

Species- and hybrid richness in spined loaches (genus *Cobitis* L.) with a checklist of the species and hybrids of Europe

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Abstract

Until recently, spined loaches of the genus *Cobitis* were thought to be represented in Europe mainly by one widespread species, the common spined loach *Cobitis taenia* L. Recent studies have shown that the diversity of spined loaches is considerably higher due to: a) presence of several species beside *C. taenia*, b) presence of gynogenetically reproducing hybrid biotypes, living together with biparentally reproducing species in mixed populations. Here we summarise the knowledge about distribution and diversity of spined loaches in Europe by combining original with published data. In southern Europe, a number of distinct species occur allopatrically, while in Central and Eastern Europe a few species have wide distribution areas. Hybrid biotypes were found exclusively in Central and Eastern Europe. They were not restricted to the contact areas between the parental species, but occur in almost the whole area. In total, 14 hybrid biotypes with different genome compositions were recorded. In the most diverse population investigated, five hybrid biotypes were associated with two species. In general, a syntopic occurrence of different species of *Cobitis* can be considered as exceptional, while the association of species with hybrid biotypes is the rule in Central and Eastern Europe. The composition of mixed populations seems to be most strongly influenced by the kind of associated species and by local history. Implications for conservation are i) not to disturb the natural patterns of diversity, ii) to consider the known richness in legislation, iii) to consider the evolutionary significance of unisexual hybrid biotypes.

Introduction

In general, freshwater fishes are suited model organisms for the investigation of zoogeography, especially if they are primary freshwater species (Banarescu, 1990). They gain additional advantages for zoogeographic models if they are widespread and man has not affected their distribution (Englbrecht et al., 2000). Spined loaches of the genus *Cobitis* provide such a suited model for zoogeographic studies. They are primary freshwater fish occurring in most of Europe despite the cold boreal areas (Banarescu, 1990). They are found in nearly all major water systems of Europe and their distribution has been hardly affected by man.

For a long time, most spined loaches in Europe were considered to belong to *Cobitis taenia* Linnaeus separated into a number of subspecies (Lelek, 1987). Nowadays, many of these subspecies are regarded to as distinct species, and several new species have been described (reviewed in Kottelat, 1997). Several surveys of regional distributions of spined

loaches have been provided (e.g. Madeira et al., 1992; Economidis & Nalbant, 1996; Povc & Šumer, 2000), but a compilation of data for whole of Europe is lacking.

Besides the diversification at the species level, the genus *Cobitis* was found to include a number of different hybrid biotypes (reviewed in Ráb & Slavik, 1996). These hybrids mainly are polyploid and are believed to perpetuate in a clonal manner by gynogenesis resulting in nearly all-female offspring (Vasil'ev et al., 1989; Saat, 1991). Therefore, two independent kinds of diversity exist within the genus *Cobitis* in Europe. The identification of these hybrid biotypes is not possible by morphology (Vasil'eva & Vasil'ev, 1998; Vasil'eva, 2000). At present, the karyotype, best combined with allozyme data, is the most reliable character for the identification of species and hybrids (Ráb et al., 2000; Vasil'eva, 2000).

In the present study we summarise own data and published information on the distribution of species and mixed populations of *Cobitis* in Europe and examine the geographic patterns of species richness and hybrid richness. To check the state of legislation, we generate a checklist of the species of *Cobitis* in Europe and compare it with the species recognised in conservation texts in Europe.

Materials and Methods

Identifications of spined loaches by karyotype data were provided by Cataudella et al. (1977), Sofradzija & Berberovic (1978), Vasil'ev et al. (1989), Boron (1992, 1995), Madeira et al. (1992), Vasil'ev (1995), Boron & Danilkiewicz (1998), Vasil'eva & Vasil'ev (1998), Boron & Kotusz (1999), Ráb et al. (2000), Šlechtová et al. (2000) and Bohlen et al. (in press). The data from Sofradzija & Berberovic (1978), Boron (1992) and Vasil'eva & Vasil'ev (1998) could have been used only partly, since the precise genomic composition of the hybrid biotypes was not stated. In addition to the listed references, unpublished results were included for the following localities: 1) spring pool at Oradea, Rumania, Danube basin, six specimens; 2) River Nera, Rumania, Danube basin, one specimen; 3) creek Polska Woda, Poland, Odra basin, nine specimens; 4) creek Szödrákos, Hungary, Danube basin, 36 specimens; 5) River Krka, Slovenia, Danube basin, eight specimens; 6) Lake Sinoe, Rumania, Black Sea basin, four specimens; 7) channel at Caraorman, Rumania, Danube basin, 12 specimens; 8) unnamed creek at Comana, Rumania, Danube basin, eight specimens; 9) Durance, France, Rhone basin, four specimens. Chromosome preparation followed Ráb & Roth (1989). Morphological and/or biochemical identification of spined loaches were taken from Doadrio (1981), Perdices et al. (1995), Economidis & Nalbant (1996), Doadrio & Perdices (1997), Vassilev (1998), Erkakan et al. (1999), Ivanova & Dobrovolov (1999), Mrakovčić et al. (2000), Povc & Šumer (2000) and Schneider et al. (2000).

Results

Species richness

In total, 23 species of *Cobitis* were recognized from the freshwaters of Europe (Table 1). Considering the remaining area of the Euro-Mediterranean subregion, four additional species (*C. satunini* Gladkov and three unnamed species) were identified by karyotype from the Caucasus (Vasil'eva & Vasil'ev, 1994; Vasil'ev, 1995), one species (*C. maroccana* Pellegrin) was recorded on the base of biochemical data from North-western Africa (Perdices et al.,

1995) and ten morphologically or genetically identified species (*C. bilseli* Battalgil, *C. elazigensis* Coad & Sarieyyüoglu, *C. fahirae* Erkakan et al., *C. levantina* Krupp & Moubayed, *C. kellei* Erkakan et al., *C. kurui* Erkakan et al., *C. puncticulata* Erkakan et al., *C. simplicispina* Hanko, *C. splendens* Erkakan et al., *C. turcica* Hanko) were listed by Erkakan et al. (1999) and Ludwig et al. (2001) for the Asian part of Turkey. No mixed populations were recorded from any of these regions. Within the outline of Europe, the species were not equally distributed, but showed a higher density in the south of Europe than in the north (Fig. 1). In the Mediterranean with the Iberian Peninsula (here referred to as ‘Southern Europe’) 17 species occur, while Central Europe, the Danube catchment area and the European part of the former USSR (here referred to as ‘Central and Eastern Europe’) contain seven species. The two regions share only one single species (*C. strumicae*).

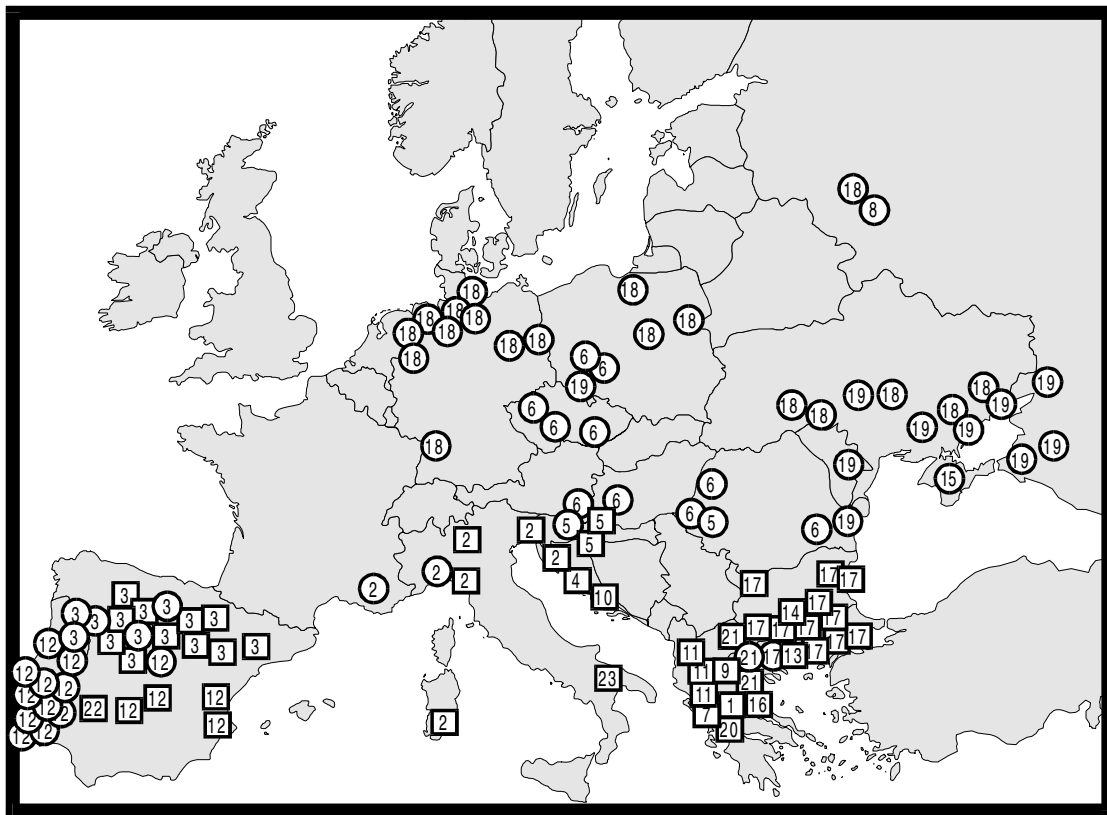


FIG. 1: Distribution of species of *Cobitis* in Europe. Records by karyotype analyses are indicated by circles, morphological and/or biochemical identifications are indicated by squares. Numbers code species: 1 - *C. arathosensis*, 2 - *C. bilineata*, 3 - *C. calderoni*, 4 - *C. dalmatina*, 5 - *C. elongata*, 6 - *C. elongatoides*, 7 - *C. hellenica*, 8 - *C. melanoleuca*, 9 - *C. meridionalis*, 10 - *C. narentana*, 11 - *C. ohridana*, 12 - *C. paludica*, 13 - *C. punctilineata*, 14 - *C. rhodopensis*, 15 - *C. spec. 'Crimea'*, 16 - *C. stephanidisi*, 17 - *C. strumicae*, 18 - *C. taenia*, 19 - *C. tanaitica*, 20 - *C. trichonica*, 21 - *C. vardarensis*, 22 - *C. vettonica*, 23 - *C. zanandrei*.

Table I: Checklist of species and hybrid biotypes of *Cobitis* of Europe. The recognition of species bases on the original description of each species, on Kottelat (1997) as well as on the references given below. Synonyms and remarks are given only if not listed by Kottelat (1997)

Species or hybrid biotype	Reference	Comments
<i>C. arathosensis</i> Economidis & Nalbant, 1996	Economidis & Nalbant (1996)	
<i>C. bilineata</i> Canestrini, 1866	Vasil'ev & Vasil'eva (1994)	
<i>C. calderoni</i> Bacescu, 1962	Perdices & Doadrio (2000)	
<i>C. dalmatina</i> Karaman, 1928	Schneider et al. (2000)	<i>C. dalmatina</i> was used by Schneider et al. (2000)
<i>C. elongata</i> Heckel & Kner, 1858	Banarescu & Nalbant (1998)	
<i>C. elongatoides</i> Bacescu & Maier, 1969	Ráb et al. (2000), Freyhof et al. (2000)	Samples of <i>C. megaspila</i> Bacescu from both localities mentioned in the first description included <i>C. elongatoides</i> and its hybrids with <i>C. tanaitica</i> (Ene, pers. comm.). <i>C. megaspila</i> therefore is tentatively taken as synonym of <i>C. elongatoides</i> .
<i>C. hellenica</i> Economidis & Nalbant, 1996	Economidis & Nalbant (1996)	
<i>C. melanoleuca</i> Nichols, 1925	Vasil'ev et al. (1989)	According to Nalbant (1993), <i>C. granoei</i> Rendahl and <i>C. sibirica</i> Gladkov are synonyms of <i>C. melanoleuca</i> .
<i>C. meridionalis</i> Karaman, 1924	Economidis & Nalbant (1996)	
<i>C. narentana</i> Karaman, 1928	Schneider et al. (2000)	<i>C. narentana</i> was used by Schneider et al. (2000)
<i>C. ohridana</i> Karaman, 1928	Economidis & Nalbant (1996)	
<i>C. paludica</i> (de Buen, 1930)	Doadrio & Perdices (1997)	
<i>C. punctilineata</i> Economidis & Nalbant, 1996	Economidis & Nalbant (1996)	
<i>C. rhodopensis</i> Vassilev, 1998	Vassilev (1998)	
<i>C. spec.</i> 'Crimea'	Vasil'ev (1995), Vasil'eva (1984)	The karyotype of specimens from Cernaja rečka on Crimea peninsula was published by Vasil'ev (1995), the morphology by Vasil'eva (1984)
<i>C. stephanidisi</i> Economidis, 1992	Economidis & Nalbant (1996)	
<i>C. strumicae</i> Karaman, 1955	Economidis & Nalbant (1996)	

<i>C. taenia</i> Linnaeus, 1758	Vasil'ev et al. (1989), Freyhof et al. (2000)	
<i>C. tanaitica</i> Bacescu & Maier, 1969	Vasil'eva & Vasil'ev (1998), Freyhof et al. (2000)	Vasil'eva & Vasil'ev (1998) gave a redescription of <i>C. tanaitica</i> , with an unnecessary replacement as <i>C. rossomeridionalis</i>
<i>C. trichonica</i> Stephanidis, 1974	Economidis & Nalbant (1996)	
<i>C. vardarensis</i> Karaman, 1928	Economidis & Nalbant (1996)	
<i>C. vettonica</i> Doadrio & Perdices, 1997	Doadrio & Perdices (1997)	
<i>C. zanandreae</i> Cavicchiola, 1965	Economidis & Nalbant (1996)	
<i>C. 1 elongatoides</i> - 1 <i>taenia</i>	Bohlen et al. (in press)	
<i>C. 2 elongatoides</i> - 1 <i>taenia</i>	Ráb et al. (2000)	
<i>C. 1 elongatoides</i> - 2 <i>taenia</i>	Bohlen et al. (in press)	
<i>C. 1 elongatoides</i> - 2 <i>tanaitica</i>	Ene, unpubl. results	
<i>C. 2 elongatoides</i> - 1 <i>tanaitica</i>	Ráb et al. (2000)	
<i>C. 1 taenia</i> - 2 spec. 'Crimea'	Vasil'ev et al. (1989)	
<i>C. 1 elongatoides</i> - 3 <i>taenia</i>	Bohlen et al. (in press)	
<i>C. 3 elongatoides</i> - 1 <i>taenia</i>	Ráb et al. (2000)	
<i>C. 3 elongatoides</i> - 1 <i>tanaitica</i>	Bohlen et al. (in press)	
<i>C. 2 elongatoides</i> - 2 <i>taenia</i>	Boron & Danilkiewicz (1998)	
<i>C. 2 elongatoides</i> - 2 <i>tanaitica</i>	Ráb, unpubl. results	
<i>C. 2 elongatoides</i> - 1 <i>tanaitica</i> - 1 spec.	Ráb et al. (2000)	
<i>C. 2 taenia</i> - 2 spec. 'Crimea'	Vasil'ev et al. (1989)	
<i>C. 1 taenia</i> - 2 spec. 'Crimea' - 1 <i>melanoleuca</i>	Vasil'ev et al. (1989)	

Table II: Comparison of the composition of mixed populations of *Cobitis* in Europe. Five cases reported in literature had to be excluded from comparison since the type of included hybrids was not identified. Highest overall richness is reached in upper Odra basin in Poland. Question marks indicate cases in which the genomic composition was reconstructed by us.

Species and hybrids	Ploidy	D-Rhine	D-Issel	D-Weser	D-Elbe	D-Elbe	CZ-Elbe	D-Odra	D-Odra	PL-Odra	D-Danube	CZ-Danube	H-Danube	RUM-Danube	RUM-Danube	RUM-Danube	BOS-Danube	RUS-Volga	PL-Vistula	PL-Bug	No. cases
<i>C. taenia</i>	2n	x	x	x	x	x		x										x	x	x	11
<i>C. elongatoides</i>	2n						x			x	x	x			?	?					7
<i>C. tanaitica</i>	2n									x											4
<i>C. melanoleuca</i>	2n																	x			1
<i>C. 1 elongatoides - 1 taenia</i>	2n			x	x			x		x											4
<i>C. 2 elongatoides - 1 taenia</i>	3n	x				x	x		x	x						?			?	?	8
<i>C. 1 elongatoides - 2 taenia</i>	3n	x	x	x	x	x		x													6
<i>C. 1 elongatoides - 2 tanaitica</i>	3n														x						1
<i>C. 2 elongatoides - 1 tanaitica</i>	3n									x	x	x	?				?				5
<i>C. 1 taenia - 2 spec. 'Crimea'</i>	3n																	x			1
<i>C. 1 elongatoides - 3 taenia</i>	4n				x			x													2
<i>C. 3 elongatoides - 1 taenia</i>	4n						x			x											2
<i>C. 3 elongatoides - 1 tanaitica</i>	4n									x	x										2
<i>C. 2 elongatoides - 2 taenia</i>	4n																			?	1
<i>C. 2 elongatoides - 2 tanaitica</i>	4n												x								1
<i>C. 2 elongatoides - 1 tanaitica - 1 spec.</i>	4n										x										1
<i>C. 2 taenia - 2 spec. 'Crimea'</i>	4n																	x			1
<i>C. 1 taenia - 2 spec. 'Crimea' - 1 melanoleuca</i>	4n																	x			1
Number of biotypes per locality		3	2	3	4	3	3	4	1	7	1	3	3	2	2	2	2	5	2	3	

Hybrid richness

The analysed set of data documented the occurrence of 24 cases of mixed populations. From these, 20 cases could be analysed according to the genomic composition of the hybrid biotypes (Table 2). In total, 14 hybrid biotypes, differing in their genomic composition, were included. Triploid hybrids were recorded from 19 mixed populations, tetraploids from 9 populations and diploids from four populations. Mixed populations consisted of one to seven different biotypes (species and hybrid biotypes combined). The pattern of presence/absence of genetic biotypes varied considerably among mixed populations. Mixed populations were found only in Central and Eastern Europe, not in Southern Europe (Fig. 2). Out of 34 reports from Central and Eastern Europe 24 turned out to include mixed populations.

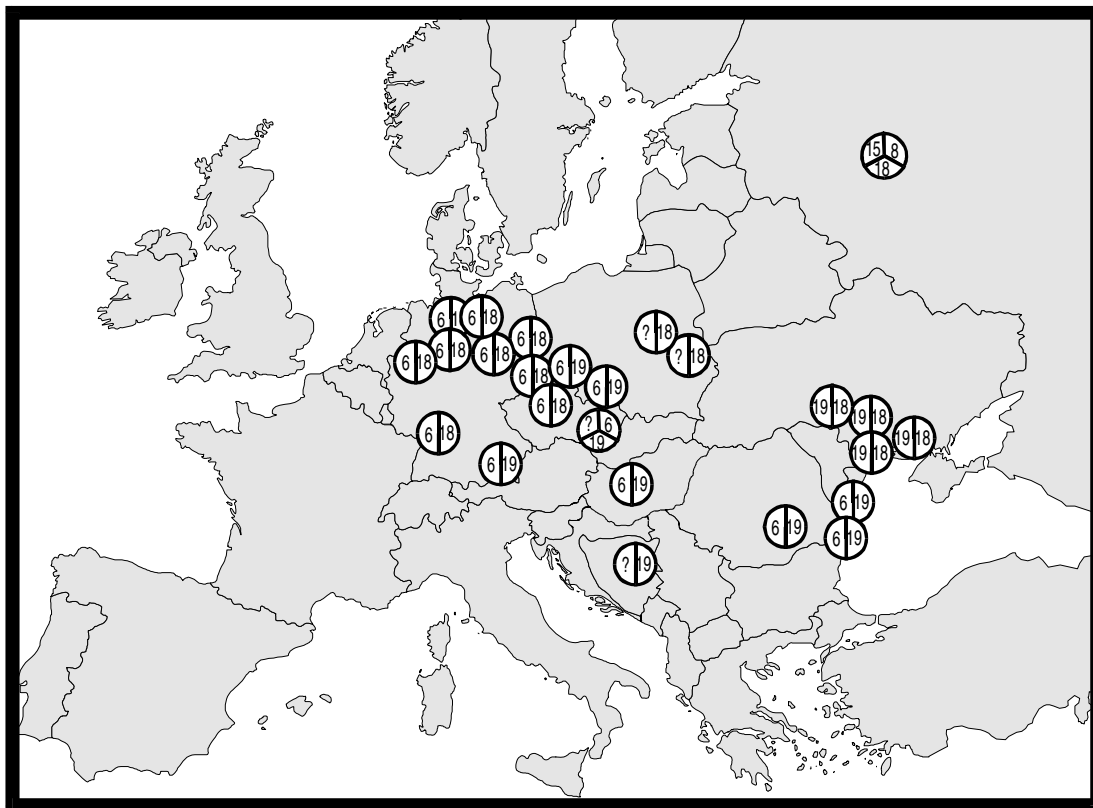


FIG. 2: Distribution of hybrid complexes of *Cobitis* in Europe as based on karyological identifications. The parental species are indicated by numbers as given in Fig. 1.

Discussion

Species richness

Our list of 23 species of the genus *Cobitis* in Europe exceeds the estimation of Kottelat (1997), who recognized 15 species for the area of Europe without the former USSR. We included two species from European part of former USSR and two species described after publishing of Kottelat (1997), considered five species as valid, which were listed as synonyms by Kottelat (1997), but excluded one species because of its probable hybrid character. Such a high fluctuation between two checklists that appeared within a few years reflects the poor

understanding of taxonomy in spined loaches of Europe and confirms the need for more detailed analysis in this field.

The remarkably higher species richness in Southern Europe than in Central and Eastern Europe is resembled by the geographic diversity in other freshwater animals, e.g. the fish genus *Barbus* (Bianco 1998) and the snail genus *Theodoxus* (Banarescu 1990). This reduction in diversity from southern to northern Europe was believed to have been caused by the effects of multiple glaciations during Pleistocene (Hewitt, 1999). The dominating ice cap and permafrost made most of the area north of the major mountain ranges unsuited for freshwater animals. Also in *Cobitis*, the division between Southern and Central and Eastern Europe is best reflected by the position of mountain ranges, following the Pyreneans, Alps, Dinaric Mountains and Balkans. The Danube basin often was assumed to have acted as a refuge area and therefore containing a high number of fish species today (Banarescu, 1990). Regarding the fauna of spined loaches, it shows somehow intermediate features. On the one hand, it contains an endemic species and shares one species with the eastern Balkan area; on the other hand it is dominated by a single species and shares this plus another species with the Central and Northeast Europe.

A phenomena expressed through whole of Europe is the rare syntopic occurrence of species of *Cobitis*. Smaller water systems usually contain only a single species, while in the larger river systems sympatric species are often separated in different river sections. For example, in the river systems of Elbe and Odra, *C. taenia* inhabits the lower stretches, while *C. elongatoides* was found exclusively in the upper part of the basins. If species occur close together, they generally differ remarkably in their ecology and are separated in a very local pattern, like *C. taenia* and *C. melanoleuca* in the Moscow River (Vasil'ev et al., 1989) or *C. elongata* and *C. elongatoides* in the Kolpa River (Povc et al., 1998).

Hybrid richness

Mixed populations were found to occur frequently and to be widespread over most of Central and Eastern Europe. Here, the co-occurrence of a species of *Cobitis* with one or more hybrid biotypes appears to be the rule rather than the exception. In opposite to the clear hybrid zones found in several plants and animals in Europe (Hewitt, 1990), mixed populations of *Cobitis* are not restricted to the contact areas between the parental species. Therefore, the mechanisms separating the species (see above) do not separate the hybrid biotypes from the species. Additionally, no exclusion between the hybrid biotypes was observed, since in many mixed populations (11 out of 20 cases) more than one hybrid biotype occurred together with the host species.

On the other hand, not all types of hybrids occurred in each mixed population, but a highly different composition of genotypes was found among localities. One factor responsible for this population diversity seems to be the kind of associated species. As a rule, the species that served as a host for the gynogenetic hybrid biotypes also dominated in the genomic composition of the associated hybrid biotypes. For example, in mixed populations from German Elbe and Odra Rivers, *C. taenia* served as host species and the associated triploid hybrids contained two genomes of *C. taenia* and one of the absent parent *C. elongatoides*. In samples from Czech stretch of Elbe River and Polish stretch of Odra River, *C. elongatoides* served as host and the associated triploid contained two genomes of *C. elongatoides* and one genome of the absent *C. taenia*. However, even between mixed populations with the same host considerable differences in the presence and absence of hybrid biotypes were observed. The most likely explanation for these effects is the local history of the populations, especially

local extremes in carrying capacity, physico-chemical conditions or biotic interactions. These factors may have eliminated or favour certain hybrid biotypes in the present or past and thus determined the present composition of the populations. Therefore, each of the mixed populations has to be assumed to be to a certain degree independent from other mixed populations.

Most likely, karyological surveys even underestimate the local hybrid richness. By analysing the maternal mtDNA lines, Janko et al. (2001) showed a multiple origin in triploid hybrid biotypes of *Cobitis* with the same genomic composition. Therefore, the actual diversity of hybrid biotypes in Europe may be considerably higher than estimated by methods applied in the present study.

Overall diversity

As stated above, two different kinds of diversity are present in the genus *Cobitis*: species richness and hybrid richness. If considering hybrids and species both as biotypes (Vrijenhoek, 1994), i.e. independent evolutionary units of more or less comparable level, one may compare the overall richness in southern Europe with that in Central and Eastern Europe. In southern Europe live 17 species and no hybrids, while in Central and Eastern Europe 21 'units' were found (seven species and 14 different hybrid biotypes). The overall richness therefore is higher in Central and Eastern Europe than in Southern Europe, contrasting sharply the picture obtained by the more classical view on species richness only. In the light of conservation of spined loaches in Europe, this fact should be considered.

Conclusions for conservation

The observed diversity of spined loaches is not at all reflected in the present legislation or conservation texts in Europe (Table 3). On the European scale, five species are considered in appendix III of Bern Convention (Council of Europe, 1979) and two species in the appendix of the FFH-directive of EU (Council of the European Communities, 1992). Only a few very recent red data lists consider the actual species occurring within the national borders. Most of the 23 species of Europe are not listed in any conservation list, even though several species in Southern Europe are locally restricted and may be in need of protection. In many cases legislation exclusively protects *C. taenia*, which sometimes not even occur in the given country. By this, none of the indigenous species gets the conservation strength it requires. A clear necessity for legislation and red data lists is to adopt conservation texts to the current state of knowledge about taxonomy and diversity.

The wide distribution and considerable impact of hybrid biotypes on the overall richness of spined loaches leads to the question how to treat hybrid biotypes in conservation. First of all, they cannot be treated in the same manner than 'normal' F₁ interspecific hybrids from whom they differ in their independent persistence over evolutionary time scales. Second, their apparent hybrid origin makes their formal taxonomy and therefore their integration into legislation difficult. Third, they certainly affect the population of the host species and sometimes clearly dominate mixed populations (Bohlen & Ritterbusch 2000), but as host specific sperm parasites they are unable to eliminate the host population. At last, hybrid biotypes may open the accompanying species ways of evolution which otherwise would stay locked as vectors for geneflow between species (Schultz, 1977) or as possible base for a reticulate speciation (Vasil'ev et al., 1989). Several, if not all, mixed loach populations have to be considered as locally unique communities. Therefore, mixed population in *Cobitis* may be treated and deserve protection like parts of existing biodiversity (Kraus, 1994).

Table III: Diversity of *Cobitis* as reflected by European conservation. Three European conservation acts as well as the red data lists for 17 countries are given. An asterisk indicate red data list in preparation.

Conservation act	<i>C. bilineata</i>	<i>C. calderoni</i>	<i>C. elongata</i>	<i>C. elongatoides</i>	<i>C. hellenica</i>	<i>C. meridionalis</i>	<i>C. paludica</i>	<i>C. stephanidisi</i>	<i>C. strumicae</i>	<i>C. taenia</i>	<i>C. trichonica</i>	<i>C. vardarensis</i>	Source
Bern Convention			+				+			+	+		Council of Europe (1979)
IUNC Red data list		VU	DD		DD	LR nt	LR nt	DD	DD		DD	DD	IUNC (1990)
EU (FFH) directive										+	+		Council of the EC (1992)
Austria										2			Herzig-Straschil (1994)
Belarus										NT			Saenko (1993)
Belgium										EN			FAO Fish. Rep. (1992)
Czech Republik										EN			Lusk (1996)
France										VU			Keith & Allardi (1996)
Germany										2			Bless et al. (1994)
Great Britain										NT			Maitland & Lyle (1996)
Hungary										DD			Keresztessy (1996)
Poland										NT			Kotusz (1996)
Portugal		DD											SNPRCN (1990)
Rumania			R							NT			Borrescu (1994)
Russia										NT			Borodin et al. (1984)
Slovakia										R			Holcik (1996)
Slovenia*	EN		EN	VU									Povc & Šumer (2000)
Spain		VU					VU						Blanco & González (1992)
Sweden										EN			FAO Fish. Rep. (1992)
Switzerland										EN			Kirchhofer et al. (1990)

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