J Insect Conserv (2011) 15:379–388
DOI 10.1007/s10841-010-9311-5

ORIGINAL PAPER

Phenotypic evidence for hidden biodiversity in the *Merodon aureus* group (Diptera, Syrphidae) on the Balkan Peninsula: conservation implication

Ljubinka Francuski · Jasmina Ludoški · Ante Vujić · Vesna Milankov

Received: 5 February 2010 / Accepted: 30 June 2010 / Published online: 16 July 2010
© Springer Science+Business Media B.V. 2010

Abstract Cryptic species and phenotypic divergent units provided useful information about hidden biodiversity in the *Merodon* genus (Diptera, Syrphidae) on the Balkan Peninsula. Discrimination three cryptic species within both the *M. aureus* (*M. aureus* A, *M. aureus* B and *M. aureus* C) and *M. cinereus* (*M. cinereus* A, *M. cinereus* B and *M. cinereus* C) complexes was done by study of subtle wing variation (wing size and shape) using a geometric morphometric approach. Observed interspecific differentiation is generally in agreement with a previous study using molecular markers (allozyme loci, COI mtDNA). A considerable evolutionary and phenotypic intraspecies diversity of the *M. aureus* A, *M. aureus* B, *M. aureus* C, *M. cinereus* A, *M. cinereus* B, *M. cinereus* C and *M. funestus* species from the Balkan Peninsula has important implications for diagnosing biodiversity, including endemic and cryptic species. Observed phenotypic divergent units within the species might be considered as an evolutionary potential of the *M. aureus* group and used for defining conservation priorities. This study has contributed to the recognition of the value of wing traits in order to decipher the hidden diversity and evolutionary diversification.

Keywords Syrphidae · *Merodon aureus* group · Cryptic species · Geometric morphometrics · Hidden phenotypic diversity · Intraspecific divergent units · Wing size · Wing shape

L. Francuski · J. Ludoški · A. Vujić · V. Milankov (✉)
Faculty of Sciences, Department of Biology and Ecology, University of Novi Sad, Trg Dositeja Obradovića 2, 21000 Novi Sad, Serbia
e-mail: vesna.milankov@dbe.uns.ac.rs

Introduction

Pleistocene glaciations have influenced evolution and differentiation of most extant species in the Northern hemisphere (Bernatchez and Wilson 1998; Hewitt 2000, 2004). Recently documented phenotypic and genetic patterns of diversity within the hoverfly taxa of the Balkans suggest a complex biogeographical history with patterns of population bottlenecks followed by range expansion (Milankov et al. 2008b; Ståhls et al. 2008; Francuski et al. 2009a; Milankov et al. 2009). The genus *Merodon* Meigen, 1803 has more than 50 European species (Speight 2008), including cryptic taxa, making it a challenging group for evolutionary and conservation biology (e.g. Mengual et al. 2006; Marcos-García et al. 2007; Milankov et al. 2008a, b, 2009; Francuski et al. 2009a; Ståhls et al. 2009).

Since distinction of genetic and phenotypic uniqueness is of great importance in studying adaptive diversification and recognition of conservation units, we studied the *M. aureus* group from the Balkan Peninsula. The *M. aureus* group included a number of species, cryptic taxa, and genetically and phenotypically divergent units. For example, in the Mediterranean region, seven Iberian species including *M. funestus, M. chalybeus* and five endemic species belong to the *M. aureus* group (Marcos-García et al. 2007). According to Milankov et al. (2008a), the *M. aureus* group included seven closely related species from the Balkans, from which six are cryptic species identified by allozyme diagnostic loci. Within the morphologically defined *Merodon aureus* Fabricius, 1805 allochronic populations originating from Morinj and Durmitor Mt were divided into two cryptic species, *M. aureus* A (spring species) and *M. aureus* C (summer species), while a population from Kopaonik Mt was identified as an evolutionarily independent unit, the

M. aureus B species. Similarly, using the species-specific alleles at the diagnostic allozyme loci, populations within *M. cinereus* (Fabricius, 1794) were identified as allopatric cryptic species *M. cinereus* A (Kopaonik Mt), *M. cinereus* B (Durmitor and Prokletije Mts) and *M. cinereus* C (Šar Mt). However, some discordance was observed among the allozyme genes and cytochrome *c* oxidase subunit I (COI) mitochondrial DNA (mtDNA) sequences used. For example, contrary to the successful delimitation by the allozyme diagnostic markers, sequence divergences at the 3′ end of mtDNA COI were unable to discriminate the *M. aureus* C (sample from Morinj), *M. cinereus* B and *M. cinereus* C species. On the other side, the cryptic species *M. aureus* A and *M. aureus* B, and *M. cinereus* A and *M. cinereus* C were considered to be morphologically inseparable taxa using a traditional taxonomic approach, in spite of allozyme loci and mtDNA by which the cryptic species were clearly diverged. Therefore, as has been already proposed (Will and Rubinoff 2004; Dayrat 2005; Will et al. 2005; Wheeler 2005; Rubinoff 2006; Roe and Sperling 2007; Agnarsson and Kuntner 2007; Padial et al. 2009; Schlick-Steiner et al. 2010), the *M. aureus* group (again) highlighted the importance of integrating multiple source data in assessing taxonomic distinctiveness, especially in case of cryptic and recently diverged species. Finally, *M. funestus* (Fabricius, 1794) is also a member of the group, it being morphologically similar to the *M. aureus* species. Within *M. funestus* an intraspecific variation in morphological traits (the length of antennae and leg coloration), allozyme loci and COI mtDNA sequences were observed (Milankov et al. 2008a) addressing questions about hidden taxonomic diversity.

Since members of the *M. aureus* group have been characterized by great morphological variability and some cryptic taxa are still remain morphologically inseparable, we performed a landmark-based geometric morphometric approach in order to quantify a subtle phenotypic variation in wing traits, such as wing size and shape, at intra- and interspecific levels. This is of great importance since geometric morphometrics was proved to be a valuable tool for detecting hidden phenotypic diversity (e.g., Debat et al. 2003; Villemant et al. 2007; Milankov et al. 2009; Francuski et al. 2009a, b; Dapporto 2010a, b). Thus, the first goal of the study was to utilize wing geometric morphometrics in delimitation and recognition of cryptic taxa, including species and intraspecific divergent units within the *M. aureus* group. Given the importance of the integrative approach in taxonomy (see above references), we compared results obtained using wing geometric morphometrics with previously explored diagnostic morphological and molecular markers. Then, we defined phenotypic uniqueness in the focal species, which provided crucial information for defining meaningful conservation units. Finally, phenetic relationships and evolutionary diversification of the *M. aureus* group were discussed.

Materials and methods

Sample collection

The samples of the *M. aureus* group species were gathered over several years (1982–1998). Wing size and shape variation was observed from 116, 221 and 64 specimens of the *M. aureus* and *M. cinereus* complexes and the *M. funestus* species, respectively, from eight localities on the Balkan Peninsula (Table 1; Fig. 1). We separately analysed 203 males and 198 females because of the wing sexual dimorphism these species exhibit. To study patterns of temporal and spatial variation of *M. funestus,* spring and summer generations from Morinj as well as spring generation from Rumija Mt, Slano and Chalkidiki were analysed. The taxonomic identification of specimens was performed using morphological characters of adults and diagnostic allozyme loci presented in Milankov et al. (2008a).

Specimen collection was limited by population size at the time of field campaigns, sometimes resulting in small sample sizes (based on field observations, sample sizes were proportional to the number of active adults at the time of sample collection). Females of *M. cinereus* B from Prokletije Mt and males of *M. funestus* from Slano and Chalkidiki were not available for analyses.

Table 1 The *M. aureus* group: species, populations and sample size

Complex	Species	Area (population)	Sample size (*N*)		
			♂	♀	∑
M. aureus	*M. aureus* A	Morinj	21	20	41
		Durmitor Mt	8	2	10
	M. aureus B	Kopaonik Mt	29	7	36
	M. aureus C	Morinj	4	2	6
		Durmitor Mt	14	9	23
M. cinereus	*M. cinereus* A	Kopaonik Mt	30	39	69
	M. cinereus B	Durmitor Mt	49	83	132
		Prokletije Mt	2	–	2
	M. cinereus C	Šar Mt	12	6	18
	M. funestus	Rumija Mt	19	11	30
		Morinj spring	13	2	15
		Morinj summer	2	5	7
		Slano	–	7	7
		Chalkidiki	–	5	5

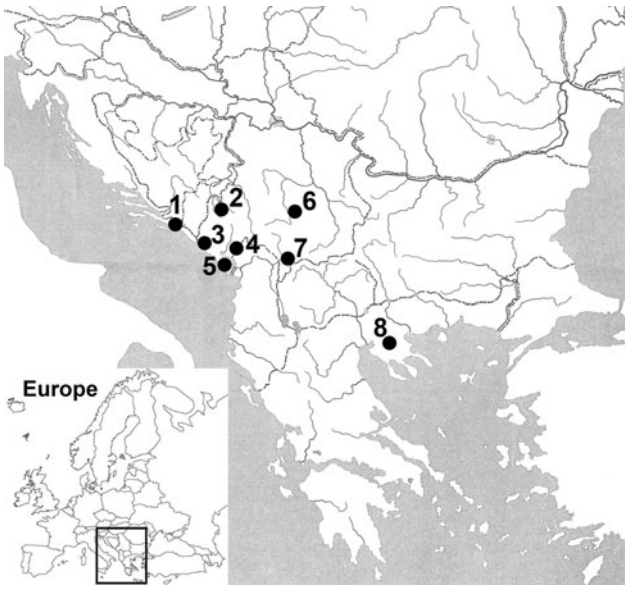

Fig. 1 Map of population sampling locations from the Balkan Peninsula: 1. Slano (Croatia, E 17°53′, N 42°46′); 2. Durmitor Mt (Montenegro, E 19°00′, N 43°11′); 3. Morinj (Montenegro, E 18°40′, N 43°29′30″); 4. Prokletije Mt (Serbia, E 19°50′, N 42°32′); 5. Rumija Mt (Montenegro, E 19°13′, N 42°05′); 6. Kopaonik Mt (Serbia, E 20°40′, N 43°15′).7. Šar Mt (Serbia, E 21°05′, N 42°12′); 8. Chalkidiki (Greece, E 23°42′, N 40°18′)

Morphometric and statistical analysis

Wing size and shape variation was observed from 401 specimens from 13 populations of the *M. aureus* group, using the landmark-based geometric morphometric method (Bookstein 1991; Rohlf and Marcus 1993). Right wings were mounted on slides with Hoyer's medium, photographed (Francuski et al. 2009a), and coordinates of 16 landmarks (Fig. 2) were digitized using TpsDig 1.40 (Rohlf 2004).

For comparison of overall wing size between species and populations in both sexes, centroid size (CS; Zelditch et al. 2004) was calculated and tested using one-way analysis of variance (ANOVA). To examine the pattern of among-species/population shape variation, the raw landmark coordinates were superimposed using a Generalized Procrustes Analysis (GPA) superimposition algorithm (Rohlf and Slice 1990). The partial warps and uniform components representing weight matrix (*w*; Rohlf et al. 1996) were obtained and used as shape variables for a discriminant analysis combined with canonical variate analysis (CVA). We used the percentages of correct classification to evaluate the discriminatory power of wing shape. Also, squared Mahalanobis distances were derived from CVA in order to quantify the interspecific phenetic relationships. Both CS and *w* matrix were obtained utilizing software TpsRelw 1.44 (Rohlf 2006). Wing shape changes associated with canonical axes were visualized as deformations grids (Rohlf et al. 1996) and were computed using TpsRegr 1.31 (Rohlf 2005). All statistical analyses were calculated using Statistica for Windows (version 8.0).

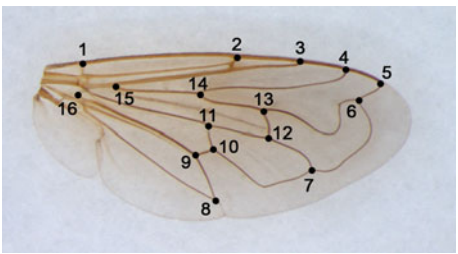

Fig. 2 The locations of 16 landmarks on wing of the *M. aureus* group selected for geometric morphometric analysis

Results

Delimitation of the *M. aureus*, *M. cinereus* complexes and *M. funestus* species

The analysis of variance (ANOVA) of wing centroid size showed highly significant differences between morphologically defined species of the *M. aureus* group when sexes were considered separately (female: $F_{(2,19)} = 9.29$, $P = 0.000$; male: $F_{(2,20)} = 27.97$, $P < 0.001$; Tukey HSD test: $P < 0.05$, except for females of the *M. cinereus* complex/*M. funestus* pair and males of the *M. aureus* complex/*M. funestus* species pair). Non-significant interspecific variation of females from the *M. aureus* and *M. cinereus* complexes and the *M. funestus* species was found for the *M. aureus* A/*M. cinereus* B, *M. aureus* A/*M. funestus*, *M. aureus* B/*M. cinereus* B, *M. aureus* B/*M. funestus*, *M. aureus* C/*M. cinereus* A, *M. aureus* C/*M. cinereus* B, *M. aureus* C/*M. cinereus* C, *M. aureus* C/*M. funestus*, *M. cinereus* B/*M. funestus* and *M. cinereus* C/*M. funestus* species pairs. Comparing the species complexes and the *M. funestus* species, significant interspecific variation in CS was found among males of the *M. aureus* A/*M. cinereus* B, *M. aureus* A/*M. cinereus* C, *M. aureus* A/*M. funestus*, *M. aureus* C/*M. cinereus* B, *M. aureus* C/*M. cinereus* C and *M. aureus* C/*M. funestus* species pairs.

Discriminant analysis combined with canonical variate analysis (CVA) of the *M. aureus* group performed over the *w* matrix of females resulted in two statistically significant canonical axes ($P < 0.001$). The first axis (CV1) accounted for 67.3% of total shape variation and clearly separated the *M. funestus* populations, while the two complexes slightly overlapped (Wilks' $\Lambda = 0.057$, $F_{(56,33)} = 19.16$, $P < 0.001$) (Fig. 3a). Similarly, scatterplot of individual scores from CVA comparing male specimens showed wing

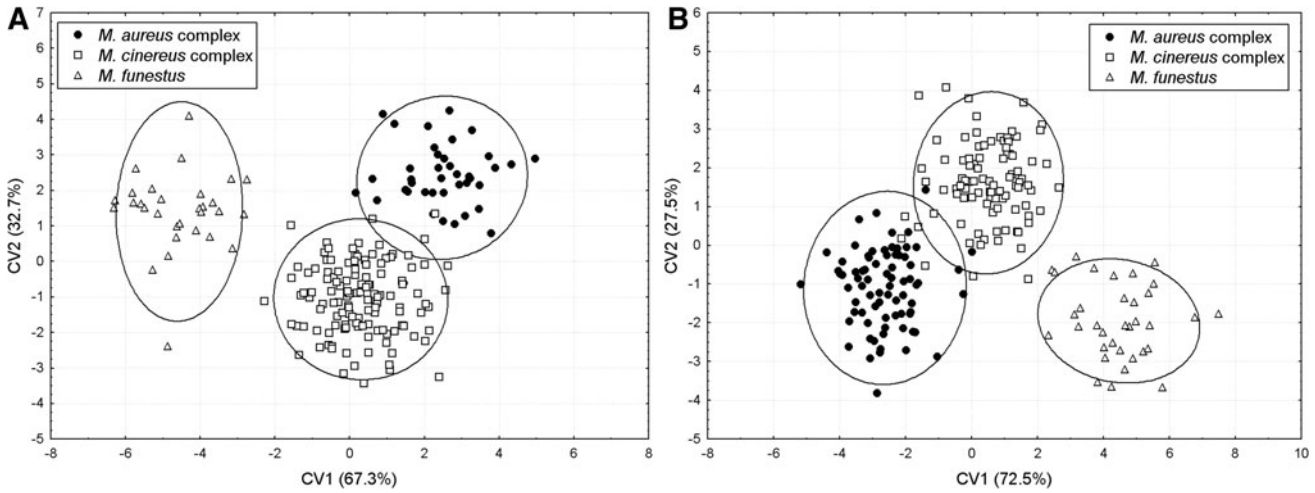

Fig. 3 Scatterplot of individual scores from CVA comparing **a** female and **b** male specimens of the *M. aureus* group for wing shape. The amount of variation explained by each axis is in parentheses

shape differences among these taxa (Wilks' $\Lambda = 0.042$, $F_{(56,35)} = 23.85$, $P < 0.001$) (Fig. 3b).

Furthermore, we explored the usefulness of wing shape in separating the *M. aureus* and *M. cinereus* complexes since they were slightly overlapped. The two axes extracted by CVA were highly significant ($P < 0.001$) and contributed to shape differentiation among species, both in females (Fig. 4a) (Wilks' $\Lambda = 0.013$, $F_{(140,67)} = 6.75$, $P < 0.001$) and males (Fig. 4b) (Wilks' $\Lambda = 0.005$, $F_{(140,68)} = 9.15$, $P < 0.001$). Male specimens of the *M. aureus* C species appeared as an intermediate group and partially overlapped with the analysed *M. cinereus* species (Fig. 4b).

Identification and delimitation of the cryptic species

The analysis of morphometric traits allowed identification of three cryptic species within the *M. aureus* complex. CS varied significantly among male specimens of the *M. aureus* complex ($F_{(2,73)} = 15.00$, $P < 0.001$). Except the *M. aureus* A/*M. aureus* C species pair, Tukey post hoc pairwise comparisons showed significant wing size differences. Contrary to the male specimens, ANOVA revealed no size differences among females ($F_{(2,37)} = 2.10$, $P = 0.14$; Tukey HSD test $P > 0.05$). Female specimens of the *M. aureus* complex were successfully differentiated using CVA indicated that interspecific differences were highly significant (Wilks' $\Lambda = 0.006$, $F_{(56,20)} = 4.34$, $P < 0.001$) (Fig. 4a). Based on wing shape, 100% of all female individuals were correctly classified. Similarly, CVA showed a clear interspecific discrimination of males within the *M. aureus* complex (Wilks' $\Lambda = 0.017$, $F_{(56,92)} = 10.79$, $P < 0.001$) (Fig. 4b). There was a substantial divergence in wing shape allowing 100% of all male individuals to be classified correctly.

It was estimated that size significantly varied among female specimens of the *M. cinereus* complex ($F_{(2,12)} = 23.32$, $P < 0.001$). Contrary to the *M. cinereus* A/*M. cinereus* C species pair, post hoc pairwise comparisons between the other species revealed statistically significant differences ($P < 0.05$, Tukey HSD test). Significant interspecific variation in CS was found (ANOVA, $F_{(2,90)} = 13.21$, $P < 0.001$) among males as well. Except for the *M. cinereus* A/*M. cinereus* B species pair, Tukey post hoc pairwise comparisons showed no significant wing size differences. Cryptic species of the *M. cinereus* complex were successfully discriminated using CVA performed on the wing shape variables (*w* matrix). In females, the first two canonical axes extracted by CVA were highly significant ($P < 0.001$) and contributed to shape differentiation among analysed species (Wilks' $\Lambda = 0.198$, $F_{(56,19)} = 4.36$, $P < 0.001$) (Fig. 5a). Despite the small morphometric overlap, 96.1% of all female individuals were classified correctly: 94.8% of *M. cinereus* A, 97.6% of *M. cinereus* B and 83.3% of *M. cinereus* C. Significant wing shape differences were found among male specimens as well (Wilks' $\Lambda = 0.142$, $F_{(56,13)} = 3.71$, $P < 0.001$). Classification results were higher for *M. cinereus* B (94.1%) and *M. cinereus* A (90%), while 75% of the *M. cinereus* C specimens were classified as such. Out of 12 *M. cinereus* C specimens used in the analysis, two were misclassified as *M. cinereus* B and one as *M. cinereus* A (Fig. 5b).

Recognition of divergent units within the species of the *M. aureus* group

The ANOVA showed no wing size differences between conspecific populations of *M. aureus* A by comparing female ($F_{(1,20)} = 0.69$, $P = 0.42$) and male specimens

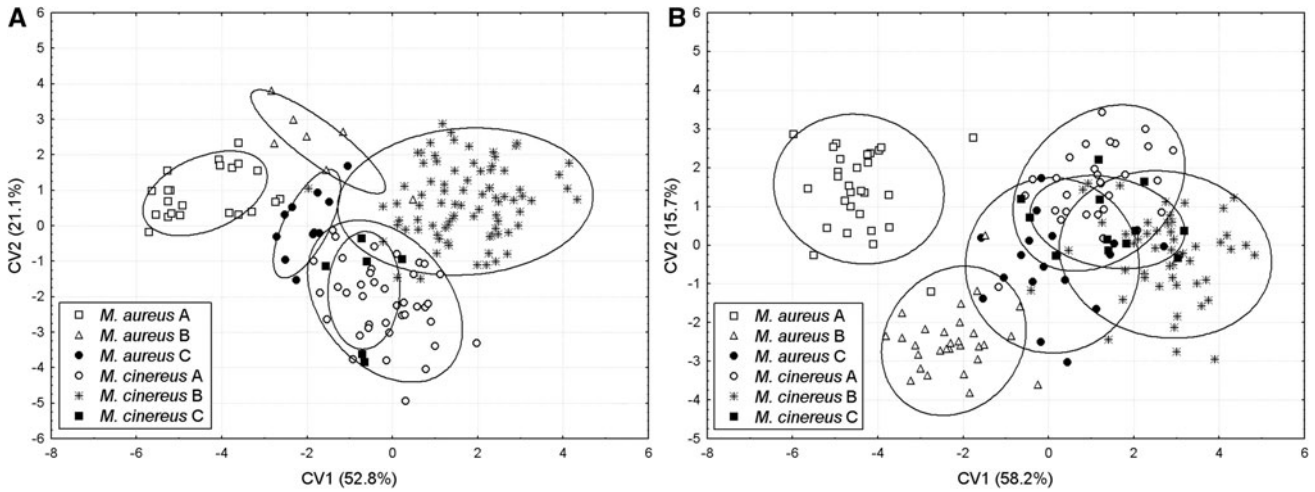

Fig. 4 Scatterplot of individual scores from CVA showing shape differentiation between female **a** and male **b** specimens of the *M. aureus* and *M. cinereus* complexes

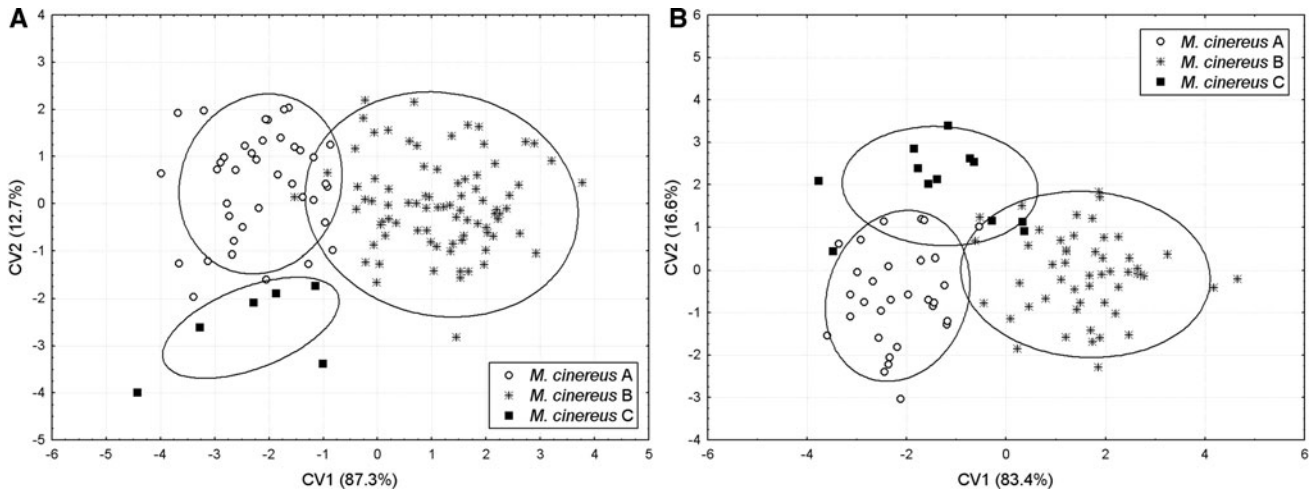

Fig. 5 Scatterplot of individual scores from CVA comparing **a** females and **b** males of the species of the *M. cinereus* complex for wing shape

($F_{(1,27)} = 2.67$, $P = 0.11$) separately. In contrast, CVA with populations and sexes as grouping variables revealed significant wing shape differences among the populations originating from Morinj and Durmitor Mt (Wilks' $\Lambda = 0.004$, $F_{(84,60)} = 4.01$, $P < 0.001$) (Fig. 6). Based on wing shape, 100% of all individuals belonging to the particular population were correctly classified. The first two canonical axes were significant ($P < 0.001$) and explained 70.4% and 20.2% of the total shape variation. The first axis (CV1) revealed shape differentiation between sexes, while the second axis (CV2) contributed to the wing shape differences between conspecific populations. Deformation grids which represents deformations along the CV2 showed that shape differences between populations were associated with displacement of landmarks 2, 3, 7, 8 and 15 (Fig. 6).

The analysis of variance of wing CS revealed non-significant differences between males of the *M. aureus* C populations ($F_{(1,16)} = 1.87$, $P = 0.19$), contrary to female specimens ($F_{(1,90)} = 9.77$, $P < 0.05$). On the other hand, CVA showed interpopulation differences for both males and females of analysed species (Wilks' $\Lambda = 0.00002$, $F_{(75,3)} = 1.92$, $P < 0.28$). The percentage of individuals originating from Morinj and Durmitor Mt correctly classified by the CVA was 100%.

Analysis of interpopulation variation revealed that there were no significant differences in wing size between male specimens of the *M. cinereus* B populations ($F_{(1,49)} = 1.08$, $P = 0.30$) (female specimens of the *M. cinereus* B populations were not available for analysis). Similarly, CVA conducted on the *w* matrix evidenced non-significant

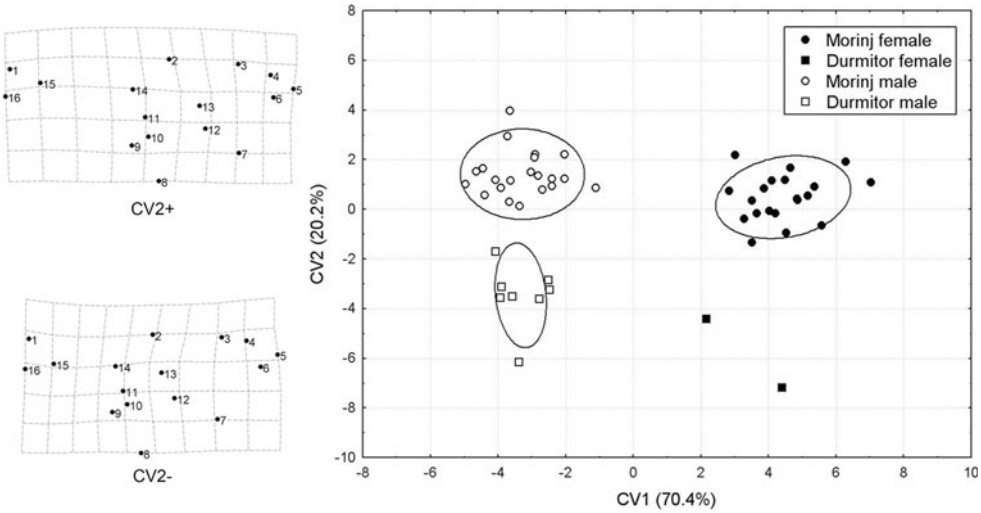

Fig. 6 Scatterplot of individual scores from CVA showing wing shape differentiation between populations and between sexes of *M. aureus* A. Thin-plate spline reconstruction representing negative and positive deformations of mean shape between conspecific populations along the CV2 axis. Deformation grids are exaggerated × 3. Numbers in the deformation grids refer to landmarks shown in Fig. 2

intraspecific differences (Wilks' $\Lambda = 0.337$, $F_{(28,22)} = 1.54$, $P < 0.15$).

Contrary to males ($F_{(2,31)} = 1.218$, $P = 0.31$; Tukey HSD test $P > 0.05$, male specimens from Chalkidiki and Slano were not available for analysis), significant wing size differences among female specimens of the *M. funestus* populations were found ($F_{(4,25)} = 13.95$, $P < 0.001$). Females from Slano had considerably larger wings than females from Rumija Mt, Chalkidiki and Morinj spring generation ($P < 0.05$, Tukey HSD test). The ANOVA also exhibit significant size variation between the Morinj spring/Chalkidiki, Morinj summer/Rumija Mt population pairs and between two generations from Morinj (Fig. 7a). CVA with the *M. funestus* populations and sexes as grouping variables revealed significant shape differences (Wilks' $\Lambda = 0.0008$, $F_{(196,21)} = 1.95$, $P < 0.001$). Two of seven axes extracted by CVA were highly significant ($P < 0.001$) and contributed to shape differentiation among populations and two Morinj generations. Individuals from Rumija Mt and spring generation from Morinj partially overlapped, but clearly separated from specimens originated from Slano, Chalkidiki and Morinj summer generation. Females of all conspecific populations were classified with 100% success, except those from Rumija Mt (90%). Percentages of correct classification for males ranged from 76.9% for Morinj spring generation, 78.9% for Rumija Mt to 100% for Morinj summer generation (Fig. 7b).

Phenotypic relationships of the species within the *M. aureus* group

The UPGMA cluster analyses based on squared Mahalanobis distances computed from *w* matrix of female shape variables grouped the species of the *M. cinereus* complex within one clade. The *M. aureus* C species was clustered in the same branch with the *M. cinereus* complex, while *M. funestus* was the most divergent taxa (Fig. 8a). The UPGMA tree derived from *w* matrix of male shape variables showed three main clusters: one composed of three *M. cinereus* species placed with *M. aureus* C, another with *M. aureus* A and *M. aureus* B and the third one with *M. funestus* as the most divergent species (Fig. 8b).

Discussion

Detection and delimitation of cryptic diversity

In this paper, wing trait analyses revealed cryptic speciation in morphologically defined *M. aureus* taxa. Based on the wing shape *M. aureus* A, *M. aureus* B and *M. aureus* C were clearly delimited. While the *M. aureus* C populations can be distinguished based on the traditional morphology, *M. aureus* A and *M. aureus* B were morphologically inseparable (Milankov et al. 2008a). Thus the only phenotypical traits that might be used in delineating the *M. aureus* A/*M. aureus* B species pair were the wing traits differences presented in this paper. Contrary to the female specimens, considerable differences in wing size between males were observed herewith. In comparison with this, wing shapes of both sexes revealed clear phenotypic interspecies differentiation of *M. aureus* A and *M. aureus* B. In the *M. aureus* A/*M. aureus* B case, wing landmark configuration differences were in accordance with diagnostic values of nuclear allozyme and COI mtDNA (Milankov et al. 2008a).

Morphological analysis of the *M. cinereus* species confirmed the existence of two morphotypes: *M. cinereus* B as one, and *M. cinereus* A/*M. cinereus* C pair as another morphotype. However, in spite of the high morphological similarity, there is a great genetic distinction between *M. cinereus* A and *M. cinereus* C (Milankov et al. 2008a).

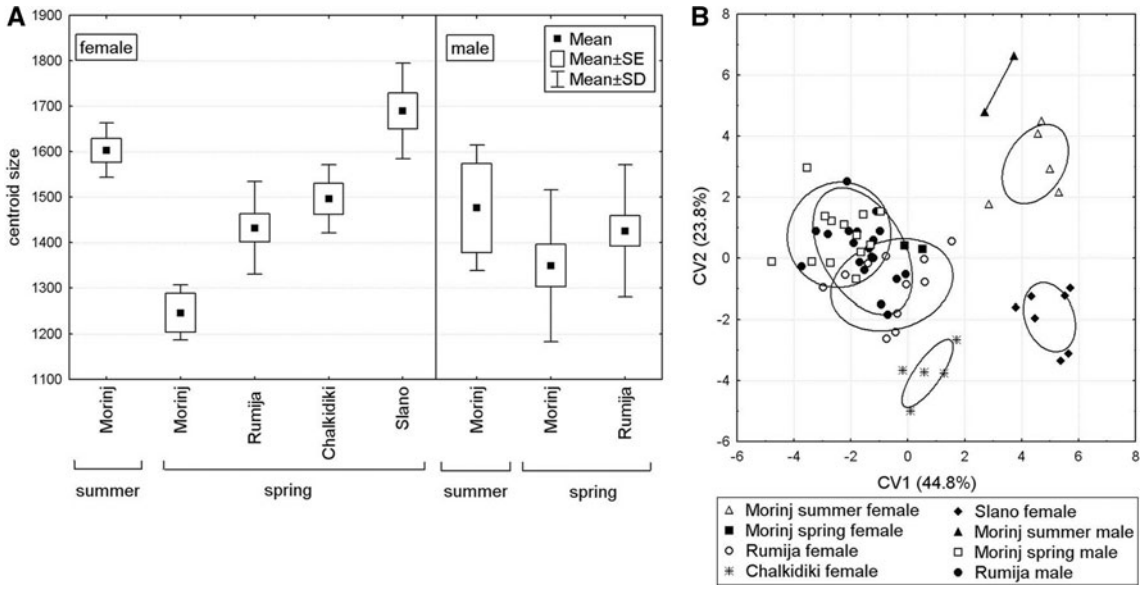

Fig. 7 Variations of wing size and shape among *M. funestus* populations. **a** Boxplot of centroid size with the mean, standard error and standard deviation illustrating intraspecific variation in wing size. **b** The relationships among populations with respect to the first two canonical variables of wing shape

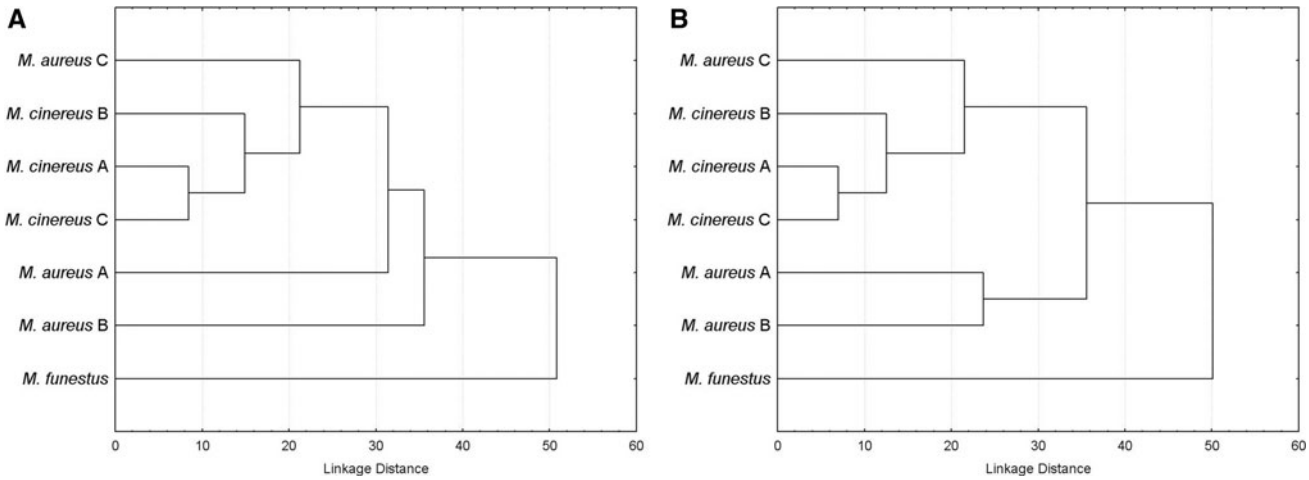

Fig. 8 UPGMA phenogram based on the squared Mahalanobis distances of **a** females and **b** males of the *M. aureus* group

The current study demonstrated phenotypic traits that delineated these two morphologically inseparable species. Although we found no significant interspecific wing size variation, *M. cinereus* A and *M. cinereus* C species were clearly delimited by wing shape. Similarly, both species were well defined by nuclear allozyme loci and mtDNA markers (Milankov et al. 2008a). Therefore, along with previous findings, this study considered allopatrically distributed *M. cinereus* taxa as evolutionarily independent entities/species and distinctive phenotypic units. Furthermore, contrary to wing size, considerable differences in wing shape between *M. cinereus* B and *M. cinereus* C of both sexes were demonstrated in this paper. The observed wing shape variation confirmed a clear separation of the species based on morphological traits and allozyme diagnostic loci. However, it was observed that both species shared identical COI mtDNA haplotype. Similarly, COI mtDNA marker was not able to resolve the species limits among *M. aureus* C, *M. cinereus* B and *M. cinereus* C, which is in discordance to the wing morphometric data presented herewith, traditional morphology and nuclear allozyme species-specific alleles (Milankov et al. 2008a).

Wing geometric morphometrics and molecular analyses demonstrated that reliance on current morphological taxonomy underestimates underlying genetic and subtle phenotypic diversity in the *M. aureus* group. Although a traditional taxonomic approach failed to discriminate some recently separated cryptic taxa within the *M. aureus* group

(Milankov et al. 2008a), they were well defined by wing landmark configurations. This study therefore has contributed to the recognition of the value of wing traits in order to decipher the hidden diversity and evolutionary diversification. Based on our results, wing traits should be integrated with molecular markers in delimitation some recently separated but reproductively isolated the *M. aureus* group taxa. Within the genus *Merodon*, wing geometric morphometrics was already used as a diagnostic tool to provide crucial information on the defining *M. bicolor* vs *M. avidus* cryptic species (Milankov et al. 2009) and species of the *M. ruficornis* group (Francuski et al. 2009a). However, it is not clear yet at what scale processes driving morphometrical diversity of the *M. aureus* group operate and the biological significance of the differences between wing patterns remain to be demonstrated. We assumed that wing interspecific differences are associated with both producing species-specific courtship song and aerodynamic performance, as already has been documented for dipteran species (Birdsall et al. 2000).

Relationships among members of the *M. aureus* group

Genetic and phenotypic relationships observed using different markers and algorithms showed different placements of the taxa within the *M. aureus* group. Comparing UPGMA dendrogram generated by allozyme data and mtDNA phylogenetic tree published in Milankov et al. (2008a) with phenogram conducted by wing shape variables presented herewith placements of the *M. aureus* A and *M. aureus* B as a monophyletic group as well as *M. funestus* as the most distinct species were the same. However, placements of *M. aureus* C and taxa of the *M. cinereus* complex were different reflecting a different pattern of evolutionary changes of phenotypic traits, allozyme loci and mtDNA sequences. Contrary to the mtDNA parsimony analysis, the *M. aureus* C, *M. cinereus* B and *M. cinereus* C taxa were well defined by allozyme markers (Milankov et al. 2008a) and wing traits. Hence, a partial congruence of relationships among members of the *M. aureus* group was found for allozyme markers and wing traits. In the wing traits generated phenogram the *M. aureus* C species was clustered in the same branch with the *M. cinereus* complex, which was inconsistent with allozyme data that showed *M. aureus* C as a distinct species to the *M. cinereus* complex and *M. aureus* A and *M. aureus* B.

Conservation implications of the recognition of phenotypic divergent units

Recognition, protection and conservation of phylogenetic lineages and meaningful conservation units is commonly based on the Evolutionarily Significant Units (ESU) concept using different molecular markers of both nuclear and mitochondrial genes in conjunction with morphological and/or ecological traits (Rayder 1986; Moritz 1994, 2002). Since consequences of counting species are crucial in detecting areas of conservation importance and species of importance (Agapow et al. 2004), detection of hidden biodiversity in the *M. aureus* group is important in its own right and has a broader implication as well. Analyses of wing traits (presented herein) and molecular markers (Milankov et al. 2008a) revealed high intra- and interpopulation genetic and phenotypic diversity within the species of the *M. aureus* group. In this paper, significant intraspecific wing shape differences in *M. aureus* A, *M. aureus* C and *M. funestus* were observed indicating a high structuring within the focal species.

Contrary to wing size differences, wing shape was found to be significantly different between the allopatric populations of the *M. aureus* A species. It was found that wing shape differences were associated with displacement of outer landmarks (landmarks 2, 3, 7, 8 and 15), all of which influence wing's width and length. Similarly, a significant interpopulation wing size (females only) and shape (both sexes) variation between populations of *M. aureus* C originating from Morinj and Durmitor Mt was observed. Additionally, population-genetic analysis of the *M. cinereus* B populations from Durmitor and Prokletije Mts revealed a great spatial variation based on differences in unique alleles at the *Gpi* (with a probability of 100%) and *Me* (with a probability >70%) loci, and thus suggested that these two populations are likely to be at an early stage of speciation (Milankov et al. 2008a). However, we found non-significant intraspecific size and shape differences between male specimens of the *M. cinereus* B populations originating from Durmitor and Prokletije Mts (female specimens were not available for analysis). In the absence of differences at mtDNA and morphological traits (Milankov et al. 2008a), and wing traits as well, they are considered as conspecific for the time being. The phenotypic variation observed in the length of antennae and leg coloration of the *M. funestus* specimens raises unresolved questions for taxonomists as well. The allozyme data observed for spring and summer generations from Morinj revealed temporal distributions of specific alleles at allozyme loci but did not conclusively resolved the status of the two generations of *M. funestus* (Milankov et al. 2008a). Analyzing spatial and temporal pattern of wing traits variation it was found a considerable differences in wing size between females (contrary to the males) between samples Slano/Rumija Mt, Slano/Chalkidiki, Slano/Morinj spring generation, Morinj spring/Chalkidiki and Morinj summer/Rumija Mt. However, wing shape of both sexes revealed phenotypic intraspecies differentiation. The two generations of *M. funestus* (spring and summer) from Morinj were significantly

distinct based on both wing traits. The existence of specific alleles (Milankov et al. 2008a) and wing traits differences (reported herein) in allochronic and sympatric samples was likely to be the results of the presence of evolutionarily independent units within *M. funestus*. Although our data provide an insight in phenotypic diversity of the *M. funestus* taxa, empirical data of a broader geographical region, more specimens and populations must be included to draw conclusions on this topic.

Acknowledgments We thank to anonymous reviewers for useful comments on the manuscript. The authors wish to thank Mike Taylor for correcting and improving the language. This work was supported in part by the Ministry of Science of Serbia No. 143006B, and the Provincial Secretariat for Science and Technological Development (Maintenance of biodiversity—"Hot spots" on the Balkan and Iberian Peninsula). Lj. Francuski is supported by a Ph.D. fellowship from Ministry of Science of Serbia.

References

Agapow PM, Bininda-Emonds ORP, Crandall KA, Gittleman JL, Mace GM, Marshall JC, Purvis A (2004) The impact of species concept on biodiversity studies. Q Rev Biol 79(2):162–354

Agnarsson I, Kuntner M (2007) Taxonomy in a changing world: seeking solutions for a science in crisis. Syst Biol 56:531–539

Bernatchez L, Wilson C (1998) Comparative phylogeography of Nearctic and Palearctic fishes. Mol Ecol 7:431–452

Birdsall K, Zimmerman E, Teeter K, Gibson G (2000) Genetic variation for the positioning of wing veins in *Drosophila melanogaster*. Evol Dev 2(1):16–24

Bookstein FL (1991) Morphometric tools for landmark data. Cambridge University Press, Cambridge

Dapporto L (2010a) Satyrinae butterflies from Sardinia and Corsica show a kaleidoscopic intraspecific biogeography (Lepidoptera, Nymphlidae). Bio J Linn Soc 100:195–212

Dapporto L (2010b) Speciation in Mediterranean refugia and post-glacial expansion of *Zerynthia polyxena* (Lepidoptera, Papilionidae). J Zool Syst Evol Res. doi: 10.1111/j.1439-0469.2009.00550.x

Dayrat B (2005) Towards integrative taxonomy. Biol J Linn Soc 85:407–415

Debat V, Bégin M, Legout H, David JR (2003) Allometric and nonallometric components of *Drosophila* wing shape respond differently to developmental temperature. Evolution 57(12): 2773–2784

Francuski Lj, Ludoški J, Vujić A, Milankov V (2009a) Wing geometric morphometric inferences on species delimitation and intraspecific divergent units in the *Merodon ruficornis* group (Diptera, Syrphidae) from the Balkan Peninsula. Zool Sci 26:301–308

Francuski Lj, Vujić A, Kovačević A, Ludoški J, Milankov V (2009b) Identification of the species of the *Cheilosia variabilis* group (Diptera, Syrphidae) from the Balkan Peninsula using wing geometric morphometrics, with the revision of status of *C. melanopa redi* Vujić, 1996. Contrib Zool 78(3):129–140

Hewitt GM (2000) The genetic legacy of the quaternary ice ages. Nature 405:907–913

Hewitt GM (2004) Genetic consequences of climatic oscillations in the quaternary. Philos T Roy Soc B 359:183–195

Marcos-García MA, Vujić A, Mengual X (2007) Revision of Iberian species of the genus *Merodon* Meigen, 1803 (Diptera: Syrphidae). Eur J Entomol 104:531–572

Mengual X, Ståhls G, Vujić A, Marcos-García M (2006) Integrative taxonomy of Iberian *Merodon* species (Diptera: Syrphidae). Zootaxa 1377:1–26

Milankov V, Ståhls G, Stamenković J, Vujić A (2008a) Genetic diversity of populations of *Merodon aureus* and *M. cinereus* species complexes (Diptera, Syrphidae): integrative taxonomy and implications for conservation priorities on the Balkan Peninsula. Conserv Genet 9(5):1125–1137

Milankov V, Ståhls G, Vujić A (2008b) Molecular diversity of populations of the *Merodon ruficornis* group (Diptera, Syrphidae) on the Balkan Peninsula. J Zool Syst Evol Res 46(2):143–152

Milankov V, Ludoški J, Ståhls G, Stamenković J, Vujić A (2009) High molecular and phenotypic diversity in the *Merodon avidus* complex (Diptera, Syrphidae): cryptic speciation in a diverse insect taxon. Zool J Linn Soc 155(4):819–833

Moritz C (1994) Defining "evolutionary significant units" for conservation. Trends Ecol Evol 9:373–375

Moritz C (2002) Strategies to protect biological diversity and evolutionary processes that sustain it. Syst Biol 51(2):238–254

Padial JM, Castroviejo-Fisher S, Köhler J, Vilá C, Chaparro JC, De la Riva I (2009) Deciphering the products of evolution at the species level: the need for an integrative taxonomy. Zool Scr 38(4):431–447

Rayder OA (1986) Species conservation and systematics: the dilemma of subspecies. Trends Ecol Evol 1:9–10

Roe AD, Sperling AH (2007) Population structure and species boundary delimitation of cryptic *Dioryctria* moths: an integrative approach. Mol Ecol 16:3617–3633

Rohlf FJ (2004) TpsDig–thin plate spline digitizer, version 1.40. Department of Ecology and Evolution, State university of New York at Stony Brook, New York

Rohlf FJ (2005) TpsRegr, version 1.31. Department of Ecology and Evolution, State university of New York at Stony Brook, New York

Rohlf FJ (2006) TpsRelw–thin plate spline relative warp, version 1.44. Department of Ecology and Evolution, State university of New York at Stony Brook, New York

Rohlf FJ, Marcus LF (1993) A revolution in morphometrics. Trends Ecol Evol 8:129–132

Rohlf FJ, Slice D (1990) Extensions of the procrustes method for the optimal superimposition of landmarks. Syst Zool 39(1):40–59

Rohlf FJ, Loy A, Corti M (1996) Morphometric analysis of old world talpidae (Mammalia, Insectivora) using partial-warp scores. Syst Biol 45(3):344–362

Rubinoff D (2006) Utility of mitochondrial DNA barcodes in species conservation. Conserv Biol 20:1026–1033

Schlick-Steiner BC, Steiner FM, Seiert B, Stauffer C, Christian E, Crozier RH (2010) Integrative taxonomy: a multisource biodiversity. Ann Rev Entomol 55:421–438

Speight MCD (2008) Species accounts of European Syrphidae (Diptera) 2008. In: Speight MCD, Castella E, Sarthou J-P, Monteil C (eds) Syrph the Net, the database of European Syrphidae. Syrph the Net publications, Dublin

Ståhls G, Vujić A, Milankov V (2008) *Cheilosia vernalis*-complex: molecular and morphological variability (Diptera, Syrphidae). Ann Zool Fenn 45:149–159

Ståhls G, Vujić A, Pérez-Bañon C, Radenković S, Rojo S, Petanidou T (2009) COI barcodes for identification of *Merodon* hoverflies (Diptera, Syrphidae) of Lesvos Island, Greece. Mol Ecol Resour 9(6):1431–1438

Villemant C, Simbolotti G, Kenis M (2007) Discrimination of *Eubazus* (Hymenoptera, Braconidae) sibling species using geometric morphometrics analysis of wing venation. Syst Entomol 32(4): 625–634

Wheeler Q (2005) Losing the plots: "barcodes" and taxonomy. Cladistics 21:405–407

Will KW, Rubinoff D (2004) Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. Cladistics 20(1):47–55

Will KW, Mishler BD, Wheeler QD (2005) The perils of DNA barcoding and the need for integrative taxonomy. Syst Biol 54(5):844–851

Zelditch ML, Swiderski DL, Sheets HD, Fink WL (2004) Geometric morphometrics for biologist a primer. Elsevier Academic Press, San Diego