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Distribution of loach fishes (Cobitidae, Nemacheilidae) in Albania, with genetic analysis of populations of *Cobitis ohridana*

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A b s t r a c t . Between 2003 and 2006, almost 80 localities in all main hydrological systems in Albania were sampled and data on the distribution of loach fishes gathered. The spined loach *Cobitis ohridana* Karaman, 1928 was found to be a common species in Albania, occurring in most of its river systems, from the Ohrid-Drin-Shkodra system in the east and north to the River Vjosë basin in the south. *Cobitis meridionalis* Karaman, 1924 occurs in Lake Prespa, while a spined loach with mtDNA of *Cobitis sensu stricto* origin is present at least in the Ohrid-Drin-Shkodra system. The most common stone loach in Albania was found to be *Oxynoemacheilus pindus* (Economidis, 2005). It was caught in the basins of the rivers Vjosë, Seman, Shkumbin and Erzen. *Barbatula sturanyi* (Steindachner, 1892) was recorded in the River Black Drin.

Key words: *Cobitis ohridana*, *Cobitis meridionalis*, *Oxynoemacheilus pindus*, *Barbatula sturanyi*, cytochrome *b*, phylogenetic relationships

Introduction

The Mediterranean freshwater ichthyofauna consists of numerous endemic species with a restricted area of distribution (C r i v e l l i & M a i t l a n d 1995). Based on their distribution, especially that of cyprinids, the Mediterranean catchments may be divided into twelve ichthyological districts (B i a n c o 1990). The district that includes Albania is one of Europe's least studied and probably least known in respect of fish distribution, taxonomy and evolutionary relationships.

There are several main, contemporarily independent, river and lake systems in Albania (Fig. 1). Listed from north to south they are the Ohrid-Drin-Shkodra system (including River Buna), rivers Mat, Ishëm, Erzen, Shkumbin, Seman (with its two large tributaries, the Devoll and Osum) and Vjosë (River Aoos in Greece), the area around Butrint Lagoon (rivers Bistrica and Pavllo) and Lake Prespa (connected underground with Lake Ohrid (A m a t a j et al. 2007)), all of which drain eventually into the Adriatic Sea. There are also other short rivers flowing directly into the Adriatic. Only a very small area in the northernmost part of Albania (in the mountains Bjeshkët e Namuna; Prokletije in Slavic) belongs to the Danube basin (D i l l 1993).

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The Ohrid-Drin-Shkodra system is composed of large lakes Shkodra and Ohrid, which are, since, connected via the River Drin, the largest river in Albania. This river is formed by the confluence of two tributaries, the Black Drin and White Drin. It has the most constant discharge of all Albanian rivers (P a n o 1985). Discharge of all other rivers is highly seasonally variable, being sometimes over ten times smaller in summer than in winter. The beds of the main rivers are usually very wide, as a great amount of gravel and pebbles is deposited around the flow itself (D i l l 1993).

Very little is known of the distribution of loach fishes (Cobitidae, Nemacheilidae) in Albania. P o l j a k o v et al. (1958) reported the presence of *Cobitis taenia meridionalis* Karaman, 1924 in Lake Prespa, *C. t. ohridana* Karaman, 1928 in Lake Ohrid, *C. t. dalmatica* Karaman, 1928 in Lake Shkodra and *Nemachilus barbatulus sturanyi* Steindachner, 1892 in Lake Ohrid. R a k a j (1995) mentioned occurrence in Albania of *Misgurnus fossilis* (Linnaeus, 1758), *Cobitis aurata balcanica* Karaman, 1922, *Cobitis taenia taenia* Linnaeus, 1758, *C. t. ohridana* and *Nemachilus barbatulus* (Linnaeus, 1758). All of the samples described in R a k a j (1995), apart from *C. t. ohridana*, came from rivers or lakes in the vicinity of Tirana. Furthermore, the limited knowledge of Albanian freshwater fishes is highlighted by recent descriptions of new species from adjacent countries (B i a n c o & K o t t e l a t 2005, E c o n o m i d i s 2005).

The aims of this paper are to update the information contained in P o l j a k o v et al. (1958) and in R a k a j (1995), to investigate more fully the distributions of loaches (Cobitidae, Nemacheilidae) in Albania and research for the first time genetic diversity and phylogenetic relationships of *C. ohridana*.

Material and Methods

In the summers between 2003 and 2006, a sampling expedition of all the main drainages in Albania was conducted. Nearly 80 localities were sampled. For catching fish, portable engine or battery electrofishing gear was used. Fish were identified by comparing them with published descriptions (I v a n o v i ć 1973, G r u p č e & D i m o v s k i 1976, Š o r i ć 2000), and using the provisional keys in E c o n o m i d i s & N a l b a n t (1996) and E c o n o m i d i s (2005). Small pieces of fin tissue were preserved in 96% ethanol for DNA analysis, while voucher specimens were labelled, preserved in 5% formaldehyde and deposited in the National Museum in Prague (Czech Republic).

To reveal the phylogenetic relationships of the *Cobitis* populations from the studied region, cytochrome *b* was analysed in one to three individuals per river system. In all, 19 specimens from ten locations were analysed. DNA was extracted from fin tissue using JETQUICK Tissue DNA Spin Kit (GENOMED) following manufacturer's instructions. The amplification primers that were used were GluF and ThrR (M a c h o r d o m & D o a d r i o 2001). PCR reactions were performed in a final volume of 25 μl containing 1–2 μl DNA, 0.5 μM of each primer, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 1 U *Taq* DNA polymerase (SEGENETIC), corresponding buffer and ddH_2O. The protocol for amplification was as follows: initial incubation of 180 s at 94°C, 35 cycles of 45 s at 94°C, 90 s at 48°C and 105 s at 72°C, and a seven-minute final extension at 72°C. PCR products were purified using ethanol precipitation. Sequencing was carried out by the Macrogen Service Centre (Seoul, South Korea) with the use of internal primers H-COB_cyt638 and L-Cyp_425 (B u j et al. 2008).

Table 1. List of analysed spined loach samples, their individual codes, sampling localities, and GenBank accession numbers. A, Albania; F, FYROM; M, Montenegro; O-D-S, Ohrid-Drin-Shkodra
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Individual code	River	Catchment	Country	Haplotype No.	Accession No.
A3011	Devoll	Seman	A	1	EF597222
A3012	Devoll	Seman	A	2	EF597223
Co1	Mat	Mat	A	3	EF597224
Co2	Mat	Mat	A	3	EF597224
Co19	Lake Ohrid	O-D-S	A	4	EF597226
Co20	Lake Ohrid	O-D-S	A	5	EF597227
Co21	Lake Ohrid	O-D-S	A	6	EF597228
Co40	Morača	O-D-S	M	5	EF597227
Co41	Shkumbin	Shkumbin	A	2	EF597223
Co42	Shkumbin	Shkumbin	A	7	EF597231
Co43	Zeze	Ishëm	A	8	EF597232
Co44	Zeze	Ishëm	A	3	EF597224
Co51	Erzen	Erzen	A	9	EF597234
Co52	Erzen	Erzen	A	9	EF597234
Co55	Vjosë	Vjosë	A	10	EF597236
Co56	Vjosë	Vjosë	A	11	EF597237
COBDRI1	Black Drin	O-D-S	F	4	EF597226
COBDRI2	Black Drin	O-D-S	F	4	EF597226
COBDRI7	Black Drin	O-D-S	F	12	EF597240

Sequences were aligned manually and revised in BioEdit (Biological sequence alignment editor v.5.0.9). The geographical origins of the samples and GenBank accession numbers are summarized in Table 1. In order to investigate the phylogenetic relationships of sampled populations, comparative sequences were downloaded from the GenBank (*Cobitis zanandreai* AF263089, AY191562; *Cobitis elongata* AF263069; *Cobitis elongatoides* AF263082, AF263081, AY191567; *Cobitis taenia* AY191565, AF263078; *Cobitis ohridana* AY191564, AY191563; *Cobitis meridionalis* AF263084; *Cobitis strumicae* DQ217373, AY191578; *Cobitis vardarensis* AY191570, AY191569, AY191568; *Cobitis bilineata* AF263090; *Cobitis hellenica* AY191583, AY191582; *Cobitis trichonica* AF263086, AF263085; *Cobitis dalmatina* EF605302, EF605303; *Cobitis narentana* EF605315, EF605316). *Sabanejewia balcanica* (GenBank Accession No. EF447289) was used as the outgroup. Prior to analysing the sequence data, the best fitting model of nucleotide substitution was assigned using Modeltest 3.06 (Posada & Crandall 1998). Under Akaike information criterion (AIC), the general time reversible model with gamma distribution of rate heterogeneity and proportion of invariable sites (GTR+I+G) was selected. Two different approaches were used: neighbour-joining (NJ) algorithm and Bayesian inference (BI). The NJ trees were constructed in PAUP* v. 4.0b10 (Swofford 2002) with parameter settings as estimated by Modeltest. Statistical support for branching nodes was estimated using 1,000 bootstrap replications. A Bayesian tree was constructed using MrBayes v. 3.0 (Huelsenbeck & Ronquist 2001). Six Monte Carlo Markov Chains were run simultaneously for 1,000,000 generations with sampling frequency of 100. Likelihood scores reached stability after about 30,000 generations. The corresponding trees were discarded as burn-in and the remaining trees were used to construct a 50 per cent majority-rule consensus tree. The posterior probabilities were used to indicate the branch supports in the final tree.

Results and Discussion

Two species of genus *Cobitis* (Cobitidae), one of genus *Barbatula* and one of genus *Oxynoemacheilus* (Nemacheilidae) were found in Albania. The most common and widespread

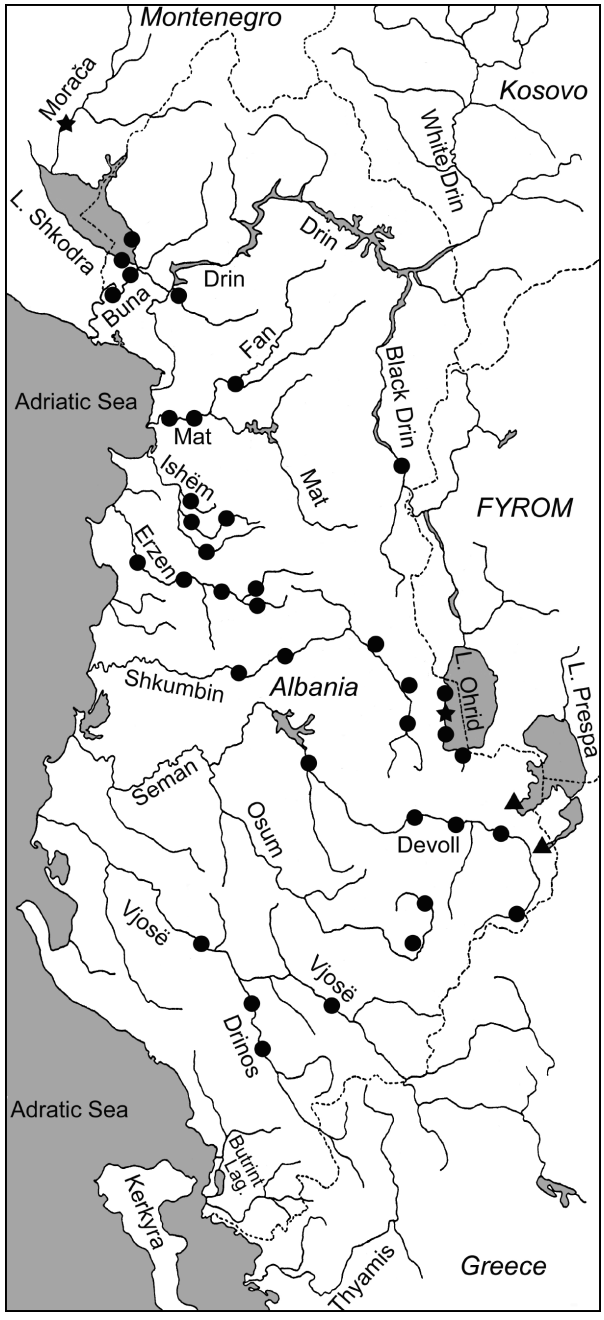

Fig. 1. Records of *Cobitis* species in Albania. ● *Cobitis ohridana*, ▲ *Cobitis meridionalis*, ★ *Cobitis* with mtDNA of *Cobitis sensu stricto* origin.

species of *Cobitis* was found to be *Cobitis ohridana*, a monocanestrinia species. It was found in most of the sampled rivers and lakes (Fig. 1). It inhabits places with slow water current and fine sediment. In places with high water velocity, such habitats were often very local and limited to a few square meters around banks or under bridges. Where suitable habitats were large, *C. ohridana* was a dominant species. The distribution area of *C. ohridana* was found to be much wider than initially thought: previously, this species had been mentioned only from Lake Ohrid (Poljakov et al. 1958, Rakaj 1995) and the River Drin system (Rakaj 1995). The record of the presence of *C. taenia taenia*, which occurs in the northern part of western, central and eastern Europe, in Çerkezë-Morinë reservoir close to Tirana (Rakaj 1995) should probably be assigned to *C. ohridana*. It is also likely that the presence of *C. dalmatina*, endemic to the River Cetina, in Lake Shkodra (Poljakov et al. 1958) refers to *C. ohridana*.

Results of the mtDNA analysis (both NJ and BI; Fig. 2) of some of the individuals collected during this study support the morphological identification and confirm that *C. ohridana* inhabits almost the whole of Albania. The tree topologies constructed by the two methods employed here were similar. Among the 19 specimens analysed, twelve different haplotypes (Table 1), occurring within two main clades, were revealed (Fig. 2). All but one of these haplotypes clustered together with the GeneBank sequence of *C. ohridana* (originating from the Greek part of the River Vjosë (Bohlen et al. 2006)) into a well-supported branch within Clade I. This clade is identical to the Adriatic lineage identified by Bohlen et al. (2006). A sister species of *C. ohridana*, *Cobitis zanandreai* Cavicchioli, 1965 (Fig 2), is found in southern Italy. The last haplotype (specimens CO20 from Lake Ohrid and CO40 from the River Morača in Montenegro), occurring inside Clade II, clustered with strong bootstrap support with *C. elongatoides* from the Danube basin (Fig. 2). Currently, it is not clear whether this haplotype belongs to a different species of *Cobitis*, is a case of past introgression or that an even more complicated diploid-polyploid complex exists in the Ohrid-Drin-Shkodra system. In any case, the presence of this haplotype indicates a past connection between the Danube basin and the Ohrid-Drin-Shkodra system.

To summarize the information on the distribution of *C. ohridana*, this species occurs in major part of Albania, in the Greek course of the River Vjosë (Aoos; Economidis & Nalbant 1996), in Lake Shkodra and its inflows in Montenegro (Ivanović 1973, Bohlen et al. 2003), in Lake Ohrid and the River Black Drin system in FYROM (Karaman 1924, Grupče & Dimovski 1976, Šorić 1990), and in the River White Drin basin in Kosovo (Šorić 1990).

The second spined loach species recorded in this study was *Cobitis meridionalis*. In Albania, this bicanestrinia species is restricted to Lake Prespa. It was found in both Mikri and Megali Prespa (Fig. 1). This result is in agreement with previous reports about occurrence of this species in the FYROM (Karaman 1924, Grupče & Dimovski 1976) and the Greek part of the Lake Prespa watershed (Economidis & Nalbant 1996, Crivelli et al. 1997, Crivelli & Lee 2000), and in Albania (Poljakov et al. 1958).

No *Cobitis* was found in the area of Butrint Lagoon, situated close to the Greek border (Fig. 1). However, in both rivers Bistrica and Pavllo there are potentially suitable habitats for spined loaches. Occurrence of a bicanestrinia *Cobitis* species is known from the River Thyamis, an adjacent river system in Greece (Economidis & Nalbant 1996, Perdices & Doadrio 2001). Bohlen et al. (2006) identified the species from this river as *Cobitis hellenica* Economidis et Nalbant, 1996. The same species might also

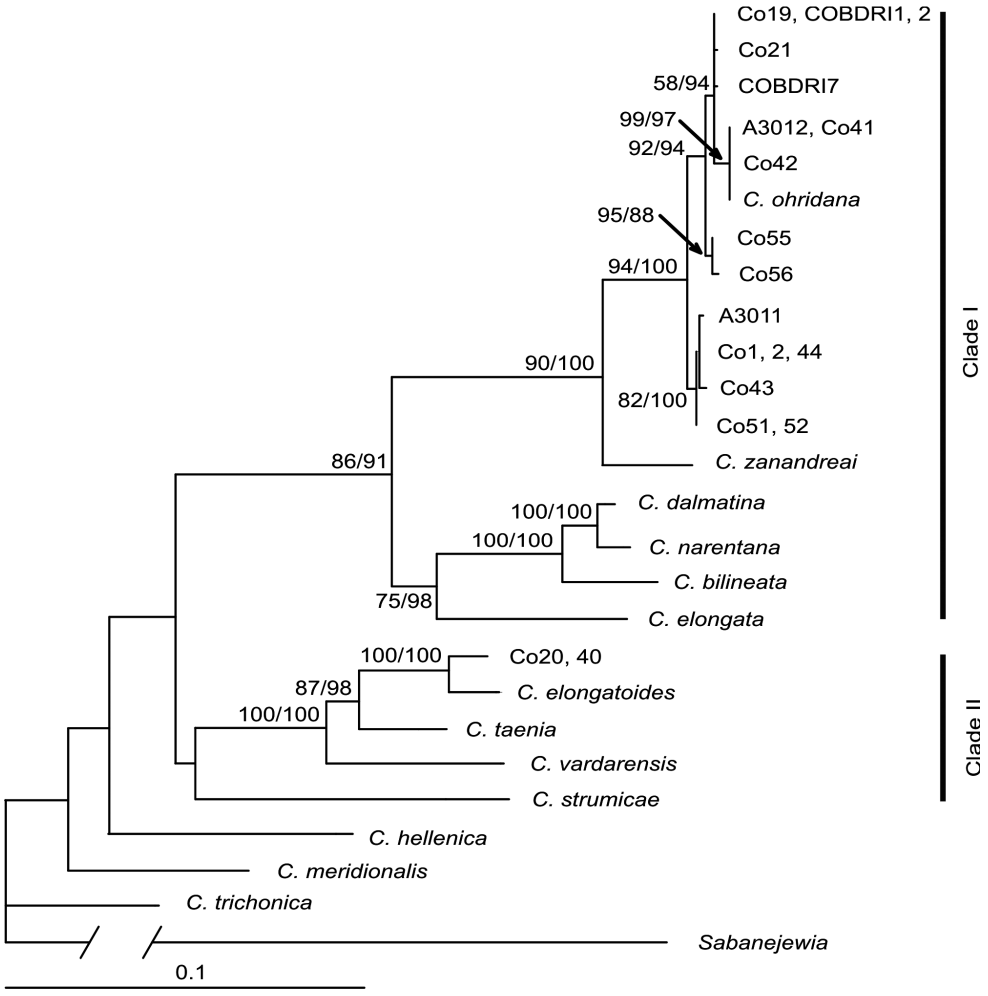

Fig. 2. Phylogenetic tree of Albanian spined loach specimens examined (code designations) and their relationships to other spined loach species. The numbers at the nodes indicate the Bayesian inference and neighbour-joining support values, respectively. Values higher than 50 per cent are shown.

be distributed in the area around Butrint Lagoon. More detailed field work is necessary to resolve this issue.

A very common and widespread species in Albania is *Oxynoemacheilus pindus*. This species inhabits drainages of the rivers Erzen, Shkumbin, Seman and Vjosë (Fig. 3). It is a rheophilic species, inhabiting main streams of the rivers with strong water current. Until now, *O. pindus* was known only from the River Aoos (E c o n o m i d i s 2005). The range of this species is thus much wider, and comparable in size to the European range of *Oxynoemacheilus bureschi* (Drenski, 1928) (Š e d i v á et al. 2008).

Barbatula sturanyi was found only in the upper part of the Albanian stretch of the River Black Drin (Fig. 3), where it inhabits stony and gravely habitats. According to Š o r i ć (1990, 2000), this species inhabits also the Black Drin drainage in FYROM and the White Drin drainage in Kosovo. However, the distribution of *Barbatula* in the rest of the Drin watershed in Albania is unknown. This uncertainty requires further detailed sampling within

the Albanian River Drin system, because another species of stone loach, *Barbatula zetensis* (Šorić, 2000), could occur here. *B. zetensis* and *B. sturanyi* are sister taxa (Š e d i v á et al. 2008) with the former abundant in the River Morača system (inflow of Lake Shkodra) in

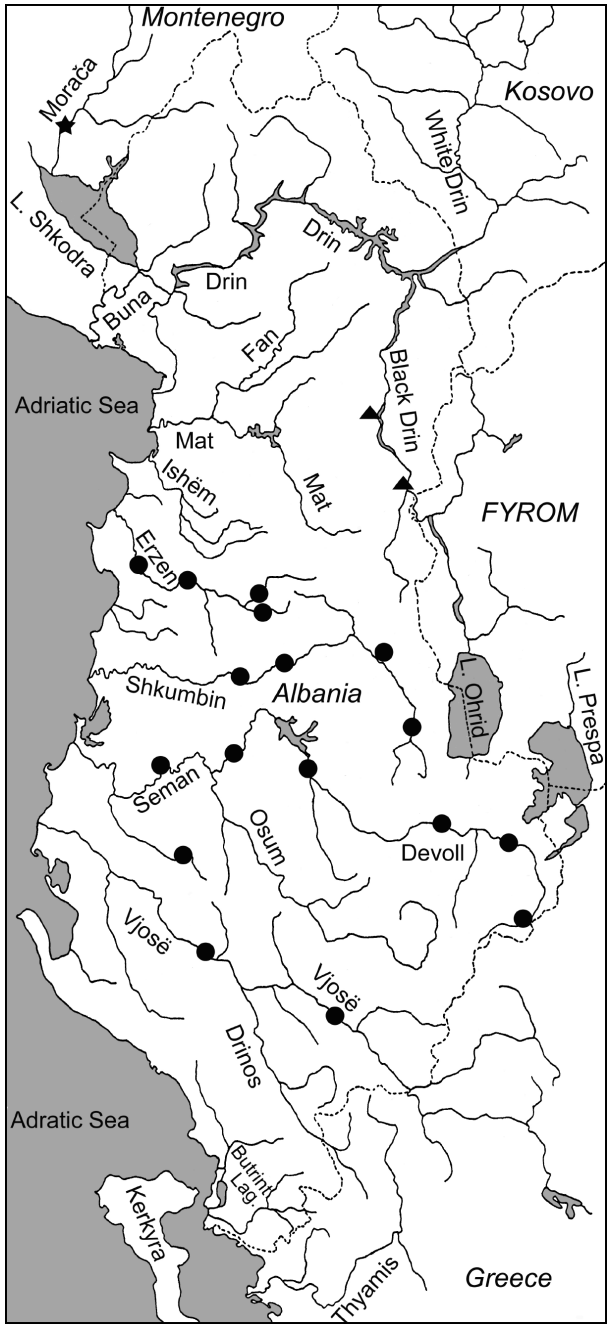

Fig. 3. Records of *Barbatula* and *Oxynoemacheilus* species in Albania. ● *Oxynoemacheilus pindus*, ▲ *Barbatula sturanyi*, ★ *Barbatula zetensis* in Montenegro.

Montenegro (B o h l e n et al. 2003). It is not clear, if any *Barbatula* species inhabits Lake Shkodra. Only I v a n o v i ć (1973) mentioned that *B. sturanyi* is rarely found in this lake. During our recent investigations, no *Barbatula* was found in Lake Shkodra, River Buna or inflows of the River Drin close to Lake Shkodra. It is likely that the formation of Lake Shkodra promoted a speciation of *B. sturanyi* and *B. zetensis* by vicariance. However, even if Lake Shkodra presents a barrier to the migration of *B. zetensis*, this species could be present in the Albanian part of the River Cijevna (Cemit), an inflow of the River Morača (Montenegro).

The reason for absence of *Barbatula* in the River Mat system is unclear (Fig. 3). Although several localities, covering the whole drainage area, were sampled, no stone loach was recorded, and nor was one found in the River Ishëm, adjacent to the River Mat.

R a k a j (1995) mentioned the occurrence of *Misgurnus fossilis* in a reservoir close to Tirana. This locality has not been sampled recently. As this locality is extremely remote from the natural distribution area of this species in Europe, the most probable explanation is an unintentional introduction along with economically important species such as common carp *Cyprinus carpio* Linnaeus, 1758. However, intentional introduction cannot be excluded.

The existence of *Sabanejewia balcanica* in Albania was reported by R a k a j (1995). The only possible natural distribution area of this species in Albania is the small northernmost part of the country, where tributaries of the River Lim (Danube drainage) originate. However, our study did not confirm its presence here, nor in other sampled localities. If *S. balcanica* occurs in any other Albanian hydrological system, it is most probably a result of introduction. On the other hand, it is also possible that R a k a j (1995) misidentified some atypical *C. ohridana* for *S. balcanica*. Colour patterns of *C. ohridana* are highly variable. Specimens with extremely large side blotches and a colour pattern resembling *S. balcanica* were observed.

In this study, four species of loaches were found in Albania: *C. ohridana*, *C. meridionalis*, *O. pindus*, and *B. sturanyi*. Preliminary analysis of the genetic diversity of the most widespread loach, *C. ohridana*, revealed a high haplotype diversity with no apparent geographic pattern, and the presence of specimens with mitochondrial DNA of *Cobitis sensu stricto*. Several important issues, including conservation status, biology, detailed distribution within different hydrological systems or relationships of populations of different drainages need to be investigated in detail.

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

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