

Genetic Polymorphism of Siberian Newt (*Salamandrella keyserlingii*, Caudata, Amphibia) in Its Range and the Cryptic Species of the Newt *S. schrenckii* from Primorie

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The Siberian newt (*Salamandrella keyserlingii* Dybowski, 1870) occupies a vast range from tundras to steppes and from the Pacific Ocean to the northwestern borders of the European part of Russia. However, its morphological characters exhibit only a slight geographic variation, so the species is considered monotypical. Taxa of different levels have repeatedly been described in various parts of the range, but their validity is not recognized at present [1]. However, there is a general notion that the Siberian newts from Primorie (the southeastern region of continental Russia near the Sea of Japan) are morphologically and biologically distinct, with populations from other parts of the range insignificantly differing from one another [1–7], although this distinction is not reflected in the taxonomic status. Siberian newts from Primorie also differ from others with respect to genome size [8].

To determine the possible genetic variation in the large range of the morphologically monomorphic species, we analyzed the variation of the nucleotide sequences of the cytochrome *b* gene in mitochondrial DNA (mtDNA) of 86 Siberian newts from five regions: Sakhalin, Chukotka, the Magadan oblast, Primorie, and the Sverdlovsk oblast (the Urals). We found considerable genetic differences (mtDNA divergence of 9.8–11.6%) between Siberian newts from Primorie and those from all other parts of the range. Such differences correspond to interspecific distinction. Therefore, it may be concluded that, although Siberian newts are morphologically monomorphic, there are two taxa of them: *S. keyserlingii* live at all the aforementioned sites

of collection except southeastern Russia, but Primorie is inhabited by another species.

The nucleotide sequences of an 825-bp region of the cytochrome *b* gene in mtDNA (between positions 14 214 and 15 308 according to the nucleotide numbering in the total mitochondrial genome of *Ranodon sibiricus* [9]) were determined by automated sequencing with the use of two pairs of primers: (1) MVZ15L and MVZ18H and (2) MVZ25L and ControlWH, which were suggested in the study [10]. We compared our data with the nucleotide sequences of the cytochrome *b* genes of different species and genera of the families Hynobiidae and Salamandridae from the database of GenBank (<http://www.ncbi.nlm.nih.gov/entrez>). In all types of phylogenetic analysis, the nucleotide sequences of the cytochrome *b* genes of three species from the genus *Euproctus* (family Salamandridae) were used as an outgroup. In addition, the available paleontological data on this species allowed us to calibrate the “molecular clock”: the rate of mutation accumulation in the cytochrome *b* gene was estimated at 0.77% of difference per million years [11].

We identified 15 variants (haplotypes) of the cytochrome *b* gene in the sample studied (Fig. 1), which exhibited marked geographic differentiation. The haplotypes of Siberian newts were grouped into two large monophyletic clusters, one of them comprising only mtDNA variants from Primorie and the other consisting of haplotypes from all other regional samples (Fig. 2). The divergence of nucleotide sequences varied between 0.1 and 11.6% (differences in 1 to 96 nucleotides), the main contribution (9.8–11.6%) being made by mtDNA haplotypes from Primorie differing from mitochondrial lineages of Siberian newts from other regions by 81–96 mutations. Siberian newts from Primorie were also characterized by a variation within the population that was considerably larger than that in the pooled Magadan, Sakhalin, Chukotka, and Ural samples (1.86 versus 0.38%).

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Haplotype	N	1111111	1112222222	2222222222	3333333333	3333444444	4444444445	5555555555	6666666666	6667777777	7777777777	88	
Sakhalin-1	(2)	TTTACCACA	GCTGTTCCCT	GTGCCGGTTC	GATAACACTG	AATATAATCT	AATCCCTCCT	TTCACCCCCC	CTTGCTACCT	CACATATACA	GACTCTTTTC	TGAATAAATC	CA
Sakhalin-2	(2)A.....
Sakhalin-3	(5)A.....
Ural-1	(8)A.....C.....T.....
Magadan-1	(23)A.....C.....T.....
Magadan-2	(2)A.....A.C.....T.....
Magadan-3	(1)A.....C.....T.....
Magadan-4	(12)A.....C.....T.....
Chukotka-1	(3)A.....C.....T.....
Vladivostok-1	(13)	CCCCCTTGGT	ATCACC.TCC	ACATAAACCC	A.CCCT.TCA	GGAGC..CTC	CGCCTTATTC	CCTGGTTTTT	T.CA.C.TTA	T..GC.C..G	ATTG.CC.CC	C.GTC.GGCT	TT
Vladivostok-2	(1)	CCCCCT.TTT	ATCACC.TTC	ACATAAACCC	A.CCCTGTCA	.GAG....TC	C.CCCTTATTC	.CTGGTTTTT	T...TCGTTA	.GT.C....G	ATTG.C..CC	...T.G.GCT	T.
Vladivostok-3	(2)	CCCCCT.TTT	ATCA.CTTC	ACATAAACCC	ATCCCT.TCA	.GAG....TC	C.CCCTTATTC	.CTGGTTTTT	T...TCGTTA	.GTGC...TG	ATTG.C..CC	...T.G.GCT	T.
Vladivostok-4	(1)	CCCCCTTGGT	ATCACC.TC.	ACATAAACCC	A.CCCT.TCA	GGA.CG.CTC	TGCCCTTATTC	CCTGGTTTTT	T.C.TC.TTA	T..GC...G	ATTG.C.CCC	...TC.GGCT	TT
Vladivostok-5	(3)	CCCCCTTGGT	ATCACC.TC.	ACATAAACCC	A.CCCT.TCA	GGA.CGGCTC	CGCCTTATTC	CCTGGTTTTT	T.CATC.TTA	T..GC..G.G	ATTG.C..CC	...TC.GGCT	TC
Vladivostok-6	(2)	CCCCCTTGGT	ATCACC.TCC	ACATAAACCC	A.CCCT.TCA	GGAGC..CTC	CGCCTTATTC	CCTGGTTTTT	T.CA.C.TTA	T..GC.C..G	ATTG.CC.CC	CAGTC.GGCT	TT
Vladivostok-7	(4)	CCCCCTTGGT	ATCACC.TCC	ACATAAACCC	A.CCCT.TCA	GGAGC..CTC	CGCCTTATTC	CCTGGTTTTT	T.CA.C.TTA	T..GC.C..G	ATTG.CC.CC	..GTC.GGCT	TT
Vladivostok-8	(2)	CCCCCTTGGT	ATCACC.TCC	ACATAAACCC	A.CCCT.TCA	GGAGC..CTC	CGCCTTATTC	CCTGGTTTTT	TCCA.C.TTA	T..GC.C..G	ATTG.CC.CC	..GTC.GGCT	TT

Fig. 1. Haplotypes of the cytochrome *b* gene in Siberian newt mtDNA. The positions of variable nucleotides are indicated according to the nucleotide sequence of the *Sakhalin-1* haplotype. *N* is the number of animals with the given haplotype. Identical haplotypes found in different regional groups of Siberian newts are highlighted.

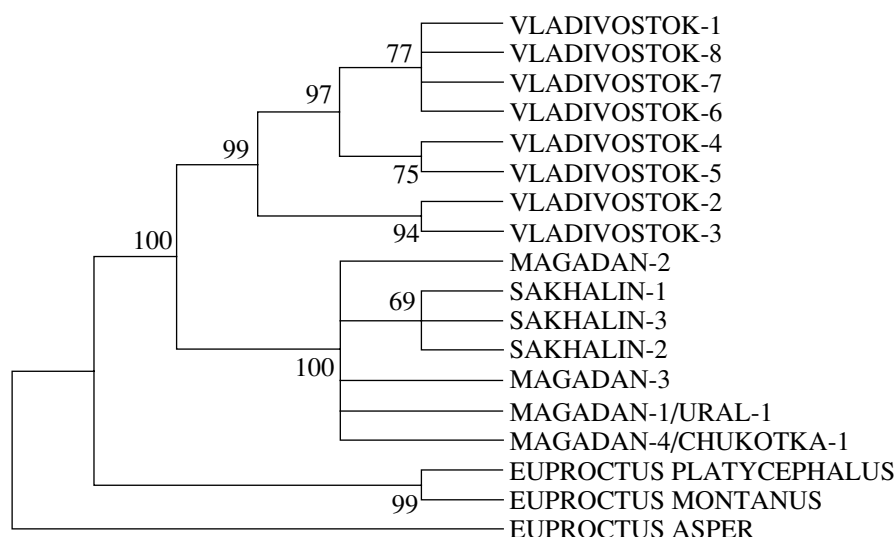


Fig. 2. The phylogenetic tree of cytochrome *b* haplotypes of Siberian newts (constructed with the use of the maximum parsimony method). The bootstrap indices are indicated at the branches.

The observed considerable differences between Siberian newts from Primorie and other regions with respect to the cytochrome *b* haplotypes are large enough to be regarded as interspecific distinction, as follows from the results of comparison of 16 species from several genera of the families Hynobiidae and Salamandridae with respect to the nucleotide sequences of this gene. For example, interspecific differences in the genus *Batrachuperus* (six species were compared) corresponded to a nucleotide sequence divergence of 4.4–9.3%; in the genus *Pseudohynobius* (two species), 15.5%; *Hynobius* (three species), 10.7–12.7%; *Euproctus* (three species), 10.7–21%.

We studied the phylogenetic relationships between the taxa of the families Hynobiidae and Salamandridae for which the nucleotide sequences of the cytochrome *b* gene were known with the use of the maximum parsimony method, with the statistical significance of clusters being estimated using bootstrap analysis. The results

obtained confirmed *Salamandrella* can be classified as a separate genus.

Representatives of *Salamandrella* were diverged from other genera of the family Hynobiidae by 15.5–18.8%, which was close to these values for other genera from the same family (14.4–18.1, 14.4–20, 14.3–18.9, 20, and 19.4% for *Pseudohynobius*, *Hynobius*, *Batrachuperus*, *Ranodon*, and *Liua*, respectively). *Salamandrella* is very similar to *Pseudohynobius* with respect to the genus scheme. Species of the genus *Euproctus* (family Salamandridae) differ from genera of the family Hynobiidae in a greater divergence (18.9–25.7%). In this tree, the sample that we studied was distinctly divided into two clusters, which further confirmed that there were two species with different haplotype sets in the genus *Salamandrella*.

The correspondence between low degrees of morphological and genetic variations of *S. keyserlingii* over the vast range may have resulted from a relatively rapid

formation of the range during the Holocene. Such a rapid postglacial spread over Eurasia was also demonstrated by the results of phylogeographic analysis of the greater spotted woodpecker [12] and other birds. On the other hand, as *S. keyserlingii* needs little heat for sustaining life (in summer and in winter), the range may have been begun to form gradually as early as in the Pleistocene [13]. This seems possible, if we take into account the age of this species, which we estimated at 490×10^3 years on the basis of the degree of mtDNA intrapopulation divergence.

The age of Siberian newts from Primorie (which had a considerably greater intrapopulation genetic variation than *S. keyserlingii*) estimated by the same method is 2.4×10^6 years. This indicates that Siberian newts have lived in the region for a long time. An increased mtDNA variation has also been found in the mouse *Apodemus peninsulae* in the same region, Primorie [14], a refugium noted for relatively stable natural environment. During the Quaternary period, Primorie escaped the catastrophes characteristic of the remaining boreal Palearctic region.

It should be remembered that the aforementioned estimates of species ages and divergence times are only conventional. However, even the orders of magnitude of the obtained values and relationships between them allow us to conclude that *S. keyserlingii* and the Siberian newt from Primorie are related to each other as two species of different ages but not as "ascendant and ancestor." Their ancestors segregated from the common evolutionary branch about 14×10^6 years ago (if we estimate the divergence using the calibration accepted in this study).

The observed genetic differences between *S. keyserlingii* and Siberian newts from Primorie agree with the conclusions of our predecessors that the latter are a distinct group (see references above). Taken together, the existing data provide sufficient grounds for classifying Siberian newts from Primorie as a separate species and restoring for them the species name *S. schrenckii*, which has been considered thus far a junior synonym of *S. keyserlingii* [15].

Salamandrella schrenckii (Strauch, 1870), sp. dist.—Schrenck Siberian newt.

Isodactylum schrenckii Strauch, 1870: 56.

Lectotype (designated): specimen with labels "No. 115. *Isodactylum Schrenckii* Str. Agdeki ad Ussuri. Dr. L. V. Schrenck. 1855," "No. 115 Trdet. O. Gumilevskii. *Hynobius keyserlingii* (Dyb.) Ogdeki Ussuri 1855. Leg.: Schrenck." Stored in the collection of Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia. Lectotype originates from the vicinity of settlement Agdeki (= Kukalevo, Khabarovsk krai) south of the Podkhorenok River mouth.

The type series described by Strauch also included specimens from Lake Baikal and the Shilka River. It is

doubtful that they belong to *S. schrenckii*, because *S. keyserlingii* was described precisely from Baikal.

S. schrenckii differs from *S. keyserlingii* in the haplotype set (mtDNA divergence of 11.6%), genome size [8], mean number of vertebrae and costal grooves [7], the shape of clutch and some other biological characteristics [5, 6], and the absence of courtship dances [3]; although the latter requires confirmation. Morphologically, the species are difficult to distinguish, and some individual animals cannot be currently identified without the use of biochemical methods. This justifies the application of the term cryptic species to *S. schrenckii*.

While the manuscript was being prepared, we analyzed five more Siberian newts that N.E. Dokuchaev collected for us in the vicinity of the Georgievka village located in the middle reaches of the Kiya River (a right tributary of the Ussuri) in the southern Khabarovsk krai. This site was located at least 50 km away (measured on a straight line) from the place where Schrenck found the type specimen. Analysis of mtDNA showed that all animals belonged to *S. schrenckii*. Thus, the spread of *S. schrenckii* to the north has been reliably established in the Ussuri River basin as far as 48° N. *S. schrenckii* is very likely to be found in the basin of the Nemta River, a right tributary of the Amur (a little north of the 48th parallel).

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REFERENCES

1. Borkin, L.Ya., *Sibirskii uglozub: Zoogeografiya, sistematika, morfologiya* (Siberian Salamander: Zoogeography, Taxonomy, and Morphology), Moscow: Nauka, 1994, pp. 54–80.
2. Ostashko, N.G., in *Voprosy gerpetologii* (Problems of Herpetology) (Abstr. V All-Union Herpetological Conference), Leningrad: Nauka, 1981, p. 98.
3. Korotkov, Yu.M., *Zool. Zh.*, 1977, vol. 56, no. 8, pp. 1258–1260.
4. Sapozhnikov, G.P., *Tr. Zool. Inst. Akad. Nauk SSSR*, 1990, vol. 213, pp. 75–90.
5. Basarukin, A.M. and Borkin, L.Ya., in *Ekologiya i faunistika amfibii i reptilii SSSR i sopredel'nykh stran* (Ecology and Faunistics of Amphibians and Reptiles of the Soviet Union and Neighboring Countries), Leningrad: Zool. Inst. Akad. Nauk SSSR, 1984, pp. 12–54.

6. Vorobyeva, E.I., Antipenkova, T.P., and Hinchliffe, J.R., *Dokl. Akad. Nauk*, 1999, vol. 364, no. 1, pp. 130–133 [*Dokl. Biol. Sci. (Engl. Transl.)*, vol. 364, no. 1, pp. 34–37].
7. Litvinchuk, S.N. and Borkin, L.J., *Contribut. Zool.*, 2003, vol. 72, no. 4, pp. 195–209.
8. Litvinchuk, S.N., Rozanov, Yu.M., Borkin, L.Ya., *et al.*, in *Voprosy gerpetologii* (Problems of Herpetology), Pushchino, 2001, pp. 168–170.
9. Zhang, P., Chen, Y.Q., Zhou, H., Wang, X.L., and Qu, L.H., *Mol. Phylogenet. Evol.*, 2003, vol. 28, pp. 620–626.
10. Goebel, A.M., Donnelly, J.M., and Atz, M.E., *Mol. Phylogenet. Evol.*, 1999, vol. 11, pp. 163–199.
11. Caccone, A., Milinkovitch, M.C., Sbordoni, V., and Powell, J.R., *Syst. Biol.*, 1997, vol. 46, pp. 126–144.
12. Zink, R.M., Drovetski, S.V., and Rohwer, S., *J. Avian Biol.*, 2002, vol. 33, pp. 175–178.
13. Berman, D.I., in *Rossiiskaya nauka: Prirodoi zdes' nam suzhdeno[...]* (*Russian Science: Nature Determined That Here We[...]*), Moscow: Oktopus, 2003, pp. 184–200.
14. Serizawa, K., Suzuki, H., Iwasa, M., *et al.*, *Biochem. Genet.*, 2002, vol. 40, no. 5/6, pp. 149–161.
15. Strauch, A., *Mem. Acad. Imp. Sci. St. Petersburg*, 1870, vol. 16, no. 4, pp. 1–110.