

molecular-phylogenetic reconstructions, which were obtained earlier at *16S rRNA* (Cooper and Chapleau, 1998) and *Cyt-b* (Kartavtsev et al, 2007 b) genes well confirm our even partial sequence data at *Co-1* gene. However, some complications are available and there will be included in the discussion.

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TOWARD A SYSTEMATICS AND PHYLOGEOGRAPHY OF EIGHT-BARBEL LOACHES OF THE GENUS *LEFUA* (COBITOIDEA: NEMACHEILIDAE) AROUND OF THE SEA OF JAPAN

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Eight-barbel loaches of the genus *Lefua* Herzenstein, 1888 are small fresh-water fishes, inhabiting the rivers and lakes of the eastern part of Asia. It is accepted (Berg, 1948; Zhu, 1989; Nakabo, 2002) that the genus consists of four species. One of these species, *L. costata* (Kessler, 1876), is widely distributed on the continent (from the Amur River basin to the Chinese province Shandong in the south). The other species are found on the islands: *L. echigonia* Jordan et Richardson, 1907, on Honshu Island; the taxonomically not yet legalized *Lefua* sp., in the southwest of Honshu Island and on Shikoky Island; *L. nikkonis* (Jordan et Flower, 1903), on Hokkaido Island, northern part of Honshu Island (Prefecture Aomori), and the southern part of Sakhalin Island. Recent studies of genetic differentiation of *Lefua* from the islands of Japanese Archipelago (Saka et al., 2003; Sakai et al., 2003; Mihara et al., 2005) generally confirmed the accepted structure of the genus. According to these studies, *L. nikkonis* was close to *L. constata* (the latter species was represented by samples from the south of the Korea Peninsula), while *L. echigonia* and *Lefua* sp. were well-differentiated lineages. Recently, it was demonstrated (Naseka, Bogutskaya, 2004) that eight-barbel loaches from the south of Primorskii krai were not morphologically identical to typical *L. costata* from the northeast of China, and should be considered as an distinct species, *Lefua pleskei* (Herzenstein, 1887). For these reasons, in the present study genetic divergence and phylogenetic position of eight-barbel loaches from different localities of Primorye (including the type locality of *L. pleskei*, Ilistaya River) among the other groups of the genus *Lefua* was evaluated based on sequence analysis of the mtDNA control region and cytochrome b gene sequences (about 2070 bp a total).

Comparative analysis of own sequence data for eight-barbel loaches from eight localities in the Amur River basin (4), the Sea of Japan (4) and the GeneBank/NCBI data for the eight-barbel loaches from the other regions of the East Asia revealed that eight-barbel loaches from Primorskii krai water basins have a specific group of mtDNA

haplotypes. This finding is considered as supporting the species status of *L. pleskei*. Genetic distances within *L. pleskei* are small (on average 0.4%) and close to those within *L. nikkonis* (on average 0.5%). The distances between this species pair are the least (on average 2.2%) among all other pair comparisons. In MP, ML, and Bayesian trees, *L. pleskei* and *L. nikkonis* haplotypes formed a common clade with high statistical support. In all tree variants, *L. costata* mtDNA haplotypes were located from outside of forementioned clade. A clade included of highly diverged lineages of *Lefua* sp. and *L. echigonia* haplotypes occupied basal position. The mtDNA haplotypes of *L. pleskei* and *L. costata* from the Amur River basin were evolutionary young and derived from the haplotypes found in these species from the Sea of Japan (*L. pleskei*) or the Yellow Sea (*L. costata*) basins. It is thereby suggested that both species rather recently migrated into the Amur River system. According to the molecular dating, basal diversification of the eight-barbel loach lineages probably took place at the end of middle Miocene (about 11 to 12 Myr ago), while divergence of *L. pleskei* and *L. nikkonis* ancestral forms probably occurred approximately, 5 Myr ago. Since all main lineages of eight-barbel loaches were found in the Sea of Japan basin (continental coastline and the islands), the divergence order and dispersal patterns of the *Lefua* species might have been largely determined by the geological development of this water body and the adjacent territories.

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