New records and molecular data of *Merodon constans* (Rossi, 1794) and *Rhingia borealis* Ringdahl, 1928 (Diptera: Syrphidae) from the Iberian Peninsula

In the early summer of 2020, fieldwork was conducted in the Spanish Pyrenees of Girona in an attempt to sample hoverflies (Diptera: Syrphidae). As a result, specimens of the Eristalinae genera *Merodon* Meigen, 1803, and *Rhingia* Scopoli, 1763 were collected (Ricarte et al. 2021). A preliminary examination of the *Merodon* specimens suggested that they belonged to the species group of *Merodon constans* (Rossi, 1794) (Vujić et al. 2020). However, given that this group consists of 15 species, of which nine were recently described, and the group did not occur in the Iberian Peninsula (understood as the territory south of the French/Spanish border) (Ricarte & Marcos-García 2017, Vujić et al. 2020), DNA was analyzed for our morphological identification to be further supported. Regarding *Rhingia*, the specimens resembled morphologically *Rhingia borealis* Ringdahl, 1928 (Barkemeyer, 1986, van Steenis, 1998). The only record of *R. borealis* from the Iberian Peninsula was a female collected in Navarra in late July 1995 (Kehlmaier 2000). To confirm the identity of the *Rhingia* specimens of Girona, their barcodes were also analyzed.

The specimens from Girona are deposited at the CEUA-CIBIO collection ('Colección Entomológica de la Universidad de Alicante', Research Institute CIBIO, University of Alicante, Spain). For the species identification of the *Merodon* material, we used Vujić et al. (2020), and for *Rhingia,* we used Barkemeyer (1986) and van Steenis (1998). The seven *Merodon* specimens (5 ♂, 2 ♀) were compared morphologically with the material of *Merodon analis* Meigen, 1822 from Serbia deposited in the same collection. The three *Rhingia* specimens from Girona (1 ♂, 2 ♀) were compared with the type material of *R. borealis* (6 ♂) from the MZLU collection (Museum of Zoology, Lund, Sweden) and with photos of two Balkan males of *R. borealis* from the FSUNS collection (Faculty of Sciences, University of Novi Sad, Serbia). CEUA-CIBIO specimens were all labeled with a unique bar code identifier (see the number in brackets in the examined material list) and databased. For the geographical coordinates of the collecting sites in Girona, see Ricarte et al. (2021). The genitalia of all studied males was dissected and stored in plastic micro vials. The photo was made following Ricarte et al. (2021), and the drawings followed the same technique as Ricarte et al. (2018). As for the drawings of the genitalia of *Eumerus azabense* Ricarte & Marcos-García, 2018, Ricarte et al. (2018) were followed.

For the molecular analyses, DNA was extracted from the meso- and metalegs of four specimens, 1 male and 1 female of *M. constans* and 1 male and 1 female of *R. borealis* from Girona, using the NZY Tissue gDNA Isolation kit. PCR amplification of the 5' end of the Cytochrome c oxidase subunit I (COI-5') region was performed with the universal primers LCO1490 and HCO2198 (Folmer et al. 1994) in a total volume of 25 µl following the protocol of Vujić et al. (2020), for *Merodon*, and Ståhls & Barkalov (2017), for *Rhingia*. PCR products were visualized in a 1% agarose gel, sequenced at Macrogen Inc. (Macrogen, Spain), and edited with Sequencher v.5.4.6 (Gene Codes Corporation 2017). Subsequently, COI-5' sequences of the *M. constans* species group (Vujić et al. 2020) and each Holarctic species of *Rhingia* available at GenBank and BOLD (Ratnasingham & Hebert 2007) were downloaded on November the 2nd, 2021. Both matrixes were aligned by eye and checked with AliView v.1.25 (Larsson 2014). Finally, Maximum Likelihood (ML) analyses with 1000 bootstrap replications were carried out in MEGA7 (Kumar et al. 2016) with the Tamura-3 parameter model (Tamura 1992) for the *Merodon* dataset and the Tamura-Nei model (Tamura & Nei 1993) for the *Rhingia* dataset (Fig. 3).

We found that the male genitalia of the studied *Merodon* specimens (Fig. 1) was most similar to *Merodon constans* (Rossi, 1794) (Vujić et al. 2020). The genitalia of the *Rhingia* specimens was similar to *Rhingia borealis* (Barkemeyer 1986), but their surstyli differed slightly in shape (Fig. 2A and figure 3b of Barkemeyer 1986, as *Rhingia austriaca* Meigen, 1830). After the morphological and molecular studies, the species *M. constans* and *R. borealis* were confirmed from Girona. The new material of *M. constans*, all from the village of Llanars, is labeled as follows: ruta Llanars-Camprodon, orillas del Ter, 29-VII-2020, leg. A. Ricarte, 1 male (108260); La Creueta, 1138 m, 29-VII-2020, leg. A. Ricarte, 1 male and 2 females (108262/CEUA_S12/OM811676; 108276; 108277/CEUA_S13 /OM811677), leg. Z. Nedeljković, 1 male (108278); orillas de la Riera de Feitús, desde Font del Tir, 29-VII-2020, leg. A. Ricarte, 2 males (108261; 108279). The new specimens of *R. borealis*, all from the village of Vilallonga de Ter, had the following data: orillas del Ter, cerca Ctra. Vilallonga-Setcases, 1166 m, 2-VIII-2020, leg. A. Ricarte, 1 male (108220/CEUA_S14/OM811678) and 2 females (108239; 108238/CEUA_S15/OM811679). Additional new material of *R. borealis* from Spain deposited in the CEUA-CIBIO collection was also examined: Asturias, Espinaredo, Piloña, 28-VIII-2019, leg. M. A. Marcos-García, 2 males (110109, 110110) and 3 females (110111-110113). The type material of *R. borealis* consisted of the male lectotype from Bräcke, 19-7-22 (MZLU-DIPT 00055786) and five other specimens, supposedly paralectotypes but unlabeled as such, with the following data: U-åker, 27-6-14, 1 male (00055789), 17-6-14, 1 male (00055788), 26-6-25, 1 male (00055790), 2-7-13, 1 male (00055787), Jmt. Undersåker, 3-7-25, leg. O. Ringdahl, 1 male (00055791). Balkan specimens of *R. borealis* at FSUNS were examined from photos of their male genitalia, and label data were as follows: Serbia, Kopaonik, 07-VI-1998, 1 male; Croatia, Gorski Kotar, 27-V-1990, 1 male.

The COI-5' sequences of the studied specimens of *Merodon* grouped with sequences of *M. constans* and *M. analis* (Fig. 3A). Thus, we excluded the possibility of our species being any other species of those reported by Vujić et al. (2020). From the pair *M. constans*/*M. analis*, the genitalia of the Girona specimens were within the variation range of *M. constans* (Vujić et al. 2020; A. Vujić, pers. comm.). The COI-5' sequences of the *R. borealis* from Girona clustered with those of other European *R. borealis* (Fig. 3B).

Figure 1. Male genitalia (right side) of a specimen of *Merodon constans* from the Pyrenees of Girona, Spain. An arrow indicates the posterior surstylar lobe. Lateral view. Scale bar = 0.5 mm.

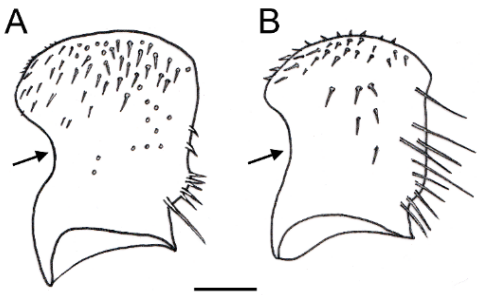

Figure 2. Male right surstyli of *Rhingia borealis*: (A) Specimen from the Pyrenees of Girona, Spain; (B) Paralectotype from Sweden. An arrow indicates the different shape of the excavation at the anterior margin of the surstyli. Lateral view. Scale bar = 0.1 mm.

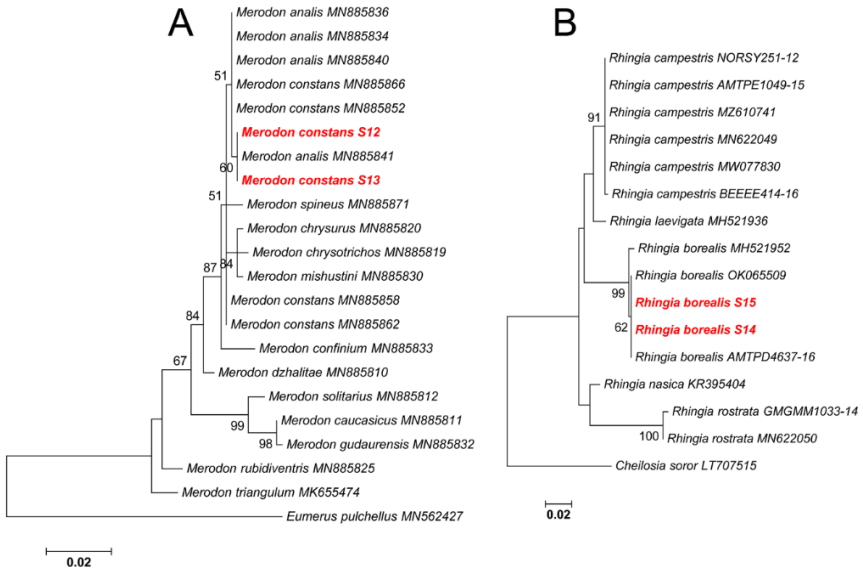

Figure 3. ML resulting trees: (A) *Merodon*; (B) *Rhingia*. Bootstrap values > 50 are shown near nodes. Branch lengths measured in number of substitutions per site. In red, analysed specimens from Girona, Spain.

The finding of the European-endemic *M. constans* in the Pyrenees of Girona is the first for the Iberian Peninsula and the westernmost within its range, suggesting that the species should also be present in France, from where only *M. analis* is confirmed (Vujić et al. 2020, Speight & Langlois 2020). As Vujić et al. (2020) and our results show, *M. constans*/*M. analis* do not always differ in COI-5' (Vujić et al. 2020), indicating the need to find additional diagnostic molecular markers for this pair of species and addressing the well-known fact that species level identification cannot always rely on COI sequences exclusively (e.g., Nedeljković et al. 2018, 2020).

Given the molecular results for the analyzed specimens of *Rhingia*, the different shape of the male surstylus (more concave anteriorly than in the type material; see Fig. 2), as well as other differences found in the color of the gena (blackish brown, while yellow in the lectotype) and the pile of thoracic scutum (all black, while intermixed yellow and blackish brown in the lectotype), should be regarded as an intraspecific variation. In general, the male from Girona is darker, but a range of variability was also observed in the colour of the scutum pile in the specimens forming the type material. The differences in the Girona specimens of *R. borealis* might be because they were found at high altitudes (> 1000 m asl), where there are colder temperatures (Dušek & Láska 1974). The specimens of *R. borealis* from Girona and Asturias represent the second confirmed record of this species from Spain, which appears to be restricted to the northern part.

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