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Morphometric measurements of *Bryodaemon* (Coleoptera: Curculionidae): contribution to phylogeny

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The aim of this study was to find patterns in morphometric diversity of three Carpathian *Bryodaemon* Podlussany, 1998 species and to distinguish characters that may be useful in further analyses. Research was conducted on 6 populations of 3 species (2 populations per species). 17 morphometric measurements were taken. Traits best corresponding to the distinction of species and populations were distinguished by Principal Component Analysis (PCA) and General Linear Model (GLM) analysis of the PCA results. Traits explaining most of variability between populations were those connected with body segments. Surprisingly, measurements connected with genitalia did not constitute important part of variation. GLM analysis suggested distinction of *B. rozneri* males but did not show any significant diversity among females. Distinction of *B. rozneri* males is partially congruent with results of a prior molecular analysis.

Keywords: weevils, phylogeny, morphometry, PCA, refugia, *Bryodaemon*

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INTRODUCTION

Morphometry has been one of first method used in biological studies but today, in "molecular era", it is still relevant for discovering biodiversity (Wanek & Sturmbauer 2015) and resolving phylogenies (Klingenberg & Marugán-Lobón 2013). Morphometric measurements are widely used in integrative approach to systematics along with molecular data (Ober & Connolly 2015) which may result in taxonomical revision (Grobler *et al.* 2006). One of the most important questions is which morphological traits should be chosen for such analysis. Variability of some traits better reflects phylogeny of taxa than variability of the others (which can be checked by comparison with DNA phylogeny). Redundancy of used traits can lead to disturbance of obtained results in attempt to resolve phylogeny based on morphological variation. There are many methods for finding appropriate measurements, for example Principal Components Analysis (PCA) (van Rensburg *et al.* 2003). When correctly chosen, morphometric measurements may be very helpful in establishing phylogeny, especially of species which are not easy to distinguish due to few

or even no diagnostic traits (De Bivort *et al.* 2010; Navia *et al.* 2015). Also, when there is a conflict between morphological and molecular data, this does not always mean that morphology is uninformative. Such kind of conflict may result from many flaws of molecular analyses (Watanabe *et al.* 2015), which are connected with such processes as introgression, incomplete lineage sorting or influence of endosymbionts (Stewart *et al.* 2014; Whitworth *et al.* 2007).

The genus *Bryodaemon* Podlussany, 1998 (Coleoptera: Curculionidae) consists of five species previously regarded as *Omiamima hanakii* Frivaldszky, 1865 and *O. brandisi* Apfelbeck, 1903. All five species are similar in appearance and difficult to distinguish without detailed examination. They inhabit mountainous areas around the Pannonian Basin: four of them (*B. boroveci*, *B. hanakii* with two subspecies – *B. hanakii hanakii* and B. *hanakii montanus*, *B. kocsirenae* and *B. rozneri*) live in the Carpathians, and one (*B. brandisi*) occupies the Bosnian part of the Dinaric Alps (Podlussany 1998). Because of high similarity in morphology and ecology, monophyly of particular species had to be confirmed, which was done with the use of molecular markers and morphometric measurements (Wacławik *et al.* 2015). Molecular phylogenies indicated monophyly of the Carpathian species and allowed to propose some phylogeny of the genus, but the results of the morphometric survey were ambiguous and suggested only clear distinction of *B. hanakii* from three other Carpathian species. Thus, the morphometric study of *Bryodaemon* has to be continued and improved. The aim of this study was to find a pattern in morphometric diversity of species and populations and to select appropriate measurements for further research.

MATERIALS AND METHODS

For this research, specimens from 6 populations of the genus *Bryodaemon* from the Carpathian Mountains, collected in years 2002-2010 were used (Table 1).

Each specimen was separately prepared on cardboard. Each body part was photographed and measured using Nikon SMZ1500 stereomicroscope with NIS Elements software. Morphological parameters connected with body features were used for measuring both sexes, and additional measurements associated with aedeagus in males, and receptaculum seminis in females were taken (Table 2).

Principal Component Analysis (PCA) was used to find traits responsible for variation of studied specimens and to enable conducting statistical analysis of obtained results. A General Linear Model (GLM) was used to find differences between populations. The factor scores of the first two axes with populations nested in species as an independent factor were analyzed. In cases when GLM showed significant differences, post-hoc analysis (Tukey's HSD) was conducted.

Additional GLM analysis of measurements of proportion: diameter of eye-distance between eye and antenna basis, with species as independents factor was also conducted.

All statistical analyses were conducted using Statistica 10 (Statsoft) and PAST 2.17c (Hammer *et al.* 2001) software. PCA was performed simultaneously in both programs. Results from Statistica (PCA, without any groups) were used for further GLM analysis, results from PAST (PCA, disregard groups) was used to visualize distribution of particular species on the plot.

RESULTS

The morphological variation of females ordinated by PCA is shown in Figs 1-2. The first two ordination axes describe 47.0% and 16.1% of variation, respectively. Traits connected with head and sternum are highly correlated with the first axis. The length and width of spermatheca correlate mostly with second axis. Visualization of the results of PCA suggested no distinction of particular species. The results of PCA conducted

Table 1. Studied populations

Species	Locality	Number of specimens
B. hanakii hanakii	Menczul Chornohora, Ukraine	10 males, 10 females
	Polonina Breskulska Chornohora, Ukraine	10 males, 10 females
B. rozneri	Sianki, Ukraine	10 males, 10 females
	Tarnawa Bieszczady Mountains, Poland	10 males, 10 females
B. boroveci	Jaworzyna Kamienicka Gorce Mountains, Poland	6 males, 7 females
	Jasiolka Lower Beskids, Poland	1 male, 2 females

Table 2. Measurements of particular body parts

Body parts	Morphometric measurements
Head	Width of head Width of rostrum Eye diameter Distance between eyes Distance: eye – basis of antennae
Left elytra	Width of elytra Height of elytra
Foreleg	Length of tibia Angle between outer edge of tibia and tarsus
Sternum	Width of sternum Height of sternum
Aedeagus (male)	In front and side view: Lenght Width Angle of apex
Spermatheca (female)	Width of spermatheca Height of spermatheca

Table 3. Results of GLM analysis performed on results of PCA conducted on females

Value	SS	Degrees of freedom	MS	F	p
Free coefficient	0.91222	1	0.912218	4.62046	0.046283
Population	2.45492	3	0.818306	4.14479	0.022417
Species	16.89801	2	8.449007	42.79498	<0.001000
Error	3.35631	17	0.197430	–	–

Table 4. Results of GLM analysis performed on results of PCA conducted on females

Value	SS	Degrees of freedom	MS	F	p
Free coefficient	0.74001	1	0.740010	1.070628	0.315299
Population	8.29529	3	2.765096	4.000470	0.025220
Species	1.85350	2	0.926751	1.340800	0.287940
Error	11.75028	17	0.691193	–	–

on male measurements are shown in Figs 3-4. First two axes describe respectively 46.1% and 13.6% of variation. Visualization of PCA results suggested distinction of particular species.

The most significant feature of both males and females is the width of sternum (load. 0.94 and 0.97) and the least important is the diameter of eye-distance between eye and antenna basis proportion.

GLM analysis of factor scores of the first axis of PCA conducted on males showed that species and populations are significantly different (Ta-

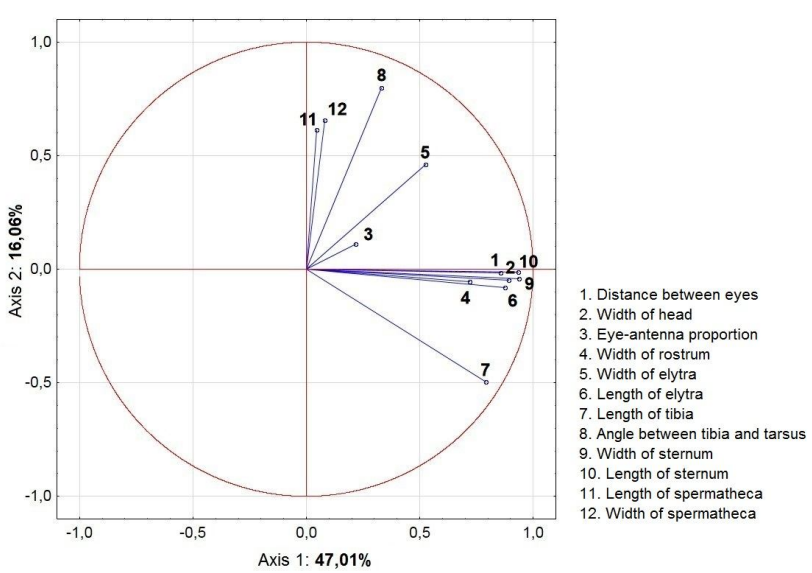

Fig. 1. Ordination plot based on Principal Components Analysis (PCA) of the morphometric measurements conducted on females (Statistica, Statsoft)

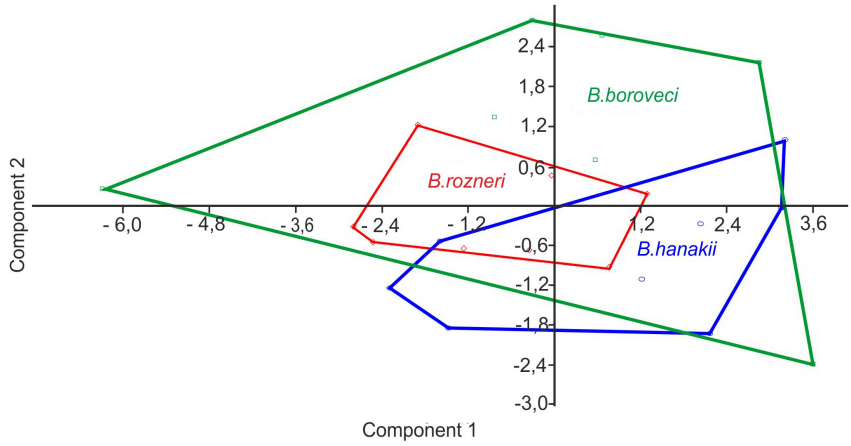

Fig. 2. Ordination plot based on Principal Components Analysis (PCA) of the morphometric measurements conducted on females, specimens from particular species in convex hulls (PAST, Hammer & Harper)

ble 3). Post-hoc test indicated very significant differences of factor scores on the first PCA axis between *B. rozneri and B. hanaki (p<0.002) and B. rozneri and B. boroveci (p<0.005)*. The differences between *B. hanaki* and *B boroveci* scores are much weaker, however still significant ($p<0.01$). Analysis of factor scores of the second axis did not show significant differences between species.

Analysis of females showed significant difference between populations but not between spe-

cies (Table 4). Post-hoc analysis did not show any significance differences.

GLM analysis of measurements of proportion: diameter of eye-distance between eye and antenna basis did not show any significant difference.

DISCUSSION

For both sexes, traits which explained most of variation in PCA analysis (thus, traits strongly correlating with the first axis), were connected with head, elytra and sternum. It is especially important when considering males. GLM and

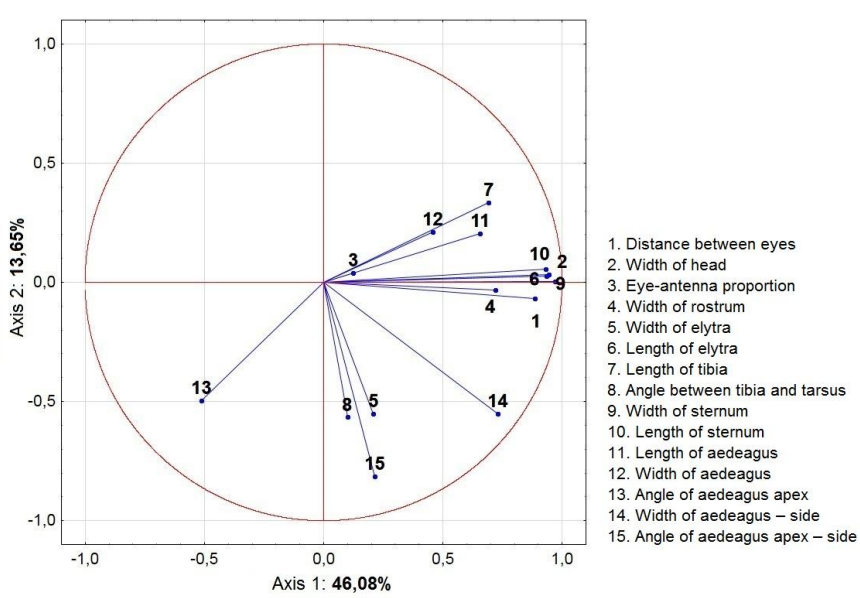

Fig. 3. Ordination plot based on Principal Components Analysis (PCA) of the morphometric measurements conducted on males (Statistica, Statsoft)

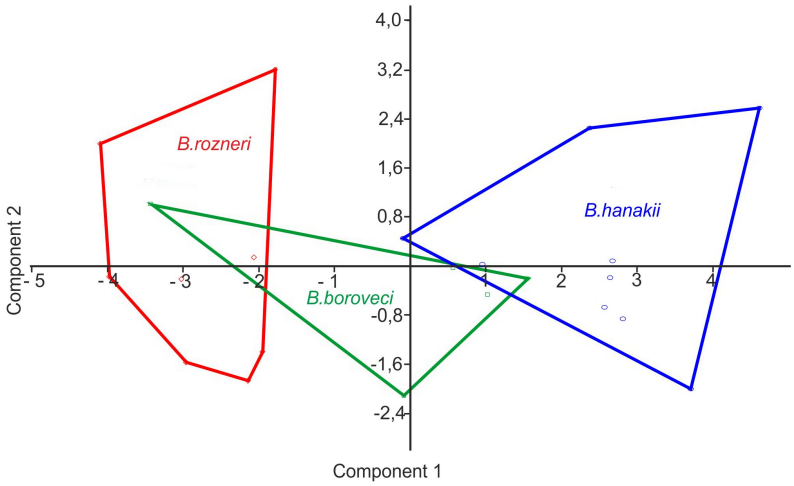

Fig. 4. Ordination plot based on Principal Components Analysis (PCA) of the morphometric measurements conducted on males, specimens from particular species in convex hulls (PAST, Hammer & Harper)

post-hoc analysis performed on the first axis of PCA conducted on males showed significant differences between species and populations. Thus, traits that strongly correlated with this axis correspond well with the differentiation between species and populations. It may be quite surprising that measurements of genitalia were not very important for explaining the diversity. In this genus the shapes of aedeagi and receptacula are quite diverged between species. Here, the small importance of explained diversity may be due to the use of measurements of length and distance, while the details of shape can be better reflected by geometric morphometry (Mutanen & Pretorius 2007). Measurements of leg did not correlate strongly with none of the first two axes. These results show that when studying the genus *Bryodaemon* using simple measurements (instead of landmarks) it is better to focus on traits connected with main parts of the body and not on the distal or smaller structures. On the other hand, probably only part of them should be used, because strong correlation of similar measurements with one (the first) PCA axis (e.g. width of head, distance between eyes), may suggest that they correlate with each other.

Measuring diameter of proportion: eye-distance between eye and antenna basis also gave surprising results. Podlussany (1998) proposed this trait as one of diagnostic character to distinguish species, while in PCA results it did not correlate strongly with none of the first two axes. Separate GLM analysis of the results of this measurement also did not show any significant differences between species.

Results of post-hoc analyses indicated that males of *B. rozneri* are distinct from the other two species, which is not observed among females. It suggests that the two sexes may have met different factors of natural selection during their evolutionary history which could have resulted in different traces of evolution.

Another important issue is related to morphological diversity and distinction of the species. Evolution of *Bryodameon* was probably connected with climate oscillations during glacial and interglacial periods. During drastic climate changes, many species and populations are able to survive only in areas with more stable climate, called refugia. Throughout Quaternary, there were many such places in the Carpathian Mountains and surrounding areas (e.g. Pannonian Basin). It is quite possible that processes of differentiation and speciation in the *Bryodameon* genus were heavily influenced by contractions and subsequent expansions of ranges of populations due to climate change. For example, two populations of one species may have been isolated in two different refugia which may lead to allopatric speciation. Isolation in mountainous refugia can lead to patterns in morphological diversity which are in many aspects consistent with phylogeny (Ober & Connolly 2015). However, all distinguished *Bryodaemon* species have similar ecological niches, which may result in similar adaptations to environment. That is why the possibility that many aspects of morphology do not reflect phylogeny but simply adaptation to the common environment, must be taken into account.

Distinction of *B. rozneri* is consistent with results of a molecular study based on *COI* marker. Yet, due to being not consistent with phylogenetic tree based on *EF1-alpha* marker, distribution of apomorphies and PCA analysis of morphological traits this version of *Bryodaemon* phylogeny was rejected (Wacławik *et al.* 2015). We argued that mtDNA phylogeny is often disturbed by incomplete lineage sorting, endosymbionts and other factors. However, these results suggest need for further analysis of the diversity of this genus.

CONLUSIONS

Traits connected with body segments (elytra, sternum etc.) are good characters for studying *Bryodaemon* diversity.

B. rozneri has been shown to differ morphologically from the other two species in males but not in females.

B. rozneri males are most distinct which is partially consistent with part of prior molecular results.

Further analysis of *Bryodaemon* diversity and phylogeny is needed.

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