

INTROGRESSION EVIDENCE AND PHYLOGENETIC RELATIONSHIPS AMONG THREE (PARA)MISGURNUS SPECIES AS REVEALED BY MITOCHONDRIAL AND NUCLEAR DNA MARKERS

I. JAKOVLIC¹, QING-JIANG WU¹, T. TREER², N. ŠPREM² and JIAN-FANG GUI¹

¹ State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Graduate School of the Chinese Academy of Sciences, Wuhan 430072, P.R. China

² University of Zagreb, Faculty of Agriculture, Department of Fisheries, Beekeeping, Game Management and Special Zoology, 10000 Zagreb, Croatia

Abstract – The taxonomy of (*Para*)*Misgurnus* genera is still debated. We therefore used mitochondrial and nuclear DNA markers to analyze the phylogenetic relationships among *Misgurnus anguillicaudatus*, *Paramisgurnus dabryanus* and *Misgurnus fossilis*. Differing phylogenetic signals from mitochondrial and nuclear marker data suggest an introgression event in the history of *M. anguillicaudatus* and *M. mohoity*. No substantial genetic evidence was found that *Paramisgurnus dabryanus* should be classified as a separate genus.

Key words: *Misgurnus anguillicaudatus*, *Paramisgurnus dabryanus*, *Misgurnus fossilis*, molecular markers, hybridization

INTRODUCTION

The taxonomy of the *Misgurnus* genus, especially in East Asia, where natural ranges of several morphologically similar species overlap, is still debated. *Misgurnus fossilis* (MF) (Lacepede) is native to Europe, while the rest of the species are native to East Asia: *M. anguillicaudatus* (MA) (Günther); *M. mohoity* (Dybowski); *M. nikolskyi* (Vasileva); *M. buphoensis* (Kim & Park); *M. tonkinensis* (Rendahl) and *M. mizolepis* (Günther). Similar to these species in its distribution and morphology is the sole representative of the related genus, *Paramisgurnus dabryanus* (PD) (Guichenot).

Only a few studies have dealt with the phylogenetic relationships among the European and East-Asian Cobitidae (Šlechtová et al., 2008; Tang et al., 2008), and no study focusing exclusively on the phylogeny

of European and East-Asian *Misgurnus* and *Paramisgurnus* genera has ever been published. As previous research has shown that incongruous results are obtained using mitochondrial and nuclear markers (Šlechtová et al., 2008), mitochondrial (cytochrome b (cyt b) and displacement loop (D-loop)) and nuclear (recombination activating gene 1 (RAG1)) markers were simultaneously used to study the evolutionary history of MF, MA and PD. All three genes have often been used in phylogenetic studies on Cypriniformes (Culling et al., 2006; Liu et al., 2003).

MATERIALS AND METHODS

Eight MF specimens, 3 *Cobitis elongata* (CE) and 2 *Barbatula barbatula* (BB), were obtained in 2007 from the River Sava, Croatia. Twelve MA, 14 PD and 3 *M. sp.* specimens were obtained in 2006 from fish markets in Wuhan, China. Specimens were determined

according to Kottelat and Freyhof (2007). The remaining sequences were obtained from *GenBank*. Along with CE and BB, *GenBank* sequences of *Gonorynchus abbreviatus*, *Leptobotia mantschurica* and *Cyprinus carpio* were used as the second outgroup.

Total DNA was extracted from fin clips preserved in ethanol (99%) by the standard phenol-chloroform extraction (Sambrook et al., 1989). Cyt b and D-loop were amplified using the primers and amplification conditions described in Culling et al. (2006) and Liu et al. (2003), respectively. 780 bp of RAG1 was amplified using the newly designed primers: Mrag1F: 5'-AGAGCTTCTCCCAGGCTACC-3' and Mrag1R: 5'-AGGGATTCGTCCCTCCAGATT-3', under the following amplification conditions: 10-100ng of DNA; 40 µl reaction volume; 1 unit of Taq DNA polymerase; initial denaturation (94°C/4 min); 34 amplification cycles: denaturation (94°C/40 s) – annealing (53°C/30 s) – extension (72°C/70 s); final extension (8 min). Sequencing was performed by commercial sequencing companies and sequences were deposited in the *GenBank* (Table 1).

Sequences were aligned and edited by *CLUSTAL W* (Thompson et al., 1997). 909 to 940 bp of D-loop, 724 bp of RAG1 and 1140 bp of the complete cyt b gene were used for further analyses. Using the Akaike Information Criterion implemented in *jModelTest* 0.1.1 (Posada, 2008), HKY+I+G was chosen for RAG1 and F81 for cyt b and D-loop datasets as the best fitting evolution model. Two independent Bayesian searches were performed using *MrBayes* (Huelsenbeck et al., 2001) under the following settings: 4 chains; 10⁶ generations; sampling frequency = 100 generations; *burnin* value = 10³. The remaining trees were used to build a consensus tree. *MEGA* (Tamura et al., 2007) and *GIMP2* (www.gimp.org) were used for displaying and editing phylogenies. In order to be able to include *GenBank* data, phylogenetic analyses were performed separately for each amplified fragment.

RESULTS AND DISCUSSION

Apart from MF and PD forming a sister group in the

former, Bayesian analyses of D-loop (Fig. 1. a) and cyt b (Fig. 1. b) datasets yielded highly congruent trees. *Misgurnus* was shown as paraphyletic with high posterior probability (PP) values. The RAG1 dataset phylogeny tree (Fig. 1. c) shows the entire (*Para*)*Misgurnus* as monophyletic. PP were high; none below 50%, the majority over 90%.

MF cluster

All datasets showed the MF cluster as very homogeneous, with the exception of the MF sequence from Ukraine in the RAG1 dataset (Fig. 1. c). It is possible that some other cobitid was mistakenly classified as MF.

PD cluster

M. mizolepis sequences were dispersed within the PD cluster in all trees, suggesting it might be just a synonym for *P. dabryanus*.

MA/M. mohoity cluster

In all of the trees, the MA cluster was shown as closely related to *M. mohoity* (*bipartitus*). In the cyt b and D-loop trees, two newly sequenced *M. sp.* specimens clustered with *M. bipartitus/mohoity* specimens from the Chinese or Russian part of the Amur drainage, corroborating that *mohoity* and *bipartitus* are synonyms (<http://fishbase.org>).

As it was previously shown that introgressive hybridization occurs between Cyprinidae genera (Freyhof et al., 2005), and an introgression event has been proposed in the evolutionary history of *Misgurnus* (Šlechtová et al., 2008; Tang et al., 2008), it can be assumed that an introgression of mtDNA from *Cobitis* by hybridization has occurred in the common ancestor of *M. anguillicaudatus* and *M. mohoity*. This research suggests that the (*Para*) *Misgurnus* clade is comprised of five species. Two of these, *Misgurnus anguillicaudatus*, with a wide native range, and *Misgurnus mohoity*, predominantly found in the Amur ecoregion and Korea, underwent mitochondrial introgression. The remaining three species are *Misgur-*

Table 1. GenBank accession numbers of the specimens sequenced for the study

<i>M. fossilis</i>	<i>Cyt b</i>	<i>D-loop</i>	<i>RAG1</i>
MF1	GU583654		GU583731
MF2	GU583655	GU583682	GU583732
MF3	GU583656	GU583683	GU583733
MF4	GU583657	GU583684	GU583734
MF5	GU583658	GU583685	GU583735
MF6	GU583659	GU583686	GU583729
MF7	GU583660	GU583687	GU583730
MF8	GU583661		GU583736
<i>M. anguillicaudatus</i>			
MA2	GU583669	GU583696	GU583738
MA3	GU583670		GU583708
MA4	GU583671	GU583697	GU583709
MA5	GU583672	GU583698	GU583710
MA6	GU583673	GU583699	GU583711
MA7	GU583674	GU583700	GU583712
MA8	GU583675	GU583701	GU583713
MA9	GU583676	GU583702	
MA10	GU583677	GU583703	
MA11		GU583704	
MA12		GU583705	
<i>P. dabryanus</i>			
PD2	GU583663	GU583689	GU583715
PD3	GU583664	GU583690	GU583716
PD4	GU583665	GU583691	GU583717
PD5	GU583666	GU583692	GU583718
PD6	GU583667	GU583693	GU583719
PD7		GU583694	GU583720
PD8	GU583668	GU583695	GU583721
PD9			GU583722
PD10	GU583662	GU583688	
PD_B1			GU583723
PD_B2			GU583724
PD_B3			GU583725
PD_M1			GU583737
<i>Misgurnus</i> sp.			
M.sp.1	GU583678	GU583706	
M.sp.2	GU583679	GU583707	
<i>Barbatula barbatula</i>			
BB1	GU583652	GU583680	
BB2	GU583653	GU583681	
<i>Cobitis elongata</i>			
CE1			GU583726
CE2			GU583727
CE3			GU583728

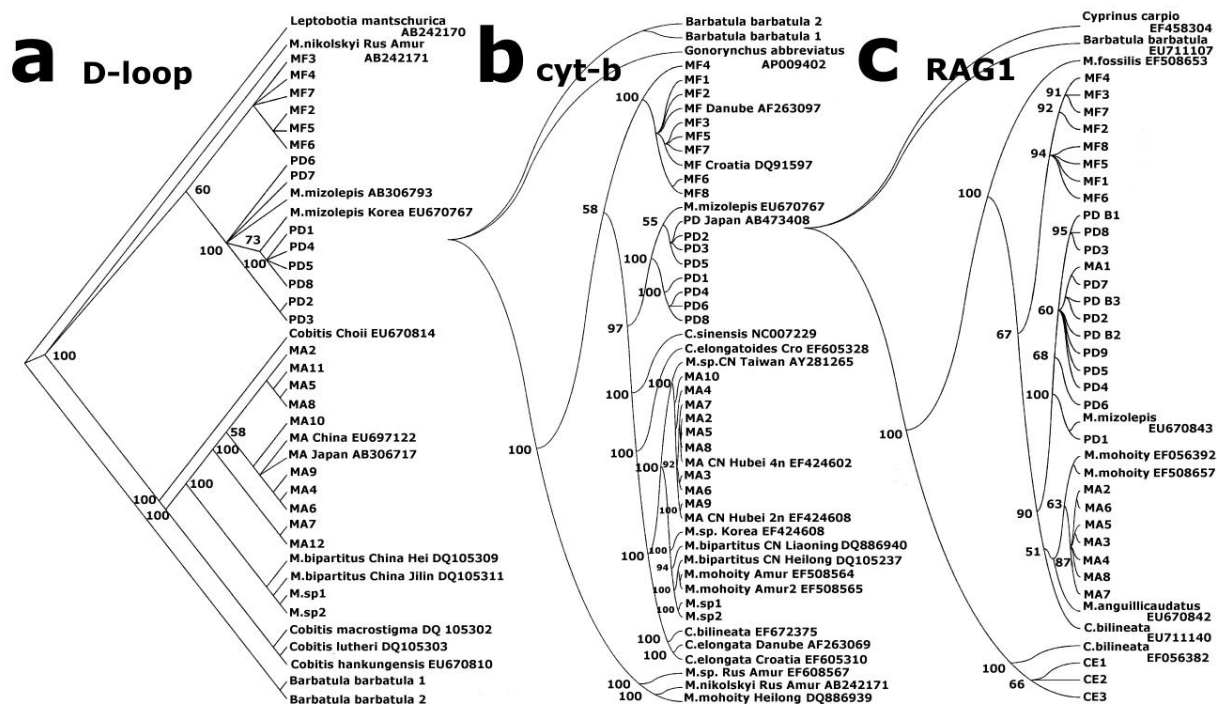


Fig. 1. a) D-loop dataset; b) Cyt b dataset; c) RAG1 dataset. Values at the nodes correspond to Bayesian posterior probabilities. Samples obtained from *GenBank* are presented with the corresponding accession numbers. ‘M’ - *Misgurnus*; ‘C’ - *Cobitis*; ‘CE’ - *Cobitis elongata*; ‘CN’ - China; ‘Rus’ - Russia; ‘Heilong’ - Heilongjiang (China).

nus fossilis, confined to European waters; *Misgurnus nikolskyi*, mostly found in the Amur river drainage and *Paramisgurnus dabryanus*, with a wide native range from South China to South Korea. As the whole (*Para*)*Misgurnus* clade is relatively new, Tang et al. (2008) suggested a conservative approach to taxonomy, but more detailed research would be necessary to corroborate these results and possibly suggest merging *Paramisgurnus* into the *Misgurnus* genus.

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