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2020-02

Zoric , L S , Acanski , J , Vujic , A , Stahls , G , Djan , M & Radenkovic , S 2020 , ' Resolving the taxonomy of the Merodon dobrogensis species subgroup (Diptera: Syrphidae), with the description of a new species ' , Canadian Entomologist , vol. 152 , no. 1 , PII S0008347X19000725 , pp. 36-59 . <https://doi.org/10.4039/tce.2019.72>

<http://hdl.handle.net/10138/314253>

<https://doi.org/10.4039/tce.2019.72>

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Resolving the taxonomy of the *Merodon dobrogensis* subgroup (Diptera: Syrphidae), with the description of a new species

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1 **Abstract**

2 The taxonomy of *Merodon dobrogensis* subgroup is reviewed. Multiple data
3 sources (morphology, geometric morphometry of wings and surstylus, molecular
4 data, distributional data) were used to investigate the subgroup in the manner of
5 integrative taxonomy. *M. dobrogensis* Bradescu, 1982 and *M. puniceus* Vujić,
6 Radenković et Pérez-Bañón, 2011 are supported as distinct species belonging to
7 the *M. dobrogensis* species complex within the *M. dobrogensis* subgroup.
8 Additionally, evidence is presented for the description of a new species, *M. rojoi*
9 Radenković et Vujić **new species** with a distribution in mainland Greece, the
10 Greek island Euboea and the Peloponnese. A short diagnosis is provided for the
11 *M. aureus* group, the *M. dobrogensis* subgroup and the newly defined *M.*
12 *dobrogensis* species complex, in addition to a description of the new species, with
13 drawings and photos of adult morphology.

14 **Introduction**

15 The *Merodon aureus* species group is a group of species within the genus
16 *Merodon* Meigen, 1803 (Diptera: Syrphidae), the most speciose hoverfly genus in
17 Europe. The species group has been morphologically defined by Radenković *et al.*
18 (2011). Molecular analyses utilizing cytochrome c oxidase I (COI) and 28S rRNA
19 gene sequences have supported it as a monophyletic lineage (Mengual *et al.* 2006;
20 Radenković *et al.* 2011; Vujić *et al.* 2012). The *M. aureus* group comprises small-
21 sized species possessing a short, rounded abdomen, metatrochanter with an inner
22 calcar with two pointed angles and a characteristic structure of the male genitalia.
23 Anterior lobe of surstylus is undeveloped, while posterior is with parallel margins
24 and rounded apex, and a narrow, elongated, sickle-shaped hypandrium without
25 lateral sclerite of aedeagus. (Radenković *et al.* 2011). Previous studies have
26 indicated that the group may have an especially large number of cryptic taxa
27 (Šašić *et al.* 2016, 2018; Veselić *et al.* 2017; Radenković *et al.* 2018).

28 Šašić *et al.* (2016) defined four subgroups within the *Merodon aureus* species
29 group (*M. aureus*, *M. bessarabicus*, *M. cinereus*, and *M. dobrogensis* subgroups
30 respectively), as well as one unplaced species complex (the *M. chalybeus*
31 complex), based upon the morphological similarity of the species. Each of these
32 subgroups contains at least one complex of cryptic species, although they may
33 contain multiple complexes of species such as the *M. bessarabicus* subgroup (see
34 Veselić *et al.* 2017).

35 Šašić *et al.* (2016) define species complexes as morphologically inseparable
36 species, which can only be resolved by employing an integrative taxonomy
37 approach including different data types such as molecular, geometric
38 morphometry and ecology. Applying this approach for the *Merodon aureus*
39 species group has led to the discovery of previously unknown species complexes.
40 For the *M. cinereus* subgroup, Šašić *et al.* (2016) resolved the *M. atratus* complex
41 of species, and found that it consisted of three species, two of which were new to
42 science. Veselić *et al.* (2017) provided evidence for the presence of four species
43 complexes in the *M. bessarabicus* subgroup. Radenković *et al.* (2018) resolved *M.*
44 *luteomaculatus* as a complex of six cryptic species. Additionally, the same
45 approach was used to resolve the *M. caeruleus* species complex (Šašić *et al.*
46 2018).

47 The taxonomy of the *Merodon dobrogensis* subgroup has not been evaluated
48 utilizing an integrative taxonomy approach. This subgroup is monotypic,
49 containing only the species *M. dobrogensis* Bradescu, 1982, first discovered in
50 Romania, and the taxon is easily recognized by its red abdomen and reddish-
51 orange tibiae. However, the description of *M. puniceus* Vujić, Radenković et
52 Pérez-Bañón, 2011, a morphologically similar species from the Aegean island of
53 Lesvos in Radenković *et al.* (2011) raises the question of whether or not *M.*
54 *dobrogensis* is a potential species complex and provides good reason to conduct
55 an in-depth taxonomic investigation, and to check for additional undiscovered
56 species within the *M. dobrogensis* subgroup.

58 **Material and methods**

59 We explored species borders within *M. dobrogensis* subgroup by applying an
60 integrative taxonomy approach using molecular data and morphology, wing and
61 surstylus geometric morphometry.

62

63 ***Taxonomy***

64 For the present study, material from continental Greece, Turkey and Romania (the
65 type locality of *Merodon dobrogensis*) has been collected in order to explore
66 overall diversity of *M. dobrogensis* subgroup. Material examined includes
67 specimens collected during the last 30 years (1982-2014) as well as any available
68 specimens including types, previously cited in the literature. Collections from
69 which specimens were obtained from are listed below.

70 The following acronyms of museums and entomological collections are used in
71 the text: Natural History Museum, London, United Kingdom (BMNH); Colección
72 Entomológica de la Universidad de Alicante, Alicante, Spain (CEUA); Faculty of
73 Sciences, Department of Biology and Ecology, University of Novi Sad, Novi Sad,
74 Serbia (FSUNS); Naturalis Biodiversity Center, Leiden, The Netherlands
75 (RMNH); World Museum Liverpool, Liverpool, United Kingdom (WML); The
76 Melissotheque of the Aegean, University of the Aegean, Mytilene, Greece
77 (MAegean) and Michael de Courcy Williams collection, Greece (M.C.W. coll.).

78 A short diagnosis is provided for the *Merodon aureus* group, the *M. dobrogensis*
79 subgroup and the newly defined *M. dobrogensis* species complex including the
80 description of a new species, with drawings and photos of adult morphology.
81 Terminology follows Thompson (1999) for non-genitalic morphology and
82 Marcos-García *et al.* (2007) for the morphology of male terminalia. Methodology
83 from Vujić *et al.* (2013) was followed for studying male genitalia. Digital photos
84 were taken with Leica DFC 320 digital camera, attached to a Leica MZ16
85 binocular microscope. Measurements were taken with an eyepiece graticule or
86 micrometer. Morphological characters were observed using a Nikon SMZ 745T
87 stereomicroscope.

88 Data on the biology of species from the *Merodon dobrogensis* subgroup,
89 presented in the database for European Syrphidae by Speight (2018) have been
90 updated.

91 ***Geometric morphometric analysis***

92 As part of an integrative taxonomic approach, two different geometric
93 morphometric analyses are employed to identify species of the *Merodon*
94 *dobrogensis* subgroup: landmark-based analysis of the wing shape and outline
95 contours of the posterior surstyle lobe of male genitalia (hereinafter referred to as
96 the surstylus).

97 High-resolution photographs of the wings and surstylus are made using a Leica
98 DFC320 video camera attached to a Leica MZ16 stereomicroscope. Landmarks

99 and semi-landmarks are drawn on every picture using the TpsDig v2.30 (Rohlf
100 2017a).

101 Principal component analysis (PCA) is carried out on the Procrustes shape
102 variables of surstylus to reduce the dimensionality of the data set. A stepwise
103 discriminant function analysis (DA) and canonical variate analysis (CVA) are
104 used to explore wing and surstylus shape variation among the taxa. Additionally, a
105 Gaussian naïve Bayes classifier is used to delimit species boundaries based on
106 wing and surstylus shape variation without a priori-defined groups. Phenetic
107 relationships among the taxa were characterised using an Unweighted Pair Group
108 Method with Arithmetic mean (UPGMA) cluster analysis based on squared
109 Mahalanobis distances computed from the discriminant function analysis applied
110 to shape variables. Superimposed outline drawings are produced using MorphoJ
111 v2.0 (Klingenberg 2011) to visualize differences in mean wing and surstylus
112 shape among species pairs. All statistical analyses are done using Statistica for
113 Windows (StatSoft Inc 2016).

114

115 Wing morphometry

116 Landmark-based geometric morphometric analysis of wing shape is conducted on
117 53 specimens of the *Merodon dobrogensis* subgroup (Table 1). The right wing of
118 each specimen is taken off by using micro-scissors and mounted in Hoyer's
119 medium on a microscopic slide. Wings are archived and labelled with a unique
120 code in the FSUNS collection, together with other data relevant to the specimens.
121 Eleven homologous landmarks at vein intersections or terminations (that could be

122 reliably identified) are selected. Due to an uneven distribution of sexes within
123 taxa, analyses are conducted on a dataset in which both sexes are pooled and for
124 the male dataset separately. Wing shape components are size-corrected to remove
125 the allometric component of wing shape.

126 Generalised least squares (GLS) Procrustes superimposition (PS) on the raw
127 coordinates is done using TpsRelw v1.68 (Rohlf 2017b) to minimize non-shape
128 variations in location, scale and orientation of wings, and to superimpose the
129 wings in a common coordinate system (Rohlf and Slice 1990; Zelditch *et al.*
130 2004).

131 Surstylus Morphometry

132 Shape analysis of the right posterior surstylus lobe of male genitalia is carried out
133 on 28 specimens of the *Merodon dobrogensis* subgroup (Table 1). The right
134 posterior surstylus lobe is removed using a scalpel and placed on its side in
135 glycerol on a microscopic slide, with a cover slip placed on top of the surstylus to
136 immobilize it.

137 15 semi-landmarks are digitized from the membranous part of the epandrium to
138 the end of the surstylus. The software CoordGen 7.14 (Sheets, 2012) with an
139 integrated Semiland module is used for semi-landmark superimposition using a
140 distance-minimizing protocol, minimizing the shape differences due to the
141 arbitrary nature of semi-landmark positions along the curve (Bookstein, 1997;
142 Zelditch et al., 2004).

143

144 ***Molecular study***

145 Total genomic DNA is extracted from mid and hind legs of 37 hoverfly specimens
146 using the SDS extraction protocol (Chen *et al.* 2010). Detailed information on
147 specimens included in molecular analyses and GenBank accession numbers are
148 presented in Table 1. Genomic DNA vouchers are conserved at the Faculty of
149 Sciences, Department of Biology and Ecology, University of Novi Sad (FSUNS).

150 The primers used for amplification of 3' and 5' ends of the mitochondrial COI
151 gene, fragment of ND4 gene, as well as the D2-3 expansion segment of the 28S
152 rRNA gene are provided in Table 2. Polymerase chain reactions (PCR) are carried
153 out in 25µl reaction volumes. The reaction mixture for 3' and 5' COI and 28S
154 rRNA gene amplification contained 1x reaction buffer (Thermo Scientific,
155 Vilnius, Lithuania), 2.5mM MgCl₂, 0.1mM of each nucleotide, 1.25U Taq
156 polymerase (Thermo Scientific, Vilnius, Lithuania), 5pmol of each primer, and
157 approximately 50-100ng of template DNA. For ND4 gene amplification we used
158 1.25U Dream Taq polymerase (Thermo Scientific, Vilnius, Lithuania) and
159 corresponding 1x Dream Taq reaction buffer (Thermo Scientific, Vilnius,
160 Lithuania) with included MgCl₂. We used the following PCR conditions for COI
161 and 28S rRNA gene amplification: 95°C for 2 min; 29 cycles of 94°C for 30s
162 each, 49°C (for 3' COI) and 50°C (for 5' COI and 28S rRNA gene) for 30 s; 72°C
163 for 2 min; with the final extension at 72°C for 8 min. The applied PCR conditions
164 for ND4 gene are: 95°C for 5 min; 35 cycles of 94°C for 1 min each, 45°C for 30
165 s; 72°C for 2 min; with the final extension at 72°C for 8 min. The purification of
166 PCR products is done using Exonuclease I and FastAP Thermosensitive Alkaline

167 Phosphatase (Thermo Scientific, Vilnius, Lithuania) according to the
168 manufacturer's instructions. The sequencing is performed in a forward direction
169 for each amplified fragment using the BigDye Terminator v.3.1 cycle sequencing
170 kit (Thermo Scientific, Vilnius, Lithuania) on an ABI3730xl Genetic Analyzer
171 (Applied Biosystems) in the Sequencing Service Laboratory of the Finnish
172 Institute for Molecular Medicine (FIMM), University of Helsinki, Finland and the
173 Macrogen EZ-Seq service, Macrogen Europe, Amsterdam, the Netherlands.

174 Molecular genetic data analyses

175 The COI and 28S rRNA gene sequences are edited for base-calling errors using
176 BioEdit 7.0.9.0., aligned manually and trimmed to final length by eye.

177 The 5'COI sequence matrix with included *Merodon puniceus* sequence (Ståhls *et*
178 *al.* 2009; Radenković *et al.* 2011; GenBank accession number: FR717827) is used
179 for uncorrected pairwise (p) distance calculation in order to assign Turkish
180 specimens to the particular species. The uncorrected p distances are calculated
181 using MEGA 7 (Kumar *et al.* 2016). In all further analysis, sequences are assigned
182 to particular species. Because of low variability, 28S rRNA gene sequences are
183 only used for genotypes determination. ND4 and COI gene sequences are
184 analysed more thoroughly. The 3' and 5' COI gene sequences are concatenated
185 and analysed together.

186 Maximum Parsimony (MP) and Maximum Likelihood (ML) analyses are applied
187 on COI and combined COI+ND4 sequence matrices. MP analysis is performed in
188 NONA (Goloboff 1999) spawned with the aid of ASADO (Nixon 2008) using the

189 heuristic search algorithm with 1,000 random addition replicates (mult*1,000),
 190 holding 100 trees per round (hold/100), maxtrees set to 100,000 and applying tree-
 191 bisection-reconnection (TBR) branch swapping. ML tree is constructed using
 192 RAxML 8.2.8 (Stamatakis 2014) using the CIPRES Science Gateway web portal
 193 (Miller *et al.* 2010) under the general time-reversible (GTR) evolutionary model
 194 with a gamma distribution (GTRGAMMA) (Rodríguez *et al.* 1990). The clade
 195 supports are calculated using 1000 bootstrap replicates for both MP and ML trees.
 196 The trees are rooted on *Eristalinus aeneus* (GenBank accession number:
 197 NC_042911). The detection of COI and ND4 haplotypes, as well as 28S rRNA
 198 gene genotypes are done in DnaSP version 5 (Librado and Rozas 2009). Average
 199 uncorrected pairwise distances (p) between taxa are calculated using program
 200 MEGA7 (Kumar *et al.* 2016).

201 ***Correlation among wing shape, surstylus shape, genetic, spatial and climatic***
 202 ***differentiation***

203 To test pairwise correlations between morphometric (wing and surstylus), genetic
 204 (COI and ND4) and geographic and climatic distances among species, two-tailed
 205 Mantel tests are performed (Mantel 1967) with 9999 permutations in PaSSaGe
 206 (Rosenberg and Anderson 2011). Additionally, partial Mantel tests are used to
 207 explore relationships between: morphometric and geographic differentiation,
 208 while accounting for the effect of climatic distances; and morphometric and
 209 climatic differentiation, while accounting for the effect of geographic distances.
 210 Morphometric distances are represented as a matrix of pairwise squared

211 Mahalanobis distances, and genetic distances as a matrix of uncorrected p
 212 distances. Geographic distances are calculated as the minimum distance between
 213 two species using QGIS (Quantum GIS Development Team, 2012). Climatic
 214 distances are represented as Euclidean distances of the factor scores calculated
 215 based on 19 bioclimatic variables generated for each locality from the current
 216 climate WorldClim dataset (2.5 arc-minutes resolution) (Hijmans *et al.* 2005).

217

218

219 **Results**

220 ***Geometric Morphometric Evidence***

221 **Wing shape**

222 Geometric morphometric analyses of wing shape show the same pattern for
 223 pooled sexes and for the males separately, thus only results based on the males'
 224 dataset are presented. Stepwise discriminant analysis provides evidence for highly
 225 significant wing shape differences among all species pairs ($p < 0.01$; *Merodon*
 226 *dobroensis* - *M. rojoi* **new species** $F_{13, 25}=45.233$; *M. dobroensis* - *M. puniceus*
 227 $F_{13, 25}= 11.875$; *M. puniceus* - *M. rojoi* **new species** $F_{13, 25}= 18.162$). Additionally,
 228 the DA with cross-validation based on wing shape shows correct species
 229 assignment for all male specimens (100%). A congruent classification is obtained
 230 by the Gaussian naive Bayes classifier through which all male specimens of *M.*

231 *puniceus* and *M. rojoi* **new species** are correctly classified, and only one out of 27
 232 specimens of *M. dobrogensis* is misclassified as *M. puniceus*.

233 The succeeding CVA conducted on shape variables of males produces two highly
 234 significant canonical axes linked to shape variation among species (CV1: Wilks'
 235 Lambda = 0.0098; $\chi^2 = 138.768$; $p < 0.01$; CV2: Wilks' Lambda = 0.2598; $\chi^2 =$
 236 40.440; $p < 0.01$). The first CV axis represents the majority of the wing shape
 237 variation (90%) and clearly differentiates all three species, with the main focus on
 238 differences between *Merodon rojoi* **new species** and the *M. dobrogensis* species
 239 complex (Fig. 1). The second canonical axis with the 10% of the wing shape
 240 variation clearly separates *M. dobrogensis* and *M. puniceus* (Fig. 1). This pattern
 241 of shape variation among species continues in superimposed outline drawings of
 242 the mean wing shapes (Figs. 2-4). The most subtle differences in mean shape are
 243 between *M. dobrogensis* and *M. puniceus* (Fig. 2), while the most obvious
 244 differences are among *M. rojoi* **new species** and *M. puniceus* (Fig. 4). Wing shape
 245 differentiation among species is most apparent in the central and distal parts of the
 246 wing (Figs. 2-4).

247 Congruent with the previous results *Merodon dobrogensis* and *M. puniceus* are
 248 closest to each other, while *M. rojoi* **new species** has the most distinct wing shape,
 249 based on the UPGMA dendrogram (Fig. 5).

250 Surstylus shape

251 PCA carried out on the Procrustes shape variables produces 26 principal
 252 components. Stepwise discriminant analysis reveals that the 12 principal

components represent the highest overall classification percentage of investigated taxa. DA shows that species within *Merodon dobrogensis* subgroup differ significantly in surstylus shape (*M. dobrogensis* - *M. rojoi* **new species** $F_{12, 15}=17.539$, $p < 0.01$; *M. dobrogensis* - *M. puniceus* $F_{12, 15}= 2.925$, $p < 0.05$; *M. puniceus* - *M. rojoi* **new species** $F_{12, 15}= 17.032$, $p < 0.01$). Importantly, all specimens are correctly classified (100%) to a priori defined groups. Moreover, a congruent classification is obtained by the Gaussian naive Bayes classifier through which all specimens are correctly classified to the belonging species, demonstrating that surstylus shape is a reliable character for interspecific discrimination. Canonical variates analysis produces two significant axes (CV1: Wilks' Lambda = 0.0167; $\chi^2 = 79.757$; $p < 0.01$; CV2: Wilks' Lambda = 0.3068; $\chi^2 = 23.039$; $p < 0.05$). As in wing shape, the first CV axis represents the majority of the surstylus shape variation (88%) and clearly differentiates *M. rojoi* **new species** from the *M. dobrogensis* species complex (Fig. 6). With 12% of total shape variation, CV2 clearly separates *M. dobrogensis* and *M. puniceus* (Fig. 6).

Differences in surstylus shape among *Merodon dobrogensis* and *M. puniceus* are mostly related to the posterior part of the posterior surstyle lobe (Fig. 7), while the greatest differences between *M. rojoi* **new species** and species from *M. dobrogensis* species complex are in anterior part of the posterior surstyle lobe (Figs. 8-9). Phenetic relationships constructed on the basis of surstylus shape follow the pattern obtained for wing shape, *M. dobrogensis* and *M. puniceus* are closest to each other, while the *M. rojoi* **new species** has the most distinct surstylus shape (Fig. 10).

276 ***Molecular data***

277 COI sequences are generated for 37 specimens belonging to the *Merodon*
 278 *dobrogensis* subgroup. Based on the uncorrected pairwise (p) distances between
 279 5' COI sequences Turkish specimens are assigned to *M. puniceus* as the p
 280 distances of specimens from Lesvos were the lowest (0-0.2%) when compared
 281 with Turkish specimens (see Supplementary Table S1). The combined 3' and 5'
 282 COI sequence alignment comprises 1400 bp. There were 144 variable positions,
 283 while 133 were parsimony informative. The resulting MP tree (Fig. 11) is a strict
 284 consensus of 46 equally parsimonious trees (length = 295, consistency index (Ci)
 285 = 95%, retention index (Ri) = 98%). It has identical topology to ML tree. The *M.*
 286 *rojoi* **new species** clade and the cluster of *M. dobrogensis* complex taxa (*M.*
 287 *puniceus* + *M. dobrogensis*) are clearly separated on both trees (Fig. 11). *M. rojoi*
 288 **new species** is resolved as a monophyletic clade with high bootstrap support
 289 (MP/ML: 100/98), while *M. dobrogensis* is resolved as nested within the *M.*
 290 *puniceus* clade. The *M. dobrogensis* clade is also supported with high bootstrap
 291 values (MP/ML: 100/95).

292 ND4 sequences are generated for 33 specimens and the alignment is 360 bp long
 293 (only one sequence was shorter, 329 bp). The number of variable positions when
 294 excluding missing data is 30 and all are parsimony informative. The combined
 295 COI+ND4 alignment used for MP and ML tree constructions is 1760 bp long. The
 296 combined COI+ND4 trees are identical in topology with COI gene trees and the
 297 main clades are supported with similar high bootstrap values (Fig. 12).

298 The average COI uncorrected pairwise distances (p^{COI}) between species pairs vary
 299 from 0.8% between *Merodon puniceus* and *M. dobrogensis*, 8.7% between *M.*
 300 *rojoi* **new species** and *M. puniceus*, and 8.8% between *M. rojoi* **new species** and
 301 *M. dobrogensis*. The average ND4 uncorrected pairwise distances (p^{ND4}) between
 302 species pairs are lower than p^{COI} values (0.4% *M. puniceus* - *M. dobrogensis*;
 303 7,8% *M. rojoi* **new species** - *M. puniceus*; 8.2% between *M. rojoi* **new species** - *M.*
 304 *dobrogensis*). Additionally, ND4 gene sequences are less variable comparing to
 305 COI gene sequences. We detected only 5 ND4 haplotypes and 19 COI haplotypes
 306 (Supplementary Table S2). The haplotypes are not shared among subgroup taxa.

307 The 28S rRNA gene sequence matrix contains 35 sequences with length varying
 308 from 578 to 579 bp, while the length of alignment is 579 bp. The total number of
 309 variable and parsimony informative sites is 3. The sequences form two genotypes
 310 from which one (I) is shared between *Merodon dobrogensis* and *M. puniceus*, and
 311 the other (II) correspond to *M. rojoi* **new species**. The genotypes differ in 3
 312 mutational steps.

313 *Correlation among wing shape, surstylus shape, genetic, spatial and climatic* 314 *differentiation*

315 Simple two-tailed Mantel tests reveals that geographic and climatic distance
 316 exhibited no association with wing and surstylus shape distances or genetic
 317 distances among *Merodon dobrogensis*, *M. puniceus* and *M. rojoi* **new species** (p
 318 > 0.05). Additionally, partial Mantel test shows that geographic distance has no

319 impact on wing and surstylus shape differentiation while accounting for climatic
320 distance, nor did climatic while accounting for geographic distance ($p > 0.05$).

321 ***Taxonomic description***

322 ***Merodon aureus* group sensu Radenković *et al.* (2011)**

323 **Diagnosis.** The following combination of characteristics distinguishes this group
324 from other *Merodon* groups: mesocoxa pilose posteriorly; anterior anepisternum
325 with pilose area ventral to postpronotum, in male genitalia anterior lobe of
326 surstylus undeveloped. Small-sized (8–13 mm) species with a short, rounded
327 abdomen, a distinct calcar on the metatrochanter in males, and a characteristic
328 structure of the male genitalia: posterior surstylus lobe with parallel margins and
329 rounded apex (Fig. 13: pl) and a narrow, elongated, sickle-shaped hypandrium
330 without lateral sclerite of aedeagus (Fig. 14).

331 ***Merodon dobrogensis* subgroup sensu Šašić *et al.* (2016)**

332 **Diagnosis.** Very distinctive species, clearly recognisable in the field by its mostly
333 reddish terga (Fig. 15) and reddish-orange tip of femora, tibiae and tarsi (except
334 two or three apical segments dark) (Figs. 16-18). Medium sized species with
335 metallic blue-green mesonotum and predominantly reddish-brown, oval abdomen
336 with purple lustre. Wings brown, darkened especially basally, anteriorly and along
337 the veins (Figs. 19-20). Species subgroup restricted to South East Europe
338 (Romania, Greece) and the western part of Turkey (Fig. 21).

339 ***Merodon dobrogensis* species complex**

340 **Diagnosis.** Eyes completely covered with black pile, as long as pedicel (greyish
341 and short in *M. rojoi* new species). Pile on mesoscutum longer than pedicel,
342 whitish, except for a patch of black pile near wing basis. Posterior quarter of
343 sternum IV in the male covered with pale pile.

344 Species distributed in Eastern Mediterranean and Balkan Peninsula. The complex
345 comprises of two species: *Merodon dobrogensis* and *M. puniceus*. Morphological
346 differences among the detected species are small.

347 Radenković *et al.* (2011) states that *Merodon puniceus* is very similar to *M.*
348 *dobrozensis* from which it supposed to differ by denser and longer pile on its
349 mesonotum, more darkened wing especially along the veins, and in the female by
350 more dense pile on the eyes. But these differences actually apply to differences
351 between *M. puniceus* and *M. rojoi*, because mentioned authors had misidentified
352 specimens from the continental part of Greece as *M. dobrozensis*, as they did not
353 have the type material of the real *M. dobrozensis* from Romania for comparison.
354 Based on the analysis of two available populations of both species, the only
355 morphological difference is the infuscation of the wings: much darker in *M.*
356 *puniceus* than in *M. dobrozensis* (Figs. 19-20).

357 ***Merodon dobrozensis* Bradescu, 1982**

358 **Types.** Bradescu, 1982: 13. Type-locality: Romania, Plateau of Dobrudja, Hagieni
359 (100m). Holotype: male, 27.viii.1977, Paratype: female 30.viii.1977 (leg. VI.
360 Bradescu) (studied).

361 **Material examined: Greece:** Evros, Mesembria: 6♂♂, 2.x.2011, 127 m.a.s.l.,
 362 leg. Michael de Coursy (M.C.W. coll. T1-T3, T14-T16), 3♂♂, 8.ix.2013, 127
 363 m.a.s.l. (FSUNS AL47, AL49, AL53); **Romania:** Mangalia: 15♂♂, Near Hagieni,
 364 28.ix.2014, 58 m.a.s.l., leg. Vujić A., Ačanski J., Šašić Zorić Lj. (FSUNS 08498-
 365 08512), 1♂, Near Liman, 28.ix.2014, 30 m.a.s.l., leg. Vujić A., Ačanski J., Šašić
 366 Zorić Lj. (FSUNS 08497), 5♂♂, Near Albești, 29.ix.2014, 50 m.a.s.l., leg. Vujić
 367 A., Ačanski J., Šašić Zorić Lj. (FSUNS 08493-08496, 08513).

368 **Diagnosis.** Differs from *Merodon puniceus* by the infuscation of the wings
 369 (lighter in *M. dobrogensis*, and much darker in *M. puniceus*, see Figs. 19-20),
 370 molecular data (COI and ND4 gene sequence variability, see Figs. 11-12), by
 371 morphometric characters of wing and male genitalia (Figs. 2, 7) and distribution.
 372 It is geographically separated from *M. puniceus*, being distributed in south-east
 373 Europe near the Black and Aegean Sea (Fig. 21).

374 **Biological data.** Preferred environment: forest; small, open areas maintained by
 375 goat-grazing, in evergreen oak matorral of *Quercus coccinea* on limestone.
 376 Flowers visited: *Prospero autumnale*. Flight period: August/October. Larva
 377 unknown (Speight 2018).

378 ***Merodon puniceus* Vujić, Radenković et Pérez-Bañón, 2011**

379 *Merodon* sp. nova 4 in Ståhls *et al.* 2009

380 **Types.** Radenković *et al.* 2011: 48. Type-locality: Greece, Lesvos.

381 **Type material (studied).** Holotype: ♂, **Greece:** Lesvos, Agiassos, 14.x.2005, 760
 382 m.a.s.l., leg. Perez-Bañón C. (MAegean 0023677). Paratypes: **Greece:** Lesvos:
 383 1♀, Agiassos, 8.x.2005, 760 m.a.s.l., leg. Perez-Bañón C. (MAegean 0023663),
 384 1♂, Polichnitos, 24.x.2008, 37 m.a.s.l., leg. Vujić A. (FSUNS 04448), 1♂, River
 385 Almiropotamos, 1.7 km WNW Vatera, from fennel, 8.x.2008, 55 m.a.s.l., leg.
 386 Hull M. (WML 1771).

387 **Additional material.** **Greece:** Lesvos, Agiassos: 1♀, vi.2005, leg. Perez-Bañón
 388 C., Rojo S., Ståhls G. (FSUNS), 2♀♀, 10.iv.2007, leg. Petanidou T. (CEUA), 1♂,
 389 23.iv.2007, leg. Perez-Bañón C. (CEUA), 1♀, 27.iv.2007, leg. Petanidou T.
 390 (CEUA). **Turkey:** 1♂, Mountain near Izmir, 16.ix.2013, leg. Vujić A. (FSUNS
 391 AL29); Bozdag mountain: 2♂♂, Sart to Bozdag 1, 16.ix.2013, 1320 m.a.s.l., leg.
 392 Vujić A. (FSUNS AJ8, AJ13), 4♂♂, Bozdag 2, after Allahdiyen, 12.x.2014, 1070
 393 m.a.s.l. leg. Vujić A., Šimić S., Radenković S. (FSUNS 08675, 08677, 08682,
 394 08687), 1♂, Bozdag 3, 12.x.2014, 1229 m.a.s.l., leg. Vujić A., Šimić S.,
 395 Radenković S. (FSUNS 08668).

396 **Diagnosis.** Differs from *Merodon dobrogensis* by the infuscation of the wings
 397 (much darker in *M. puniceus* and lighter in *M. dobrogensis*, see Figs. 19-20), by
 398 morphometric characters of wing and male genitalia (Figs. 2, 7), distribution in
 399 western Anatolia and Lesvos Island, as well in altitudinal range (Figs. 21-22). The
 400 two species differ also based on COI and COI+ND4 tree topologies (Figs. 11-12).

401 **Biological data.** Preferred environment: forest; small, open areas in *Castanea* and
 402 evergreen *Quercus* and *Pinus* forests. Flowers visited: *Prospero autumnale*,

403 *Foeniculum vulgare* Mill. Flight period: spring (April to Jun) and autumn
 404 (September/October). Larva unknown (Speight 2018).

405 ***New species description***

406 ***Merodon rojoi* Radenković et Vujić new species**

407 **Type material.** Holotype: ♂, **Greece:** Mountain Erymanthos, Kalentzi, [37.95039
 408 N, 37.95039 E], 2.ix.2012, 1212 m.a.s.l., leg. Vujić A. (FSUNS G2385).
 409 Paratypes: Greece: 1♂, Euboea, Nea Artaki, 18.ix.1982, leg. Bette (BMNH)
 410 (misidentified as *M. dobrogensis* in Radenković *et al.* 2011); 2♀♀, Attiki, Dafni,
 411 10 km W from Athena, 28.ix.1985, 199 m.a.s.l., (RMNH 03074) (misidentified as
 412 *M. dobrogensis* in Radenković *et al.* 2011 and Petanidou *et al.* 2011); 1♂,
 413 Argolida, Korfos-Epidavros, 15-27.ix.1986, 136 m.a.s.l., leg. Wolf (RMNH
 414 04157) (misidentified as *M. dobrogensis* in Radenković *et al.* 2011); 8♂♂, 10♀♀,
 415 Mountain Erymanthos, Kalentzi, 2.ix.2012, 1212 m.a.s.l., leg. Vujić A. (FSUNS
 416 G2374-G2384, G2386-G2391, G2393); 1♀, Mountain Pindos, above Katara,
 417 3.ix.2012, 1200 m.a.s.l., leg. Vujić A. (FSUNS G2392); 1♀, Mountain Olympus
 418 (on *Prospero autumnale* (L.) Speta), 22.ix.2013, 632 m.a.s.l., leg. Vujić A.
 419 (FSUNS AL12).

420 **Diagnosis.** Eyes covered with sparse, greyish pile, shorter than pedicel (Fig. 23),
 421 mostly black in *Merodon dobrogensis* species complex (as on Fig. 25). In the
 422 male, pile on scutum is as long as pedicel; mostly pale, with two maculae of black
 423 pile around wing basis and transverse suture; in the female short black pile (as
 424 long as half of pedicel) cover most of the scutum (Figs. 29-30). Wing infuscation

425 similar to *M. dobrogensis*. Posterior quarter of sternum IV in the male with few
426 black pile. Additionally, it differs from taxa belonging to the *M. dobrogensis*
427 species complex by morphometric characters of wing and male genitalia (Figs. 3-
428 4, 8-9), distribution (Fig. 21), COI and COI+ND4 tree topologies (Figs. 11-12),
429 COI and ND4 unique haplotypes, as well as unique 28S rRNA gene genotype.
430 Differs from *M. dobrogensis* in its altitudinal range (Fig. 22).

431 **Male (Fig. 32)**

432 **Head (Figs. 23, 26).** Antenna (Fig. 26) reddish-brown, basoflagellomere 2.5 times
433 as long as wide, 1.6–1.7 longer than pedicel, concave and darkened dorsally, apex
434 rectangular; arista dark brown and thickened basally, slightly shorter than the
435 basoflagellomere. Face and frons black with bluish lustre, covered with long
436 whitish-yellow pile. Oral margin bare with black lustre. Vertical triangle
437 isosceles, shiny black, predominantly covered with black pile, and posteriorly
438 with long, white pile. Ocellar triangle equilateral. Eye contiguity about 11
439 ommatidia long. Vertical triangle: eye contiguity: ocellar triangle = 1.5 : 1.5 : 1.
440 Eye pile sparse, greyish. Occiput with whitish-yellow pile, along the eye margin
441 with dense white microtrichia and posteriorly with metallic bluish-greenish lustre.

442 **Thorax.** Postpronotum with conspicuous tooth-like process posteriorly. Scutum
443 and scutellum black with metallic blue lustre, covered with short, erect white to
444 yellowish pile, except black pile around wing basis and transverse suture.
445 Posterior anepisternum, anepimeron and posterodorsal part of katepisternum with
446 long whitish-yellow pilosity and metallic blue lustre. Wing with dense

447 microtrichia, brown, infuscated especially basally, anteriorly and along veins;
448 veins dark brown except light brown C, Sc and basal part of R1. Calypter grey
449 yellow. Halter with light brown pedicel and dark brown capitulum. Femora dark
450 brown with pale apex; pro- and mesofemur with a stripe of light yellow pile
451 posteriorly and very short, black pile anteriorly and dorsally; metafemur with very
452 short, black pile. Tibiae and tarsi orange, except 2 (3) apical tarsomeres darkened
453 (fourth tarsomere distinctly brown), covered with yellow pilosity and some black
454 pile. Metatrochanter with an inner calcar with two pointed angles.

455 **Abdomen (Fig. 33).** Oval, slightly longer than mesonotum; reddish-brown;
456 tergum I black with golden lustre; tergum II reddish-brown with small black
457 marking medially, and two lateral indistinct white microtrichose spots near the
458 black marking; tergum III reddish-brown; tergum IV reddish-brown, black
459 posteriorly; pile on terga predominantly short golden, except black ones on black
460 markings and posterior margins of terga II and III. Sterna translucent, from
461 reddish-yellow to reddish-brown towards the end of abdomen, black lined,
462 covered with long whitish-yellow pile, except few black pile on posterior quarter
463 of sternum IV.

464 **Male genitalia.** Similar to all other species of the *Merodon aureus* group (Figs.
465 13-14): posterior surstylar lobe with parallel margins and rounded apex (Fig. 13)
466 and a narrow, elongated, sickle-shaped hypandrium without lateral sclerite of
467 aedeagus (Fig. 14).

468 **Female (Figs. 17, 24, 27, 34).** Similar to the male except for the normal sexual
469 dimorphism and for the following characteristics: metatrochanter (Fig. 17)
470 without thorn; black markings on terga (Fig. 34) narrower or absent; tergum II
471 with more conspicuous two lateral white microtrichose spots and terga III and IV
472 with distinct stripes of white pilosity; tergum V and sternum V black..

473 Length: Body 11–12 mm, wing 8–9 mm.

474 **Etymology.** The new species is named after the Spanish entomologist Santos
475 Rojo and is dedicated to his comprehensive work on syrphids. Additionally, "rojo"
476 in Spanish language means red, indicating the red abdomen in this species.

477 **Biological data.** Preferred environment: forest; small, open areas in evergreen
478 *Quercus* and *Pinus* forests. Flowers visited: *Prospero autumnale*. Flight period:
479 Autumn (September). Larva unknown.

480

481

482 **Discussion**

483 With their red abdomen, species from the *Merodon dobrogensis* subgroup have
484 clearly different morphological features compared to all other species within the
485 *M. aureus* species group. *M. dobrogensis* was described from Romania (Bradescu,
486 1982), and for a long time, there were no additional records of this species.
487 Radenković *et al.* (2011) described the morphologically very similar species *M.*
488 *puniceus* from the island of Lesvos, but as there were no genetic data on *M.*

489 *dobrogensis* it was impossible to unambiguously resolve the taxonomy of this
490 species. Continental material from central, south and west of Greece referred to as
491 *M. dobrogensis* in Petanidou *et al.* (2011) as well as Radenković *et al.* (2011) are
492 the here described *M. rojoi* **new species**. During 2013 and 2014, we collected new
493 specimens of *M. dobrogensis* in Romania and eastern Greece and here we confirm
494 that they belong to one nominal species based on molecular data, morphology,
495 wing geometric morphometry and distribution. Additionally, we validate *M.*
496 *puniceus* as a separate species based on genetic and, wing and surstylus
497 morphometry differentiations. These two species form the *M. dobrogensis* species
498 complex.

499 New distributional data of *Merodon puniceus* have been recorded in the western
500 part of the Anatolian Peninsula, representing the first known mainland sites for
501 this species. Previously, this species has been listed as endemic for Lesvos Island,
502 Greece (Radenković *et al.* 2011). The specimens from Turkey are firstly assigned
503 to *M. puniceus* based on similarity of the DNA barcode sequences to one from
504 Lesvos Island (Supplementary Table S1) what is then supported based on
505 integrative taxonomy approach applied here. The distributional pattern of *M.*
506 *puniceus* is in agreement with the earlier study of *Merodon* fauna in the Aegean
507 region (Vujić *et al.* 2016). Namely, the fauna of large islands not very remote
508 from nearest mainland, such as Lesvos or Samos is closely related to the adjacent
509 mainland fauna. These two islands host approximately half of the *Merodon*
510 species previously classified as Anatolian (Ståhls *et al.*, 2016; Vujić *et al.* 2016).

511 *Merodon rojoi* **new species**, which is morphologically similar to both above-
 512 mentioned taxa, has been discovered in the central, south and west of Greece. This
 513 species has some unique morphological features which separates it from the *M.*
 514 *dobrogensis* species complex and it is resolved as a separate monophyletic clade,
 515 supported with high bootstrap values on COI and COI+ND4 trees. Additionally, it
 516 has unique COI and ND4 haplotypes as well as 28S rRNA genotype. Moreover,
 517 *M. rojoi* **new species** differs significantly in its wing and surstylus shape from
 518 species from the *M. dobrogensis* species complex, with an excellent percentage of
 519 correct species classification by both discriminant analysis (both wing and
 520 surstylus 100%) and the Gaussian naïve Bayes classifier analyses (wing: 97%
 521 surstylus: 100%). Apart from the significant differences in wing and surstylus
 522 shape among all three species, the position of specimens in both CVA scatter plot
 523 clearly illustrates the division of the *M. dobrogensis* subgroup into *M. rojoi* **new**
 524 **species** and *M. dobrogensis* species complex. This segregation is also noticeable
 525 when comparing species mean wing and surstylus shapes, with the lowest
 526 differences within the *M. dobrogensis* species complex.

527 In general, the molecular findings on the *Merodon dobrogensis* subgroup species
 528 support wing and surstylus shape differentiation among them. These results are
 529 not surprising, given that congruence between molecular and geometric
 530 morphometrics results in recent taxonomic studies of hoverfly species become
 531 standard (Vujić *et al.* 2013; Nedeljković *et al.* 2013, 2015; Ačanski *et al.* 2016;
 532 Šašić *et al.* 2016, 2018; Radenković *et al.* 2018; Chroni *et al.* 2018; Kočiš Tubić
 533 *et al.* 2018). Moreover, based on both simple and partial Mantel tests, there is no

534 significant correlation of wing and surstylus shape and genetic differentiation with
 535 climate and geographic proximity among investigated species.

536 The low average uncorrected p distances (p^{COI} , p^{ND4}) between the two species
 537 from the *Merodon dobrogensis* species complex, suggests that they diverged
 538 recently and probably during the late Pleistocene. However, high COI sequence
 539 divergence between *M. rojoi* **new species** and the *M. dobrogensis* complex
 540 species indicates a much earlier divergence of the *M. rojoi* **new species** lineage
 541 from the *M. dobrogensis* complex ancestor lineage. The speciation process is
 542 probably related to the complex geological history of the Aegean region and
 543 severe climatic oscillation during the Pleistocene epoch, as it is suggested for
 544 many other *Merodon* taxa inhabiting Mediterranean and sub-Mediterranean
 545 regions (e.g. Petanidou *et al.* 2011; Ačanski *et al.* 2016; Šašić *et al.* 2016; Vujić *et*
 546 *al.* 2016; Radenković *et al.* 2018). Also, it may be connected to their larval
 547 biology/host plant relationships which remain unknown.

548 The applied integrative approach once again proved useful in resolving the
 549 taxonomy of *Merodon* species. The *M. dobrogensis* subgroup was successfully
 550 resolved using molecular data, geometric morphometry and distributional data in
 551 addition to morphological character states analysis.

552

553

554 **Acknowledgements**

555 This work was funded by the Ministry of Education, Science and Technological

556 Development of the Republic of Serbia Grant Nos. OI173002 and III43002, the
 557 Provincial Secretariat for Science and Technological Development (0601-504/3)
 558 and H2020 Project “ANTARES” (664387).

559

560 **Supplementary material**

561 Supplementary Table S1. The matrix of uncorrected pairwise (p) distances
 562 between 5' COI sequences of the *Merodon dobrogensis* subgroup. The colours
 563 depict species: light green - *M. puniceus*, dark green - *M. dobrogensis*, red - *M.*
 564 *rojoi* **new species**. [MS Excel Worksheet (.xlsx), 14KB]

565 Supplementary Table S2. List of COI and ND4 sequence haplotypes of the
 566 *Merodon dobrogensis* subgroup. [MS Excel Worksheet (.xlsx), 11KB]

567

568

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742 Table 1. List of the *Merodon dobrogensis* subgroup specimens used in molecular and geometric morphometry analyses.

Collection	Species	Locality	Sex	ID	Wing ID	Surstylus ID	DNA ID	COI	ND4	28S
								GenBank	GenBank	GenBank
								accession no.	accession no.	accession no.
FSUNS	<i>M. dobrogensis</i>	Romania, Mangalia, near Alberti	male	08494	WM1857					
FSUNS	<i>M. dobrogensis</i>	Romania, Mangalia, near Liman	male	08497	WM1861	08497				
FSUNS	<i>M. dobrogensis</i>	Romania, Mangalia, near Hagieni	male	08498	WM1862	08498				
FSUNS	<i>M. dobrogensis</i>	Romania, Mangalia, near Hagieni	male	08499	WM1863					
FSUNS	<i>M. dobrogensis</i>	Romania, Mangalia, near Hagieni	male	08501	WM1865	08501				
FSUNS	<i>M. dobrogensis</i>	Romania, Mangalia, near Hagieni	male	08502	WM1866	08502				

FSUNS	<i>M. dobrogensis</i>	Romania, Mangalia, near Hagieni	male	08503	WM1867	08503	
FSUNS	<i>M. dobrogensis</i>	Romania, Mangalia, near Hagieni	male	08504	WM1868	08504	
FSUNS	<i>M. dobrogensis</i>	Greece, Evros, Mesembria	male	AL47	WM1883		
FSUNS	<i>M. dobrogensis</i>	Greece, Evros, Mesembria	male	AL53	WM1884		
FSUNS	<i>M. dobrogensis</i>	Greece, Evros, Mesembria	male	AL49			AU35
FSUNS	<i>M. dobrogensis</i>	Greece, Evros, Mesembria	male	T15	WM1881		AU36
FSUNS	<i>M. dobrogensis</i>	Greece, Evros Mesembria	male	T14	WM1880		AU37
FSUNS	<i>M. dobrogensis</i>	Greece, Evros Mesembria	male	T3	WM1879		AU38
FSUNS	<i>M. dobrogensis</i>	Greece, Evros	male	T2	WM1878	T2	AU39

		Mesembria					
FSUNS	<i>M. dobrogensis</i>	Greece, Evros, Mesembria	male	T1	WM1877	T1	AU40
FSUNS	<i>M. dobrogensis</i>	Greece, Evros, Mesembria	male	T16	WM1876		AU41
FSUNS	<i>M. dobrogensis</i>	Romania, Mangalia, near Albești	male	08493	WM1856	08493	AU415
FSUNS	<i>M. dobrogensis</i>	Romania, Mangalia, near Albești	male	08495	WM1858		AU417
FSUNS	<i>M. dobrogensis</i>	Romania, Mangalia, near Albești	male	08496	WM1859		AU418
FSUNS	<i>M. dobrogensis</i>	Romania, Mangalia, near Albești	male	08513	WM1860	08513	AU419
FSUNS	<i>M. dobrogensis</i>	Romania, Mangalia, near Hagieni	male	08500	WM1864		AU423
FSUNS	<i>M. dobrogensis</i>	Romania, Mangalia, near Hagieni	male	08512	WM1875	08512	AU632

FSUNS	<i>M. dobrogensis</i>	Romania, Mangalia, near Hagieni	male	08511		08511	AU633
FSUNS	<i>M. dobrogensis</i>	Romania, Mangalia, near Hagieni	male	08510	WM1874	08510	AU634
FSUNS	<i>M. dobrogensis</i>	Romania, Mangalia, near Hagieni	male	08509	WM1873		AU635
FSUNS	<i>M. dobrogensis</i>	Romania, Mangalia, near Hagieni	male	08508	WM1872	08508	AU636
FSUNS	<i>M. dobrogensis</i>	Romania, Mangalia, near Hagieni	male	08507	WM1871	08507	AU637
FSUNS	<i>M. dobrogensis</i>	Romania, Mangalia, near Hagieni	male	08506			AU638
FSUNS	<i>M. dobrogensis</i>	Romania, Mangalia, near Hagieni	male	08505	WM1869	08505	AU639
FSUNS	<i>M. puniceus</i>	Greece, Lesvos, Polichnitos	male	04448	WM1894	04448	
MAegean	<i>M. puniceus</i>	Greece, Lesvos,	male	0023677		03075	

		Agiassos						
		Greece, Lesvos,						
FSUNS	<i>M. puniceus</i>	Agiassos	female	03076	WM1893			
FSUNS	<i>M. puniceus</i>	Turkey, Bozdag Mt.	male	08687	WM1892	08687		
FSUNS	<i>M. puniceus</i>	Turkey, Bozdag Mt.	male	AJ8	WM1885	AJ8	AU32	
FSUNS	<i>M. puniceus</i>	Turkey, mountain near Izmir	male	AL29	WM1886	AL29	AU33	
FSUNS	<i>M. puniceus</i>	Turkey, Bozdag Mt.	male	AJ13	WM1887	AJ13	AU34	
FSUNS	<i>M. puniceus</i>	Turkey, Bozdag Mt.	male	08668	WM1888	08668	AU410	
FSUNS	<i>M. puniceus</i>	Turkey, Bozdag Mt.	male	08675	WM1889		AU411	
FSUNS	<i>M. puniceus</i>	Turkey, Bozdag Mt.	male	08677	WM1890		AU412	
FSUNS	<i>M. puniceus</i>	Turkey, Bozdag Mt.	male	08682			AU413	
FSUNS	<i>M. rojoi</i> new species	Greece, Erymanthos Mt., Kalentzi	male	G2387	WM1896	G2387		
FSUNS	<i>M. rojoi</i> new species	Greece, Erymanthos Mt., Kalentzi	female	G2391	WM1897			
FSUNS	<i>M. rojoi</i> new	Greece, Erymanthos	female	G2389	WM1898			

	species	Mt., Kalentzi				
FSUNS	<i>M. rojoi</i> new	Greece, Erymanthos	female	G2383	WM1899	
	species	Mt., Kalentzi				
FSUNS	<i>M. rojoi</i> new	Greece, Erymanthos	female	G2388	WM1900	
	species	Mt., Kalentzi				
FSUNS	<i>M. rojoi</i> new	Greece, Erymanthos	female	G2393	WM1901	
	species	Mt., Kalentzi				
FSUNS	<i>M. rojoi</i> new	Greece, Erymanthos	male	G2376		G2376
	species	Mt., Kalentzi				
FSUNS	<i>M. rojoi</i> new	Greece, Erymanthos	female	G2374	WM1902	
	species	Mt., Kalentzi				
FSUNS	<i>M. rojoi</i> new	Greece, Erymanthos	female	G2390	WM1903	
	species	Mt., Kalentzi				
FSUNS	<i>M. rojoi</i> new	Greece, Pindos Mt.,	female	G2392	WM1911	AU42
	species	ispod Katare				
FSUNS	<i>M. rojoi</i> new	Greece, Olympus Mt.	female	AL12	WM1912	AU43
	species					

FSUNS	<i>M. rojoi</i> new species	Greece, Erymanthos Mt., Kalentzi	male	G2377		G2377	AU44
FSUNS	<i>M. rojoi</i> new species	Greece, Erymanthos Mt., Kalentzi	male	G2378	WM1905		AU45
FSUNS	<i>M. rojoi</i> new species	Greece, Erymanthos Mt., Kalentzi	female	G2379	WM1895		AU46
FSUNS	<i>M. rojoi</i> new species	Greece, Erymanthos Mt., Kalentzi	female	G2380	WM1906		AU47
FSUNS	<i>M. rojoi</i> new species	Greece, Erymanthos Mt., Kalentzi	male	G2381	WM1907	G2381	AU48
FSUNS	<i>M. rojoi</i> new species	Greece, Erymanthos Mt., Kalentzi	female	G2382	WM1908		AU49
FSUNS	<i>M. rojoi</i> new species	Greece, Erymanthos Mt., Kalentzi	male	G2385	WM1909	G2385	AU50
FSUNS	<i>M. rojoi</i> new species	Greece, Erymanthos Mt., Kalentzi	male	G2384	WM1910		AU51

744 Table 2. Primers used for amplification of COI, ND4 and 28S rRNA gene
 745 fragments.

	Primer	Source
3' COI gene	C1-J-2183 (Jerry)	Simon <i>et al.</i> (1994)
	TL2-N-3014 (Pat)	Simon <i>et al.</i> (1994)
5' COI gene	LCO-1490	Folmer <i>et al.</i> (1994)
	HCO-2198	Folmer <i>et al.</i> (1994)
ND4 gene	ND4+	Gorochotegui-Escalante <i>et al.</i> (2000)
	ND4-	Gorochotegui-Escalante <i>et al.</i> (2000)
28S rRNA gene	28S F2	Belshaw <i>et al.</i> (2001)
	28S 3DR	Belshaw <i>et al.</i> (2001)

746 **Figure captions**

747 **Figs. 1-5.** Geometric morphometric analysis of the wing shape of the *Merodon*
 748 *dobrogensis* subgroup. **1**, Scatter plot of individual scores of CV1 and CV2; **2-4**,
 749 superimposed outline drawings of average wing shape for each species.
 750 Differences between the species were exaggerated threefold to make them more
 751 visible; **5**, UPGMA phenogram constructed using Mahalanobis square distances
 752 of wing shape.

753 **Figs. 6-10.** Geometric morphometric analysis of the surstylus shape of the
 754 *Merodon dobrogensis* subgroup. **6**, Scatter plot of individual scores of CV1 and
 755 CV2; **7-9**, superimposed outline drawings of average surstylus shape for each
 756 species. Differences between the species were exaggerated twofold to make them
 757 more visible; **10**, UPGMA phenogram constructed using Mahalanobis square
 758 distances of surstylus shape.

759 **Figs. 11-12.** The Maximum parsimony trees of the *Merodon dobrogensis*
 760 subgroup. (filled circles ● stand for unique changes, open circles ○ stand for non-
 761 unique; bootstrap values ≥ 50 are presented near nodes; bootstrap values in red
 762 correspond to those from Maximum likelihood trees). **11**, The COI strict
 763 consensus tree of 46 equally parsimonious trees; **12**, The COI+ND4 strict
 764 consensus tree of 23 equally parsimonious trees.

765 **Figs. 13-14.** *Merodon rojoi* **new species**, male genitalia, lateral view. **13**,
 766 epandrium; **14**, hypandrium. pl = posterior surstyle lobe; c = cercus.

767 **Fig. 15.** Habitus of *Merodon puniceus*, male, dorsal view. (from: Radenković *et*
768 *al.* 2011, p. 55, Fig. 66).

769 **Figs. 16-18.** Metaleg, lateral view. **16**, *Merodon rojoi* **new species** male; **17**, *M.*
770 *rojoi* **new species** female; **18**, *M. dobrogensis* male (genitalia dissected). t = calcar
771 on metatrochanter.

772 **Figs. 19-20.** Right wing, dorsal view, male. **19**, *Merodon dobrogensis*; **20**, *M.*
773 *puniceus*.

774 **Figs. 21-22.** The distribution range of the *Merodon dobrogensis* subgroup. **21**,
775 Species distribution ● *M. dobrogensis* (N=31), ▲ *M. puniceus* (N=16), ■ *M. rojoi*
776 **new species** (N=25); **22**, variability plot of species altitudinal gradients (N=31).

777 **Figs. 23-28.** Head, the *Merodon dobrogensis* subgroup. **23-25**, dorsal view. **26-28**,
778 lateral view. **23**, **26**, *M. rojoi* **new species**, male; **24**, **27**, *M. rojoi* **new species**,
779 female; **25**, **28**, *M. dobrogensis*, male.

780 **Figs. 29-31.** Mesoscutum, lateral view. **29**, *Merodon rojoi* **new species**, male; **30**,
781 *M. rojoi* **new species**, female; **31**, *M. dobrogensis*, male.

782 **Fig. 32.** Habitus of *Merodon rojoi* **new species**, male, lateral view.

783 **Figs. 33-34.** *Merodon rojoi* **new species**, abdomen, dorsal view. **33**, male; **34**,
784 female.

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