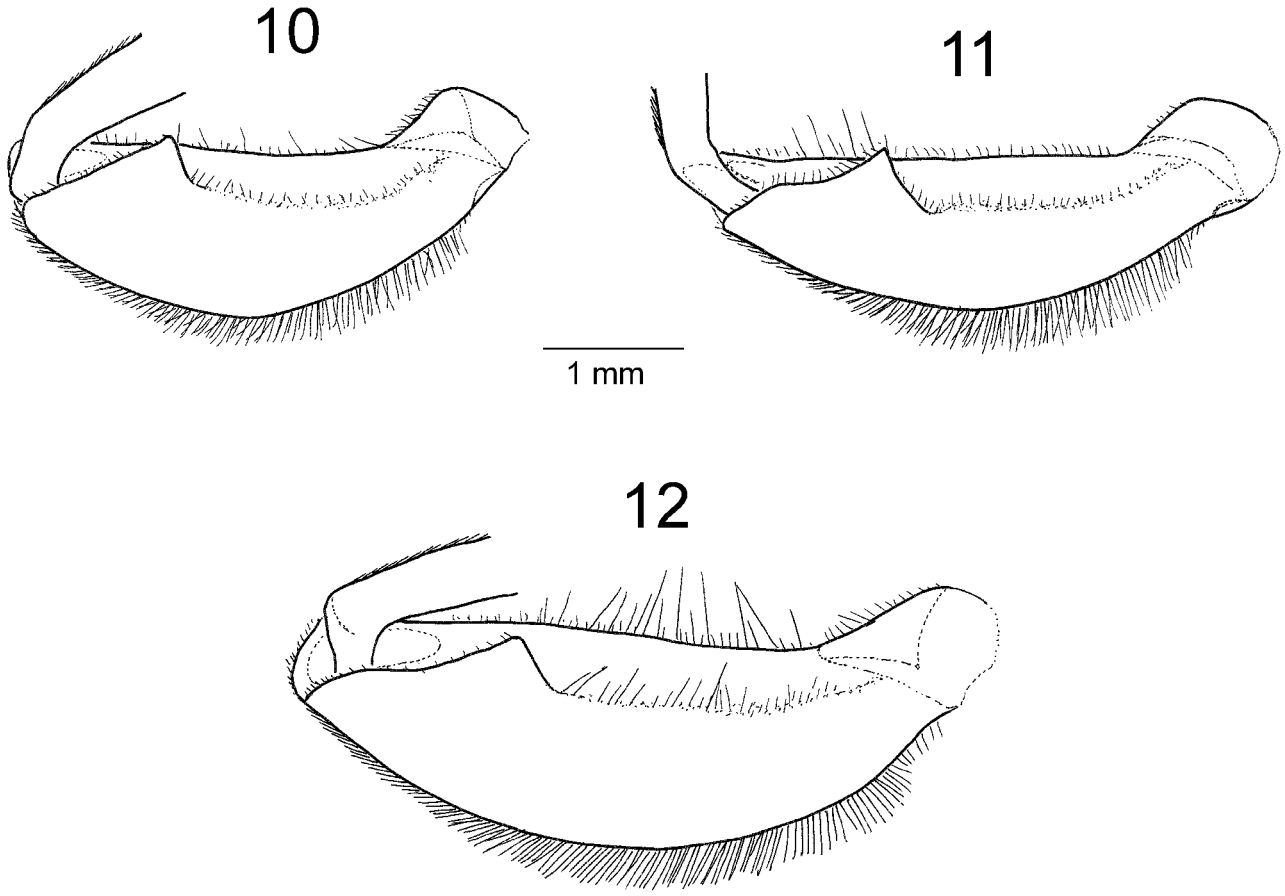
Abdomen (fig. 16). Orange-black with white microtrichose stripes, tapering, 1.4 times longer than mesonotum (including scutellum). Tergites orange-brown except black tergite 1 and median part of tergite 2 goblet-shaped. Tergites 2–4 with pair of white microtrichose marks: triangular spots in tergite 2; wide, oblique bands on tergites 3-4. Hairs on tergites yellow except short black ones on postero-medial part of tergite 3 and below microtrichose stripes on tergite 4 medially. Sternites translucent, orange to brown towards the tip of abdomen, covered with long whitish-yellow hairs.

Male genitalia (figs. 18–20). Similar to species *M. nigritarsis* and *M. femoratoides*. Anterior surstyle lobe (fig. 19: a) covered with dense short hairs, 1.66–1.7 times shorter than posterior one; posterior surstyle lobe (fig. 19: p) not tapering, ellipsoidal at posterior end, 2.5–2.73 times longer than wider; interior accessory lobe (fig. 19: i) of posterior surstyle lobe narrow and long (in lateral view); cercus (fig. 19: c) rectangular, without prominences. Hypandrium (fig. 18) narrow, elongated and sickle–shaped; lateral sclerite of aedeagus (figs. 18, 20: la) with short posterior end; hypandrium with a pair of large finger-like lateral projections (figs. 18, 20: lt); lingula (figs. 18, 20: l) short, sometimes embedded into hypandrium, but not curved up.

Female (figs. 3, 4, 6, 13, 17). Similar to the male except normal sexual dimorphism and for the following characteristics: white microtrichose longitudinal stripes on mesoscutum more visible; tergites (fig. 17) predominately black except most of the tergite 2 orange (only narrow antero-median part black), lateral margins

and two small triangular spots on anterior margin of tergite 3 orange as well as ground color of cuticle under the white microtrichose transverse stripes on tergites 3–4; sternites orange-brown except anterior part of sternites 1 and the whole sternites 4–5 black (sometimes sternites 2–3 darkened medially and posteriorly). Black hairs on tergites more present than in males: posterior margin of tergite 2, almost whole tergites 3–4 except lateral sides and white microtrichose stripes (and on tergite 4 also posterior margin) with pale hairs. First flagellomere (fig. 6) 1.8 times as long as wide, 1.7–1.85 longer than pedicel.

Length: Body 11–14 mm, wing 8–9 mm.



FIGURES 10–12. Hind femur of male, ventral view. 10. *Merodon latifemoris* **n. sp.**; 11. *Merodon nigritarsis*; 12. *Merodon femoratoides.*

Merodon pulveris Vujić et Radenković, n. sp.

(Figs. 27–28, 31–32, 35–36, 39–40, 43, 46)

Type material. Holotype: ♂, GREECE: **Lesvos**: 5.4 km SSE Agiassos, Alt. 760 m, 39° 3' 17" N, 26° 23' 50" E, Maquis mixed, 8.10.2005, leg. C. Perez-Bañón; AEU_0023644; FSUNS_03293; (FSUNS).

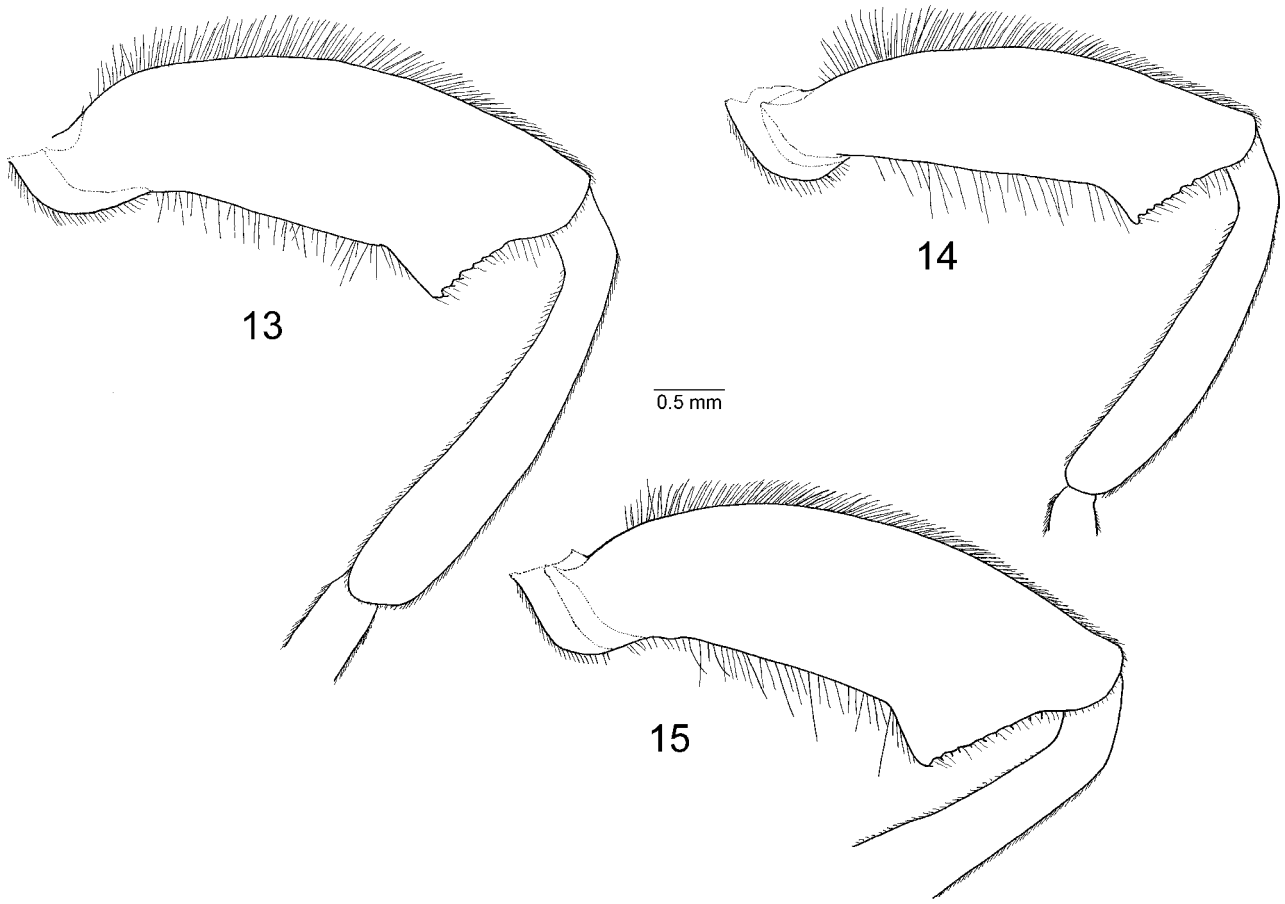
Paratypes: GREECE: **Lesvos**: ♂, 5.4 km SSE Agiassos, Alt. 760 m, 39° 3' 17" N, 26° 23' 50" E, mixed maquis, 06.10.2005, leg. C. Perez-Bañón; AEU 0023647; DNA voucher specimen, lab. No. MZH_S569, G. Ståhls, FMNH, Helsinki, Finland. ♂, 5.4 km SSE Agiassos, Alt. 760 m, 39° 3' 17" N, 26° 23' 50" E, mixed maquis, 06.10.2005, leg. C. Perez-Bañón; AEU 0023670; DNA voucher specimen, lab. No. MZH_S567, G. Ståhls, FMNH, Helsinki, Finland. 19♂, 5.2 km SSE Agiassos, Alt. 746m, 39° 02' 46" N, 26° 24' 35" E, 23.09.2009, leg. M. Hull; (NML). 2♂, 5.2 km SSE Agiassos, Alt. 746m, 39° 02' 46" N, 26° 24' 35" E, 05.10.2009, leg. M. Hull; (NML). ♀, 5.4 km SSE Agiassos, Alt. 760 m, 39° 3' 17" N; 26° 23' 50" E, mixed maquis, 08. 10. 2005, leg. C. Perez-Bañón; AEU_0023646; FSUNS_03292; (FSUNS). ♀, 5.4 km SSE Agiasos; Alt. 760 m, 39° 3' 17" N 26 23' 50" E, mixed maquis, 06.10.2005, leg. C. Perez-Bañón; AEU 0023650; DNA voucher specimen, lab. No. MZH_S568, G. Ståhls,

FMNH, Helsinki, Finland, EMBL COI barcode accession number FR717826. ♀, 5.4 km SSE Agiasos; Alt. 760 m, 39° 3' 17" N 26 23' 50" E, mixed maquis, 06.10.2005, leg. C. Perez-Bañón; AEU 0023662; DNA voucher specimen, lab. No. MZH_S570, G. Ståhls, FMNH, Helsinki, Finland. ♀, 5.2 km SSE Agiassos, Alt. 746m, 39° 02' 46" N, 26° 24' 35" E, 05.10.2009, leg. M. Hull; (NML). 3♂ ♀, TURKEY: Antalya, Finike, 08.04.1962, Alt. 50m, leg. Guichard & Harvey, det. Hurkmans W. as *Merodon natans*; B.M. 1962-299; (NHML). ♀, Aspendos, Finike, 04.04.1962, Alt. 50m, leg. Guichard & Harvey, det. W. Hurkmans as *Merodon tricinctus*; B.M. 1962-299; (NHML). ♂ 2♀, Izmir, Selcuk, 14.10.1990, det. W. Hurkmans as *Merodon natans*; FSUNS_3288, FSUNS_3289, FSUNS_3290; (ZMA). 8♀, Izmir, Selcuk, 14.10.1990; (ZMA).

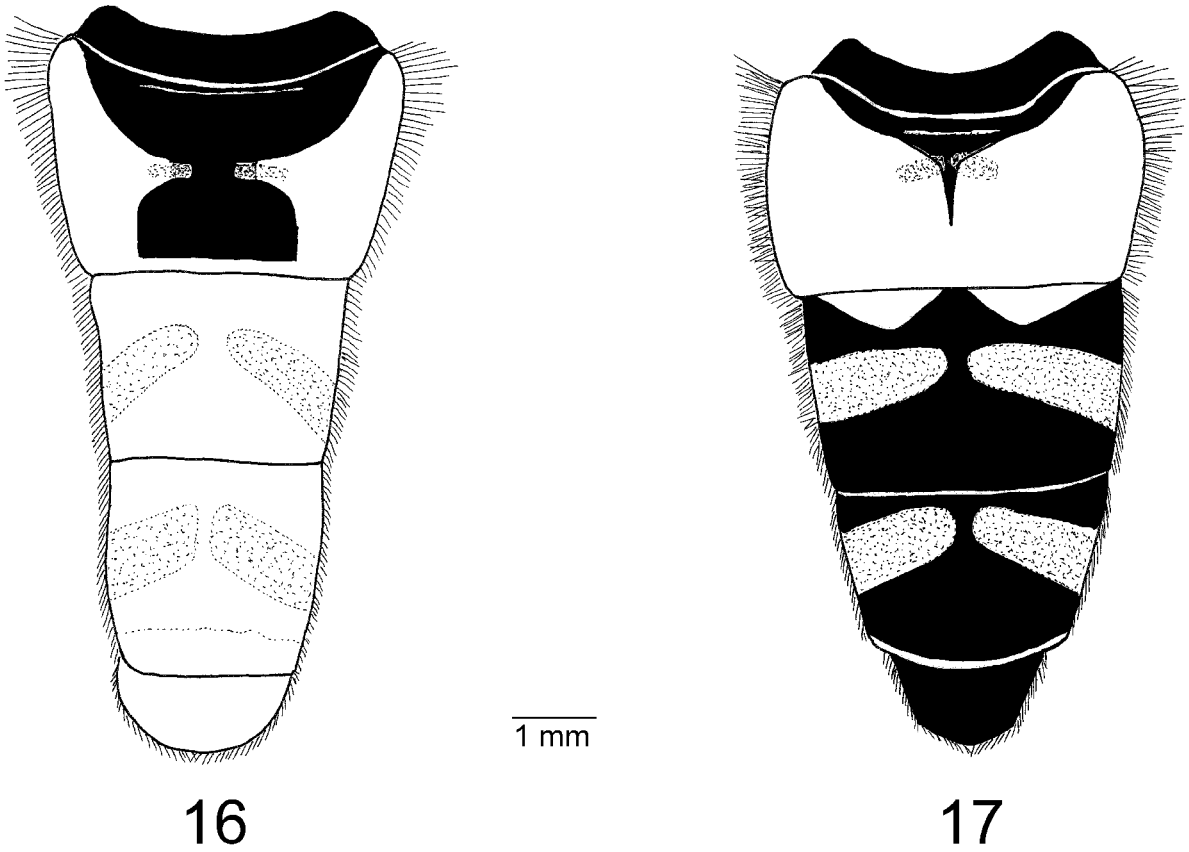
Etymology. The epithet *pulveris* is a Latin word meaning dusted (nom. *pulvis*, gen. *pulveris*) refers to white microtrichose longitudinal stripes on mesoscutum and transversal bands on tergites.

Diagnosis. Black, short haired species. Mesoscutum (fig. 35) with five distinct longitudinal microtrichose stripes. Tergites (fig. 36) with white microtrichose transverse bands and inconspicuous reddish antero-lateral spots on tergite 2. Legs black except reddish–brown tibia darkened in the middle and reddish–brown first and second tarsomeres.

This species belongs to the *natans* (Fabricius) species group with following diagnostic features: posterior side of mid coxa with hairs; hairs on anterior anepisternum reduced; anterior lobe of surstylus oval, rounded, hairy and not curved innerly (figs. 44, 46, 48, 50, 52, 53: a). It is closely related to *M. natans*, but separable by the characters of male genitalia: ventral margin of posterior surstyle lobe with wart–like prominence (figs. 44, 46: v), absent in *M. natans* (figs. 48, 50: v). Females of these two species are very similar, but first tarsomere in *M. natans*, besides yellow, also covered with black hairs, while in *M. pulveris* black hairs absent (or present only basally on metaleg). *Merodon pulveris* **n. sp.** can be easily distinguished from related species *M. calcaratus* (Fabricius) by longer antenna (figs. 31–34), broader metafemur (figs. 39–42) and large, rounded posterior surstyle lobe (figs. 44, 46: p) of male genitalia (in *M. calcaratus* narrow, fingerlike; figs. 52, 53: p).



FIGURES 13–15. Hind leg of female, lateral view. 13. *Merodon latifemoris* **n. sp.**, 14. *Merodon nigritarsis*; 15. *Merodon femoratoides.*



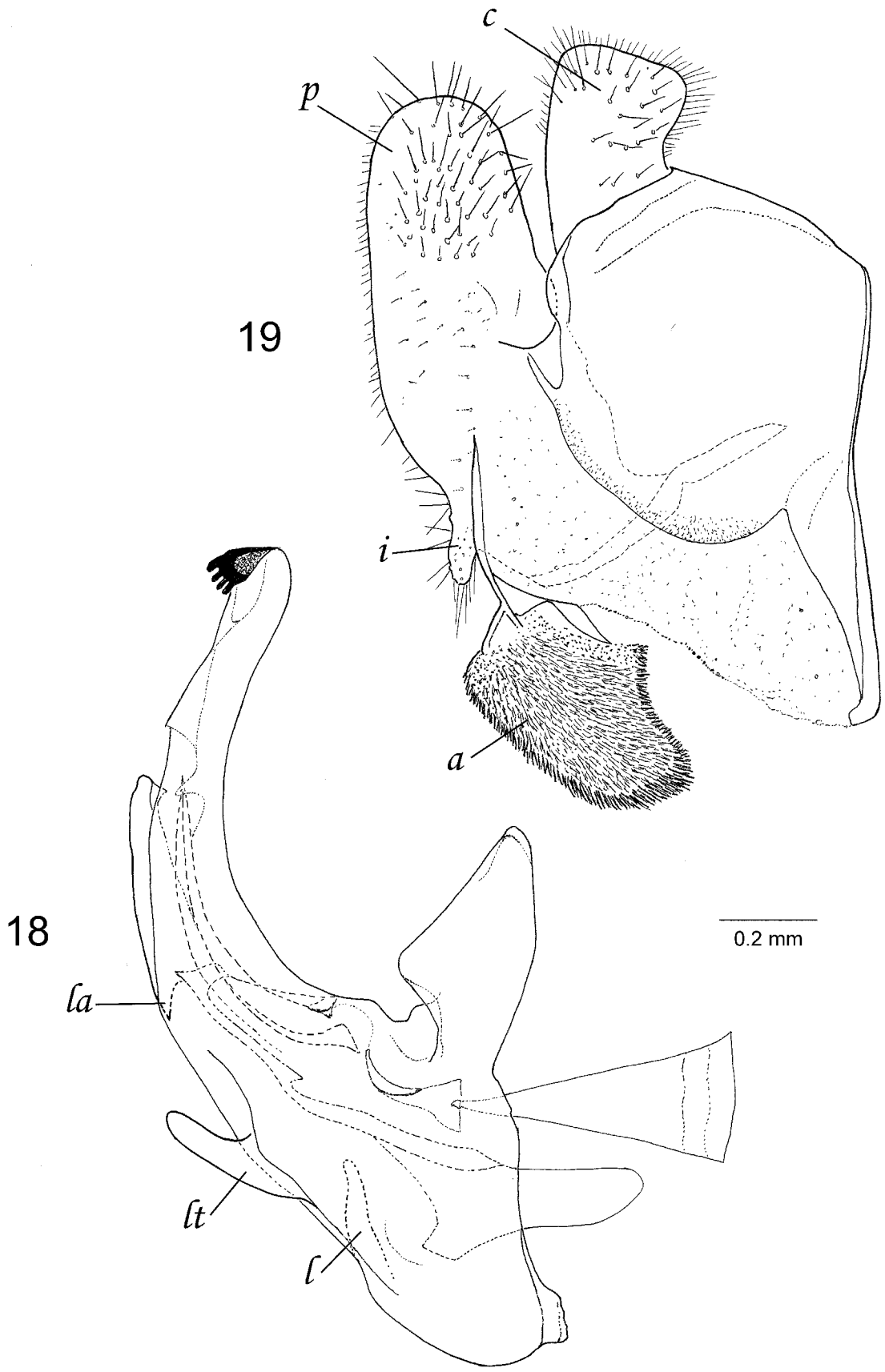
FIGURES 16, 17. Abdomen of *Merodon latifemoris* **n. sp.**, dorsal view, 16. male, 17. female.

Description. Male.

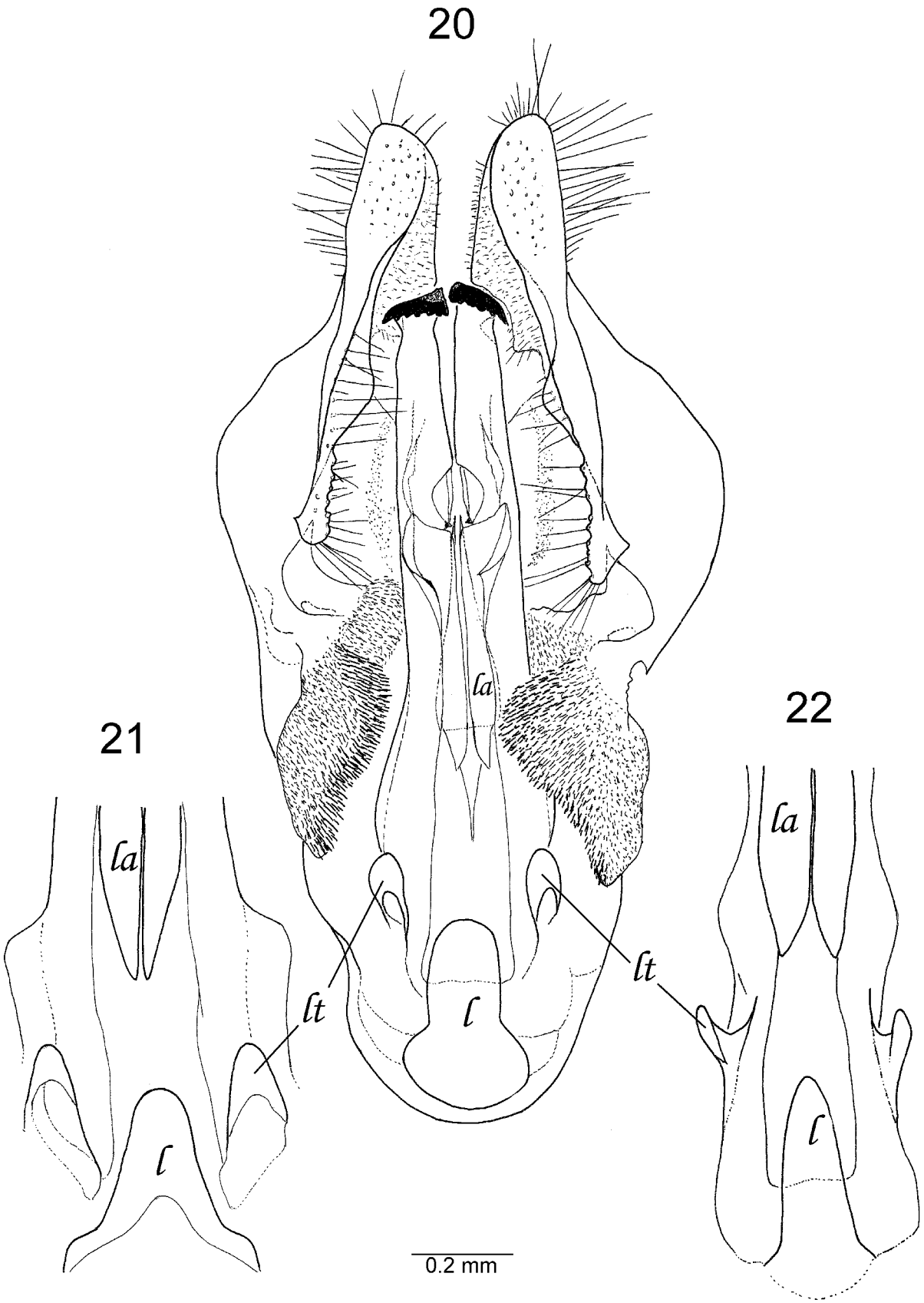
Head (fig. 27). Antenna (fig. 31) dark brown; first flagellomere light brown basally, 2.2 times as long as wide, dorsal margin concave, apex acute; arista: second and third flagellomeres light brown; fourth flagellomere dark brown except pale base, 1.2 times as long as first flagellomere. Face black, shiny, covered with long white hairs; frons black with thick white microtrichia and long, dense, white hairs. Oral margin bare with black luster. Vertical triangle isosceles, with white microtrichia anteriorly and posteriorly, shiny black in the ocellar triangle, covered with long white hairs, except few black ones in the ocellar triangle. Eye contiguity about 10 ommatidia long. Ocellar triangle equilateral. Vertical triangle: eye contiguity: ocellar triangle = 3.7 : 1 : 1.5. Eye hairs short, scarse and white. Occiput covered with thick whitish-golden microtrichia and hairs .

Thorax. Mesonotum black, covered with long white-yellow hairs. Scutum (fig. 35) with three medial golden microtrichose longitudinal stripes connected with two lateral longitudinal ones by microtrichose transverse suture. Posterior anepisternum, anepimeron and postero-dorsal part of katepisternum with golden microtrichia and long white-yellow hairs. Wing hyaline with dense microtrichia, dark veins, except light basal parts and most parts of C, Sc and R1. Calypter and halter light yellow. Legs black, except following parts: paler base and yellow apex of femora; reddish-brown tibiae, darkened medially; reddish-brown first, second and third tarsomeres (in metaleg dorsally slightly darkened); metafemur (fig. 39) thickened, about 2.93 times as long as deep. Legs with yellow hairs, except short black hairs on apico-dorsal surface of pro- and mesofemora and postero-dorsal surface of metafemur.

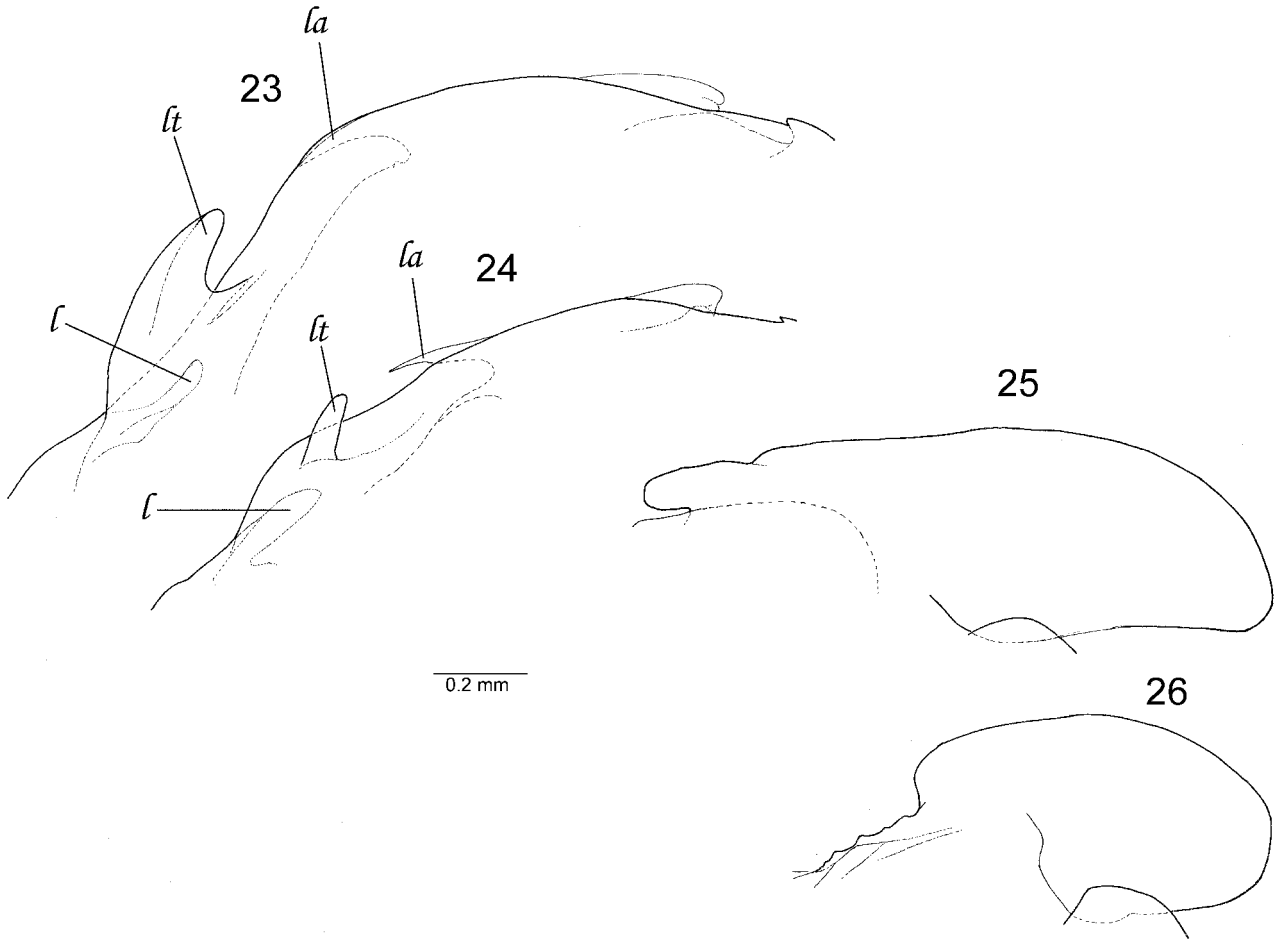
Abdomen (fig. 36). Black, slightly tapering, as long as mesonotum. Tergite 1 with white microtrichia; tergite 2 with two triangular antero-lateral reddish spots, white microtrichose transverse band interrupted in the middle and white microtrichose posterior margin; tergite 3 with white microtrichose transverse band narrowly interrupted in the middle and white microtrichia on a pale reddish posterior margin; tergite 4 with arcuate white microtrichose transverse band and white microtrichia on wide, pale reddish posterior margin (in some specimens posterior margins, lateral sides and transverse bands of tergites more reddish translucent). Hairs on tergites predominately whitish except black ones on black areas. Sternites brown, white-grayish microtrichose, covered with long white hairs.



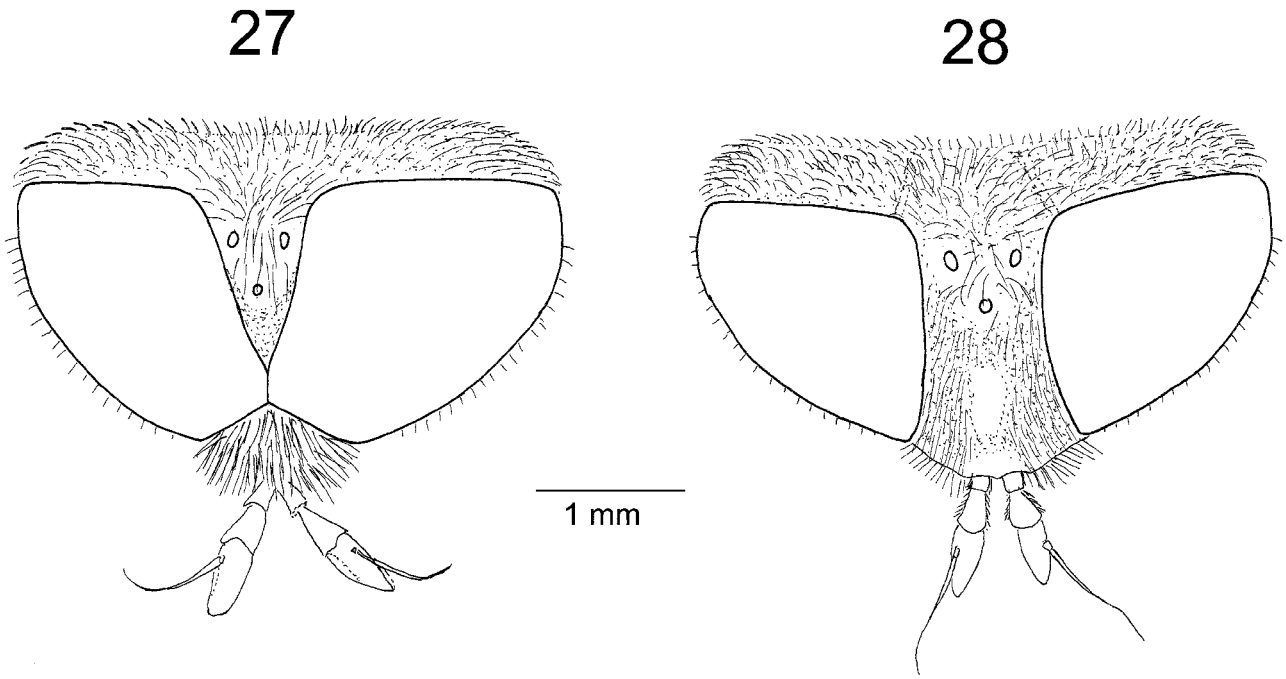
FIGURES 18, 19. Male genitalia of *Merodon latifemoris* **n. sp.**, lateral view. 18. hypandrium; 19. epandrium; a—anterior surstyle lobe; c—cercus; i—interior accessory lobe of posterior surstyle lobe; l—lingula; la—lateral sclerite of aedeagus; lt—lateral projection of hypandrium; p - posterior surstyle lobe.



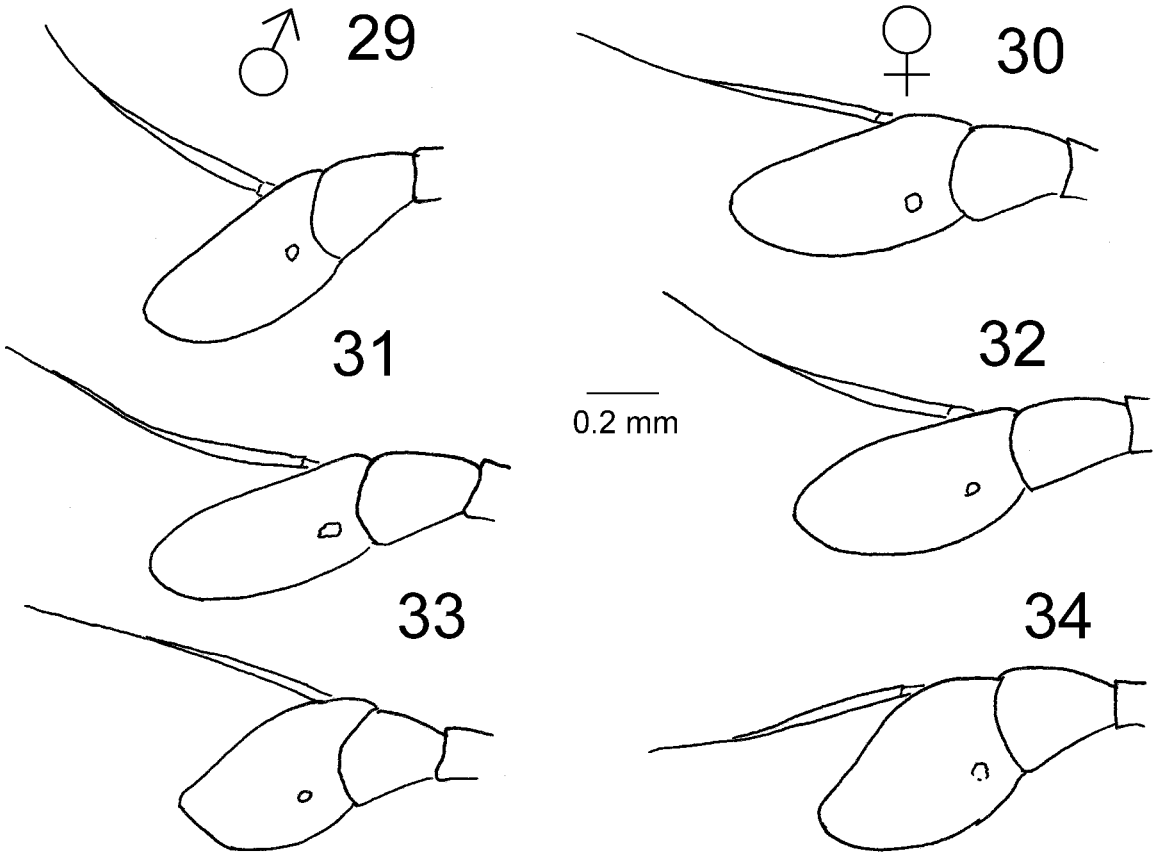
FIGURES 20–22. Male genitalia, ventral view, 20. *Merodon latifemoris* **n. sp.**; 21. *Merodon femoratoides*; 22. *Merodon nigritarsis*; l—lingula; la—lateral sclerite of aedeagus; lt—lateral projection of hypandrium.



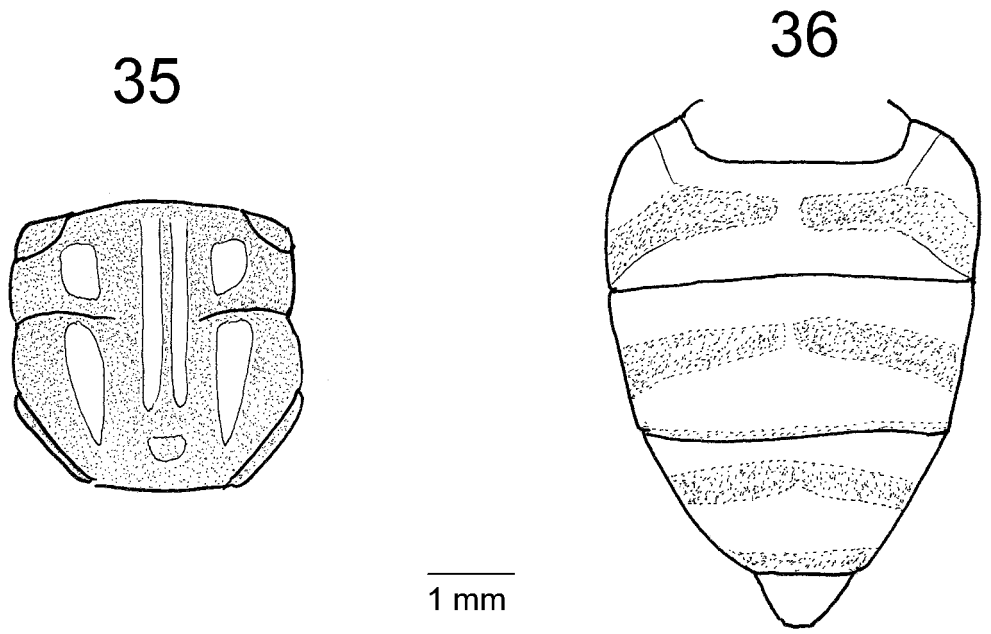
FIGURES 23–26. Male genitalia, lateral view. 23, 24. ventral part of hypandrium; 25, 26. posterior surstyle lobe; 23, 25. *Merodon femoratoides*; 24, 26. *Merodon nigritarsis*; l—lingula; la—lateral sclerite of aedeagus; lt—lateral projection of hypandrium.



FIGURES 27, 28. Head of *Merodon pulveris* **n. sp.**, dorsal view. 27—male; 28—female.



FIGURES 29–34. Antenna (first flagellomere and pedicel), lateral view; 29, 30. *Merodon natans*; 31, 32. *Merodon pulveris* **n. sp.**; 33, 34. *Merodon calcaratus*; 29, 31, 33. male; 30, 32, 34. female.

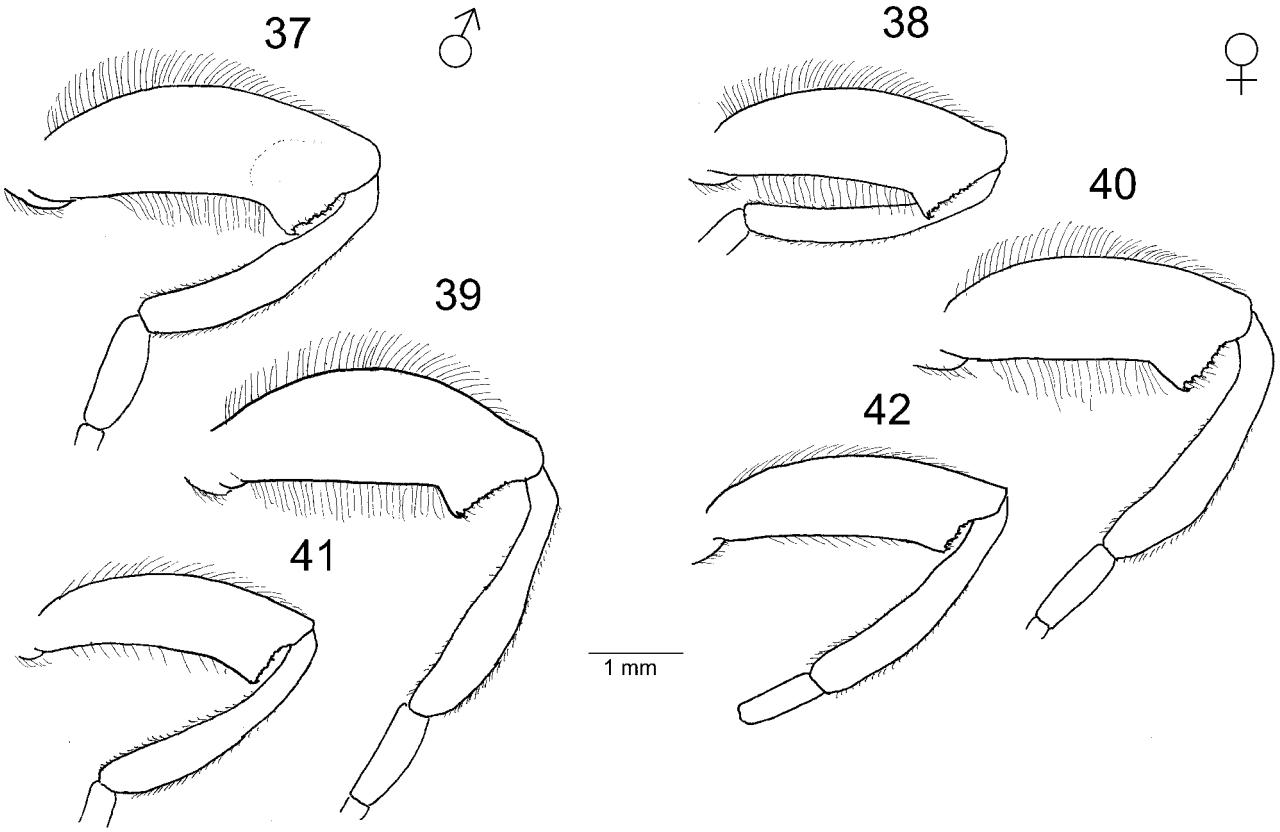


FIGURES 35, 36. Male of *Merodon pulveris* **n. sp.**, dorsal view. 35. mesoscutum; 36. abdomen.

Male genitalia (figs. 43–46). Ventral margin of posterior surstyle lobe (figs. 44, 46: v) concave with hairy, wart-like prominence. Anterior surstyle lobe (fig. 44, 46: a) oval with short, dense pubescence. Posterior surstyle lobe (figs. 44, 46: p) large, nearly perpendicular. Cercus (fig. 44: c) rounded rectangular. Hypandrium (figs. 43, 45) stout, very broad medially. Lateral sclerite of aedeagus (figs. 43, 45: la) long, finger-like.

Female (figs. 28, 32, 40). Similar to the male except normal sexual dimorphism. Frons black with thick white microtrichia except on shiny central stripe. Vertex completely white-yellow microtrichose except area around ocelli. Tergite 5 black with white microtrichose lateral and posterior margins, and with more or less distinctive two antero-medial white microtrichose spots.

Length: Body 11–12 mm; wing 7–8 mm.



FIGURES 37–42. Hind leg, lateral view. 37, 38. *Merodon natans*; 39, 40. *Merodon pulveris* **n. sp.**; 41, 42. *Merodon calcaratus*; 37, 39, 41. male; 38, 40, 42. female.

Merodon puniceus Vujić, Radenković et Pérez-Bañón, n. sp.

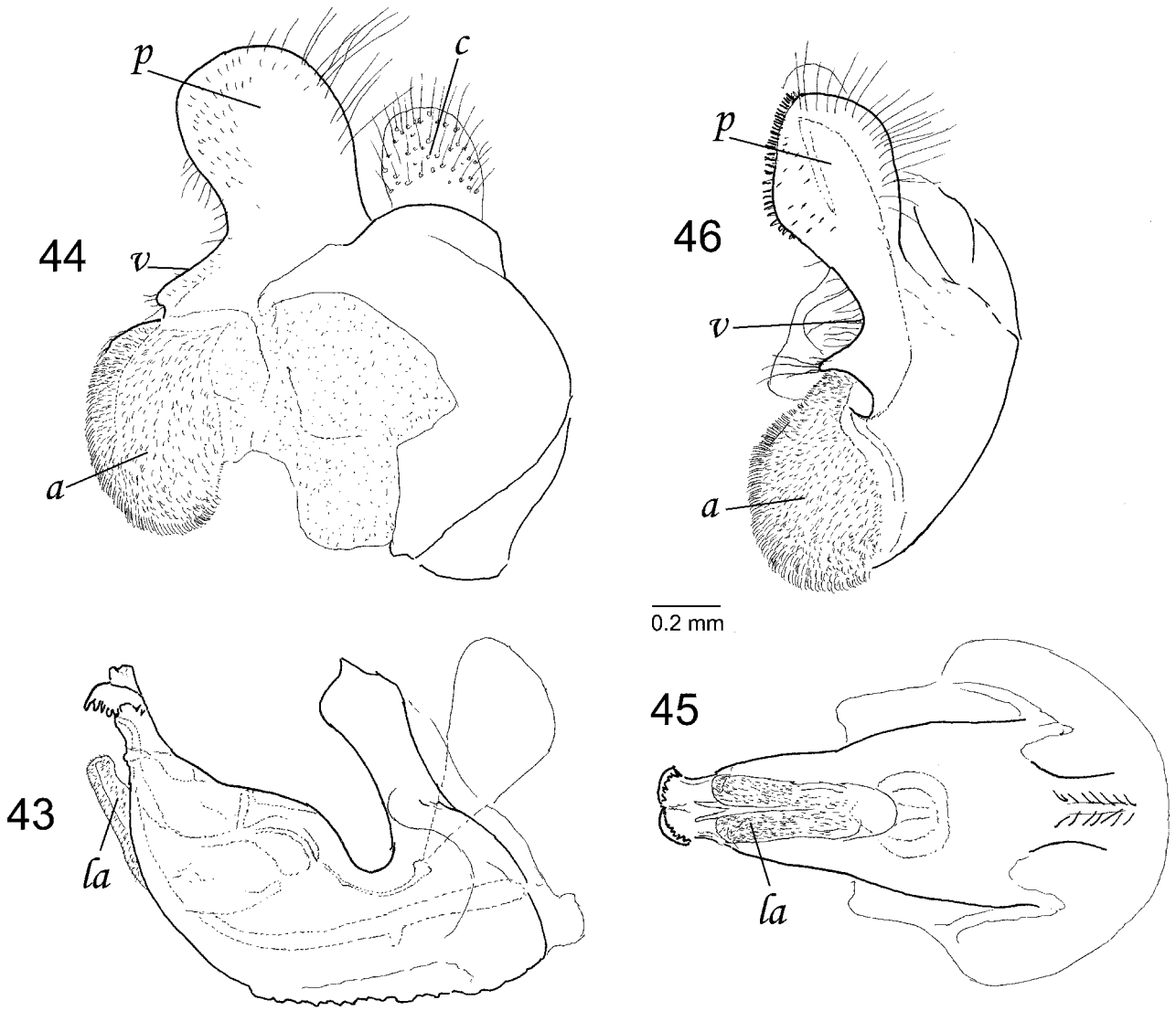
(Figs. 55–66)

Type material. Holotype: ♂, GREECE: **Lesvos**, 5.4 km SSE Agiassos; Alt. 760 m, 39° 3' 17" N; 26° 23' 50" E; mixed maquis; 14. 10.2005; leg. C. Perez-Bañón; AEU_0023677; FSUNS_03075; (CEUA).

Paratypes: GREECE: **Lesvos**: ♂, Scala, Polihnitos, 24.10.2008, leg. A. Vujić; FSUNS_04448; (FSUNS). ♂, 1.7 km WWN Vatera, 39° 01' 49" N, 26° 10' 30" E, 18.08.2009, leg. M. Hull, FSUNS_1771; (NML). ♀, 5,4 km SSE Agiassos; Alt. 760 m, 39° 3' 17" N; 26° 23' 50" E; mixed maquis; 08. 10. 2005; leg. C. Perez-Bañón; DNA voucher specimen, lab. No. MZH_S574, G. Ståhls, FMNH; AEU_0023663; FSUNS_03076; (MZH), EMBL COI barcode accession number FR717827. Studied material of *Merodon dobrogensis* Bradescu: GREECE: ♂, Euboea, Nea Artaki, 18.09.1982, leg. Bette, det. W. Hurkmans; (NHML). ♂, Korfos, Epidaurus, 15–27.09.1986; FSUNS_04157; (ZMA). ♀, Attiki, Dafni 10km W of Athena, 28.09.1985; (ZMA).

Etymology. The epithet, *puniceus* which is a Latin word meaning purple–red color, refers to the red tergites with purple luster.

Diagnosis (fig. 66). Medium sized species with metallic blue-green mesoscutum and predominately reddish-brown, oval abdomen with purple luster. Wing brown, darkened especially basally, anteriorly and along veins. Eyes completely black haired. Femur dark brown with yellow apex; tibia and tarsus orange except apical two (three) tarsomeres darkened.



FIGURES 43–46. Male genitalia of *Merodon pulveris* **n. sp.**; 43, 45. hypandrium; 44. epandrium; 46. left surstyle; 43, 44. lateral view; 45, 46. ventral view; a—anterior surstyle lobe; c- cercus; la—lateral sclerite of aedeagus; p—posterior surstyle lobe; v—ventral margin of posterior surstyle lobe.

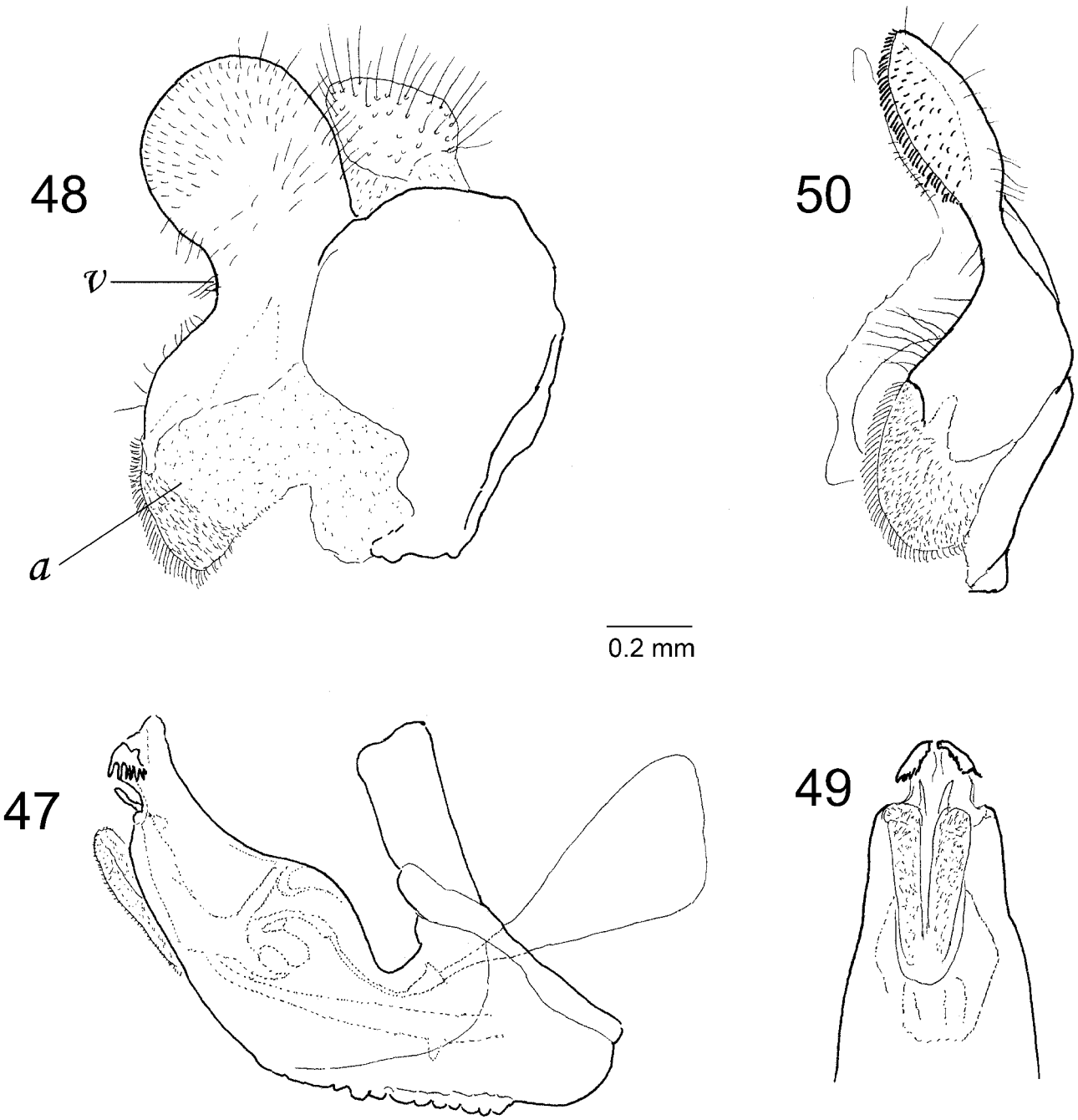
The species belongs to the *aureus* group and is closely related to *Merodon aureus* Fabricius. This group comprises small (8–13 mm) species with hairy posterior part of mid coxa and many hairs on posterior anepisternum below postpronotum; very short rounded abdomen; distinct thorn on metatrochanter in male; first flagellomere reddish to dark-brown; knees, tibiae and basal two tarsomeres pale; male genitalia (figs. 63–65) is almost identical in all species within the *aureus* group: anterior surstyle lobe undeveloped; posterior surstyle lobe with parallel margins and rounded apex; hypandrium narrow, elongated and sickle-shaped; lateral sclerite of aedeagus reduced.

M. puniceus **n. sp.** is very similar to *M. dobrogensis* Bradescu from which it differs by dense and longer hairs on mesonotum, more darkened wing especially along veins, and in female by more dense hairs on eyes.

Description. Male.

Head (fig. 55). Antenna (fig. 57) reddish-brown, first flagellomere 2.5 times as long as wide, 1.6–1.7 longer than pedicel, concave and darkened dorsally, apex rectangular; arista: second and third flagellomeres pale, fourth flagellomere dark brown and thickened basally, slightly shorter than first flagellomere. Face and frons black with bluish luster, covered with long whitish-yellow hairs. Oral margin bare with black luster. Vertical triangle isosceles, shiny black, anteriorly (in the ocellar triangle) predominantly covered with black hairs, and posteriorly with long, white hairs. Ocellar triangle equilateral. Eye contiguity about 11 ommatidia long. Vertical triangle: eye contiguity:

ocellar triangle = 1.8 : 1.5 : 1. Eye hairs long, dense, black. Occiput with whitish-yellow hairs, along the eye margin with dense white microtrichia and posteriorly with metallic bluish-greenish luster.

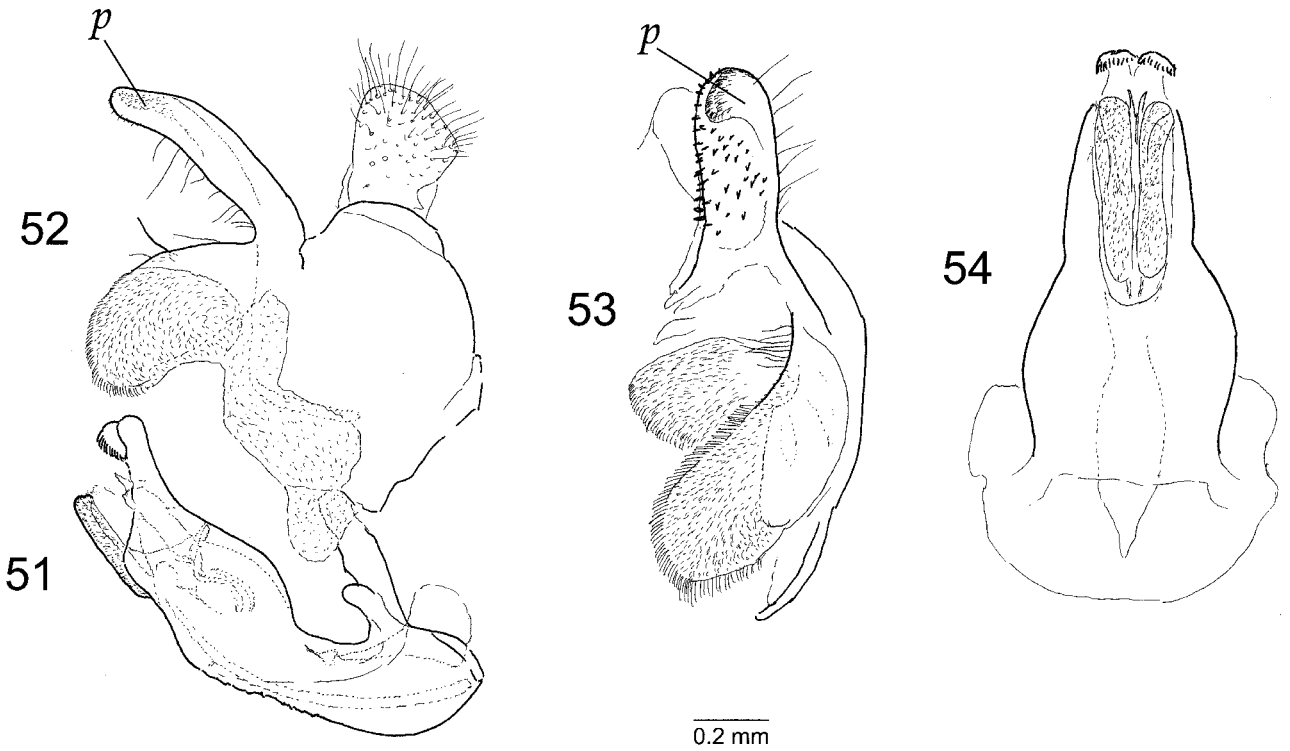


FIGURES 47–50. Male genitalia of *Merodon natans*. 47. hypandrium, lateral view; 48. epandrium, lateral view; 49. apical part of hypandrium, ventral view; 50. left surstyle, ventral view; a—anterior surstyle lobe; v—ventral margin of posterior surstyle lobe.

Thorax. Postpronotum with conspicuous tooth-like process posteriorly. Scutum and scutellum black with metallic blue luster, covered with long, dense, erect white to yellowish hairs. Posterior anepisternum, anepimeron and postero-dorsal part of katepisternum with long whitish-yellow hairs and metallic blue luster. Wing with dense microtrichia, brown, darkened especially basally, anteriorly and along veins; veins dark brown except light brown C, Sc and basal part of R1. Calypter pale yellow. Halter with light brown pedicel and dark brown capitulum. Femora dark brown with pale apex; pro- and mesofemur with long light yellow hairs posteriorly and short, black hairs anteriorly and dorsally; metafemur with long reddish-brown hairs antero-dorsally, short ones apico-dorsally,

on triangular projection and a few longer ones ventrally, with short black hairs baso-dorsally, antero-ventrally and sparse ones postero-dorsally. Tibiae and tarsi orange, except 2 (3) apical tarsomeres darkened (fourth tarsomere distinctly brown), covered with yellow hairs and some black ones. Metatrochanter (fig. 59: tr) with an inner thorn with two pointed angles.

Abdomen (fig. 61). Oval, slightly longer than mesonotum; reddish-brown with purple luster laterally and on black markings medially; tergite 1 black with golden luster; tergite 2 reddish-brown with black goblet-like marking medially, black posterior margin and two lateral indistinct white microtrichose spots near the narrowest part of the goblet-like black marking; tergite 3 reddish-brown with triangular black marking postero-medially and black lateral and posterior margins; tergite 4 reddish-brown antero-laterally, black medially and posteriorly ('hour-glass' shaped); hairs on tergites predominantly short golden, except black ones on black markings and posterior margins of tergites 2 and 3. Sternites translucent, from reddish-yellow to reddish-brown towards the end of abdomen, black lined, covered with long whitish-yellow hairs.



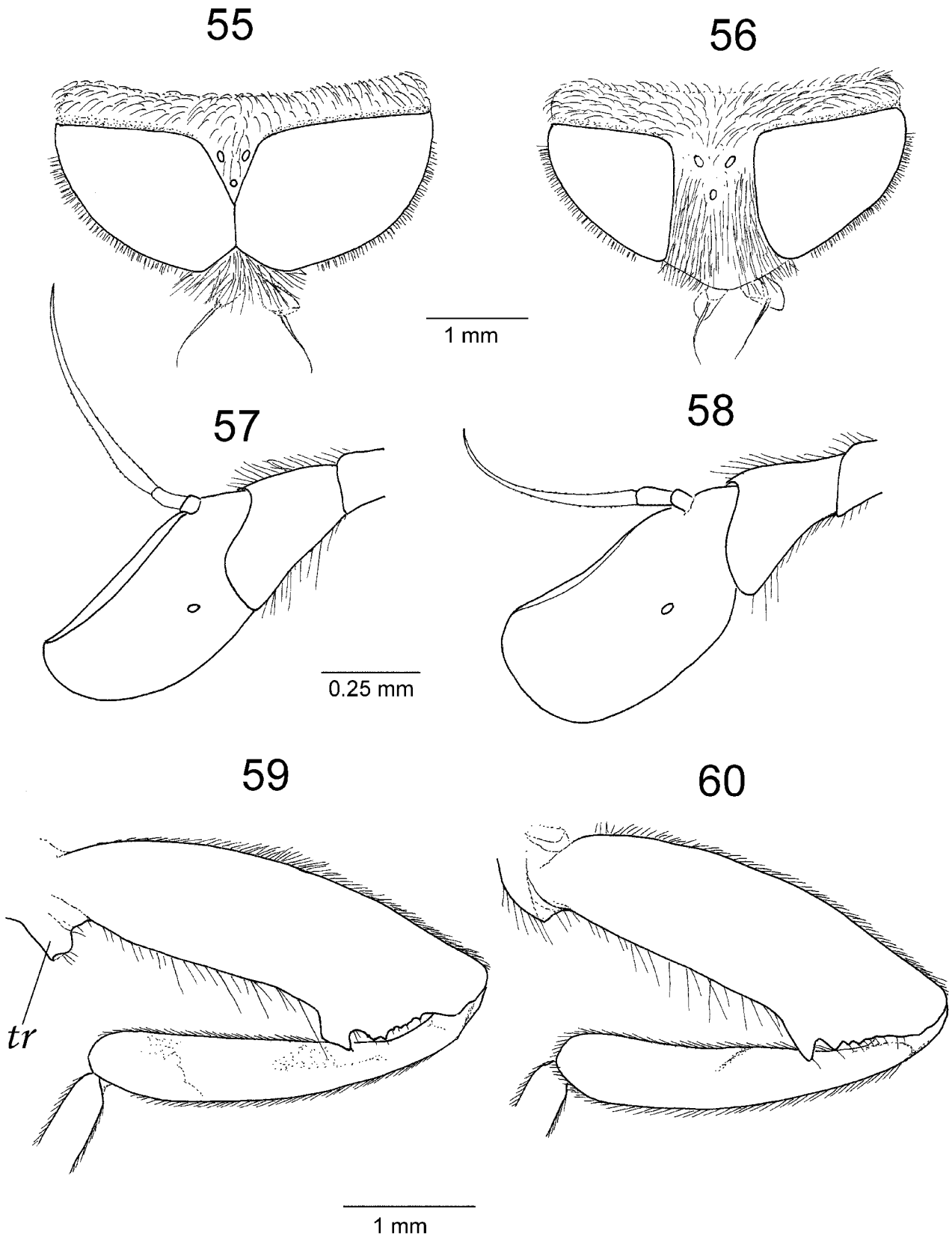
FIGURES 51–54. Male genitalia of *Merodon calcaratus*. 51, 54. hypandrium; 52. epandrium; 53. left surstyle; 51, 52. lateral view; 53, 54. ventral view; p—posterior surstyle lobe.

Female (figs. 56, 58, 60, 62). Similar to the male except normal sexual dimorphism and for the following characteristics: metatrochanter (fig. 60) without thorn; black markings on tergites (fig. 62) narrower; tergite 2 with more conspicuous two lateral white microtrichose spots near the narrowest part of the goblet-like black marking; tergite 5 and sternite 5 black.

Length: Body 11–12 mm, wing 8–9 mm.

Discussion

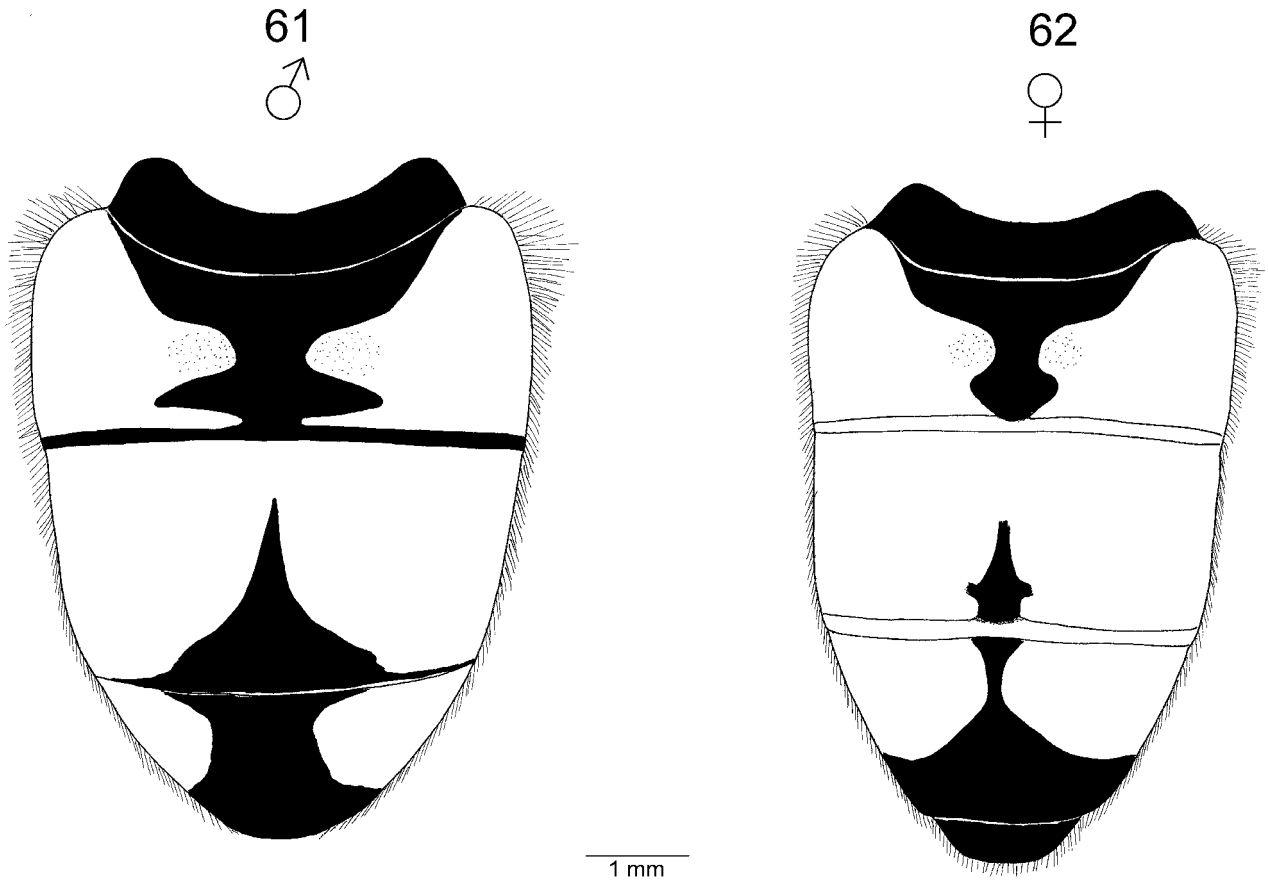
Molecular techniques (especially DNA sequencing) increase our capacity to describe biological diversity. The discovery of putatively new species based on DNA sequences has created new opportunities in taxonomy (Meier 2008). Once flagged as being genetically distinct, it will allow a taxonomist to focus on populations that are likely to represent new species. All cases of conflict between the DNA tree and morphological assignment deserve additional investigation using additional samples and other independent gene regions. Barcoding can bring part of the information necessary to study questions that have high value for biodiversity and conservation studies.



FIGURES 55–60. *Merodon puniceus* **n. sp.** 55,56. head, dorsal view; 57, 58. antenna, lateral view; 59, 60. hind leg, lateral view; 55, 57, 59. male; 56, 58, 60. female; tr—trochanter.

Integrating COI barcoding into morphological and ecological studies of *Merodon* spp. throughout the Mediterranean region will eventually enable identification of samples of all the life stages using molecular and/or

morphological characters. Moreover, numerous non-morphological characters (as mating behavior, biochemical characters, biogeography, phenology etc.) can be used to distinguish cryptic or sibling species. As Bickford *et al.* (2007) suggest, we incorporated in the research of alpha taxonomy of genus *Merodon* (ie. the description of new taxa) molecular data of the new species studied here and almost all of the species cited from Lesvos island (Ståhls *et al.* 2009). Some authors consider that cryptic species are almost evenly distributed among major metazoan taxa and biogeographical regions (Pfenninger & Schwenk 2007). On the contrary, other studies support the idea that the proportion of cryptic species could be related with multiple mechanisms generating hidden species diversity. Every new genus or specific habitat could have their own pattern of cryptic diversity (Trontelj & Fišer 2009). This could be probably the case in the genus *Merodon*, with six cryptic species among about 23 species occurring on the island of Lesvos (unpublished data).

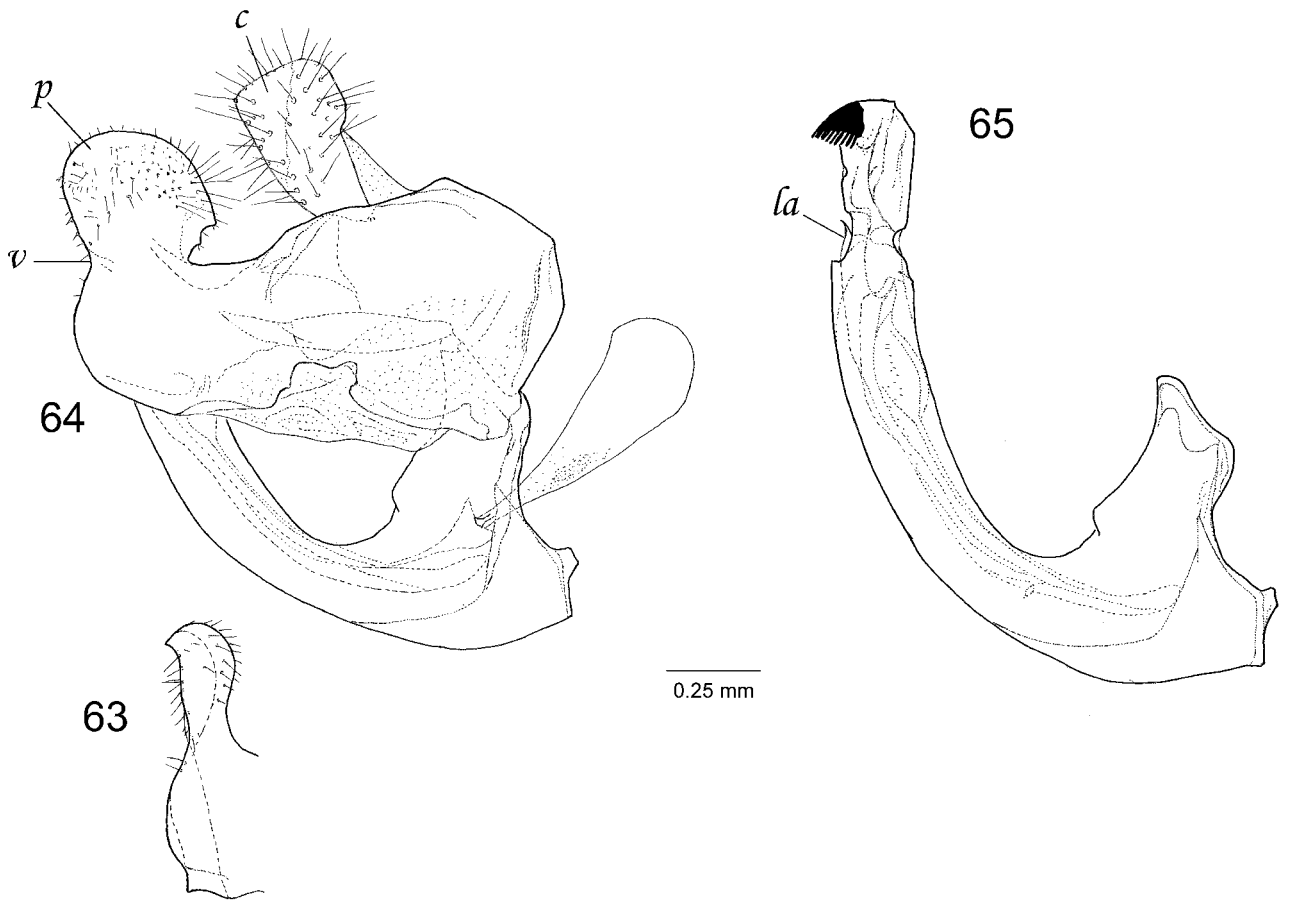


FIGURES 61, 62. Abdomen of *Merodon puniceus* **n. sp.**, dorsal view; 61. male; 62. female.

The recently described *Merodon sapphous* Vujić, Radenković et Pérez-Bañon and *M. puniceus* Vujić, Radenković et Pérez-Bañón **n. sp.** belong to the *aureus* species group, of which a very high number of localized endemic species are known from around the Mediterranean Basin (Vujić *et al.* 2007). The uncorrected sequence divergence for the COI gene between *M. sapphous* (GB accession no FM206491) and *M. puniceus* is 6.77%. *M. puniceus* is a distinctive, endemic species of Lesvos island, but very similar to *M. dobrogensis* Bradescu (no other species of *Merodon* show such a reddish-brown color pattern on the abdomen, fig. 66). These taxa are morphologically very similar, and probably sibling species, but can be separated by the darkened wings and the different pilosity patterns. Unfortunately there are no molecular data available for *M. dobrogensis* due to the fact that it is a very rare species, known only from one locality in Roumania (Dobroudja) (Bradescu 1982) and in Greece (Athens) (Petanidou *et al*. in prep.).

Merodon pulveris **n. sp.** is another cryptic species closely related to the South-European species *M. natans* (Fabricius), but separable due to characters of male genitalia and specific chaetotaxy. The uncorrected sequence difference for the COI gene between these species is 4.77%. *M. pulveris* **n. sp.** is to date found on the island of Lesvos (Greece) and along the west coast of Turkey. Although there are a number of records of *M. natans* from

west and central Aegean islands (unpublished data), on the island of Lesvos only this closely related Anatolian endemic species, *M. pulveris* **n. sp.**, is present.



FIGURES 63–65. Male genitalia of *Merodon puniceus* **n. sp.** 63. left surstyle, ventral view; 64. epandrium with basal part of hypandrium, lateral view; 65. hypandrium, lateral view; c—cercus; la—lateral sclerite of aedeagus; p—posterior surstyle lobe; v—ventral margin of posterior surstyle lobe.

Merodon latifemoris **n. sp.** is a cryptic species related with *M. nigritarsis* Rondani and *M. femoratoides*. The three taxa are closely related, as indicated by both morphological and molecular characters (Ståhls *et al.* 2009). The uncorrected sequence divergence for the COI gene between *M. latifemoris* and *M. nigritarsis* (GB accession no FM206494) is 2.0%, and between *M. latifemoris* and *M. femoratoides* (GB accession no FM206501) is 2.31%. Here we present a set of combined morphological data that can be used to distinguish between these taxa. Among these taxa, *M. nigritarsis* is the most morphologically variable species and with the largest distribution that includes Central Europe, the North Mediterranean and Western Asia (around Black and Caspian Sea). Although it appears in the Mediterranean region, *M. nigritarsis* also prefers high mountains. *M. femoratoides* is an Eastern Mediterranean species that ranges up to the Black Sea, while *M. latifemoris* **n. sp.** is found only in Greece, on the island of Lesvos and on the Greek Pindos Mountains at Katara pass. All three species occur on the island of Lesvos, *M. latifemoris* **n. sp.** appears synchronically and sympatrically only with *M. nigritarsis* in June, while *M. femoratoides* occurs earlier, during May.

This paper is an additional contribution to illustrate the great diversity of the genus *Merodon* in the Eastern Mediterranean (on Mediterranean islands). The data support the conclusions of previous studies on other *Merodon* species (Milankov *et al.* 2008 a,b,c), which indicate the necessity to define effective conservation units and, the importance of protection of phylogenetic lineages within endemic taxa and species groups and conservation of hidden biodiversity. Cryptic speciation could be especially important in conservation planning as previously demonstrated for hoverflies (Schönrogge *et al.* 2002), because endangered taxa can constitute two or more cryptic species, with different ecological requirements.

FIGURE 66. Male of *Merodon puniceus* **n. sp.**, color pattern.

Acknowledgements

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