

Status of populations of the genus *Cobitis* in Slovakia

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Genetic and karyological analyses of specimens of 15 populations of the genus *Cobitis* revealed that the species *Cobitis taenia* did not occur in the hydrographic system of Slovakia, until recently. Five populations in the Danube drainage area were identified as hybrid diploid-polyploid complex *Cobitis elongatoides* × *C. tanaitica*. Five populations in the Slaná river drainage area were found belonging to the diploid pure species *Cobitis elongatoides*. Specimens of four populations of the Bodrog river drainage area were analyzed and assessed as hybrid diploid-polyploid complex *C. elongatoides* × *C. tanaitica*. Similarly, a hybrid complex of analogical karyological characteristics was registered in the Tisza river drainage area. It might seem improbable that the species *C. taenia* would occur in Slovakia (Central Europe) as it was presented until recently or, that it would be represented in the hybrid complexes.

Key words: *Cobitis*, taxonomic status, *Cobitis elongatoides*, ploidy, hybrid complex, Slovakia.

Introduction

There are major changes in the recent knowledge of the species composition of the genus *Cobitis* in Europe. The original view that the populations of *Cobitis* living in Europe belonged to the species *Cobitis taenia* L., 1758 which in the respective areas has been yet divided into subspecies (LELEK, 1987; HOLČÍK, 1995) has changed radically. The

principal change was brought about by knowledge on the occurrence of hybrid diploid – polyploid complexes apart from pure species within the genus *Cobitis* in Europe (VASILEV & VASILEVA, 1982; VASILEV et al., 1989, 1990; BORŇ, 1992 and others). The situation was also complicated by the promotion of the subspecies category to the species level of this genus (KOTTELAT, 1997). This entrenched more than 10 species within the

Table 1. Samples taken from *Cobitis* populations examined in Slovakia.

No. of population Stream	Males/ Females	Flow cytometry	Allozyme analysis	Chromosome analysis	2 n	3 n	4 n
1. Morava, r.km 72.5	3 M/48 F	15	17	14	2 M	1 M/27 F	
2. Hron, r.km 0.5	7 F		7			7 F	
3. Nitra channel	21 F		21	5		21 F	
4. Ipeľ r.km 69.0	2 M/22 F		24	4	1 M	1 M/21 F	
5. Paríž	4 F	4	4	4		4 F	
6. Blh	12 M/8 F	14	16	7	12 M/8 F		
7. Ida	18 M/20 F	23	17	5	8 M/15 F		
8. Belžanský	13 M/16 F	18	17	6	8 M/10 F		
9. Torysa	7 M/6 F	13	13	2	7 M/6 F		
10. Olšava	23 M/31 F	31	17	11	14 M/17 F		
11. Bodrog	9 M/54 F	44	21	14	8 M/5 F	36	1 M/2 F
12. Latorica	3 M/27 F	20	21	4	1 M/2 F	1 M/17 F	
13. Ondava	28 F	26	24	5	1 F	25 F	
14. Uh (ch. Revištia)	8 M/97 F	22	8	5	3 M	1 M/17 F	1 M
15. Tisza	2 M/19 F	5	9	7	2 M	17 F	1 F

range originally attributed to *C. taenia*. The application of genetic methods in the research of populations of the genus *Cobitis* within the last decade brought a lot of knowledge on structure of this genus in Europe. It was found that apart from pure species there were populations, so-called hybrid diploid-polyploid complexes consisting of species with a genome composed of sets of at least two species, as well as individuals of the donor species. The following four species share the creation of these complexes: *Cobitis taenia* L., 1758, *Cobitis elongatoides* Bacescu et Maier, 1969, *Cobitis tanaitica* Bacescu et Maier 1969 and *Cobitis melanoleuca* Nichols, 1925 (see reviews in FREYHOF et al., 2000; BOHLEN & RÁB, 2001). At present, 23 species of *Cobitis* and about 16 various hybrid complexes have been identified in Europe (BOHLEN & RÁB, 2001). Populations of *Cobitis* in the hydrological system of Slovakia (Central Europe) were classified as *C. taenia* (HOLČÍK, 1995, 1996; HENSEL & MUŽÍK, 2001) until recently. This study brings new knowledge on the status of populations of the genus *Cobitis* from Danube river and Tisza river drainage areas in Slovakia.

Material and methods

Specimens of 15 populations of the genus *Cobitis* from hydrological system of Slovakia (see Fig. 1 and Tab. 1) were sampled in 1999–2003. The sex of the captured specimens was identified according to lamina circularis, an oval bony formation on the IInd. ray of pectoral fin. As evident in Table 1, only some of the specimens were sampled for laboratory analyses, the rest were

released after identification of sex. Karyological analyses, allozyme analyses and flow cytometry were performed for taxonomic identification and ploidy level assessment. These methods were not applied parallelly to all specimens studied.

Chromosome preparations were made from kidney using standard methods according to BORON & KOTUSZ (2000). Air-dried slides were stained with 4% Giemsa solution. The chromosome classification of LEVAN et al. (1964) was adopted. Allozyme analysis was based upon protein electrophoresis (ŠLECHTOVÁ et al., 2000). Diagnostic alleles with the following patterns were used for identification of genomes of *C. elongatoides* and *C. tanaitica*: *Sod** 60, 100, *Gpi-A** 88, 100, 110, *sAat 1** 74, 85, 100. Tetraploid status was assessed by means of a diagnostic allele *Gpi-A** 88, 100, 100, 113. Ploidy analysis was performed also on Partec CCA 1 flow cytometer (Partec GmbH, Germany) according to VINDELOV & CHRISTENSEN (1994) by determination of the relative DNA content in muscle tissue cells fixed in ethanol, using High Resolution DNA kit T (Partec GmbH, Germany) with 4', 6-diamidino-2-phenylindol (DAPI; excitation/emmission maxima 358/461 nm) for nuclear DNA staining (OTTO, 1994). Diploid form confirmed by karyotype analysis was used as reference standard.

Results

Danube drainage area (Tab. 1, populations No. 1–5)

The occurrence of diagnostic alleles either for *C. elongatoides*, or for *C. tanaitica* confirmed that females in samples Nos. 2 (Hron), 3 (Nitra) and 5 (Paríž) were exclusively triploid 2 *C. elongatoides* × 1 *C. tanaitica*. This was confirmed by karyolog-

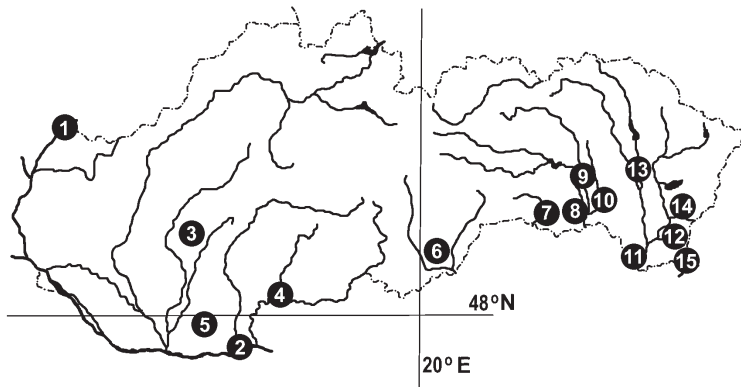


Fig. 1. Localization of the studied populations of the genus *Cobitis* on the territory of Slovakia: 1 – Morava, r.km 72.5; 2 – Hron, r.km 0.5; 3 – Nitra (Dlhý canal); 4. – Ipeľ, r.km 69.0; 5. – Paríž (Nitra); 6 – Blh; 7 – Ida; 8 – Belžanský; 9 – Torrysa; 10 – Olšava; 11 – Bodrog; 12 – Latorica; 13 – Ondava; 14 – Uh (canal); 15 – Tisza.

ical analysis of part of the specimens, as well as by flow cytometric results (Tab. 1). Diploid males with *C. elongatoides* genome were found in samples No. 1 from Morava river and No. 4 from Ipeľ river but females were found expressively dominating in both samples (No. 1–94.12% and No. 4–91.67%).

Slaná drainage area (Tab. 1, populations No. 6–10)

Allozyme analysis revealed diploid specimens belonging to *C. elongatoides* in all cases. This conclusion was confirmed also by karyological analysis in part of specimens, which showed classic karyotype structure: 30 m + 16 sm + 2 st + 2 a. Similarly, flow cytometry confirmed the diploid status (Fig. 2 a). There were high frequencies of males in the respective samples: No. 6 – Blh (60%), No. 7 – Ida (47.4%), No. 8 – Belžanský (44.8%), No. 9 – Torrysa (53.8%) and No. 10 – Olšava (42.6%).

Bodrog drainage area (Tab. 1, populations No. 11–14)

Allozyme analysis of specimens of all 4 populations showed that these populations studied represented the so-called hybrid complex. Apart from triploids, diploid specimens showing the characteristics of *C. elongatoides* were found in all samples of the populations studied. One male and two females of the Bodrog population were identified as tetraploid 2 *C. elongatoides* × 2 *C. tanaitica*. Tetraploid status was also confirmed by flow cytometry (Fig. 2c). Low frequency of males in samples was found characteristic for the hybrid complexes: No. 11 – Bodrog (16.28%), No. 12 – Latorica (10%), No. 13 – Ondava (0%) and No. 14 – Uh (7.62%).

Tisza drainage area

One sample (Tab. 1, population No. 15) studied

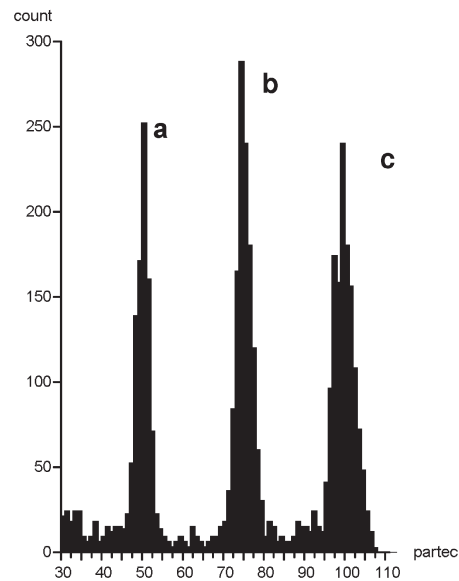


Fig. 2. Histogramme of distribution of relative DNA content in cells of muscle tissue of: a – diploid *C. elongatoides* from Ida river; b – triploid 2 *C. elongatoides* × 1 *C. tanaitica* from Ondava river; c – tetraploid 2 *C. elongatoides* × 2 *C. tanaitica* from Bodrog river.

was gained from an old watercourse of the Tisza river near by Velké Trakany village. The population was found to be a hybrid complex *C. elongatoides* × *C. tanaitica*. Two diploid males had the characteristic chromosome set of *C. elongatoides*. Triploid females had the genome of 2 *C. elongatoides* × 1 *C. tanaitica*. The flow cytometric assessment of relative DNA content in muscle tissue cells found one female (SL 78 mm) to be a tri-/tetraploid mosaic individual. The frequency of males in the sample was 9.52%.

Discussion

All data on the occurrence of populations of the genus *Cobitis* in the Danube and Tisza river drainage areas in Slovakia were hitherto diagnosed as *Cobitis taenia* (KUX & WEISZ, 1964; KIRKA et al., 1981; HOLČÍK, 1995). Similarly, the last version of the Red list of lampreys and fishes of Slovakia reports solely on *Cobitis taenia* (HOLČÍK, 1996; HENSEL & MUŽÍK, 2001). Our results demonstrate that this species does not occur in Slovakia. *Cobitis taenia* occurs either as a pure species or in hybrid complexes in northern parts of Europe, in the Baltic Sea and North Sea basins (ŠLECHTOVÁ et al., 2000; BOHLEN et al., 2002; BOROŇ & KOTUSZ, 2000), in England (our unpublished data) and in Ukraine and Russia (VASILEVA & VASILEV, 1998). In the territory of the Czech Republic, the haploid set of genome of *C. taenia* occurs in the hybrid type in the Elbe river drainage area (RÁB et al., 2000). A possibility of the occurrence of the *C. taenia* genome could be in streams belonging to the Baltic Sea basin (Poprad) but no occurrence of *Cobitis* was recorded there in the territory of Slovakia (WEISZ & KUX, 1959; KUX & WEISZ, 1960; KIRKA et al. 1978). For the time being, there is no information from the Danube drainage area about any recordings of *C. taenia* either as a pure species, or as a part of a hybrid type (RÁB et al., 2000; BOHLEN & RÁB, 2001). From the territory of Hungary where the studied hydrological system of Slovakia passes, there is only one genetically verified record on the occurrence of the hybrid complex *C. elongatoides* × *C. tanaitica* from Szodrakós stream, a tributary of the Danube (BOHLEN & RÁB, 2001; ERÖS, 2003). From the Dyje river (a tributary of Morava river on the territory of the Czech Republic), the occurrence of diploid *C. elongatoides* was identified from the spring zone, as well as the occurrence of the hybrid complex *C. elongatoides* × *C. tanaitica* from the middle- and lower reach (RÁB et al., 2000; ŠLECHTOVÁ et al., 2000). Site No. 2 Morava stream, where the same result was gained, is related to the lower reach of Dyje. Nevertheless, it is true that with regards to the range of Danube drainage area, knowledge on the status of populations determined by means of genetic methods is currently insufficient.

The hybrid complexes have been found to contain in addition to the predominant triploid individuals, also diploid ones that, with regard to the structure of their karyotype, are either the pure donor species or less frequent hybrids. Diploid hybrids *C. taenia* × *C. elongatoides* have been re-

ported from Germany (BOHLEN et al., 2002), the same as from the drainage area of the Odra river in Poland (BOROŇ & KOTUSZ, 2000). The occurrence of tetraploid individuals in the hybrid complexes is the least frequent. From the upper part of the Danube drainage area there have been reports of tetraploid individuals 2 *C. elongatoides* × 2 *C. tanaitica* (BOHLEN et al. 2002). We found the same genome combination in the hybrid complexes in the drainage areas of the rivers Bodrog and Tisza. Likewise, tetraploid combinations of the genomes of two species have been reported from Poland, Russia and Ukraine (VASILEVA, 2000; BOROŇ, 2001). Evaluating the representation of ploidy in the hybrid complexes, our own results show differences between the sexes. In most cases, the males are diploid or, in single cases only, triploid or even tetraploid. The females are predominantly triploid and just occasionally diploid or tetraploid. In a hybrid complex produced by two species, one could find theoretically eight genome variations, consisting of genome parts of the two initial species, the occurrence of which could be 2n, 3n, and 4n. Some observations indicate that a hybrid complex may even contain sets of genomes of three species (VASILEV et al., 1989; RÁB et al., 2000), which leads to the possible occurrence of additional variations in the genome structure. The above tends to show that the hybrid complexes within the genus *Cobitis* present very complex biological units.

A correct identification of the taxonomic status of populations of the genus *Cobitis* is a necessary prerequisite for effective application of conservation arrangements. Presently valid legislative conservation of *Cobitis* either on a European level (Council Directive No. 92/43/EEC), or on a national level (Czech Republic, Slovak Republic and other states) arose at a time when most populations were considered a part of species *Cobitis taenia* (LELEK, 1987).

In order to provide effective national legislative conservation, it is necessary to know about particular species and the hybrid status of *Cobitis* populations (LUSK et al., 2002). As the determination by means of karyological and other genetic methods is time-demanding and professionally exigent, it would be useful to employ some basic knowledge for preliminary identification whether a population of diploid individuals or a hybrid complex is concerned. To gain the necessary knowledge of the status of *Cobitis* populations, we recommend the following approach:

1. To perform sex determination of the highest possible number of live specimens in the field.

A high proportion of males (above 40%) provides a signal that it might concern a population of diploid bisexual fish of a pure species. In this case it is enough to sample several (up to 10) specimens for the determination of species specificity. If females dominate (80% and more), it is necessary to sample more specimens for the determination of ploidy level and identification of the hybrid complex.

2. Flow cytometry reveals the ploidy level of the sampled fish. The number of samples for the karyotype analysis can be derived from these data.

3. Karyotype analysis or, preferably, protein electrophoresis allows identification of species specificity and of hybrid composition of the population sample studied.

It seems necessary to obtain more data on the status of *Cobitis* populations in the Danube drainage area, with regards to its size (817,000 km²). These data should answer the question if, apart from pure diploid populations of *C. elongatoides*, any diploid pure populations of *C. tanaitica* occur there. Currently, there is just one record of the occurrence of diploid individuals of *C. tanaitica* from the lower part of the drainage area in Romania (BOHLEN & RÁB, 2001). Our current knowledge (ŠLECHTOVÁ et al., 2000; LUSK et al., 2000, these results) indicates that populations of diploid pure species *C. elongatoides* were maintained only in the terminal parts of the drainage areas of individual tributaries of main rivers. Central and lower parts are inhabited by the hybrid complex. The hybrid complex *C. elongatoides* × *C. tanaitica* arose in the zone of contact between these two species. If the populations of pure *C. tanaitica* did not occur in other parts of the Danube drainage area, it might be presumed that the hybrid complex *C. elongatoides* × *C. tanaitica* inhabited the Danube drainage area later. It would overlap and fuse with the original populations of *C. elongatoides*. *C. elongatoides* served as host, if polyploid individuals had at least 2 sets of the genome of this species.

The present legal tools protecting biodiversity are based on the concept of a species as the basic unit in the sense of the Linnaean classification of organisms. Data obtained by the application of genetic methods in recent years have posed the problem of sibling species, hybrid complexes, and even populations themselves as the objects of protection (TAYLOR, 1999; VASILEVA, 2000; BOHLEN & RÁB, 2001). In order to secure effective legal protection of fish biodiversity, it is necessary to enlarge the specification of the object of this protection, besides the classical concept of species, by additional natural developmental units, as indicated

above. In our case it would appear necessary, for the populations of *Cobitis* spp. living in the waters of Slovakia, to specify the object of protection as “*Cobitis elongatoides* and hybrid complexes”.

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