

SHORT
COMMUNICATIONS

Molecular Evolution of the Lake Baikal Endemic Caddisflies (Trichoptera)

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Abstract—Phylogenetic relationships of the nine Lake Baikal endemic caddisfly species with the representatives of the genus *Apatania* (Apataniidae) were reconstructed on the basis on nucleotide sequences of the mitochondrial *COI* gene. The results of Bayesian analysis pointed to the relationships of the endemics with the North American *Apatania incerta* (Banks, 1897) and *A. sorex* (Ross, 1941). The divergence of the genetic lineages of the Baikalian and Nearctic apataniids occurred in the Late Oligocene–Early Miocene. On the dendrogram, endemic species formed two monophyletic clades corresponding to the tribes Thamastini and Baicalinini. It was also found that the genus *Protobaicalina* was polyphyletic, while the genus *Baicalina* was a species flock. Our analysis showed that the nearest common ancestor of the studied Baikal caddisfly species existed about 21 Mya, while the modern species composition of endemic fauna dates back to Pliocene–Pleistocene age (2.5–0.7 million years).

Keywords: Lake Baikal, Apataniidae, endemic caddisflies, phylogenetic analysis

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Lake Baikal is the largest ancient lake located in the south of Eastern Siberia. Over 25–30 million years of its existence, it has formed taxonomically rich and unique fauna; the species diversity of some of its groups (amphipods, mollusks) is comparable to the species diversity of the fauna of all water bodies in Eastern Siberia and Eurasia together. Moreover, 60% of the animal species inhabiting Baikal are found only in this lake [1].

Endemic trichoptero fauna of Lake Baikal is represented by seven genera (four of which are monotypic) of the family Apataniidae. A.V. Martynov [2] was the first to subdivide the then subfamily Apataniidae into two tribes, Apataniini and Baicalinini, which included endemic species. V.D. Ivanov and T.V. Menshutkina [3] on the basis of morphological traits combined endemic species into the tribes Thamastini (*Baicalodes ovalis* Martynov, 1924, *Baicalinella foliata* Martynov, 1924, *Protoradema baicalensis* (Martynov, 1914), *Protoradema setosum* (Martynov, 1924), *Radema infernale* Hagen, 1864, *Thamastes dipterus* Hagen, 1958) and Baicalinini (*Protobaicalina miltispinosa* (Mey, 1994), *Protobaicalina tallingi* (Rozhkova, 1996), *Protobaicalina spinosa* (Martynov, 1914), *Protobaicalina nigrostriata* (Martynov, 1914), *Baicalina bellicosa* Martynov, 1914, *Baicalina reducta* Martynov, 1924, *Baicalina thamastoides* Martynov, 1914, *Baicalina*

levanidovae Ivanov et Menshutkina, 1996). All these species live exclusively in the Baikal open waters and are not found outside of the native lake. In Lake Baikal itself, the larvae in mass inhabit the rocky littoral of the lake, and in this zone, they make up to 20% of the macrozoobenthos abundance and biomass [4].

Morphological analysis of adult individuals enabled A.V. Martynov [5] to suggest that endemic species in Lake Baikal derived from primitive forms similar to species of genus *Apatania* Kolenati, 1848. Currently, the water bodies around the Lake Baikal are inhabited by five species of the genus *Apatania*: *A. crymophila* MacLachlan, 1880, *A. stigmatella* (Zetterstedt, 1840), *A. zonella* (Zetterstedt, 1840), *A. döhleri* Schmid, 1954, and *A. majuscula* MacLachlan, 1872. The latter species is also found in the bays of the lake.

Our study was aimed at solving two problems: first, on the basis of molecular data for the mitochondrial cytochrome *c* oxidase subunit I gene (*COI*), to establish phylogenetic relationships within the group of Lake Baikal endemic caddisflies and their relationships with the other members of the family Apataniidae; second, to determine the age of endemic caddisfly genera and the time of their divergence from the sister taxa.

The material was collected in the period from 2002 through 2011 at twelve localities of Lake Baikal and the

Angara River. The larvae were fixed in 70% ethanol and the adult insects were dried. Endemic caddisflies were assigned to the species according to identification keys [2, 3, 6]. Isolation of genomic DNA was performed by the modified method of J.J. Doyle and E.E. Dickon [7]. The 710-bp fragment of the mitochondrial *COI* gene was amplified with standard primers LCO1490 and HCO2198 [8]. The amplification conditions consisted of denaturation at 95°C (5 min); followed by 40 cycles at 95°C (30 s), 50°C (30 s), 72°C (50 s); and final elongation at 72°C (8 min) [9]. The structure of PCR products was determined on an ABI 3130XL genetic analyzer at the DNA Sequencing Center for Collective Use, Siberian Branch of the Russian Academy of Sciences, Novosibirsk. The obtained sequences were edited and aligned in the BioEdit 7.0.7 software program [10].

The calculation of genetic distances was performed using the APE package functions [11] in the R 2.14.1 programming language. The HKY + I + Γ best-fit nucleotide substitution model was chosen using the BIC criterion in the jModelTest 2.1.3 software program [12].

The dataset for phylogenetic analysis consisted of unique haplotypes of the species of the genus *Apatania* and nine species of Baikalian caddisflies. Seven representatives of different genera of the sister family Limnephilidae were added to the dataset for calibration of the molecular clock. Phylogenetic analysis was performed with the parallel version of the MrBayes 3.2.0 software program [13]. The choice of molecular clock model was made with the help of the Bayesian approach to statistical evaluation in the same program. The model with relaxed molecular clock was used in subsequent phylogenetic reconstruction. The dataset was divided into three parts in accordance with the nucleotide position in the codon, and the nucleotide substitution models were chosen in the jModelTest 2.1.3 software program with the BIC criterion (TN93 + Γ , F81, and GTR + Γ , for the first, second, and third nucleotide position, respectively). The calibration of the phylogenetic tree was performed on the basis of the age of fossil representatives of the families Limnephilidae and Apataniidae from the Eocene Baltic amber [14]. The time of occurrence of each family was set by a shifted exponential distribution with a minimum value of 34 million years and the expectation of 56 million years. Two independent analysis with four Markov chains in each were conducted to reconstruct the phylogenetic trees. The length of chains was 45 million generations, with sampling every 2000 generations. The first 25% of the tree samples were excluded from further analysis. Convergence of the results of Bayesian analysis was evaluated by the values of the effective sample size (ESS > 200). The calculations were performed on the Blackford computing cluster of the Irkutsk Supercomputer Center of the Russian Academy of Sciences [15].

The nucleotide sequences were obtained for the *COI* gene fragments isolated from 64 specimens belonging to nine endemic species and one Palearctic species, *A. majuscula*. After editing and alignment, the nucleotide sequences were deposited in the GenBank database. The length of the alignment was 591 bp.

The mean value of interspecific divergence between the studied Baikalian apataniids was 8.75% substitutions, ranging from 2.61% (between *B. thamastoides* and *B. reducta*) to 12.58% (between *B. bellincola* and *R. infernale*). Two Baikalian tribes demonstrated different levels of interspecific genetic polymorphism. It was in the range of 2.61–6.12% substitutions for the species of the tribe Baicalinini and from 4.57 to 12.58% for the species of the tribe Thamastini. Intraspecific and interspecific distances within the genus calculated for the Baikalian endemics and *Apatania* are shown in the table.

Analysis of the phylogeny show that none of the apataniid species living at present in the water bodies around Lake Baikal are the sister species to the Baikalian caddisflies. On the dendrogram, apataniids are divided into two groups. The first group includes the species of the genus *Apatania*, except for *A. incerta* (Banks, 1897) and *A. sorex* (Ross, 1941), which together with the Baikalian endemic species form a second group (Fig. 1a). This group corresponds to the phyletic section complexa, subsection complexa, which is the most ancient and primitive, and to which, in addition to the North American *A. nigra* (Walker, 1852), *A. alberta* Nimmo, 1971, *A. comosa* (Denning, 1949), *A. incerta*, and *A. sorex*, were attributed the Baikalian endemic species *Protoradema baicalensis* and *Protoaicalina nigrostriata* [17]. Similar relationships between the Baikalian endemics and North American species are also found in other amphibiotic insects, chironomids of the genus *Orthocladius* [18]. The relatedness of the Baikal and Nearctic faunas is obviously the result of the ancient relationships during the existence of intercontinental bridges in the Beringia [17]. According to molecular data, the divergence between the studied Baikalian species and North American species occurred 25 Mya (95% CI: 20–31 Mya).

The representatives of the tribe Thamastini can be considered the most ancient endemic caddisflies. The common ancestor of the genera *Baicalinella*, *Radema*, *Baicalodes*, and *Thamastes* lived about 16 Mya (95% CI: 12–21 Mya). The stage of the geological development of the Baikal Rift, which began 30 Mya and lasted for 20 million years, is associated with global cooling, the beginning of orogeny, and the appearance at the site of the present-day Lake Baikal, in the contours of its Southern and Central depressions, of a large and deep (up to 500 m) water body, Southern–Central proto Lake Baikal. These factors led to the zoogeographic isolation of the Baikal Depression and the development of the Baikalian endemic hydrobiota [19].

Pairwise genetic distances for the species of the genus *Apatania* and the Baikalian endemics

Genus	Species	GenBank acc. no. ¹	Number of nucleotide sequences	Mean genetic distances, % (min–max)		Number of species in the genus [16]
				intraspecific	interspecific (within one genus)	
<i>Apatania</i>	<i>A. stigmatella</i>	KC559549, KJ675332, GU114429, GU114428	4	1.08 (0.18–1.82)	11.80 (0.84–14.79)	~100
	<i>A. zonella</i>	JQ907592, JQ907593, GU114430, KJ674860	4	0 (0.00–0.00)		
	<i>A. incerta</i>	HM102534–HM102540	7	2.29 (0–4.26)		
	<i>A. majuscula</i>	KR153079–KR153087	9	0.71 (0–1.63)		
	<i>A. wallengreni</i>	KJ675047	1	–		
	<i>A. sorex</i>	JQ935298	1	–		
	<i>A. helvetica</i>	KT613192	1	–		
<i>Protobaicalina</i>	<i>P. spinosa</i>	KR153124	1	–	4.71	4
	<i>P. tallingi</i>	KR153115–KR153123	9	0.07 (0–0.18)		
<i>Baicalina</i>	<i>B. thamastoides</i>	KR153144	1	–	2.94 (2.61–3.45)	4
	<i>B. bellicosa</i>	KR153125, KR153127–KR153132	7	0.20 (0–0.72)		
	<i>B. reducta</i>	KR153133–KR153139, KR153141–KR153143	10	0.80 (0–1.81)		
<i>Baicalodes</i>	<i>B. ovalis</i>	KR153105–KR153114	10	1.30 (0–2.56)	2.56	1
<i>Thamastes</i>	<i>Th. dipterus</i>	KR153094–KR153097	4	0.81 (0–1.63)	1.63	1
<i>Baicalinella</i>	<i>B. foliata</i>	KR153098–KR153104	7	0.15 (0–0.54)	0.54	1
<i>Radema</i>	<i>R. infernale</i>	KR153090–KR153093	4	1.60 (0.54–2.56)	2.56	1

¹ GenBank acc. no., sequence accession number in the GenBank database.

The time of existence of the nearest common ancestor of the genera *Protobaicalina* and *Baicalina* (tribe Baicalinini) falls within the Late Miocene (95% CI: 5.5–10.6 Mya) and coincides with the filling of the Northern Baikal Depression by the waters of the Southern–Central proto Lake Baikal. Along with the segregation of the genetic lineages of two genera, the divergence within *Protobaicalina* took place. The node combining both genera is characterized by polytomy,

which makes *Protobaicalina* a polyphyletic genus. Another genus of this tribe, *Baicalina*, by contrast, is monophyletic and a flock of species that have minimum interspecific distances among the studied Baikalian caddisflies, with the mean value of 2.9% substitutions.

The time of appearance of modern Baikalian species is consistent with the earlier supposition on the

Pliocene–Pleistocene age of endemic caddisfly fauna [3]. This age is associated with the beginning of glaciation and the death of thermophile caddisfly species. Population of the free niches by cryophilic apataniids could have served as a trigger for the appearance of new species in the Lake Baikal. On the phylogenetic tree, the expected time of existence of the ancestors of *B. bellicosa*, *B. reducta*, *P. tallingi*, *B. foliata*, *R. infernale*, *T. dipterus*, and *B. ovalis* falls within the period between 0.7 and 2.5 million years. Thus, the observed diversity of Baikalian species can be treated as a result of recent explosive speciation [20].

Our preliminary data on the evolution of the Baikal caddisflies are generally consistent with the concepts of development of this group of invertebrates based on the analysis of morphological characters. The differences of the molecular phylogenetic tree topology from classical taxonomy are minor and have a posteriori probabilities close to 0.50.

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