



Review

of the doctoral dissertation by MSc Alexandra Tokareva, titled: "*A comprehensive genus-level reconstruction phylogeny of Paederinae (Coleoptera: Staphylinidae) based on combined genomic and morphological data*", conducted at Museum and Institute of Zoology of the Polish Academy of Sciences, Polish Academy of Sciences, under the supervision of Prof. dr. hab. K. Wioletta Tomaszewska and Dr. Dagmara Żyła

1. Introduction.

The research presented in this dissertation focuses on the subfamily Paederinae within the megadiverse family Staphylinidae. Paederinae is one of the largest among the 37 recognized subfamilies of rove beetles, yet it has received limited attention over the past century. This has complicated both its taxonomy and classification. The phylogeny of Paederinae remains largely unexplored, hindered by outdated species descriptions, the need for classification of new material, and numerous large, unrevised genera. Previous studies have suffered from an incomplete taxonomic and geographic scope, as well as a restriction to either morphological or molecular data alone. Most available molecular data originate from broader phylogenetic studies on Coleoptera, which include only a limited number of Paederinae genera and sequences. To address these challenges, the Candidate has proposed a total-evidence approach, integrating both molecular and morphological data to infer relationships and evolutionary processes within Paederinae while also establishing a new systematic framework for the studied taxon. Given the group's diversity, the limited knowledge of its phylogeny and taxonomy, the poor prior recognition of its fauna, and the necessity of integrating both 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 Alexandra Tokareva is an original and valuable study, with the Candidate's leading role in preparing its most critical parts clearly confirmed by statements from co-authors. The published papers and manuscripts included in the dissertation propose a



reconstruction of the phylogeny of Paederinae based on total-evidence data, followed by a revision of the subtribal system within this subfamily. The scope of the research, the complexity of the scientific problems addressed, and the extensive morphological documentation presented in chapters 4 and 5 of the thesis alone meet the criteria required for a doctoral dissertation.

Structured into five separate article- or manuscript-based chapters, this work makes a significant contribution to the ongoing discussion on the phylogeny, systematics, and taxonomy of the subfamily Paederinae, a large and diverse group of Coleoptera. The morphological revisions, in-depth analysis of thoracic morphology, and phylogenetic reconstruction supported by fossil data and NGS UCE methods provide a strong foundation for substantial of Paederinae. Notably, this dissertation is the first to use genomic data with such extensive taxon sampling for phylogenetic reconstruction in this group. An undeniable aspect of originality is also evident in the dissertation's section dedicated to alpha taxonomy (where the Candidate had a more limited role), which includes numerous species descriptions and redescriptions. The dissertation represents a modern study that meets high scientific standards in Coleoptera taxonomy, systematics and phylogeny research. It is fully comparable to other high-quality studies addressing similar topics in different invertebrate groups.

b. Scientific Value of the Dissertation

Each of the five extensive article-based chapters—three of which are already published papers, while the remaining two are manuscripts prepared for journal submission—addresses taxonomic or evolutionary issues related to the subfamily Paederinae. Using a total-evidence approach, the Candidate reconstructed the phylogeny of Paederinae and proposed a new subtribal system. The scope of the research, the complexity of the scientific problems tackled, and the extensive morphological documentation presented in the dissertation fully meet the criteria required for a doctoral dissertation. MSc Alexandra Tokareva based her research on an extensive dataset. A comparative analysis of thoracic morphology was conducted on 155 representatives, with two to five specimens of each species sourced from 14 museum collections. The study focused on examining the details of the mesoventral plate (preepisternum, prepectus, anapleural suture, anapisterno-basisternal connection, furcal arms). Dissected structures were examined and documented using light microscopy and scanning electron microscopy (SEM). The documentation is of high quality, with reproducible drawings and images that ensure reliable comparisons of



illustrated structures across species. A small but important contribution is the comparison and unification of terminology used by different authors to describe the mesoventral plate (Table 1, chapter 4). The morphological data matrix included 204 characters, most of which were adopted from previous studies, with a significant original contribution of 29 newly added characters related to the mesoventral plate (chapter 4). The molecular studies involved specimens from 190 different species across 157 genera of Paederinae (145 specimens preserved in ethanol, 56 dry-pinned), sourced from 17 museum collections. A custom group-specific UCE bait set was designed to target 2,143 UCE loci, with a median of 657 UCEs recovered per taxon. The phylogenetic analysis, based on a total-evidence dataset comprising 204 morphological characters and 196,896 bp of molecular data, was performed using IQ-TREE v2.3.4 software.

The results led to a thorough and well-supported revision of both the tribal and subtribal systems. The monophyly of Paederinae and their division into four tribes were confirmed. The new tribe, Sphaeronini, was strongly supported in all analyses, and the monophyly of the remaining tribes also received high support. The most important changes in the tribal and subtribal system include: 1) elevation of the subtribe Sphaeronina Casey to the tribal level (Sphaeronini); 2) designation of the new subtribe Scymbaliina, along with its diagnosis; 3) redefinition of six subtribes, with revised genus compositions and updated diagnoses. The Candidate has definitively demonstrated that ultraconserved elements (UCEs) serve as a valuable source of data for reconstructing phylogenetic relationships within the studied taxon. Additionally, the Candidate found that the morphology of secondary structures of the mesoventral plate exhibits a stable pattern of variation across larger taxonomic groups. A comparative analysis of phylogenetic trees before and after incorporating mesoventral plate data into the morphological matrix highlighted the significance of these characters in providing a phylogenetic signal at the subtribal level. The total-evidence approach proved highly effective, as the inclusion of morphological data significantly increased resolution at the subtribal level and provided additional support for certain lineages.

Unfortunately, attempts to date the phylogeny using Fossilized Birth-Death (FBD) analysis were not entirely successful. The Candidate assessed the results as unreliable. However, despite these limitations, the obtained topology and age estimations may still be informative for future analyses. The Candidate intends to continue these studies in collaboration with the second supervisor, Dr. Dagmara Żyła.



3. Substantive Value of the Dissertation

I assess the substantive value of the dissertation as high. The study's aims are clearly formulated, and the proposed research methods are well-suited to achieving them. The foundation of the taxonomic and phylogenetic analyses lies in refining methods that ensure the acquisition of reproducible and comparable data. Given the complexity of the chosen research subject, this presents a considerable challenge. The mesoventral plate, selected by the Candidate for an in-depth morphological analysis, is a highly complex three-dimensional structure of the thorax. Its preparation requires careful extraction from specimens in a consistent manner while preserving its overall shape. Sclerotized, three-dimensional structures such as the mesoventral plate are particularly challenging to document reproducibly using light microscopy, schematic drawings, or scanning electron microscopy (SEM). It should be noted that the Candidate has mastered these techniques to a very high standard, likely benefiting from the expertise of her second supervisor. The morphological characteristics of the species studied in the dissertation are extensively documented through schematic drawings and SEM images on a large scale. This provides a solid and reliable foundation for phylogenetic and evolutionary analyses, as well as for the definitions of newly established or redefined taxa. Phylogenetic relationships were reconstructed using ultraconserved elements (UCEs) for the first time in this group, with the application of appropriate, up-to-date software and first applied so high taxon sampling. The interpretation of the results is precise, and the conclusions—particularly regarding the reclassification of Paederinae into four tribes and 16 subtribes—are well-founded.

4. Editorial Correctness of the Dissertation

The dissertation is extensive (293 pages) and well-structured. The main part is preceded by a abstract in both Polish and English, as well as acknowledgements. The next chapter, "Summary", follows the structure of typical scientific article. After a brief "Introduction" (3 pages), the Candidate clearly defines the research objectives in the "Aims of the study" chapter (1 page). The "Materials and Methods" chapter (5 pages) provides a comprehensive summary of the methodologies applied across all studies in the dissertation. Similarly, the "Result" chapter (4 page) offer a collective synthesis of findings. These are followed by a concise "Conclusion" (1 page) and



six-page-long "References". The "Summary" is followed by a full text articles/manuscripts included in the dissertation, designated as "chapters 1-5". These articles/manuscripts are large, consisting of 24, 16, 78, 49 and 86 pages, respectively. Each of these follows the typical structure of a scientific publication, including an introduction, materials and methods, results, and discussion. The final two sections (manuscripts) also contains extensive supplementary materials. The esthetical presentation of morphological documentation and phylogenetic trees deserves special recognition. The dissertation concludes with "chapter 6", which outlines further research plans and perspectives.

5. Critical Comments

The presented dissertation is an large work of high scientific merit. The critical remarks provided should be regarded primarily as suggestions for modifications or updates to some of the thesis.

The first remark concerns the dissertation's volume. In my opinion, there is no justified reason to include papers (chapters) 1 and 3 in the dissertation. Given the unsuccessful attempt of dating the final phylogenetic tree (UCE), there was no need to include paper 2 either. The scope of the content, applied research methods, presented morphological documentation, and obtained phylogenetic reconstructions in chapters 4 and 5 are sufficient in themselves as a dissertation. If structured this way, the Candidate (and the supervisors) would have achieved a more cohesive and well-rounded body of work rather than an oversized form that appears to be assembled from everything "at hand".

One of the most important parts of the dissertation, the analysis of mesoventral plate structure (chapter 4), is presented in an exceptionally hermetic manner. It lacks an introductory schematic drawing and SEM image of a sample species with ALL analysed mesoventral plate elements clearly marked. In subsequent figures and illustrations, the abbreviations are sparse, making significantly more difficult assessment of the homology and similarity of structures across different taxa/types. The schematic drawings are visually appealing but very difficult to analyse and compare due to the lack of clear abbreviations. All presented mesoventral plate structure types should be documented with the following: drawing of outer side, SEM images of outer and inner side (for each of the 23 types). To be frank, I was unable in some cases to determine whether the designated character states are indeed reliable.



I was particularly surprised by the identification of as many as 23 mesoventral plate types. I hope that during the public defence, the Candidate will convincingly justify this typology by clearly presenting and explaining the differences between the proposed types. In my opinion, based on the provided documentation, we are certainly dealing with a continuous variation of forms and shapes, and the presented classification/division into "types" lacks justification. I have similar concerns regarding a significant portion of the designated character states, which, in my view, do not exhibit clear polarization to justify their inclusion in the matrix for phylogenetic analyses (e.g., characters 7, 9, 10, 13, 14, 17, 21, 23, 24). I also have a question: did the Candidate attempt to analyse matrix characters for potential correlation? The application of correlated characters in analyses is a common artefact in classical cladistics when trying to extract the maximum number of traits from studied structures.

Fortunately, the Candidate based the phylogenetic reconstruction largely on molecular data, using a modern method that provides a large amount of information (UCE). However, the obtained tree contains numerous (seven) monophyletic clades that are only provisionally named as "lineages". Why, in the case of these clades, many of which are species-rich, did the Candidate not formally establish additional subtribes?

A technical note regarding the illustrative documentation – the background in SEM images should be cleaned. In its current form, it disrupts visual perception and makes it difficult to observe the illustrated features.

6. Summary

The presented dissertation provides new, original morphological and genetic data for the poorly studied rove beetles of subfamily Paederinae. Through the successful application of total-evidence methods, the Candidate aligns with modern approaches and standards in the research of insect taxonomy, systematics, and phylogeny. Her leading role in the preparation of the dissertation is beyond doubt. I believe that MSc Alexandra Tokareva has demonstrated the ability to conduct effective scientific research, as well as the skill to process results and present them in the form of a scientific dissertation.

In view of the above, I conclude that the evaluated doctoral dissertation of Alexandra Tokareva fully meets the conditions specified in art. 187 of Act of 20 July 2018, Law on Higher Education and



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Science (Journal of Laws of 2018, item 1668, as amended). I therefore request the Scientific Council of the Museum and Institute of Zoology of the Polish Academy of Sciences to admit Alexandra Tokareva to the further stages of the doctoral dissertation.