

# 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

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## Abstract

The present study investigates phenotypic differentiation patterns among four species of the *Cheilosia variabilis* group (Diptera, Syrphidae) using a landmark-based geometric morphometric approach. Herein, wing geometric morphometrics established species boundaries that confirm *C. melanopa* and *C. redi* stat. nov. as evolutionarily independent entities or separate species within the previously described *C. melanopa* species. Seventeen samples of *C. lasiopa*, *C. variabilis*, *C. melanopa* and *C. redi* from 11 localities on the Balkan Peninsula were analysed. Based on wing shape and size, both species boundaries and intraspecific divergent units were delineated indicating the possible presence of evolutionarily independent units within the analysed taxa. Significant differences in wing size were obtained among the analysed species and canonical variate analysis showed that wing shape was sufficiently different to allow the correct classification of 99.7% and 100% individuals of males and females, respectively. Considerable wing size variation was detected among males and females of conspecific populations of *C. melanopa* and males of *C. variabilis*. Contrary to partial discrimination based on the male wing shape among conspecific populations of *C. redi* and *C. variabilis*, clear separation of conspecific populations of *C. melanopa* (for both sexes) and females of *C. redi* and *C. variabilis* was observed. The UPGMA cluster analysis based on squared Mahalanobis distances revealed a close similarity between *C. melanopa* and *C. redi*, whereas *C. lasiopa* was the most divergent species. Results presented in this study utilize wing shape and size as new taxonomic characters in delimitating the closely related species and populations of the *Cheilosia variabilis* group.

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## Introduction

The Balkan Peninsula harbours ecologically, morphologically and genetically diverse species of hoverflies (Diptera, Syrphidae), including a number of endemic and cryptic species (e.g., Milankov *et al.*, 2008, 2009). A pattern of genetic and phenotypic divergence of taxa reflects the complex biogeographical history of the region (Hewitt, 2000, 2004; Pincell *et al.*, 2005) coupled with the evolutionary history of population bottlenecks followed by range expansion (Milankov *et al.*, 2008, 2009; Ståhls *et al.*, 2008). In addition, hidden phenotypic and genetic units and evolutionary diversification among populations of the hoverflies on the Balkan Peninsula have been observed (Milankov *et al.*, 2008, 2009; Francuski *et al.*, 2009). Therefore, study of hoverfly taxa subdivided into genetically and phenotypically distinct populations has implications in taxonomy, systematics and conservation.

The genus *Cheilosia* Meigen, 1822 (Diptera, Syrphidae) comprises 300 species distributed in the Palearctic region, more than 80 Nearctic and at least 50 from the Oriental region (Ståhls *et al.*, 2004). High intraspecific diversity, morphological similarity of the *Cheilosia* species, presence of cryptic species and numerous synonyms create various problems in taxonomy and systematics studies (e.g., Ståhls *et al.*, 2008; Ludoški *et al.*, 2008).

The subjects of the present study are *C. lasiopa* Kowarz, 1885 and *C. variabilis* (Panzer, 1798) and two Balkan subspecies of *C. melanopa* (Zetterstedt, 1843) (Vujić, 1996), all of which form the *C. variabilis* group of species (van Veen, 2004). Using a combination of morphological characters for subgeneric classification of the genus *Cheilosia*, Barkalov (2002) placed the four closely related taxa into subgenus *Cheilosia s. str.*, while some authors kept these taxa in another *Cheilosia* group (Becker, 1894; Haarto and Kerppola, 2007). The parsimony analysis of the mitochondrial DNA (mt-DNA) sequences of the cytochrome *c* oxidase subunit I (COI) and nuclear 28S ribosomal RNA gene (Ståhls et al., 2004) revealed subdivision of the genus *Cheilosia* into 13 subgenera according to Barkalov (2002). The phylogenetic analysis classified the *C. variabilis* and *C. melanopa* species within the monophyletic clade of the *Cheilosia s. str.* subgenus (Ståhls et al., 2004). Van Veen (2004) provided a morphology-based key for accurate identification of the *C. variabilis* group taxa. The taxa belonging to the *C. variabilis* group share the following characters: eyes haired, face with long hairs on the sides of the facial knob, equal to or longer than hairs on eye rim, and black legs (van Veen, 2004). The *C. variabilis* group has been known as a challenging group of hoverflies for studies from taxonomical and biogeographical perspectives. For example, the analysis of the *C. melanopa* specimens from the Balkans and adjacent regions indicated the existence of two subspecies: *C. melanopa melanopa* described as a smaller subspecies predominantly covered with black hairs, and *C. melanopa redi* which is larger and covered with reddish hairs (Vujić, 1996). *Cheilosia m. melanopa* is distributed in areas that are more northern and at higher altitudes in the southern part of this range, while *C. m. redi* appears in Central and Southern Europe at low altitudes (Vujić, 1996). Both *C. variabilis* and *C. lasiopa* are distributed in Northern, Central and Western Europe, Siberia and Mongolia, while *C. variabilis* also occupies some areas in Africa (Morocco) (Speight, 2007). While the larval development of the subspecies is unknown, the larva of *C. variabilis* have been described in the roots of *Scrophularia nodosa* (Dušek, 1962). The immature stages of *C. lasiopa* initially live in the leaf and stem bases of *Plantago lanceolata* and later in the rootstock of that plant (Stuke and Carstensen, 2000).

Landmark-based geometric morphometric technique is considered to be the most rigorous morphometric method (Gilchrist et al., 2000; Debat et al., 2003) and a useful tool in accurate identification and delimitation of numerous insect species (e.g. Debat et

al., 2006; Jirakanjanakit et al., 2008; Marsteller et al., 2009). Since it has been known that wing shape exhibits a high heritability in nature (Bitner-Mathé and Klaczko, 1999; Moraes et al., 2004), wing morphology is of a primary importance to entomologists interested in systematics. Wing size and shape have different genetic properties, size heritability being generally low, while wing shape is less sensitive to environmental changes and highly heritable (Bithner-Mathé and Klaczko, 1999; Birdsall et al., 2000). However, wing size is usually associated with body size, and thus potentially linked to a number of fitness components (Reeve et al., 2000). Assuming that wing shape is related to flight ability, wing shape might also influence fitness (Kölliker-Ott et al., 2003). Although the genetic determination of the final details of morphometric characters is still relatively poorly known, Birdsall et al. (2000) suggested that genes that regulate wing shape are more tightly connected than those for wing size, and the former are probably associated with gene determination of wing veins. Therefore, investigation of the wing measurements has both taxonomic value (cf. cryptic species of the *Drosophila buzzatii* Patterson & Wheeler, 1942 cluster, Moraes et al., 2004), and importance in study of adaptive evolution (Soto et al., 2008; Bischoff et al., 2009).

To date, wing geometric morphometrics have been successfully used to resolve taxonomic uncertainties of some species groups (*Merodon avidus* (Rossi, 1790), Milankov et al., 2009; *Cheilosia canicularis* (Panzer, 1801), Ludoški et al., 2008), and to define divergent phenotypic units in hoverflies (the *Merodon ruficornis* Meigen, 1822 group, Francuski, et al., 2009). Moreover, the only morphological traits that might be used in delineating some cryptic species (a group of reproductively isolated but morphologically inseparable species) such as *Merodon bicolor* Gill Collado, 1930 vs *M. avidus* (Milankov et al., 2009), *Merodon aureus* Fabricius, 1805 and *M. cinereus* (Fabricius, 1794) complexes (Francuski, unpublished) were wing landmark configurations.

Considering all of these, we examined the usefulness of wing landmarks for species delimitation and for quantification of intra- and interspecific variation of species of the *C. variabilis* group on the Balkan Peninsula. The goal of the present study was identification of taxa within the *C. variabilis* group using landmark-based geometric morphometrics on wing traits. By introducing morphometric techniques, we also re-evaluated the taxonomic status of *C. melanopa melanopa* and *C. melanopa redi*. Then, we used wing traits to test the

differences among conspecific populations and recognize phenotypic units within the species. Thus, phenotypic variation across spatially and temporally fragmented populations was used to examine the utility of wing size and shape for detecting levels of intraspecific variation as well. Finally, phenetic relationships among species of the *C. variabilis* group based on our findings were discussed. The results reported herein provide a taxonomic framework and give us better insight into intra- and interspecific variation of the *C. variabilis* group.

Material and methods

Sample collection

During the period 1981-2000, 475 specimens of the *C. variabilis* group species were collected from 11 localities on the Balkan Peninsula (Fig. 1, Table 1) for use in morphological studies. The study material considered 371 male and 104 female specimens from 17 populations. Due to the sexual wing shape dimorphism

Area (population)			<i>C. lasiopa</i>		<i>C. melanopa</i>		<i>C. redi</i>		<i>C. variabilis</i>	
L	Name	Code	♂	♀	♂	♀	♂	♀	♂	♀
1	Eastern Alps	ALP	-	-	-	-	-	-	48	11
2	Fruška Gora Mt	FG	-	-	-	-	8	7	57	6
3	Vršačke Mt	VPL	-	-	-	-	-	-	10	1
4	Cer Mt	CER	-	-	-	-	-	-	12	-
5	Tara Mt	TAR	-	-	-	-	-	-	14	4
6	Dubašnica Mt	DUB	-	-	-	-	13	18	21	10
7	Stara Mt	STA	-	-	-	-	-	-	12	2
8	Suva Mt	SUV	-	-	-	-	-	-	15	-
9	Kopaonik Mt	KOP	-	-	-	-	-	-	82	10
10	Šar Mt	ŠAR	-	-	7	8	-	-	3	2
11	Durmitor Mt	DUR	9	6	12	6	1	2	47	11
Total			9	6	19	14	22	27	321	57

Table 1. The *Cheilosia variabilis* group: species, populations and sample size (L-locality, see Fig. 1)

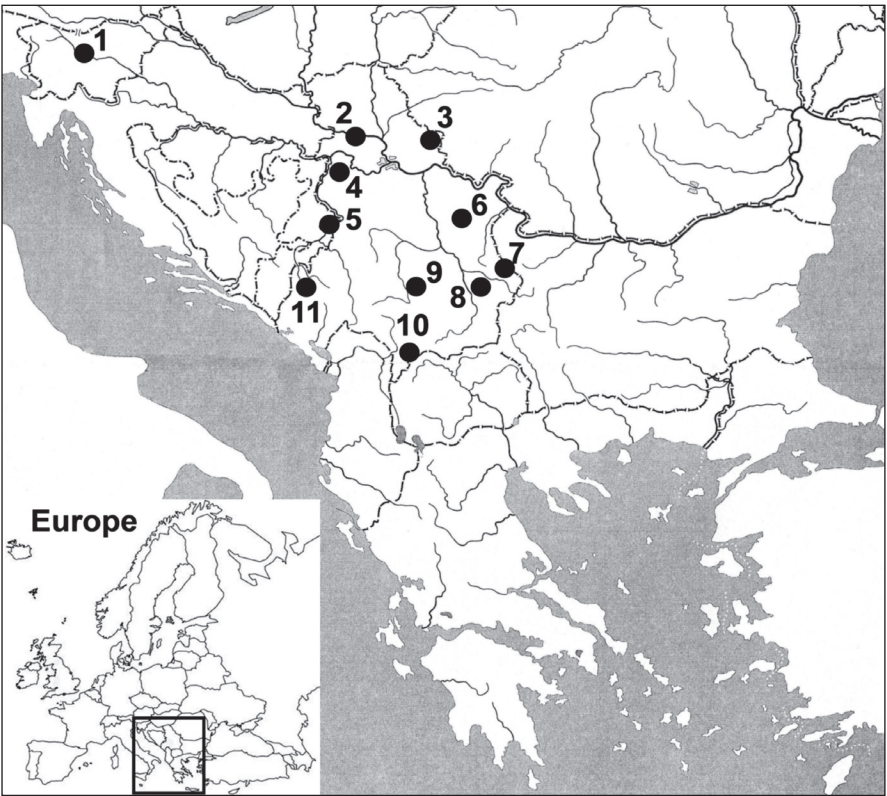


Fig 1. Map of the Balkan Peninsula. Origin of the analysed populations: 1. Easter Alps (Slovenia, ALP; E 13°30'-14°40', N 46°15'); 2. Fruška Gora Mt (Serbia, FG; E 19°50', N 45°10'); 3. Vršačke Mt (Serbia, VPL; E 21°20', N 45°08'); 4. Cer Mt (Serbia, CER; E 19°26', N 44°37'); 5. Tara Mt (Serbia, TAR; E 19°24', N 43°55'); 6. Dubašnica Mt (Serbia, DUB; E 21°59', N 44°01'); 7. Stara Mt (Serbia, STA; E 22°40', N 43°23'); 8. Suva Mt (Serbia, SUV; E 22°10', N 43°10'); 9. Kopaonik Mt (Serbia, KOP; E 20°40', N 43°15'); 10. Šar Mt (Serbia, ŠAR; E 20°44', N 42°00'); 11. Durmitor Mt (Montenegro, DUR; E 19°00', N 43°11').



these species exhibit, analyses were performed separately on males and females.

In the preliminary analyses of phenotypic variability, morphological diagnostic traits (see Results) allowed identification of *C. melanopa melanopa* and *C. m. redi* as two separate species. Morphological differences in wing shape and size were considered substantial for species level differentiation. Prior to geometric morphometric analysis, identification of specimens was performed using other morphological characters of adults (Vujić, 1996).

#### *Morphometric and statistical analysis*

Wing size and shape variation was observed from 475 specimens of the *C. variabilis* group, using the landmark-based geometric morphometric method (Bookstein, 1991; Rohlf and Marcus, 1993; Marcus *et al.*, 1996; Adams *et al.*, 2004). The right wing of each specimen was mounted separately in Hoyer's medium between microscope slides and digital images were captured using a stereomicroscope Leica MZ12.5 and attached video camera Leica DFC320 connected to a PC computer. Sixteen landmarks positioned at vein intersections or terminations were collected using TpsDig 1.40 and expressed as  $x,y$  coordinates in a Cartesian space (Rohlf, 2004) (Fig. 2).

Wing size was computed as centroid size (CS; the square root of the sum of squared distance between each landmark and the wing centroid), an isometric

estimator of size. One-way analysis of variance (ANOVA with post hoc Tukey HSD test) was used to test differences in CS among species and populations. To examine wing shape variation, the digitized landmark data were subjected to Generalized Procrustean superimpositions to standardise size of the landmark configurations and remove differences due to translation and rotation (Rohlf and Slice, 1990), and then a thin plate spline analysis was done. The resulting matrix ( $w$ ; 'weight matrix' of Rohlf *et al.*, 1996) containing the non-uniform and uniform shape variables were calculated was used for a discriminant analysis combined with canonical variate analysis (CVA) to examine the pattern of among and within species/population variation in total shape space. We used the percentages of correct classification to evaluate the discriminating power of wing shape. Both centroid size and  $w$  matrix were obtained utilizing TpsRelw software (version 1.44; Rohlf, 2006) and all statistical analyses were calculated using Statistica for Windows (version 8.0).

Shape changes associated with canonical axes were visualized as deformations obtained by regressing  $w$  matrix onto individual canonical axis scores (Rohlf *et al.*, 1996). Multiple regressions and visualization of deformation grids were computed using TpsRegr 1.31 (Rohlf, 2005). The phenetic relationships among species were determined by UPGMA cluster analysis (Sneath and Sokal, 1973) based on squared Mahalanobis distances derived from CVA.

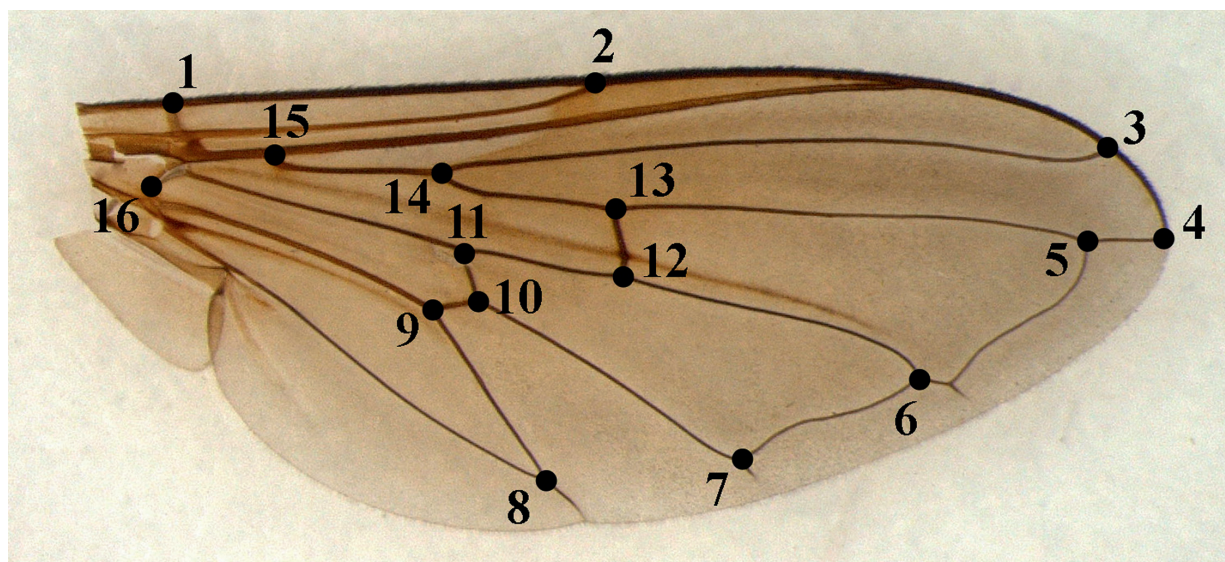


Fig. 2. The locations of 16 landmarks on a right wing of the *C. variabilis* group selected for geometric morphometric analysis.

Results

*Cheilosia redi* Vujić 1996 stat. nov.

*Cheilosia melanopa redi* was described from Central Europe and the Balkan Peninsula as a subspecies of *C. melanopa* based on clear differences in coloration of hairs on the body. Comparing to *C. melanopa* (8–10mm), *C. redi* is a larger species (10–12mm). In addition, diagnostic characters of *C. redi* are: males – body hairs reddish (from yellow-reddish to brown-reddish), black hairs can be found on tergites, sometimes on mesoscutum (on postalar calli) and scutellum margin, wings with brown veins; females – body covered with adpressed yellow-reddish hairs, legs paler than in *C. melanopa*, tibiae pale in basal third and on apex. Specimens of *C. melanopa* are characterized by the following features: males – body hairs black, except the combination of black and pale hairs on tergites, differently arranged, and pale hairs laterally and adpressed black hairs in the middle of tergites, tibiae brownish basally; females – face with pale hairs, mesoscutum with pale and black hairs (longer are black), abdomen with pale hairs laterally and adpressed black hairs in the middle of tergites; tibiae brownish basally (Vujić, 1996). Contrary to differences of body hairs, differential features in male genitalia structure, which was proved to be the main taxonomic character for recognizing the species of the genus *Cheilosia* (Vujić, 1996), appeared to be identical in both subspecies (Table 2; Fig. 3).

*Cheilosia m. redi* is distributed in the Pannonian plain, lowlands and hills of the central Balkan Peninsula. *Cheilosia m. melanopa* occurs on high mountains in Northern and Central Europe. A study conducted during 1998 discovered sympatric and sinchronic occurrence of both, *C. m. melanopa* and *C. m. redi* 'subspecies', only on the Durmitor Mt in Montenegro (locality Kanjon Sušice). Results of wing geometric morphometrics obtained in this paper (see below) strongly support an independent taxonomic position at the species level of *C. melanopa* and *C. redi*. Based on the

observed morphometric data, previously recognized morphological differences (Table 2) and co-existence of both taxa, we propose formal elevation of *C. redi* to the species level.

Diagnostic characters

The analysis of variance (ANOVA) of wing centroid size (CS) revealed significant differences between the

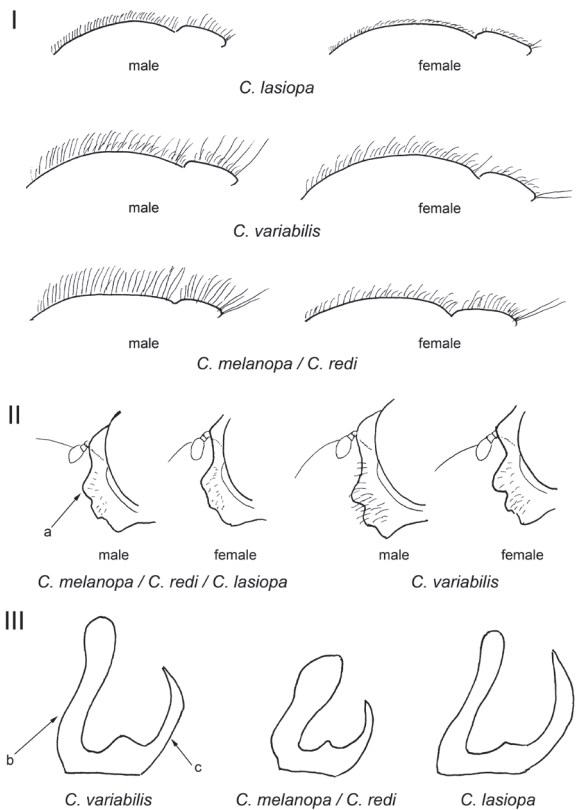


Fig 3. Diagnostic morphological traits of the *C. variabilis* group. I: hairs on mesonotum, lateral view; II: face, lateral view: a – facial tubercle; III: male genitalia – gonostylus: b – ventral lobe of gonostylus, c – dorsal lobe of gonostylus.

Table 2. Diagnostic morphological traits of the *C. variabilis* group (see Fig. 3)

Species	Body hairs			Facial tubercle	Male genitalia – gonostylus
	Length	Color on mesonotum	Color on tergites (males)		
<i>C. melanopa</i>	long	predominantly black	mixed pale and black	rounded	dorsal lobe two times shorter than ventral
<i>C. redi</i>	long	reddish	pale	rounded	dorsal lobe two times shorter than ventral
<i>C. lasiopa</i>	short	pale with black on central part	pale	rounded	dorsal lobe as long as ventral
<i>C. variabilis</i>	long	mixed pale and black	mixed pale and black	snout-like	dorsal lobe 2/3 length of ventral

*C. melanopa* and *C. redi* species. *Cheilosia redi* had bigger wings than *C. melanopa*, both in males ( $F_{1,39} = 32.07$ ,  $P < 0.001$ ) and females ( $F_{1,39} = 51.33$ ,  $P < 0.001$ ) (Fig. 4, Table 3).

*Cheilosia melanopa* and *C. redi* were successfully discriminated using CVA performed on the wing shape variables ( $w$  matrix). In males, the first canonical axis extracted by CVA was highly significant and contributed to shape differentiation between analysed species (Wilks'  $\Lambda = 0.001$ ,  $F_{84,27} = 2.55$ ,  $P < 0.01$ ) (Fig. 5a). Significant wing shape differences were found also among female specimens (Wilks'  $\Lambda = 0.0001$ ,  $F_{112,38} = 2.22$ ,  $P < 0.01$ ) (Fig. 5b).

The first canonical axis accounting 77.8% of total shape variation clearly separated *C. melanopa* from *C. redi* with 100% classification success. The thin plate spline visualization showed that male shape differences were associated with displacement of landmarks 5, 6, 7 and 8 (Fig. 5c), while for female specimens, landmarks 1, 2, 6, 7 and 8 have the highest contributions for *C. melanopa*/*C. redi* wing shape differences (Fig. 5d).

#### Identification of species

The ANOVA on CS of the wings showed a highly significant result among species of the *C. variabilis* group when sexes were considered separately (male:  $F_{3,367} = 131.41$ ,  $P < 0.001$ ; female:  $F_{3,100} = 72.47$ ;  $P < 0.001$ ; Tukey HSD test:  $P < 0.001$ , except for males of the *C. lasiopa*/*C. melanopa* species pair) (Fig. 4, Table 3).

CVA with species as grouping variable performed on the  $w$  matrix gave satisfactory results for overall species comparison. In males, the three axes extracted by CVA were highly significant and contribute to shape differentiation among species (Wilks'  $\Lambda = 0.059$ ,  $F_{84,10} = 19.04$ ,  $P < 0.001$ ) (Fig. 6a). The percentage of individuals correctly classified was in total 99.7%; except for the *C. melanopa* species (94.7%) analysed species were classified with rate of 100%. Similarly, CVA showed a clear interspecific discrimination of females within the *C. variabilis* group (Wilks'  $\Lambda = 0.003$ ,  $F_{84,22} = 15.87$ ,  $P < 0.001$ ) (Fig. 6b).

There was a substantial divergence in wing shape to classify correctly 100% of all female individuals.

#### Recognition of phenotypic units

The character CS varied significantly between two *C. melanopa* populations; wings of Durmitor Mt specimens were considerably larger than *C. melanopa* wings from Šar Mt, both in males ( $F_{1,17} = 13.04$ ,  $P < 0.01$ ) and females ( $F_{1,12} = 93.69$ ,  $P < 0.001$ ). To assess the wing shape variation between studied populations, we used CVA conducted on the  $w$  matrix. Although CVA evidenced no significant population differences (Wilks'  $\Lambda = 0.00003$ ,  $F_{84,6} = 2.59$ ,  $P = 0.09$ ), percentages of correct classification were 100% (Fig. 7a). The first two canonical axes were significant and explained 87.6% and 10.2% of the total shape variation. The second axis (CV2) contributed to shape differentiation between two populations. Deformation grids which represents deformations along the CV2 demonstrated that population differences primarily associated with displacement of landmarks 4, 7, 8 and 12 (Figs 7b, 7c).

The ANOVA showed no wing size differences between conspecific populations of *C. redi* (DUB, FG,

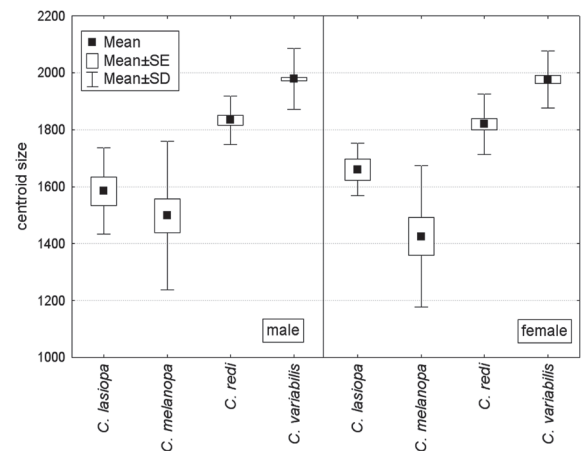


Fig. 4. Boxplot of centroid size of species of the *C. variabilis* group with the mean, standard error and standard deviation illustrating interspecific variation in wing size.

Table 3. Wing size differences (+ significant; - non-significant) (above diagonal) and squared Mahalanobis distance ( $D_{Mah}$ ) estimated by wing shape parameters (below diagonal) (female/male) among species pairs of the *C. variabilis* group.

Species	<i>C. melanopa</i>	<i>C. redi</i>	<i>C. lasiopa</i>	<i>C. variabilis</i>
<i>C. melanopa</i>		+/+	+/-	+/+
<i>C. redi</i>	40.62/29.91		+/+	+/+
<i>C. lasiopa</i>	86.11/35.59	140.56/55.56		+/+
<i>C. variabilis</i>	53.65/42.05	74.42/45.93	97.49/67.13	

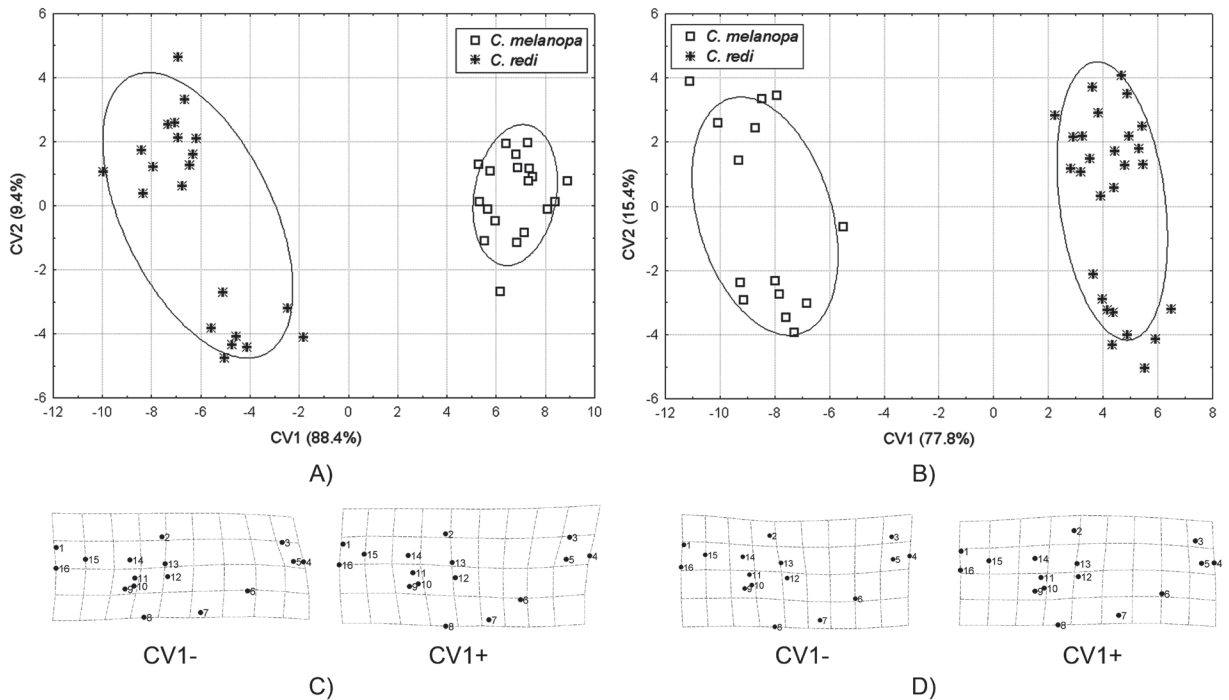


Fig. 5. Scatterplot of individual scores from the CVA showing shape differentiation between A) male and B) female specimens of the *C. melanopa* and *C. redi* species. The amount of variation explained by each axis is in parentheses. Shape changes are shown as deformations using thin plate spline representing negative and positive deformations of mean shape between C) male and D) female specimens along the CV1 axis. Deformation grids are exaggerated by a factor three. Numbers in the deformation grids refer to landmarks shown in figure 2.

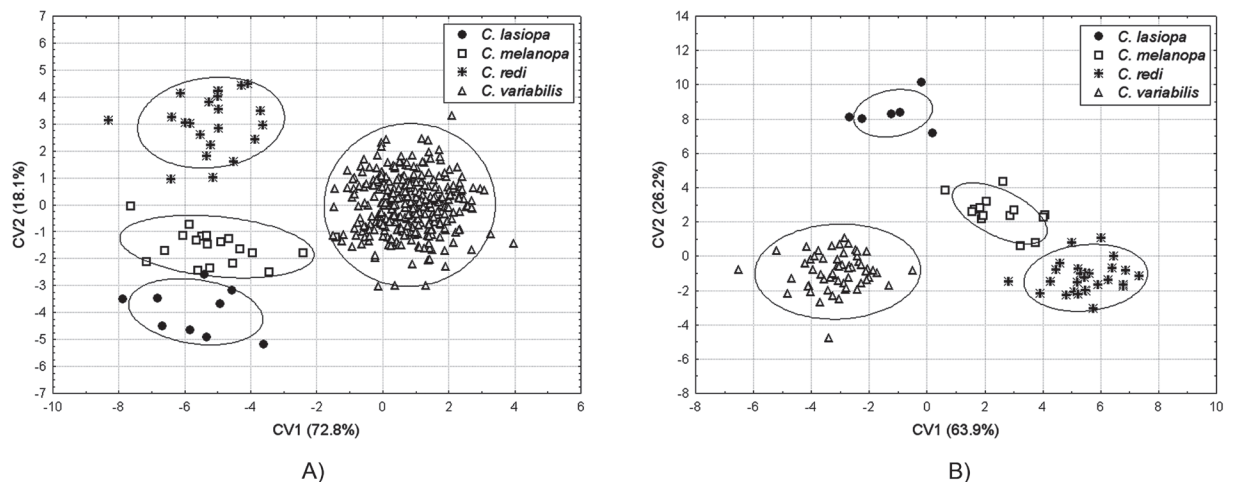


Fig. 6. Scatterplot of individual scores from CVA comparing A) male and B) female specimens of the *C. variabilis* group for wing shape. The amount of variation explained by each axis is in parentheses.

DUR) by comparing male ( $F_{2,19} = 0.54$ ,  $P = 0.59$ ) and female specimens ( $F_{2,24} = 0.12$ ,  $P = 0.88$ ) separately. Contrary to size, CVA with populations and sexes as grouping variable revealed significant shape differences (Wilks'  $\Lambda = 0.0004$ ,  $F_{112,66} = 3.64$ ,  $P < 0.001$ ).

Females from three analysed populations clearly differed in their wing shape, while male specimens were partially overlapped (Fig. 8).

Contrary to females ( $F_{8,48} = 2.11$ ,  $P = 0.53$ ), significant wing size differences between male specimens of



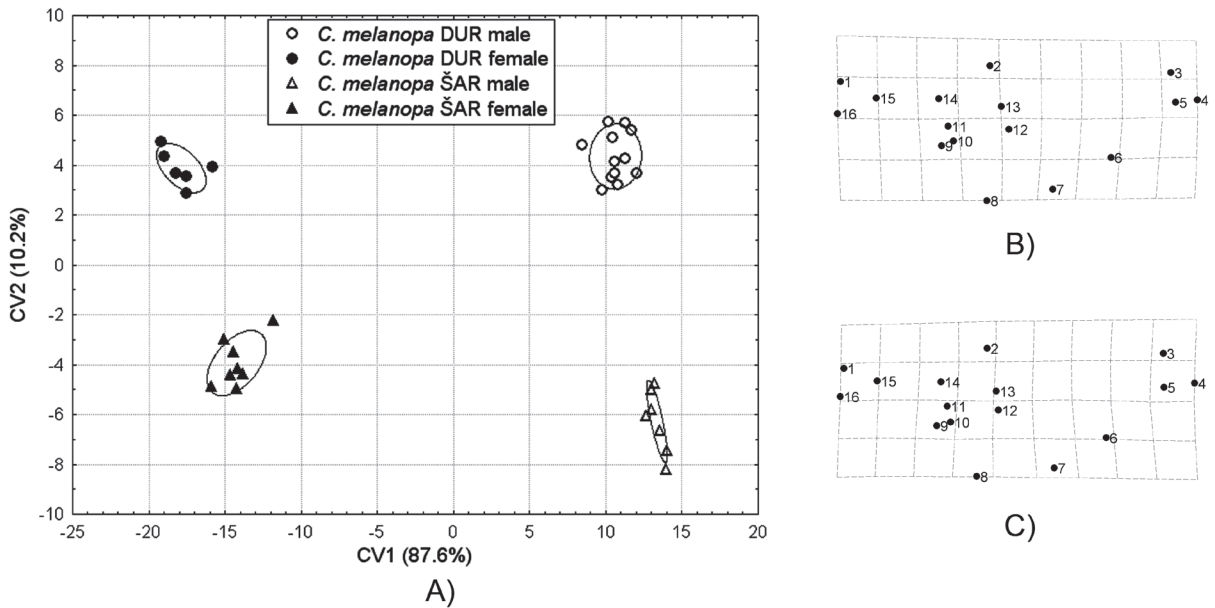


Fig. 7. Scatterplot of individual scores from CVA comparing male and female specimens of the *C. melanopa* conspecific populations for wing shape (DUR, Durmitor Mt; ŠAR, Šar Mt). Shape changes are shown as deformations using thin plate splines (deformation grids are exaggerated  $\times 3$ ). Numbers in the deformation grids refer to landmarks shown in figure 2. Grids B) and C) represents positive and negative deformations, respectively, over the CV2. The amount of variation explained by each axis is in parentheses.

the *C. variabilis* populations were found ( $F_{10,31} = 12.51$ ,  $P < 0.001$ ). Males from Kopaonik and Suva Mts had considerably larger wings than males from Eastern Alps, Dubašnica, Durmitor and Fruška Gora Mts ( $P < 0.05$ , Tukey HSD test). The ANOVA also exhibit significant size variation ( $P < 0.05$ , Tukey HSD

test) between the populations of the Fruška Gora Mt/ Stara Mt and Vršacke Mt/Suva Mt pairs (not shown). CVA revealed population differences within both sexes. In males, six of ten axes extracted by CVA were highly significant, contributed to shape differentiation among populations (Wilks'  $\Lambda = 0.141$ ,  $F_{280,27} = 2.22$ ,  $P < 0.001$ ) and led to correct classification rates between 20% and 100% (not shown). When female specimens were compared (Wilks'  $\Lambda = 0.0001$ ,  $F_{224,19} = 2.03$ ,  $P < 0.001$ ), significant wing shape differences allowed 98.3% of individuals to classify correctly with 100% classification success for individuals of all analysed populations except those from Fruška Gora Mt (83.3%) (Fig. 9).

#### Phenotypic relationships

The UPGMA cluster analyses of the squared Mahalanobis distances computed from  $w$  matrix of female shape parameters (Table 3) clustered *C. melanopa* and *C. redi* in the same branch, while *C. lasiopa* was the most divergent species (Fig. 10). The UPGMA dendrogram obtained from male shape parameters provided the same phenetic relationships among analysed species (not shown).

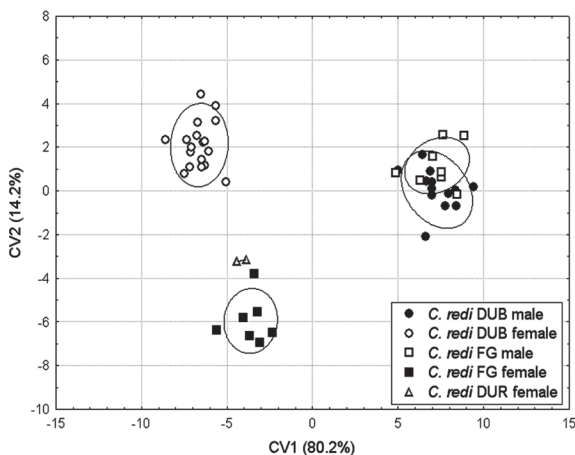


Fig. 8. The relationships among populations of the *C. redi* species with respect to the first two canonical variables (DUB, Dubašnica Mt; FG, Fruška Gora Mt; DUR, Durmitor Mt).



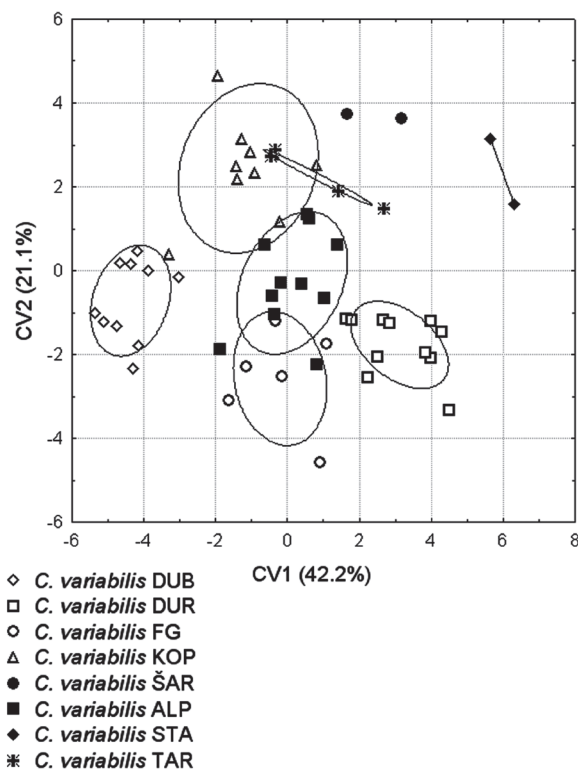


Fig. 9. Scatterplot of individual scores from CVA comparing female specimens of the *C. variabilis* conspecific populations for wing shape (DUB, Dubašnica Mt; DUR, Durmitor Mt; FG, Fruška Gora Mt; KOP, Kopaonik Mt; ŠAR, Šar Mt; ALP, Eastern Alps; STA, Stara Mt; TAR, Tara Mt).

## Discussion

### Species delimitation

This study is the first to quantify subtle wing geometric morphometric traits among populations of two previously nominated subspecies, *C. melanopa melanopa* and *C. m. redi* from the Balkans (Vujić, 1996). To date, subspecies discrimination was solely based on morphological characters such as body hairs (black and reddish in *C. melanopa melanopa* and *C. melanopa redi*, respectively) and wing veins (dark and brown in *C. m. melanopa* and *C. m. redi*, respectively) (Vujić, 1996). Although the morphology of male genitalia has been widely used in identification of hoverfly species, the diagnostic value of it was not recognized to be important in delimitation of the previously nominated subspecies. Similarly, lack of taxonomic value of male genitalia has been observed for cryptic taxa within the morphologically defined spe-

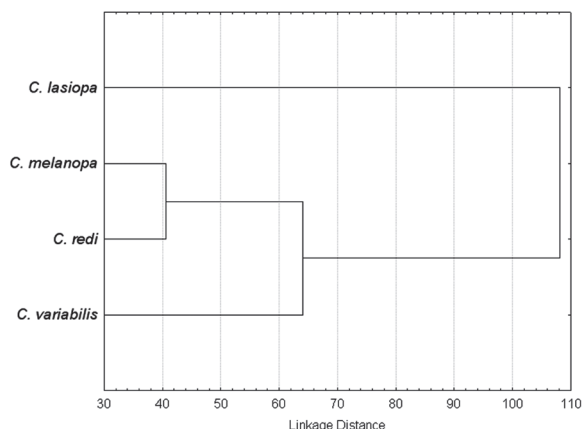


Fig. 10. UPGMA phenogram based on the squared Mahalanobis distances of females of the *C. variabilis* group.

cies *Cheilosia vernalis* (Fallen, 1817) (Ståhls *et al.*, 2008) and closely related species pair *Cheilosia nigripes* (Meigen, 1822) and *C. vicina* (Zetterstedt, 1849) (Barkalov and Ståhls, 1997). However, based on wing shape and size presented herein the status of the focal taxa was clarified as evolutionarily independent biological entities, the *C. melanopa* and *C. redi* species. Wing centroid size (*C. melanopa* – smaller, and *C. redi* – larger wings) and wing shape were found to be significantly different between the analysed species (Table 3). Moreover, observed divergent wing shape phenotypes in sympatric populations from Durmitor Mt clearly demonstrated *C. melanopa* and *C. redi* as evolutionarily independent entities. Wing shape differences were associated with displacement of outer landmarks in females (landmarks 5, 6, 7, 8) and landmarks that located at proximal and posterior parts of wing in males (1, 2, 6, 7, 8), all which influence wing's width and length. The consistency of this pattern of interspecies variation is also evident in other *Cheilosia* species (J. Ludoški, unpublished data). Therefore, associated with previous findings, the current study has located the wing's region of taxonomic importance. The occurrence of similar patterns of wing variation in different species of the genus *Cheilosia* might reflect conservative genetic constraints, but only further analysis will offer a precise explanation.

We quantified morphological differences of the species within the *C. variabilis* group using wing geometric morphometrics as well. Considerable interspecific differences in wing size were estimated; *C. melanopa* had the smallest wings, followed by *C. lasiopa*, *C. redi* and *C. variabilis*. Documented wing

shape variation also revealed clear phenotypic differentiation among the species of the *C. variabilis* group. Such subtle wing morphometric distinctiveness among closely related species was also found in other species groups within the family Syrphidae (Ludoški et al., 2008; Francuski et al., 2009; Milankov et al., 2009), and demonstrated wing traits as a powerful taxonomic tool.

#### *Intraspecies phenotypic diversity*

Our findings revealed high phenotypic divergence among conspecific populations within the *C. melanopa*, *C. redi* and *C. variabilis* species. Contrary to wing size, wing shape was found to be significantly different between females of *C. redi*. The extent of wing variation between populations of *C. melanopa* originating from Durmitor and Šar Mts included discrete variation of wing shape primarily associated with displacement of landmarks 4, 7, 8 and 12. Deformation grids showed that most morphological variation occurred in the distal-posterior part of wing, a region that has been supposed to be important in aerodynamic performance and courtship song in dipteran species (Birdsall et al., 2000). We also found significant interpopulation wing size variation between males of *C. variabilis*. In comparison, the wing shape of females of *C. variabilis* revealed phenotypic interpopulation differentiation. Hence, we considered that allopatrically distributed populations of the *C. melanopa*, *C. redi* and *C. variabilis* species have likely maintained a separate and distinct evolutionary history that is probably the result of isolation in refugia such as Balkan mountains (Dubašnica, Fruška Gora, Durmitor and Šar Mts). Likewise, intraspecific morphological variability and distinctive phenotypic units were also observed in some species of the *Cheilosia* (J. Ludoški, unpublished data) and *Merodon* genera (Francuski et al., 2009; Milankov et al., 2009). Among hoverfly taxa, especially those originating from the Balkans, the presence of divergent units and cryptic diversity is likely to be influenced by an assortment of environmental factors and biogeographical processes (Francuski et al., 2009; Milankov et al., 2009).

In summary, in order to derive a conclusion about the status of divergent phenotypic units it would be necessary to include ecological traits and molecular markers, and more sampling. Bearing in mind that recognition and delimitation of distinctive phenotypic and genetic units are essential in detecting species and genetic diversity, our findings need to be taken into ac-

count in taxonomic revisions and ongoing conservation plans. Indeed, determination of species borders as well as intraspecies evolutionarily significant units (ESU, according to Ryder, 1986) is proposed to be crucial for identifying areas of endemism, biodiversity hotspots, and consequently, areas of conservation priority (Myers et al., 2000; Moritz, 2002). Finally, considering that phenotypic diversity presented herein shed light on morphological evolution, we would like to address the use of subtle morphometric traits in the study of intraspecies diversity of other insect species as well.

#### *Phenetic relationships*

Analysis of wing geometric morphometric traits in populations from the Balkans has provided phenetic relationships among members of the *C. variabilis* group. The UPGMA cluster analysis presented a close relationship between the *C. melanopa*/*C. redi* species pair indicating that these are recently diverged taxa, while *C. lasiopa* was the most distant species. The wing differences may indicate phylogenetic patterns but this should be proven by further detailed phylogenetic classification of the *C. variabilis* group species and evaluation of the phylogenetic significance of wing shape. Results from this study are of interest in their own right, and would, also, be highly useful in understanding the biogeographic history of the region. Finally, observed phenotypic diversity along with ongoing molecular analysis would provide broader insights into the phylogeny of the genus *Cheilosia*. This is of special interest since phylogenetic relationships among members of the genus *Cheilosia* was inferred using only two members of the *C. variabilis* group. In that paper, Ståhls et al. (2004) using cytochrome *c* oxidase subunit I mitochondrial DNA (COI mtDNA) and nuclear 28S rRNA gene, placed *C. variabilis* and *C. melanopa redi* as closely related taxa within the *Cheilosia* s. str. subgenus.

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