

Review of Doctoral Thesis

Name and surname of the candidate: **Drashti R. Parmar**

Thesis title: **Reconstructing Evolutionary Histories of Calliphoridae (Diptera: Cyclorrhapha: Oestroidea) at Different Taxonomic Levels Using Phylogenomic Methods**

Supervisor: **Prof. dr hab. Krzysztof Szpila**

Co-supervisor: **Dr Nikolas P. Johnston**

Reviewer: **Dr hab. Tomasz Mamos, prof. University of Lodz**

1. Scientific Value of the Thesis

I. Originality of the Research:

This thesis represents an important advancement in dipteran phylogenomics by assembling the largest mitochondrial genome dataset for Calliphoridae to date, covering 107 species. The candidate successfully addresses critical knowledge gaps regarding the evolutionary history of *Luciliinae* and *Calliphorinae*, particularly through the inclusion of previously underrepresented Afrotropical taxa. The discovery and formal description of two new species (*Calliphora teraramma* and *C. mesay*) from the Ethiopian Highlands highlights the study's original contribution to biodiversity. Furthermore, by challenging the traditional "stepwise" model of parasitism evolution and demonstrating the flexible, reversible nature of larval feeding strategies, the research offers novel evolutionary insights that challenge established dogmas in the field.

II. Scientific Value of the Chapters/Articles

The chapters constitute a high quality studies that effectively bridges systematics, evolutionary biology, and applied entomology. The rigorous characterization of mitogenome architecture provides foundational data on gene rearrangements and codon usage biases. The phylogenetic analyses resolve subfamily relationships, validating Rhiniinae and Rhinophorinae while exposing significant genus-level instability in *Calliphora* and *Lucilia*. The integration of molecular dating with biogeographic analysis offers a compelling narrative for the Miocene-Pliocene radiation of blowflies. Additionally, the updated barcode libraries have immediate practical value for forensic and veterinary fields, enhancing species identification accuracy in regions where data was previously sparse.

2. Substantive Value of the Dissertation

The candidate demonstrates a high level of methodological competence, effectively utilizing genome skimming to bypass traditional sequencing limitations and unlock museum specimens. The introduction clearly formulates the research hypotheses regarding phylogenetic resolution and trait evolution. The selection of tools—ranging from genome skimming and Bayesian inference for divergence dating to ancestral state reconstruction is appropriate and state-of-the-art. The statistical analysis of codon usage and strand asymmetry is robust. The interpretation of results is mature; the candidate critically analyzes conflict between datasets (e.g., the unstable placement of *Eurychaeta palpalis*) and contextualizes findings well within existing literature. The conclusions regarding the independent origins of obligate parasitism are logically derived from the data and clearly presented.

3. Editorial Correctness of the Dissertation

The thesis constitute of three published and one submitted paper with proper abstract (in Polish and English) and extended summary of the thesis. The dissertation is written in clear, precise academic English with appropriate use of specialized terminology. The structure is logical, moving seamlessly from broad introductions to specific genomic analyses, taxonomic descriptions and evolutionary discoveries. The text is well-referenced, demonstrating a wide knowledge of the field. The descriptions of figures (phylogenies, mitogenome maps) and tables show a high standard of graphic presentation and data visualization. The taxonomic nomenclature follows the International Code of Zoological Nomenclature conventions rigorously. The naming of new species using Amharic roots (*teraramma*, *mesay*) shows cultural sensitivity and editorial thoughtfulness regarding the type locality.

4. Critical Comments

While the dissertation is scientifically sound, I need to rise a few points, especially focusing on the fourth article.

I. The study relies heavily on mitochondrial genomes. While cost-effective, mtDNA is a single locus subject to introgression and maternal inheritance biases. The candidate acknowledges this, noting that nuclear data is needed to resolve deep nodes; however, the inclusion of nuclear markers (such as USCOs) for the problematic genera would have strengthened the conclusions. Such an addition to the fourth paper would be highly welcome and is feasible through the assembly of short reads, even if only by mapping to candidate genes.

II. The lack of nuclear genes is particularly evident in the species delimitation methods. Furthermore, delimitation was based solely on barcode-gap-based methods (ABGD, ASAP). Current

standards typically require a comparison of multiple methods, especially phylogeny-based ones (e.g., mPTP) and multi-gene approaches (e.g., STACEY or BPP).

III. For the mitochondrial-based phylogeny, RNA genes could also have been utilized using a dedicated aligner such as LocARNA. Furthermore, in such reconstructions, it is advisable to test whether (and which) genes can be concatenated, rather than defaulting to concatenating them all.

IV. What is the justification for the concatenation of all genes? Did they all share the same substitution model?

V. In my opinion, a 0.6–1.2% genetic distance and 98 SNPs across the whole mitochondrial genome are insufficient for differentiating new species, particularly in insects. Larger population sampling and the inclusion of nuclear markers should be performed to better justify this decision.

VI. For the time-calibrated phylogeny reconstruction, the Yule species model and a relaxed clock were used as priors. This selection should be justified. In my opinion, the Yule model specifically should be replaced with a Birth-Death prior, as some level of extinction is expected in such a deep-level phylogeny. Additionally, direct priors for MRCA calibration (distribution, median, 95% HPD) should be provided in the supporting material.

VII. Regarding the fourth, unpublished study, beyond the inclusion of nuclear data, I strongly suggest analysing diversification rate shifts (e.g., using BAMM) to substantiate the temporal arguments. Furthermore, I recommend applying comparative methods (e.g., MCMCglmm or PGLS) to rigorously model the evolution of larval feeding habits.

5. Final Assessment

This dissertation represents an important contribution to entomology and evolutionary biology, and reading it was very interesting for me. The structure, the well-made summary, and the author's personal touch made a very good impression. The PhD candidate successfully achieved the research goals, filling major gaps in the mitogenomic coverage of Calliphoridae and providing a robust phylogenetic framework for the family. I am strongly convinced that in future, the gaps in nuclear data will be successfully mitigated.

The discovery of new species and the elucidation of the evolutionary history of parasitism demonstrate both technical skill and scientific creativity. The work is methodologically sound, substantively rich, and clearly presented. Furthermore, the candidate's role as first and corresponding author on the published studies proves their ability to conduct independent, high-quality research. I strongly recommend the acceptance of this thesis and consider it a significant step forward in the study of Oestroidea.

I, the undersigned, state that the reviewed doctoral dissertation of Drashti R. Parmar meets the conditions specified in Act of 20 July 2018, Law on Higher Education and Science (Academic Degrees and Title and on Degrees and Title in the Arts (Journal of Laws 2018 item 1668 with amendments), and I request that the Faculty of Biological and Veterinary Sciences at Nicolaus Copernicus University in Toruń admit Drashti R. Parmar to the further stages of the doctoral proceedings.

Ja, niżej podpisany stwierdzam, że recenzowana rozprawa doktorska Drashti R. Parmar spełnia warunki określone ustawą z dnia 20 lipca 2018 r. – Prawo o szkolnictwie wyższym i nauce (Dz.U. 2018 poz. 1668 ze zm.) i wnioskuję do Rady Wydziału Nauk Biologicznych i Weterynaryjnych Uniwersytetu Mikołaja Kopernika w Toruniu o dopuszczenie Drashti R. Parmar do dalszych etapów przewodu doktorskiego.

05.12.2025

Date



Tomasz Mamos