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

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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.

Introduction

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

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

57

Material and methods

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

Taxonomy

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

The following acronyms of museums and entomological collections are used in the text: Natural History Museum, London, United Kingdom (BMNH); Colección Entomológica de la Universidad de Alicante, Alicante, Spain (CEUA); Faculty of Sciences, Department of Biology and Ecology, University of Novi Sad, Novi Sad, Serbia (FSUNS); Naturalis Biodiversity Center, Leiden, The Netherlands (RMNH); World Museum Liverpool, Liverpool, United Kingdom (WML); The Melissotheque of the Aegean, University of the Aegean, Mytilene, Greece (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 $MgCl_2$, 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 $MgCl_2$. 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 *dobrogensis - M. rojoi* **new species** $F_{13, 25}$=45.233; *M. dobrogensis - M. puniceus*

227 $F_{13, 25}$= 11.875; *M. punicues - 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; χ2 = 138.768; p < 0.01; CV2: Wilks' Lambda = 0.2598; χ2 =

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 reveales that the 12 principal

253 components represent the highest overall classification percentage of investigated

254 taxa. DA shows that species within *Merodon dobrogensis* subgroup differ

255 significantly in surstylus shape (*M. dobrogensis* - *M. rojoi* **new species** $F_{12,}$

256 $_{15}$=17.539, p < 0.01; *M. dobrogensis* - *M. puniceus* $F_{12, 15}$= 2.925, p < 0.05; *M.*

257 *puniceus* - *M. rojoi* **new species** $F_{12, 15}$= 17.032, p < 0.01). Importantly, all

258 specimens are correctly classified (100%) to a priori defined groups. Moreover, a

259 congruent classification is obtained by the Gaussian naive Bayes classifier

260 through which all specimens are correctly classified to the belonging species,

261 demonstrating that surstylus shape is a reliable character for interspecific

262 discrimination. Canonical variates analysis produces two significant axes (CV1:

263 Wilks' Lambda = 0.0167; $\chi 2$ = 79.757; p < 0.01; CV2: Wilks' Lambda = 0.3068;

264 $\chi 2$ = 23.039; p < 0.05). As in wing shape, the first CV axis represents the majority

265 of the surstylus shape variation (88%) and clearly differentiates *M. rojoi* **new**

266 **species** from the *M. dobrogensis* species complex (Fig. 6). With 12% of total

267 shape variation, CV2 clearly separates *M. dobrogensis* and *M. puniceus* (Fig. 6).

268 Differences in surstylus shape among *Merodon dobrogensis* and *M. puniceus* are

269 mostly related to the posterior part of the posterior surstyle lobe (Fig. 7), while the

270 greatest differences between *M. rojoi* **new species** and species from *M.*

271 *dobrogensis* species complex are in anterior part of the posterior surstyle lobe

272 (Figs. 8-9). Phenetic relationships constructed on the basis of surstylus shape

273 follow the pattern obtained for wing shape, *M. dobrogensis* and *M. puniceus* are

274 closest to each other, while the *M. rojoi* **new species** has the most distinct

275 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 *dobrogensis* 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. dobrogensis*, as they did not

353 have the type material of the real *M. dobrogensis* 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. dobrogensis* (Figs. 19-20).

357 *Merodon dobrogensis* **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. Vl.

360 Bradescu) (studied).

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

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

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

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

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

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

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

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

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

Biological data. Preferred environment: forest; small, open areas in *Castanea* and 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**

559

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 GenBank accession no.	ND4 GenBank accession no.	28S GenBank accession no.
FSUNS	*M. dobrogensis*	Romania, Mangalia, near Alberti	male	08494	WM1857					
FSUNS	*M. dobrogensis*	Romania, Mangalia, near Liman	male	08497	WM1861	08497				
FSUNS	*M. dobrogensis*	Romania, Mangalia, near Hagieni	male	08498	WM1862	08498				
FSUNS	*M. dobrogensis*	Romania, Mangalia, near Hagieni	male	08499	WM1863					
FSUNS	*M. dobrogensis*	Romania, Mangalia, near Hagieni	male	08501	WM1865	08501				
FSUNS	*M. dobrogensis*	Romania, Mangalia, near Hagieni	male	08502	WM1866	08502				

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

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

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

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

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

FSUNS	*M. rojoi* **new species**	Greece, Erymanthos Mt., Kalentzi	male	G2377		G2377	AU44
FSUNS	*M. rojoi* **new species**	Greece, Erymanthos Mt., Kalentzi	male	G2378	WM1905		AU45
FSUNS	*M. rojoi* **new species**	Greece, Erymanthos Mt., Kalentzi	female	G2379	WM1895		AU46
FSUNS	*M. rojoi* **new species**	Greece, Erymanthos Mt., Kalentzi	female	G2380	WM1906		AU47
FSUNS	*M. rojoi* **new species**	Greece, Erymanthos Mt., Kalentzi	male	G2381	WM1907	G2381	AU48
FSUNS	*M. rojoi* **new species**	Greece, Erymanthos Mt., Kalentzi	female	G2382	WM1908		AU49
FSUNS	*M. rojoi* **new species**	Greece, Erymanthos Mt., Kalentzi	male	G2385	WM1909	G2385	AU50
FSUNS	*M. rojoi* **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 *et al.* (1994)
	TL2-N-3014 (Pat)	Simon *et al.* (1994)
5' COI gene	LCO-1490	Folmer *et al.* (1994)
	HCO-2198	Folmer *et al.* (1994)
ND4 gene	ND4+	Gorrochotegui-Escalante *et al.* (2000)
	ND4-	Gorrochotegui-Escalante *et al.* (2000)
28S rRNA gene	28S F2	Belshaw *et al.* (2001)
	28S 3DR	Belshaw *et al.* (2001)

Figure captions

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

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

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

Figs. 13-14. *Merodon rojoi* **new species**, male genitalia, lateral view. **13**, 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 disected). 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.

785

786