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Species Relationships in the Genus *Bryodaemon* (Coleoptera: Curculionidae)*

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Establishing reliable taxonomy and phylogeny of similar, evolutionarily young species is among the greatest challenges in biology. Clearly the best approach is to use a combination of informative traits, including molecular markers and morphometric measurements. The objective of this study was to verify the taxonomy and phylogeny of four morphologically similar Carpathian species of *Bryodaemon* Podlussany, 1998 (Coleoptera: Curculionidae). Species relationships were studied using three molecular markers: two nuclear (*ITS-2* and *EF1-α*) and one mitochondrial (*COI*, barcoding marker). We also took morphometric measurements of 35 taxonomically derived characteristics of body parts and genital apparatus. The potential presence of apomorphic features also was determined. We then compared our results with data concerning the ecology and geography of previously studied species. Our analyses confirmed the monophyly of this group and established a phylogeny for the genus. We propose that *B. hanakii* is the earliest derived species, based on morphometric measurements, apomorphies and the *EF-1α* phylogeny. The pattern of nucleotide variation in this marker also indicates that *B. rozneri* and *B. boroveci* are the youngest species. This hypothesis is consistent with geographical ranges and ecological preferences of Carpathian *Bryodaemon* species. We also considered an alternative hypothesis based on the *COI* gene tree which indicated that *B. rozneri* was the oldest species. However, this arrangement is inconsistent with our morphological data.

Key words: Phylogeny, weevils, Carpathian Mountains, morphometry, molecular markers, apomorphies.

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Discovering and describing biodiversity is a critical task for biologists because it aids in understanding processes that occur in the environment, evolutionary mechanisms, and the influence of human actions on nature (GROOMBRIDGE 1992). With increasing knowledge about the variety of species at a global scale and an awareness of the decline of many habitats with a concomitant extinction of species, it becomes clear that there is a significant component of biodiversity that we may never discover (TREFAUT *et al.* 2014). Thus, it is especially important to study events of recent speciation and young species. However, young species may be difficult to distinguish and their similarity may lead to the omission of significant components of biodiversity (BEHEREGARAY & CACCONE 2007; ELMER *et al.* 2007; WANG *et al.* 2008). The best approach for studying young, similar species is an integrative approach that uses a wide spectrum of data instead of focusing on only a few measurements or traits (DAYRAT 2005).

Bryodaemon Podlussany, 1998 (Coleoptera: Curculionidae) is a genus of weevils consisting of five species which previously were regarded as two: *Omiamima hanakii* Frivaldszky, 1865 and *Omiamima brandisi* Apfelbeck, 1903. *Bryodaemon* species are similar in appearance and are difficult to distinguish without detailed examination. All five species are essentially shiny with reduced, decumbent hairs that differentiate them from the genus *Omiamima*, which is characterized by erect, distinct hairs. Also, all *Bryodaemon* spp. possess oval-shaped elytra without a humeral angle which separates them from the genus *Humeronima* (1998) as determined by PODLUSSANY (1998). All five species of *Bryodaemon* 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.

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Systematic classification of the genus *Bryodaemon* was based primarily on the shape of the aedeagus, but also included shape of the head and tarsi as well as the number of claws (PODLUSSANY 1998). All five species are herbivores and prefer moist subalpine forest habitats, but can also be found in subalpine meadows (especially *B. hanakii*). The species distributed within the Carpathian Mountains occupy mostly small, isolated and fragmented patches, with *B. hanakii* occupying the most non--disjunct range (PETRYSZAK 2002; PODLUSSANY 1998). All possible pairs of Carpathian species, with the exception of *B. hanakii* and *B. kocsirenae*, are sympatric in some areas. Morphological similarities, close but fragmented geographical distributions, and similar habitat associations indicate that these weevils could represent evolutionarily young species and it is possible that divergence and dispersal processes among *Bryodaemon* populations were associated with recent climatic oscillations and subsequent environmental change (AFZAL-RAFII & DODD 2007; BABIK *et al*. 2005; PROVAN & BENNETT 2008; RONIKIER *et al.* 2008; WANG *et al.* 2008). The survival of these species during periods of suboptimal conditions was probably facilitated by occupying refugia in or near the Carpathian Mountains, indicated by their fragmented distribution in the Carpathian region. However, to date no morphometric or comprehensive molecular research has been conducted on the genus *Bryodaemon.* Because of this lack of research along with high similarity of particular species, it is possible that the purported species in this genus are not correctly distinguished. Cryptic but currently undescribed species may exist among *Bryodaemon* populations, or some currently described species may actually be only distinct populations of one species.

Therefore, the objective of this work was to contribute to a preliminary understanding of the phylogeny and relationships among populations of Carpathian *Bryodaemon* species. We aimed at confirming the monophyly of the studied species and proposing a phylogenetic hypothesis. To provide reliable results, we used both molecular markers and morphological features.

Material and Methods

In this research we collected specimens from the four Carpathian *Bryodaemon* species. Data for the molecular research were collected between 2002-2010 from Poland, Ukraine, and Slovakia (Table 1). There were 1-6 specimens analyzed for each population. Specimens of *Otiorhynchus coecus* (Oc)*, Omiamima mollina* (Om) and *Omias winkelmanni* (Ow) were used as outgroups. The latter two are presumably closely related to *Bryodaemon*. They belong to the same tribe (Omiini) and weevils from *Omias* are quite similar to those from the former *Omiamima* complex (PODLUSSANY 1998). Also *Otiorhynchus* belongs to the same subfamily as *Bryodaemon* (Entiminae). Attempts to collect the fifth species, the non-Carpathian *B. brandisi*, were unsuccessful.

Three molecular markers were used, two nuclear (internal transcribed spacer 2 *ITS-2* and elongation factor 1-α*: EF1*-α) and one mitochondrial (mitochondrial cytochrome oxidase I *COI*).

DNA was isolated from whole bodies using the NucleoSpin Tissue kit (Machery Nagel Düren, Germany) according to established protocols. Amplifications of three markers were performed by PCR with primers (*ITS-2: ITS3, ITS4; EF1-α: M3,* rcM44.9; *COI*: C1-J-2183, TL2-N-3014). Reaction components were: 3 μl DNA, 3 μl 10xbufor, 3 μl MgCl2, 6 μl reagent Q, 0.6 μl dNTP, 0.6 μl starter F, 0.6 μl starter R, 0.2 μl polymerase Taq and 12 μl water. Amplification was performed in a Mastercycler Epigradient S (Eppendorf) with profile: 95°C for 4 min, 35 cycles of 95°C for 30 s, 52°C for 1 min, 72°C for 2 min and a final extension period of 72°C for 10 min. Results of amplification were checked by electrophoresis on 1% agarose gels stained with midori green. After purification (NucleoSpin Extract II (Macherey-Nagel), the PCR products were sequenced using the BigDye Terminator v.3.1. Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA, USA). Sequences were examined in BioEdit 7.1.3.0. (HALL 1999). For each population consensus sequences were obtained using this software. Alignment was performed using ClustalW (THOMPSON

Table 1

The location of examined material for molecular and morphological data

Species	Locality	Habitat
Bryodaemon hanakii hanakii (Bh1)	Ukraine, Chornohora, Menczul	Beech wood and alpine meadow
Bryodaemon hanakii hanakii (Bh2)	Ukraine, Chornohora, Polonina Breskulska	Beech wood and alpine meadow
Bryodaemon boroveci (Bb1)	Poland, Gorce Mountains	Spruce lower supalpine forest, beech wood, beech-spruce forest
Bryodaemon boroveci (Bb2)	Poland, Lower Beskids	Beech wood
Bryodaemon boroveci (Bb3)	Slovakia, Niżna Polianka	Beech wood
Bryodaemon rozneri (Br1)	Ukraine, Borzawa	Beech forest, alpine meadow
Bryodaemon rozneri (Br2)	Ukraine, Zakarpattia, Chertezh	Subalpine meadows
Bryodaemon rozneri (Br3)	Poland, Bieszczady Mountains	Spruce forest
Bryodaemon rozneri (Br4)	Ukraine, Zakarpattia, Zabrid	Beech wood
Bryodaemon kocsirenae (Bk1)	Poland, Bieszczady Mountains	Alpine meadow

et al. 2002). The Akaike Information Criterion in MrModeltest 2.3 (NYLANDER 2004) and PAUP* (SWOFFORD 2002) were used to determine the best-fitting nucleotide substitution model (KIMURA 1980). Phylogenetic trees were constructed using software: PAUP* 4.10b using maximum parsimony trees and MrBayes 3.1 1 (HUELSENBECK & RONQUIST 2001) for Bayesian inference. For all MP analyses, we conducted a heuristic search with tree bisection and reconnection (TBR) branch swapping and random addition sequences (MaxTrees = 500), with 500 random addition replicates. Node support was assessed with the bootstrap technique using 5 000 pseudoreplicates and TBR branch swapping. Bayesian analysis was conducted with 1 cold and 3 heated Markov chains for 3 000 000 generations, with trees sampled every 100th generation and each simulation conducted twice. To determine stationarity of the MCMC chains, variation in log-likelihood scores was examined graphically using Tracer software (RAMBAUT & DRUMMOND 2007). The appropriate number of sampled trees was discarded. The remainder was used to reconstruct the 50% majority rule consensus tree. All trees were visualized with TreeView1.6.6 (PAGE 1996). All obtained sequences were deposited in GenBank (Accession nr: KJ699357-KJ699377 and KJ801835-KJ801844).

Table 2

Mean values of male and female morphological measurements (Bb – *Bryodaemon boroveci,* Bh – *Bryodaemon hanakii hanakii*, Bk – *Bryodaemon kocsirenae,* Br – *Bryodaemon rozneri*)

Morphometric measure	Abre-viation	Bb	Bh	Bk	Br	Bb	Bh	Bk	Br
		Males				Females			
Body length (mm)	C1	2.81	3.25	3.02	3	2.9	3.24	3.07	3.27
Elytra length (mm)	C2	1.75	2.02	1.85	1.84	1.89	2.1	1.94	2.05
Elytra width (mm)	C3	1.29	1.55	1.39	1.42	1.47	1.62	1.54	1.66
Elytra height (mm)	C4	0.92	1.15	0.94	0.95	1.03	1.2	1.01	1.11
Hind angle of elytra	C5	117.09	120.15	126.24	114.52	131.34	119.49	128.2	126.89
Lateral angle of elytra	C6	76.32	88.09	79.76	79.76	83.8	85.47	82.12	82.12
Prothorax length (mm)	C7	0.61	0.71	0.7	0.67	0.59	0.7	0.66	0.71
Prothorax width (mm)	C8	0.7	0.86	0.8	0.8	0.73	0.86	0.81	0.85
Prothorax height (mm)	C9	0.65	0.77	0.72	0.75	0.67	0.83	0.73	0.82
Head length (mm)	C10	0.69	0.65	0.69	0.61	0.65	0.62	0.69	0.58
Head width (mm)	C11	0.47	0.56	0.54	0.46	0.51	0.52	0.55	0.51
Head height (mm)	C12	0.33	0.36	0.36	0.36	0.32	0.39	0.37	0.35
Length of snout (mm)	C13	0.4	0.43	0.43	0.37	0.39	0.4	0.44	0.35
Width of basal snout (mm)	C14	0.28	0.3	0.34	0.25	0.31	0.28	0.34	0.29
Snout width at eye basis (mm)	C15	0.32	0.32	0.37	0.29	0.35	0.32	0.37	0.32
Distance between eye (mm)	C16	0.27	0.31	0.31	0.28	0.31	0.31	0.31	0.31
Distance: eye fringe – prothorax (mm)	C17	0.12	0.13	0.11	0.11	0.1	0.12	0.09	0.1
Distance: eye – basis of antennae (mm)	C18	0.27	0.29	0.28	0.25	0.26	0.27	0.26	0.25
Eye diameter (mm)	C19	0.15	0.14	0.18	0.14	0.15	0.14	0.17	0.15
Length of stipes (mm)	C20	0.51	0.53	0.56	0.48	0.49	0.5	0.52	0.49
Width of stipes (mm)	C21	0.1	0.11	0.12	0.09	0.1	0.1	0.1	0.09
Length of club (mm)	C22	0.23	0.24	0.25	0.21	0.22	0.22	0.24	0.21
Width of club (mm)	C23	0.13	0.14	0.15	0.12	0.12	0.13	0.14	0.12
Length of tarsus (mm)	C24	0.13	0.15	0.15	0.15	0.11	0.12	0.12	0.13
Width of tarsus (mm)	C25	0.15	0.18	0.19	0.17	0.14	0.15	0.14	0.14
Length of tarsal claw (mm)	C26	0.15	0.15	0.17	0.13	0.13	0.15	0.14	0.13
Width of tarsal claw	C27	0.04	0.04	0.03	0.03	0.04	0.03	0.03	0.04
Length of tibia (mm)	C28	0.67	0.7	0.69	0.59	0.6	0.6	0.61	0.56
Width of tarsal basis (mm)	C29	0.09	0.1	0.12	0.09	0.09	0.08	0.09	0.09
Width of tarsal apex (mm)	C30	0.15	0.14	0.17	0.15	0.15	0.13	0.14	0.14
Length of penis (mm)	C31	0.55	0.75	0.59	0.62				
Maximal width of penis (mm)	C32	0.15	0.17	0.15	0.16				
Angle of penis apex	C33	101.71	59.97	99.36	94.05				
Length of penis process (mm)	C34	0.06	0.07	0.06	0.06				
Width of penis process (mm)	C35	0.03	0.03	0.03	0.02				
Length of spermatheca (mm)	C31					0.33	0.32	0.32	0.35
Length of medial lobe of spermatheca (mm)	C32					0.07	0	0.07	0.09
Length of hind lobe of spermatheca (mm)	C33					0.09	0.07	0.07	0.07
Angle of medial lobe of spermatheca (mm)	C34					137.66	0	138.09	74.62
Angle of hind lobe of spermatheca (mm)	C35					93.18	47.12	82.36	36.3

Thirty-five morphological parameters were chosen for morphometric analysis (Table 2). Five parameters were associated with male genitalia and female receptaculum seminis. In each morphometric sample ten specimens belonging to each species were chosen. Each body element was measured using a Nikon SMZ1500 stereo microscope with NIS Elements software. Measured distances were assessed five times and the average size for each specimen was counted. For each species, ten males and ten females were chosen randomly. The relationships between body size variation and classification to the entire species were compared by principal component analysis derived from Canoco for Windows 4.52 (TER BRAAK & SMILAUER 2003).

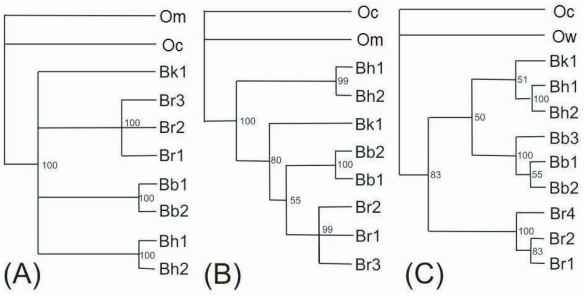

Fig. 1. Phylogenetic tree of Carpathian *Bryodaemon* populations constructed using *ITS-2* (A), *EF1-α* (B) and *COI* (C) markers with maximum parsimony.

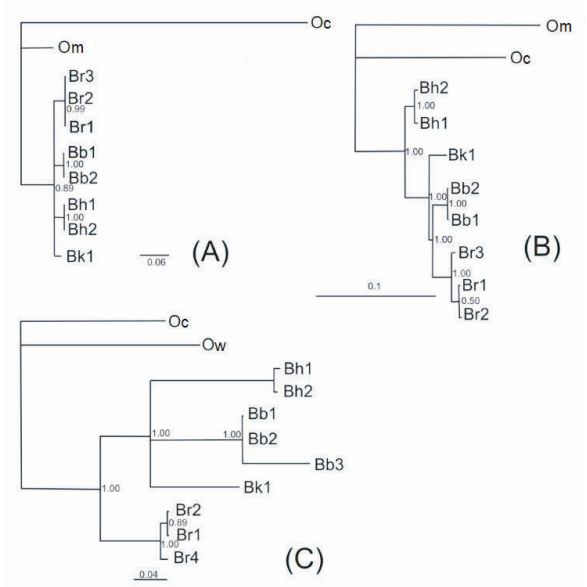

Fig. 2. Phylogenetic tree of Carpathian *Bryodaemon* populations constructed using *ITS-2* (A), *EF1-α* (B) and *COI* (C) markers with Bayesian analysis.

Results

The GTR+I+G model was chosen for *COI* (gamma distribution shape parameter G = 0.4426; -lnL = 3793.1868; AIC = 7606.3735), the SYM+G model for *ITS2* (gamma distribution shape parameter G = 0.3150; -lnL = 1974.4475; AIC = 3966.8950), and the GTR+G for EF1-α (gamma distribution shape parameter G = 0.3775; -lnL = 1600.6150; AIC = 3219.2300).

The derived MP trees of three individual genes had similar topology and populations of distinct species were grouped in separate clades. The *ITS2* gene tree had the strongest support with values of 100 for all major clades (Fig. 1a). Although the internal relationships among studied populations were unresolved in this tree, it confirmed the monophyly of all studied species.

The *EF* gene tree (Fig. 1b) indicated *B. hanakii* as the most distinct species, and was generally well-supported with the exception of the split between *B. boroveci* and *B. rozneri* (bootstrap value = 55). The *COI* gene tree (Fig. 1c) had poorly supported clades and indicated *B. rozneri* as the most distinct species.

Bayesian analysis also indicated monophyly of the four species, with populations of one species separated in different clades or subclades. The *ITS*2 gene tree (Fig. 2a) was poorly resolved but confirmed that the species comprise a monophyletic genus. The *EF* gene tree (Fig. 2b) indicated that *B. hanakii* was the most phylogenetically distinct species. The *COI* gene tree (Fig. 2c) contained two clades: one consisting of *B. rozneri* populations and a second with populations from the three other species, with the latter divided into smaller species-specific subclades. Bootstrap values were high for essentially every tree, with lower values only for two *B. rozneri* populations in the *EF* tree.

Principal component analysis revealed variation in male morphology among the species. The first, second, and third axes of the principal component analysis described 70%, 12.4%, and 10.6 % of the variation in the measured characters, respectively. However, only axis one and three contributed to the division of some species based on morphology (Fig. 3A). The increasing elevation of the elytra (C4) (load. 0.95), lateral angle of the elytral apex (C6) (load. 0.71), elytra length (C2) (load. 0.64), total body length (C1) (load. 0.59), and decreasing angle of the penis apex (C33) (load. -0.81) clearly separated specimens of *B. hanakii* from the other species along the first ordination axis. However, there was large variation among specimens in the posterior angle of the penis apex along axis 3. (C33) (load. 0.58).

The morphological variation of females ordinated by principal component analysis is shown in figure 3B. The first ordination axis, describing 93% of the measurement variation, clearly divides *B. hanakii* females from the rest of the group. The angle of the middle apex decreased along the first axis (load. 0.99). Along the second axis, which described only 3.7% of overall variation, *B. rozneri* is separated from the rest of the *Bryodaemon* complex. The morphometric measure that differentiates this species is decreasing angle of distal apex of spermatheca (load. 0.87).

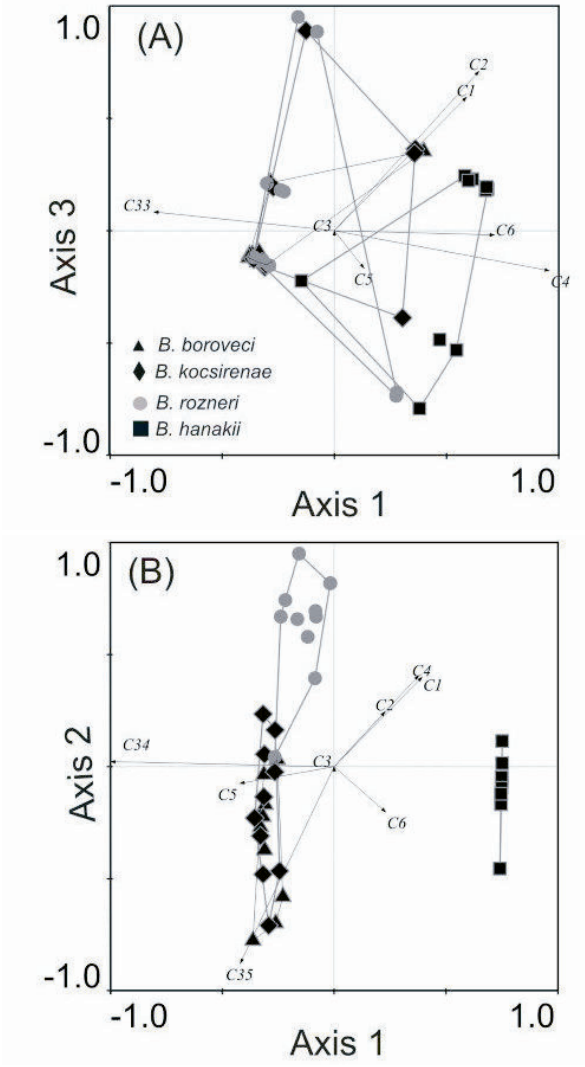

Fig. 3. (A) Principal Component Analysis for morphological characters (arrows) and populations of males. (B) PCA analysis for morphological characters (arrows) and populations of females. C1-C33 – characters in which variation was detected (see Table 2).

Discussion

Our phylogenetic trees constructed from molecular data confirm the monophyly of each of the Carpathian *Bryodaemon* species. The molecular data are also consistent with the results of morphometric analyses which indicated a clear delineation of three groups: *B. hanakii*, *B. rozneri* and a group consisting of *B. kocsirenae* and *B. boroveci.* Morphological features independently confirm that these species are significantly different.

However, each molecular marker appeared to indicate a different history of divergence among Carpathian *Bryodaemon* species. The *COI* trees indicated the following order of species formation: *B. rozneri* (*B. boroveci* (*B. kocsirenae*: *B. hanakii*). However, the *EF* trees indicated a different evolutionary history: *B. hanakii* (*B. kocsirenae* (*B. boroveci:B. rozneri*). The *ITS* trees confirm species monophyly but do not indicate which species was earliest or most recently derived. Thus, these trees were not considered during the reconstruction of the phylogeny of *Bryodaemon*. Therefore, the histories suggested by *EF* trees and *COI* trees should be considered as two hypotheses which should be tested against results from morphological surveys and complimentary ecological and geographical data. This approach to integrating data from studies based on different methods has been shown as reliable and successful in many surveys involving insect taxonomy and phylogeny (GEBIOLA *et al.* 2012; STÜBEN & ASTRIN 2012). The history of divergence based on the *COI* trees indicates that *B. rozneri* is the earliest derived species, whereas *B. hanakii* and *B. kocsirenae* are the youngest and sister species. However, this explanation conflicts with the results of our morphometric surveys. Measurements and inter-species comparisons of most morphometric features indicate that *B. hanakii* is the most distinct species whereas *B. boroveci* with *B. kocsirenae* are very similar to each other. This result is clearly visible from the comparisons of males and females and is inconsistent with the phylogeny suggested by *COI* analysis. Furthermore, morphometric analyses indicated the distinctive nature of *B. hanakii* when compared to the other species. We would expect that a species so distinct from the others would not be one of two young sister species as indicated by the *COI* tree (KIRKENDALE & MEYER 2004).

Moreover, a closer look at the diversity of apomorphies also contradicts results of the *COI* tree. *B. rozneri* has one visible trait absent in other species: a sharp groove from the frontal pit to apex (PODLUSSANY 1998). Determining which features are ancestral is valuable for reconstructing species phylogenies. However, for traits which are present only in single species (like the groove in *B. rozneri*) it is difficult to determine because these characters cannot be compared with closely related genera or with insects in general. However, one apomorphy that could be considered in relation to other insects is the number of claws. Both *Humeromima* and *Omiamima* (distinguished by PODLUSSANY (1998) along with *Bryodaemon*) have pairs of tarsal claws which are also a common feature in the *Omiini* tribe (BOROVEC 2010; PODLUSSANY 1998). Thus, it is expected that a pair of claws will be an ancestral feature among *Bryodaemon* species. This feature is shared by *B. hanakii*, *B. kocsirenae* and also the fifth species *B. brandisi*, while *B. rozneri* and *B. boroveci* are united by having a single claw on the tarsus. If we assume that the latter species are older, the presence of two claws is a trait that had to evolve twice: first among ancestors of *Bryodaemon*, and then a second time in a common ancestor of *B. hanakii* and *B. kocisrenae*. Another possibility is that this trait was lost independently in two lineages (of *B. boroveci* and *B. rozneri*). However, the loss of

one claw among the common ancestor of *B. rozneri* and *B. hanakii* is the most parsimonious explanation. The available ecological and geographic data also do not support an earlier split of *B. rozneri* than the other studied species.

When comparing the *EF* tree with other data, some of the previously mentioned problems remain, but there is some congruence between this tree and the morphological and ecological data. Although the history of the genus from this tree is still inconsistent with the morphometric results, it does identify *B. hanakii* as the oldest species. The morphometric results indicate that this species displays extensive divergence from the other three. This divergence is evident when comparing the entire dataset and individual features. Moreover, evaluation of apomorphies indicates its distinctiveness from the other species. *B. hanakii* weevils have slightly different genitalia than other species-both penis and spermatheca (PODLUSSANY 1998). These apomorphies are particularly significant adaptations because the shape of the genitalia is an important pre-zygotic barrier to successful mating (i.e., "lock and key" model; (KUBOTA *et al.* 2012).

The ecology and geography of *B. hanakii* also varies slightly from the other species. All Carpathian *Bryodaemon* species prefer forests with beech, spruce, and fir, but are also collected from subalpine meadows. *B. hanakii* occurs in this latter habitat significantly more often than the three other species. Furthermore, the distribution of this species in the Carpathian Mountains is less disjunct than the other three species.

In summary, the morphological and ecological evidence, in combination with the *EF* tree, indicates that *B. hanakii* may be the oldest among the studied species. Results from the *EF* tree are also consistent with the apomorphies, including the number of claws that divides the species into two groups. This hypothesis is consistent with the geographical distribution of *B. kocsirenae*, *B. rozneri* and *B. boroveci.* All three species exhibit more northern and western distributions than *B. hanakii*. *B. boroveci,* identified as potentially one of the youngest species, occupies areas most to the north and west when compared to the other species, suggesting that migration of the genus has occurred in a northwestern direction.

However, other information contradicts this hypothesis. The morphometric data indicate a high degree of similarity between *B. kocsirenae* and *B. boroveci*, whereas the *EF* tree indicates *B. rozneri* and *B. boroveci* as the youngest pair. This could result from the very high similarity of *Bryodaemon* species which are young species that inhabit very similar biotopes. Thus, some similarities among species could be the result of analogous adaptations to the environment.

The inconsistency of the *COI* tree is problematic, particularly with respect to discrepancies between the nuclear and mitochondrial DNA phylogenies. Different topologies for different markers are often the result of ancestral polymorphism, incomplete lineage sorting or events of gene flow that occur during the speciation process (POLLARD *et al.* 2006). Discrepancies between nuclear and mitochondrial DNA surveys may also be related to the higher mutation rate of mtDNA (JOHNSON *et al.* 2003). Alternatively, because mtDNA is inherited maternally without recombination, it evolves differently than nuclear DNA. For example, mtDNA can be influenced by maternally inherited endosymbionts that may increase or decrease diversity or even lead to paraphyly of mtDNA, thus making results unreliable (GOMPERT *et al.* 2008; HURST & JIGGINS 2005; WHITWORTH *et al.* 2007). In fact, because maternally inherited endosymbionts are very common among insects, analyses of insect mtDNA are often problematic (ZUG & HAMMERSTEIN 2012). Because our mtDNA assays were incongruent with our other results, it is possible that this factor may at least partially explain the discrepancies.

Disjunct ranges of species with sympatric overlap in some areas indicate that their evolution was connected with climate oscillations that cause range contractions and expansions (HEWITT 1999). European mountains often worked as barriers for expanding populations, but also as refugia during cold or warm stages (MARYAŃSKA-NADACHOWSKA *et al.* 2012; PROVAN & BENNETT 2008) As previously discussed, the distribution of Carpathian *Bryodaemon* species indicates an expansion of the genus in a northwesterly direction. The distribution of the fifth species, *B. brandisi*, within the mountains of Bosnia suggests independent dispersal of some ancestral populations in a western direction to the Dinaric Alps. Therefore, the evolutionary origin of the genus *Bryodaemon* could be located in or near the southeastern Carpathians. Among the four species, *B. hanakii* is the only one commonly collected from alpine meadows. This indicates that divergence between *B. hanakii* and the three other Carpathian species was connected with substantial environmental changes, including the possibility of forest expansion during warm stage (GÖMÖRY *et al.* 2012; KAJTOCH *et al.* 2014; SLOVAK *et al.* 2012). It is quite possible that the other three species, adapted to the forest habitat, have undergone multiple contractions and expansions of their ranges during a generally northwestern radiation, in response to glacial and interglacial periods (FIJARCZYK *et al.* 2011; KNOWLES 2001). We suggest that *Bryodaemon* weevils could be model organisms for future research investigating glacial refugia located in the Carpathian Mountains and adjacent regions.

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