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OXFORD

Original Article

Is Dorcadionini monophyletic? First phylogeny of the genus *Eodorcadion* reveals startling relationships in Central Asian flightless lamiines (Coleoptera: Cerambycidae)

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ABSTRACT

The flightless tribe Dorcadionini is one of the most species-rich and taxonomically challenging groups in the family Cerambycidae. *Eodorcadion* is one of five genera included in Dorcadionini, and one of three distributed in Central Asia. The current classification of the genus is based on taxonomic works pre-dating the application of cladistic methods. We used molecular data from one mitochondrial and three nuclear markers (*COI*, *CAD*, *ITS2*, and *Histone 3*) for 70 specimens in 23 species and subspecies, representing all subgenera and most species groups of *Eodorcadion* to build its first phylogeny. By including some taxa of Central Asian *Dorcadion* and *Lamia textor* (type genus of Lamiini and Lamiinae), we assessed the monophyly of the tribe. The results of our analyses are the first to fully support the synonymy of Dorcadionini with Lamiini and confirm the morphology-inferred monophyly of *Eodorcadion*. Consequently, Lamiini = Dorcadionini **syn. nov**. Moreover, based on the obtained phylogeny and morphological differences, we diagnosed and subsequently described two new subgenera of *Eodorcadion: Altaidorcadion* Karpiński **subgen. nov**. and *Arenodorcadion* Karpiński **subgen. nov**., while the currently recognized subgenus *Humerodorcadion* **stat. nov**. was elevated to the genus rank. Our phylogeny is remarkably consistent with results previously obtained by other authors that used endophallic morphology.

Keywords: *COI* genetic distance, integrative taxonomy, phylogenetic systematics, rapid speciation, revised classification, synonymy

INTRODUCTION

Lamiinae Latreille, 1825, with about 20000 described species, is the most diverse subfamily of longhorned beetles, while the most species-rich and taxonomically challenging Palaearctic tribe is Dorcadionini Swainson, 1840, with nearly 900 species and subspecies classified into five genera and 14 subgenera (Tavakilian and Chevillotte 2024). Initial molecular data (Souza *et al.* 2020, Soydabaş-Ayoub and Uçkan 2024) suggested that this tribe should be synonymized with Lamiini Latreille, 1825. However, this synonymy was based on very limited taxon sampling and Dorcadionini remained monophyletic (see Discussion for more details). Leading authors working on Dorcadionini retained the tribal rank in their recent works (e.g. Dascălu *et al.* 2022, Danilevsky 2022, Karpiński *et al.* 2023, Caba and Dascălu 2024).

The genus *Eodorcadion* Breuning, 1947 (Fig. 1) includes nearly 70 species and subspecies distributed in southern Russia (between the Southern Ural Mts. and the Pacific Ocean), eastern Kazakhstan, the whole territory of Mongolia, and northern China. Currently, three subgenera are distinguished within the genus: *Eodorcadion s.s.*; *Humerodorcadion* Danilevsky *et al.*, 2005; and *Ornatodorcadion* Breuning, 1947 (Tavakilian and Chevillotte 2024). Although *Eodorcadion* was revised by Danilevsky (2007), this work was based on morphological and zoogeographic data, without any phylogenetic reconstruction. Moreover, almost no molecular data have been published so far for representatives of this genus—the only work was published by Karpiński *et al.* (2023), however, this paper focused only on the so-called '*Eodorcadion intermedium* species-group', which constitutes a small part

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Figure 1. Genus *Eodorcadion*. A–I, adults *in situ*. A, B, *E. gorbunovi*; C, *E. exaratum argali*; D, *E. kozlovi*; E, *E. intermedium*; F, *E. oryx*; G, *E. consentaneum*; H, *E. dorcas scabrosum*; I, *E. maurum australe*. J–L, habitat diversity in Mongolia. J, *E. consentaneum*; K, *E. dorcas scabrosum*; L, *E. dorcas dorcas*.

of the subgenus *Ornatodorcadion* and was shown to be a polyphyletic species' assemblage, established based on the similarity of the elytral stripes. Elytral pattern is a trait probably resulting from ecological adaptation and is not of high taxonomic value (Karpiński *et al.* 2023). Reconstruction of a molecular phylogeny is undoubtedly hampered by the fact that representatives of the genus are distributed in a very poorly explored and difficult to access region of Central Asia, where the coverage of barcoded taxa in most invertebrate groups is very low due to the lack of DNA-grade samples.

The systematics of the genus *Eodorcadion*, especially at the subgeneric level, seems questionable, or rather incomplete, and molecular data are essential to solve several taxonomic issues. Its division is based mainly on the morphology of the endophallic structures (Danilevsky *et al.* 2005), which, although very informative, (i) does not allow for precise determination of differences between subgeneric and generic rank, and (ii) is extremely time-consuming and difficult to study (preparation, inversion, and inflation of fragile membranous structures required), and, therefore, is inaccessible to most entomologists. The authors retained the two existing subgenera and established the third one (*Humerodorcadion*). However, they also mentioned the possibility of the existence of one or two more subgenera, natural groups with distinctive endophallic morphology. It is important to note that the newly described subgenus *Humerodorcadion* stood out exceptionally from the remaining groups, which may suggest differences at a higher taxonomic level. It should also be emphasized that the authors did not examine all of the species and subspecies, leaving the possibility that some taxa not included in the study represent further natural groups.

Karpiński *et al.* (2023) for *Eodorcadion* and Dascălu *et al.* (2022) for *Dorcadion* have already demonstrated that using cytochrome *c* oxidase subunit I (*COI*) data alone to infer phylogeny in such a taxonomically challenging and recently derived flightless group is not sufficient at the genus or even species-group level. These authors hypothesized that introgression was responsible for part of the great morphological variability in Dorcadionini. However, these are not the only complex evolutionary mechanisms that make understanding this group extremely difficult. For instance, the similarity in pattern of elytral stripes (serving as

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camouflage) appears to be a case of homoplasy resulting from ecological adaptation, so very similar patterns may occur across species groups and even subgenera (Karpiński *et al.* 2023). Moreover, in some species both sexes show the same elytral pattern, while in others the males are only black and the females are also black (androchromic) or striped (autochromic); or other variants: males are only striped with two types of females, or both sexes have two colour variants. This may indicate parallel evolution driven by ecological adaptation. Rapid speciation may occur in such a diverse group of flightless beetles living in such an easily changing environment (desertification, drying up of natural barriers such as lakes and rivers), leading to the so-called 'phylogenetic pitchfork', polytomies in phylogenies.

This work aimed to reveal the first phylogeny of the genus *Eodorcadion* and to confront it with the current classification based mainly on morphological data derived from the analysis of endophallic structures. We hypothesize that the current system is not sufficient due to the complex evolutionary history of these beetles and that only molecular data (supported by morphology) can show a complete phylogenetic picture. In particular, the subgeneric structure should be further investigated, as suggested in the morphological study, because some groups clearly differ. Hence, the next goal of this paper was to verify the rank of *Humerodorcadion* as a potential separate genus due to the strong distinctiveness of the endophallus. Finally, we wanted to investigate the relationship between *Eodorcadion*, *Dorcadion*, and *Lamia* Fabricius, 1775, to test the monophyly of the tribe Dorcadionini.

MATERIALS AND METHODS

Material examined

This study is based on an examination of approx. 400 specimens of the genus *Eodorcadion* representing all currently recognized subgenera and most species groups, including possible new subgenera (based on morphological data). Most of the research material was collected during the first author's sampling campaigns in Mongolia (in 2015, 2019, 2022, 2023 and 2024), organised thanks to the financial support listed in the acknowledgments. We studied taxa of the subgenera: *Eodorcadion s.s.*, *Humerodorcadion*, and *Ornatodorcadion*, including the complex and morphologically distinctive (endophallic morphology; Danilevsky *et al.* 2005) group of *Eodorcadion maurum* (Jakovlev, 1889), as well as the morphologically untested, in Danilevsky *et al.* (2005), *Eodorcadion egregium* (Reitter, 1897). Special attention was paid to the taxa representing *Humerodorcadion*, as there is significant dissimilarity of endophallic structures (Danilevsky *et al.* 2005) possibly indicating a genus rank. Geographically, the taxon sampling spanned most of Mongolia, eastern Kazakhstan (East Kazakhstan Region), southern Russia (Republic of Tuva), and some regions of China (for morphological analysis only). The studied taxa were identified by the first author based on his own data and information, as well as some literature (Danilevsky 2007, Danilevsky and Lin 2012a, b). The nomenclature and classification follow Tavakilian and Chevillotte (2024).

The beetles were examined using an Olympus SZX7 stereomicroscope and a Keyence VHX-7000 digital microscope at the Museum and Institute of Zoology, Polish Academy of Sciences, Poland (MIZ). Additionally, several specimens were examined and imaged using an Hitachi S-3400 N SEM at MIZ. Most of the photographs of the habitus were taken with a Keyence VHX-7000 digital microscope, the remaining ones were taken with a Canon EOS 50D digital SLR equipped with a Canon 100 mm f/2.8 USM Macro lens and subsequently stacked using Zerene Stacker v.1.04 (www.zerenesystems.com) software. All plates were prepared using GIMP v.2.10.10 (www.gimp.org).

Molecular analysis

Seventy specimens of *Eodorcadion* (23 taxa), three of *Dorcadion* (three species), and one of *Lamia* were successfully sequenced for one to four of the following molecular markers: *COI*, *CAD*, *ITS2*, and *Histone 3* (Supporting Information, Table S1). Sequences for all four markers were obtained for 30 specimens representing 16 taxa and all subgenera of *Eodorcadion*. In total, 110 new sequences were produced in this study; for 15 taxa these are the first available *COI* data.

Part of the laboratory work (barcoding of the specimens with vouchers between LK0019 and LK0084) for extracting, purifying, amplifying, and sequencing the DNA was performed at the Canadian Centre for DNA Barcoding (CCDB, http://www.ccdb.ca), University of Guelph, Ontario, Canada, following their standard protocol. For the tissue submission, a meso-femur was cut open on both ends to expose the muscle tissue and then partly crushed with forceps and placed in a sealed well that contained two drops of 95% ethanol on a standard 96-well microplate. Thirty-four specimens from both ethanol-preserved and dried materials were successfully sequenced for a 570–658 bp DNA barcoding fragment. The obtained sequences and additional relevant information, such as the specimen images, primers, gel images, and trace files, were uploaded to the 'Barcode of Life Database' (BOLD, http://www.boldsystems.org).

The authors performed additional laboratory work to attain *COI* sequences and the three nuclear markers from the remaining samples. We used the DNeasy column extraction kit (Qiagen, Hilden, Germany) to extract DNA from ethanol-preserved and dried samples. Usually, whole-leg tissue was taken from adult beetles, dried to remove ethanol, and incubated overnight at 55°C with lysis buffer. DNA was eluted twice into 150 µL (for dried specimens, first into 100 µL, then into 50 µL) with Qiagen buffer AE. Four gene segments were sequenced: ~940 bp of nuclear protein-coding rudimentary (CAD), ~660 bp of mitochondrial protein-coding *COI*, ~490 bp of nuclear ribosomal internal transcribed spacer 2 (ITS2), and ~330 bp of nuclear Histone 3 (*H3*), totalling ~2500 bp of aligned sequence data. These gene regions have proven useful for taxonomic discrimination in Cerambycidae and, especially, in Dorcadionini (Dascălu *et al.*, 2022, Gorring and Cognato 2023, Karpiński *et al.* 2023, Caba and Dascălu 2024). Polymerase chain reaction (PCR) amplification was carried out using published primers and newly designed primers specific to the Cerambycidae *COI* barcode region (Table 1). The PCR conditions for *CAD* followed Karpiński *et al.* 2021. For the *ITS2* and *H3* master mix, we used 2 µL of 10× PCR buffer, 2 µL of 25 mM $MgCl_2$, 4 µL of Promega 5× green GoTaq flexi buffer, 0.3 µL of 100 mM dNTP solution, 1.25 µL of each primer (10 µM), 0.2 µL Taq polymerase (5 u/µL, Qiagen Hot Start Taq DNA Polymerase), 1 µL DNA template, and 13 µL of molecular-grade water. For several samples that failed on the first attempt, a second attempt was made by using a higher volume of DNA template

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Table 1. Primer sequences used for PCR amplification.

Gene	Primer name	Direction	Sequence	Reference
COI	LCO1490	F	GGTCAACAAATCATAAAGATATTGG	Folmer *et al.* 1994
COI	HCO2198	R	TAAACTTCAGGGTGACCAAAAATCA	Folmer *et al.* 1994
COI	bycF	F	TTTCAACWAACCAYAAAGATATTGG	Karpiński *et al.* 2023
COI	bycR	R	TAAACTTCWGGATGWCCAAAAAATC	Karpiński *et al.* 2023
CAD	CD338F	F	ATGAARTAYGGYAATCGTGGHCAYAA	Moulton and Wiegmann 2004
CAD	CD668R	R	ACGACTTCATAYTCNACYTCYTTCCA	Wild and Maddison 2008
CAD	CD688R	R	TGTATACCTAGAGGATCDACRTTYTCCATRTTRCA	Wild and Maddison 2008
ITS2	5.8S_cbgp_F	F	TCGATGAARRMCGCAGYDAAHTG	Dascălu *et al.* 2022
ITS2	28S_cbgp_R1	R	GATATGYTTAAATTCRGSGGGT	Germain *et al.* 2013
H 3	H3F	F	ATGGCTCGTACCAAGCAGACVGC	Colgan *et al.* 1998, 2000
H 3	H3R	R	ATATCCTTRGGCATRATRGTGAC	Colgan *et al.* 1998, 2000

(2 µL) and 12 µL of water. Thermal cycler conditions for ITS2 followed Dascălu *et al.*, 2022. For *H3*, we used the following PCR conditions: 95°C for 15 min, followed by 40 cycles of 94°C for 30 s, 49°C for 30 s, and 72°C for 60 s, with a final extension at 72°C for 10 min. The PCR primers were used for sequencing in all cases, with CD338F and CD668R used for *CAD*. Sequencing was performed at the Research Technology Support Facility at Michigan State University, East Lansing, MI, USA. Some specimens whose sequences appeared problematic were re-sequenced at MIZ using a very similar procedure, for additional verification. The resulting chromatograms were loaded into SEQUENCHER software v.5.1 (Gene Codes Corporation, http://www.genecodes.com) to assemble, manually edit, and export consensus gene sequences.

All the sequences produced in this study are available in BOLD, in the public online dataset '*Eodorcadion III Central Asia LK*' (DS-LKCCAEIII; https://dx.doi.org/10.5883/DS-LKCCAE). They were also submitted to GenBank under the accession numbers: PX108174–PX108254 (Supporting Information, Table S1). All of the voucher specimens reported in this paper (except PSG945, PSG1825, and PSG1826) were deposited and kept frozen in L.K.'s Cerambycid DNA-grade specimen bank at MIZ.

Phylogeny

Taxon coverage

The ingroup included the following taxa of the genus *Eodorcadion*: (in *Ornatodorcadion*) *E. kozlovi* (Suvorov, 1912), *E. oryx* (Jakovlev, 1895), *E. exaratum argali* (Jakovlev, 1889), *E. gorbunovi* Danilevsky, 2004, *E. zichyi* (Csiki, 1901), *E. intermedium* (Jakovlev, 1889), *E. consentaneum* (Jakovlev, 1899), *E. dorcas dorcas* (Jakovlev, 1901), *E. dorcas scabrosum* Namhaidorzh, 1972, *E. egregium egregium* (Reitter, 1897), and *E.* cf. *kaznakovi* (Suvorov, 1912) (this specimen needs to be carefully diagnosed as *E. kaznakovi* is known only from China, while our specimen was collected in Mongolia); (in *Eodorcadion s.s.*) *E. carinatum involvens* (Fischer-Waldheim, 1823), *E. carinatum* cf. *blessigi* (Ganglbauer, 1884), *E. altaicum* (Suvorov, 1909), *E. rubrosuturale kerulenum* Danilevsky, 2007, *E. maurum maurum* (Jakovlev, 1889), *E. maurum australe* Danilevsky, 2014, *E. maurum katharinae* (Reitter, 1898), *E. maurum quinquevittatum* (Hammarström, 1892), *E. maurum sajanicum* (Hammarström, 1892), and *E. tuvense* Plavilstshikov, 1958; (in *Humerodorcadion*) *E. humerale humerale* (Gebler, 1823) and *E. humerale impluviatum* (Faldermann, 1833). *Lamia textor* (Linnaeus, 1758), *Dorcadion* (*Acutodorcadion*) *alexandris* Pic, 1900, and *Dorcadion* (*Acutodorcadion*) *darjae* Danilevsky, 2001 were used as the closest related outgroup, and *Dorcadion* (*Cribridorcadion*) *sokolowi* Jakovlev, 1899 was used to root the trees (see Discussion for more details). The individuals collected in the field were preserved in 96% ethanol. In some cases, dried specimens that had been collected a few years before sequencing and killed with ethyl acetate, were utilized.

Data alignment, partitioning, and phasing

There were several processing and quality checks performed on the gene data. Within SEQUENCHER, chromatograms were assembled into contigs, and the primer regions were trimmed. Bases of low quality or that conflicted between forward and reverse reads were manually edited, and IUPAC ambiguity codes were added at ambiguous or heterozygous sites. After export from SEQUENCHER, each gene was aligned using MAFFT v.7 (Katoh and Standley 2013). Gene matrices were then constructed by importing the aligned sequence files into MESQUITE v.3.7 (Maddison and Maddison 2019). Here the protein-coding genes were also translated to check for stop codons. The concatenated dataset of ~2500 bp sites was analysed in PartitionFinder v.2.1.1 (Lanfear *et al.* 2017), using the greedy search algorithm with AICc as the selection metric, and comparing unlinked and linked branch lengths. Models were reduced to those not combining gamma+I, as that interaction may cause problems (Yang 2014). The lowest AICc scheme partitioned the data into nine subsets with six models—TRN+G: COI_pos1, COI_pos3, CAD_pos1, H3_pos1; TRN+I: COI_pos2; TRN: H3_pos3; HKY+I: CAD_pos2, CAD_pos3; JC: H3_pos2; HKY+G: ITS2.

Phylogenetic analysis

Bayesian inference (BI) for the concatenated *COI*, *CAD*, *ITS2*, and *H3* dataset was run with MrBayes v.3.2.6 (Ronquist *et al.* 2012) on CIPRES Science Gateway v.3.3 (Miller *et al.* 2010), using two runs with four Markov chain Monte Carlo (MCMC) chains each (one cold). Sequences were partitioned according to the best partitions and model determined with PartitionFinder 2. It was run for 10 million generations, with sampling every 1000 generations. Stationarity and convergence were evaluated by deviation of split frequencies <0.01, potential scale reduction factor values ~1.00, and effective sample size (ESS) >200 as measured in TRACER v.1.6 (http://beast.community/tracer). The tree files were summarized with the sumt command in MrBayes with a

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burn-in of 25% to produce a consensus tree. The obtained tree was viewed and manipulated in FigTree v.1.4 (https://github.com/rambaut/figtree). We considered Bayesian posterior probability from MrBayes (PP) 0.95–1 to be strongly supported and 0.85–0.94 to be moderately supported. Nodes with PP < 0.85 were considered to be unsupported. Any lower (unsupported) values and values for internal clades (for specimens of the same species/subspecies) have been removed from the edited tree for clarity.

Maximum likelihood (ML) analysis for the concatenated *COI*, *CAD*, *ITS2*, and *H3* dataset was conducted using IQ-TREE v.1.6.10 (Nguyen *et al.* 2015) on the IQ-TREE web-server (Trifinopoulos *et al.* 2016). The run was conducted with edge-proportional partition models (–spp). Branch support was estimated using 1000 ultrafast bootstrap replicates (Minh *et al.* 2013), with the 'bnni' approach to reduce the risk of overestimating support values (Hoang *et al.* 2018) and an increased value of maximum number of iterations to stop (–nm 10000). Data partitions and models of sequence evolution for ML analysis were also assessed in IQ-TREE prior to phylogenetic analysis. We considered ML bootstrap values (BS) 90–100 to be strongly supported and 75–89 to be moderately supported. Nodes with BS < 75 were considered to be unsupported. Any lower (unsupported) values and any values for internal clades (for specimens of the same species/subspecies) have been removed from the edited tree.

In addition, maximum parsimony (MP) analysis for the concatenated *COI*, *CAD*, *ITS2*, and *H3* was performed with PAUP* v.4.0b10 (Swofford 2002) using heuristic searches with tree bisection-reconnection (TBR) branch swapping and 100 random addition sequence replicates with 500 trees limit. Bootstrap support (mpBS; Felsenstein 1985) was estimated with 1000 bootstrap replicates, each with simple addition sequence searches with 500 trees limit.

Analyses of the intra-and interspecific *COI* genetic distances were conducted in Mesquite using a *p*-distance model.

RESULTS

Phylogenetic estimation

All three inference methods (ML, BI, and MP) returned similar topologies (Figs 2, 3; Supporting Information, Fig. S1, respectively). Monophyly of the genus *Eodorcadion* (in accordance with the current nomenclature, i.e. with inclusion of *Humerodorcadion*) was supported in all analyses; in BI with strong support (PP 0.95) and in ML with moderate support (BS 87). However, if *Humerodorcadion* were to be treated as a separate genus (see subsection 3.3 for more details), the support for *Eodorcadion* sensu nov. would be stronger, ML (BS 98). While BI analysis failed to resolve most *Eodorcadion* subgenera, *Humerodorcadion* has been clearly separated from them (Fig. 3), which is another signal to elevate

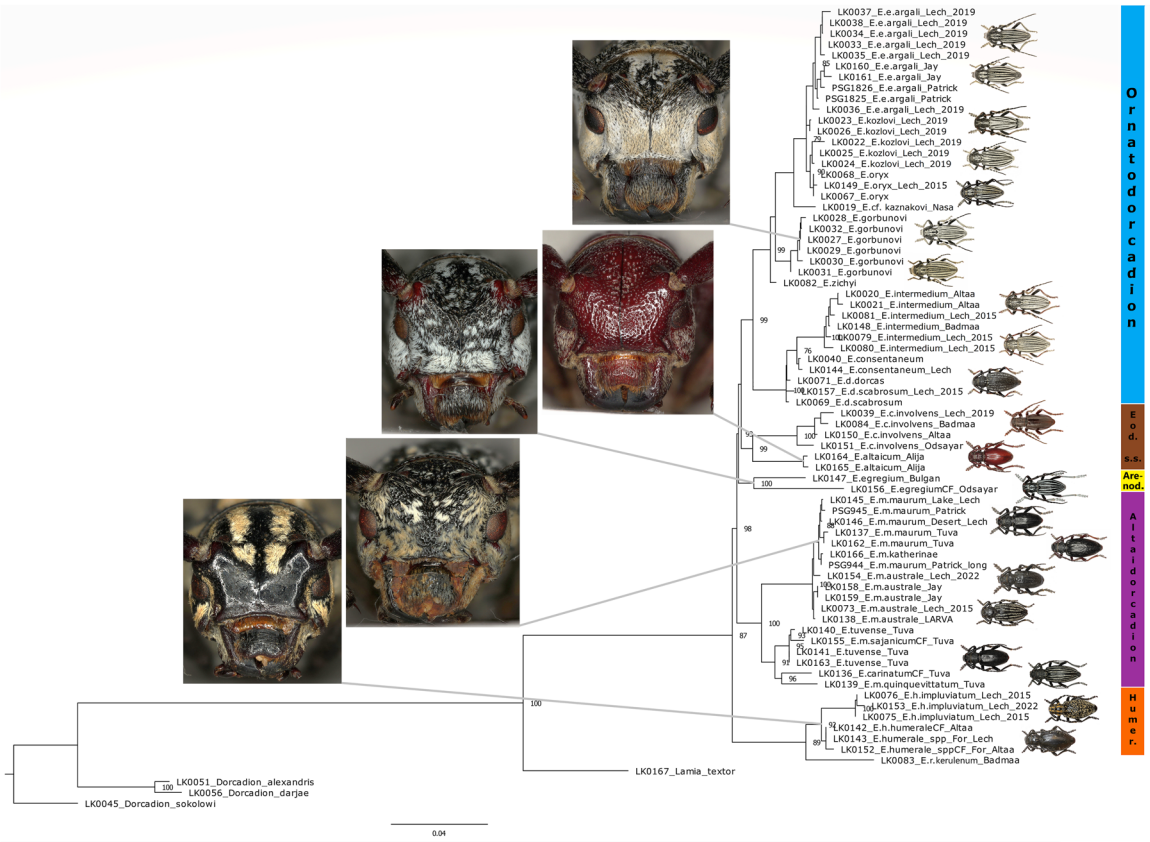

Figure 2. Maximum likelihood phylogeny of the examined *Eodorcadion* taxa obtained from analysis of the concatenated *COI*, *CAD*, *ITS2*, and *H3* matrix. ML bootstrap scores (BS) are indicated adjacent to main nodes [nodes with BS < 75 (unsupported) and values for internal clades for specimens of the same species/subspecies are not presented]. Subgenera of *Eodorcadion* sensu nov. and *Humerodorcadion* **stat. nov.** are marked by colours: blue—*Ornatodorcadion*; brown—*Eodorcadion s.s.*; yellow—*Arenodorcadion* Karpiński **subgen. nov.**; purple—*Altaidorcadion* Karpiński **subgen. nov.**; orange—*Humerodorcadion* **stat. nov.**

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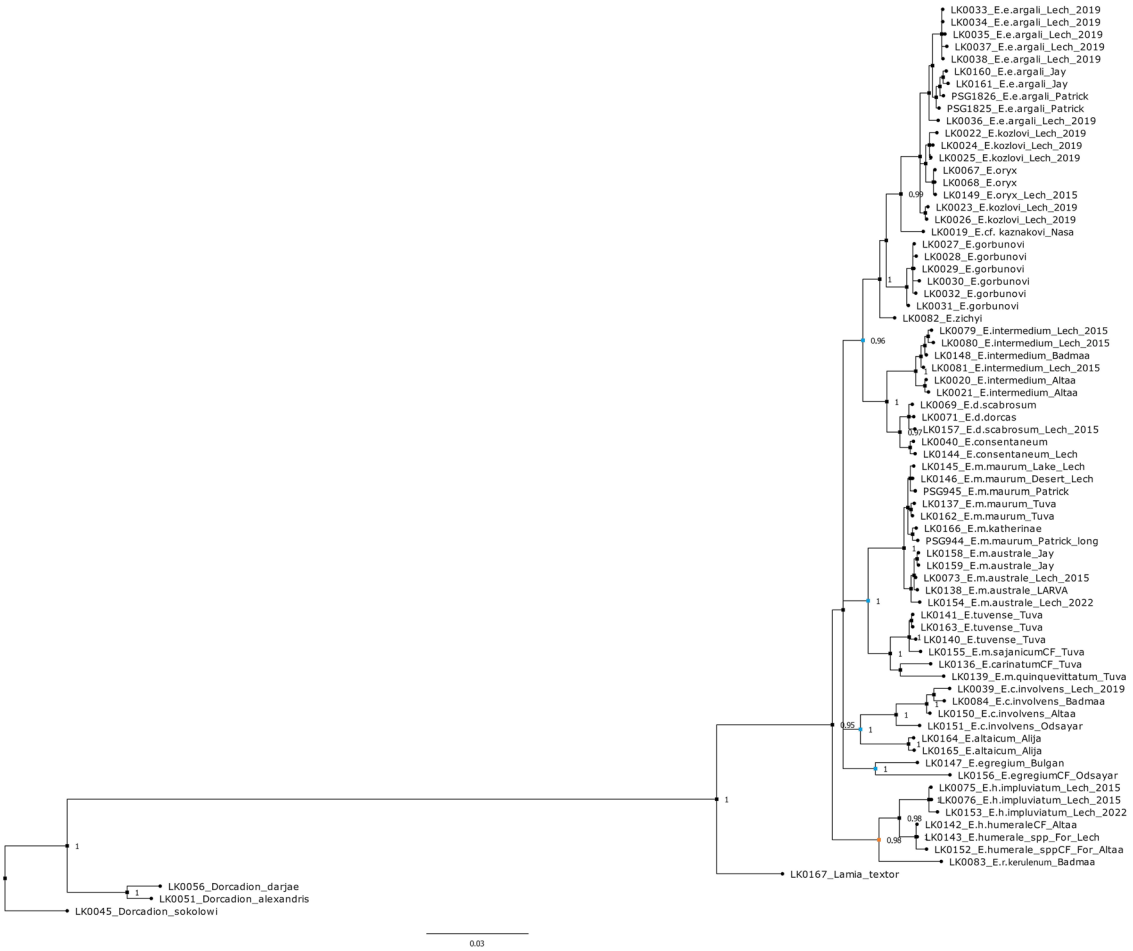

Figure 3. Bayesian phylogeny of the examined *Eodorcadion* taxa obtained from analysis of the concatenated *COI*, *CAD*, *ITS2*, and *H3* matrix. BI posterior probability (PP) is indicated adjacent to main nodes [nodes with PP < 0.85 (unsupported) and values for internal clades for specimens of the same species/subspecies are not presented].

this taxon to genus rank. *Humerodorcadion* was recovered as monophyletic with strong support in BI (PP 0.98) and moderate support in ML (BS 89). Surprisingly, besides two subspecies of the type species *E. humerale*, this group also included *E. rubrosuturale*, which is morphologically close to *E. carinatum* (type species of the subgenus *Eodorcadion s.s.*). When this species was excluded (see subsection 3.2 for more details), also *Humerodorcadion* received strong support in ML (BS 92).

A few strongly supported clades emerged: clade I (corresponding to the currently recognized subgenus *Ornatodorcadion*), clade II (corresponding to the nominate subgenus *Eodorcadion s.s.*), and two further groups: clade III comprising '*E. maurum* species-group', and clade IV comprising only *E. egregium*. In ML inference (Fig. 2), all these groups were resolved and received strong support (BS: 99, 99, 100, 100, respectively). The topology was as follows: (*E. maurum* group + (*E. egregium* group + (*Ornatodorcadion* + *Eodorcadion s.s.*))). In BI inference (Fig. 3), the same main clades were found as a polytomy (see Discussion for more details). In MP inference (Supporting Information, Fig. S1), the same clades were recovered, and similarly *Humerodorcadion* was sister to all remaining *Eodorcadion* groups.

In clade I (*Ornatodorcadion*), two distinct groups emerged with strong support (BS 99/100; PP 1/1). The first one included *E. exaratum*, *E. kozlovi*, *E. oryx*, *E. gorbunovi*, *E. zichyi*, and one more taxon (cf. *E. kaznakovi*). *Eodorcadion zichyi* was revealed to be the most distantly related species in this group, followed by *E. gorbunovi*. All three analyses showed the same topology for this clade, except for one difference in MP analysis—*E. gorbunovi*, not *E. zichyi*, was placed as sister to all remaining taxa. Furthermore, three morphologically distinguishable species (*E. exaratum*, *E. kozlovi*, and *E. oryx*) showed a polytomy at their shared node, whereas the latter two species were additionally intermixed. The second distinct group in *Ornatodorcadion* consisted of *E. intermedium*, *E. consentaneum*, and *E. dorcas*. In this case, the main difference between ML and BI analyses was a polytomy at the initial node in the former analysis, whereas in the latter, *E. consentaneum* was clustered with two subspecies of *E. dorcas*, sister to *E. intermedium*. MP inference supported BI phylogeny.

In clade II (*Eodorcadion s.s.*), all three methods returned the same topology—*E. carinatum* (type species of the genus) was clustered together with morphologically similar *E. altaicum* (BS 99; PP 1). It is worth emphasizing that for *E. carinatum*, the Mongolian subspecies *E. c. involvens* (from central-northern Mongolia) was tested, while *E. altaicum* is distributed over a thousand kilometres to the west, in eastern Kazakhstan. Interestingly, in the MP analysis, the discussed clade was recovered as sister to the

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combined clades I (*Ornatodorcadion*) and III (represented by *E. egregium*) (Supporting Information, Fig. S1), even though morphologically representatives of *Eodorcadion s.s.* are significantly different from the above-mentioned groups. Moreover, in the ML analysis, *Eodorcadion s.s.* was revealed as closest to *Ornatodorcadion*, while the *E. egregium* clade was found to be sister to the two former clades (Fig. 2). Although the first hypothesis did not receive sufficient support, the second one, (*E. egregium* + (*Ornatodorcadion* + *Eodorcadion s.s.*)), was strongly supported (BS 93). On the other hand, BI analysis did not resolve the relationships between these major clades (Fig. 3).

Clade III included only one species (*E. egregium*) represented by two specimens and, although the difference in *COI* genetic distance between them was exceptionally large (see Discussion for more details), they were clearly clustered together in all analyses. The phylogenetic relationship of clade III to clades I and II was discussed above.

Clade IV consisted of the '*E. maurum* species-group', distributed in north-western Mongolia and Tuva in southern Russia. It was recovered as sister to all remaining clades in ML and MP inferences, whereas in BI a polytomy was obtained at the major node for *Eodorcadion* sensu nov. Two distinct groups emerged within the clade, receiving strong support. The first (BS 100, PP 1) included *E. maurum maurum*, as well as two more of its six (five tested) currently recognized subspecies—*E. m. australe* and *E. m. katharinae*. The second (BS 91, PP 1) group consisted of *E. tuvense* and two further subspecies of *E. maurum: E. m. quinquevittatum* and *E. m. sajanicum*. Interestingly, the latter taxon was found to be much more closely related to *E. tuvense* than to *E. maurum*, which indicates the need to elevate *E. sajanicum* to the rank of species or consider a subspecific relationship with *E. tuvense*. On the other hand, *E. m. quinquevittatum* was shown to be strongly distinct from both *E. maurum* and *E. tuvense*. Although it was clustered together with one specimen representing '*E. carinatum* species-group' (LK0136), due to a potential problem with this sample, the latter should be ignored in this study (see Discussion for more details).

One of the most significant results of this study was revealing that *Lamia* (*L. textor*, type species of Lamiini and Lamiinae) renders Dorcadionini paraphyletic. *Eodorcadion* is more closely related to *Lamia* than to *Dorcadion* (subgenera *Acutodorcadion* and *Cribridorcadion* were tested), even for taxa from a geographically close region (*Eodorcadion* taxa from Mongolia and *Dorcadion* taxa from Kazakhstan and Kyrgyzstan). The findings of this study expand the evidence supporting the need for synonymy of a non-monophyletic Dorcadionini with Lamiini (see Discussion for more details).

Cytochrome c oxidase subunit I genetic distances

The *COI* genetic distances within and between all studied taxa calculated using uncorrected *p*-distance are presented in the Supporting Information, Table S2. *Humerodorcadion* was found to be significantly less closely related to all other *Eodorcadion* taxa (mean distance: 8.94%; median distance: 9.11%) than they were to each other (mean distance: 5.79%; median distance: 6.56%). These calculations did not include *E. rubrosuturale* as, although they were grouped in the same clade in the phylogeny, this species was also not closely related to the remaining *Humerodorcadion* taxa studied (mean distance: 8.35%). Within *Eodorcadion* sensu nov., the most closely related were clade I (*Ornatodorcadion*) and clade II (*Eodorcadion s.s.*) (mean distance: 7.05%; median distance: 7.05%), followed by clade I and clade IV ('*E. maurum* species-group') (mean distance: 7.08%; median distance: 7.06%), while the highest genetic distance was found between clade I and clade III (*E. egregium*) (mean distance: 7.97%; median distance: 7.89%).

As a reference, *Eodorcadion* sensu Danilevsky *et al.* (2005) (including *Humerodorcadion*) was clearly less closely related to *Dorcadion* (mean distance: 13.58%; median distance: 13.55%), even compared to *Lamia* (mean distance: 9.95%; median distance: 9.63%). Thus, the genetic distance between *Eodorcadion* sensu nov. and *Humerodorcadion* was comparable to that between *Eodorcadion* sensu Danilevsky *et al.* (2005) and *Lamia* (mean distance: 8.94% vs. 9.95%, respectively). Finally, the *COI* genetic distance between *Lamia* and *Dorcadion* was 13.51% (mean and median), almost the same as that between *Eodorcadion* sensu Danilevsky (2020) and *Dorcadion*. This is another clear indication (obviously related to the phylogenetic position discussed before) for the synonymy of the tribe Dorcadionini. The remaining genetic distances concerning lower-level taxa (species groups, species, and subspecies), along with relevant taxonomic changes, will be discussed in subsequent works.

Taxonomy changes

Synonymy of Dorcadionini

The results obtained in this study, particularly the phylogenetic position of the genus *Lamia* (type genus of Lamiini and Lamiinae) within Dorcadionini, as well as the clearly lower *COI* genetic distance between *Lamia* and *Eodorcadion* (with or without *Humerodorcadion*) than between *Eodorcadion* and *Dorcadion*, unequivocally support for the first time the synonymy of the tribe Dorcadionini:

Tribe: Lamiini Latreille, 1825. Type genus: *Lamia* Fabricius, 1775.
= Dorcadionini Swainson, 1840. Type genus: *Dorcadion* Dalman, 1817. **syn. nov**.

Elevation of Humerodorcadion

We propose to elevate *Humerodorcadion* to generic rank based on its position in the obtained phylogeny (sister to all remaining *Eodorcadion* clades in all analyses), as well as its clearly higher *COI* genetic distance to *Eodorcadion* sensu nov. compared to that within remaining taxa of *Eodorcadion* (among other main clades = subgenera of *Eodorcadion*). Moreover, after analysing the obtained phylogeny, we performed a morphological analysis to confirm its molecular distinctiveness. We found that *Humerodorcadion humerale* **comb. nov.** had the frons covered with punctures of a completely different nature compared to other studied *Eodorcadion* species. The punctation was definitely smaller, denser, and more regular (Fig. 4A). In addition, this species had a unique type of coloration of the elytra, which did not form the longitudinal stripes very common in *Eodorcadion* sensu nov., but instead irregular clusters of yellow or whitish pubescence. An important characteristic for the elevation of *Humerodorcadion* is the structure of its endophallus presented and discussed in another work (see Discussion for more details).

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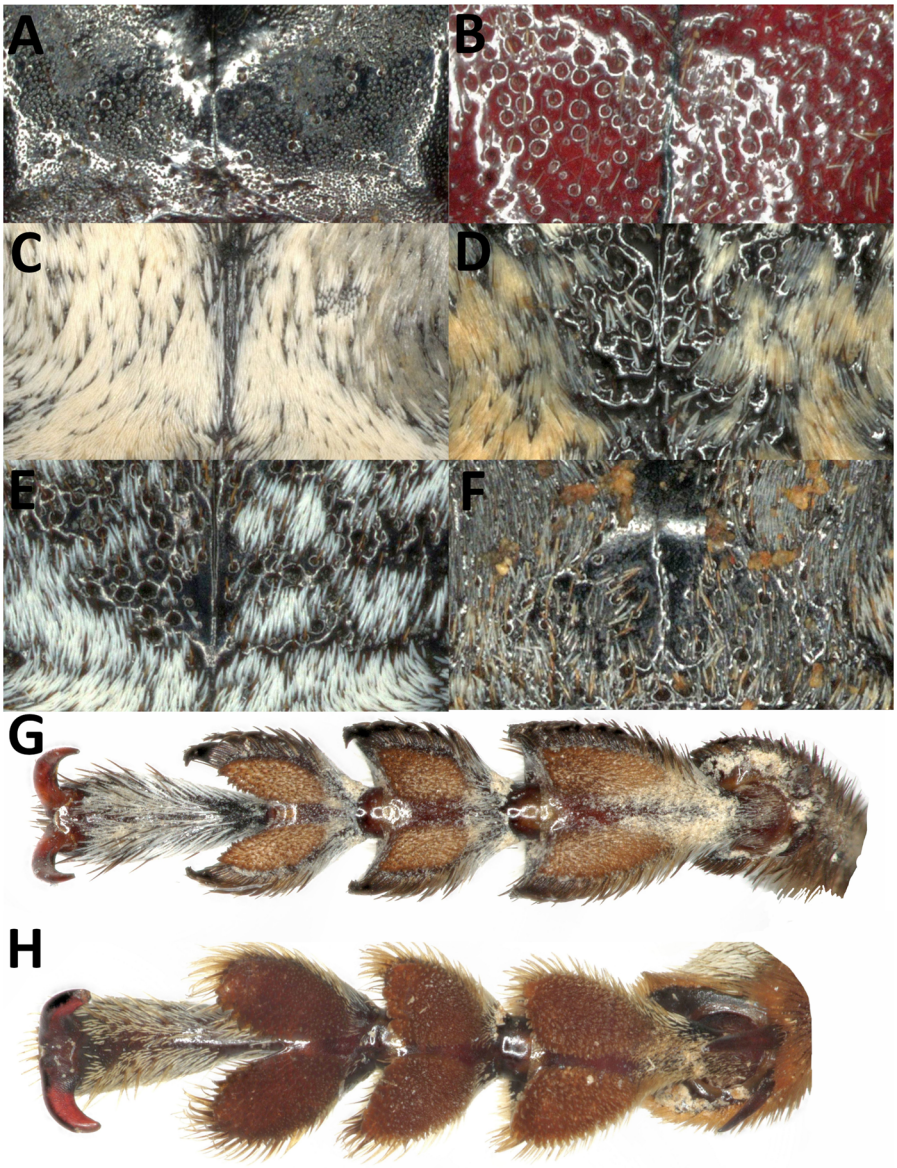

Figure 4. Diagnostic morphological characters in the genera *Eodorcadion* sensu nov. and *Humerodorcadion* **stat. nov.** A–F, punctation of frons. A, *H. humerale* **comb. nov.**; B, *E.* (*E. s.s.*) *altaicum*; C, *E.* (*Ornatodorcadion*) *intermedium*; D, *E.* (*Altaidorcadion* Karpiński **subgen. nov.**) *maurum katharinae*; E, *E.* (*Arenodorcadion* Karpiński **subgen. nov.**) *egregium*; F, *E.* (*E. s.s.*) *glaucopterum*. G, H, front tarsal pads. G, *E.* (*Arenodorcadion* Karpiński **subgen. nov.**) *egregium*; H, *E.* (*Ornatodorcadion*) *gorbunovi*.

Genus: *Humerodorcadion* Danilevsky *et al.*, 2005. **stat. nov**.
= *Eodorcadion* (*Humerodorcadion*) Danilevsky *et al.*, 2005.
Type species: *Dorcadion humerale* Gebler, 1823 (raised to genus rank).

Establishment of two new subgenera

Four well-defined clades were revealed in the phylogeny. In ML inference, all of them received strong support; at the same time, in BI inference, the same main clades could not be resolved (unlike *Humerodorcadion*), which may be due to rapid speciation and indicate a roughly similar time of separation. Two of these clades are consistent with the current taxonomy, while the other two should be considered as new subgenera. In order to verify this hypothesis and to diagnose new taxa, we performed a morphological analysis. In addition, we analysed the differences in endophallic structures presented by other authors, along with related comments regarding the taxa proposed herein for the new subgenera (see Discussion for more details).

Genus *Eodorcadion* Breuning, 1947
Subgenus *Altaidorcadion* Karpiński **subgen. nov.**
ZooBank [urn:lsid:zoobank.org:act:77A0219B-B30C-4367-9443-EF18ED3A7F18]
Type species: Eodorcadion maurum (Jakovlev, 1889).

Diagnosis: Altaidorcadion Karpiński **subgen. nov**. can be distinguished from most subgenera of *Eodorcadion* (except *Eodorcadion*

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s.s.) by its distinctly transverse, very wide and short, strongly rugose pronotum, typically without smooth area along the suture, as well as by its rugose, dense, and strong punctation of the head, usually including the frons (Fig. 4D). The new subgenus differs in external morphology from the nominate subgenus (where it was previously placed) primarily in its larger and more robust body, longer and thicker antennae, and more rugose head and pronotum. The elytra of the latter is smooth, leather-like in texture, with almost invisible punctures, which is absent in the new subgenus. Moreover, all taxa of *Eodorcadion s.s.* have a brown body, while representatives of *Altaidorcadion* Karpiński **subgen. nov**. are black (only a small proportion of individuals in several taxa are brownish). Regarding the internal characters, the new subgenus clearly differs in endophallic morphology: the apical mace of the endophallus is exceptionally large, whereas the central trunk is relatively short (Danilevsky *et al.* 2005) (see Discussion for more details).

Etymology: Latin: *Altai*, Altai and *Dorcadion* (generic name; although it should be *Eodorcadion*, all names of previously described subgenera in this genus have been derived from *Dorcadion*). The name is a toponym referring to the Altai Mountains as taxa of this subgenus are generally distributed along the eastern border of the Altai Mountains, in Mongolia and Russia.

Distribution: North-western Mongolia (the Great Lakes Basin of Mongolia) and southern Russia (Tuva). The distribution of *Altaidorcadion* Karpiński **subgen. nov**. appears to almost exactly coincide with the Great Lakes Basin desert steppe ecoregion (157212 km^2; WWF ID: PA1316), which covers the enclosed basin centred on Uvs Lake, a saline, endorheic basin in north-western Mongolia. Part of the ecoregion extends through the region into Russia, but the range of the new subgenus extends even further north into central Tuva.

Species and subspecies included (8): Eodorcadion maurum maurum (Jakovlev, 1889), *E. maurum australe* Danilevsky, 2014, *E. maurum katharinae* (Reitter, 1898), *E. maurum otgonum* Danilevsky, 2022, *E. maurum quinquevittatum* (Hammarström, 1892), *E. maurum sajanicum* (Hammarström, 1892), *E. ptyalopleurum* (Suvorov, 1909), and *E. tuvense* Plavilstshikov, 1958.

Remarks: The new subgenus is established primarily based on its distinct phylogenetic position and the morphological characters listed above. The average genetic distance of *COI* to the remaining subgenera of *Eodorcadion* was 7.6%, while the average distance to *Eodorcadion s.s.* was 7.0%. Although the distance to the remaining subgenera was slightly lower than in the case of *Arenodorcadion* Karpiński **subgen. nov**., the discussed group differs in several aspects from the nominate subgenus, in which it is currently placed. It is worth noting that in the obtained phylogeny, the discussed clade was sister to *Eodorcadion s.s.* + *Ornatodorcadion* (see Discussion for more details).

Genus *Eodorcadion* Breuning, 1947
Subgenus *Arenodorcadion* Karpiński **subgen. nov**.
ZooBank [urn:lsid:zoobank.org:act:93FA60DC-D02F-4ABE-812E-C712E7E792FD]
Type species: Eodorcadion egregium (Reitter, 1897).

Diagnosis: Arenodorcadion Karpiński **subgen. nov**. can be distinguished from other subgenera of *Eodorcadion* by its overall body shape and very long hindlegs (especially in males), and unique and highly modified tarsal pads. The pads are clearly distinct by the presence of rows of long, dense, thick, stiff setae on their margins, forming a brush, causing the pads to be raised (Fig. 4G). Representatives of the other subgenera have the pads flat and clearly wider, with much shorter and sparser outer bristles (Fig. 4H; *E. gorbunovi* and *Ornatodorcadion*), not forming a compact brush structure, or barely distinguishable from the structure of the pads themselves (*E. carinatum* and *Eodorcadion s.s.*). Moreover, compared to *E. egregium*, the tarsal pads are rather similar among all the remaining subgenera. The new subgenus is clearly distinguished by its characteristic body shape, i.e. short and strongly convex elytra, with very long hindlegs, especially in males. The ratio of the length of the elytra to their width in males was on average 1.67, while in the morphologically closest subgenus *Ornatodorcadion* the elytra were much more slender (1.84, in *E. gorbunovi*). Additionally, the pronotum is relatively very narrow and long (average W : L ratio in males was 1.42; measured to the base of the pronotal tubercles), which differentiates it in particular from *Altaidorcadion* Karpiński **subgen. nov**. (1.6, in *E. maurum maurum*) and *Eodorcadion s.s.*, in which the pronotum is very wide and short, but also from *Ornatodorcadion* (1.51, in *E. gorbunovi*).

Etymology: Latin: *arēnōsus*, sandy or related to sand and *Dorcadion* (generic name; although it should be *Eodorcadion*, all names of previously described subgenera in this genus have been derived from *Dorcadion*). The name is a neuter noun and refers to the species' occurrence in sandy areas and to the morphological adaptations facilitating life in desert and semi-desert regions.

Distribution: South-western Mongolia and north-western China (Xinjiang).

Species and subspecies included (2): Eodorcadion egregium egregium (Reitter, 1897) and *E. egregium kabaki* Kadyrbekov, 2004.

Remarks: The new subgenus is established primarily on the basis of its distinct phylogenetic position and the morphological characters listed above. The average genetic distance of *COI* to the remaining subgenera of *Eodorcadion* was 8.5%, while the average distance among all studied taxa of *Eodorcadion* sensu nov. was 5.8%. Endophallic structures of *E. egregium* were not investigated by Danilevsky *et al.* (2005), therefore, there are no data allowing their comparison with other groups (see Discussion for more details). Tarsal pads and long hindlegs (especially in males that actively seek out females in the habitat) appear to be adaptations to life in desert areas. Interestingly, according to Hua (2002), *E. egregium* is associated with a completely different host plant—*Euphorbia pekinensis* Rupr. (Euphorbiaceae), which emphasizes its bionomical distinctiveness. However, our own observations of this species in Mongolia, in a habitat with grasses of the genus *Achnatherum* P. Beauv., typical for most *Eodorcadion* species, contradict this information.

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DISCUSSION

Molecular-based phylogeny vs. morphology of endophallus

The current classification of the genus *Eodorcadion* is based on taxonomic works pre-dating the application of cladistic methods. The initial division into subgenera was originally based on the presence (in *Ornatodorcadion*) or absence (in *Eodorcadion s.s.*) of a short transverse ridge (cicatrix) near the apex of the first antennomere. According to Danilevsky *et al.* (2005), this character is not very reliable as the cicatrix is barely visible in some species traditionally placed in *Eodorcadion s.s.*or *Ornatodorcadion*. For this reason, the subgeneric division of *Eodorcadion* was not accepted by Plavilstshikov (1958). However, the current organization hinges mainly on the morphology of the endophallic structures (Danilevsky *et al.* 2005), which, although informative and quite reliable, does not allow for a precise determination of a boundary between subgeneric and generic rank, nor for a solid estimation of the relationships between individual natural groups. It should be emphasized that the authors examined only a proportion of the species and subspecies, hence there was a possibility that some taxa not included in the study represent subsequent natural groups (see below). After that publication, no additional changes were made to the subgeneric-level taxonomy of *Eodorcadion*, despite the publication of a morphology-based revision of the entire genus a few years later (Danilevsky 2007).

No phylogeny of *Eodorcadion* has been reconstructed, so our phylogeny is the first picture of the relationships within the genus. Karpiński *et al.* (2023) presented the first molecular data, but this work focused exclusively on the so-called '*Eodorcadion intermedium* species-group', which constitutes a small part of the subgenus *Ornatodorcadion* and turned out to be a polyphyletic species complex, established based on the similarity of the elytral stripes. Interestingly, regarding the number of natural groups (potentially subgenera), our results are remarkably consistent with those obtained earlier by Danilevsky *et al.* (2005), based solely on endophallic morphology. These authors retained two previously functioning subgenera (*Eodorcadion s.s.* and *Ornatodorcadion*) and established a third one—*Humerodorcadion*. However, they also mentioned the possible need to distinguish at least one more subgenus, a natural group with a distinctive morphology of the endophallus.

Regarding *Eodorcadion s.s.* (13 taxa examined, but according to the current taxonomy only eight species), Danilevsky *et al.* (2005) found that in five species traditionally placed in *Ornatodorcadion* (*E. quinquevittatum*, *E. leucogrammum*, *E. maurum*, *E. tuvense*, and *E. ptyalopleurum*, currently representing three valid species), the endophallic structures showed no connection with this subgenus but were overall similar to *E. carinatum* and were, therefore, moved to *Eodorcadion s.s.* At the same time, these authors stated that this group may constitute a separate subgenus (see below). Furthermore, three other species from China (*E. virgatum*, *E. sifanicum*, and *E. glaucopterum*), similar to each other in terms of endophallic morphology, could be assigned to another new subgenus. Because the overall shape of the endophallus in this small group was similar to that of the nominate subspecies, the authors decided to accept all three taxa in *Eodorcadion s.s.*, despite the fact that they had previously been placed in *Ornatodorcadion*. None of these three species were examined in our study (due to significant problems in obtaining DNA-grade samples of Chinese taxa), thus it is possible that at least one more subgenus needs to be defined. In our phylogeny, the subgenus *Eodorcadion s.s.* was identified as clade II, with strong support—*E. carinatum* (the type species of the genus) was clustered together with the morphologically similar *E. altaicum*.

In *Ornatodorcadion* (only eight taxa examined; currently seven valid species), Danilevsky *et al.* (2005) found that the endophallus was quite homogeneous—long and narrow, about as long as the elytra—and, therefore, they did not speculate on possible further subdivisions. However, in a later work, Danilevsky (2007) divided this subgenus into three main groups (based on the elytral pattern): *E. brandti* and *E. egregium* (discussed below), '*Eodorcadion intermedium* species-group', and '*Eodorcadion ornatum* species-group'. Karpiński *et al.* (2023) have already shown that such a division, at least in the case of the second group, is unjustified, since *E. dorcas* and *E. consentaneum*, and, most importantly, *E. intermedium* (initial species for the group, which turned out to be a separate species from *E. kozlovi*), clearly formed a separate clade from all other species included in this group by Danilevsky. In our phylogeny, these results were confirmed, with the additional consideration of more taxa. We identified the subgenus *Ornatodorcadion* as clade I, with strong support—*E. exaratum*, *E. kozlovi*, *E. oryx*, *E. gorbunovi*, *E. zichyi*, and *E.* cf. *kaznakovi* formed the major group, while the second group consisted of *E. intermedium*, *E. consentaneum*, and *E. dorcas*.

The group that, according to Danilevsky *et al.* (2005), was particularly distinctive in endophallic morphology and probably should be considered as a separate subgenus was the already mentioned group of five (currently three) species (*E. quinquevittatum*, *E. leucogrammum*, *E. maurum*, *E. tuvense*, and *E. ptyalopleurum*), herein designated by us as '*E. maurum* species-group'. These authors found that in this group, the apical mace of the endophallus was exceptionally large, whereas the central trunk was relatively short. Traditionally placed in *Ornatodorcadion*, transferred by Danilevsky *et al.* (2005) to *Eodorcadion s.s.*, the '*E. maurum* species-group' was identified in our phylogeny as clade IV (again with strong support) and after expanding with a few additional taxa, was herein described as the subgenus *Altaidorcadion* Karpiński **subgen. nov.** Moreover, the new subgenus was recovered in ML and MP inferences as sister to all remaining clades of *Eodorcadion* sensu nov., which means that *Eodorcadion s.s.* is more closely related to *Ornatodorcadion* than to '*E. maurum* species-group'—this confirms its distinctiveness from the nominate subgenus. The separation of this subgenus also seems to be supported by zoogeographical data; Danilevsky *et al.* (2005), based on many years of field observations, concluded that *Eodorcadion* species of the same subgenus are never sympatric (although two or even three different subgenera may co-occur), and yet species of the '*E. maurum* species-group' were often found together with *E. carinatum involvens*, which represents the nominate subgenus. Our field observations also confirm such relationships. This group included one specimen (LK0136) morphologically resembling the nominate subgenus ('*E. carinatum* species group'). This is currently difficult to explain—we initially suspected contamination with genetic material from another individual, but after careful analysis of the *COI* genetic distance, it turned out that the specimen was not very close to any of the sequenced specimens (the closest

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distance was 4% to *E. tuvense*), although it was clearly *Eodorcadion* DNA. As only *COI* and *H3* sequences were utilized for this sample, there is also a possibility that this individual is, in fact, a hybrid of species from *E. carinatum* and *E. maurum* species-groups (often sympatric), where a female from the latter group bred with a male from the former, hence this individual has mtDNA of a species from '*E. maurum* species-group'. This specimen, ideally along with additional samples, will be examined in detail in subsequent studies, but for now it must be considered a contaminated sample.

The last natural group found in our study was clade III, which included only one species (*E. egregium*) represented by two specimens. It was described in this work as the subgenus *Arenodorcadion* Karpiński **subgen. nov**. Danilevsky *et al.* (2005) did not examine this species, thus there is no data on the morphology of its endophallus. However, these authors did examine *E. brandti*, which, along with *E. egregium*, is distributed in the westernmost part of the genus' range (except *E. carinatum*), and *E. brandti*, and *E. egregium* are relatively similar in external morphology (both lack a humeral hair stripe), leading Danilevsky (2007) to consider them as part of the same group—one of the three groups in the subgenus *Ornatodorcadion* discussed above. Therefore, it is possible that *E. brandti* also belongs to the subgenus *Arenodorcadion* Karpiński **subgen. nov**. Regarding our phylogeny, we consider both examined specimens as *E. egregium* because they were almost identical in external morphology; however, their sequences differed substantially. Although the difference in *COI* genetic distance between them was exceptionally large, reaching 10.2%, they were clearly clustered together in all analyses, with strong support. The DNA material was of very good quality and the sequences obtained were clean. The sequencing of both individuals was repeated and the results were the same. This is a very interesting case, which is currently difficult to explain, having only one individual from each of these two populations. It is possible that these are two copies of the mtDNA genome. It is worth emphasizing that the individuals were collected at sites located several hundred kilometres apart and additionally separated by mountain ranges, but both are located within, or on the edge of, the species' known range. It is possible that a species distributed in such a large area actually represents a complex of cryptic taxa, but even then such a large difference in genetic distance would be exceptional.

It is possible that at least three species from China mentioned by Danilevsky *et al.* (2005) (*E. virgatum*, *E. sifanicum*, and *E. glaucopterum*) may represent another undiagnosed subgenus. In addition to the differences revealed in the endophallic structures, they also differ significantly in their external morphology (especially *E. glaucopterum* with its very short and strongly rounded elytra). However, none of these species were examined in this study and to verify this hypothesis it is necessary to obtain DNA-grade material from China, which seems very difficult in the current geopolitical situation.

Regarding *Humerodorcadion*, we decided to elevate this taxon to generic rank based on the phylogenetic and *COI* genetic distance data, as well as its relatively high morphological distinctiveness. Another important indication was the distinctiveness of its endophallic structures, as shown by Danilevsky *et al.* (2005). This taxon was originally diagnosed and described at subgeneric rank, with the statement that *E. humerale* and *E. lutshniki* constituted a very distinct natural group, characterized by a short, thick endophallus without a central trunk, the pre-apical bulb connected directly to the medial tube, and the apical phallomere not elongated and without appendages (Danilevsky *et al.* 2005, Danilevsky 2007). It should be noted that in the images presented by these authors, taxa of *Humerodorcadion* stand out exceptionally compared to the remaining *Eodorcadion* groups, suggesting differences at a higher taxonomic level.

Monophyly of Dorcadionini

Due to the great similarity in external morphology among the genera of Dorcadionini, e.g. between *Dorcadion* and *Eodorcadion*, until recently there have been no indications or discussions about the possible polyphyly of the tribe, although according to Löbl and Smetana (2010), Dorcadionini seemed to be morphologically differentiated from Lamiini only by adaptive characters. Even other flightless genera of the subfamily Lamiinae, such as *Lamia*, *Morimus* Brullé, 1832, or *Herophila* Mulsant, 1862, clearly differ morphologically among themselves more than Dorcadionini. Only recently Souza *et al.* (2020) addressed this issue and attempted to synonymize Dorcadionini (along with three other tribes) with Lamiini. There are, however, three significant problems with this synonymy. First, in their phylogeny, *Lamia textor* formed a clade with another Lamiini species, *Lamiomimus gottschei* Kolbe, 1886, and this clade was revealed as a sister-clade to a clade consisting of three species of Dorcadionini. This means that Dorcadionini remained monophyletic (which the authors even emphasize on pages 8 and 11) and, therefore, there was not enough evidence to recognize this tribe as a synonym of Lamiini. This leads to the next problem, which was the very poor taxon sampling. In order to make such important taxonomic decisions, the tribes studied should be represented by at least a few species per genus and include representatives of most genera (ideally also subgenera). Only three species of Dorcadionini were included: two species of *Dorcadion*, one of which represented the subgenus *Carinatodorcadion* and the other was an unidentified taxon from Azerbaijan, and one, also unidentified, species of *Iberodorcadion* Breuning, 1943. This seems to be far from sufficient taxon sampling to make a decision to synonymize the tribe. The publication also lacks a formal act of synonymy, in accordance with the International Code of Zoological Nomenclature, which in our opinion makes it null and void. Moreover, the authors stated that there is a possible alternative to retaining Lamiini and Dorcadionini and preserving tribal monophyly—by dividing the current Monochamini into at least three different tribes. This latter argument is not realistic in the context of expanded sampling of the tribe, which would spawn many additional tribes, as shown in Gorring (2019).

The results obtained in this study alone, i.e. the phylogenetic position of the genus *Lamia* within Dorcadionini and the clearly lower *COI* genetic distance between *Lamia* and *Eodorcadion* than between *Eodorcadion* and *Dorcadion*, unequivocally support the synonymy of the tribe Dorcadionini. Nevertheless, it is also worth discussing morphological and bionomic aspects of these groups, starting with a very informative trait, which is the endophallic morphology. Danilevsky *et al.* (2005) have already verified these structures in the flightless genera *Lamia* and *Morimus*. The former genus was found, surprisingly, to be very close to *Iberodorcadion*, while the latter was shown to be very close to *Dorcadion*

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(*Cribridorcadion*). The authors even concluded that *Iberodorcadion* may be descended from Lamiinae other than Dorcadionini. Despite these observations, no decision was made to synonymize the tribe, which could have been a result of a loyalty to the taxonomic system that has been in use for decades, and from the traditional use of external morphology, not allowing for mechanisms of adaptation to environmental conditions. Karpiński *et al.* (2023) concluded that some of the diagnosed 'species-groups' in *Eodorcadion* are polyphyletic assemblages, established on the basis of the pattern of elytral stripes, which appears to be a by-product of ecological adaptation, and does not necessarily reflect phylogenetic relationships. Danilevsky *et al.* (2005) also found, based on the simplicity of the endophallus, that the phylogenetically youngest Dorcadionini was *Politodorcadion* (no differences in the apical phallomere), followed by *Eodorcadion* (lack of the apical membrane).

Considering ecological aspects, it is quite likely that most genera of former Dorcadionini are similar in external morphology because, being flightless, they independently adapted to live in analogous non-forest habitats. Given the presented results, it is reasonable to assume that *Lamia*, a taxon occurring in woodland habitats, shared a common ancestor with *Eodorcadion* after its separation from *Dorcadion*. It is likely that *Lamia* evolved by adapting to development in woody plants (mostly willows) and life in woodland habitats, which is not observed in Central Asian Dorcadionini (except *Humerodorcadion humerale* **comb. nov.**, occurring in very sparse forest steppe zone, but still developing on grass roots). The fact that *Eodorcadion* is considered one of the evolutionarily youngest genera of Dorcadionini seems to support the hypothesis of a recent split from *Lamia*. What also seems significant is the fact that, despite a similar development strategy on similar plants, *Dorcadion* species have a completely different phenology compared to *Eodorcadion* (Danilevsky 2007, Karpiński *et al.* 2018a, b), and even in the same area (e.g. eastern Kazakhstan) they occur in very different seasons (April–June vs. July–August, respectively).

Phylogenetic pitchfork

Bayesian inference performed in this study revealed the same four major clades in *Eodorcadion* (putative subgenera) as in the maximum likelihood inference, but failed to resolve relationships among these lineages. This could have resulted from insufficient data or due to rapid speciation. As for possible lack of data, we obtained a large basal polytomy, a so-called 'phylogenetic pitchfork' on this important branch of the tree, even though four highly informative molecular markers for the group should be sufficient to reconstruct the phylogeny of a single genus, especially for taxa from one relatively small geographical region. Four molecular markers almost identical to ours (with RpP0 instead of *H3*) were used, for instance, to fully resolve the phylogeny of a more diverse (14 described subgenera) and species-rich genus *Mylabris* Fabricius, 1775 (Coleoptera: Meloidae) (Salvi *et al.* 2019). Interestingly, the polytomy occurred at the exact location in the tree where the putative subgenera were found. Therefore, we are more inclined to the phenomenon of rapid speciation rather than to the lack of data.

The term 'rapid speciation' means that multiple speciation events occurred at the same time. One of the best examples of this phenomenon is the cichlid fishes in Lake Malawi—hundreds of species living in a single lake constitute the most extensive recent vertebrate adaptive radiation, with average sequence divergence between species pairs being only 0.1%–0.25% (Malinsky *et al.* 2018). According to Scherz *et al.* (2022) even whole genome sequences can fail to resolve the evolutionary history of the most rapidly radiating lineages in this group, mostly due to incomplete lineage sorting, standing genetic variation, and introgression. These authors, assuming that introgression between clades apparently contributed to the difficulties in phylogenetic reconstruction, could not resolve the basal polytomy, even after restricting the dataset to non-introgressed sites. Taking into account the taxonomic diversity of *Eodorcadion* and other genera of the former Dorcadionini, it can be assumed that the problem in resolving their phylogenies may also be related to the rapid species' diversification, driven by hybridization and introgression (Dascălu *et al.* 2022, Caba and Dascălu 2024), ecological adaptation (Karpiński *et al.*, 2023), and the fact that living over such a vast area (parapatric mechanisms), this flightless group is exceptionally susceptible to relatively small geographical barriers (allopatric mechanisms).

CONCLUSION

Our results are the first to fully support the synonymy of the tribe Dorcadionini with Lamiini and confirm the morphology-inferred monophyly of the genus *Eodorcadion*. However, based on the obtained phylogeny and morphological differences, we have elevated the subgenus *Humerodorcadion* **stat. nov.** to the genus rank, and diagnosed and subsequently described two new subgenera of *Eodorcadion: Altaidorcadion* Karpiński **subgen. nov**. and *Arenodorcadion* Karpiński **subgen. nov**. Molecular data leave no doubt that *Eodorcadion* is more closely related to *Lamia* than to *Dorcadion*. It is worth emphasizing that our phylogeny is remarkably consistent with results previously obtained by other authors based on endophallic morphology. Given the taxonomic complexity of *Eodorcadion* and other genera of the former Dorcadionini, it can be assumed that this group is undergoing a so-called rapid speciation, driven by hybridization and introgression, as well as ecological adaptation, which causes difficulties in resolving their phylogeny.

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SUPPLEMENTARY DATA

Supplementary data are available at *Zoological Journal of the Linnean Society* online.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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DATA AVAILABILITY

The data underlying this article are available in the Barcode of Life Database (BOLD) at https://boldsystems.org/, and can be accessed with https://dx.doi.org/10.5883/DS-LKCCAE.

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