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*MERODON NATAN*S AND *M. PULVERIS* (DIPTERA: SYRPHIDAE): DELIMITATING CRYPTIC HOVERFLY SPECIES USING THE GEOMETRIC MORPHOMETRIC METHOD

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Abstract

A landmark-based geometric morphometric method was employed to delimitate two morphologically closely-related species of the hoverfly *Merodon natans* species group based on wing parameters. The studied species, *Merodon natans* and *M. pulveris*, were successfully determined using this method. Analyses revealed a significant difference in wing size and shape between individuals of the two species, thereby proving that these are two distinct species, even though, morphologically very similar. Discriminant and cluster analyses also uncovered a subtle difference in wing shape between *M. pulveris* populations, indicating intraspecific differentiation with potential conservation implications. The results of the morphological study were supplemented with known distribution data of the investigated species.

KEY WORDS: hoverflies, *Merodon natans* group, species distribution, integrative taxonomy, Aegean archipelago

Introduction

The family Syrphidae (hoverflies) of the order Diptera is one of the most species-abundant families of the order, with around 6000 species across 188 genera. Hoverflies occupy every continent on the planet, except Antarctica, and have multiple roles in the ecosystem. Potentially the most important is their pollination ability (Markov *et al.*, 2016; Klecka *et al.*, 2018). Some groups and species can also act as bioindicators (Sommaggio & Burgio, 2014) and regulate the numbers of pest species (Thompson & Rotheray, 1998).

The genus *Merodon* Meigen, 1803 comprises around 160 taxa residing throughout the Palearctic and, in lesser numbers, the Afrotropical area (Hurkmans 1993; Ståhls *et al.*, 2009; Vujić *et al.*, 2011; 2016). It is one of the most abundant hoverfly genera in the Mediterranean (Dirickx, 1994), most likely because of the high diversity of bulb plant species (Ricarte *et al.*, 2008; Andrić *et al.*, 2014; Preradović *et al.*, 2018). Larvae of the genus *Merodon* feed on bulbs and other underground organs of the plants from the geophyte families, such as Amaryllidaceae and Iridaceae (Andrić *et al.*, 2014; Ricarte *et al.*, 2017; Preradović *et al.*, 2018). Different species of the genus have been the subject of multiple genetic, systematic, taxonomic and phylogenetic studies in recent years (Vujić *et al.*, 2007; 2012; 2013; 2016; 2019; Milankov *et al.*, 2008a, b; 2009; 2013; Francuski *et al.*, 2009; 2011; Ståhls *et al.*, 2009; 2016; Radenković *et al.*, 2011; 2018a,b; Popović *et al.*, 2015; Ačanski *et al.*, 2016; Šašić *et al.*, 2016, 2018; Kočiš Tubić *et al.*, 2018). However, some groups and complexes are still understudied and offer an exceptional opportunity to gain insight into the puzzling relationships among species, especially considering the high diversity within the genus and the complex history of the Mediterranean.

The use of an integrative taxonomic approach resulted in the identification of about 20 complexes of cryptic species within the genus (Ačanski *et al.*, 2016; Šašić *et al.*, 2016, 2018; Kočiš Tubić *et al.*, 2018; Radenković *et al.*, 2018a,b; Vujić *et al.*, 2018; 2019). The *Merodon natans* group consists of three species, *M. natans* (Fabricius, 1794), *M. pulveris* Vujić et Radenković, 2011 and *Merodon calcaratus* (Fabricius, 1794) (Radenković *et al.*, 2011). Specimens belonging to *Merodon natans* group are medium-sized, black and short pilose species (Fig. 1). Diagnostic characters such as: posterior side of mid coxa covered in pilosity; reduced pile on the anterior anepisternum; oval, rounded, pilose and innerly not curved anterior lobe of surstylus distinguish this group from other groups of the genus (Radenković *et al.*, 2011). This species group was identified as a new putative subgenus by using genetic data, (Radenković et al., 2018b). To distinguish *M. natans* from the almost cryptic species *M. pulveris*, some subtle differences in the shape of genitalia can be used to separate males, while females, although quite similar, can be identified by the colorization of the hairs covering the hind legs. On the other hand, *M. calcaratus* can be easily recognized by its shorter antennae and specific characters of the male genitalia (Radenković *et al.*, 2011). Within the *Merodon natans* group, the species *M. natans* has the widest distribution. It occurs in most southern European countries and in the western and eastern parts of Europe, unlike *M. pulveris,* which is only registered in Greece and Turkey and is considered an Anatolian endemic (Radenković *et al.*, 2011). According to Speight (2018), the distribution of *M. natans* in the eastern parts of Europe requires reappraisal due to potential confusion with the very similar *M. pulveris*. To date, *M. calcaratus* is recorded in Portugal (van Eck, 2016) and Algeria (North Africa) (Haffaressas *et al.*, 2017).

Modern-day taxonomy is based on combining multiple different methods to identify, delineate and describe different taxa. This approach, called integrative taxonomy, is widely accepted (Bluemel *et al.*, 2014; Miraldo *et al.*, 2014; Šašić *et al.*, 2016, 2018; Kočiš Tubić *et al.*, 2018; Radenković *et al.*, 2018a,b) as it supposedly provides a thorough insight into taxonomic problems by combining genetic information with morphology and data on the distribution of the species. When it comes to the taxonomy and systematics of insects, despite their seemingly simple structure, the wings are of primary interest. Owing to its high heritability (Birdsall *et al.*, 2000), wing shape is a stable taxonomic character. Wing-size heritability is, generally, low, and is usually connected to body size and, thus, to different environmental components. Together, these two characteristics allow a detailed insight into the phenotypic diversity of Dipterans in general (Bitner-Mathé & Klaczko, 1999; Calle *et al.*, 2002; Moraes *et al.*, 2004; Dujardin et al., 2005; Jirakanjanakit *et al.*, 2008; Vujić *et al.*, 2013; Nedeljković *et al.*, 2013, 2015; Ačanski *et al.*, 2016).

In this study, we analyzed morphological differences between *Merodon natans* and *M. pulveris* from the Balkan Peninsula and Turkey (Fig. 2) (Appendix 1) using the geometric morphometric analysis of wing parameters in order to accurately delineate the two species. We compared our results with distributional data to obtain a comprehensive insight into the relationships among these species.

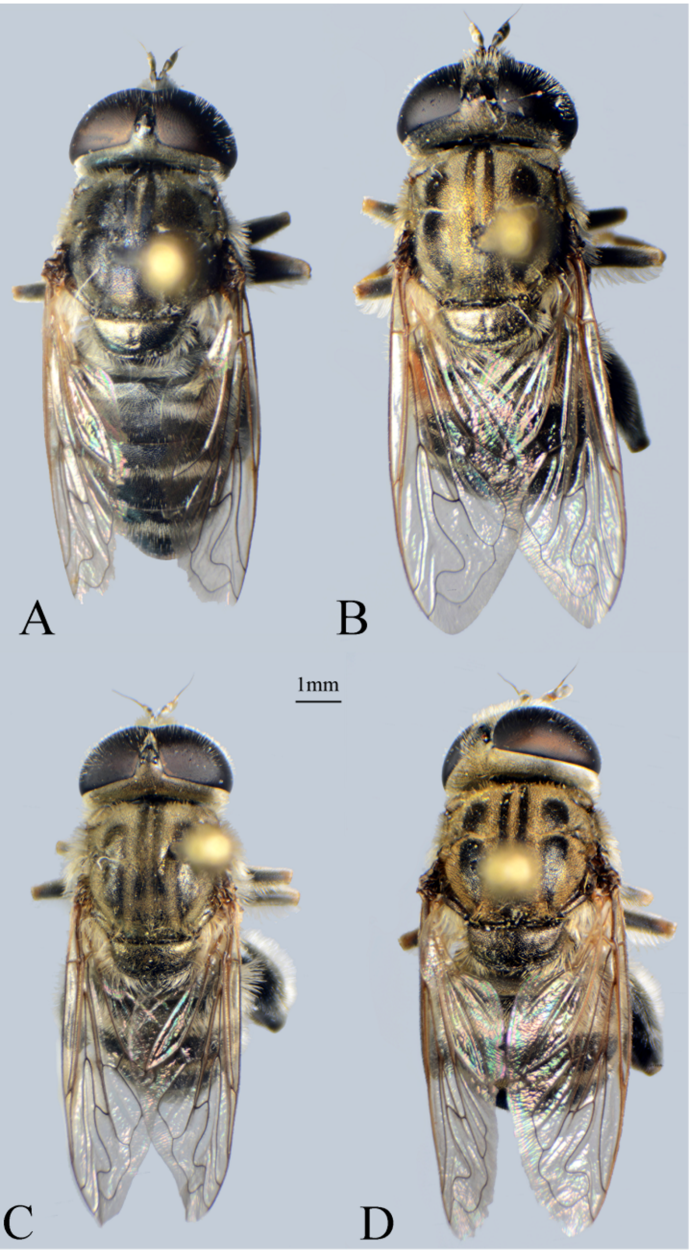

Figure 1. Dorsal view: *Merodon natans* male (A), *Merodon natans* female (B), *Merodon pulveris* male (C), *Merodon pulveris* female (D).

Materials and methods

Taxonomic study

The present study was based on an examination of all available adult specimens of the species *M.* and *M. pulveris* from the Balkan Peninsula and Turkey that are deposited in the collections of the Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad (FSUNS). Additional material was borrowed from the following institutions and private collections: Naturalis Biodiversity Center, Leiden, Netherlands (NBCN, former RMNH); Zoological Museum, Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark (ZMUC); World Museum Liverpool, Liverpool, UK (WML); Natural History Museum, London, UK (BMNH); Musee National d'Histoire Naturelle, Paris, France (MNHN); Museum of Zoology Lund University, Lund, Sweden (MZLU); Naturhistorisches Museum Wien, Vienna, Austria (NHMW); National Museums Liverpool, Liverpool, UK (NML); Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, Germany (ZFMK); National Museum of Bosnia and Herzegovina, Sarajevo, Bosnia and Herzegovina (ZHMB); Naturhistorisches Museum Wien, Vienna, Austria (NHMW); MAegean – The Melissotheque of the Aegean, University of the Aegean, Mytilene, Greece; Michael de Courcy Williams collection, Greece (M.d.C.W.coll.); Gunilla Ståhls-Mäkelä collection, Finland (G.S. coll.); Jeroen van Steenis collection, the Netherlands (J.v.S.coll.); Dieter Doczkal collection, Germany (D.D.coll.); Axel Ssymank collection, Germany (A.S.coll.); Miroslav Barták collection, Czech Republic (M.B.coll.). Specimens were identified by Ante Vujić and Snežana Radenković.

Photographs of the available specimens were taken with a Nikon Coolpix D7100 camera attached to Nikon SMZ745T stereomicroscope. Photos were processed using the software CombineZ5 (Hadley, 2006) and edited in Adobe Photoshop CS3 v10.0 (Adobe Systems) (2008). The species distribution map (Fig. 2) was created in DIVA-GIS software, version 7.1.7 (Hijmans *et al.*, 2001).

Figure 2. Distribution of *Merodon natans* (green circle) and *Merodon pulveris* (red triangle) in southeastern Europe.

Geometric morphometric analysis

Landmark-based geometric morphometric analysis of wing parameters was conducted on 231 specimens of the *Merodon natans* group; 118 *M. natans* and 113 *M. pulveris* (Appendix 1). Sampling localities of specimens used in geometric morphometric analyses are shown in Fig. 3. The right wing of each specimen was removed using microscissors and mounted on a slide using Hoyer's medium. Wings were photographed with a Leica DFC320 camera attached to a Leica MZ16 stereomicroscope. Eleven homologous landmarks, representing wing shape, were drawn on each wing using TpsDig 2.31 (Rohlf, 2017). To minimize the non-shape variations, such as scale, orientation and location, and to superimpose the wings in a common coordinate system, we applied generalized least squares (GLS) superimposition (Rohlf & Slice, 1990; Zelditch *et al.*, 2004) on the digitized data. For the wing-shape analysis, partial warp scores were calculated using TpsRelv 1.69 (Rohlf, 2017). To test for differences in wing size, isometric estimator known as centroid size (CS) were used. All wings used in the analysis were labeled and stored in the FSUNS collection, along with other data relevant to each specimen.

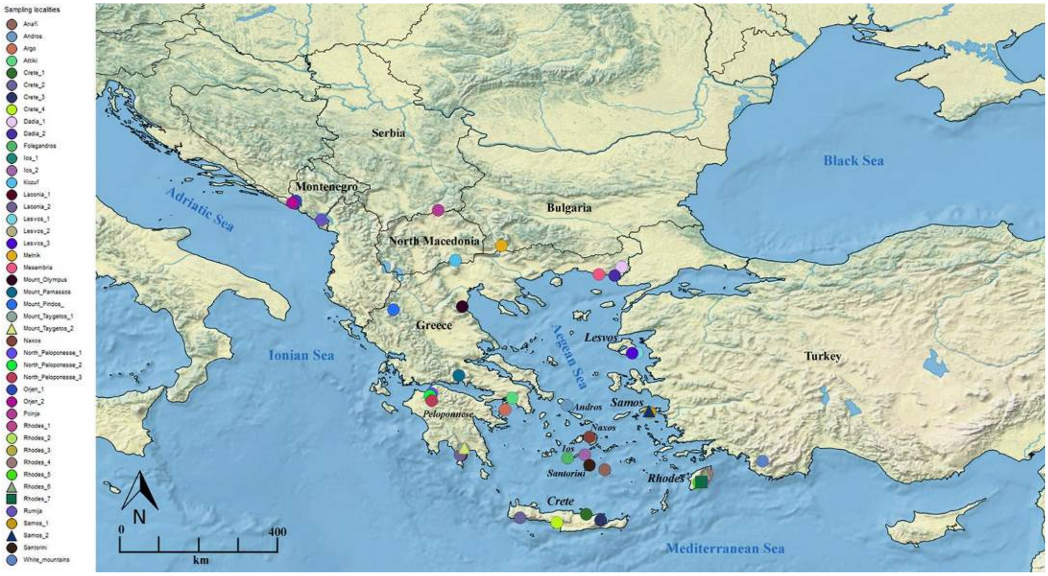

Figure 3. Sampling localities of specimens used in geometric morphometric analyses.

Data analysis

Sexual dimorphism in wing size was analyzed using one-way ANOVA, while the two – way multivariate analysis of variance (MANOVA) was used to test differences in wing shape between sexes and taxa. In addition, discriminant function analysis (DA) and canonical variate analysis (CVA) were employed to analyze the shape differences between the studied taxa. UPGMA cluster analysis based on squared Mahalanobis distances generated by the DA was used to assess differences in wing shape among individuals of different populations of the two examined species of the *Merodon natans* group. All statistical analyses were performed using Statistica software (Tibco Software Inc, 2017).

Results

Examined material

Merodon natans (Fabricius, 1794)

BULGARIA: 1 ♂, 1 ♀, Melnik, Vinograd, 41.497905N 23.383349E, 09.09.2012, leg. Vujić A., FSUNS_G2675, G2682; CROATIA: 1 ♀, “Splitsko-Dalmatinska”, Spalato, 1862, leg. Mann C.H., NHMW; 1 ♂, 2 ♀♀, Umag, Kaldama, 45.43138892N 13.52388889E, 07.09.1955, FSUNS_01568–01570; GREECE: 1 ♂, Central Greece and Euboea, Poros, 37.499457N 23.458444E, leg. Hermann, MNHN; 1 ♂, 3 ♀♀, Olympia, Ilis, 37.6422549N 21.60182502E, 03.10.1962, NBCN; 2 ♂♂, Thesprotia, Igoumenitsa, 39.5038889N 20.2655556E, 17.10.1962, NBCN; 1 ♂, Lasithi, Kritsa, 35.153868N 25.6515929E, 17.05.1971, leg. Gross, ZFMK; 3 ♂♂, 6 ♀♀, Corfu, Dassia 5 km SE Korakiana, 39.6833N 19.8333E, 30.05.1971, NBCN; 2 ♀♀, Epta Pigai, 36.24451899N 28.120301E, 17.05.1983, leg. Danielsson R., MZLU; 1 ♀, Chania, Vryses, 35.383N 24.2E, 30.06.1985, NBCN; 1 ♂, Attiki, Daphni, 38.008056N 23.641944E, 01.11.1985, NBCN; 3 ♀♀, Lasithi, Mesa, 35.1833333N 25.5166667E, 29.10.1986, NBCN; 2 ♂♂, Chania, Omalos Plain, 35.3N 23.9E, 08.10.1987, NBCN; 14 ♂♂, 12 ♀♀, Attiki, Daphni, 38.008056N 23.641944E, 30.09.1991, NBCN; 14 ♂♂, 2 ♀♀, Attiki, Daphni, 38.008056N 23.641944E, 03.10.1991, NBCN; 16 ♂♂, 9 ♀♀, Attiki, Daphni, 38.008056N 23.641944E, 10.10.1991, NBCN; 5 ♂♂, 4 ♀♀, Attiki, Daphni, 38.008056N 23.641944E, 14.10.1991, NBCN; 9 ♂♂, 13 ♀♀, Attiki, Daphni, 38.008056N 23.641944E, 24.10.1991, NBCN; 1 ♀, Attiki, Daphni, 38.008056N 23.641944E, 29.10.1991, NBCN; 1 ♂, 3 ♀♀, Attiki, Daphni, 38.008056N 23.641944E, 30.04.1992, NBCN; 8 ♂♂, 3 ♀♀, Attiki, Daphni, 38.008056N 23.641944E, 28.09.1992, NBCN; 1 ♂, 1 ♀, Attiki, Daphni, 38.008056N 23.641944E, 01.10.1992, NBCN; 3 ♂♂, 1 ♀, Attiki, Daphni, 38.008056N 23.641944E, 05.10.1992, NBCN; 23 ♂♂, 6 ♀♀, Attiki, Daphni, 38.008056N 23.641944E, 08.10.1992, NBCN; 14 ♂♂, 8 ♀♀, Attiki, Daphni, 38.008056N 23.641944E, 12.10.1992, NBCN; 16 ♂♂, 23 ♀♀, Attiki, Daphni, 38.008056N 23.641944E, 19.10.1992, NBCN; 1 ♀, Mountain Pindos, Katara Pass, 39.7968N 21.2292E, 20.05.1997, FSUNS_03273; 1 ♂, Magnisias, Platania, 39.1500N 23.3167E, 16.09.1999, D.D. coll._03286; Chania, Sougia, 35.25N 23.8166667E, 20–22.04.2001, leg. Michelsen, ZMUC_00515891; 1 ♂, Arcadia, Paleochori 2.5 km S Loussios river, 37.152009N 22.718297E, 28.04.2005, leg. Jeroen van Steenis, J.v.S. coll.; 1 ♂, Evros, Mesembria-Zoni, 40.870639N 25.630667E, 28.09.2007, M.d.C.W. coll.; 5 ♂♂, 5 ♀♀, Evros, Mesembria, 40.862219N 25.640589E, 02.10.2011, leg. Michael de Coursy, M.d.C.W. coll._T4–T13; 4 ♂♂, Evros, Mesembria, 40.862219N 25.640589E, 15.10.2011, leg. Michael de Coursy, M.d.C.W. coll._T4–T13; 1 ♂, Mountain Erymanthos, Kalenzi, 37.95039N 21.776045E, 02.09.2012, leg. Vujić, FSUNS_G2677; 11 ♂♂, Attiki, Manastir Daphni, 38.0108881N 23.6357631E, 07.10.2012, leg. Vujić, Radenković, FSUNS_2641, 2647–2652, 2654–2655, 2674, 2680; 1 ♀, Andros, Pithara, near Apikia, 37.842993N 24.901763E, 08.10.2012, leg. Vujić, FSUNS_G2699; 1 ♀, Andros, Vory, 37.893197N 24.890773E, 09.10.2012, leg Vujić, Radenković, FSUNS_G2707; 1 ♂, Naxos, near Skeponi, 37.13777778N 25.46750000E, 13.10.2012, leg. Gunilla Ståhls, G.S. coll._08868; 1 ♂, 2 ♀♀, Lasithi, Schisma, 35.189494N 25.680493E, 14.10.2012, leg. Vujić, FSUNS_2646, 2678, 2480; 1 ♂, Heraclion, Ierapetra-Analipsi, 35.32727702N 25.3466659E, 14.10.2012, leg. Vujić, FSUNS; 1 ♀, Chania, Georgiopoulis, 35.3652223N 24.257583E, 16.10.2012, leg. Ssymank, A.S. coll._05858; 1 ♀, Santorini, Panagia Kalou, 36.463N 25.4238E, 05–07.04.2013, leg. Petanidou, Devalez, FSUNS_ G2829; 1 ♀, Anafi, Helicodrome, 36.3565N 25.7736E, 10–13.04.2013, leg. Petanidou, MAegean_UOTA_MEL_075121; 1 ♂, 2 ♀♀, Ios, Mersinia Rema, 36.701097N 25.30901E, 14–16.04.2013, leg. Petanidou, Devalez, Maegean_UOTA MEL_ 075185, 075224, 075241; 1 ♂, Argo-Saronic island, Aegina, Ag. Asm – Kontos, 37.744598N 23.469101E, 12–14.07.2013, leg. Margaroni S., MAegean_ UOTA MEL_ 094722; 6 ♂♂, 8 ♀♀, Evros, Dadia, Delta (West), 40.835N 26.001111E, 08.09.2013, FSUNS_AL43–AL59; 4 ♂♂, 6 ♀♀, Mountain

Olympus, 40.11834498N 22.4792689E, 22.09.2013, FSUNS_AL5–6, AL10, AL13, AL15–16, AL21, AL26, AL35; 2 ♂♂, Achaia, near Erymanthos, 38.115177N 21.772531E, 20.04.2014, leg. Vujić, FSUNS_06488, 06485; 2 ♂♂, 1 ♀, Rethymnon, Orne-Agia Galini, 35.13851401N 24.67051E, 25.04.2014, leg. Vujić, FSUNS_06422, 06429, 06435; 1 ♂, 5 ♀♀, Achaia, after Patra, 38.0602651N 21.733867E, 04.10.2014, leg. Vujić A., Šimić S., Radenković S., FSUNS_08550–08552, 08557, 08563, 08564; 1 ♂, 1 ♀, Mountain Parnassos, Itea, 38.45178097N 22.4021071E, 04.10.2014, leg. Vujić A., Šimić S., Radenković S., FSUNS_08534; Mountain Parnassos, near Amfissa, 38.531551N 22.402258E, 04.10.2014, leg. Vujić A., Šimić S., Radenković S., FSUNS_08529, 08530; 2 ♂♂, 3 ♀♀, Laconia, Aeropoli, 36.6932942N 22.4276651E, 06.10.2014, leg. Vujić A., Šimić S., Radenković S., FSUNS_08576, 08586, 08599, 08601, 08605; 1 ♂, Mountain Taygetos, Sparta 2, 36.862378N 22.5284319E, 06.10.2014, leg. Vujić A., Šimić S., Radenković S., FSUNS_08575; 1 ♂, Mountain Taygetos, Sparta 3, 36.834941N 22.5175489E, 06.10.2014, leg. Šimić S., FSUNS_08606; 1 ♂, Mountain Mainalo, Kardaras, 37.1866522N 22.3118909E, 07.10.2014, leg. Vujić A., Šimić S., Radenković S., FSUNS_08612; 1 ♀, Naxos, near Eggares, 37.1141097N 25.421671E, 10.10.2014, leg. Vujić, Šimić, Radenković, FSUNS_08630; 1 ♂, Folegandros, Chora, 36.6244N 24.916898E, 18.05.2015, leg. Petanidou, MAegean_UOTA MEL_115051; 2 ♂♂, Achaia, Patra, 38.060468N 21.734067E, 05.09.2018, leg. Vujić *et al.*, FSUNS_20962, 20963; 11 ♂♂, 7 ♀♀, Achaia, Anastasi, 37.91005N 21.996523E, 06.09.2018, leg. Vujić *et al.*, FSUNS_21025,21027, 21030–21033, 21043, 21048, 21049; 21060–21063, 21068–21071; 7 ♂♂, Kea, Kato Meria, 37.596080N 24.332772E, 11.09.2018, leg. Vujić *et al.*, FSUNS_21284–21288, 21290, 21310; MONTENEGRO: 2 ♂♂, Skadarsko jezero, 42.123062N 19.274921E, 12.05.1982, leg. Vujić, FSUNS_01566, 01567; 3 ♂♂, 1 ♀, Skadarsko jezero, Vranjina, 42.279163N 19.134177E, 13.05.1982, FSUNS_01572–01575; 1 ♂, Boka Kotorska, Morinj, 42.490394N 18.648914E, 07.05.1994, leg. Vujić, FSUNS_03732; 1 ♂, 1 ♀, Boka Kotorska, Morinj, 42.490394N 18.648914E, 25–27.04.1998, FSUNS_01576, 03731; 3 ♂♂, Montenegro, Rumija, 42.11201N 19.21739E, 02.05.2011, leg. Vujić, FSUNS_G0250–G0252; 2 ♂♂, Orjen, Planinarski dom, 42.51210798N 18.5570939E, 27.08.2011, FSUNS_R59, R81; 1 ♀, Orjen, Crkvica, 42.561N 18.630E, 28.08.2011, leg. Vujić A., Radišić P., FSUNS_R6; 6 ♂♂, 5 ♀♀, Risan, Risan 2, 42.527750N 18.706383E, 22.09.2018, leg. Vujić *et al.*, FSUNS_21427–21436, 21440; 7 ♂♂, 2 ♀♀, Risan, Risan 1, 42.532066N 18.699094E, 22.09.2018, leg. Vujić *et al.*, FSUNS_21443, 21451, 21455, 21459, 21461, 21468, 21469, 21471; 2 ♀♀, Risan, Risan a, b, 42.527750N 18.706383E, 23.09.2018, leg. Vujić *et al.*, FSUNS_21751, 21831; NORTH MACEDONIA: 4 ♂♂, 8 ♀♀, Kožuf, Konsko, 41.185625N 22.329156E, 05.09.2012, leg. Vujić A., FSUNS_G2642–G2645, G2656–G2661, G2665, G2676; 2 ♀♀, Kožuf, Smrdljive vode, Gevgelija, 41.193086N 22.283187E, 05.10.2012, leg. Vujić A., FSUNS_G2662, G2663; SERBIA: 9 ♂♂, 1 ♀, Pčinja, Vogance, 42.34295197N 21.9244209E, 06.09.2012, leg. Vujić A., FSUNS_G2666–G2673, G2681, G2664; 1 ♂, Pčinja, Trgovište, 42.38264N 22.05010E, 07.09.2012, leg. Vujić A., FSUNS_2683.

Merodon pulveris Vujić et Radenković, 2011

Material studied: Type material published in Radenković *et al.*, 2011;

New data. GREECE: Samos: 1 ♂, Pyrgos, 37.712686N 26.79914E, 15.04.2011, FSUNS_F48; 2 ♂♂, Spatharaioi- Paghondhas, 37.675049N 26.816749E, 15.04.2011, FSUNS_F27, F29; 3 ♂♂, 14 ♀♀, Ag. Kiriaki, 37.70012298N 26.6129409E, 18.10.2012, leg. Vujić A, FSUNS_2686–G2706; Lesvos: 29 ♂♂, 4 ♀♀, Agiassos, 39.046267N 26.409955E, 19.09.2009, leg. Vujić A., FSUNS_Q64–Q95; 1 ♂, 1 ♀, Agiassos, 39.0525N 26.3825E, 03.10.2018, leg. Vujić A., FSUNS_21626–21627; 4 ♀♀, Koundouroudia, 39.0514N 26.5311E, leg. Devalez J., MAegean UOTA MEL_095156, 095157, 095160, 095162; Rhodes: 1 ♀, Lindos, 36.0905556N 28.084E, 15.04.1970, NBCN; 2 ♂♂, 4 ♀♀, Lindos, 36.0905556N 28.084E, 17.04.1970, NBCN; 18 ♀♀, Lindos, 36.0905556N 28.0844444E, 18.04.1970, NBCN; 2 ♂♂, 3 ♀♀, Lindos, 36.0905556N 28.0844444E, 20.04.1970, NBCN; 1 ♂, Lindos, 36.0905556N 28.084E, 29.03.1970, NBCN; 2 ♂, 2 ♀♀, Lindos, 36.0905556N 28.084E, 30.03.1970, NBCN; 1 ♂, 1 ♀, Lindos, 36.0905556N 28.084E, 01.04.1971, NBCN; 6 ♂♂, Lindos, 36.0905556N 28.084E, 04.04.1971, NBCN; 1 ♀, Lindos, 36.0905556N 28.084E, 05.05.1971, NBCN; 29 ♂♂, 1 ♀, Lindos, 36.0905556N 28.084E, 06.04.1971, NBCN; 1 ♂, Lindos, 36.0905556N 28.084E, 07.04.1971, NBCN; 57 ♂♂, Lindos, 36.0905556N 28.084E, 08.04.1971, NBCN; 10 ♂♂, Lindos, 36.0905556N 28.084E, 09.04.1971, NBCN; 2 ♂♂, Kattavia, 35.95N 27.7666667E, 09.04.1971, NBCN; 1 ♂, 2 ♀♀, Loutanis river, Afantou – Archangelos, 36.259778N 28.138889E, 08.04.2012, leg. Vujić A., FSUNS_T49–T51; 1 ♂, Lardos, 36.073722N 28.012944E, 15.04.2012, leg. Vujić A., FSUNS_G1089; 1 ♂, Kiotari, 36.048722N 27.951083E, 15.04.2012, leg. Vujić A., FSUNS_G1090; 13 ♂♂, 9 ♀♀, Loutanis river, Afantou – Archangelos, 36.259778N 28.138889E, 15.04.2012, leg. Vujić A., FSUNS_G1076–G1082; 16 ♂♂, 9 ♀♀ near Seven Spring Valley, 36.259127N 28.111188E, 17.04.2012, leg. Vujić, A., FSUNS_G1117–G1142; 4 ♂♂, 1 ♀, Kalathos, 36.116748N 28.058878E, 16.10.2012, leg. Vujić A., FSUNS_2684, 2685, 2697, 2698, 2700; TURKEY: 1 ♀, Toparlar, Toparlar Şelalesi, 37.003611N 28.635556E, 30.05.2009, leg. Dursun O., M.B. coll._10484; 12 ♂♂, 7 ♀♀, Muğla, University Campus, 37.160833N 28.372222E, 03–11.2013, leg. Bartak M., M.B. coll._10465–10483; 1 ♂, 1 ♀, White mountains, Tlos, 36.554167N 29.4209439E, 05.01.2014, leg. Vujić A., FSUNS_06277, 06278.

Geometric morphometric analysis

Sexual dimorphism

We tested for sexual dimorphism using two-way MANOVA of wing shape. The results showed highly significant differences in shape among species ($F_{1,\,227}=9.5862$, $p<0.01$) and sexes ($F_{1,\,227}=24.114$, $p<0.01$). Furthermore, the wings of females were significantly larger than those of males (one-way ANOVA of CS: $F_{1,\,229}=22.994$, $p<0.01$) (Fig. 4). Thus, all of the results clearly point to the existence of sexual dimorphism in the group. Due to this result, subsequent analyses were conducted on males and females separately.

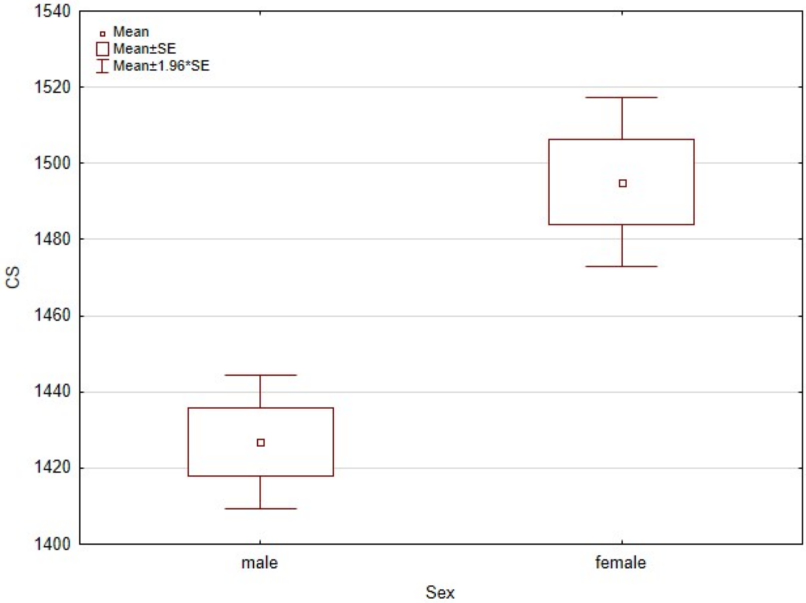

Figure 4. Box plot showing the differences in wing size between males and females of

Merodon natans and *M. pulveris*.

Males

Stepwise discriminant analysis conducted on wing shape variables showed that males of *M. natans* and *M. pulveris* differed highly significantly in wing shape ($F_{18,\ 210}=38.233$, $p<0.01$), with an overall classification success of 98.46% (98.48% correct classification for *M. natans* and 98.43% correct classification for *M. pulveris* males). Only one specimen of *M. natans* was misclassified as *M. pulveris* and *vice versa.* Results from the Gaussian Naive Bayes Classifier were in concordance with the discriminant analysis: 3 *M. natans* and 2 *M. pulveris* males were incorrectly classified, resulting in 96.17% correct classification.

Females

Stepwise DA showed that females of *M. natans* and *M. pulveris* differed highly significantly in wing shape ($F_{18,\ 210}=31.6$, $p<0.01$), with the overall classification success of 100%, i.e. all females were correctly classified. Results of the Naive Bayes Classifier were, again, similar to those from DA: all *Merodon natans* females were correctly classified, while 2 *M. pulveris* females were classified as *M. natans*, resulting in 98.035% correct classification.

Canonical variate analysis (CVA)

We designated species*sex groups *a priori* (i.e. *M. natans* male, *M. natans* female, *M. pulveris* male, *M. pulveris* female) and performed the CVA analysis on wing shape parameters of the entire sample. CVA produced 2 highly significant canonical axes. CV1 (85.2% of wing shape variation) distinctly separated individuals of *M. natans* from *M. pulveris* (regardless of the sex), while CV2 (13% of wing shape variation) separated the sexes within the respective species (Figure 5). Results of the CVA are given in Table 1.

Interpopulation variability of the *Merodon natans* group

For statistical accuracy purposes, the population study of the *Merodon natans* group comprised 9 populations with 10 or more individuals in the study. These originated from Serbia (Pčinja), North Macedonia (Kožuf) and Greece (Attiki, Mount Olympus, Mesembria, Dadia, the islands of Lesvos, Samos and Rhodes).

Discriminant analysis revealed significant differences between pairs of populations of the two species, mirroring differences shown in Fig. 6 (Table 2). The overall classification success was 82.55%. Succeeding CVA produced 5 significant canonical axes, with the first two accounting for 85.1% of shape variation in the sample. The first CV (63.3%) separated inland Balkan (*Merodon natans*) from Greek island populations (*M. pulveris*), while the second CV (21.8%) separated Rhodes population of *M. pulveris* from all other populations (Fig. 6). UPGMA clustering based on squared Mahalanobis distances confirmed the CVA results, with island populations (*M. pulveris*) closely clustered and standing opposed to mainland populations (*M. natans*). There is also branching within the *M. pulveris* populations, again showing the distinctiveness of the Rhodes island population (Fig. 7).

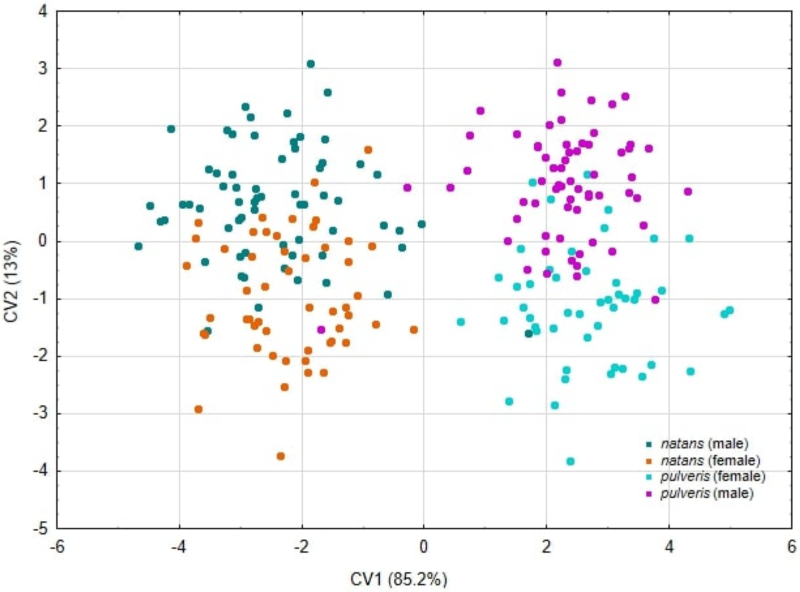

Figure 5. Grouping of individuals along canonical axes based on wing shape. CV1 separates *Merodon natan*s from *M. pulveris*, CV2 separates sexes within the species.

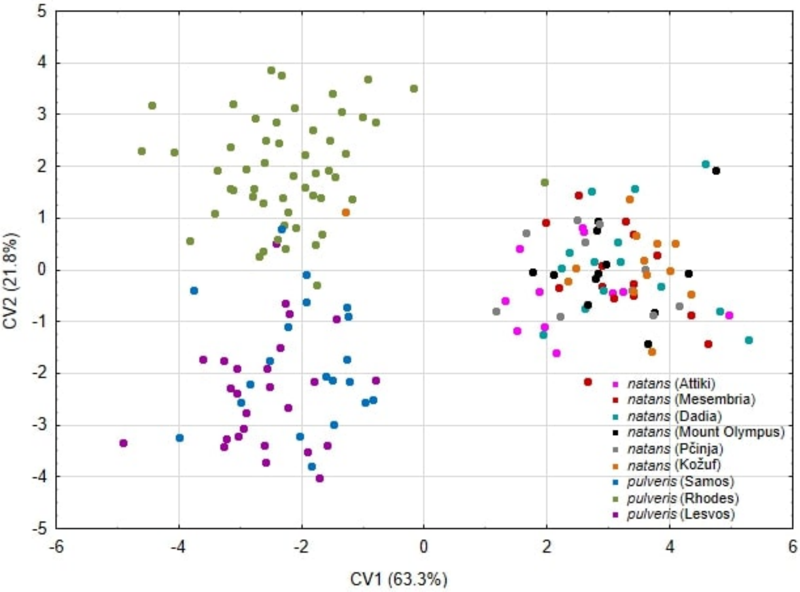

Figure 6. Population variability of the *Merodon natans* group based on wing shape. CV1 separates the two species, while CV2 shows a divergence of the Rhodes population within the *M. pulveris* populations.

Table I. Results of CV analysis of wing shape variables in the *Merodon natans* group.

	CV axis	**Eigenvalue**	**Canonical R**	**Wilks' λ**	**Chi² test**	***df***	***p***	**% variance**
Males	CV1	5.45646	0.919302	0.154884	226.6074	13	0	100
Females	CV1	8.40572	0.945347	0.106318	201.7186	18	0	100
Entire sample	CV1	5.868028	0.924336	0.068765	586.2759	54	0	85.2
	CV2	0.894443	0.687125	0.472281	164.2899	34	0	13
	CV3	0.117683	0.324487	0.894708	24.3654	16	0.081823	1.8
Populations	CV1	7.275151	0.937633	0.008307	754.5329	144	0	63.3
	CV2	2.505482	0.845419	0.06874	421.6949	119	0	21.8
	CV3	0.694808	0.640283	0.240966	224.1382	96	0	6
	CV4	0.373402	0.521422	0.408391	141.046	75	0.000006	3.3
	CV5	0.306571	0.484394	0.560885	91.0727	56	0.002117	2.7
	CV6	0.187226	0.397115	0.732836	48.9563	39	0.131859	1.6
	CV7	0.083833	0.278116	0.870042	21.9262	24	0.583673	0.7
	CV8	0.060467	0.238788	0.94298	9.2468	11	0.599124	0.6

Discussion

Numerous studies have confirmed the usefulness of geometric morphometric methods for species delimitation (e.g. Debat *et al.*, 2006; Ibañez *et al.*, 2007; Jirakanjanakit *et al.*, 2008; Ludoški *et al.*, 2008; Leaché *et al.*, 2009; Schwarzfeld & Sperling, 2014; Karanović *et al.*, 2015; Ruane 2015; Sontigun *et al.*, 2017). Moreover, a number of taxa from the hoverfly family were described using, among other methods, landmark-based geometric morphometrics (Vujić *et al.*, 2007; 2013; Ludoški *et al.*, 2008; Milankov *et al.*, 2008a,b; 2013; Radenković *et al.*, 2011; 2018a,b; Nedeljković *et al.*, 2013; 2015; Ačanski *et al.*, 2016; Chroni *et al.*, 2018; Kočiš Tubić *et al.*, 2018; Šašić *et al.*, 2016; 2018). In the present study, this method was applied to the *Merodon natans* group to accurately delineate two of the most similar species of the group.

Wing-size analysis showed clear sexual dimorphism, with females having larger wings; this is not surprising, considering that about 80% of insects (Teder & Tammaru, 2005) in general, 75% of species from the Syrphidae family (Gilbert, 1985) and other species from the genus *Merodon* itself (Milankov *et al.*, 2009; 2013) share this phenomenon. As already stated, wing size is connected to body size and larger-winged females should, therefore, have the reproductive edge when it comes to the number of eggs laid (Teder & Tammaru, 2005; Stillwell *et al.*, 2010), as well as being preferred as a reproductive partner by the male (Isaac, 2005). The sexes also differed in wing shape. Different wing shape between the sexes arises from the different roles assumed by males and females during the reproductive period. As already proven, flying ability and species recognition are correlated with wing shape (Birdsall *et al.*, 2000). Intrasexual differences in wing shape (especially in males) point to the existence of a premating barrier (promoting sexual and reproductive isolation) through species-specific courtship song (which is affected by wing shape) in sympatric species (Cowling & Burnet., 1981; Ritchie & Gleason, 1995; Tauber & Eberl, 2003; Menezes *et al.*, 2013). The present study covered two non-sympatric species. Therefore, the differences in wing shape among individuals of the same sex probably stem from a long period of isolation, which again points to the conclusion that these are, in fact, two separate species. Furthermore, geographical distribution and environmental factors have no impact on wing shape (Bitner-Mathé & Klaczko, 1999; Birdsall *et al.*, 2000;

Moraes *et al.*, 2004; Mezey & Houle, 2005; Dworkin & Gibson, 2006; Yeaman *et al.*, 2010), which is highly heritable and, therefore, important for species delimitation.

With its numerous islands and islets, the Mediterranean is geographically one of the most interesting areas in the world. The Aegean archipelago along the Greek and Turkish coast comprises almost 8000 islands, most of which have an area smaller than 1 km² (Triantis et al., 2008). Such diverse geomorphology, complex geological history and the existence of a closely positioned mainland that acts as a source area have resulted in high biodiversity and endemism of the area (Lymberakis & Poulakakis, 2010). The geographical distribution of populations of *M. natans* and *M. pulveris* also favors the fact that these are two distinct species (Fig. 2). The two species are divided by the so-called “Mid Aegean dividing line” (see Vujić *et al.*, 2016); *M. natans* is geographically described as a “Balkan” species since it is distributed over the Balkans and the eastern Mediterranean islands, but not on the Anatolian peninsula, as opposed to *M. pulveris*, which assumes an “Anatolian” distribution, inhabiting the Anatolian peninsula and the eastern Mediterranean islands, but is absent on the Balkan Peninsula. This kind of geographical pattern is probably a result of the aforementioned history of the area, which has experienced recurring changes in land and sea configuration and connectivity over time (Anastasakis & Dermitzakis, 1990). Specifically, two geomorphological events shaped the area and, probably, species distributions into their recent forms (Blondel *et al.*, 2010): the opening of the Mid-Aegean Trench (MAT), positively separating the eastern from the western part of the archipelago (12 mya) and the so-called Messinian Salinity Crisis (5.9-5.3 mya), when a major drop in sea level allowed land masses to emerge (Krijgsman *et al.*, 1999). Moreover, evidence shows that the formation of the MAT is thought to have had a major influence in determining biogeographical patterns of reptiles (Poulakakis et al., 2003; 2005a,b,c; 2008), coleopterans (Fattorini, 2002), isopods (Sfenthourakis, 1996), butterflies (Petsopoulos *et al.*, 2018) and hoverflies (Vujić *et al.*, 2016; Chroni *et al.*, 2018).

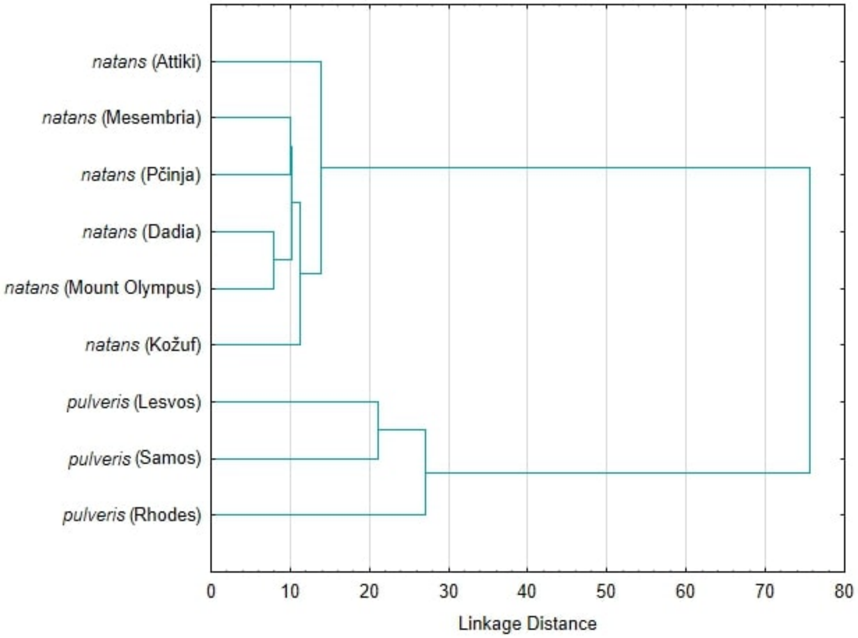

Figure 7. UPGMA phenogram showing relationships among populations of the *M. natans* group based on wing shape.

Table II. F values obtained by the DA showing differences between pairs of populations in the *Merodon natans* group (**p*<0.000001) and the percent correct classification of every population.

	Attiki	Mesembria	Dadia	Lesvos	Mount Olympus	Pčinja	Rhodes	Samos	Kožuf	% correct
Attiki		3.28604	1.90603	13.63322*	1.28389	2.15701	14.04225*	9.46010*	3.31537	72.72727
Mesembria	3.28604		2.62273	18.88283*	1.90962	0.81541	19.89634*	14.75621*	2.56111	85.71429
Dadia	1.90603	2.62273		20.24717*	1.03354	2.04393	19.60751*	14.33603*	2.37253	71.42857
Lesvos	13.63322*	18.88283*	20.24717*		16.55986*	13.12270*	17.04885*	4.64111*	18.25623*	96.15385
Mount Olympus	1.28389	1.90962	1.03354	16.55986*		1.27143	16.05836*	12.41279*	1.58782	50
Pčinja	2.15701	0.81541	2.04393	13.12270*	1.27143		12.91768*	10.68922*	1.37503	50
Rhodes	14.04225*	19.89634*	19.60751*	17.04885*	16.05836*	12.91768*		12.18759*	18.20799*	96.15385
Samos	9.46010*	14.75621*	14.33603*	4.64111*	12.41279*	10.68922*	12.18759*		14.60009*	85
Kožuf	3.31537	2.56111	2.37253	18.25623*	1.58782	1.37503	18.20799*	14.60009*		69.23077
Total										82.55814

Islands are known to be natural laboratories for diversification and natural selection. Island biodiversity can be explained as depending on the island area and its geographical isolation (MacArthur & Wilson, 1963; 1967). Most of the islands of the Aegean archipelago started forming during the Middle to Upper Miocene, i.e. 12 to 11 mya, and some, like Crete, were isolated since that time (Sondaar & van der Geer, 2002). Considering the complex paleogeographical history of the Aegean and its long geographical isolation, it is not surprising that a certain degree of divergence is present among the *M. pulveris* populations. Rhodes, with its 1400 km^2, is the largest of the Dodecanese islands (southeastern Aegean). Individuals of the *M. pulveris* population from Rhodes were found to have significantly different wing shape not just in comparison to *M. natans* populations, but also to other *M. pulveris* populations (Table 2, Fig. 6, 7). It is likely that the long-term isolation combined with the size of the island influenced the divergence of this population. Moreover, there are known endemics from the genus *Merodon* (Vujić *et al.*, 2016) and divergent populations of other taxa (Masseti *et al.*, 2006) on Rhodes. This uniqueness makes these populations not only taxonomically interesting but also important from a conservation point of view.

This study confirmed the power of wing characteristics as taxonomic characters and the geometric morphometric method as a powerful tool for species delimitation. Although morphologically quite similar, the two investigated species, *M. natans* and *M. pulveris*, were successfully delineated using this method. Discriminant analysis and canonical variate analysis of wing shape distinctly separated the individuals into their respective species, with high percentages of correct classification, employed, both to, separate sexes of the two species or the entire sample. Our study also uncovered some hidden divergence among the *M. pulveris* populations that could have important taxonomic and conservation implications. These results also point to the need for further research into the studied species (especially their genetic structure) that could give a complete insight into the complex relationships among them.

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Appendix 1

Name	Latitude	Longitude	Species	Country	No of individuals
Anafi	36.3565	25.7736	*natans*	Greece	1
Andros	37.842993	24.901763	*natans*	Greece	1
Andros	37.893197	24.890773	*natans*	Greece	1
Argo	37.744599999999998	23.469100000000001	*natans*	Greece	1
Attiki	38.010888000000001	23.635763000000001	*natans*	Greece	11
Crete 1	35.327277000000002	25.346665999999999	*natans*	Greece	1
Crete 2	35.25	23.8166667	*natans*	Greece	1
Crete 3	35.189494	25.680493	*natans*	Greece	2
Crete 4	35.138514000000001	24.67051	*natans*	Greece	3
Dadia 1	41.0299	26.1617	*natans*	Greece	1
Dadia 2	40.835	26.001111	*natans*	Greece	13
Folegandros	36.624400000000001	24.916899999999998	*natans*	Greece	1
Ios 1	36.7011	25.309	*natans*	Greece	2
Ios 2	36.701099999999997	25.309000000000001	*natans*	Greece	1
Kožuf	41.187632	22.320312	*natans*	North Macedonia	13
Laconia 1	36.696489	22.462720999999998	*natans*	Greece	1
Laconia 2	36.693294000000002	22.427665000000001	*natans*	Greece	4
Lesvos 1	39.0514	26.5311	*pulveris*	Greece	2
Lesvos 2	39.046266	26.409954	*pulveris*	Greece	1
Lesvos 3	39.046267	26.409955	*pulveris*	Greece	23
Melnik	41.525709	23.389677	*natans*	Bulgaria	2
Mesembria	40.862219	25.640589	*natans*	Greece	14
Mount Pindus	40.048420999999998	20.889747	*natans*	Greece	1
Mount Olympus	40.118344999999998	22.479268999999999	*natans*	Greece	12
Mount Parnassos	38.531551	22.402258	*natans*	Greece	3
Mount Taygetus 1	36.834941000000001	22.517548999999999	*natans*	Greece	1
Mount Taygetus 2	36.862378	22.528431999999999	*natans*	Greece	1
Naxos	37.114109999999997	25.421671	*natans*	Greece	2
North Peloponesse 1	38.115177	21.772531	*natans*	Greece	2
North Peloponesse 2	38.060265000000001	21.733867	*natans*	Greece	4
North Peloponesse 3	37.95039	21.776045	*natans*	Greece	1
Orjen 1	42.561	18.630	*natans*	Montenegro	1
Orjen 2	42.512107999999998	18.557093999999999	*natans*	Montenegro	2
Pčinja	42.342951999999997	21.924420999999999	*natans*	Serbia	10
Rhodes 1	36.116748	28.058878	*pulveris*	Greece	5
Rhodes 2	36.244518999999997	28.120301000000001	*pulveris*	Greece	2
Rhodes 3	36.259721999999996	28.138888999999999	*pulveris*	Greece	2
Rhodes 4	36.259778	28.138889	*pulveris*	Greece	15
Rhodes 5	36.048722	27.951083	*pulveris*	Greece	2
Rhodes 6	36.259127	28.111188	*pulveris*	Greece	25

Appendix 1 - continued

Name	Latitude	Longitude	Species	Country	No of individuals
Rhodes 7	36.073722	28.012944	*pulveris*	Greece	1
Rumija	42.11201	19.21739	*natans*	Montenegro	3
Samos 1	37.675049999999999	26.816749999999999	*pulveris*	Greece	19
Samos 2	37.712686	26.79914	*pulveris*	Greece	1
Santorini	36.463	25.4238	*natans*	Greece	1
White mountains	36.554167	29.420943999999999	*pulveris*	Turkey	2
Unknown	Unknown	Unknown	*pulveris*	Unknown	1
Total					231

MERODON NATANS И *M. PULVERIS* (DIPTERA: SYRPHIDAE): РАЗГРАНИЧАВАЊЕ КРИПТИЧНИХ ВРСТА ПОМОЋУ МЕТОДЕ ГЕОМЕТРИЈСКЕ МОРФОМЕТРИЈЕ

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Извод

Геометријско - морфометријски метод, базиран на положају тачака на крилима, кориштен је у сврху разграничавања блиско сродних врста из *Merodon natans* групе. Две проучаване врсте: *Merodon natans* и *M. pulveris* су успешно раздвојене помоћу ове методе. Резултати анализа показали су значајне разлике у облику и величини крила између јединки две дате врсте, потврђујући тако да су у питању 2 раздвојене, иако, морфолошки, веома сличне, врсте. Дискриминантна и кластер анализа, такође, су показале суптилне разлике у облику крила између популација врсте *M. pulveris,* указујући тако на интраспецијску диференцијацију са потенцијалним конзервационим импликацијама. Резултати морфолошке студије, допуњени су доступним подацима о дистрибуцији анализираних врста.

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