

Molecular phylogeny of the genus *Choanomphalus* (Pulmonata: Planorbidae)

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ABSTRACT. Phylogenetic relationships of the genus *Choanomphalus* were analyzed by comparison of partial nucleotide sequences of mitochondrial gene coding for I subunit of cytochrom C oxydase. [CO I] The degree of nucleotide diversity accumulated within Baikalian species suggests that the common ancestor of all Recent Baikalian species existed approximately 3.5 MY ago. It gave rise to two major clades which most likely originated from small sand-dwellers and underwent a secondary radiation approximately 2.5 MY ago. The non-Baikalian species *Ch. mongolicus*, which is endemic to Lake Hövsgöl (North-western Mongolia), appears to be a valid member of one of the lineages of Baikalian endemics.

hoy, 1946. There are also 3 subgenera (*Lamorbis* Starobogatov, 1967, *Vitreoplanorbis* Moskvicheva in Zatarawkin, 1985, *Pseudogyraulus* Prozorova et Starobogatov, 1996) from Europe, Altai and Russian Far East. Starobogatov and Sitnikova [1992] have outlined several possible evolutionary scenarios for the Baikalian group. Keeping in mind the necessity to revise completely the taxonomy of the genus, the authors mentioned 22 species. They argued that the first stage of the genus evolution was a split between small and large forms, the larger ones crawling on stone surface, the smaller ones "fitting well into small cavities". Then both groups occupied new substrates: sand and soft silt. The process of adaptation of these two substrates generated new adaptive shell traits.

Here we attempt to clarify the evolutionary history of this group based on the comparison of nucleotide sequences of the fragment of mitochondrial gene coding for subunit I of cytochrom=C=oxydase.

Introduction

The systematics of the genus *Choanomphalus* Gerstfeldt, 1859 is contradictory and unclear. Dybowski [1875] attributed 3 species to this genus, Lindholm [1909] added 13 new species and divided all known species into three subgenera, believing that *Ch. aorus* Boettger is a variety of *Ch. amauro-nius*. He was followed by Dybowski and Grochmalicki [1925] who proposed to isolate the choanomphalans as a separate family Wladislawidae while increasing the amount of species of the Baikalian choanomphalans to 22-24 and subdividing them into six large groups. According to Kozhov [1936], the genus *Choanomphalus* in Lake Baikal includes 2 subgenera and 8 species. He treated many of previously described taxa as subspecies or varieties and described a new species *Ch. annuliformis*. Kozhov proposed to treat the Baikalian choanomphalans as a separate subfamily Choanomphalinae within the family Planorbidae. Beckman and Starobogatov [1975] have described five new deep-water species and proposed to subdivide the genus *Choanomphalus* (Planorbidae) into 8 subgenera, including some taxa treated by Kozhov as synonyms. Seven subgenera are Baikalian endemics and one (*Achoanomphalus* Lindholm, 1909), besides Baikalian species, includes Hövsgölian endemic *Ch. mongolicus* Koz-

Material and methods

Samples. Gastropod were collected from lakes Baikal, Hövsgöl (Mongolia), and Biwa (Japan) (Table 1). Snails were either preserved in 96% ethanol or kept alive. Shells from the samples subjected to further analysis have been kept and used as a control of species identity. Baikalian and Hövsgölian species were identified by T. Sitnikova by conchological characters, according to an unpublished key composed by Ya. Starobogatov. Species from Biwa were identified according to Mori [1938].

DNA extraction. Foot muscle tissue was used for DNA extraction. For both fresh and ethanol fixed tissues the same slightly modified Cetyl trimethyl ammonium bromide [CTAB] extraction by modified method [Doyle, Dixon, 1987] was employed. The homogenisation was performed in buffer containing 100 mM Tris-HCl, 1.4M NaCl, 20mM EDTA, 2% CTAB, 0.2% β-merkaptoethanol. After a 30-min incubation at 60°C in a water bath and a twice repeated extraction by equal volume of chloroform, DNA was precipitated by iso-propanol. Only single individuals have been used for analysis.