Genetic diversity of the flat leeches (Hirudinea, Glossiphoniidae) in Western Siberia

Nadezhda Bolbat
Irkutsk State University
Irkutsk, Russia
nadya.mandzyak@mail.ru

Lyudmila Fedorova
Surgut State University
Surgut, Russia
ludiko@list.ru

Irina Kaygorodova
Limnological institute SB RAS
Irkutsk, Russia
irina@lin.irk.ru

Introduction

Glossiphoniidae is a family of parasitic annelids that plays an important role in the environment, providing nutrition to aquatic vertebrates, a regulator of the number of hydrobionts, being parasites of mainly mollusks, as well as carriers of helminths and blood parasites of vertebrates, as well as an indicator of environmental stress. The history of the study of flat leeches in Western Siberia originates in the first half of the 20th century. Recent study of the Ob River macrozoobenthos have revealed six species of flat leeches - *Glossiphonia complanata* (L., 1758), *Alboglossiphonia heteroclita* (L., 1761), *Glossiphonia concolor* (Apáthy, 1888), *Helobdella stagnalis* (L., 1758), *Hemiclepsis marginata* (Müller, 1774), *Theromyzon tessulatum* (Müller, 1774). Earlier, in the Kazakhstan part of the Irtysh River Basin, five species of flat leeches were recorded - *H. stagnalis*, *H. marginata*, *G. complanata*, *T. tessulatum* and *Alboglossiphonia* sp.; moreover, 3 distinct morphotypes were found within *G. complanata*.

The faunistic studies of flat leeches are complicated because of lack of distinct taxonomic system. The scarcity of external morphological features and, at the same time, high intraspecific variability makes it difficult to determine the taxonomic informativity of morphological diagnostics.

For taxa boundaries assessment, as well as for new species identification, DNA barcoding is increasingly being used. Based on the results of similar studies, we decided to use DNA barcoding in conjunction with classical morphological analysis to delimit species of flat leeches from Western Siberia.

Materials and methods

Biological material was collected in freshwater bodies of the Irtysh and the Ob Rivers Basins in 2014-2018. Morphological analysis of flat leeches was carried out in accordance with modern zoological identification keys. Molecular analysis was conducted though a standard phenol-free DNA extraction with subsequent amplification with universal mtCOI primers. Sanger's sequencing was performed at the «Syntol» Company (Moscow, Russia). Phylogeny reconstruction was carried out with the Tamura-Nei model using the Maximum likelihood (ML) method implemented in MEGA 7.0.

Results and discussion

Morphological analysis

T. tessulatum

H. marginata

H. stagnalis

***Alboglossiphonia* sp.**

3 distinct complanata-like morphotypes

***Glossiphonia* sp. 1**

***Glossiphonia* sp. 2**

***Glossiphonia* sp. 3**

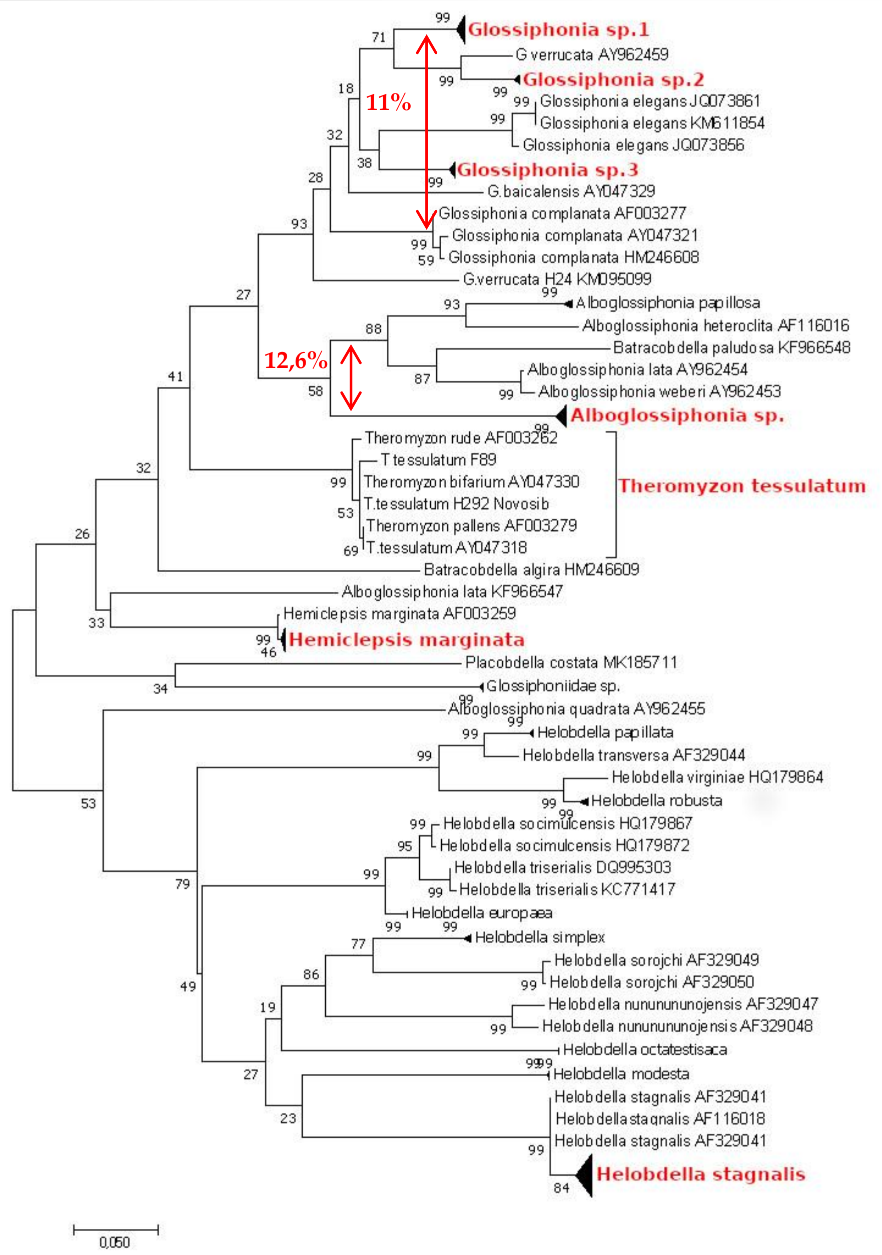

Fig. 1. ML-tree (lnL =-20102,61), based on 119 COI nucleotide sequences with length of 709 bp

Molecular genetic analisys

Sequence analysis revealed that the Western Siberian flat leeches split into 7 phylogenetic lineages (Fig. 1), genetic pairwise distances between which vary from 3.7% to 18.2%. These values exceed the threshold value for interspecific variability, which may indicate their independent species status. In addition to Theromyzon tessulatum, Helobdella stagnalis and Hemiclepsis marginata, previously detected in the region, four potentially new species - *Glossiphonia* sp. 1, *Glossiphonia* sp. 2, *Glossiphonia* sp. 3, and *Alboglossiphonia* sp. were found. Based on the COI-phylogeny, it can be argued that 3 species, which morphologically fit the *G. complanata* description, which indicates the presence of a cryptic species complex. P-distances between them and the *G. complanata* clade are 8-11 %. Nucleotide sequences of the Western Siberian *Alboglossiphonia* sp. formed a separate branch, distant from representatives of the congeneric European fauna, with a significant genetic distance of 12.6%. The results obtained for the Western Siberian leeches once again confirm the advantage of the DNA barcoding application, as one of the molecular methods for species delimitation, used in an integrated approach to the study of taxonomic composition of a fauna.

This work was supported by RFBR grant 17-29-05097.