ISSN 0096-3925, Moscow University Biological Sciences Bulletin, 2010, Vol. 65, No. 1, pp. 34–39. © Allerton Press, Inc., 2010.
Original Russian Text © S.L. Kuzmin, N.A. Poyarkov, I.V. Maslova, 2010, published in Vestnik Moskovskogo Universiteta. Biologiya, 2010, No. 1, pp. 40–45.

On the Variability of Fire-Bellied Toads in the Far East

S. L. Kuzmin[a], N. A. Poyarkov[b], and I. V. Maslova[c]

[a] *Institute of Ecology and Evolution, Russian Academy of Sciences, Leninskii pr. 33, Moscow, 119071 Russia*
[b] *Department of Vertebrate Zoology, Faculty of Biology, Moscow State University, Moscow, 119991 Russia*
e-mail: n.poyarkov@gmail.com
[c] *Institute of Marine Biology, Far East Branch, Russian Academy of Sciences, Vladivostok, 690041 Russia*

Received May 25, 2008

Abstract—We studied external morphology and molecular genetics of two forms of the oriental fire-bellied toad, described as separate subspecies *Bombina orientalis silvatica* Korotkov, 1972, and *Bombina* orientalis practicola Korotkov, 1972. We have found significant differences between them in body size, coloration and some morphometric parameters but not in genetics. Taking into account their geographical separation, one can consider them as forest and meadow ecological morphs but not different taxa. Therefore, the names *Bombina orientalis practicola* Korotkov, 1972, and *B. orientalis silvatica* Korotkov, 1972, should be considered as junior synonyms of the name *Bombina orientalis* (Boulenger, 1980). We suppose that these morphs have been isolated from each other recently, and their morphological differences at genetic similarity are related to high plasticity of the species, when ecologically determined morphological differentiation precedes genetic differentiation.

Key words: *Bombina orientalis*, systenatics, subspecies, genetics, morphology, zoogeography, populations

DOI: 10.3103/S0096392510010074

The Far East of Russia (Primorye and the south of Khabarovsk Territory) is inhabited by the oriental fire-bellied toad *Bombina orientalis* (Boulenger, 1980), which is also encountered in the north-east of China and Korea. This species is morphologically very variable though this variability is poorly studied.

Yu.M. Korotkov [1] described two forms of fire-bellied toads from different types of biotopes of Primorye: *Bombina orientalis silvatica* and *Bombina orientalis praticola* (there is a misprint in the original: *Bomhiba*). According to Yu.M. Korotkov [1], the body length of female *silvatica* reaches 53 mm and mass reaches 11 g (male toads are in general 48 mm and 8.7 g and female toads 48 mm and 8 g). Dorsal surface is brown or green and the ventral is orange or yellow with black spots. This form inhabits mixed cedar-broad-leaved forests in the whole territory of Primorye. Sexual maturity occurs under the body length not less than 39–40 mm. Female toad lays up to 200 eggs with general mass up to 1.2 g. This form of fire-bellied toad leads an amphibiont way of life in the reproduction period and then terrestrial; water production makes 42% of diet. Female *practicola* toads reach body length 42 mm and mass 4.2 g (male toads, on average, are 35 mm and 3.3 g and female are 36 mm and 3.3 g). The dorsal surface is black-and-brown, practically black or green; the ventral is bright red (sometimes yellow) with black spots. This form was encountered only on sedge-reedy meadows and in grass associations in the mouth of Kievka River (Sudzukhe) and near railway station Khasan near the Vodonupty Bay in the South Primorye. Sexual maturity is reached under the body length not less than 30 mm. Female toad lays up to 150 eggs with mass 0.42 g. This form leads a mainly water way of life during the whole season of activity; hydrobionts make 80% of diet. In the valley of Kievka River both forms are separated by the distance 15–20 km. Yu.M. Korotkov and E.B. Korotkova [2] gave later some additional information on morphology of these two forms. But up to recently nobody either admitted or disproved their taxonomic validity.

Subspecies status of these two forms has been doubtful up to recently and the question of its sympatric distribution has not been clarified yet. The article is devoted to this problem.

MATERIAL AND METHODS

Field study was conducted in 1986–1999 years in Primorye near the Khasan settlement (coordinates 42°26' N, 130°39' EL), in the Zapovednik Kedrovaya Pad' (43°4' N, 131°33' EL) and in Ussuriysky

Zapovednik (cordon no. 1: 43°40' N, 132°25v EL, Anikin cordon: 43°39' N, 132°27' EL, and near the Kaimanovka settlement: 43°38' N, 132°14' EL). Besides, root study of the south of the Khasan District and Lazovsky Zapovednik was conducted. Probes of individuals for genetic analysis were obtained also from Khanka Lake and in province Heilongjiang (PRC). All in all 127 adult individuals were used.

Linear measurements were conducted by a trammel to 0.1 mm, using a Tanita 1476 balance. We used standard measurements and morphometric indices used in the systematic of amphibians [3]. For morphoemtric indices, we determined mean and error of mean (M±SE); reliability of differences of means was assessed by Student's test (t). Statistical treatment was conducted using the STATISTICA 5.0A program. For mapping of areas and analyzing biological parameters we used information form the "Amphibians of USSR" (®0229803415, State Register of databases of RF) database.

For genetic analysis, we used the ends of fingers of fair-bellied toads. This is a relatively harmless method, which allows animals to survive. Total genome DNA was extracted from small tissue samples fixed in ethanol using filtration on glass-fiber plates (GF) by silicate method of extraction of DNA and NucleoSpin® 96 Tissue kit (Macherey-Nagel Duren, Germany) by protocol of producer.

Final volume of all extracts of DNA was 60 μm. Extracts were suspended in 40 μL of distilled water and the region 600 bp near the 5'-end of the CO1 gene was amplified by standard protocol (Herbert et al., 2003). The total value of PCR was 12.5 μL and contained 2 μL of DNA extract (details: [4]).

Sequences of CO1 of full length were amplified with primers LepF1-5'-ATTCAACCAATCATAAAGATATTGG-3' and LepR1 5'-TAAACTTCTGGATGTCCAAAAAATCA-3' [5] or VF1-d (TTCTCAACCAACCACAARGAYATYGG) and VR1-d (TAGACTTCTGGGTGGCCRAARAAYCA). PCR was conducted in total volume 12.5 μl, which contained 2.5 mM $MgCl_2$, 5 pmol of each primer, 20 μM dNTP, 10 mM Tris HCl (pH 8.3), 50 mM KCl, 10-20 ng (1–2 μl) of genome DNA and one unit of TaqDNA-polimerase using termocyclic profile with one cycle 2 min under 94°, five cycles by 40 s under 94°, 40 s under 45°, and 1 min under 72° and then 35 cycles by 40 s under 94°, 40 s under 51°, and 1 min under 72° with the final stage 5 min under 72°. Products were visualized in 2° agarose E-Gel® 96-well system (Invitrogen) cleared by set PCR-purification kit (Millipore, Bedford, MA, United States). Probes were sequenced in two directions using the Big-Dye-Reaction kit (P.E., United States) on the DNA ABI 3730 analyzer (Applied Biosystems). Details of laboratory protocols are presented in article [6].

Construction of dendrogramms by algorithm of the nearest neighbor on the basis of genetic distance by the Kimura method [7] was conducted using the *ForcCon* v. 1.0, *TreeCon* v. 1.3b, MEGA 3.0 [8], *TreeView* v. 1.6.6 and BOLD (www.barcodinglife.org – [9]) programs. For assessment of level of differences of fire-bellied toads in and between species, two sisterly forms, which inhabit the former USSR, ordinary (*Bombina bombina*) and yellow-bellied (*B. variegata*) toads from Transkarpatie (Uzhgorod, Lumshory), were used. As external group anurans *Alytes obsteticans pertinax* and *Discoglossus galganoi* were used (numbers of sequences in GenBank are presented in Fig. 2). As a marker for assessment of genetic differentiation we used widely applied in systematic and diagnostic of amphibian cytochrome oxidase I gene (COI) with length 660 basepairs. Obtained sequences of fragment of cytochrome oxidase I gene are available at the COI Barcoding Amphibians page (www.barcodinglife.org). Stability of branches was assessed with the help of 1000 boot-strap (BS) replicas. Reconstruction of MP-dendrogram was conducted using MEGA 3.0 [8] and PAUP v. 4.1 [10].

RESULTS AND DISCUSSION

Morphological Differentiation of Oriental Fire-Bellied Toad. The obtained results (Table 1) indicated the following differences of *practicola* (population 1) from *silvatica* (populations 2–5). *Practicola* is smaller: it has smaller mass and body length, which is most vividly expressed in the comparison of maximums. However, female toads are reliably different from male and female toads of all populations by average length, besides population 2; male toads are different only from populations 3 and 5. According to average body mass, male toads differ reliably only from female toads from population 3 and male toads from population 5; male toads differ from all except male and female toads of population 5. By average ratio of head length to body length male *practicola* differ reliably from individuals of all studied populations. But some *silvatica* have a head respectively longer than *practicola* (population 5) and some have a shorter head (all other). Female toads by relative head length, on average, differ reliably both from male toads of their own population and male and female toads of population 5.

By average thigh length, female and male toads *practicola* differ reliably only from population 5. By leg length, male toads differ from all other except populations 2 and 5; female toads differ from all except 3 and 4. Average ratio of body length to thigh length and leg length to thigh length differ reliably from male toads *practicola* from female toads of population 2. The other differences are unreliable ($p > 0.01$). Thus, our data agree with the data of Korotkov [1, 11] about the smaller size of this form.

By a number of features, there are differences between studied populations *sivatica* (Table 1). Reliable differences of means are established by all studied parameters of morphology but no regularities were revealed. We may point out the only tendency to the

```
1 AACCTATATTTAGTCTTTGGTGCCTGAGCCGGAATGGTTGGAACTGCTCTCAGCTTGCTAATTCGAGCAGAGCTAAGC
2 ..............................................................................
3 ..............................................................................
4 .C...G.......A........................G........C..............................
5 .C...T...C...T............G....................C..T...C.AT..........G..A..G...

1 CAGCCTGGAACCTTGCTTGGAGACGACCAGATTTATAATGTAATCGTTACCGCCCACGCCTTCGTAATAATCTTCTTT
2 ...................................C..........................................
3 ..............................................................................
4 .....C..............G...........C..C..............T........T..T........T.....C
5 .....C.....AC................A.....C......G.T........T...T.............T..T...

1 ATAGTTATACCCATCATGATCGGCGGATTTGGTAACTGACTAGTTCCACTAATAATTGGAGCCCCAGACATAGCCTTC
2 ..............................................................................
3 ..............................................................................
4 .....C..G..T.....A.....G.................GA....CT......................G......
5 ..G..A..G........A..G..A..C.G...A........T..C..T..............T..G.....G......

1 CCTCGAATAAACAACATAAGCTTTTGACTTCTTCCCCCCTCGTTCCTACTACTTTTAGCATCATCAGGTGTGGAGGCG
2 ........G..T..................................................................
3 ...........AC.................................................................
4 ........G..T..T........C........C..T..A..A.....GT.G...........C.....C..A.....A
5 ........G..T.....G....................A..A.....CT....C........C..G.....A.....C

1 GGAGCCGGGACGGGTTGAACTGTCTATCCGCCATTGGCAGGAAACTTGGCCCACGCCGGAGCATCAGTAGACTTAACC
2 ..............................................................................
3 ..............................................................................
4 ........A..C..............C..A..CC.A..G..G...C.......T..A..............TC.G..T
5 ..G.....A..C...........T..C.....T..A..G......C.A.....T..A...............C....T

1 ATTTTTTCTTTACACCTTGCCGGAGTGTCCTCAATTCTAGGCGCTATTAATTTTATTACAACAACAATCAACATAAAA
2 ..................................................C...........................
3 ..................................................C........G..................
4 ..............T........G....................C.....C.................T.....G...
5 ..............T.....T..G..A........C..G........C..C.................T.....G...

1 CCACCAGCAATATCACAATACCAAACGCCATTATTTGTGTGATCCGTGCTAATTACAGCTATTCTCTTACTCCTCTCG
2 ............................................T.................................
3 ..............................................................................
4 ..C........G.....G..T.....A.....G.....A.....T....................TC....T..T..A
5 ..C........G.....G..T.....A.....G...........T..A.................TC.......T..A

1 CTTCCCGTTCTTGCTGCAGGAATCACCATACTTCTCACGGACCGCAATTTAAATACAACCTTTTTCGACCCTGCCGGG
2 ...................................T..........................................
3 .................................................................T............
4 ..C..T.................T.....G........A.....T........C...........T........T..A
5 .....T.....G..C........T..T..G.....T..A.....T........C...........T........T..A

1 GGAGGAGACCCTGTACTGTATCAACACTTATTC
2 .................................
3 .................................
4 ..G..C..T........A..C..G..TC.G...
5 ..G.....T........A..C......C.....
```

Fig. 1. Sequences of studied fragment of cytochrome-oxidase I gene for five forms of *Bombina*: 1, *Bombina orientalis* ssp., prov. Haylunzzyan, PRC; 2, *Bombina orientalis practicola*, Khasan, Primorye, Russia; 3, *Bombina orientalis silvatica*, Zapovednik Kedrovaya Pad', Primorye, Russia; 4, *Bombina orientalis variegata*, s. Lumshory, Transkarpatye, Ukraine; 5, *Bombina bombina bombina*, Minai, Transkarpatye, Ukraine.

increase of body sizes in south populations in comparison with north.

Coloration of the oriental fire-bellied toads varies significantly: the dorsal surface is brown-grey up to grey-green or bright green with dark spots; on the belly, bright red or orange coloration prevails dark by surface and forms an irregular picture. Individuals with two-color back (for example, with half green or half brown) coexist with one-color [12]. All studied individuals of *practicola* were brown from the dorsal side; one individual had both red but yellow spot on the belly. Among 229 studied *silvatica*, 24% were brown, 55% were green (several individuals were light green), 21% were mixed (a half of the back was green and a half was brown; in the middle it was green and on the sides it was brown or grey; green spots of medial green line were on the brown back). Coloration of *silvatica* could change in one individual to different casts of brown imitating color of tree waste [2]. Thus, the share of brown individuals in population allows to differ both forms.

Genetic differentiation according to data of COI gene fragment of mitochondrial DNA. Examples of obtained sequences of COI gene fragment for three species of genus *Bombina* are presented in Fig. 1. Genetic distances revealed between different populations *B. orientalis*, which were related by Korotkov to different subspecies, turned out to be extremely small (Table 2) and do not reach subspecies level of differences. The obtained dendrogram indicated clear differences of three nominal species: *Bombina bombina*, *B. variegata*, and *B. orientalis* (Fig. 2). At the same time, in layering of *B. orientalis*, genetic differences are insignificant and do not reach the subspecies level. Moreover, some individuals determined as *practicola* are united with *silvatica*. Thus, according to data of studied fragment

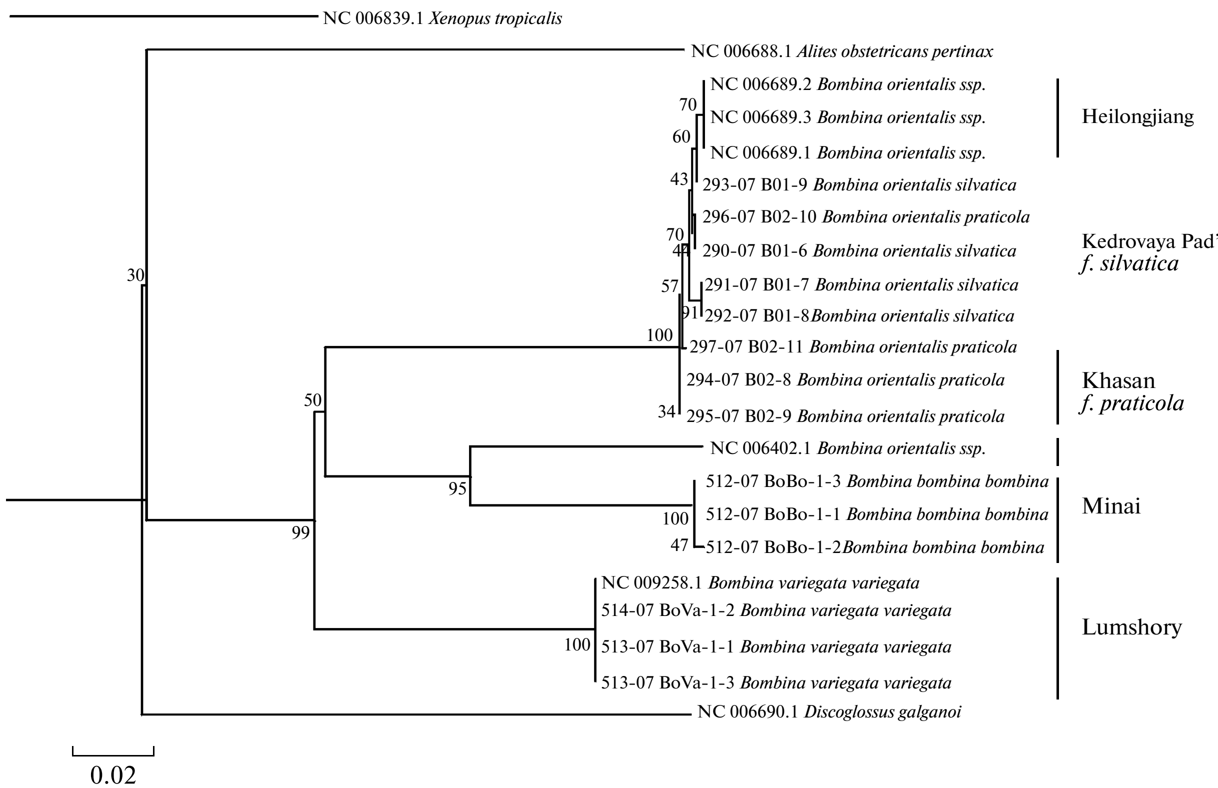

Fig. 2. NJ-dendrogram of interactions of studied populatiosn of genus *Bombina* built by data of fragments of fragment of cytochrome-oxidase CI (mitochondrial DNA) with length 660 bp.

of mitochondrial DNA, *practicola* and *silvatica* do not form genetically discrete layerings. Our results agree with data on the Korean fire-bellied toad [13] in reference to relatively low changeability of the oriental fire-bellied toad by mitochondrial DNA.

Distribution and biotopes of oriental fire-bellied toad. It was pointed out before that some populations of fire-bellied toads have intermediate morphology between *silvatica* and *practicola* [2]. In addition, the geographical border between them is unclear. That is why the "border" region between areas was studied in August 1999. It goes approximately in the region of Sukhanov range in the south of the Khasan District. *Silvatica* form is more widely spread to the north whereas *practicola* is to the south. No zone of sympatry was revealed. Moreover, the study of the floodplain of the Gladkaya River (to the south from Sukhanov range) did not reveal the presence of fair-bellied toads. According to our data *practicola* inhabits only sedge-reed and grass meadows of the Khasan plain, which lacks not only trees but even bushes. This form is kept only in water. Similar conditions of living of this form are also in the mouth of the Kievka River. *Silvatica* is encountered only in forest biotopes and never in ponds situated at open areas at the distance more than 200 m from a forest border. It leads a half-water way of life in the reproductive period and then lives overland.

According to our and literary data (see references: [12]), the area of form *practicola* is limited by the following points: Khasan District: Golubiny Utyos Mtn., 42°25' N, 130°45' EL; surroundings of Vodonupta Bay, region of st. Khasan, 42°26' N, 130°37' EL; Khasan honeydew, 42°25' N, 130°39' EL; surroundings of st. Khasan, 42°26' N, 130°39' EL; Swan Lake, 42°20' N, 130°42' EL, Lazovsky region: Melkovodnaya Bay, 42°51' N, 133°36' EL; mouth of Kievka River, 42°52' N, 133°40' EL; Kievka Settlement, Lazovsky Zapovednik, 42°54' N, 133°41' EL. The other points of findings [12, p. 120–180] apparently belong to *silvatica*. Apparently, this very form inhabits also on the north of Khasan District but this question needs further research. Taking into consideration the fact that Yu.M. Korotkov has been working for a long period of time in Lazovsky Zapovednik and gives good comparative data, their information on *practicola* in that region must be reliable. Consequently, in the species area of the oriental fire-bellied toad *practicola* occupies two isolated regions: on the south of Khasan District and in maritime part of Lazovsky Zapovednik. In both places no sympatry with *silvatica* was present.

In is interesting that *Quercus dentata* with the main area in Korea and Japan is encountered in the Russian Far East only on the extreme South of Primorye in the Khasan District and isolated population is in Lazovsky Zapovednik. It may be just a coincidence but it could

Table 1. Morphometric features of individuals from different populations: (1) *Bombina orientalis praticola* and (2–5) *B. orientalis silvatica*; M ± SE, min–max

Parameters	Populations							
	1		2	3	4		5	
n	16 male toads	5 female toads	25 female toads	25 female toads	15 male toads	13 female toads	25 male toads	13 female toads
L	37.03 ± 0.66	37.18 ± 0.7	37.85 ± 0.4	41.76 ± 0.42	39.41 ± 0.74	40.2 ± 1.01	43.59 ± 0.75	44.1 ± 0.46
	33.8–41.6	35.5–38.5	33.0–42.0	37.4–45.2	32.5–43.0	32.5–46.4	35.6–52.1	40.9–46.5
L.c	11.89 ± 0.45	10.58 ± 0.08	10.12 ± 0.17	11.71 ± 0.14	11.16 ± 0.44	11.27 ± 0.26	15.88 ± 0.21	15.49 ± 0.27
	9.2–15.4	10.5–10.9	9.0–12.5	9.6–13.0	9.1–15.5	9.8–12.8	13.9–17.8	14.0–17.1
T	14.78 ± 0.18	14.84 ± 0.32	15.04 ± 0.17	16.04 ± 0.13	16.87 ± 0.3	15.68 ± 0.24	–	–
	14.1–15.4	14.1–15.5	13.2–16.0	14.2–17.2	14.4–19.0	14.1–17.1		
F	15.99 ± 0.35	15.12 ± 0.36	14.6 ± 0.24	16.13 ± 0.17	16.55 ± 0.39	15.55 ± 0.30	18.66 ± 0.22	17.92 ± 0.34
	14.2–19.4	14.3–15.9	12.8–17.0	14.4–18.5	13.8–19.0	13.5–16.9	15.9–21.0	16.4–20.0
M	4.64 ± 0.42	3.35 ± 0.2	6.44 ± 0.21	7.02 ± 0.39	5.09 ± 0.27	5.02 ± 0.34	6.84 ± 0.25	6.86 ± 0.3
	3.4–6.6	3.0–3.7	4.3–8.0	4.3–10.7	3.4–6.8	2.9–7.4	4.0–9.6	5.4–8.6
L.c/L	0.32 ± 0.01	0.28 ± 0.01	0.27 ± 0.004	0.28 ± 0.003	0.28 ± 0.01	0.28 ± 0.01	0.37 ± 0.01	0.35 ± 0.001
	0.27–0.38	0.27–0.3	0.23–0.32	0.25–0.32	0.24–0.37	0.25–0.37	0.32–0.41	0.32–0.40
L/F	2.33 ± 0.3	2.46 ± 0.02	2.61 ± 0.04	2.6 ± 0.04	2.39 ± 0.04	2.59 ± 0.07	2.34 ± 0.03	2.47 ± 0.03
	2.09–2.51	2.42–2.49	2.24–3.08	2.23–3.02	2.19–2.61	2.27–3.09	1.98–2.59	2.28–2.66
T/F	0.98 ± 0.01	0.98 ± 0.02	1.03 ± 0.02	1.0 ± 0.01	1.02 ± 0.02	1.03 ± 0.03	–	–
	0.98–0.99	0.94–0.99	0.89–1.15	0.89–1.09	0.93–1.21	0.89–1.15		

Note: Populations: 1 – Surroundings of s. Khasan, Aug. 16, 1999, wasteland; 2 – Kedrovaya Pad', valley, broad-leaved forest, July 16, 1986; 3 – Ussuriysky Zapovednik, first cordon, mountain cedar-broad-leaved forest, June 18, 1986; 4 – Ussuriysky Zapovednik, valley cedar, broad-leaved forest, s. Kaimanovka, May 30, 1990; 5 – Ussuriysky Zapovednik, cedar, broad-leaved dark coniferous forest, Anikin cordon, May 27, 1997.

Table 2. Average *p*-distances (%) of comparison of sequences of fragment of COI gene for the study of samples (below the diagonal) and error (according to data of 50 bootstrap-replicas) (above the diagonal)

Sample	1	2	3	4	5
1		0.003	0.002	0.020	0.022
2	0.007		0.003	0.019	0.020
3	0.006	0.007		0.021	0.021
4	0.162	0.157	0.162		0.015
5	0.188	0.182	0.188	0.135	

Note: Samples: 1 – *Bombina orientalis* ssp., prov. Haylunzzyan, PRC; 2 – *Bombina orientalis practicola*, Khasan, Primorye, Russia; 3 – *Bombina orientalis silvatica*, Zapovednik Kedrovaya Pad', Primorye, Russia; 4 – *Bombina variegata variegata*, s. Lumshory, Zakarpatye, Ukraine; 5 – *Bombina bombina bombina*, Minai, Zararpatye, Ukraine.

not be eliminated that it reflects similarities of ecological demands and conditions of habitation on the local level.

CONCLUSIONS

There are reliable morphological differences in body size and coloration between forms *praticola* and *silvatica* of oriental fire-bellied toad but not by studied genetic features. Taking into consideration their geographical isolation, we may speak about the presence of two ecological morphs: forest and meadow but not about different taxons. Therefore, the names *Bombina orientalis practicola* Korotkov, 1972, and *B. orientalis silvatica* Korotkov, 1972, may be regarded as younger synonyms of *Bombina orientalis* [14]. Apparently the meadow morph originated from the forest as a result of settling or deforestation of landscape. These populations had to stay at open area in water when they lost forest biotopes typical for this species.

Reliable biotopic differences may be a reason for the formation of two morphs. These morphs have apparently been isolated recently, and morphological differences with genetic similarity are connected with high plasticilty of species when ecologically mediated morphological differentiation precedes genetic.

The work is supported by the Naturalis National Museum of Natural History, Leiden, Netherlands, and the international program of barcoding (Barcoding of Life Initiative, ABAAP) in particular, under support of the Canadian Center of DNA-barcoding, Biodiversity Institute of Ontario, Canada, and Paul D.N. Hebert personally, Alex Borisenko, Natali Ivanova and Alex Smith. We are very grateful to all the above mentioned people and also to B.D. Vasil'ev, professor from the Biological Department, Moscow State University.

REFERENCES

1. Korotkov, Yu.M., Biology of Oriental Fire-Bellied Toad *Bombina orientalis*), *Agkistrodon blomhoffi*, and *Agkistrodon halys* in Primorski Krai, in *Zoologicheskie problemy Sibiri* (Zoological Problems of Siberia), Novosibirsk, 1972, p. 302.
2. Korotkov, Yu.M. and Korotkova, E.B., Ecology of Oriental Fire-Bellied Toad (*Bombinf orientalis*), in *Redkie i ischezayushchie zhivotnye sushi Dal'nego Vostoka* (Rare and Endangered Terrestrial Animals of the Far East of Russia), Vladivostok, 1981, pp. 46–51.
3. Kuz'min, S.L., *Zemnovodnye byvshego SSSR* (Amphibians of the Former USSR), Moscow: KMK, 1999 [in Russian].
4. Hajibabaei, M. Ivanova, N.V., et al., Critical Factors for Assembling a High Volume of DNA Barcodes, *Philos. Transact. Roy. Soc. London, Ser. B*, 2005, vol. 360, pp. 1959–1967.
5. Herbert, P.D.N., Cywinska, A., Ball, S.L., and De Waars, J.L., Biological Identification through DNA Barcodes, *Proc. Roy. Soc. London, Ser. B,* 2003, vol. 270, pp. 313–321.
6. Smith, M.A., Poyarkov, N.A., and Hebert, P.D.N., CO1 DNA Barcoding and Amphibians: Take the Chance, Meet the Challenge, *Mol. Ecol. Resources*, 2007, vol. 8, no. 2, pp. 235–246.
7. Kimura, M., A Simple Method for Estimating Evolutionary Rate of Base Substitutions Through Comparative Studies of Nucleotide Sequences, *J. Mol. Evol.*, 1980, vol. 16, pp. 111–120.
8. Kumar, S., Tamura, K., Jakobsen, I.B., and Nei, M., MEGA3: Molecular Evolutionary Genetics Analysis Software. Tempe, Arizona: Arizona Sate Univ., 2005, pp. 150–163.
9. Ratnasingham, S. and Hebert, P.D.N., Bold: The Barcode of Life Data System (www.barcodinglife.org), *Mol. Ecol. Notes,* 2007, vol. 7, pp. 255–364.
10. Swofford, D.L., *PAUR* (and Other methods). Phylogenetic Analysis Using Parsimony, Version, 4.0. Sinauer Associates, Sunderland, MA, 2002.
11. Korotkov, Yu.M. and Korotkova, E.B., Ecology of the Oriental Fire-Bellied Toad (*Bombina orientalis*), in *Redkie i ischezayushchie zhivotnye sushi Dal'nego Vostoka* (Rare and Endangered Terrestrial Animals of the Far East of Russia), Vladivostok, 1981, pp. 46–51.
12. Kuz'min, S.L. and Maslova, I.V., *Zemnovodnye rossiiskogo Dal'nego Vostoka* (Amphibians of the Far East of Russia), Moscow: KMK, 2005 [in Russian].
13. Lee, H.Y., Park, O.Y., Jin, J.H., Oh, S.J., and Yang, S.Y., Fragment Analysis and Variation in Mitochondrial DNA of Korean Toad *Bombina orientalis*, *Korean J. Genetics*, 1996, vol. 18, no. 2, pp. 93–102.
14. Boulenger, G.A., A List of the Reptiles and Batrachians of Amoorland, *Ann. Mag. Nat. Hist.,* 1890, vol. 5, no. 6, pp. 137–144.