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MORPHOLOGICAL VARIABILITY IN WING SIZE AND WING SHAPE AMONG SPECIES *Aphidius absinthi* Marshall, *A. rosae* Haliday AND *A. urticae* Haliday (HYMENOPTERA: BRACONIDAE: APHIDIINAE)

Ana S. Mitrovski-Bogdanović* and Petar M. Radojičić

University of Kragujevac, Faculty of Science, Institute of Biology and Ecology, Radoja Domanovića 12, 34000 Kragujevac, Republic of Serbia
*Corresponding author; E-mail: amitrovski@kg.ac.rs

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ABSTRACT. In this paper, we applied traditional and geometric morphometric methods to analyze variability in wing size and wing shape among species *Aphidius absinthi* Marshall, *A. rosae* Haliday and *A. urticae* Haliday. These taxa represent closely related species with different biological and ecological characters. For the morphometric analyses, we used a sample of 52 female specimens that were collected during the period 2009-2013, on different localities in Serbia. Traditional morphometric analyses revealed statistical significance in stigma shape discrimination of analysed taxa. Our geometric morphometric analyses also confirmed that major contribution to the wing shape variation had the changes in length of the radial sector and stigma shape. Combining the traditional and geometric morphometric analyses, we confirmed the validity of the wing characters previously used in taxonomic studies of the genus *Aphidius*.

Key words: *Aphidius absinthi*, *A. rosae*, *A. urticae*, geometric morphometry.

INTRODUCTION

The genus *Aphidius* Nees, with about 100 described species worldwide (TOMANOVIĆ *et al.*, 2007) and about 35 detected species in Europe (STARÝ, 1970), is one of the the largest within the subfamily Aphidiinae (Hymenoptera: Braconidae). All species are solitary endoparasitoids of aphids. Many *Aphidius* species have a great potential as biocontrol agents in biological control programs (HAGVAR and HOFSVANG, 1991), so a success of these programs depends on their correct identification. Because of that, there are many papers relating to the taxonomy of *Aphidius* species (EADY, 1969; STARÝ, 1973; PUNGERL, 1983; PENNACCHIO, 1989; MESCHELOFF and ROSEN, 1990; TAKADA, 1998; TOMANOVIĆ and STARÝ, 2001; KAVALLIERATOS *et al.*, 2001, 2006; TOMANOVIĆ *et al*., 2003, 2004, 2007, 2013; KOS *et al*., 2011; JAMHOUR *et al*., 2016). However, due to a great variability of morphological characters, many taxonomic problems were encountered in the genus *Aphidius*. One of them is taxonomical position of *Aphidius absinthi* Marshall, *A. rosae* Haliday and *A. urticae* Haliday, which represent closely related species with different biological and ecological characters. *A. absinthi* is parasitoid of *Macrosiphoniella* Del Guecio species, *A. rosae* represents a highly specialized species restricted to *Macrosiphum rosae* Linnaeus, while *A. urticae* has wide host range and parasitizes on *Acyrthosiphon* Mordvilko, *Amphorophora* Buckton, *Macrosiphum* Passerini and *Microlophium* Mordvilko species. According to the last

revision, these taxa mostly differ from each other by the number of antennal segments, length of metacarpal vein, number of costulae on anterolateral area of petiole and host range (STARÝ, 1973).

The purpose of this study was to analyse morphological differentiation in the forewing size and shape among species *A. absinthi*, *A. rosae* and *A. urticae* by traditional morphometry and geometric morphometric analyses and to test the validity of morphological characteristics, such as wing venation, previously used for their identification (STARÝ, 1973; PENNACCHIO, 1989; TOMANOVIĆ *et al*., 2003, 2007).

MATERIAL AND METHODS

Traditional morphometry

For the morphometric analyses, we used a sample of 52 female specimens that were collected during the period 2009-2013, on different localities in Serbia (Table 1). Plant samples bearing both live and mummified aphid hosts, were collected for parasitoids rearing. Samples of live aphids were preserved in 90% ethanol and 75% lactic acid at a ratio of 2:1 (EASTOP and VAN EMDEN, 1972) for later identification. The remaining aphids were maintained in the laboratory until parasitoid emergence. Mummies, each attached to a small leaf piece, were placed separately in small plastic boxes and put inside a growth cabinet. On the lid of each box there was a circular opening covered with muslin for ventilation in order to maintain the conditions inside the boxes similar to those in the growth cabinet (22.5^{o}C, relative humidity 65%, 16L:8D) (KAVALLIERATOS *et al*., 2001). All analyzed specimens were boiled in 10% KOH, dissected, and mounted in Canada balsam (STARÝ, 1970). The external structure of emerged parasitoids was studied using a ZEISS Discovery V8 stereomicroscope.

Three continuous characters were used for the morphological characterization of the analyzed specimens, as follows: stigma length (STL), stigma width (STW) and the length of R1 vein = metacarpal (R1L) (Fig. 1). All the characters were presented in terms of a ratio in order to eliminate effect of size, also allowing direct comparison of the obtained results with other analyses (Table 2). Morphological terminology for wing diagnostic characters used in this study is based on SHARKEY and WHARTON (1997).

Table 1. List of the *Aphidius* species used in analyses.

Parasitoid	Host aphid	Host plant	Country	No. of specimens
Aphidius absinthi	*Macrosiphoniella* sp.	*Centaurea rhenana*	Serbia	16
Aphidius rosae	*Macrosiphum rosae*	*Rosa* sp.	Serbia	19
Aphidius urticae	*Macrosiphum euphorbiae*	*Euphorbia esula*	Serbia	17

Analysis of variance (ANOVA) was used to test the statistical significance of differences in variation of STL/STW and STL/R1L, while Tukey's test was done for their pairwise comparison. Canonical Variate Analysis (CVA) was performed to determine which of analysed ratio characters would contribute significantly to species discrimination. Percentage of the correct identification was calculated by Discriminant Function Analysis

(DFA). All standard statistical analyses were performed in Statistica 6 software package (STATSOFT, 2001).

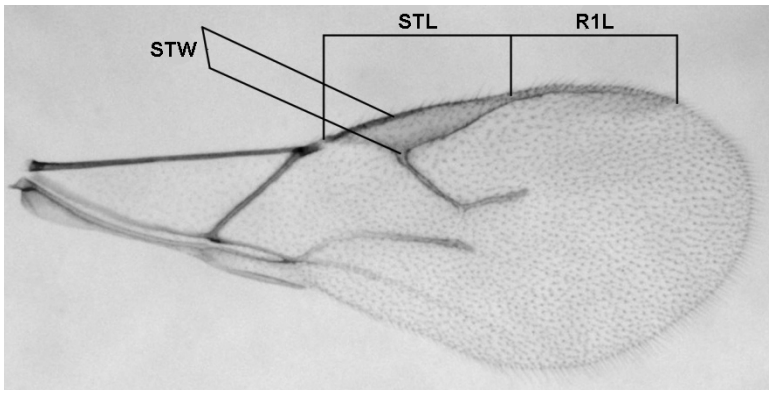

Fig. 1. Analysed characters on a left forewing of *Aphidius absinthi* female (STL – stigma length, STW – stigma width, R1L – length of metacarpal (R1) vein).

Table 2. List of characters for morphometric analyses.

Character code	Type	Description
STL/STW	Ratio	Stigma length/stigma width
STL/R1L	Ratio	Stigma length/length of R1 vein

Geometric morphometrics

The geometric morphometrics approach was applied to explore and quantify variations in wing size and wing shape of 52 female specimens (ZELDITCH *et al*., 2012). The same sample was used for both, traditional morphometry and geometric morphometric methods (Table 1). Left forewing of each specimen was detached, mounted in Canada balsam and photographed using a Leica System Microscope DM2500 with a Leica DFC490 Digital Camera. We selected 13 specific landmarks to describe the wing size and shape. The positions of the landmarks are given in Fig. 2, while their definitions are presented in Table 3. All landmarks were digitized using TpsDig software (ROHLF, 2005). Landmarks were superimposed by the Generalized Procrustes Analysis (ROHLF and SLICE, 1990; BOOKSTEIN, 1991). Procrustes coordinates were used as shape variables in following statistical analyses.

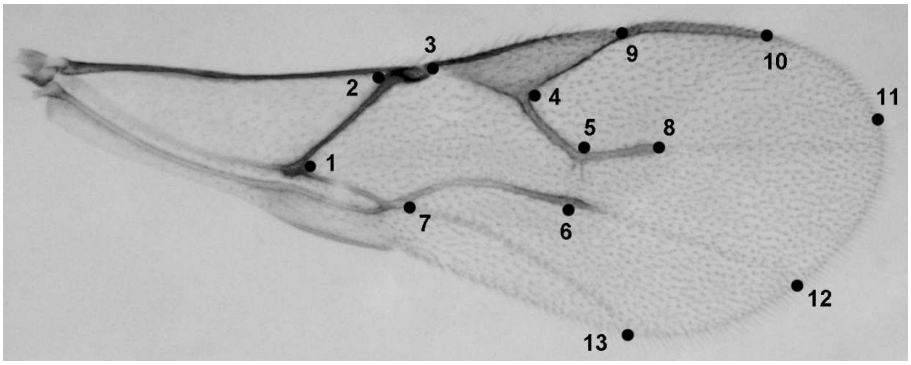

Fig. 2. Landmarks scored on a left forewing of *Aphidius absinthi* female.

To estimate wing size, we computed the centroid size (CS), a geometric measure of the size which reflects the amount of dispersion around the centroid of the landmark configuration (BOOKSTEIN, 1991). The variation in the wing size (CS) among different species

from the genus *Aphidius* was analyzed by one-way ANOVA. Post-hoc pairwise comparison for wing size was done by Tukey's test. Multivariate analysis of variance (MANOVA) on the full set of the shape variables was performed to analyze a differences in the wing shape of parasitoids belonging to different species (ZELDITCH *et al.*, 2012). All statistical analyses were performed with the Statistica 6 software package (STATSOFT, 2001).

Table 3. Landmarks descriptions

Landmarks	Description
1, 2, 7	proximal part of the forewing
3, 4, 9	length and width of the stigma
4, 5, 8	radial sector
5, 6	length of r-m vein
9, 10	length of R1 vein (= metacarpal)
11, 12, 13	distal part of the forewing (projections of the radial sector, medial and cubital vein to the edge of the wing)

Canonical variate analysis (CVA), which reduces within group variances and increases between group divergences, was performed to explore a divergence of the wing shape among the three species using software MorphoJ (KLINGENBERG, 2011). Discriminant function analysis (DFA) was used to evaluate the accuracy of classsification by original and cross-validation percentages of the cases (MANLY, 1997).

RESULTS AND DISCUSSION

Traditional morphometry

The Analysis of variance showed statistically significant differences in stigma shape described by STL/STW (ANOVA: F=4.302, MS=0.4606, df=2, P=0.01), in contrast to the other ratio character STL/R1l (ANOVA: F=0.1015, MS=0.01050, df=2, P=0.90). The results of Tukey's test indicated on statisticaly significant differences in STL/STW character only between species *A. absinthi* and *A. urticae* (p=0.0142).

Also, the results of Canonical Variate Analysis confirmed that ratio character STL/STW has higher contribution to the species discrimination (Table 4). The results of Discriminant function analysis (DFA) based on analysed ratio characters, indicate about 50% correct assignment of specimens to the *a priori* designated species. The following percentages for the correct classification of individuals per species were found: *A. absinthi* 50.00% , *A. rosae* 47.37% and *A. urticae* 52.94%.

Table 4. Standardized canonical discriminant function coefficients for *Aphidius* species

Character code	CV1	CV2
STL/STW	**1.183856**	-0.059801
STL/R1L	-0.686106	-0.966617
Eigenval	0.262315	0.003484
Cum.Prop	0.986892	1.000000

Geometric morphometrics

A significant variation in the wing centroid size was found among the *Aphidius* species (ANOVA: $F = 9.146$, df = 2, $P < 0.0001$). We found that *A. absinthi* females have larger wings (mean wing CS = 1379.38 ± 44.65) than *A. rosae* females (mean wing CS = 1292.31 ± 182.48) and *A. urticae* females with the smallest wing size (mean wing CS = 1171.65 ± 144.96). Tukey's test indicated statisticaly significant differentiation in wing size between *A. absinthi* and *A. urticae* (p=0.0003), as well as between *A. rosae* and *A. urticae* (p=0.0345). A significant difference in the wing shape (MANOVA: Wilks' Lambda = 0.017149, $F = 8.4$, df1 = 44, df2 = 56, $P < 0.0001$) among species was also found.

Correct classification of individuals per species based on wing shape was provided as the following percentages (the first and the second values in brackets represent the original and cross-validation, respectively): *A. absinthi* 100% (62.5%), *A. rosae* 100% (100%) and *A. urticae* 100% (65%).

Canonical variate analysis (CVA) revealed that the first canonical axis explained 90.60% of the total variability in wing shape. *A. absinthi* and *A. urticae* were clustered together and clearly discriminated from *A. rosae* by the position of radial sector and r-m vein (Fig. 3). Specimens of *A. rosae* have elongated radial sector (described by landmarks 4, 5 and 8) and shorter r-m vein (described by landmarks 5 and 6). However, *A. absinthi* and *A. urticae* specimens are separated along the second canonical axis, which explained 9.40% of the total variability in wing shape. The main shape changes that discriminate these species are related to the stigma shape (described by landmarks 3, 4 and 9) and R1 vein (described by landmarks 9 and 10). In contrast to *A. urticae*, the specimens of *A. absinthi* have wider stigma, shorter R1 vein and wider distal part of the wing (described by landmarks 11, 12 and 13).

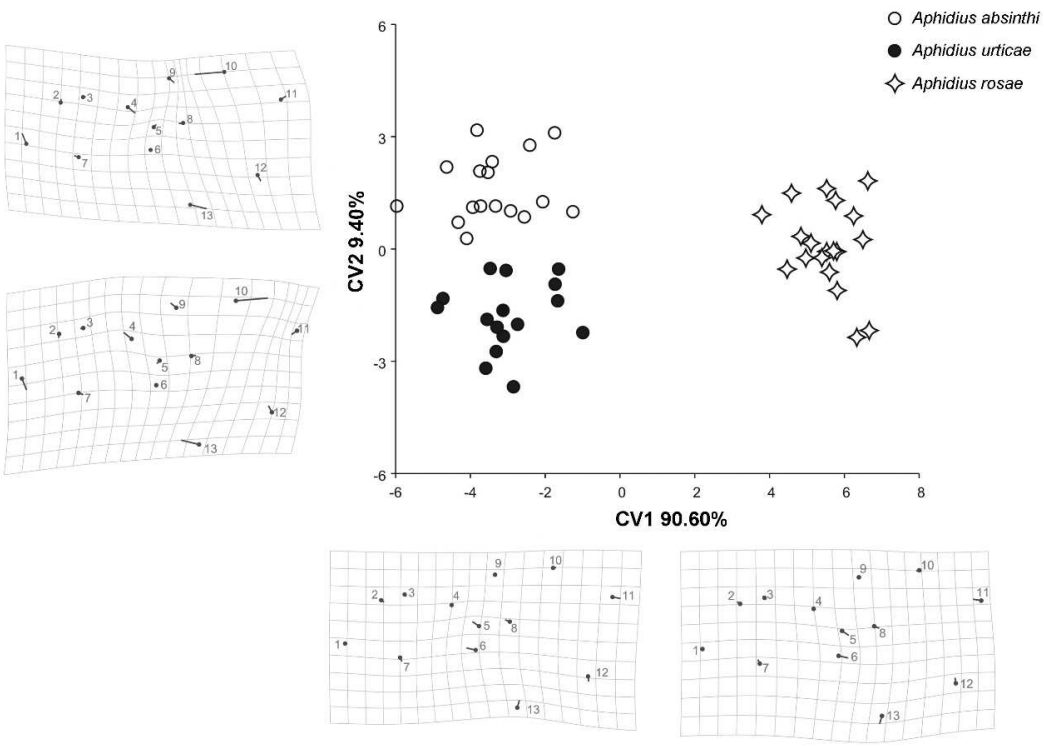

Fig. 3. Ordination of the *Aphidius* specimens in the morpho-space. The thin-plate spline deformation grids illustrate the wing shape changes correlated with the first and the second canonical axis.

Previously, taxonomic studies within a genus *Aphidius* were based mainly on a few morphological characters such as: wing venation, sculpturing on the anterolateral area of

petiole, number of antennal segments, shape and chaetotaxy of the female genitalia, tentorial index, number of maxillary and labial palpomeres (SMITH, 1944; EADY, 1969; STARÝ, 1973; TOMANOVIĆ *et al*., 2003, 2007).

Based on traditional and geometric morphometric analyses our results confirmed that all three analyzed taxa, *A. absinthi, A. rosae and A. urticae* are true species. According to the results of traditional morphometry, we found that the stigma shape has statistically significant influence on the species discrimination. Geometric morphometric analysis also confirmed that the major variation in the wing shape consisted of changes in the length of the radial sector and r-m vein, as well as in the stigma shape itself. The shape of the stigma, the length of R1 vein and the ratio between the length of stigma and the length of the R1 vein were comonly used as valid characters for the morphological characterization and separation within the genus *Aphidius* (STARÝ, 1973; PENNACCHIO, 1989; KAVALLIERATOS *et al*., 2001; RAKHSHANI *et al.*, 2008; PETROVIĆ *et al*., 2010; KOS *et al*., 2011; TOMANOVIĆ *et al*., 2003, 2013, 2014).

In contrast to the last revision of the genus *Aphidius*, where *A. absinthi* and *A. rosae* belong to the *A.rosae* group (STARÝ, 1973), we found that *A. absinthi* has clustered together with *A. urticae* and clearly discriminate from *A. rosae*, which specimens have elongated radial sector and shorter r-m vein. However, we also found clear differences between *A. absinthi* and *A. urticae* in stigma shape, length of R1 vein, as well as in wing size.

CONCLUSIONS

We found clear differences among species *A. absinthi, A. rosae and A. urticae* in the wing size, as well as in the wing shape by the position of radial sector and r-m vein, stigma shape and the ratio between the length of stigma and the length of the R1 vein.

Combining the traditional and geometric morphometric analyses, we confirmed the reliability of previosly used wing characters for *Aphidius* identification, also indicating that r-m vein could be used as a new character in identification keys.

This paper represents a contribution to the resolving of some taxonomical problems within the genus *Aphidius*, but the clarification of the status of many other aggregations or cryptic species requires further morphological and molecular researches.

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