

Original Article

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## Morphological discrimination of the genera *Binodoxys* Mackauer and *Trioxys* Haliday (Hymenoptera: Braconidae: Aphidiinae) based on the general shape of forewings

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### Abstract:

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The genera *Binodoxys* and *Trioxys*, members of the Aphidiinae subfamily, are classified in the same subtribe Trioxina within the tribe Trioxini. These two genera are morphologically very similar, but differ in a large degree in their ecology. Generally, *Binodoxys* species are specialized in parasitizing aphids on herbaceous plants, unlike *Trioxys* species, which have co-evolved with the tree-attacking aphids. For this study, we analyzed the right forewings of four *Binodoxys* and two *Trioxys* species, using the method of geometric morphometrics. Sixteen specific landmarks were digitalized for each forewing of 80 parasitoid individuals. The results of two statistical methods ANOVA and MANOVA demonstrated statistically significant differences of the wing shapes, especially between the genera. Visualizing the results of the dispersion of specimens in the morphospace via multivariate tests PCA and CVA, we found that the most variable character in wing structure was the radial sector, differentiating *Binodoxys* from *Trioxys*.

**Key words:** Trioxina, geometric morphometrics, variability, parasitoids

### Apstrakt:

Lazarević, M., Ilić Milošević, M., Stanković, S.S., Žikić, V.: *Morfološko razdvajanje rodova Binodoxys Mackauer i Trioxys Haliday (Hymenoptera: Braconidae: Aphidiinae) na osnovu oblika krila. Biologica Nyssana, 8 (1), Septembar 2017: 105-111.*

Rodovi *Binodoxys* i *Trioxys*, članovi podfamilije Aphidiinae, su klasifikovani u isti podtribus Trioxina u okviru tribusa Trioxini. Pomenuta dva roda su morfološki veoma slična, ali se ekološki dosta razlikuju. Generalno posmatrajući, vrste roda *Binodoxys* su se specijalizovale za parazitiranje biljnih vaši koje se hrane zeljastim biljkama, za razliku od vrsta roda *Trioxys*, koje su koevoluirale sa biljnim vašima koje žive na drvenastim biljkama. Za ovu studiju, analizirana su desna krila četiri vrste roda *Binodoxys* i dve *Trioxys* vrste, koristeći metod geometrijske morfometrije. Za svako krilo, od ukupno 80 analiziranih jedinki parazitoidea, izabrano je šesnaest tačaka. Rezultati dve statističke metode, ANOVA i MANOVA, pokazali su postojanje statistički značajnih razlika u obliku krila, naročito između rodova. Vizuelizacijom rezultata multivarijantnim testovima,

PCA i CVA, uočeno je da je najvarijabilnija struktura radijalni sektor, na osnovu koga se rodovi *Binodoxys* i *Trioxys* razdvajaju.

**Ključne reči:** Trioxina, geometrijska morfometrija, varijabilnost, parazitoidi

## Introduction

The Aphidiinae subfamily can be distinguished from other members of the Braconidae family for being the exclusive parasitoids of aphids (Hemiptera: Aphididae). They are solitary koinobiont endoparasitoids. Uniquely parasitizing aphids was one of the reasons why Starý (1981) recognized this group as a separate entity, raising it to the family level – Aphidiidae, which can be found in older literature. Nowadays, this group of parasitoids is usually considered as one of the subfamilies of braconids (Quicke & van Achterberg, 1990; Wharton et al., 1992). This is a moderately large subfamily with about 500 described species worldwide (Yu et al., 2012). The subfamily is divided into five tribes, where the tribe Trioxini in Europe comprises nine genera: *Betuloxys* Mackauer 1960, *Binodoxys* Mackauer 1960, *Calaphidius* Mackauer 1961, *Falciconus* Mackauer 1959, *Harkeria* Cameron 1900, *Lipolexis* Förster 1862, *Monoctonia* Starý 1962, *Monoctonus* Haliday 1833, and *Trioxys* Haliday 1833 (Mackauer & Starý, 1967; Mackauer et al., 1968). The tribe Trioxini consists of two subtribes, Trioxina and Monoctonina. Subfamily Aphidiinae have a diverse forewing venation, which is often quite a useful trait for determination of parasitoids on the generic level. Among the subfamily, several members of the subtribe Trioxina possess highly reduced forewing venation, with only the stigma, radial vein and radial sector vein. Beside wings, which are the most useful trait, other morphological structures are important for determination of aphidiines on lower taxonomic levels, such as species (Wharton et al., 1997). Several genera of the subtribe Trioxina contain paired prongs, which are extensions of the female's seventh metasomal sternite. In European fauna, only the species of the genera *Betuloxys*, *Binodoxys* and *Trioxys* possess those abdominal excrescences. These structures are very important for the identification of specimens on the generic and also species level; especially the shape and the curvature of prongs, the number and disposition of setae on them (Starý, 1981). Furthermore, European genera which have prongs can be differentiated from one another by the morphology of the second metasomal segment, petiole. While the genera *Betuloxys* and *Trioxys* have one pair of tubercles with spiracles, the genus *Binodoxys* possesses two pairs, primary and secondary. In this genus, the primary tubercles are

with spiracles, while the secondary are without them. The position of the secondary tubercles on petiole is variable and highly important for the identification of *Binodoxys* at the species level (Mackauer, 1959, 1960; Fulbright et al., 2007).

Knowing that the variability of the forewings in shape and size, and also in the wing venation can be a significant, often not conspicuous, characteristic to separate two morphologically similar species, there were a lot of studies where the geometric shape of the wing was used to accent that variability and those differences. In contrast to Monoctonina, Trioxina can be easily recognized by the forewing venation which is significantly reduced, but all minute differences between genera and among species of the same genus can be explained using the method of geometric morphometrics (Zelditch et al., 2012). This particular method has already been successfully applied in many previous studies on Aphidiinae wings (Žikić et al., 2009, 2014; Mitrovski-Bogdanović et al., 2009, 2014; Kos et al., 2011; Tomanović et al., 2014; Ilić Milošević et al., 2015; Stanković et al., 2015), sometimes revealing a new species, such as *Ephedrus loniceriae* Tomanović, Kavallieratos & Starý 2009 in Žikić et al. (2009).

Beside the morphological differences, there is also a biological dissimilarity between these genera, as their hosts feed on different plants. The species of the genus *Binodoxys* attack aphids on herbaceous plants, while *Betuloxys* and *Trioxys* species are known mostly from the hosts feeding on trees and shrubs (Mescheloff & Rosen, 1993; Pike et al., 1996, 2000; Akhtar et al., 2011; Žikić et al., 2012).

The aim of this study was to explore the variability of the shape and size of forewings, comparing the wing venation using the method of geometric morphometrics, and to determine whether there was a significant difference that would help in the further application of geometric morphometrics on the species of both investigated genera *Binodoxys* and *Trioxys*.

## Material and methods

### Sampled material

The specimens for this study were collected in the period between 2010 and 2013 on the territory of Serbia and Montenegro (Tab. 1). Plant parts infested

**Table 1.** The list of sampled material used in the analysis; abbreviations: SRB – Serbia, MNE – Montenegro; MJ – M. Janković, SS – S. Stanković, ZK – Z. Kojičić, VŽ – V. Žikić

Parasitoid species	No. of individuals	Country, locality, date, legator
<i>B. acalephae</i> (Marshall 1896)	14	SRB, Niš, Popovac, 04.07.2010. VŽ
<i>B. angelicae</i> (Haliday 1833)	15	SRB, Sićevačka klisura (gorge) 12.05.2013. SS
<i>B. brevicornis</i> (Haliday 1833)	10	MNE, Durmitor Mt., Crno jezero (lake), 25.07.2012. VŽ
<i>B. heraclei</i> (Haliday 1833)	11	SRB, Dukat Mt, 29.06.2012. SS
<i>T. complanatus</i> Quilis Perez 1931	16	SRB, Smederevo, Malo Orašje, 10.06.2012. MJ
<i>T. pallidus</i> (Haliday 1833)	14	SRB, Kruševac, 19.05.2013. ZK

with aphids were cut and placed into plastic containers and covered with a muslin cloth. According to the instructions given in Kavallieratos et al. (2010), these containers were placed in a growing cabinet and kept at the following conditions: 22.5 °C, 65% relative humidity and photo period of 16L:8D for 3-4 weeks until parasitoid emergence. Thus collected parasitoids were placed in vials containing 96% ethanol. Several wingless adult aphids were selected, put in 70% ethanol and sent to a specialist for identification.

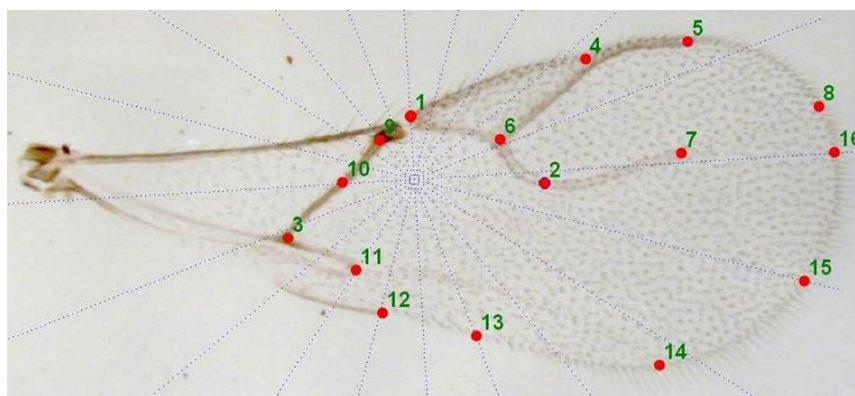
#### Wing preparation, selection of landmarks and geometric morphometrics

The method of geometric morphometrics was applied to explore the morphological differences in wing size and shape between the genera *Binodoxys* and *Trioxys* (Zelditch et al., 2012). We examined 80 right forewings of females in total; four *Binodoxys* species: *B. acalephae* (Marshall 1896), *B. angelicae* (Haliday 1833), *B. brevicornis* (Haliday 1833) and *B. heraclei* (Haliday 1833), and two species of *Trioxys*: *T. complanatus* Quilis Perez 1931 and *T. pallidus* (Haliday 1833) (see **Tab. 1**). The right forewings of each specimen were detached, mounted on microscopic slides in Berlese solution and photographed using Leica system DM2500 microscope with Leica DFC490 digital camera (Leica Microsystems©, Wetzlar, Germany).

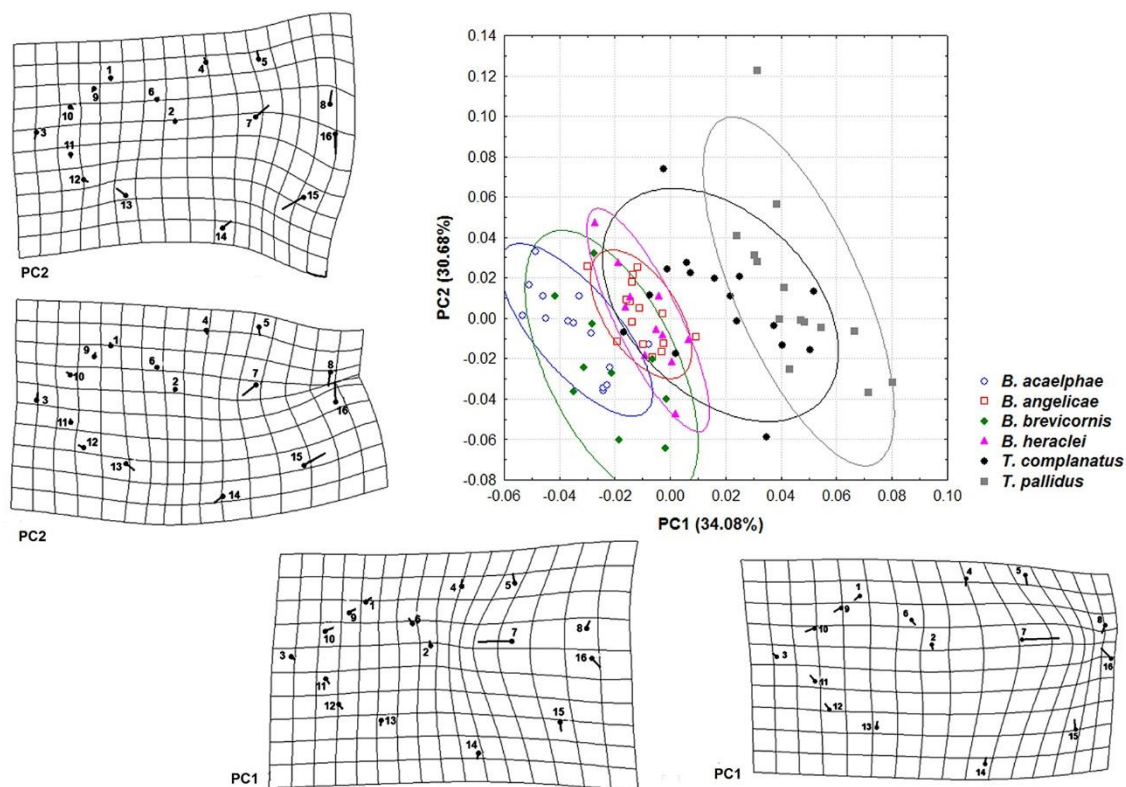
The specific landmarks from 1 to 9, together with the landmarks 11, 12 and 14, represent true landmarks (LM), while the other four (10, 13, 15 and 16) were set as semi-landmarks (S-LM). These semi-landmarks are used to better describe the distal part of the wing which lacks wing venation (**Fig. 1**). The landmarks 1, 4 and

6 define the length and width of the stigma; LM 4 and 5 represent the metacarpal (R1) vein; LM 2, 6 and 7 describe the radial sector, while LM 8 represents the projection of the radial sector to the edge of the wing. The landmarks 3, 9, 10, 11 and 12, together with the S-LM 13, delineate the proximal part of the wing, while LM 5, 8, 14, and S-LM 15 and 16 define the distal part of the wing.

The constellation of selected landmarks and the semi-landmarks was positioned and processed using the software MakeFun6 (Sheet, 2003) and tpsDig2 (Rohlf, 2005). The General Procrustes Analysis was applied to eliminate variation which can be caused due to the differences in the wing size, orientation and/or positioning during the digitalization (Bookstein, 1991) and for each wing to compute the centroid size (CS) as a geometric measure of size. Based on the CS, the differences in the wing size were analyzed using the analysis of variance (ANOVA), while the shape variability was computed using the multivariate analysis of variance (MANOVA). All statistical methods were done in Statistica software package (Stat Soft Inc. 7.0). To visualize the differences in the wing shape and size, the Principal Component Analysis (PCA) and Canonical Variate Analysis (CVA) were done using the software MorphoJ (Klingenberg, 2011).



**Fig. 1** Set of specific landmarks and semi-landmarks on the forewing of *Binodoxys angelicae*



**Fig. 2** Ordination of the *Binodoxys* and *Trioxy* species in the morphospace defined by the first two principal axes, for an interval of confidence of 0.8. The thin-plate transformation grids illustrate forewing changes along PC1 and PC2

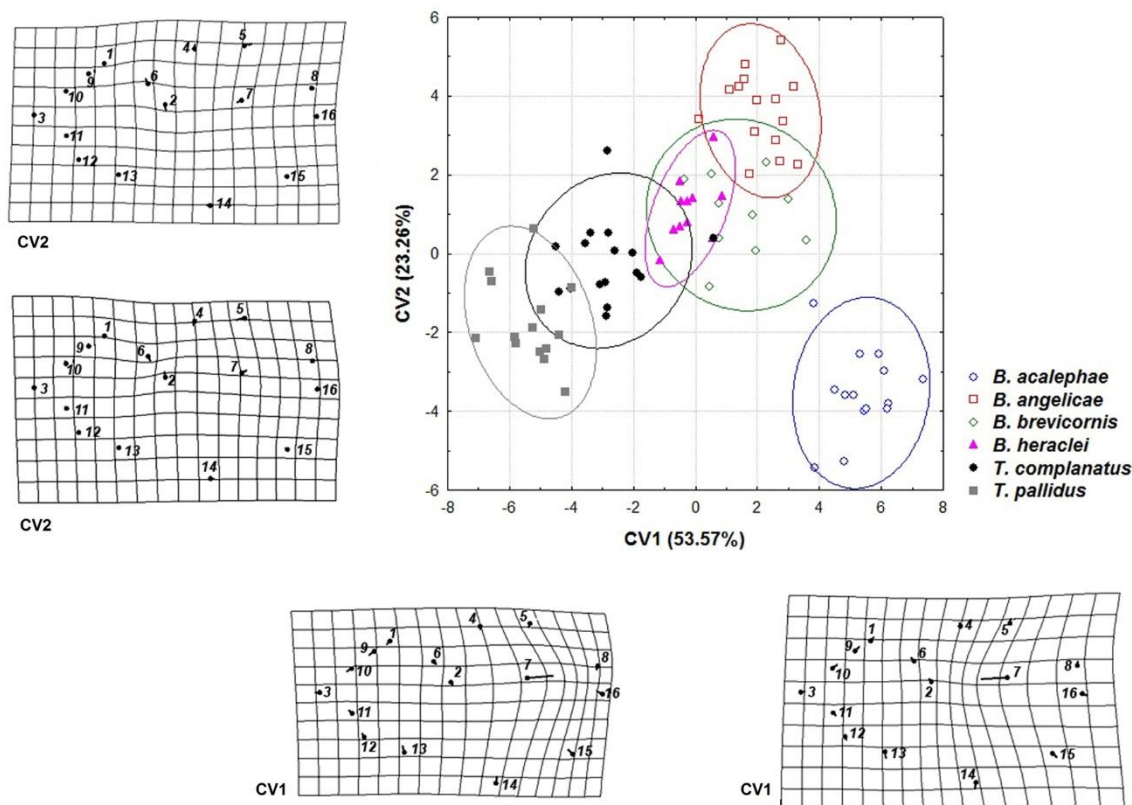
**Results and discussion**

For the variability among and between the selected groups, ANOVA test showed statistically significant differences in wing size ( $F_{(452|16,25)} = 3.62$ ;  $p < 0.006$ ). The wing shape variability which was tested by MANOVA also displayed statistically significant differences:  $Wilks\lambda = 0.000444$ ;  $F_{(11363|1211)} = 6$ ;  $p < 0.001$ . Delineation of the genera in morphospace is presented over Principal component analysis defined by the first two PC axes (**Fig. 2**). The discrimination between *Binodoxys* and *Trioxy* in the analysis of the forewing shape is evident, with a very small overlapping between the two genera (**Fig. 2**), where the first two PC axes accounted for 64.76% of the total variability. The analyzed species are separated along PC1 = 34.08%, according to the length of the radial sector (**Fig. 1, 2**) which was calculated as the most variable part of the wing. Placing all *Binodoxys* species in the negative part of PC1, this structure points a shorter radial sector than in *Trioxy*. To a lesser extent, all other parts of the forewing contribute to the separation of the analyzed genera. As a consequence of all shape changing in the wing along the positive and the negative senses of the PC1, the wings in *Binodoxys* species are generally shorter and broader in their distal part, LM (5, 7, 8, 14, 15 and 16), unlike *Trioxy* species, which wings are

elongated. Changes in wing morphology illustrated by PC2 = 30.68% are reflected not so much in the length of the wings as much as in the width. Thus, these kinds of changes explained by PC2 are more related to intragroup variability than the differences between the two genera.

Although there is a strong trend of delimitation of *Binodoxys* and *Trioxy* applying PCA, to obtain a clearer view of the discrimination of these two genera, we used Canonical variate analysis (CVA). After applying CVA, the ordination of analyzed specimens in the morphospace, which was defined by the first two canonical axes, has pointed out the interpreted morphological differences in wings much better (**Fig. 3**). The first canonical axis (CV1) explained 53.57% of the total variability of the wing shape, thus splitting *Binodoxys* and *Trioxy* species in the two distinct groups. Both analyzed species of the genus *Trioxy*, *T. complanatus* and *T. pallidus*, are positioned in the negative part of the CV1, while the four species of *Binodoxys* are distributed along the positive part of the CV1 axis. As it can be seen from the CVA graph, most of the differences are caused by the variability of the distal part of the wing, where the LM 7 contributes most to the differences of the wing shape with respect to all other landmarks in this region. Unlike both species of *Trioxy*, the species of





**Fig. 3** Distribution of the species of *Binodoxys* and *Trioxyx* in the morphospace defined by the CV1 and CV2 axes, for an interval of confidence of 0.85

the genus *Binodoxys* have a much shorter radial sector and rather wider wings. Also, the wings of *Binodoxys* are little larger (in average) than in *Trioxyx*.

Changes in wing size and shape are presented via transformation grids. The best discrimination was shown in *B. acalephae*, where this species is abstracted in the positive part of CV1, and the negative CV2 = 23.26% (**Fig. 3**). The other species, *B. angelicae*, is clearly separated from the other three *Binodoxys* species, being positioned in the positive part of the CV2. The other two species of *Binodoxys*, *B. brevicornis* and *B. heraclei*, mostly overlap in the center of the morphospace defined by CV1 x CV2, sharing more or less the similar shape of the wings. In this analysis, both *Trioxyx* species are well discriminated. The reason of the partial overlapping lies in the morphology of the forewing, especially in the variation of the radial sector (Fulbright et al., 2007). Since the genus *Trioxyx* is very large, comprising about 60 species (Žikić et al., 2017), the radial sector can be variable. For example, it can be extremely short like in *T. bonnevillensis* (Smith 1944), medium-sized in *T. ibis* (Mackauer 1961) and very long in *T. curvicaudus* (Mackauer 1967). Having this in mind, it should be noted that the use of geometric morphometrics is suitable only for the

species with similar wing armature. Contrariwise, the majority of *Binodoxys* species have a less variable radial sector on the forewings, and in comparison to the *Trioxyx* species listed above, we can say that it is of a medium size. Therefore, we can see much greater overlapping among the *Binodoxys* species.

### Conclusion

For a very long time, the taxonomic status of the genus *Binodoxys* was considered as a subgenus within the genus *Trioxyx*. Since Mackauer (1959) separated these two genera, there was a need for the revision of both genera. Most keys for determination of aphidiines are based on the morphology of petiole and shape of ovipositor and accessory prongs. Adding the geometric shape of the wings to the characters listed above, it is confirmed that *Binodoxys* and *Trioxyx* are two genera.

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