UNIVERSITATEA „ALEXANDRU IOAN CUZA” DIN IAŞI
FACULTATEA DE BIOLOGIE
ȘCOALA DOCTORALĂ DE BIOLOGIE

Multilocus phylogeny of the tribe Dorcadionini (Coleoptera, Cerambycidae): a taxonomic reassessment and implications for biogeography and species diversity

Summary of doctoral thesis

Scientific advisor:
PhD. habil. Mircea Dan MITROIU

PhD student:
Florina Georgiana CABA

Iași
2025

Table of Contents

INTRODUCTION

The family Cerambycidae is included in the order Coleoptera, one of the more diverse group of organisms with over 390000 described species, while the real number of species is very hard to estimate (Bouchard *et al*. 2017). Cerambycidae is one of the biggest family in the order with around 4000 to 5000 genera (Wang, 2017). Cerambycidaes are separated in 9 subfamilies, with almost 90% of the species being classified in the subfamilies Lamiiane and Cerambycinae (Rossa & Goczał, 2021).

Most of the taxa from the family Cerambycidae are in two subfamilies: Lamiinae with over 20000 species and Cerambycinae with over 11000 species, these two subfamilies representing over 90.5% of the diversity of the family. The most speciouse 5 genera from Lamiinae are: *Pterolophia* Newman; *Glenea* Newman; *Sybra* Pascoe; *Dorcadion* Dalman și *Exocentrus* Dejean (Rossa & Goczał, 2021).

Dorcadionini is a hyperdiverse group, representing almost 40% of the longhorned beetles from Europe (Sama *et al*., 2010), in Romania these species are bioindicators for Ponto-Sarmatian steppe habitats unaltered by anthropogenic factors, habitats that are protected by the European Union legislation.

The purpose of this thesis is to reconstruct the phylogeny of the tribe Dorcadionini, to have a better understanding of the interspecific relationships and the evolution of the group. These could show us if the evolutive history is reflected in the actual taxonomic classification.

1. THE STUDIED GROUP AND ASPECTS OF MOLECULAR TAXONOMY

1.1. The tribe Dorcadionini

Dorcadionini is a morphologically diverse Palaearctic tribe in the subfamily Lamiinae (Cerambycidae) and consists of six genera (Danilevsky, 2006, 2020; Danilevsky & Kasatkin, 2007; Danilevsky *et al*., 2005). It is a specious group of flightless coleopterans, representing about 40% of the European longhorn beetles fauna (Sama *et al.,* 2010). Dorcadionini has around 915 described taxa (species and subspecies) (Danilevsky & Lazarev, 2025) that are found mostly in the south of the Palaearctic: from the Iberic Peninsula (*Iberodorcadion* Breuning) to the Korean Peninsula (*Eodorcadion* Breuning). These longhorned beetles are found in steppe-like habitats and prefer open spaces, the adults feed on leaves of grasses (*Poaceae*), and the larvae live in the soil and feed on the roots. Their size is relatively small, from 8 mm to 31 mm.

Presently, the taxonomy of Dorcadionini is stable, with no major changes since 2005 and 2007 when Danilevsky *et al*. (2005) and Danilevsky & Kasatkin (2007), based on the morphology of endophallus from 130 taxa have restructured this tribe in six genera and 12 subgenera (Danilevsky & Lazarev, 2025).

1.2.1. DNA barcoding

DNA barcoding is widely recognised as a valuable molecular tool for a fast and accurate identification of species and biodiversity assessment (Hebert *et al*., 2016; Hendrich *et al*., 2015). In animals, it is based on sequencing and analysing a comparatively short, standardised fragment from the 5′ end of the mitochondrial cytochrome c oxidase I (*COI*) gene (Hebert *et al*., 2003; Valentini *et al*., 2009). This technique relies on the legacy of morphology-based taxonomy as, for the purpose of specimen identification, the barcodes are linked with already known species (Gibbs, 2018). For the method to work well in the identification process, the taxonomic coverage of the sequence database must be almost exhaustive (Ekrem *et al.,* 2007) and the identification success must be tested with new sequences. With the rapid increase in the number of available barcodes, at least theoretically identifications become straightforward: if a specimen is included in a known Barcode Identification Number (BIN), it is then accurately matched to a corresponding species (except cases of barcode sharing); if not, it may represent a potentially new or overlooked species (Gibbs, 2018; Ratnasingham & Hebert, 2013).

1.2.2. Single locus species delimitation methods

Single locus species delimitation methods can be separated in three big categories (Flot, 2025) based on the criteria that they use for species identification: methods based on interspecific distances – ABGD (Automatic Barcode Gap Discovery, Puillandre *et al*., 2012) and ASAP (Assemble Species by Automatic Partitioning, Puillandre, Brouillet & Achaz, 2021); methods based on shared alleles – haplowebs (Flot *et al*., 2010) and methods based on phylogenetic trees – GMYC (generalized mixed Yule-coalescent, Pons *et al*., 2006 și Fontaneto *et al*., 2007) and PTP (Poisson tree processes, Zhang *et al*., 2013) (Dellicour & Flot, 2018).

1.3. Hybridization in evolution

In its broadest sense, natural hybridisation is the reproduction between individuals from genetically distinct populations, resulting in a single or multiple generations of hybrids. Taxonomically these populations may belong to different species (interspecific hybridisation) or can be part of the same species (intraspecific hybridisation), sometimes involving subspecies if these populations are described and named within this taxonomic rank (Chan *et al*., 2019). Hybridisation can also be an intentional process when used by humans, for example, in plant breeding (Kopecky *et al*., 2022), and in this context, the term can be used for the crossing of minimally distinct individuals (Harrison, 1993). This subchapter is discussing the role of interspecific hybridization in generating biological diversity.

1.4. Molecular phylogeny studies in Lamiinae and Dorcadionini

The first paper that analyses the phylogeny of the Lamiinae subfamily was published by Santana-Souza *et al.* (2020) and has a multilocus approach. Later Ashman *et al*. (2022) publishes the first phylogenetic analyses of the Australian and Asian Lamiinae. Two yers later, Soydabaş-Ayoub & Uçkan (2024) reconstructs the phylogeny of Lamiinae from the east of Marmara Basin. Li et al., studies in 2024 the monophyly of the subfamily and it's tribes analysing 158 mitochondrial genomes from eleven subfamilies of cerambycides. Lastly, Song et al in January of 2025 published a pylogenomic analysis where they include species from twelve Lamiinae tribes. All these studies have reached the same conclusions as de Santana Souza in 2020 and suggest that the tribe Monochamini should be synonimised with the tribe Lamiini.

1.5. Molecular markers in our study

For a broad and clear image of the evolution of Dorcadionini we have used for our phylogenetic reconstruction both fast evolving molecular markers to see recent species and relatively new events and slow evolving molecular markers to check the relationship between genera and subgenera and resolve old phylogenetic events. The mitochondrial gene fragments (*COI* and *Cytb*) and the Internal Transcribed Spacer 2 (*ITS2*) – a fragment of noncoding ribosomal DNA, are our fast evolving molecular markers. The single copy nuclear markers, being of coding genes – *Wingless*, *Histone 3* and *Elongation Factor 1α* are our slow evolving markers, also the ribosomal gene fragments *18S* and *28S* are slow evolving in this group.

2. MATERIAL AND METHODS

Specimens

The samples used have been collected since 2007, most of them by dr. M.-M. Dascălu and several other researchers from Ukraine, Hungary, Moldova, Bulgaria, Greece, Czechia, Azerbaijan, China, Kazakhstan, Iran, Georgia, Armenia, Turkey, Russia and Croatia. The complete list of the 263 analysed samples is presented in Table 2.1. in the thesis, 17 of those have been eliminated from our study because of low quality DNA.

DNA amplification, sequencing and data depository

Being comparatively large beetles, we used tissue samples extracted from each specimen: the abdomen was dissected, and the muscular tissue and gonads were stored in 96% ethanol. DNA was extracted using Chelex 100 resin (Bio-Rad Laboratories) from a small piece of tissue as described in Fusu and Ribes (2017). When dry mounted specimens were used, DNA was extracted from one leg using a nondestructive protocol based on the DNeasy Blood & Tissue Kit (Qiagen) as described in Cruaud *et al.* (2019). After extraction, the leg was glued back to the voucher.

All the samples were amplified using standard 25 ul reactions, using the primers from Table 2.2 from the thesis. If the sample was low quality and it could not be amplified using the standard reaction, we optimised the PCR by using more or less DNA solution (between 1 and 6 ul), or we added 6,25 ul trehalose 25% for each sample to improve the reaction, or for samples with degraded DNA we used a different type of Master Mix with a different enzyme – LongAmp ® Taq 2× Master Mix from NEB. We also did semi-nested PCR to get short fragments of COI sand 28S, and nested PCR for short ITS2 fragments using original primers. All reactions were checked with a 1% agarose electrophoresis, and for some samples we observed that we also amplified the pseudogene because there were multiple bands for sample on the electrophoresis. For these samples, we used a gel purification kit – Zymoclean™ Gel DNA Recovery Kit from Zymo Research, and after we reamplified the samples with a standard reaction and 8 ul purified PCR product and we lowered the number of thermal cycles from 40 to 15 or 20.

The PCR products were purified with polyethyleneglycol (PEG) using a protocol based on Paithankar and Prasad (1991).

Sequence alignment and genetic distances

PCR products were sequenced at Macrogen Europe, and double-stranded sequences were assembled using Pregap4 v.1.5 and Gap v.4.10 in the Staden Package (Bonfield *et al*., 1995). For three specimens that showed on the ITS2 trace files double-peaks characteristic of heterozygous indels, individual haplotypes were resolved using Indelligent v.1.2 (Dmitriev & Rakitov, 2008) and by direct comparison with homozygous individuals.

All the sequences were aligned with the Clustal W algorithm as implemented in MEGA X (Kumar *et al*., 2018) and pairwise distances and K2P distances were calculated for the COI sequences also in MEGA X. For ITS2, the alignment was done in MAFFT v.7 (Katoh & Standley, 2013) on the website of the European Bioinformatics Institute (Madeira *et al*., 2019), followed by minor manual adjustments. The alignments were double checked with SequenceMatrix (Vaidya *et al.,* 2011) and assembled in concatenated nucleotide matrices, one for the mitochondrial markers and for the nuclear markers using Mesquite v.3.70 (Maddison & Maddison, 2021).

For COI, a substitution saturation analysis was performed in DAMBE v.7 (Xia, 2018) as described in Fusu (2017) based on unique sequences and fully resolved sites only. For phylogenetic inferences, we analysed our data under both maximum likelihood (ML) and Bayesian inference (BI), the alignment was delimited by codon position and the best substitution model and partitioning scheme identified using PartitionFinder 2 (Lanfear *et al*., 2016). For the ML analysis, the results from PartitionFinder v. 2.1.1 were used as input for a partitioned analysis with scaled branch lengths in RAxML-NG v.1.1.0 (Kozlov *et al*., 2019). The BI analysis was performed with MrBayes v.3.2.7a (Ronquist et al., 2012). The convergence of parameters was assessed with Tracer v.1.7.2. (Rambaut *et al.,* 2018), ensuring that all ESS were >200. The final trees were imported in FigTree v.1.4.4 (Rambaut, 2010) and TreeViewer (Bianchini & Sánchez-Baracaldo, 2024) and edited in Adobe Illustrator, Inkscape and Adobe Photoshop.

The haplotype networks were reconstructed in PopART v.1.7 (Leigh & Bryant, 2015) with the maximum statistical parsimony method (aka TCS method) (Clement *et al*., 2000).

Molecular clock calibrations

To estimate the time frame for the diversification of Dorcadionini in the study region, we used BEAST v.1.10.4. The model used for the expected branching pattern was either a Yule model (applied to the complete dataset or to a reduced dataset containing one

representative per species) or a coalescent model with constant population size applied to the complete dataset.

Single-locus species delimitations

We used the two popular methods (Luo *et al*., 2018) of single-locus species delimitation, namely General Mixed Yule Coalescent (GMYC) (Fontaneto *et al*., 2007) and Poisson Tree Processes (PTP) (Zhang *et al.,* 2013), both with two versions: single threshold (Pons *et al*., 2006) and multiple threshold (Fujisawa & Barraclough, 2013) versions of GMYC, and both original PTP (Zhang *et al*., 2013) and multi-rate PTP (mPTP) (Kapli *et al*., 2017).

3. RESULTS AND DISSCUTIONS

3.1. Generating and analysing DNA barcode sequences of Dorcadionini from Romania and nearby countries

3.1.1. Results

Alignments

Overall, our DNA barcode library comprises 152 sequences from 24 species of Dorcadionini. For six polytypic species, we barcoded two to three different subspecies (hence, our dataset covers a total of 33 newly barcoded taxa plus other two mined from GenBank. All newly generated barcode sequences, except four, were full length barcodes of 658 bp (the incomplete sequences were 629–654 bp). For *28S*, the alignment contained 29 sequences and 842 positions with sequences varying in length from 783 to 842 and a single indel of 1 bp or 2 bp in the outgroup taxa. For *ITS2*, the alignment of the 31 sequences had 636 positions including gaps, with complete sequences varying in length from 507 to 523 bp.

Phylogenetic analyses

Both the ML and BI trees based on *COI* had similar topologies, with a few notable exceptions. In the BI tree, *D. decipiens* is sister to nominotypical *D. pusillum*, while in the ML tree, it is nested within it. Sister to the (*D. decipiens* + *D. murrayi* + *D. pusillum*) clade is *D. axillare axillare* in the ML tree, while in the BI tree, it is *D. scopolii*. The position of *D. tuleskovi* varies radically between the two trees. Most species were recovered as monophyletic with some exceptions. In the BI tree, all *D. pusillum pusillum* sequences are part of the same clade (though it also includes one *D. pusillum ochrolineatum* and three *D. axillare moldavicum*), while *D. pusillum vasiliscus* and *D. pusillum ochrolineatum* are nested within *D. murrayi*. In the ML tree, *D. pusillum pusillum* is not monophyletic: specimens from Ukraine (Od14 and Od09) are recovered as a distinct basal lineage, while the species is paraphyletic relative to *D. murrayi* and *D. decipiens*.

Dorcadion axillare axillare is monophyletic in both trees, while *D. axillare moldavicum* is polyphyletic since it is nested within both *D. murrayi* and *D. pusillum pusillum*. One specimen, that was identified initially as *D. lineatocolle* and on second thought as *D. lugubre* (lin-Pro1001), is grouped with *D. lineatocolle* instead of clustering with *D. lugubre lugubre*.

Both trees also recover three highly supported, though the exact relationships between the constituent species vary slightly between the two approaches. The first clade has a posterior probability of 1 and a bootstrap probability of 95%, the second a posterior probability of 1 and a bootstrap probability of 100% and the third a posterior probability of 0.97 but a bootstrap probability below 50%.

The *28S* tree recovered only the second group; however, the alignment had low information content. The *ITS2* tree and the concatenated nuclear tree recovered both the first and second groups with high support at least on one tree. No species of the third group was sequenced for the nuclear markers, since they were not involved in mitochondrial-morphological discordances. Similar to the *COI* tree, members of the subgenus *Carinatodorcadion* are nested within the subgenus *Cribridorcadion* with 99 BP and 1 PP. However, the species that were polyphyletic or paraphyletic on the *COI* tree are monophyletic on the concatenated nuclear tree. The exception is *D. etruscum* [sensu Pesarini & Sabbadini (2007)] that is very distinct on *COI* but appears conspecific with *D. lugubre* on the nuclear gene tree. Also, the specimen of *D. lugubre* lin-Pro1001 that clustered with *D. lineatocolle* on the *COI* tree is heterozygous for ITS2, being represented on the tree twice.

Divergence times

On the *COI* dataset including one sequence per species and a Yule model, the substitution rate was estimated (mean ± SD) at 0.0127} 0.0034 substituted sites per million year per lineage (2.54% divergence rate). When all sequences are included, both under a coalescent model with constant population size and under a Yule model, the estimate was 0.0119} 0.0032 (2.38% divergence). Both values are close to the rate provided by Brower (1994), which is considered the universal arthropod mtDNA rate. We obtained quite different age estimates for nodes depending on the composition of the alignment and the modelling of the branching patterns. Most accurate are likely those obtained on the reduced dataset under a Yule model, and the most erroneous are those obtained using the complete dataset under the same Yule model, because most branching events would be better described by the coalescent. The first analysis estimated the root of the tree (the last common ancestor of *Dorcadion*, *Iberodorcadion* and *Neodorcadion*) at 11.8 Mya (95% highest posterior density (HPD) interval 6.05–19.95) while the youngest split, the one between *D. murrayi* and nominotypical *D. pusillum*, was dated at 0.47 Mya (95% HPD interval 0.1–1.06).

Haplotype sharing

On the *COI* phylogenetic trees, there is extensive haplotype sharing between *D. murrayi*, *D. pusillum* and *D. axillare*. However, this involves only three subspecies of the latter two species: *D. pusillum ochrolineatum*, *D. pusillum vasiliscus* and *D. axillare moldavicum*.

In the haplotype network, the haplotypes of *D. murrayi* are connected to the haplotypes of *D. pusillum pusillum* through only four mutational steps, while the other two species included in the analysis are more divergent: a minimum of 19 substitutions separate *D. axillare axillare* from both *D. murrayi* and *D. pusillum pusillum* while *D. decipiens* is 12 substitutions apart from *D. pusillum pusillum*. Five specimens of *D. pusillum ochrolineatum* and *D. pusillum vasiliscus* have mitochondria from the same haplogroup as *D. murrayi*. In *D. pusillum vasiliscus*, we detected only *D. murrayi* mitochondria, while in *D. pusillum ochrolineatum*, one specimen (puSG03) has a haplotype that clustered with those of the nominotypical subspecies. All 16 barcoded specimens of *D. axillare moldavicum* harbour mitochondria that are not of their own species but were captured either from *D. murrayi* (most populations, 13 specimens) or from *D. pusillum pusillum* (the Chirceşti population, specimens axCh1201–axCh1203). In both cases, they have the most common haplotype of the donor species.

Divergence and barcoding gap

Mean intraspecific K2P distances based on *COI* ranged from 0.04% in *D. aethiops* to a maximum of 1.61% in *D. equestre* followed closely by *D. lugubre* at 1.53% and *D. axillare* at 1.34%. The mean divergence between species varies from 0.62% between *D. pusillum* and *D. murrayi* to 16.85% between *D. holosericeum* and *I. fuliginator*. This overlap between the intraspecific and interspecific genetic distances clearly shows that, unfortunately, there is no barcoding gap.

The overlap is even bigger if the maximum intraspecific pairwise distance is used because it can be as high as 5.8% (in *D. equestre*), followed by *D. axillare* (3.65%), *D. lugubre* (2.79%), *D. pedestre* (2.17%) and *D. fulvum* (1.38%). The maximum value is more meaningful for molecular species delimitations than the mean intraspecific distance that is strongly influenced (lowered) by the inclusion in the analysis of numerous identical or very similar sequences. A similar problem that artificially broadens the barcoding gap is its estimation using mean between-species distances rather than minimum distances (Meier *et al*., 2008).

When taxa involved in haplotype sharing are removed from the analysis, the smallest and the largest mean intraspecific distances remain the same, but there is a noticeable drop in the molecular variability for *D. lugubre* to 0.36% and *D. axillare* to 0.40%. The smallest mean distance between species (the one between *D. pusillum pusillum* and *D. murrayi*) increases to 0.77%, but it is still very small and comparable to intraspecific distances; the next smallest distance (the one between *D. murrayi* and *D. axillare*) increases significantly from 0.9 to 3.2%.

Species delimitation approaches

When comparing the various species delimitation scenarios with the morphology and current taxonomy, it appears that out of the two approaches, PTP lumps more, sometimes rightfully so: *D. equestre* is recognised by PTP as a singles species except for *Dorcadion equestre reclinatum*. More frequently, the lumping is unjustified: *D. murrayi* and nominotypical *D. pusillum* are considered as potentially one species by original PTP, plus *D. decipiens* being also included in the same putative species in mPTP. The whole group consisting of *D. aethiops*, *D. etruscum*, *D. pedestre* and *D. kozanii* is considered as one species in mPTP. The original PTP method oversplit only once, in an instance where all other methods identified the sequences correctly as a single species: it delimited a pair of basal sequences from the middle of the distribution area of *D. tauricum* as a potentially distinct species.

On the contrary, the GMYC method tends to oversplit, and especially its multiple threshold variant, that identified as putative species several unique basal but not particularly divergent sequences of *D. pusillum ochrolineatum*, *D. scopolii*, *D. equestre equestre* and *N. exornatum* (GMYCm). Strangely enough, the multiple threshold method outperformed the singe threshold method in correctly assigning one basal sequence of *D. fulvum* to the species and not splitting it (GMYCs and GMYCm).

Discussion

Phylogenetic reconstructions

The substitution saturation analysis of the *COI* sequences indicated limited substitution saturation. Also, there is good correlation between the inferred trees and some of the previously recognised species groups. Depending on the type of BI analysis, *Iberodorcadion* is sister to *Dorcadion* while *Neodorcadion* is a separate clade or *Iberodorcadion* and *Neodorcadion* are sister groups. Members of the subgenus

Cribridorcadion of *Dorcadion* are divided in three highly supported clades, recovered by both inference methods.

The first clade contains species explicitly included by Pesarini and Sabbadini (2007) in the *Dorcadion minutum* species group or morphologically close to them (*D. axillare*, *D. decipiens*, *D. litigiosum*, *D. murrayi*, *D. pusillum*). Resolution is poor for the three species involved in extensive haplotype sharing (*D. axillare*, *D. murrayi* and *D. pusillum*). The group was also recovered on the *ITS2* tree and on the concatenated tree, though species sampling was less dense compared to the *COI* tree.

The second well-supported clade contains species that at least in males are mostly devoid of elytral pubescence: *D. etruscum*, *D. kozanii* and *D. pedestre* species groups proposed by Pesarini and Sabbadini (2007, 2010). This clade surprisingly also contains both species of the subgenus *Carinatodorcadion* included in our study: *D. fulvum* and *D. aethiops* that are thus nested within the subgenus *Cribridorcadion*. They do not form a monophyletic group, but instead, *D. aethiops* is much closer to *D. pedestre*, *D. etruscum* and *D. kozanii*, while *D. fulvum* is well apart. The mean pairwise distance between *D. aethiops* and *D. etruscum* is 3.43%, and that between *D. aethiops* and *D. kozanii* is only 2.66%. This is significantly lower than the maximum intraspecific distance in *D. equestre* (5.8%) and close to that in *D. pedestre* (2.17%). The divergence between *D. aethiops* and (*D. kozanii* + *D. pedestre*) is estimated at 1.6 Mya (95% HPD interval 0.52–2.66 Mya) . On the *28S* tree, *D. aethiops*, *D. fulvum*, *D. etruscum*, *D. lineatocolle* and *D. lugubre* form a polytomy. The tree is not fully resolved because, contrary to our expectations, the fragment was found to be too conserved. On the concatenated nuclear tree, however, *D. aethiops* is sister to *D. fulvum*, as expected from the morphology and from their classification in the same subgenus, indicating its hybrid origin.

The third clade on the *COI* tree contains all other taxa of the subgenus *Cribridorcadion* included in the study, but this group is very heterogeneous, likely because it contains a low sampling of many other species groups. For example, the position of *D. tuleskovi* (*Dorcadion peloponesiumgroup*) on the tree varies greatly between the Bayesian and the maximum likelihood approaches.

Hybridization and haplotype sharing

Most frequently, haplotype sharing between species can be either the result of recent speciation and incomplete lineage sorting or the result of introgressive hybridisation (Funk & Omland, 2003; Sloan et al., 2017; Toews & Brelsford, 2012; Wirtz, 1999). In *D. murrayi*,

D. pusillum and *D. axillare*, the mitochondrial-morphological discordance between species is present only in the areas of range overlap between the taxa. While the populations of *D. pusillum* and *D. axillare* sampled from localities outside the distribution range of *D. murrayi* are genetically distinct (*D. pusillum pusillum* from Republic of Moldova and Ukraine and *D. axillare axillare* from Bulgaria), those in Romania (where the distribution of the three species overlaps) have mostly *D. murrayi* haplotypes.

Evolutionary implications

Introgressive hybridization has been earlier suggested as another potential mechanism that generates diversity in other flightless Cerambycidae like *Mesechthistatus* (Nakamine & Takeda, 2008) or *Morimus* (Hardersen *et al*., 2017), we are providing new evidence in support of this hypothesis. Otherwise, genetic evidence of hybridisation following the secondary contact was revealed for two species or subspecies of *Monochamus* based on microsatellite data (Goczał *et al*., 2020. Hybridisation and possibly introgression was recently documented for two species of *Cerambyx* as shown by the incongruence between mitochondrial sequences and morphology (Torres-Vila & Bonal, 2019).

Even distantly related Dorcadionini species, belonging to distinct lineages and classified in different subgenera, can hybridise. This is strongly suspected in *Iberodorcadion* (Bahillo de la Puebla, 1999), while in *Dorcadion*, it appears that *D. aethiops* is the result of the hybridization between a species of *Dorcadion* (*Carinatodorcadion*) and a yet unknown species of *Dorcadion* (*Cribridorcadion*), since its nuclear sequences are grouped with one subgenus, while its mitochondrial sequence beyond any doubt belongs to the other subgenus.

Small interspecific distances

Introgression is not the only factor making molecular identifications or taxonomic decisions in Dorcadionini challenging. Even if introgression followed by mitochondrial capture is removed from the analyses, geographic structuring within species or presence of distinct species cannot be distinguished based solely on molecules, because some species pairs have very low between-species distances. A threshold of 2–3% was suggested as indicative for species-level divergence (Mutanen *et al*., 2012; Ratnasingham & Hebert, 2007, 2013; Smith *et al*., 2005). In our study, certain sequences from known, well-differentiated species are not sufficiently distinct genetically according to this threshold. Between species distances are comparatively small for the trio *D. aethiops*, *D. pedestre* and *D. kozanii* (2.66–2.88%), and they are even smaller for the trio *D. murrayi*, *D. pusillum pusillum* and *D.*

decipiens (0.77–2.38%). These distances are comparable or below the largest intraspecific distances found in *D. equestre* (5.8%) and *D. pedestre* (2.17%).

There are other documented cases concerning reciprocally monophyletic species with low divergence: *Rhynchites bacchus* (L.) and *Rhynchites auratus* (Scopoli) (Coleoptera, Attelabidae) have a minimum K2P distance of 1.86%; *Harpalus attenuatus* Stephens and *Harpalus rubripes* (Duftschmid) (Coleoptera, Carabidae) show a minimum K2P distance of 1.07% (Hendrich *et al*., 2015); *Bembidion ascendens* K. Daniel and *Bembidion fasciolatum* (Duftschmid) (Coleoptera, Carabidae) show interspecific distances values ranging from 0.49 to 0.82% (Raupach *et al.,* 2016).

Genetic distinctiveness of subspecies

Concerning the ability of the DNA barcodes to distinguish subspecies, we identified both, cases in which subspecies are genetically distinct and cases where they are identical. *Dorcadion equestre* has a very pronounced genetic substructuring that follows closely the accepted subspecific structure, while in *D. axillare* and *D. pusillum*, even if subspecies are genetically distinct from the nominotypical ones, this is not because of genetic divergence but the result of their origin through introgression.

On the other hand, in other three species where we analysed more than one subspecies, no differentiation was found between them. For *D. fulvum*, we sampled from five countries and covered three subspecies but found no geographic substructure, the same for *D. aethiops* sampled from distant areas (Romania and Greece) with representatives of three subspecies. In the case of *I. perezi*, we included only one specimen per subspecies, and they were from the contact area. The morphological differentiation in this case is much larger than that between the subspecies of *D. fulvum* or *D. aethiops*, and they are considered either good species or subspecies depending on the author (Hernandez, 2000; Tome, 2004, 2012), but still the DNA barcode is almost identical. Since low genetic divergence in the DNA barcode between taxa or specimens is not always a proof of conspecificity, due to the possibility of introgression and mitochondrial capture, our treatment of *I. perezi hispanicum* and *I. perezi ghilianii* as subspecies is purely arbitrary and follows Danilevsky (2020). If they are treated as distinct species, we have yet another case of interspecific hybridisation and mitochondrial- morphological discordance.

In the thesis we discused in more details the cases where we found discordances between the taxonomy and genetic data.

3.2. A natural hybrid between *Dorcadion lineatocolle* and *Dorcadion lugubre*

3.2.1. Results

Alignments and haplotype networks

Complete sets of seven sequences were generated for the hybrid, *D. lineatocolle* and both subspecies of *D. lugubre*, for a total of 15 specimens.

The *ITS2* alignment has 521 positions including 23 variable positions, of which all but one are informative. Due to indels, the sequences vary from 508 to 512 nucleotides. There are four indels of a single nucleotide, an indel of two nucleotides, and four indels of three nucleotides. Two of the indels of a single nucleotide are superposed with two of the indels of three nucleotides. *Dorcadion lugubre minkovae* is separated by only two substitutions from *D. lugubre lugubre* and both subspecies are highly divergent from *D. lineatocolle*. The hybrid is heterozygous at this locus, with one allele from *D. lugubre minkovae* and one allele from *D. lineatocolle*.

The *Wg* alignment has 469 positions, 12 are variable and 11 informative. All three taxa are distinct but *D. lugubre minkovae* is closer to *D. lineatocolle* than to *D. lugubre lugubre* (four vs. seven mutational steps, respectively). The hybrid is heterozygous at this locus, with one allele from *D. lugubre minkovae* and one allele from *D. lineatocolle*.

The *EF-1α* alignment has 348 positions, and four of them are both variable and informative. *Dorcadion lugubre minkovae* is closer to *D. lineatocolle* (one mutation apart) than to *D. lugubre lugubre* (two mutations apart); there is also haplotype sharing between the two subspecies of *D. lugubre*. The hybrid is homozygous with both alleles from *D. lineatocolle*.

The *H3* alignment has 328 positions with a single position variable and informative as well. The two subspecies of *D. lugubre* share the same haplotype and are separated from *D. lineatocolle* by a single substitution. The hybrid is heterozygous with one allele from *D. lugubre* and one from *D. lineatocolle*.

The *28S* alignment has 842 positions of which three are variable but none of them is informative. Most beetles, regardless of the species, have the same haplotype, with single individuals from each species being differentiated by a single mutation. The hybrid is homozygous and possesses the most common haplotype.

The two mitochondrial genes are similarly informative. The *COI* alignment has 658 positions, with 67 positions variable and only one non informative. The *Cytb* alignment has

433 positions; 43 positions are variable and 41 of them are informative as well. Due to the high number of substitutions, the mitochondrial genes were not analysed as a haplotype network but only with phylogenetic methods.

Phylogenetic analysis

The mitochondrial phylogenetic trees reconstructed using the concatenated *COI* and *Cytb* markers (both ML and BI), have similar topologies, where *D. lugubre minkovae* is separated into a distinct and very divergent clade, with a posterior probability (PP) of 1 and a bootstrap (BP) of 100. *Dorcadion lineatocolle* and *D. lugubre lugubre* are positioned in two distinct sister clades (BP = 100, PP = 1), with the hybrid individual (linPro1001) included in the *D. lineatocolle* clade. *Dorcadion lugubre lugubre* specimens are genetically very similar, while there is a large variability in *D. lineatocolle*. In the ML tree, the support for the nodes of *D. lineatocolle* and *D. lugubre lugubre* has the BP value of 72 for both, while the PP values in the BI tree are 0.74 and 1, respectively. The only difference between the ML and BI trees is within the *D. lineatocolle* clade, but it involves only sequences on very short branches. All three taxa are monophyletic in both ML and BI phylogenetic trees, and *D. lugubre lugubre* is much more genetically similar to *D. lineatocolle* than to *D. lugubre minkovae*.

In the nuclear concatenated phylogenetic trees (ML and BI) generated using the five molecular markers, the parental species are clustered in two distinct clades. *Dorcadion lugubre* and *D. lineatocolle* are monophyletic with *D. lugubre lugubre* placed together with *D. lugubre minkovae* which is in accordance with the morphology-based taxonomy. The support for the two clades is strong in both the ML and BI tree with a bootstrap value of 100 and a PP value of 1, respectively. The hybrid is present twice on the trees, being heterozygote, with an allele from *D. lineatocolle* and one from *D. lugubre minkovae* for all molecular markers, except for *Ef-1α*. This gene is known to have several copies, but the very small differences between the sequenced alleles make us believe that they are orthologous and not paralogous, especially because in Coleoptera, the paralogous copy is very divergent (Simon et al., 2010). The topologies of the ML and BI trees are very similar, with respect to *D. lugubre*: In both trees, *D. lugubre lugubre* is monophyletic (P = 100, PP = 1); however, it renders *D. lugubre minkovae* paraphyletic.

Genetic distances

The intraspecific genetic distance based on *COI* for *D. lineatocolle* varies from 0% to 1.82%, showing a relatively high divergence between localities. For *D. lugubre lugubre*,

the intraspecific distances are small, 0%–0.46%, presumably because all individuals were collected from the same area and are part of the same meta-population. The two specimens of *D. lugubre minkovae* had identical haplotypes and differ from *D. lugubre lugubre* by 7.3%. Following a BLAST search, the closest species to *D. lugubre minkovae* is *D. aethiops*, GenBank having seven identical or almost identical sequences of this species from Dascălu *et al*. (2022).

Discussion

The parental taxa

The parental species of the hybrid have a distribution confined to the Balkan peninsula: Both species are present in Bulgaria, Greece and Macedonia, but *D. lineatocolle* has a wider distribution, additionally including Albania, Serbia and Montenegro (Danilevsky, 2020).

The hybrid: genetic data

The hybrid has a *D. lineatocolle* mitochondrial haplotype indicating that a female of this species bred with a male of *D. lugubre minkovae* (in animals, mitochondria being inherited only from the mother). In nuclear DNA, the hybrid is mostly heterozygous, with copies originating from the two parental species. In the concatenated phylogenetic tree, the hybrid is present in the middle of the *D. lineatocolle* clade, but is basal in the *D.* lugubre clade, probably because both EF-1α alleles are from *D. lineatocolle.* This suggests that his *D. lugubre minkovae* parent was already slightly introgressed and explains why the hybrid is more similar *to D. lineatocolle*. Further proof for the existence of isolation mechanisms between *D. lugubre* and *D. lineatocolle* is that hybrids are rare: We did not detect ongoing introgression between the parental species, with all 15 examined specimens, except for the hybrid, being concordant for their nuclear genotype, mitochondria, morphological assignment to a species and distribution.

Taxonomy of *Dorcadion lugubre lugubre*, yet another taxon originated from introgression?

The integrative approach to the hybrid and its parental taxa brought new insights on the taxonomy of *D. lugubre*, which depending on the taxonomic authority is vastly different (Danilevsky, 2020; Lazarev, 2023; Pesarini & Sabbadini, 2007, 2010).

Dorcadion lugubre lugubre represents yet another case of mitochondrial–morphological discordance which was only recently discovered in Dorcadionini (Dascălu *et al*., 2022; Karpiński, Gorring, & Cognato, 2023). The nuclear tree is concordant with the

morphology and taxonomy, the two subspecies of *D. lugubre* being grouped together, while in the mitochondrial tree, *D. lugubre minkovae* is very distinct whereas *D. lugubre lugubre* is grouped with *D. lineatocolle* at small genetic distance. The taxonomic rank of *D. lugubre minkovae* as a subspecies of *D. lugubre* (according to Danilevsky, 2020) is sustained by the nuclear genes, all the specimens being placed in the same clade with little divergence. Also, the endophallus of the two subspecies is the same. On *ITS2*, *D. lugubre lugubre* and *D. lugubre minkovae* are very close even if they are distinct, on *H3* and *EF-1α*, the two subspecies share alleles. On *Wg* on the other hand, *D. lugubre minkovae* is closer to *D. lineatocolle* than to *D. lugubre lugubre* (four substitutions vs. seven). On the two mitochondrial genes, *D. lugubre lugubre* is close to *D. lineatocolle* even if it is distinct. The minimum difference in *COI* is 2.58% situated within the murky interval between species and intraspecific variability. For comparison, the largest intraspecific differences in *D. lineatocolle* are 1.82%. These discrepancies likely point to past hybridisation and reticulate evolution of the three taxa. Given the comparatively large divergence in mtDNA, the introgression is not recent or ongoing.

Despite the large divergence in *COI*, we prefer to treat *lugubre* and *minkovae* as subspecies because of the identical endophallus and high similarity or identity on several nuclear markers. We give priority to the nuclear genome and morphology because mtDNA is prone to crossing interspecific boundaries and be misleading as shown in this paper and in Dascălu *et al*. (2022).

Reticulate evolution in Dorcadionini

The mtDNA of *D. lugubre minkovae* is very divergent from the nominal subspecies (at 7.1%), which would mean that these are two different and very distinct species—according to Hebert *et al.* (2003) between two species the value of the genetic distance in *COI* is larger than 2%–3%. Consequently, we searched in GenBank for sequences similar to our *COI* sequences of *D. lugubre minkovae*; the nearest match was *D. aethiops* sequences from Dascălu *et al*. (2022). These results are not surprising because, in our previous study, we discovered that *D. aethiops* (from subgenus *Carinatodorcadion*) has captured mitochondria from an unknown species in the subgenus *Cribridorcadion* (Dascălu *et al.,* 2022). The exact origin of mtDNA of *D. aethiops* remained unknown because of conflicting information between the phylogenetic analyses and genetic distances. The present study narrowed down the `circle of suspects` to the mitochondrial lineage of *D. lugubre minkovae*

with whom *D. aethiops* has a similarity of 97.57% (2.43% divergence), the two being recovered as sister taxa.

This work demonstrates for the first time using molecular markers that a specimen with an atypical and intermediate phenotype is a hybrid, and that *D. lugubre lugubre* is very likely a hybridogenic taxon. Not long ago, Dascălu *et al.* (2022) showed that *D. axillare moldavicum* Dascălu & Fusu, *D. pusillum ochrolineatum* Dascălu and *D. pusillum vasiliscus* Dascălu are hybridogenic taxa. We believe that the morphologically distinct populations originated from introgression with another species are best classified as subspecies to highlight their distinctiveness but at the same time to emphasise that they are not reproductively isolated.

This phenomenon is not limited to the western Palearctic, as Karpiński, Gorring, and Cognato (2023) described the case of introgression in the Mongolian *Eodorcadion intermedium* species group. While in taxa with distinct morphology hybridisation is a well-known fact, in those with cryptic morphologies (such as *Dorcadion*), this phenomenon is much more difficult to detect (Macholan, 2013; Mallet, 2005), and information only starts to accumulate through the introduction of genetic techniques.

3.3. The reconstruction of the multilocus phylogeny of Dorcadionini

3.3.1. Results

Alignments used to test the monophyly of Dorcadionini, Lamiini and Monochamini

We have created a multilocus matrix to determine the monophyly of Dorcadionini, consisting of an alignment of 27 original sequences, with minimum one representalive from each genus and subgenus of Dorcadionini from the larger analysis and 40 sequences imported from GenBank (Table 2.4 from the thesis).

The *COI* alignment has 1548 bp length (shortest sequences have 418 bp), no indels, with 870 conserved sites, 678 variable sites and 587 sites that are informative for parsimony.

The *Cytb* alignment has 1143 bp length, no indels, the shortest sequences have 404 bp, there are 644 conserved sites, 496 variable sites and 395 parsimony informative sites.

The *Elongation factor 1α* alignment has a length of 1326 bp (shortest sequences have 203 bp), with 4 to 25 indels (in the intron), 980 conserved sites, 338 variable sites and 202 parsimony informative sites.

The *Wingless* alignment has a length of 480 bp (the shortest sequences have 431 bp) with 6 to 9 indels (a multiple of 3, so the reading frame of is the same), 288 conserved sites, 184 variable sites and 145 that are parsimony informative sites.

The *Histone 3* alignment has a length of 328 bp (the shortest sequences have 225 and 285 bp), no indels, 210 conserved sites, 184 variable sites and 95 sites informative for parsimony.

The *ITS2* alignment has a length of 677 bp (shortest sequences have 512 bp), with indels typical for a noncoding sequence, 264 conserved sites, 342 variable sites and 215 parsimony informative sites.

The *28S* alignment has a length of 1445 bp (shortest sequences have 334 bp), with 32 to 125 indels, 1118 conserved sites, 273 variable sites and 111 parsimony informative sites.

The *18S* alignment has a length of 1361 bp (shortest sequences have 559 bp) with 1 or 2 indels, 1348 conserved sites, 11 variable sites and 3 parsimony informative sites.

Alignments used to analyse the ingroup relationships

This matrix was constructed from 2 mitochondrial alignments (*COI* and *Cytb*), 3 single copy nuclear alignments (*Ef-1a*, *Wingless* and *H3*) and 3 nuclear ribosomal alignments (*ITS2*, *28S* and *18S*). We have eliminated from this analysis Al2101 (*Acrocinus longimanus*) because it was not close enough phylogeneticly to the rest of our samples.

The mitochondrial alignments are almost complete; *COI* has 112 original sequences plus 15 sequences downloaded from GenBank (Table 2.5 from the thesis). Two sequences are very short – 261 and 317 bp and 75 sequences are complete with 658 bp. This indel originates in a sequence we imported from GenBank. In this alignment there are 343 conserved sites, 316 variable sites and 294 parsimony informative sites.

The *Cytb* alignment has 98 original sequences (one sequence has 405 bp and the rest are 436 bp with a 3 bp insertion and a 1 bp deletion in one sample), with one sequence downloaded from GenBank (Table 2.5 from the thesis). In this alignment there are 186 conserved sites, 247 variable sites and 202 parsimony informative sites.

We have 53 sequences of *Elongation factor 1a*, three of them are incomplete with a length of 248, 304 and 334 bp and 50 complete sequences of 348 bp. From GenBank we downloaded 4 sequences (Table 2.5 from the thesis), the resulting alignment has 222 conserved sites, 126 variable sites and 84 parsimony informative sites.

For *Wingless* we used 64 original sequences, with lengths between 457 and 475 bp with no indels or 3 to 6 indels, and 4 sequences were imported from GenBank (Table 2.5 from the thesis). The alignment has 326 conserved sites, 143 variable sites and 105 parsimony informative sites.

The most nuclear sequences we obtained were of *Histone 3*, more exactly 102 sequences with lengths between 246 and 328 bp with no indels. We also got 5 sequences from GenBank (Table 2.5 from the thesis) and the final alignment has 214 conserved sites, 114 variable sites and 99 parsimony informative sites.

The *ITS2* alignment has 102 sequences with lengths between 484 and 831 bp and with 195 up to 430 indels. We have added 2 sequences from GenBank (Table 2.5 from the thesis), the alignment has 320 conserved sites, 410 variable sites and 265 parsimony informative sites.

The *28S* alignment has 84 original sequences, 25 of them are short (314 to 480 bp, with 13 to 22 indels), the other 60 sequences have between 798 and 862 bp with 18 – 22 indels. We downloaded 7 sequences from Genbank (Table 2.5 from the thesis) that made the total length of the alignment equal to 1084 bp, with 948 conserved sites, 130 variable sites and 64 parsimony informative sites.

The smallest alignment is the *18S* one with 44 sequences, with a short one (448 bp), and the rest with lengths between 874 and 1361 bp, some sequences have 2 indels. There are 1345 conserved sites, 14 variable sites and 4 parsimony informative sites.

Degree of completeness of the dataset

We generated and analysed sequences for 1 to 8 molecular markers from 114 samples. We amplified all 8 markers for 32 samples, 7 markers for 18 samples, 6 markers for 16 samples, 5 and 4 markers for 18 different samples, 3 and 2 markers for 4 different samples and lastly only 4 samples have only one marker. These samples with only one marker were included in the analysis because they were important taxa for the systematics and taxonomy of the group. From GenBank we downloaded a total of 40 sequences (Table 2.4. from the thesis).

The phylogenetic analysis of the individual molecular markers

Before we could reconstruct the multilocus phylogeny, we constructed for each molecular marker phylogenetic trees. The purpose of this is to check for cross contamination or pseudogenes.

Testing the monophyly of Dorcadionini, Lamiini and Monochamini

To test the monophyly of Dorcadionini we generated multiple multilocus phylogenetic trees ML (with RAxML-NG and IQTree) and BI (with MrBayes) – Figures 3.22. and 3.23. (from the thesis) using a smaller data set for Dorcadionini but larger for Lamiini and Monochamini. All trees have similar topologies.

The phylogeny of the Dorcadionini tribe: mitochondrial vs nuclear

The phylogenetic trees resulted from the concatenated mitochondrial dataset and the concatenated nuclear data set have the same general topology as the trees for testing the monophyly of Dorcadionini, and also between the nuclear and mitochondrial trees there are no significant differences.

The phylogeny of the Dorcadionini tribe: concatenated data

The phylogenetic trees reconstructed from the concatenated matrix (mitochondrial and nuclear) have a very similar topology regardless of the method used to reconstruct them (ML with RAxML-NG (Fig. 3.27 thesis) or IQTree (Fig. 3.28 thesis) on one side and BI with MrBayes on the other side.

Our Dorcadionini samples are separated in two clades, one with the genus *Eodorcadion* next to *Lamia*, and the other clade with *Dorcadion*, *Iberodorcadion*, *Neodorcadion* and *Politodorcadion.*

CONCLUSIONS

- Dorcadionini are a good model for testing the success of DNA Barcoding for identification and species separation based on molecular methods. This is due to the high number of species and subspecies, the probable result of allopatric speciation (they are flightless longhorened beetles), but also of reticulate evolution. Because they have low mobility the intraspecific phylogeographic structure can be significant. The sequences published already in GenBank generated by our analyses are 307 out of the total of 835 published Dorcadionini sequences in BOLD and GenBank.
- At the time of publishing the sequences from the DNA barcoding paper (Dascălu *et al.*, 2022) these were the first Dorcadionini sequences published with the exception of the few sequences from the German Barcoding of Life project. These sequences cover the Romanian Dorcadionini fauna with multiple sequences per species.
- We have generated 659 new sequences that will be published soon, from them 112 are *COI*, the standard DNA Barcoding marker.
- We have created and tested a new set of specific Dorcadionini primers that generate a "minibarcode" compatible with the standard sequence, that could be used for samples with a degraded DNA.
- We have created a new set of primers for a shorter fragment of *ITS2* for Dorcadionini with degraded DNA – they could be used for DNA extracts contaminated with fungi.
- *Dorcadion axillare*, *D. murrayi* and *D. pusillum* represent an interesting case of hybridisation followed by mitochondrial capture; both the morphology and the analysis of the nuclear genes confirm that *D. axillare moldavicum*, *D. pusillum ochrolineatum* and *D. pusillum vasiliscus* are indeed subspecies of *D. axillare* and *D. pusillum* as originally described, and not of *D. murrayi.*
- *Dorcadion aethiops* belongs to the subgenus *Carinatodorcadion*, but its mitochondrial DNA is much closer to species of *Cribridorcadion* than to *D.* (*Carinatodorcadion*) *fulvum*. The exact origin of the mtDNA of *D. aethiops* remaines unknown because of conflicting information between the phylogenetic analyses and genetic distances, the mitochondrial lineage of *D. aethiops* is the same with that of *D. lugubre minkovae.*

- *Dorcadion lugubre lugubre* represents yet another case of mitochondrial-morphological discordance which was only recently discovered in Dorcadionini and is most likely a hybridogenic taxon.
- We have demonstrated using molecular methods, that an individual with atypical and intermediary characters is a hybrid. According to the mitochondrial haplotype and the analysis of the nuclear alleles the hybrid is the result of the mating between a female of *D. lineatocolle* with a male of *D. lugubre minkovae*.
- DNA Barcoding can be used in Dorcadionini only correlated with morphology, distribution data, and more than one nuclear molecular marker because of the frequency of hibridisation and mitochondrial capture.
- Monochamini is a paraphyletic group even if inside of it there is a monophyletic group that includes the type genus *Monochamus*, the genus *Anoplophora* and the related genera. The tribe Lamiini *senso stricto* is also paraphyletic, because Dorcadionini derives from it. The genera *Morimus* and *Herophila* are more closer to Dorcadionini than to *Lamia*.
- Dorcadionini is monophyletic if we exclude the genus *Eodorcadion*.
- The genus *Eodorcadion* should be transferred to the tribe Lamiini. This is the only option for taxonomists that accept paraphyletic groups. For a monophyletic taxonomy Monochamini and Dorcadionini are synonymous with Lamiini.

BIBLIOGRAPHY

1. Ashman, L. G., Shin, S., Zwick, A., Ślipiński, A., & McKenna, D. D. (2022). The first phylogeny of Australasian Lamiinae longhorn beetles (Coleoptera: Cerambycidae) reveals poor tribal classification and a complex biogeographic history. *Systematic Entomology*, *47*(1), 213-230.
2. Baur, B., Coray, A., Lenzin, H., & Schmera, D. (2020). Factors contributing to the decline of an endangered flightless longhorn beetle: A 20-year study. *Insect Conservation and Diversity, 13*(2), 175‐186.
3. Baur, B., Coray, A., Minoretti, N., & Zschokke, S. (2005). Dispersal of the endangered flightless beetle *Dorcadion fuliginator* (Coleoptera: Cerambycidae) in spatially realistic landscapes. *Biological Conservation, 124*, 49‐61.
4. Bianchini, G., & Sánchez-Baracaldo, P. (2024). TreeViewer: Flexible, modular software to visualise and manipulate phylogenetic trees. *Ecology and Evolution*, 14(2), e10873.
5. Bonfield, J. K., Smith, K. F., & Staden, R. (1995). A new DNA sequence assembly program. *Nucleic Acids Research, 24*, 4992‐4999.
6. Bonnet, T., Leblois, R., Rousset, F., & Crochet, P. A. (2017). A reassessment of explanations for discordant introgressions of mitochondrial and nuclear genomes. *Evolution*, 71(9), 2140–2158.
7. Borchsenius, F. (2009). *FastGap, version 1.2*. Department of Biosciences, Aarhus University. http:// www. aubot. dk/ FastGap_ home. Htm
8. Bouchard, P., Smith, A. B., Douglas, H., Gimmel, M. L., Brunke, A. J., & Kanda, K. (2017). Biodiversity of coleoptera. *Insect biodiversity: science and society*, 337-417.
9. Breuning, S. (1943a). Nouveaux cerambycides palearctiques (1re note). *Miscellanea Entomologica, 40,* 89‐104.
10. Breuning, S. (1943b). Beitrag zur Wertung der Geschlechtorgane für die Systematik. *Zeitschrift für Morphologie und Ökologie der Tiere*, 39, 523–526.
11. Breuning, S. (1947) Nouveaux Cérambycidés paléarctiques (4e note). *Miscellanea Entomologica 43*, [1946] 141–147.
12. Breuning, S. (1948). Notes concernant la repartition geografique et les tendances de specialisations chez les Dorcadionini (Col. Cerambycidae). *EOS,* 24 (4).
13. Breuning, S. (1962). Revision der Dorcadionini (Coleoptera, Cerambycidae). *Entomologische Abhandlungen und Berichte aus dem Staatlichen Museum für Tierkunde in Dresden, 27*, 1‐665.
14. Breuning, S., & Villiers, A. (1967). Cérambycides de Turquie (2. note). *L'entomologiste*, *23*(3), 59-63.
15. Brower, A. V. Z. (1994). Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Sciences, 91*, 6491‐6495.
16. **Caba, F. G**., & Dascălu, M. M. (2024). Dissecting natural hybridisation in longhorned beetles through an integrative approach: Further proof of reticulate

evolution in Dorcadionini (Cerambycidae, Lamiinae). *Zoologica Scripta*, *53*(5), 665-687.

17. Chan, W. Y., Hoffmann, A. A., & van Oppen, M. J. (2019). Hybridisation as a conservation management tool. *Conservation Letters*, 12(5), e12652.

18. Clement, M., Posada, D. C. K. A., & Crandall, K. A. (2000). TCS: A computer program to estimate gene genealogies. *MolecularEcology, 9*(10), 1657‑1659.

19. Cruaud, A., Nidelet, S., Arnal, P., Weber, A., Fusu, L., Gumovsky, A., Huber, J., Polaszek, A., & Rasplus, J. Y. (2019). Optimized DNA extraction and library preparation for minute arthropods: Application to target enrichment in chalcid wasps used for biocontrol. *Molecular Ecology Resources, 19*, 702‑710.

20. Danilevsky, M. L. (1993). Some preliminary results of the type-material study of the Cerambycidae (Coleoptera) collection of the National Museum of Natural History, Paris. *Russian Entomological Journal,* 2(1), 47-50.

21. Danilevsky, M. L. (1995). New *Dorcadion* Dalman, 1817 from Kazakhstan (Coleoptera, Cerambycidae). *Lambillionea*, *95*(3), 420- 428.

22. Danilevsky, M. L. (1996). New taxa of the genus *Dorcadion* Dalman from Asia (Coleoptera, Cerambycidae). *Lambillionea*, *96*(2), 407-420.

23. Danilevsky, M. L. (1998). A review of subspecific configuration of *Dorcadion (Carinatodorcadion) carinatum* (Pallas, 1771) with a description of new subspecies (Coleoptera: Cerambycidae). *Acta ent. Slovenica*, *6*, 135-142.

24. Danilevsky, M. L. (2001). *Review of subspecific structure of Dorcadion (s. str) glicyrrhizae (Pallas, 1771) (Coleoptera, Cerambycidae)*. Les cahiers Magellanes, 5: 1-27.

25. Danilevsky, M. L. (2002).Subspecific structure of *Dorcadion (Dzhungarodorcadion) semenovi* Ganglbauer, 1884 (Coleoptera, Cerambycidae). - *Les cahiers Magellanes*, *10* (2001): 1-18.

26. Danilevsky, M. L. (2007). *Revision of the Genus Eodorcadion Breuning, 1947 (Coleoptera, Cerambycidae).* Magellanes. Collection systematique, vol. 16. Andresy (France).

27. Danilevsky, M. L. (2010) Tribe Dorcadionini. In: Löbl, I. & Smetana, A. (Eds.), Catalogue of Palaearctic Coleoptera. Vol. 6. Chrysomeloidea. Apollo Books, Stenstrup, pp. 241–264.

28. Danilevsky, M. L. (2014). Two new subspecies of *Dorcadion aethiops* (Scopoli, 1763) from Bulgaria (Coleoptera, Cerambycidae). *Human. Space. Int. Almanac*, *3*, 251-254.

29. Danilevsky, M. L. (2020). (ed). Catalogue of Palaearctic Coleoptera, vol. 6 (1), Chrysomeloidea I (Vesperidae, Disteniidae, Cerambycidae). Revised and updated edition. Leiden / Boston: Brill, i-xxii, 1-712.

30. Danilevsky, M. L. (2021). A new species of the genus *Dorcadion* Dalman, 1917 (Coleoptera, Cerambycidae) from Xinjiang, China. *Гуманитарное пространство*, *10*(8), 1063-1073.

31. Danilevsky, M. L. (author) & Lazarev M. A. (software) 2025. Catalogue of Palaearctic Chrysomeloidea (Vesperidae, Disteniidae, Cerambycidae). Version. 19.02.2025. - http://cerambycidae.net. Accesed 01.03.2025

32. Danilevsky, M. L., & Kasatkin, D. G. (2007). Further investigation of Dorcadionini (Coleoptera: Cerambycidae) endophallus with a revision of taxonomical position of the genus *Trichodorcadion* Breuning, 1942. *Russian Entomological Journal*, *15*(4) [2006], 401‑407.

33. Danilevsky, M. L., & Murzin, S. V. (2009). A revue of *Dorcadion* Dalman, 1817 species of "*laeve*-group". Part 2 (Coleoptera, Cerambycidae, Lamiinae). *Les cahiers Magellanes*, *93*, 1-22.

34. Danilevsky, M. L., Kasatkin, D. G., & Rubenyan, A. A. (2005). Revision of the taxonomic structure of the tribe Dorcadionini (Coleoptera: Cerambycidae) on the base of endophallic morphology. *Russian Entomological Journal, 13* [2004], 127‑149.

35. Dascălu, M. M. (2018). The subspecific structure of *Dorcadion* (*Cribridorcadion*) *pusillum* Kuster (Coleoptera: Cerambycidae) with description of two new subspecies from Romania. *Zootaxa, 4442*(1), 43‑62.

36. Dascălu, M. M., & Fusu, L. (2012). *Dorcadion axillare* Kuster, 1847 (Coleoptera, Cerambycidae): Distribution, morphometrics, karyotype and description of a new subspecies from Romania. *Zootaxa, 3322*, 35‑48.

37. Dascălu, M. M., **Caba**, **F. G.**, & Fusu, L. (2022). DNA barcoding in Dorcadionini (Coleoptera, Cerambycidae) uncovers mitochondrial-morphological discordance and the hybridogenic origin of several subspecies. *Organisms Diversity & Evolution*, 1-25.

38. de Santana Souza, D., Marinoni, L., Monné, M. L., & Gómez-Zurita, J. (2020). Molecular phylogenetic assessment of the tribal classification of Lamiinae (Coleoptera: Cerambycidae). *Molecular Phylogenetics and Evolution*, 145, 106736

39. Dellicour, S., & Flot, J. F. (2018). The hitchhiker's guide to single-locus species delimitation. *Molecular Ecology Resources*, 18(6), 1234-1246.

40. Dmitriev, D. A., & Rakitov R. A. (2008). Decoding of superimposed traces produced by direct sequencing of heterozygous indels. *PLoS Computational Biology*, *4*(7), e1000113.

41. Ezard, T., Fujisawa, T., & Barraclough, T. (2014). SPLITS: SPecies' LImits by Threshold Statistics. R package version 1.0–19/r51. https://R- Forge.R- proje ct. org/ proje cts/ splits/

42. Farrell, B. D. (1998). " Inordinate Fondness" explained: why are there So many beetles?. *Science*, *281*(5376), 555-559.

43. Flot, J. F. (2015). Species delimitation's coming of age. *Systematic Biology*, 64(6), 897-899.

44. Flot, J. F., Couloux, A., & Tillier, S. (2010). Haplowebs as a graphical tool for delimiting species: a revival of Doyle's" field for recombination" approach and its application to the coral genus Pocillopora in Clipperton. *BMC Evolutionary Biology*, *10*(1), 1-14.

45. Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology, 3*(5), 294‐299.

46. Fontaneto, D., Herniou, E. A., Boschetti, C., Caprioli, M., Melone, G., Ricci, C., & Barraclough, T. G. (2007). Independently evolving species in asexual bdelloid rotifers. *PLoS Biology*, *5*(4), e87.

47. Funk, D. J., & Omland, K. E. (2003). Species-level paraphyly and polyphyly: Frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology Evolution & Systematics, 34*, 397‐423.

48. Fusu, L., & Ribes, A. (2017). Description of the first Palaearctic species of *Tineobius* Ashmead with DNA data, a checklist of world species, and nomenclatural changes in Eupelmidae (Hymenoptera, Chalcidoidea). *European Journal of Taxonomy, 263*, 1‐19.

49. Giannoulis, T., Dutrillaux, A., Sarri, C., Mamuris, Z., & Dutrillaux, B. (2020). Phylogenetic relationships between genera *Dorcadion*, *Lamia*, *Morimus*, *Herophila* and some other Lamiinae (Coleoptera: Cerambycidae) based on chromosome and CO1 gene sequence comparison. *Bulletin of Entomological Research, 110*(3), 321‐327.

50. Gorring, P. S., & Farrell, B. D. (2023). Evaluating species boundaries using coalescent delimitation in pine-killing *Monochamus* (Coleoptera: Cerambycidae) sawyer beetles. *Molecular Phylogenetics and Evolution*, 184, 107777.

51. Hajibabaei, M., Singer, G. A., Hebert, P. D., & Hickey, D. A. (2007). DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. *TRENDS in Genetics*, 23(4), 167-172.

52. Hebert, P. D. N., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(1512), 313‐321.

53. Hebert, P. D., Ratnasingham, S., & De Waard, J. R. (2003). Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270(suppl_1), S96-S99.

54. Hendrich, L., Moriniere, J., Haszprunar, G., Hebert, P. D. N., Hausmann, A., Kohler, F., & Balke, M. (2015). A comprehensive DNA barcode database for Central European beetles with a focus on Germany: Adding more than 3500 identified species to BOLD. *Molecular Ecology Resources, 15*, 795‐818.

55. Hernández, J. M. (2000). Estudio multivariante de la genitalia masculine y femenina en seis especies de *Iberodorcadion* Breuning, 1943 (Coleoptera, Cerambycidae, Lamiinae) de la Comunidad de Madrid (Espana) y propuesta de nuevas sinonimias para el grupo. *Boletin Asociacion Espanola de Entomologia, 24*(1‐2), 97‐129.

56. Ilić, N., & Ćurčić, S. (2015). A checklist of longhorn beetles (Coleoptera: Cerambycidae) of Serbia. *Zootaxa, 4026*(1), 1‐97.

57. Jakovlev, B. E. (1897). Un nouveau *Dorcadion* du Caucase. *Horae Soc. Entomol. Ross.*, *31*(1–2), 245-247.

58. Karpiński, L., Gorring, P., & Cognato, A. I. (2023). DNA vs. morphology in delineating species boundaries of endemic Mongolian *Eodorcadion* taxa (Coleoptera: Cerambycidae). *Diversity*, *15*(5), 662.

59. Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution, 30*(4), 772‑780.

60. Kirschner, P., Záveská, E., Gamisch, A., Hilpold, A., Trucchi, E., Paun, O., ... & Schoenswetter, P. (2020). Long-term isolation of European steppe outposts boosts the biome's conservation value. *Nature communications*, *11*(1), 1968.

61. Kopecký, D., Martín, A., & Smýkal, P. (2022). Interspecific hybridisation and plant breeding: From historical retrospective through work of Mendel to current crops. *Czech Journal of Genetics and Plant Breeding,* 58(3), 113–126.

62. Kozlov, A. M., Darriba, D., Flouri, T., Morel, B., & Stamatakis, A. (2019). RAxML-NG: A fast, scalable, and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics, 35*(21), 4453‑4455.

63. Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular biology and evolution*, 35(6), 1547–1549.

64. Lanfear, R., Frandsen, P. B., Wright, A. M., Senfeld, T., & Calcott, B. (2016). PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution, 34*(3), 772‑773.

65. Lazarev, M. A. (2023). The subspecies taxonomy of *Dorcadion (Cribridorcadion) lugubre* Kraatz, 1873 and *D. (C.) etruscum* (Rossi, 1790) (Coleoptera, Cerambycidae), *Гуманитарное пространство*, 12(4), 427–448.

66. Leigh, J. W., & Bryant, D. (2015). PopART: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution, 6*(9), 1110‑1116.

67. Li, K., Yu, S. W., Hu, H., Feng, Y. F., Storey, K. B., Ma, Y., Zhang, J.Y. & Yu, D. N. (2024). The phylogenetic relationship of Lamiinae (Coleoptera: Cerambycidae) using mitochondrial genomes. *Genes*, *15*(1), 13.

68. Maddison, W. P. & Maddison, D.R. (2021). Mesquite: a modular system for evolutionary analysis. Version 3.70 http://www.mesquiteproject.org

69. Mallet, J. (2005). Hybridization as an invasion of the genome. *Trends in Ecology & Evolution, 20*(5), 229‑237.

70. Marshall, D. C., Hill, K. B. R., Moulds, M., Vanderpool, D., Cooley, J. R., Mohagan, A. B., & Simon, C. (2016). Inflation of molecular clock rates and dates: Molecular phylogenetics, biogeography, and diversification of a global cicada radiation from Australasia (Hemiptera: Cicadidae: Cicadettini). *Systematic Biology, 65*(1), 16‑34.

71. Meier, R., Zhang, G., & Ali, F. (2008). The use of mean instead of smallest interspecific distances exaggerates the size of the barcoding gap and leads to misidentification. *Systematic Biology, 57*(5), 809‑813.

72. Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., Von Haeseler, A., & Lanfear, R. (2020). IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular biology and evolution*, *37*(5), 1530-1534.

73. Minkova, S. (1961). Untersuchungen uber die Artenzusammensetzung der Tribus Dorcadionini (Col. Cerambycidae) in Bulgarien. *Bulletin de l'Institut de Zoologie et Musée, Sofia, 10,* 293‑309.

74. Moritz, C., & Cicero, C. (2004). DNA barcoding: Promise and pitfalls. *PLoS Biology*, *2*(10), e354.

75. Mutanen, M., Hausmann, A., Hebert, P. D., Landry, J. F., de Waard, J. R., & Huemer, P. (2012). Allopatry as a Gordian knot for taxonomists: Patterns of DNA barcode divergence in Arctic-Alpine Lepidoptera. *PLoS One*, *7*(10), e47214.

76. Özdikmen, H., & Kaya, G. (2015). A proposed new arrangement of some Dorcadionini (Coleoptera: Cerambycidae). *Munis Entomology and Zoology*, *10*, 1-10 .

77. Paithankar, K. R., & Prasad, K. (1991). Precipitation of DNA by polyethylene glycol and ethanol. *Nucleic acids research*, 19(6), 1346.

78. Pesarini, C., & Sabbadini, A. (2007). Ricerche sui Dorcadiini di Grecia. II. Le specie della Grecia centromeridionale e quelle del gruppo di *Dorcadion kozanii* (Coleoptera Cerambycidae). *Atti della Societa Italiana di Scienze Naturali e del Museo Civico di Storia Naturale in Milano*, *148*(1), 35‑83.

79. Pesarini, C., & Sabbadini, A. (2010). Ricerche sui Dorcadionini di Grecia IV. Le specie della Macedonia e della Tracia (Coleoptera Cerambycidae). *Atti della Societa Italiana di Scienze Naturali e del Museo Civico di Storia Naturale in Milano*, *151*(2), 179‑216.

80. Pesarini, C., & Sabbadini, A. (2013). Note su *Dorcadion turchi*, con descrizione di cinque nuove specie e tre nuove sottospecie (Coleoptera Cerambycidae). *Annali del Museo Civico di Storia Naturale di Ferrara*, *14/15* [2011/2012], 51‑64.

81. Plavilstshikov, N. N. (1958). *Timber Beetles. P.3. Subfamily Lamiinae. P.1. Fauna of the USSR, Beetles 23(1)*. Akademija Nauk SSSR, Moskow, Leningrad.

82. Pons, J., Barraclough, T. G., Gomez-Zurita, J., Cardoso, A., Duran, D. P., Hazell, S., Kamoun, S., Sumlin, W.D & Vogler, A. P. (2006). Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic biology*, 55(4), 595-609.

83. Puillandre, N., Brouillet, S., & Achaz, G. (2021). ASAP: assemble species by automatic partitioning. *Molecular Ecology Resources*, 21(2), 609-620.

84. Puillandre, N., Lambert, A., Brouillet, S., & Achaz, G. J. M. E. (2012). ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular ecology*, 21(8), 1864-1877.

85. Rambaut, A. (2010). FigTree v1.3.1. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh. http://tree.bio.ed.ac.uk/software/figtree/

86. Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, *67*(5), 901.

87. Ratnasingham, S., & Hebert, P. D. (2013). A DNA-based registry for all animal species: The barcode index number (BIN) system. *PLoS One*, *8*(7), e66213.

88. Ratnasingham, S., & Hebert, P. D. N. (2007). BOLD: The Barcode of Life Data System. *Molecular Ecology Notes, 7*, 355‐364.

89. Richmond, M. P. (2014). The role of aedeagus size and shape in failed mating interactions among recently diverged taxa in the *Drosophila mojavensis* species cluster. *BMC Evolutionary Biology, 14*, 255.

90. Ronquist, F., & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics, 19*, 1572‐1574.

91. Rossa, R., & Goczał, J. (2021). Global diversity and distribution of longhorn beetles (Coleoptera: Cerambycidae). *The European Zoological Journal*, *88*(1), 289-302.

92. Rougerie, R., Lopez-Vaamonde, C., Barnouin, T., Delnatte, J., Moulin, N., Noblecourt, T., Nusillard, B., Parmain, G., Soldati, F., & Bouget, C. (2015). PASSIFOR: A reference library of DNA barcodes for French saproxylic beetles (Insecta, Coleoptera). *Biodiversity Data Journal*, *3*, e4078.

93. Rulik, B., Eberle, J., von der Mark, L., Thormann, J., Jung, M., Kohler, F., Apfel, W., Weigel, A., Kopetz, A., Kohler, J., & Fritzlar, F. (2017). Using taxonomic consistency with semi-automated data pre-processing for high quality DNA barcodes. *Methods in Ecology and Evolution, 8*(12), 1878‐1887.

94. Sama, G. (2002). *Atlas of the Cerambycidae of Europe and the Mediterranean Area* (Vol. 1). Kabourek.

95. Sama, G., Dascălu, M. M., & Pesarini, C. (2010). Description of *Dorcadion gashtarovi* n. sp. (Coleoptera, Cerambycidae) from Romania and Bulgaria with review of the closely related species. *North- Western Journal of Zoology*, *6*(2), 286‐293.

96. Simmons, M. P., & Ochoterena, H. (2000). Gaps as characters in sequence-based phylogenetic analyses. *Systematic biology*, 49(2), 369–381.

97. Sloan, D. B., Havird, J. C., & Sharbrough, J. (2017). The on-again, offagain relationship between mitochondrial genomes and species boundaries. *Molecular Ecology, 26*(8), 2212‐2236.

98. Smith, M. A., Fisher, B. L., & Hebert, P. D. (2005). DNA barcoding for effective biodiversity assessment of a hyperdiverse arthropod group: The ants of Madagascar. *Philosophical Transactions of the Royal Society B: Biological Sciences, 360*(1462), 1825‐1834.

99. Song, N., Wang, M., Zhai, Q., & Zhang, H. (2025). Insights into the phylogeny of longhorn beetles from phylogenomic data. *Zoological Journal of the Linnean Society*, *203*(1), zlae174.

100. Soydabaş-Ayoub, H. K., & Uçkan, F. (2024). Time-scaled phylogenetic analysis of some extant Lamiinae (Coleoptera, Cerambycidae) species of East of Marmara Basin, Türkiye. *Biologia*, *79*(2), 545-555.

101. Srivathsan, A., & Meier, R. (2012). On the inappropriate use of Kimura- 2-parameter (K2P) divergences in the DNA-barcoding literature. *Cladistics, 28*(2), 190‐194.

102. Sukumaran, J., & Knowles, L. L. (2017). Multispecies coalescent delimits structure, not species. *Proceedings of the National Academy of Sciences, 114*(7), 1607‐1612.

103. Taberlet, P., Fumagalli, L., Wust-Saucy, A. G., & Cosson, J. F. (1998). Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology, 7*(4), 453‐464.

104. Toews, D. P. L., & Brelsford, A. (2012). The biogeography of mitochondrial and nuclear discordance in animals. *Molecular Ecology, 21*, 3907‐3930.

105. Tomé, M. (2002). Revisión y propuesta de sinonimia de los subgéneros *Iberodorcadion*, *Hispanodorcadion* y *Baeticodorcadion* (Coleoptera, Cerambycidae). *Boletin de la Sociedad Entomológica Aragonesa*, (31), 77-81.

106. Tomé, M. (2004). Rehabilitacion de *Dorcadion* (*Iberodorcadion*) *nudipenne* Escalera (Coleoptera, Cerambycidae). *Boletín de la S.E.A.*, *35*, 247‐249.

107. Tomé, M. (2012). Conexiones geologicas del *Dorcadion* (*Iberodorcadion*) *perezi* Graells, 1849 en la Sierra de Guadarrama (Espana). *Revista Gaditana de Entomología, 3*(1‐2), 41‐50.

108. Török, P., Ambarlı, D., Kamp, J., Wesche, K., & Dengler, J. (2016). Step (pe) up! Raising the profile of the Palaearctic natural grasslands. *Biodiversity and Conservation*, *25*, 2187-2195.

109. Vaidya, G., Lohman, D. J., & Meier, R. (2011). SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics*, *27*(2), 171–180.

110. Vives, E. (1976). Contribución al conocimiento de los *Iberodorcadion* Breuning (Col. Cerambycidae). *Miscel· lània Zoològica*, *3*(5), 163-168.

111. Wang, Q. (Ed.). (2017). *Cerambycidae of the world: biology and pest management*. CRC press.

112. Willis, P. M. (2013). Why do animals hybridize? *Acta Ethologica, 16*(3), 127‐134.

113. Wirtz, P. (1999). Mother species-father species: Unidirectional hybridization in animals with female choice. *Animal Behaviour*, *58*(1), 1‐12.

114. Wood, J. R. (2018). DNA barcoding of ancient parasites. *Parasitology*, *145*(5), 646-655.

115. Xia, X. (2018). DAMBE7: New and improved tools for data analysis in molecular biology and evolution. *Molecular Biology and Evolution, 35*, 1550‐1552.

116. Zagaykevich, I. K., & Puchkov, O. V. (2009). *Dorcadion equestre*. In I.A Akimov (Ed.), *Red book of Ukraine. The animal world*. (pp. 119). Global Consulting, Kiev.

117. Zamoroka, A. M. (2019a). A new subspecies of *Dorcadion fulvum* (Scopoli, 1763) (Coleoptera: Cerambycidae) from Western Ukraine. *Polish Journal of Entomology, 88*(4), 363‐378.

118. Zhang, J., Kapli, P., Pavlidis, P., & Stamatakis, A. (2013). A general species delimitation method with applications to phylogenetic placements. *Bioinformatics, 29*(22), 2869‐2876.