

## DNA barcode-based delimitation of the *Glossiphonia* species

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**Key words:** species delimitation, molecular taxonomy, *Glossiphonia*, Eastern Siberia

**Motivation and Aim:** Our knowledge about global hirudofauna is getting continuously enriched, however the taxonomic uncertainty of the *Glossiphonia* genus still persists. To resolve the issues associated with the determination of species status, molecular and bioinformational methods must be integrated into the practice of taxonomic research along with classical analysis of morphological features. In present study we utilized a combination of different approaches to solve the problem of leech species delimitation within the *Glossiphonia* genus.

**Methods and Algorithms:** Morphological analysis was carried out in accordance with the existent classification [1, 2]. Molecular analysis was performed by the standard phenol-free extraction and the subsequent amplification with universal COI primers. Phylogenetic analysis was performed with the use of Maximum Likelihood (ML) method implemented in MEGA 7.0 [3], to determine the threshold value of intraspecific genetic variations we used GMYC method based on the analysis of ultrametric tree obtained from Bayesian inference (BI) in BEAST v.1.8.4 [4]. Additionally, ABGD program was used to determine the barcode gap [5].

**Results:** The results of morphological, molecular and 4 bioinformational methods (ML, BI, GMYC и ABGD) indicate the independent taxonomic status of at least eight species within the *Glossiphonia* genus, including two Eastern Siberian species – *G. verrucata* and *Glossiphonia* sp.

**Conclusion:** The combination of results of present study points out the increased objectivity of biodiversity estimation within the *Glossiphonia* leech genus in case of implementing classical methods in conjunction with molecular, phylogenetic and bioinformational methods of analysis.

**Acknowledgements:** The study was performed in frame of the state assignment No. 0345-2016-0002 (AAAA-A16-116122110066-1), and in part of the Baikal fauna investigation was financially supported by the Russian Foundation for Basic Research (RFBR), project No. 17-2900097.

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