

## Genetic and morphological analyses of 50-chromosome spined loaches (*Cobitis*, Cobitidae, Pisces) from the Black Sea basin that are morphologically similar to *C. taenia*, with the description of a new species

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**Abstract.** Karyotyped specimens from three populations of spined loaches, genus *Cobitis*, that occurred in the Veleka (Bulgaria), Chernaya (Crimean Peninsula) and Southern Bug (Ukraine) Rivers in north-western Pontic region were subjected to enzyme electrophoresis (for 5 loci), comparative morphological studies, and phylogenetic analysis (based on PCR of a 1230 bp fragment of mtDNA and the cytochrome *b* gene). These studies resulted in the description of loaches from the Crimean Peninsula as a new species *Cobitis taurica*, while the taxonomic status of populations from the Veleka and S. Bug rivers appeared to be controversial and in need of further investigation.

**Key words:** “*Cobitis taenia* complex”, karyotype, nuclear and mtDNA markers, biogeography

### Introduction

Several species of European spined loaches of the genus *Cobitis* correspond to the Mayr' (1969) concept of sibling species (Vasil'eva & Vasil'ev 1998, Vasil'eva 2000). One group of such loaches, “the *C. taenia* complex”, contains diploid as well as hybrid polyploid forms, which differ significantly at the genetic level, but are nearly indistinguishable from *C. taenia* Linnaeus, 1758 by all major diagnostic morphological characters. For example, the presence of only one elongated dark spot at the base of a caudal fin, broad axe-shaped *lamina Canestrini* in males and more or less rounded scales with a relatively small central part devoid of cross furrows (see Vasil'eva 2000). One of these forms has been discovered in the Chernaya River, the south-western part of the Crimean Peninsula, the Black Sea basin, in 1981 (see Vasil'eva 1984). Loaches from this river displayed external morphological characters that were quite similar to those of *C. taenia* (see Vasil'eva 1984, 2000), but their karyotype was entirely different (2n=50 with chromosome arms, NF=96; Vasil'ev 1985, 1995, Vasil'ev & Vasil'eva 1994, Rábová et al. 2004) from any other European *Cobitis* species studied to date (see Vasil'ev & Vasil'eva 1982, Ráb & Slavík 1996, Borón 2003). Vasil'ev (1995) therefore hypothesized the separate specific status for loaches from the Chernaya River.

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Further extensive genetic screening of loach populations from south-eastern Europe using karyotyping, flow cytometry (FC), mtDNA and nuclear markers revealed other populations of spined loaches with  $2n = 50$  chromosomes and very similar karyotype structure in the Southern Bug R. (Ukraine) and the Veleka R. (southern Bulgaria). This discovery stimulated our research into this new form with such an unusual karyotype and the comparison of all three populations with other members of “the *C. taenia* complex” based on morphological characters as well as genetic diagnostic markers. The present paper deals with the formal description of the spined loach from the Chernaya River as a new species and discusses mutual relationships among populations of loaches with 50 chromosomes and the unusual karyotype in the north-western Pontic region.

## Materials and Methods

### Materials

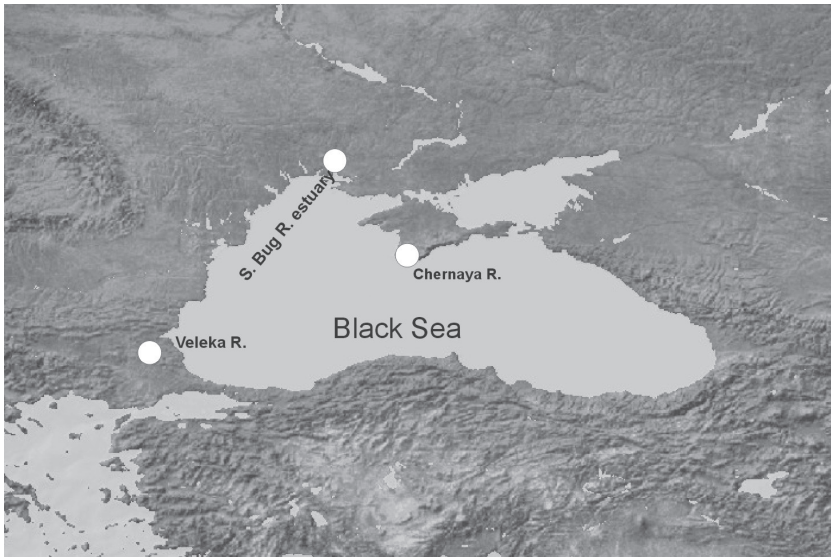
50-chromosome spined loaches were collected during expeditions in 2002–2003 at three localities (Table 1, Fig.1): the Chernaya River, the Crimean Peninsula (5♀, 1♂), the Veleka R., southern Bulgaria (4♀, 3♂), and the mouth of the Southern Bug R., Ukraine (9♀, 10♂). All these specimens were subsequently identified genetically and all but 8 individuals, from the latter locality, were morphologically examined. For morphological investigation, we further used loach samples collected in the Chernaya R. in 1985 (37 spec.) and in 2004 (1 spec.). All these voucher specimens are kept in the Zoological Museum of the Moscow State University (ZMMU), and detailed information about samples used for the description of a new species are given below. Karyological, morphological and genetic data from our previous studies on European loaches (Vasil'eva 1984, Vasil'ev 1995, Vasil'eva & Vasil'ev 1998, Ráb et al. 2000, Janko et al. 2003, 2005, Rábová et al. 2004, Culling et al. 2005) were also used as a comparative data-set, as well as partially described (Vasil'ev 1985, 1995) karyological data obtained in 1981 (20 karyotyped specimens) and in 1985 (69 karyotyped specimens) from spined loaches occurring in the Chernaya R.

### Karyological analysis

We followed standard procedures described earlier (Vasil'ev 1978, Ráb & Roth 1988). Chromosomes were classified according to Levan et al. (1964).

**Table 1.** Allozyme alleles found in studied samples of spined loaches (for the new form, the numbers of samples are given in parentheses; data for *C. taenia*, *C. elongatoides* and *C. tanaitica* are taken from Šlechťová et al. 2000).

Species or morph	Loci	Gpi-A*	s-Aat*	s-Mdh-A*	Ldh-B*	Sod*
<i>Cobitis taenia</i>		87	71	60, 100	100, 117	60
<i>C. elongatoides</i>		100, 113	100	100, 70, 40	100, 117	100
50-chromosome morph from the Chernaya River (N=6)		87	71	100, 60	100	60
50-chromosome morph from the Southern Bug (N=19) and Veleka (N=7) rivers		87	71	60	100	60
“ <i>C. tanaitica</i> ”		87	71	100, 60	100, 117	60



**Fig. 1.** Map of distribution of the *C. taurica* sp. nova and similar forms used for this study.

### Allozyme analysis

The skeletal muscles of the examined specimens were stored at  $-40^{\circ}$  before being electrophoretically analysed. The five diagnostic loci (i.e. glucosephosphate isomerase A, GpiA\*; superoxide dismutase, Sod\*; s-aspartate amino transferase, sAat\*; malate dehydrogenase, Mdh\*; and lactate dehydrogenase, Ldh\*) were scored, using the same analyses protocols and enzyme nomenclature as in Šlechťova et al. (2000). Allele homologies were determined by comparing electrophoretic mobility of the studied samples to specimens analysed previously. Presence / absence of alleles were put into a matrix and overlaid on a data matrix from Šlechťova et al. (2000).

### Morphological analysis

We included characters consistently used in the taxonomy of spined loaches that have been previously described (Vasil'eva 1984, 1988, Vasil'eva et al. 1989, Vasil'eva & Vasil'ev 1998). All morphometric data were subject to standard statistic univariate analysis.

### Phylogenetic relationships

Relationships between 50-chromosome specimens with peculiar karyotype examined in this study and some other European loaches were assessed using sequences of mitochondrial cytochrome b gene (*cyt b*) from Culling et al. (2005) and Janko et al. (2005) (Accession numbers: AY706159- AY706203, AF263083 & AF263084), which included the sequences from three specimens from the Chernaya R., one specimen from the S. Bug R. and three specimens from the Veleka R.

Nucleotide divergences between haplotypes were estimated with the PAUP\* software package, version 4.0b10 (Swofford 1999) using the HKY 85 + Gamma model of DNA substitution (Hasegawa et al. 1985). The HKY 85 + Gamma model was selected as an

appropriate model for our dataset using the hierarchical likelihood ratio test implemented in the MODELTEST program, version 3.06 (P o s a d a & C r a n d a l l 1998). Phylogenetic relationships among haplotypes were reconstructed by the neighbour-joining algorithm (NJ) using the corrected distance matrix, and by the maximum likelihood (ML) criterion using heuristic search and parameter settings as calculated with MODELTEST (base frequencies: A = 0.2608, C = 0.2397, G = 0.1612, T = 0.3383, transition: transversion ratio = 7.1405). Both analyses were performed with PAUP\*. To infer statistical support for the internal branches of the NJ tree, nonparametric bootstrap resampling with 1000 replicates was performed. We used a sequence of *C. albicoloris* Chichkoff, 1932 as an outgroup which was justified by the phylogenetic relationships within the genus (P e r d i c e s & D o a d r i o 2001).

## Results

### Karyological analysis

We revealed 50-chromosome spined loaches from the Chernaya, Veleka and S. Bug rivers to have the same karyotype composed of 5 pairs of meta-, 15 pairs of submeta-, 4 pairs of subtelo- and 1 pair of acrocentric chromosomes, NF=90<sup>1</sup> (Fig. 2). There were no polyploid specimens among the karyotyped loaches collected in the Chernaya R. in 1981 and in 1985 (89 adult and juvenile specimens), and both males (47 adult specimens) and females (23 adult specimens) had the same karyotype structure. This karyotype differs remarkably from the karyotypes of other diploid bisexual species from “the *Cobitis taenia* complex”: the karyotype of *C. taenia* s. stricto consists of 48 chromosomes with NF=76 (V a s i l ’ e v & V a s i l ’ e v a 1982, V a s i l ’ e v 1985, R á b et al. 2000, B o r o n 2003); the karyotype of *C. elongatoides* Băcescu in Băcescu et Maier, 1969<sup>2</sup> includes 50 chromosomes with NF=96 (R á b et al. 2000, L u s k et al. 2003, B o r o n 2003); the so called “*C. tanaitica* Băcescu et Maier, 1969” from Central Europe has 2n=50 chromosomes and NF=82–84 (R á b et al. 2000, L u s k et al. 2003), while East European *C. rossomeridionalis* Vasil’eva et Vasil’ev, 1998 is characterized by the presence of fixed Y-autosome translocation (centric fusion) resulting in 2n=50 and NF=86 in females and 2n=49 and NF=86 in males (V a s i l ’ e v 1995, V a s i l ’ e v a & V a s i l ’ e v 1998)<sup>3</sup>.

### Allozyme analysis

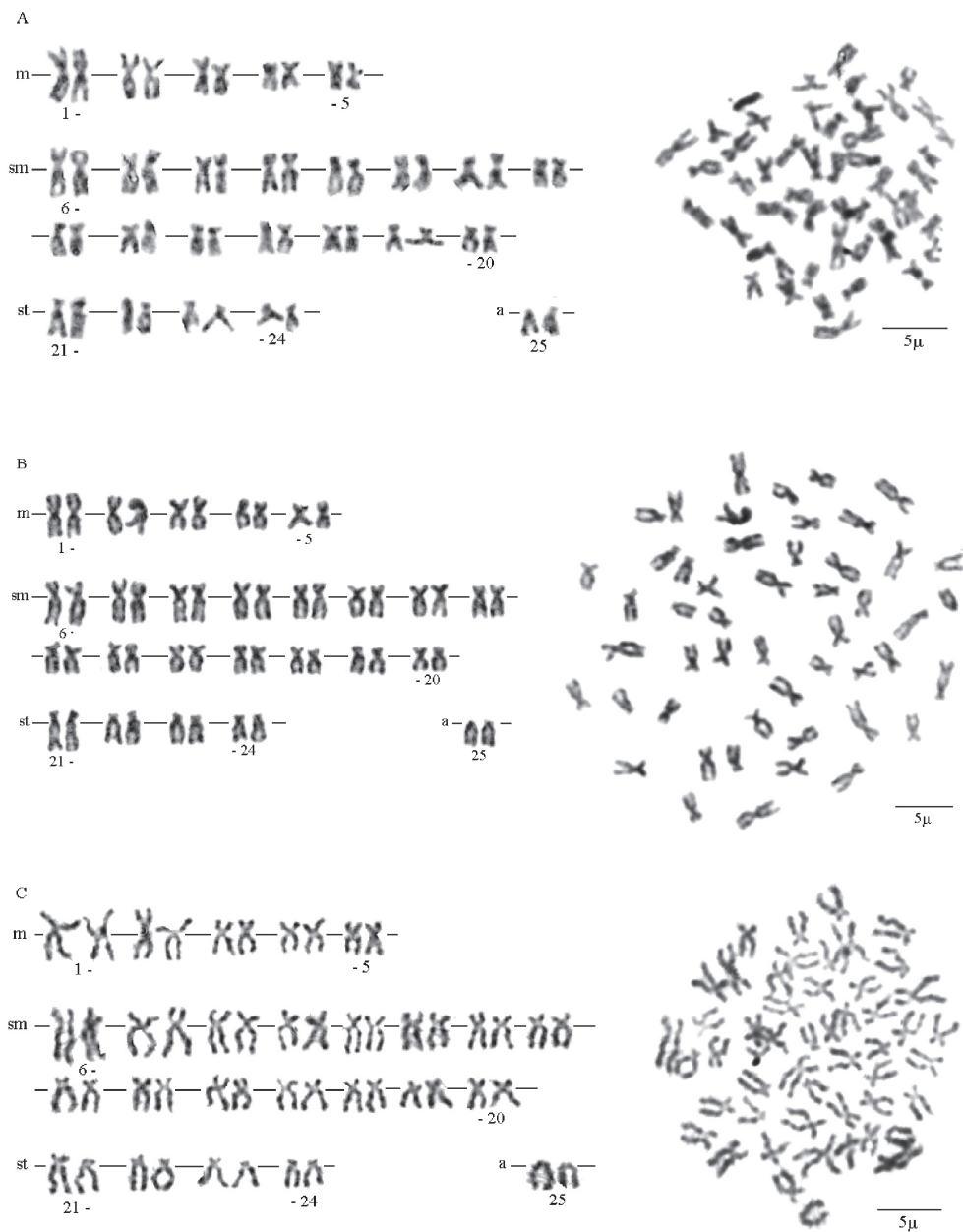
For three (Gpi-A\*, s-Aat\*, Sod\*) out of the five studied loci, the alleles found in 50-chromosome specimens from the Chernaya, Veleka and S. Bug rivers were identical to those found in

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<sup>1</sup> In this work we used a chromosome classification that differed from those presented for loaches from the Chernaya River in a previous study (V a s i l ’ e v 1995) to compare the data with that obtained later for different European loaches.

<sup>2</sup> Some authors (F r e y h o f et al. 2000, N a l b a n t et al. 2001) believe Băcescu & Maier to be authors of the name *elongatoides* because this name was available from their publication (Băcescu & Maier 1969). But Băcescu & Maier (1969) undoubtedly considered only Băcescu to be responsible for the name *elongatoides* (pp. 39, 40, 43). Other nomenclature problems for this species were discussed in F r e y h o f et al. 2000.

<sup>3</sup> The name “*C. tanaitica*” is usually applied to species with 2n=50 and NF=82–84 (L u s k et al. 2003, Š l e c h t o v á et al. 2003, J a n k o et al. 2003, 2005). But nomenclature problems remain in discussion (see V a s i l ’ e v a & V a s i l ’ e v 1998, F r e y h o f et al. 2000, B o h l e n & R á b 2001), and the taxonomic relationship between populations from the Don River (typical locality) and Central European fresh waters seem to be unresolved.



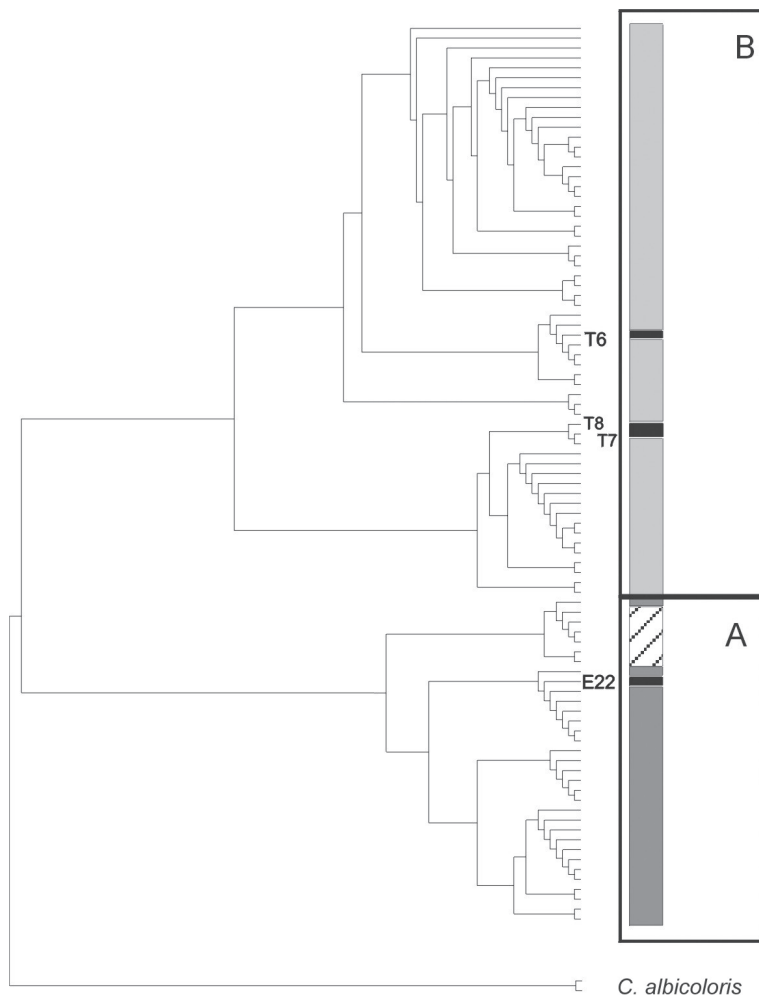
**Fig. 2.** Karyotypes of *Cobitis taurica* sp. nova from the Chernaya River (a) and the similar forms from the Veleka (b) and the Southern Bug (c) rivers.

*C. taenia* and “*C. tanaïtica*”, while *C. elongatoides* was characterized by fixation of alternative alleles (Table 1). For locus *Ldh-B\** all of the compared species and populations possessed allele \*100, but populations from the Chernaya, Veleka and S. Bug rivers missed the allele \*117, although it is relatively frequent among other species described previously. All three populations of the 50-chromosome form possessed allele \*60 at the *s-Mdh-A\** locus, which

was otherwise characteristic for *C. taenia*, but we also encountered allele \*100 fixed in *C. elongatoides* and *C. tanaitica* and also found in a single *C. taenia* specimen from the Alma R. in Crimean Peninsula (Šlechťová et al. 2000, 2003).

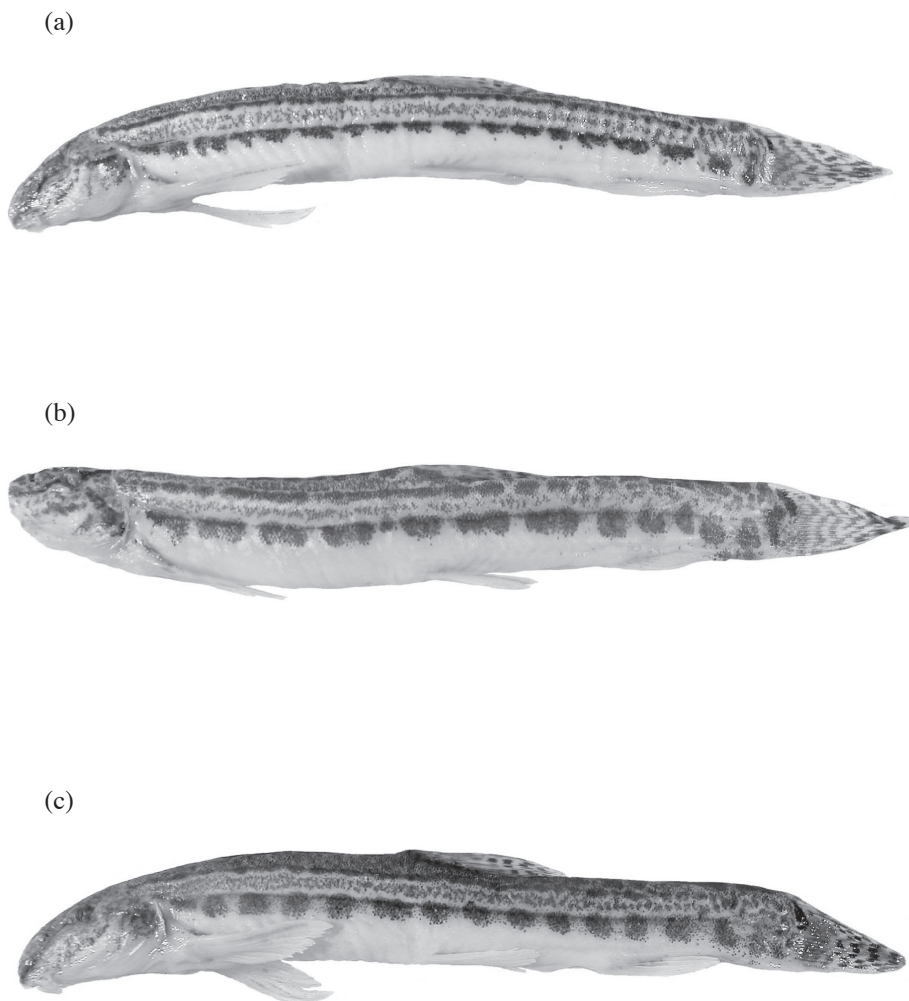
### Phylogenetic analysis

The 1088 bp sequences of the *cyt b* gene were determined for 7 individuals from the three studied populations. A total of 191 sites were variable. All observed polymorphisms were single nucleotide substitutions. The combination of nucleotide states at variable positions defined 4 distinct haplotypes encountered among the individuals of the studied 50-chromosome form, designated as T6-T8, E22 according to Janík et al. (2005; accession numbers: AY706162,



**Fig. 3.** ML tree topology of phylogenetic relationships among analysed individuals from the *C. taenia* hybrid complex. Two major clades referred in the text are denoted by boxes A and B, respectively. Light grey lines indicate the positions of haplotypes encountered either in *C. taenia* or in asexual hybrids with *taenia*-type mtDNA, dark grey denotes haplotypes of *C. elongatoides* or hybrids with *elongatoides*-type mtDNA, stripped bars indicate the haplotypes of *C. tanaitica* and black symbols indicate the haplotypes encountered in the new 50-chromosome form.

AY706165, AY706166 and AY706184). NJ and ML phylogenetic methods resulted in slightly differing tree topologies, however the positioning of the T6-T8, E22 among haplotypes, found in *C. taenia*, *C. elongatoides*, "*C. tanaitica*" and the clonal hybrids, didn't differ. The sequences, in the NJ tree, clustered in two major clades (Fig. 3), corresponding to clades A and B from J a n k o et al. (2003) with high bootstrap support (100%). The average pairwise sequence divergence between the clades is 5.2%. Sequences of the studied 50-chromosome form did not represent a monophyletic lineage, but specimens from the Veleka R. (haplotype E22) clustered in clade A composed of haplotypes found in *C. elongatoides*, and "*C. tanaitica*" (and also polyploids and hybrids with *elongatoides*-type mtDNA). Whereas, haplotypes from the Crimean Peninsula (T7 and T8) and the Southern Bug R. (T6) clustered in clade B consisting of haplotypes encountered in *C. taenia* specimens (and also polyploids with *taenia*-type mtDNA). Neither of the two Ukrainian populations form a monophyletic lineage within clade



**Fig. 4.** Holotype of *Cobitis taurica* sp. nova, male, SL 60.0 mm, the Chernaya River, ZMMU P-21358 (a); spined loach male, SL 62.6 mm, from the Veleka River, ZMMU P-21363 (b); spined loach male, SL 62.6 mm, from the Southern Bug River, ZMMU P-21365 (c).

Table 2. Several morphometric characters of different spined loach species.

Species, sample	<i>Cobitis taurica</i> sp. nova, Chernaya R., type specimens		<i>C. taurica</i> sp. nova, Chernaya R. (from Vasil'eva, 1984)		Spined loaches from the Voleka R. (ZMMU P-21363, P-21364)		Spined loaches from the Southern Bug R. (ZMMU P-21365, P-21366)	
	Females (n=6)	Males (n=5)	Females (n=7)	Males (n=7)	Females (n=4)	Males (n=3)	Females (n=4)	Males (n=4)
Character	Ranges / M	Ranges / M	Ranges / M	Ranges / M	Ranges / M	Ranges / M	Ranges / M	Ranges / M
TL (mm)	89.3-120.0 102.8	60.5-70.5 66.2	46.0-84.2 67.7	49.5-66.0 59.3	83.2-114.3 95.8	67.0-74.0 69.8	79.3-92.5 85.3	63.0-79.3 70.9
SL (mm)	78.6-94.0 87.1	51.5-60.0 56.6	40.0-71.2 57.7	42.3-56.0 50.3	71.5-100.5 83.4	58.2-62.6 59.7	69.0-81.0 74.3	54.2-69.5 61.5
In % of SL								
aD	49.9-53.6 51.0	50.3-53.0 51.9	49.5-53.9 51.6	50.0-55.3 52.0	50.8-52.7 51.5	49.5-51.1 50.5	50.6-52.5 51.9	49.8-52.8 51.1
pD	39.3-42.1 40.2	37.3-40.3 39.0	36.8-43.1 40.8	37.5-42.1 40.3	39.7-41.5 40.7	39.5-41.9 40.4	39.8-42.0 40.8	39.4-41.6 40.6
aV	48.9-50.8 49.9	50.0-52.7 51.5	49.5-54.8 52.7	51.3-57.0 54.2	49.6-51.1 50.6	51.4-52.9 52.2	48.0-50.4 49.5	49.7-50.8 50.4
aA	70.4-76.4 73.5	73.5-76.3 75.3	73.8-78.7 76.8	74.6-78.9 77.3	73.5-76.5 74.6	74.6-80.8 77.0	70.6-75.4 73.8	70.1-73.1 71.8
pl	15.3-18.5 16.4	14.9-17.1 15.9	15.7-19.7 17.6	12.2-18.9 15.8	15.7-16.8 16.1	15.8-16.5 16.2	15.2-18.8 17.2	17.0-18.6 17.8
H	14.2-15.5 14.9	12.5-15.5 13.6	12.6-17.1 15.1	11.3-16.4 14.7	15.8-17.4 16.3	13.6-14.1 13.8	12.6-15.6 14.2	12.4-13.1 12.9
h	7.5-8.9 8.2	8.3-9.9 9.1	6.9-9.1 8.2	7.4-10.5 9.1	8.3-9.8 9.0	9.3-9.8 9.6	8.2-8.7 8.6	8.8-9.7 9.3
Cr	6.6-8.1 7.4	6.1-9.1 7.0	6.5-9.3 8.0	6.9-8.0 7.3	7.6-8.2 7.8	7.4	7.0-8.3 7.7	6.7-7.2 7.0
Cr <sub>mm</sub>	6.4-7.0 6.8	5.2-8.4 6.4	7.4-9.3 8.2	6.0-8.0 6.9	7.2-8.1 7.6	7.4	6.2-7.5 6.8	6.6-6.9 6.7



cr	$\frac{2.2-2.9}{2.5}$	$\frac{1.8-3.1}{2.2}$	$\frac{2.4-3.8}{3.2}$	$\frac{2.3-3.3}{2.7}$	$\frac{2.1-2.7}{2.4}$	$\frac{1.9-3.4}{2.5}$	$\frac{2.3-2.9}{2.6}$	$\frac{1.9-2.6}{2.2}$
PV	$\frac{30.6-33.2}{31.8}$	$\frac{29.7-32.6}{31.4}$	$\frac{30.7-35.5}{33.2}$	$\frac{31.4-36.8}{34.0}$	$\frac{30.0-33.1}{31.1}$	$\frac{33.0-34.7}{33.9}$	$\frac{29.0-30.8}{29.7}$	$\frac{27.2-30.5}{29.1}$
IP	$\frac{10.6-14.1}{12.0}$	$\frac{17.1-21.8}{18.7}$	$\frac{10.8-13.8}{12.3}$	$\frac{14.5-19.6^*}{17.1}$	$\frac{11.9-14.3}{12.9}$	$\frac{18.9-20.0}{19.3}$	$\frac{12.0-13.2}{12.7}$	$\frac{17.3-19.7}{18.9}$
IV	$\frac{9.6-12.5}{11.2}$	$\frac{12.1-15.3}{14.1}$	$\frac{11.3-14.3}{12.4}$	$\frac{12.4-15.8}{14.2}$	$\frac{10.2-12.9}{11.4}$	$\frac{13.9-14.7}{14.3}$	$\frac{12.1-12.8}{12.5}$	$\frac{14.1-14.8}{14.5}$
ID	$\frac{9.6-12.2}{11.0}$	$\frac{9.9-11.6}{10.8}$	$\frac{8.4-11.5}{10.7}$	$\frac{9.0-11.6}{10.8}$	$\frac{9.5-10.9}{10.3}$	$\frac{9.1-10.3}{9.7}$	$\frac{9.1-9.8}{9.5}$	$\frac{9.2-11.3}{10.2}$
hD	$\frac{13.4-15.0}{14.3}$	$\frac{15.7-19.7}{17.2}$	-	-	$\frac{13.1-15.1}{13.8}$	$\frac{15.6-16.1}{15.8}$	$\frac{13.8-14.6}{14.2}$	$\frac{15.6-16.3}{16.1}$
IA	$\frac{7.5-8.7}{8.1}$	$\frac{7.8-8.8}{8.5}$	$\frac{6.8-8.8}{7.5}$	$\frac{5.5-7.6}{7.1}$	$\frac{7.0-9.0}{7.9}$	$\frac{7.2-8.1}{7.7}$	$\frac{7.7-8.3}{8.0}$	$\frac{8.1-9.6}{8.8}$
hA	$\frac{10.2-12.1}{11.2}$	$\frac{12.2-15.0}{13.5}$	-	-	$\frac{11.1-11.5}{11.2}$	$\frac{12.5-13.7}{13.1}$	$\frac{10.5-11.9}{11.2}$	$\frac{11.8-12.8}{12.2}$
c	$\frac{17.0-18.4}{17.9}$	$\frac{19.9-21.3}{20.4}$	$\frac{18.5-20.5}{19.2}$	$\frac{18.6-21.0}{19.9}$	$\frac{17.8-19.3}{18.6}$	$\frac{18.4-20.6}{19.2}$	$\frac{18.5-20.9}{19.7}$	$\frac{19.9-22.3}{21.1}$
In % of c								
IB1	$\frac{0-8.5}{3.2}$	$\frac{0-10.7}{7.2}$	$\frac{8.8-12.8^*}{10.6}$	$\frac{5.2-9.4^*}{7.3}$	$\frac{6.8-11.1}{8.5}$	9.2	$\frac{7.6-11.1}{8.8}$	$\frac{8.0-9.1}{8.5}$
IB3	$\frac{6.6-14.2}{9.8}$	$\frac{13.0-20.4}{16.2}$	$\frac{7.5-18.9^*}{15.1}$	$\frac{11.2-18.2^*}{13.9}$	$\frac{11.1-15.9}{14.2}$	$\frac{15.0-20.0}{17.5}$	$\frac{11.4-16.0}{13.5}$	$\frac{12.4-16.4}{15.0}$
r	$\frac{41.9-46.1}{43.1}$	$\frac{39.7-43.5}{41.2}$	$\frac{39.0-47.1}{42.8}$	$\frac{34.6-45.8}{40.3}$	$\frac{42.0-43.5}{42.6}$	$\frac{35.2-41.7}{39.1}$	$\frac{43.1-46.3}{44.5}$	$\frac{38.0-44.9}{41.7}$
o	$\frac{15.6-21.3}{17.6}$	$\frac{17.7-24.4}{20.1}$	$\frac{15.0-20.0}{17.7}$	$\frac{15.5-18.5}{16.8}$	$\frac{16.7-18.9}{17.8}$	$\frac{15.9-19.0}{17.5}$	$\frac{15.6-19.4}{17.5}$	$\frac{16.2-18.8}{17.4}$

po	$\frac{50.4-55.1}{53.0}$	$\frac{46.6-52.4}{49.9}$	$\frac{44.4-54.1}{49.8}$	$\frac{50.5-57.0^*}{53.1}$	$\frac{44.9-52.3}{49.1}$	$\frac{50.0-55.1}{51.7}$	$\frac{48.3-53.2}{50.1}$	$\frac{49.2-51.2}{49.9}$
hc	$\frac{51.5-57.9}{54.6}$	$\frac{50.0-58.3}{55.0}$	$\frac{57.8-67.7}{60.2}$	$\frac{52.8-67.3}{61.3}$	$\frac{52.4-57.3}{54.8}$	$\frac{51.7-59.5}{56.1}$	$\frac{50.0-55.0}{52.4}$	$\frac{46.3-54.4}{50.5}$
io	$\frac{9.2-17.4}{13.6}$	$\frac{9.5-15.8}{12.2}$	$\frac{12.2-19.5}{15.6}$	$\frac{13.6-17.3}{15.7}$	$\frac{13.0-15.9}{14.4}$	$\frac{10.0-15.5}{13.2}$	$\frac{12.6-15.3}{13.9}$	$\frac{10.7-14.8}{13.5}$
In % of PV								
IP	$\frac{33.6-44.8}{37.8}$	$\frac{52.7-70.8}{59.8}$			$\frac{36.0-46.8}{41.7}$	$\frac{57.3-57.6}{57.5}$	$\frac{41.0-44.4}{42.9}$	$\frac{56.6-70.6}{65.3}$
IV	$\frac{30.6-37.5}{35.0}$	$\frac{37.2-49.7}{44.9}$			$\frac{30.6-42.2}{36.9}$	$\frac{42.2-42.4}{42.3}$	$\frac{40.0-42.9}{41.9}$	$\frac{46.2-53.5}{50.0}$
D	$\frac{7-8}{7.7}$	$\frac{7-8}{7.8}$	8	8	8	7	$\frac{7-8}{7.5}$	8
A	6	$\frac{5-6}{5.8}$	6	6	$\frac{5-6}{5.8}$	6	$\frac{5-6}{5.8}$	6
P	$\frac{7-8}{7.3}$	$\frac{6-8}{7.0}$	$\frac{7-8}{7.3}$	$\frac{7-8}{7.3}$	$\frac{7-8}{7.5}$	$\frac{7-8}{7.3}$	$\frac{7-8}{7.5}$	$\frac{7-8}{7.3}$
V	$\frac{5-6}{5.8}$	6		$\frac{5-6}{5.3}$ $\frac{5-6}{5.3}$	$\frac{5-6}{5.5}$	5	6	6
C	$\frac{13-14}{13.7}$	14	14	14	14	14	$\frac{12-14}{13.5}$	14
sp	$\frac{17-28}{22.0}$	$\frac{19-24}{20.8}$	15 – 26		$\frac{14-17}{15.7}$	$\frac{13-16}{15.0}$	$\frac{15-18}{16.8}$	$\frac{13-18}{15.8}$

Abbreviations: TL – total body length; SL – body length (from the anterior part of the head to visible base of the caudal fin); aD – predorsal, pD – postdorsal, aV – preventral, aA – preanal distances, pl – length of caudal peduncle, H – maximum body depth, h – least depth of caudal peduncle, Cr – body width before dorsal fin, Crm – body width at the level of dorsal fin, cr – least body width, PV – distance between pectoral and ventral fins, IP – pectoral fin length, IV – ventral fin length, ID – length of dorsal fin base, hD – dorsal fin depth, lA – length of anal fin base, hA – anal fin depth, c – head length, lB1 – rostral barbel length, lB3 – mandibular barbel length, r – preorbital distance, o – horizontal eye diameter, po – postorbital distance, hc – maximum head depth, io – interorbital distance, D – branched rays in dorsal fin, A – in anal fin, P – in pectoral fin, V – in ventral fin, C – in caudal fin, sp – spots in the fourth Gambetta's zone; n – number of specimens.

B, since the haplotype from the S. Bug R. (T6) clustered together with local populations of *C. taenia*, whereas haplotypes from the Chernaya R. (T7 and T8) clustered with *C. taenia* populations occurring in the Dnieper R.

### Morphological analysis

Spined loaches from the Chernaya River diverged from populations occurring in the Veleka and S. Bug rivers by the size and number of spots in the fourth Gambetta's zone of pigmentation (spots along the side midline) and by the position of the tip of suborbital spine, as well as the tip of its outer branch. The fourth Gambetta's zone of specimens from the Chernaya River consists of relatively small and low 15–28, usually more than 18, spots whose depth is about two times smaller than the horizontal eye diameter (Fig. 4a), their suborbital spine always reaches the posterior edge of the pupil and usually beyond, and its outer branch usually goes as far as the centre of the eye and often even further (see description). At the same time, the fourth Gambetta's zone of the specimens from the Veleka and S. Bug rivers consists of relatively large 13–18, more often 15–18, spots whose depth is about equal to the horizontal eye diameter (Fig. 4b,c). Their suborbital spine usually does not reach the posterior edge of the pupil (it goes as far as the posterior edge of the pupil or further in small males from the Veleka River only), and its outer branch usually (85.7 % in the Veleka R. and 100 % in the S. Bug R.) does not reach the centre of the eye. The diagnostic characters differentiating between spined loaches of the Chernaya River and other European species are given below.

The differences between populations from the Veleka and the S. Bug rivers are not so prominent being revealed mainly in the head shape. The head of specimens from the Veleka R. is somewhat shortened and deep, with a more or less straight profile. While specimens from the S. Bug R. have a somewhat elongated head that is not so deep, with an obviously hooked and overhanging nose; Fig. 4b,c). Significant differences between these populations (slight overlap between the ranges of characters) were also revealed for 5 morphometric characters, namely maximum body depth, distance between paired fins, preorbital distance, head depth and the relationship between ventral fin length and the distance between paired fins. Some characters demonstrated prominent differences between the two species in males only (Table 2).

### Discussion

Three newly discovered populations of 50-chromosome spined loaches from the north-western Pontic region studied by combined comparative morphological and genetic analyses turned out to differ from all other known forms of European loaches of the genus *Cobitis* s. stricto. The population of this form dwelling in the Chernaya R. apparently represents a bisexual diploid species, since we have recorded individuals of both sexes (23 males among 34 adult specimens collected in 1981 – 20 of them were used for karyology and osteology and 14 for external morphology, and 46 males among 73 adult specimens collected in 1985 – 36 specimens karyotyped and 37 specimens kept in ZMMU; sex-ratio 1.7: 1 in favor of males). The loaches inhabiting these locations share a common karyotype not observed in any other loach species studied to-date.

The hypothesized ancestral karyotype for *Cobitis* species is characterized by  $2n=50$  with 32–34 acrocentric chromosomes and  $NF=66–68$  because this karyotype is the least structured (the most simple from a cytogenetic point of view) and is observed in species occurring in the Far East, Korea and Japan as well as in European species (Hitotsu et al. 1969,

Ueno & Ojima 1976, Cataudella et al. 1977, Ueno et al. 1985, Vasil'eva et al. 1992, Kim et al. 1999). Thus, the karyotypes of European *Cobitis* species are represented by at least two different evolutionary lineages: 1) "the *C. taenia*" lineage with karyotype ( $2n=48$ ,  $NF=76$ ) arose by centric fusion resulting in the origin of large metacentric pair and several pericentric inversions and 2) one or several lineages with karyotypes characterized by high NF that arose by pericentric inversions. The newly discovered 50-chromosome form of loaches belongs to the second lineage.

Our results suggest some differences between unique 50-chromosome populations and other members of "the *C. taenia* complex" in s-Mdh-A\* and Ldh-B\* loci, but this must be considered with caution since a restricted number of samples were studied (Table 1). Similarly, divergences in several enzyme and nonenzyme protein systems including malate dehydrogenase were found by the isoelectric focusing analysis between the loaches from the Veleka R. and Middle Danubian specimens (Danailov et al. 1998, Ivanova et al. 2003). These fishes were determined by the authors as *C. taenia*, but recent investigations confirm the Danube River basin to be inhabited by *C. elongatoides* and "*C. tanaitica*", while *C. taenia* is apparently absent in this river system (see Janako et al. 2005, Culling et al. 2005). Based on the presence of the same allele in the superoxide dismutase locus in the Veleka R. and Danubian samples (Ivanova et al. 2003) we suggest the latter population was represented by "*C. tanaitica*" and not *C. elongatoides*, which is fixed for a different allele at this locus (Šlechťov et al. 2000).

Our results also suggest genetic heterogeneity among populations of the unique 50-chromosome form themselves: the Chernaya River population harbours an Mdh\* allele (\*100) missing in the other populations and the results of mtDNA analysis placed all three populations into different lineages; the haplotype of the Bulgarian population even being derived from *C. elongatoides*.

The Chernaya River belongs to the territory of so called Mountain Crimea representing the northern remains of the hypothetical large Pontic Land supposed to exist at the site of the contemporary deep Black Sea hollow until the end of Tertiary period and to be the most developed of the Sarmat age. The Dobruzha region in the west (coastal part of Roumania and northern Bulgaria), the coastal zone of Anatolia in the south and the Meskhi plateau in Transcaucasia in the east are considered to be the other remains of this Land (see Puzanov 1929, 1949). According to biogeographic data, the hydrofauna of the Chernaya River is of ancient origin from this Pontic Land fauna and thus is the most related to its remains in Balkan and Minor Asia (Puzanov 1949). After the Pontic Land dipped into the sea at the beginning of the first glacial period, the Mountain Crimea island is supposed to have been connected with Roumania, southern Ukraine and the Novorossiysk coast of the Caucasus by a broad land strip (see Puzanov 1929, 1949). Furthermore, the main immigration wave of Pleistocene fauna from the southern Ukraine and Russia were given the possibility to survive in the refuge of the so-called Steppe Crimea. This immigration wave, however, did not affect the Chernaya R. that was supposed to be isolated from the recent fauna of the northern coast of the Black Sea basin since the Paleocene period (Puzanov 1949), about 60 My., and is represented by several endemic species (see Vasil'eva et al. 2005). Such processes may explain the disjointed distribution of the studied form of loaches, which have also been observed in other freshwater fishes (e.g. *Barbus escherichii* lineage II from Kotlík et al. (2004).

At the moment, since the present results of genetic, morphological and biogeographic analyses yield controversial solutions to the relationship of the 50-chromosome spined loaches from the Chernaya, Southern Bug and Veleka Rivers, we consider further comprehensive investigations

of the spined loaches from the Black Sea basin crucial for more advanced taxonomic conclusions. In any case, the description of the spined loaches from the Crimean Peninsula as a separate species should be considered as an essential procedure for the taxonomic resolution of diploid spined loach species as well as for further studies of polyploid hybrid loaches possessing haploid chromosome sets of such diploids. We therefore present formal a description here.

***Cobitis taurica* Vasil'eva, Vasil'ev, Janko, Ráb et Rábová sp. nova (Fig. 4a).**

*Cobitis taenia* (not of Linnaeus, 1758): P u z a n o v 1929: 19; T s e e b 1929: 115; B e r g 1949: 890 (part.: Chernaya R. in Crimea); Delyamure 1964: 45; V a s i l ' e v 1985: 172 (part.: Crimea); P r o k o p o v 2004: 165.

*Cobitis taenia taenia* (not of Linnaeus, 1758): M o v c h a n 1988: (part.: Crimea).

Holotype – ZMMU P-21358, male TL 70.5 mm, SL 60.0 mm, Chernaya River, Crimean Peninsula, 14.06.2002, collector K. J a n k o (karyotyped, electrophoretically and DNA-studied). Paratypes: ZMMU P-21359, 1 female TL 96.1 mm, SL 82.5 mm and 3 males TL 63.7–69.2 mm, SL 27.4–31.4 mm, Chernaya R., 5.07.1985, collector V. V a s i l ' e v ; P-21360, 5 females TL 89.3–120.0 mm, SL 78.6–91.5 mm, collected together with holotype; P-21361, male TL 60.5 mm, SL 51.5 mm, Chernaya R., 11.07.2004, collectors V. V a s i l ' e v & E. V a s i l ' e v a . Additional materials: P-21362, 33 specimens (21 males), collected together with P-21359.

Diagnosis: One elongated narrow dark spot in the upper part of the base of the caudal fin; males with one *lamina Canestrini* in the pectoral fin, usually broad, axe-shaped; more or less rounded scales with a relatively small central part devoid of cross furrows; Gambetta's zones of pigmentation are well developed, the fourth zone consists of relatively small and low 15–28, usually more than 18 spots whose depth is about two times smaller than the horizontal eye diameter (the largest spots on the caudal peduncle are also noticeably smaller than the horizontal eye diameter); the suborbital spine always reaches the posterior edge of the pupil and usually goes further and in small specimens (with TL less than 65 mm), usually reaches the posterior edge of the eye; the outer branch of this spine usually (95.5 %, n=44) goes as far as centre of eye and often further; the head is moderately long and deep with a more or less straight profile; 2n=50 (5 pairs of meta-, 15 pairs of submeta-, 4 pairs of subtelo- and 1 pair of acrocentrics), NF=90.

Description.<sup>4</sup> D II–III 6–8 (more often 7), A (II) III 5–6 (more often 6), P I (5) 6–8 (more often 7), V I–II (5) 6, C I 13–14 I. Morphometric characters are presented in Table 2. Head without overhanging nose; dorsal fin begins at the level of the origin of the pelvic fin or somewhat anteriorly (pelvic fins are situated at the level of the last unbranched dorsal ray); barbels short: the mandibular barbels never reach the anterior edge of the eye, more often they do not go as far as the posterior edge of the nostrils, samples collected in 2002 included fishes with completely or noticeably reduced rostral barbels; descriptions of some other morphological characters, as well as some craniological features and drawings of specimens, lips, *lamina Canestrini* and bones were presented earlier (V a s i l ' e v a 1984). Maximum body length (TL) comprises 109 mm in females and 73 mm (V a s i l ' e v a 1984) in males; the smallest male with developed *lamina Canestrini* was 49 mm in length.

Distribution. Recently we consider this species to be endemic of the Chernaya River in the Crimean Peninsula. But we can't completely reject its possible conspecificity with spined loach populations from the Veleka and S. Bug rivers and thus its wider distribution within the Black Sea basin.

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<sup>4</sup> In our paper we present very short descriptions of the new species due to their external resemblance with the well-known *C. taenia*.

Etymology. The species name *taurica* is derived from Tauris-Taurida, the ancient Greek and Latin name of the Crimean Peninsula.

Comparative remarks. As mentioned above, *C. taurica* belongs to “the *C. taenia* complex”, but it differs (as well as spined loaches from the Veleka and S. Bug rivers) from all other members of this group by the unique karyotype structure. Furthermore, this species can be distinguished from every other diploid bisexual species (including the populations from the Veleka and S. Bug rivers with the same karyotype), as well as from polyploids attributed to this complex, by the smaller and more abundant spots in the fourth Gambetta’s zone of pigmentation. This last character, as well as the position of the suborbital spine separate *C. taurica* from *C. taenia* also found in the Crimean Peninsula (the Alma River): *C. taenia* usually has less than 15 large spots in the fourth Gambetta’s zone, its suborbital spine usually (about 70 %) does not reach the posterior edge of the pupil, and the outer branch of the spine usually (about 80 %) does not reach the centre of the eye.

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#### LITERATURE

- BĂCESCU M. & MAIER R. 1969: The *Cobitis* of the Don and Volga. *J. Ichthyol.* 9: 38–44.
- BERG L.S. 1949: [Freshwater fishes of the USSR and neighbouring countries] Vol. 2. *Academy Science of USSR, Moscow – Leningrad (in Russian)*.
- BOHLEN J. & RÁB P. 2001: Species and hybrid richness in spined loaches of the genus *Cobitis* (Teleostei, Cobitidae) with a checklist of European forms and suggestions for conservation. *J. Fish Biol.* 59 (Suppl. A): 75–89.
- BOROŇ A. 2003: Karyotypes and cytogenetic diversity of the genus *Cobitis* (Pisces, Cobitidae) in Poland: a review. Cytogenetic evidence for a hybrid origin of some *Cobitis* triploids. *Folia biologica (Kraków)* 51 (Suppl.): 49–54.
- CATAUDELLA S., SOLA L., ACCAME MURATORI R. & CAPANNA E. 1977: The chromosomes of 11 species of Cyprinidae and one Cobitidae from Italy, with some remarks on the problem of polyploidy in the Cypriniformes. *Genetica* 47 (3): 161–171.
- CULLING M.A., JANKO K., BOROŇ A., VASIL’EV V.P., CÔTÉ I.M. & HEWITT G.M. 2005: European colonisation of the spined loach *Cobitis taenia* from Ponto-Caspian refugia based on mitochondrial DNA variation. *Molecular Ecology (in press)*.
- DANAĬLOV M., TERZIEVA P. & DOBROVOLOV I. 1998: Electrophoretical survey of *Cobitis taenia*, *Cobitis peshevi* and their hybrid from some Bulgarian rivers. *Acta Zool. Bulg.* 50 (2/3): 127–132.
- DELYAMURE S.L. 1964: [Fishes from the freshwaters]. *Izdatel’stvo “Krym”, Simferopol (in Russian)*.
- FREYHOF J., RÁB P. & BOHLEN J. 2000: The valid names of some European species of the genus *Cobitis* (Teleostei, Cobitidae). *Folia Zool.* 49 (Suppl. 1): 3–7.
- HASEGAWA M., KISHINO H. & YANO T. 1985: Dating of the human-ape splitting by molecular clock of mitochondrial DNA. *J. Molecular Evolution* 11: 160–174.
- HITOTSUMACHI S., SASAKI M. & OJIMA Y. 1969: A comparative karyotype study in several species of Japanese loaches (Pisces, Cobitidae). *Jap. J. Genet.* 44 (3): 157–161.

- IVANOVA P., ERK'AKAN F., ÖZEREN S.C. & DOBROVOLOV I. 2003: Biochemical-genetic comparison of *Cobitis simplicispina* from Turkey, and *C. taenia*, *C. albicoloris* and *C. elongata* from Bulgarian freshwaters. *Folia Biologica (Kraków) 51 (Suppl.): 79–84*.
- JANKO K., CULLING M.A., RÁB P. & KOTLÍK P. 2005: Ice age cloning – comparison of the Quaternary evolutionary histories of sexual and clonal forms of spiny loaches (*Cobitis*; Teleostei) using the analysis of mitochondrial DNA variation. *Molecular Ecology 14*: 2991–3004.
- JANKO K., KOTLÍK P. & RÁB P. 2003: Evolutionary history of asexual hybrid loaches (*Cobitis*; Teleostei) inferred from phylogenetic analysis of mitochondrial DNA variation. *Journal Evol. Biol. 16*: 1280–1287.
- KIM S.-Y., PARK J.-Y. & KIM I.-S. 1999: Chromosome of spined loach, *Iksookimia yongdokensis* (Pisces: Cobitidae) from Korea. *Korean J. Ichthyol. 11 (2)*: 172–176.
- KOTLÍK P., BOGUTSKAYA N.G. & EKMEKCI F.G. 2004: Circum Black Sea phylogeography of *Barbus* freshwater species: divergence in the Pontic glacial refugium. *Molecular Ecology 13*: 87–95.
- LEVAN A., FREDGA K. & SANDBERG A.A. 1964: Nomenclature for centromeric position on chromosomes. *Hereditas 52*: 201–220.
- LUSK S., KOŠČO J., HALAČKA K., LUSKOVÁ V. & FLAJŠHANS M. 2003: Identification of *Cobitis* from the Slovakian part of the Tisza basin. *Folia Biologica (Kraków) 51 (Suppl.): 61–65*.
- MAYR L. 1969: Principles of Systematic Zoology. McGraw-Hill Book Company. N.Y., St. Louis, San Francisco, Toronto, London, Sydney.
- MOVCHAN Yu.V. 1988: [Cobitidae, Siluridae, Ictaluridae...]. In: Fauna Ukraini 8. Ribi. 3. *Naukova dumka, Kiev (in Russian)*.
- NALBANT T.T., RÁB P., BÖHLEN J. & SAITOH K. 2001: Evolutionary success of the loaches of the genus *Cobitis* (Pisces: Ostariophysi: Cobitidae). *Trav. Mus. Nat. d'Hist. Natur. "Grigore Antipa" 43*: 277–289.
- PERDICES A. & DOADRIO I. 2001: The molecular systematics and biogeography of the European Cobitids based on mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution 19*: 468–478.
- POSADA D. & CRANDALL K. A. 1998: MODELTEST: testing the model of DNA substitution. *Bioinformatics 14*: 817–818.
- PROKOPOV G.A. 2004: Fauna of fresh waters of pool Black river. *Voprosy rasvityi Kryma. Nauchno-praktich. diskussijnno-analitich. sbornik. 15. Problemy inventariz. krymskoi bioty. Tavriya-Plus, Simferopol: 151–174 (in Russian with English summary)*.
- PUZANOV I.I. 1929: [Animal world of Crimea]. *Krymosizdat, Simferopol (in Russian)*.
- PUZANOV I.I. 1949: [The peculiarity of Crimean fauna and its origin]. *Uchenye zapiski Gor'kovskogo gos. universiteta 14*: 5–32 (in Russian).
- RÁB P., RÁBOVÁ M., BOHLEN J. & LUSK S. 2000: Genetic differentiation of the two hybrid diploid-polyploid complexes of loaches, genus *Cobitis* (Cobitidae) involving *C. taenia*, *C. elongatoides* and *C. spp.* in the Czech Republic: karyotypes and cytogenetic diversity. *Folia Zool. 49 (Suppl. 1)*: 55–66.
- RÁB P. & ROTH P. 1988: *Cold-blooded vertebrates*. In: Balíček P, Forejt J. & Rubeš J. (eds), [Methods of chromosome analysis]. *Czechoslovak. Biol. Soc. Publishers, Brno: 115–124 (in Czech)*.
- RÁB P. & SLAVÍK O. 1996: Diploid-triploid-tetraploid complex of the spined loach, genus *Cobitis* in Pšovka creek: the first evidence of the new species of *Cobitis* in the ichthyofauna of the Czech Republic. *Acta Univ. Carolinae, Biologica 39 (1995): 3–4: 201–214*.
- RÁBOVÁ M., RÁB P., BOROŇ A., BOHLEN J., JANKO K., ŠLECHTOVÁ V. & FLAJŠHANS M. 2004: Cytogenetics of bisexual species and their asexual hybrid clones in European spined loaches, genus *Cobitis*. I. Karyotypes and extensive polymorphism of major ribosomal sites in four parental species. *Abstract: Cytogenetic and Genome Research 106*: p. 16.
- ŠLECHTOVÁ V., LUSKOVÁ V., ŠLECHTA V., LUSK S., HALAČKA K. & BOHLEN J. 2000: Genetic differentiation of two diploid-polyploid complexes of spined loach, genus *Cobitis* (Cobitidae), in the Czech Republic, involving *C. taenia*, *C. elongatoides* and *C. spp.*: allozyme interpopulation and interspecific differences. *Folia Zool. 49 (Suppl. 1)*: 67–78.
- ŠLECHTOVÁ V., LUSKOVÁ V., ŠLECHTA V., LUSK S. & PIVOŇKOVÁ J. 2003: Potential species identification by allozyme/protein markers in European spined loaches. *Folia Biologica (Kraków) 51 (Suppl.): 43–47*.
- SWOFFORD D.L. 1999. PAUP\*. Phylogenetic Analysis Using Parsimony (\*and other methods). Version 4.0. *Sinauer Associates, Sunderland, Massachusetts*.
- TSEEB Ya. 1929: [Preliminary results of studies on ichthyofauna of Crimean rivers]. *Trudy Krymskogo nauchno-issledov. instit. 2 (2)*: 113–123 (in Russian)
- UENO K. & OJIMA Y. 1976: Karyotypes and geographical distribution in the genus *Cobitis* (Cobitidae). *Bull. Japan. Soc. Sci. Fish. 46*: 9–18.

- UENO K., SENOU H. & KIM I.S. 1985: A chromosome study of five species of Korean cobitid fish. *Japan. J. Genet.* 60: 539–544.
- VASIL'EV V.P. 1978: [Chromosomal polymorphism in *Spicara smarvis* (Pisces, Centranchidae)]. *Zool. Zhurn.* 57: 1276–1278 (in Russian).
- VASIL'EV V.P. 1985: [Evolution karyology of fishes]. *Nauka, Moscow (in Russian)*.
- VASIL'EV V.P. 1995: [Karyological diversity and taxonomic heterogeneity in *Cobitis* “*taenia*” (Pisces, Cobitidae)]. *Dokl. Akad. Nauk SSSR* 342: 839–842 (in Russian).
- VASILEV V.P. & VASIL'EVA E.D. 1982: [A new diploid-polyploid complex in fish]. *Dokl. Akad. Nauk SSSR.* 266: 250–252 (in Russian).
- VASILEV V.P. & VASIL'EVA E.D. 1994: The karyological diversity in spined loaches from genera *Cobitis* and *Sabanejewia*. *Fishes and their environment. VIII Congr. Europ. Ichthyol. Oviedo, Spain:* 67.
- VASIL'EVA E.D. 1984: [The comparative morphological analysis of two populations of spined loaches (genus *Cobitis*, Cobitidae) differed with the number of spots at the base of caudal fin]. *Vopr. Ikhtiolog.* 24: 43–53 (in Russian).
- VASIL'EVA E.D. 1988: [Redescription and morphoecological characteristics and distribution of *Cobitis granoei* (Teleostei, Cobitidae)]. *Zool. Zhurn.* 67: 1025–1036 (in Russian).
- VASIL'EVA E.D. 2000: Sibling species in the genus *Cobitis* (Cobitidae, Pisces). *Folia Zool.* 49 (Suppl. 1): 23–30.
- VASIL'EVA E.D., COLLARES-PEREIRA M.J. & MADEIRA J. 1992: Variability, divergence, and taxonomy of loach on the Iberian Peninsula. *J. Ichthyol.* 32 (4): 69–84.
- VASIL'EVA E.D., OSINOV A.G. & VASIL'EV V.P. 1989: The problem of reticulate speciation in vertebrates: the diploid-triploid-tetraploid complex in the genus *Cobitis* (Cobitidae). I. Diploid species. *J. Ichthyol.* 29 (7): 35–47.
- VASIL'EVA E.D. & VASIL'EV V.P. 1998: Sibling species in genus *Cobitis* (Cobitidae). *Cobitis rossomeridionalis* sp. nova. *J. Ichthyol.* 38: 580–590.
- VASIL'EVA E.D., VASIL'EV V.P. & BOLTACHEV A.R.: 2005: [Taxonomic relations of gudgeons from the Crimean Peninsula]. *Voprosy Ikhtiolog.* 45 (6): 768–781 (in Russian).