



Review

of the doctoral dissertation by MSc Ryan Lumen, titled: "*Phylogeny and Taxonomic Revision of Subtribe Eurynotina (Coleoptera: Tenebrionidae)*", conducted at Museum and Institute of Zoology of the Polish Academy of Sciences, Polish Academy of Sciences, under the supervision of Prof. dr. hab. Marcin Kamiński

1. Introduction.

The research presented in this dissertation focuses on the subtribe Eurynotina within the diverse family Tenebrionidae. Eurynotina represents one of the relatively neglected lineages of darkling beetles, with current knowledge of its phylogeny and systematics remaining outdated and previous classifications relying predominantly on morphological characters. This taxon, comprising approximately 100 species, is characterised by a preference for extreme, sparsely inhabited arid environments. Consequently, Eurynotina is poorly represented in scientific collections, with more than 75% of its species known only from type series. Very little is also known about the reproductive biology of the group, although a few reports suggest the occurrence of possible ovoviviparity, a relatively rare phenomenon among Coleoptera.

Eurynotina therefore clearly required a modern integrative study combining molecular and morphological data. The most important objectives included establishing a well-supported phylogenetic hypothesis, testing the monophyly of the entire subtribe and its constituent genera, and conducting a comprehensive taxonomic revision. To address these objectives, the Candidate adopted an integrative approach combining molecular and morphological data to infer phylogenetic relationships and evolutionary processes within Eurynotina, while simultaneously establishing a new systematic framework for the group.

Given the previously limited knowledge of the group's diversity, phylogeny, and taxonomy, as well as the need to integrate traditional and modern research methods, the chosen topic is undeniably ambitious.



2. Scientific Value of the Dissertation

a. Originality of the Research

The dissertation presented by MSc Ryan Lumen is an original and valuable scientific study, with the Candidate's leading role in the preparation of its most important components clearly confirmed by statements from the co-authors. The journal articles and monograph included in the dissertation present a reconstruction of the phylogeny of Eurynotina based on genomic methods integrated with morphological data, followed by a revision of the generic classification within the subtribe. The scope of the research, the complexity of the scientific problems addressed, and the extensive morphological documentation undoubtedly meet the standards expected of a doctoral dissertation.

Structured into five separate chapters based on published articles and a monograph, the dissertation makes a significant contribution to our understanding of the phylogeny, systematics, and taxonomy of Eurynotina, an important lineage within the large and diverse family Tenebrionidae. The taxonomic revisions, detailed analyses of adult-external, genital, and preimaginal morphology, and phylogenetic reconstruction supported by genomic data provide a solid foundation for a substantial revision of the systematics of Eurynotina. Notably, this dissertation is the first study to apply genomic data to the phylogenetic reconstruction of the group with such extensive taxon sampling.

The originality of the dissertation is also clearly demonstrated by its substantial contribution to alpha taxonomy, including numerous species descriptions and redescriptions, synonymisations, and lecto- and neotype designations. Overall, the dissertation represents a modern and comprehensive study that meets high scientific standards in the fields of Coleoptera taxonomy, systematics, and phylogenetics. Its scientific quality is fully comparable to that of other high-quality studies addressing similar questions in different groups of invertebrates.

b. Scientific Value of the Dissertation

Each of the five extensive article-based chapters, all of which have already been published, addresses taxonomic, evolutionary, or reproductive issues related to the subtribe Eurynotina. Using two molecular approaches, the Candidate reconstructed the phylogeny of Eurynotina and proposed a new generic classification. The scope of the research, the complexity of the scientific



problems addressed, and the extensive morphological documentation presented in the dissertation fully meet the standards expected of a doctoral dissertation. MSc Ryan Lumen based his research on an extensive dataset comprising material both recently collected in natural habitats and obtained from a wide range of museum collections (19 in total).

The Candidate revised all groups traditionally recognised within the subtribe. As part of his doctoral research, nearly all available type material representing the species-group names within Eurynotina was examined (121 specimens in total). These revisions resulted in the description of new genera, the elevation of former subgenera to genus rank, the description of 12 new species, and descriptions of preimaginal stages. In addition to the description of new taxa, synonymisation represents an equally important component of taxonomic revision. The Candidate made several such taxonomic decisions, including six at the genus level and 16 at the species level, and also designated lectotypes and neotypes for historical type series.

Throughout the taxonomic revisions, morphological structures were examined and documented using light microscopy and scanning electron microscopy (SEM). The resulting documentation is of high quality, with detailed and reproducible drawings and images that allow reliable comparisons of morphological structures across species. MSc Ryan Lumen has mastered the techniques of morphological documentation to an excellent degree, undoubtedly benefiting from the extensive experience of his supervisor in this field. This provides a reliable foundation for both the description and delimitation of taxa and broader evolutionary considerations. The Candidate's excellent taxonomic skills culminate in the final work of the series, a comprehensive monograph.

A relatively small but important additional contribution is the proposed revision of the terminology used by different authors to describe particular structures of the genital apparatus in the studied group.

Building on a solid foundation of classical taxonomy, MSc Ryan Lumen carried out the remaining research tasks in an exemplary manner: he presented a well-supported phylogenetic hypothesis, tested hypotheses concerning the evolution of morphological characters and reproductive strategies, and proposed a new systematic framework for the subtribe.

The molecular component of the dissertation involved two approaches. Targeted enrichment next-generation sequencing (TE-NGS) of nuclear loci was applied to well-preserved,



relatively fresh material. As this approach produced poor results for historical pinned specimens, the Candidate employed a DNA bycatch approach to obtain mitochondrial sequence data (12 mtDNA loci). This represents a well-established approach in museomics, where the quality and quantity of DNA extracted from historical specimens are often severely limited. Combined with a morphological character matrix, the molecular dataset was used to reconstruct the phylogenetic relationships within Eurynotina, with a total of 60 species included in the analyses. Mitochondrial DNA data were also used to support the delimitation of three new species, for which clear genetic differentiation among the respective OTUs was recovered.

Finally, the Candidate proposed a new classification based on five major phylogenetic lineages recovered in the analyses. The results provided the basis for a thorough and well-supported revision of the generic classification of Eurynotina. They also supported the monophyly of the subtribe and its sister-group relationship with Platynotina.

3. Substantive Value of the Dissertation

I assess the scientific merit of the dissertation as high. The aims of the study are clearly formulated, and the research methods employed are well suited to achieving them. The taxonomic and phylogenetic analyses are founded on carefully refined methodological approaches that ensure the acquisition of reproducible and comparable datasets, encompassing both molecular and morphological data. Given the complexity of the research subject, this represents a considerable methodological challenge.

The morphological structures identified by the Candidate as diagnostic for particular taxa are often complex and three-dimensional, as exemplified by the aedeagus. Their preparation and visual documentation require careful and consistent extraction from specimens while preserving their original shape and spatial configuration. Sclerotised, three-dimensional structures such as the aedeagus are particularly challenging to document in a reproducible manner using light microscopy or scanning electron microscopy (SEM). It should be emphasised that the Candidate has mastered these techniques to a very high standard.

The morphology of the species studied in the dissertation is extensively documented using both image stacking in light microscopy and SEM. This comprehensive documentation provides a solid and reliable foundation for phylogenetic and evolutionary analyses, as well as for the



diagnosis and definition of newly established or redefined taxa. Phylogenetic relationships within Eurynotina were reconstructed using targeted enrichment sequencing (TE-NGS), applied to this group for the first time, together with appropriate and up-to-date analytical software. Also noteworthy is the extensive taxon sampling achieved in the molecular analyses, which is particularly impressive given the substantial reliance on material from museum collections.

The results are interpreted carefully and convincingly, and the conclusions, particularly those concerning the reclassification of Eurynotina into five major lineages, are well supported by the evidence presented. Overall, the dissertation represents a comprehensive, methodologically rigorous, and modern treatment of a large and taxonomically challenging group.

4. Editorial Correctness of the Dissertation

The dissertation is extensive (373 pages) and well structured. The main part is preceded by abstracts in both Polish and English, as well as a list of publications included in the dissertation. Following a well-composed "Introduction" (5 pages), the Candidate clearly defines the research objectives in the "Aims of the Study" chapter (1 page). The "Materials and Methods" chapter (8 pages) provides a comprehensive overview of the methodologies applied across the studies comprising the dissertation. Similarly, the "Summary of Thesis and Publication Results" chapter (4 pages) provides a synthesis of the major findings. These sections are followed by a synthetic "Discussion" (3 pages) and concise "Conclusions" (1 page). The "References" section comprises eight pages. The introductory part is followed by the full texts of the publications included in the dissertation, presented as subchapters 8.1–8.5. These are substantial contributions, comprising 27, 29, 21, 25, and 219 pages, respectively. The first four articles follow the conventional structure of a scientific paper, including an introduction, materials and methods, results, and discussion. The final contribution, a comprehensive taxonomic monograph, has a structure appropriate to this type of publication, comprising an introduction, materials and methods, taxonomy, photographs of type specimens, acknowledgements, references, and an index of Eurynotina species names. The aesthetic quality and clarity of the morphological documentation and phylogenetic trees deserve special recognition.



5. Critical Comments

The presented dissertation is an extensive study of high scientific merit. I do not identify any major weaknesses. The critical remarks presented below should therefore be regarded primarily as suggestions and points intended to stimulate further discussion.

The Candidate recovered five major lineages within Eurynotina in phylogenetic analyses. Nevertheless, a considerably larger number of genera (19) is retained in the proposed classification, including three monotypic genera. This raises the question of whether such extensive generic subdivision is necessary. Why not synonymise the remaining genera within the *Oncotus*, *Psectrapus*, and *Schyzoschelus* lineages and recognise five broadly defined genera corresponding to the five major phylogenetic lineages? In my view, the Candidate may be unnecessarily multiplying informal "lineages" rather than replacing them with a simpler and phylogenetically supported classification comprising five genera. Such a solution could also be more practical for potential users of the classification working outside specialist taxonomy, for example in community ecology, nature conservation, or various fields of applied science.

I would also welcome a broader discussion of the evolution of reproductive strategies and larval morphology within Eurynotina. I understand that the available data are relatively sparse, but can any further conclusions be drawn regarding the evolution of ovoviviparity in these beetles? Are there any morphological features that characterise the larvae of ovoviviparous species? Does their morphology differ significantly from that of oviparous species? Could any such differences be associated with the exploitation of different food resources or with different ways of utilising the same type of food? Finally, how does the situation observed in Eurynotina compare with patterns known from other Tenebrionidae and from Coleoptera more generally?

6. Summary

The presented dissertation provides new and original morphological and genomic data on the poorly studied darkling beetles of the subtribe Eurynotina. Through the successful application of an integrative approach, the Candidate follows modern standards in research on insect taxonomy, systematics, and phylogeny. His leading role in the preparation of the studies comprising the dissertation is beyond doubt. I believe that MSc Ryan Lumen has demonstrated both the ability to conduct independent and effective scientific research and the skills necessary



to analyse and interpret the results and present them in the form of a coherent scientific dissertation.

In view of the above, I conclude that the doctoral dissertation submitted by MSc Ryan Lumen fully meets the requirements specified in Article 187 of the Act of 20 July 2018 – Law on Higher Education and Science (Journal of Laws of 2018, item 1668, as amended). I therefore request that the Scientific Council of the Museum and Institute of Zoology of the Polish Academy of Sciences admit MSc Ryan Lumen to the further stages of the doctoral proceedings.

At the same time, in recognition of the high scientific quality, originality, and comprehensive scope of the research presented, I recommend that the dissertation be awarded a distinction, in accordance with the regulations of the Museum and Institute of Zoology of the Polish Academy of Sciences.