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Comparative karyological analysis of mud loach and spined loach species (genera *Misgurnus* and *Cobitis*) from the Far East region of Russia

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A b s t r a c t . The karyotypes of one mud loach and three spined loach species occurring in the Far East region of Russia are presented. *Misgurnus nikolskyi* has 2n=50 with NF=64, *Cobitis lutheri* has 2n=50 and NF=70, *C. choii* has 2n=50 and NF=68, and *C. melanoleuca* has 2n=50 and NF=72. The karyotype of *M. mohoity* is proved to consist of 50 chromosomes. These results are discussed in relation with some taxonomic and evolution problems in loaches.

Key words: karyotypes, taxonomic problems, evolution

Introduction

The analysis of recent publications on the taxonomy and zoogeography of freshwater fish of Russia demonstrates significant changes in the list of the Amur River ichthyofauna presented by N i k o l s k y (1956). The most important results are obtained in the cobitid fishes. First of all, it is proven that the European spined loach *Cobitis taenia* Linnaeus, 1758 is absent in this area that is instead populated by three distinct species, namely *C. melanoleuca* Nichols, 1925, *C. lutheri* Rendahl, 1925, and the third species described as *C. lebedevi* Vasiľeva et Vasiľev, 1985, that some later detected as a junior synonym of the Korean *C. choii* Kim et Son, 1984 (V a s i ľ e v 1985, V a s i ľ e v a 1998). Moreover, the taxonomic revision of mud loaches conducted in the Amur River basin (V a s i ľ e v a 2001) revealed the presence of two species different from China's *Misgurnus anguillicaudatus* (Cantor, 1842): *M. mohoity* (Dybowski, 1869) and the newly described *M. nikolskyi* Vasiľeva, 2001, which some authors (S h e d k o & S h e d k o 2003) considered to be a junior synonym of the Korean *M. buphoensis* Kim et Pak, 1995. In addition to the aforementioned species, N o v o m o d n y et al. (2004) suppose the recent penetration of three of China's loaches: *M. anguillicaudatus, Paramisgurnus dabryanus* Guichenot, 1872, and *C. sinensis* Sauvage et Dabry de Thiersant, 1874. However, the situation with mud loaches is more complex, since *M. anguillicaudatus* is represented by two independent species (diploid and tetraploid) in China (Y u et al. 1989).

Thus, some problems of the taxonomy of cobitid species and their distribution in the Amur River basin remain unresolved, as do the relations between the ichthyofauna of the Amur River basin and the neighbouring Chinese, Korean, and Japanese faunas. These problems should be investigated by different methods, including recent genetic ones. This study presents karyological data for four cobitid species from the Amur River basin (*M. nikolskyi*, *C. lutheri*, *C. choii,* and *C. melanoleuca*), as well as data on chromosome number

for *M. mohoity*, which shed light on some taxonomic and evolutionary problems in the genera *Cobitis* and *Misgurnus*.

Materials and Methods

Materials for karyological analysis were collected in 1996 in the Khanka Lake basin (Amur River basin) and the Razdol'naya River (Japan Sea basin), and in 2004–2005 in the Amur River basin (Fig. 1). The total number of specimens is presented in Table 1. The karyotype of *Misgurnus nikolskyi* was studied in specimens from the Komissarovka (Khanka Lake basin, 4 spec.) and Kupriyanikha (Amur River basin, 6 spec.) Rivers, and from the channel connecting the Amur lagoon (the Great Peter Bay) and unnamed lake on the isthmus of Peschanyi at Beregovoje village (1 spec.). *M. mohoity* – was investigated from the spring at Bogorodskoje

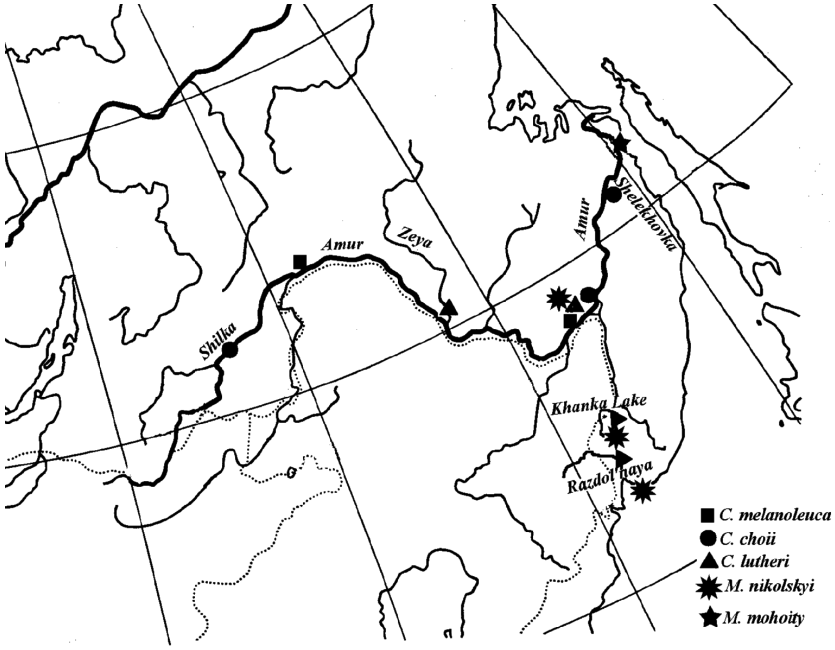

Fig. 1. A map showing the collection sites of studied species.

Table 1. Karyotypes of studied loach species from genera *Misgurnus* and *Cobitis*.

Species	Localities	2n	Karyotype structure	NF	N
M. nikolskyi	Amur River basin, Amur lagoon basin	50	10m+4sm+36sta	64	11
M. mohoity	Amur River basin	50	-	-	1
C. lutheri	Amur River basin, Razdol'naya River	50	12m+8sm+30sta	70	19
C. choii	Amur River basin	50	8m+10sm+8st+24a	68	5
C. melanoleuca	Amur River basin	50	6m+16sm+28sta	72	10

N, number of studied specimens; 2n, chromosome number in diploid set; NF, number of chromosome arms. Chromosome complement: m, metacentric; sm, submetacentric; st, subtelocentric; a, acrocentric.

village (Amur River basin). Specimens of *Cobitis lutheri* were collected in the Komissarovka (11 spec.), Razdol'naya (2 spec.), Kupriyanikha (2 spec.), and Zeya (Amur River basin, 2 spec.) Rivers, while *C. choii* – in the Amur River (3 spec.) and its tributaries, the Shilka (1 spec.) and Shelekhovka (1 spec.) Rivers, and *C. melanoleuca* – in the Amur River (10 spec.). The samples of spined loaches and *M. nikolskyi* included both males and females; karyotypes were studied in metaphases from kidney cells. Karyological data for the only male of *M. mohoity* was obtained in the metaphase of the first meiotic division. Karyological analysis was prepared using standard procedures (V a s i l' e v et al. 1989).

Results and Discussion

Results of karyological studies are presented in Table 1 and in Figs. 2 – 6. The conspecific specimens from different localities have the same karyotype structure. A diploid chromosome number 2n=50 and 64 chromosome arms (NF) were found for both sexes of *Misgurnus nikolskyi* (Fig. 2). This karyotype significantly differs from the karyotype described for the Korean mud loach *M. bufoensis* (Table 2), which completely rejects the hypothetical conspecificity of the two mentioned mud loach species (S h e d k o & S h e d k o 2003). Moreover, according to karyological data (Table 2), *M. bufoensis* seems to be phyletically more related to the Chinese species *Paramisgurnus dabryanus*, which is confirmed to be

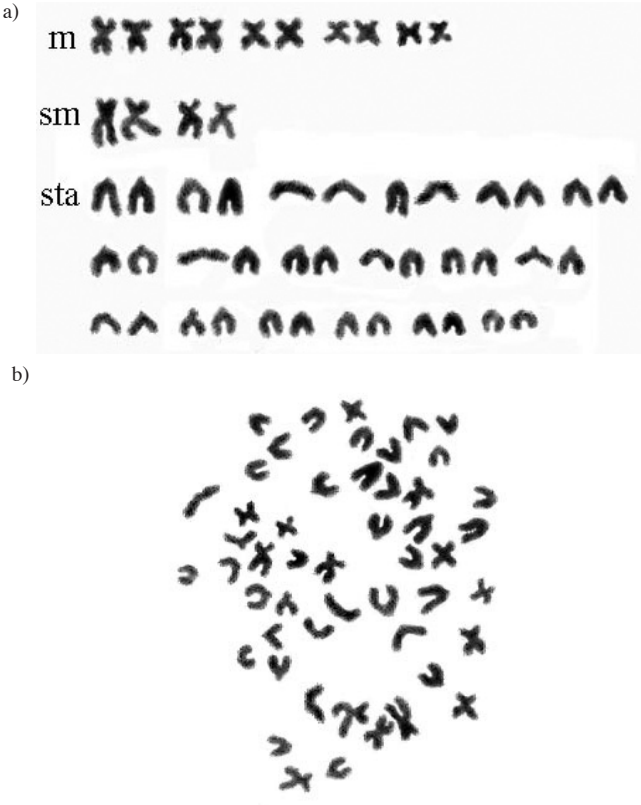

Fig. 2. Karyotype (a) and metaphase cell (b) of *Misgurnus nikolskyi*.

Table 2. Karyotypes of *Misgurnus* and *Cobitis* species from the Far East and Japan based on literature data.

Species	2n	Karyotype structure	NF	Reference
*Misgurnus anguillicaudatus**	50	8m+6sm+36sta	64	Yu et al. 1989
	100	16m+12sm+72sta	128	
	50	10m+4sm+36sta	64	Kim & Pak 1995, Ojima & Takai 1979
	50	14msm+36sta	64	Ojima & Hitotsumachi 1969
	50	8m+6sm-st+36a	64	Suzuki 1992
	48	12m+4sm+32sta	64	Ojima & Takai 1979
	100	20m+8sm+72sta	128	
	75	15m+6sm+54sta	96	
M. mizolepis	48	12m+4sm+32sta	64	Ueno et al. 1985
	50	10m+2sm+38sta	62	Kim & Pak 1995
M. bufoensis	48	10m+2sm+36sta	60	Kim & Pak 1995
Paramisgurnus dabryanus	48	12m+4sm+32a	64	Li et al. 1983
*Cobitis "taenia taenia"**	50	12m+4smst+34a	66	Ueno & Ojima 1976
	86	32m+32smst+22a	150	
	94	26m+32smst+36a	152	
*C. longicorpus***	50	20msm+30sta	70	Kim et al. 1999b
	50	12m+8sm+30sta	70	Ueno et al. 1985
	50	20msm+30sta	70	Kim & Lee 1990
*C. koreensis***	50	22msm+28sta	females 72 males 71	Lee et al. 1983
	50	12m+8sm+30sta	70	Ueno et al. 1985
*C. pumila **	50	22msm+28 sta	females 72 males 71	Kim et al. 1999b
*C. choii***	50	18msm+32sta	68	Kim et al. 1999b
*C. yongdokensis***	100	44msm+56sta	144	Kim et al. 1999b
C. sinensis	48	18msm+30sta	66	Kim & Lee 1986
	40	20m+8sm+4st+8a	68	Yu et al. 1989
	100	26m+18sm+16st+30a	134	Yu et al. 1989
	48	18msm+30sta	70	Kim & Lee 1990
C. tetralineata	50	10m+6sm+34sta	66	Kim et al. 1999a
Cobitis cf. *laosensis*	48	14m+4sm+30sta	66	Kim et al. 1999a
C. lutheri	50	12m+4sm+34sta	66	Ueno et al. 1985
	49	11m+6sm+32sta	66	Kim et al. 1999a
	50	10m+6sm+34sta	66	
	51	9m+6sm+36sta	66	
	50	16msm+34sta	66	Kim et al. 1999b
C. striata	50	16msm+34 sta	66	Kim & Lee 1986
*C."taenia striata"**	50	12m+4sm+34a	66	Ueno & Ojima 1976
	50♀	12m+4sm+34a	66	Saitoh et al. 1984
	50♂	12m+4sm+34a	66	
	49♂	13m+4sm+32a	32	
	98	20m+22smst+56a	140	Ueno & Ojima 1976
C. biwae	48	20sm+22smst+6a	90	Ueno et al. 1980
	48	16m+24smst+8a	88	
	48	16m+22smst+10a	86	
	96	32m+54smst+10a	180	
	96	58msm+38sta	154	Ojima & Hitotsumachi 1969
C. takatsuensis	50	12m+18sm+20sta	80	Kimizuka et al. 1982
*C. melanoleuca****	50	30msm+20 sta	80	Lee et al. 1983
	50	24msm+26sta	74	Kim et al. 1999b

Explanations are the same as in Table 1.
* The taxonomic status is presented according to cited papers.
** The species is separated in the genus *Iksookimia* by some authors (Kim et al. 1999a,b).
*** Korean authors present this loach as species *C. granoei (= C. melanoleuca).*

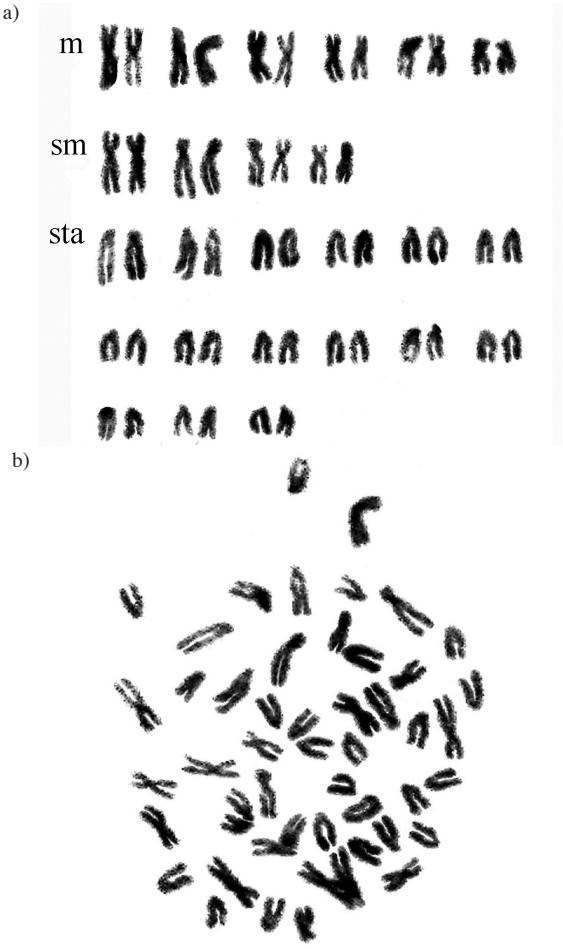

Fig. 3. Karyotype (a) and metaphase cell (b) of *Cobitis lutheri*.

a senior synonym of *M. mizolepis* Günther, 1888 (V a s i l'e v a 2001), although some authors wrongly classify the 50-chromosome mud loach as *M. mizolepis* (see Table 2). The 50-chromosome mud loach species have very similar karyotype structures (Table 2). However, according to recent karyological data, the mud loach from Korea with 10 metacentrics classified as *M. anguillicaudatus* by K i m & P a k (1995) (Table 2), seems to be conspecific with *M. nikolskyi*.

Specimens of *Cobitis lutheri* studied from 4 localities (Fig. 1) have the same karyotype, significantly different from the karyotype of spined loaches from Korea classified as the same species (Tables 1 and 2). Namely, specimens from both the Amur and Razdol'naya Rivers have 20 biarmed chromosomes (Fig. 3) and NF=70, while Korean spined loaches have 16 biarmed chromosomes and NF=66. These differences correspond to two pericentric inversions. Moreover, chromosomal polymorphism with chromosome numbers varying from 49 to 51 and the same NF was revealed in some Korean populations (K i m et al. 1999a). The nature of this polymorphism is unclear (the number of studied specimens was not noted), but another nominal species *C. tetralineata* Kim, Park et Nalbant, 1999, characterised

by a very restricted range, likely represents a local conspecific population, since both Korean taxa have similar karyotypes (Table 2) and external morphology (K i m & P a r k 2002). In this case, *C. tetralineata* should be considered as an available name for the aforementioned Korean species.

As mentioned above, *C. lebedevi* described as a new species from the Amur River basin, was proven to be a junior synonym of the Korean species *choii* (V a s i l'e v a 1998). However, some authors (K i m et al. 1999a) were not sure in their conspecificity, confirmed in this study based on the revealed similarity in the main karyological characters of both nominal taxa (Fig. 4, Tables 1 and 2).

It should be added that most *Misgurnus* and *Cobitis* species from the Far East and Japan have 2n=50 (Tables 1 and 2), and loaches which have another diploid chromosome number represent distinct "morphological" species. The species with 2n=48 have one pair of large metacentrics testifying to its origin from the 50-chromosome set by one centric fusion. At the same time, the centric fusion of acrocentric chromosomes was not the main mechanism of karyotype evolution in loaches. The only case of significant distinction in chromosome numbers was found among spined loaches from China and Korea classified as *C. sinensis* Sauvage et Dabry de Thiersant, 1874 (Table 2). Two karyomorphs with 2n=48 and 2n=40 revealed that these loaches should be considered different species; the karyotype with 2n=40

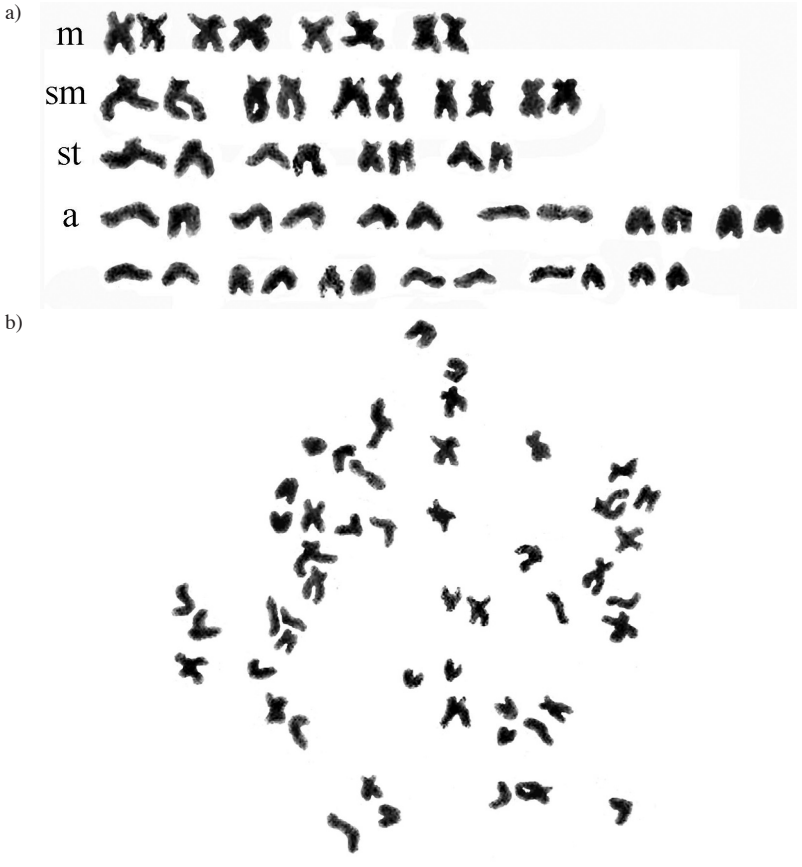

Fig. 4. Karyotype (a) and metaphase cell (b) of *C. choii*.

Fig. 5. Karyotype (a) and metaphase cell (b) of *C. melanoleuca*.

originated from the karyotype with 2n=48 or 50 by four / five centric fusions. Usually, loach species differ with chromosome morphology and NF values. For example, among diploid *Misgurnus* species, NF values vary from 60 to 64, while among diploid *Cobitis* species – from 64 to 90 (Tables 1 and 2). The karyotypes with NF 64–66 are more frequent, whereas species with the lower NF value, *M. bufoensis* and the Korean 50-chromosome mud loach classified as *M. mizolepis* (Table 2), have small areas and are probably derived from widely distributed species, such as *M. anguillicaudatus*.

Cobitis melanoleuca is the only spined loach with a continuous area from the Far East and China to European water bodies (Vasil'eva 1998). The European populations of this species from the Don, Kuban and Volga River basins, as well as from the Malyi and Bol'shoi Uzen' Rivers have the same karyotype with 2n=50 and NF=76 (8m+18sm+24sta) (Vasil'ev 1985, Vasil'ev et al. 1989, Vasil'ev & Vasil'eva 2008). The population of the same species from the Selenga River (Siberia) is characterized by a lower number of submetacentrics and NF=74 (8m+16sm+26sta) (Vasil'ev & Vasil'eva 2008). According to this study, the populations of *C. melanoleuca* from the Amur River basin have the lowest value of NF=72. Their karyotype consists of 6 meta-, 16 submeta- and 28 subtelo- and acrocentric chromosomes (Table 1, Fig. 5). Thus, a gradual transition from these populations to Siberian (by one chromosome mutation resulted in a new pair of metacentrics) and then to European ones (with the origin of a new pair of submetacentrics) is observed. The distinguished karyotype with 30 biarmed chromosomes and NF=80 was presented earlier for *C. melanoleuca* from Korea (Table 2). However, further investigations (Kim et al. 1999a, Kim & Park 2002) demonstrate that the Korean spined loach, which has an external morphology similar to that of China's *C. melanoleuca,* represents an

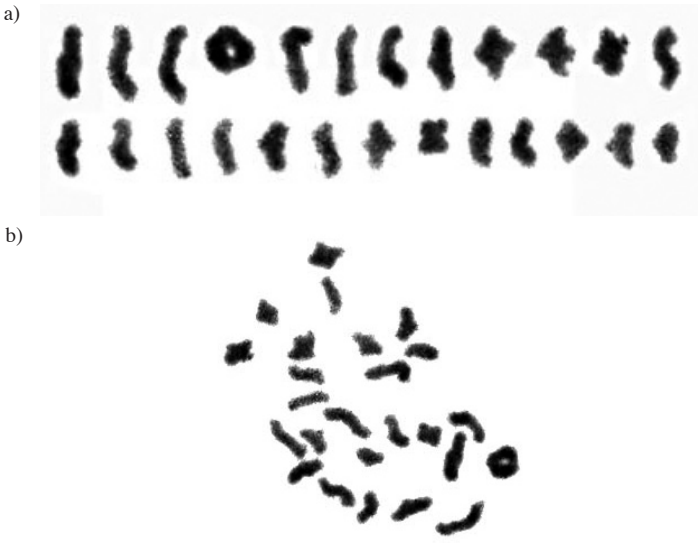

Fig. 6. Chromosome bivalents (a) and M I (b) of *Misgurnus mohoity.*

independent species, *C. pacifica* Kim, Park et Nalbant, 1999. Karyological data from this study confirm this conclusion. At the same time, the karyological diversity revealed within *C. melanoleuca* s. stricto in this study should be interpreted in relation to the polytypic structure of this species.

We did not find any species with polyploid origin among loaches karyotyped in this study, although such species with 2n about 100 are often observed in genera *Misgurnus* and *Cobitis* (Table 2). Our preliminary results for *M. mohoity* also confirm this conclusion. The meiosis of this species is characterised by the presence of not more than 25 bivalents, thus the chromosome number in this species should not exceed 2n=50 (Fig. 6).

A c k n o w l e d g e m e n t s

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