Ital. J. Zool., *65, Suppl.*: 219-221 (1998)

Differences in clutch size, egg size and larval pigmentation between *Cobitis taenia* and *C. bilineata* (Cobitidae)

JÖRG BOHLEN
Institute of Freshwater Ecology and Inland Fisheries,
Dept. IV: Biology and Ecology of Fish,
Müggelseedamm 310, D-12587 Berlin (Germany)

ABSTRACT

A comparison of spawning features, eggs and larvae between spined loach from northern Italy and northern Germany revealed differences in egg size, clutch size and number of portions per female. Larvae differed in the presence of a ventral pigment row in *C. taenia* which was absent in *C. bilineata*. Two pigmentation intensity morphs occurred in *C. bilineata* larvae while in *C. taenia* larvae only one morph was observed. These differences (and the observations of others) suggest that the two taxa are different species.

KEY WORDS: *Cobitis* - Larval fish systematics - Reproductive biology.

(Received 28 August 1997 - Accepted 15 January 1998)

INTRODUCTION

The North-Italian spined loach was originally described as *Cobitis taenia bilineata* by Canestrini in 1866. Later on several forms have been described. However, more recently, all of them and particularly the forms '*puta*' and '*bilineata*' were shown to be expressions of genetic polymorphism and hormonal activity (Lodi, 1968; Lodi & Malacarne, 1991). Consequently, it is widely accepted that there is only one species of *Cobitis* in northern Italy (Bacescu, 1961; Lodi, 1976; Lodi & Malacarne, 1991; Bianco, 1994) and this is referred to as *C. taenia bilineata*, Canestrini, 1866.

Kottelat (1997) considered *C. bilineata* Canestrini, 1866 as a species separate from *C. taenia* (L.). He based his assumption on differences in the number of diploid chromosomes between the species. Cataudella *et al.* (1977) noted 50 diploid chromosomes in spined loach from Italy while *C. taenia* has 48 chromosomes (Vasilev *et al.*, 1989; Boron, 1992). Another difference between the North-Italian and the Middle-European populations was the presence of two black spots on the base of the caudal fin in *C. bilineata* versus only one spot in *C. taenia* (Bacescu, 1961). The number of caudal spots has often been used in the taxonomy of spined loaches (Vasileva, 1984; Saitoh & Aizawa, 1987; Economidis & Nalbant, 1996). Furthermore, *C. bilineata* males display a cyclic change in body pigmentation. During breeding season they exhibit two black longitudinal stripes on their body sides while the stripes are reduced to rows of spots during the non-breeding season (Lodi & Malacarne, 1991). By contrast, *C. taenia* displays rows of spots along the body regardless of breeding condition.

The objective of the present study was to compare the reproductive biology and some characters of eggs and larvae of both populations to determine the utility of these characters in distinguishing between *C. taenia* and *C. bilineata*.

MATERIALS AND METHODS

Spined loach from Lake Garda, northern Italy, and from River Haaren, northern Germany, were bred in aquaria under similar conditions. All females measured 100-110 mm in total length (TL) and had bred successfully in the past year. For each species, three replicates of one female and one or two males were placed together in an aquarium approximately half a year before breeding seasons. Temperature, food and other conditions were similar in all aquaria. When spawning occurred eggs fell through a metal grate covering a plastic box. Thus eggs could be readily obtained and quantified. At blastula stage, eggs were removed and counted, while yolk and chorion diameter were measured. Eggs were incubated at 20-24° C in transparent plastic boxes on a dark background with indirect illumination (room with large windows). Total length of embryos was measured immediately after hatching. In some clutches preservation of larvae occurred every two days after hatching. Tricaine was used for killing and 4% fomaldehyde solution for fixing and storing larvae. Besides killing the fish Tricaine ensured that the maximum intensity of pigmentation was expressed in the preserved specimens.

RESULTS AND DISCUSSION

During spawning season, *C. bilineata* males expressed the *'bilineata'* pattern of coloration as described by Lodi & Malacarne (1991), and the *'puta'* pattern during the non-breeding season. Male *C. taenia* did not alter their colour pattern with regard to reproductive season

The number of eggs per spawning act was from 89 to 431 in *C. taenia* and from 212 to 1650 in *C. bilineata* (Table I). Since conditions in all tanks were identical, these data may be indicative of different reproductive strategies between the two taxa. While the North-Italian loach releases relatively larger numbers of eggs on a few dates per season, the North-German loach spawns more frequently but releases fewer eggs per date. *C. taenia* had a longer reproductive season (15 to 17 weeks, mean 16 weeks) than *C. bilineata* (six weeks in two females, one female with isolated spawning acts) and produced higher numbers of eggs per female (*C. taenia* 2905 to 4285, mean 3615; C. *bilineata* 785 to 3059, mean 2048 (Table I).

Yolk and chorion diameters were significantly smaller in *C. taenia* than in *C. bilineata* (Students-t, both $P < 0.001$). No differences in size of hatchlings were observed (Students-t, $P = 0.130$).

Differences between the taxa were also recorded with regard to larval pigmentation. Body pigmentation developed about two days after hatching. From this stage until end of larval period, a light and a dark type of larvae could easily be distinguished in *C. bilineata.* While the first type shows nearly no pigmentation, the second type was intensely pigmented. Pigmentation did not change during ontogeny in any individual. Both pigmentation types were observed within all clutches examined. Lateral pigment intensity in larval *C. taenia* was similar in all individuals. A character exclusively found in larval *C. taenia* was a ventral row of pigment cells (Fig. 1). This pattern was expressed in larvae 3 to 4 days old until the end of the larval period. All investigated *C. taenia* larvae ($n = 63$) displayed this marked character while none of *C. bilineata* larvae ($n = 69$) had any pigmentation on the ventral region.

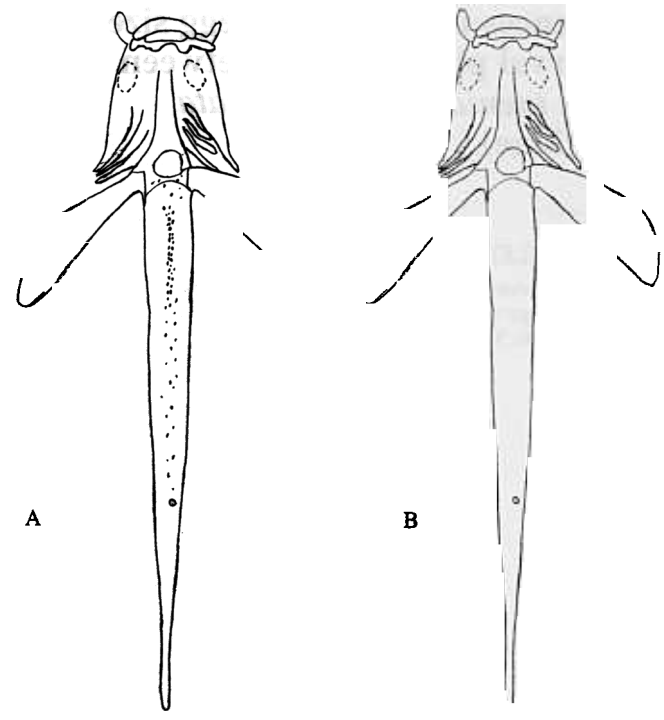

Fig. 1 - Ventral view of two *Cobitis* larvae (both 8 mm TL). A, *C. teania*, presence of rows of pigments; B, *C. bilineata*, absence of pigments.

Pigmentation patterns are often used to identify larval fish (Koblickaya, 1981). The differences in reproductive biology and larval characteristics described in this study clearly permit identification of the two spiny loach populations. These character differences may indicate different evolutionary trends within the two populations. Separately, these characters are insufficient to draw taxonomic conclusions, however, when aligned with the differences in coloration and the number of chromosomes (see above), they facilitate taxonomic separation. The authors agree with Kottelat (1997), and confirm the rehabilitation of *C. bilineata* as distinct species from *C. taenia.*

TABLE I - *Number of eggs per spawning act, mean diameter of yolk and chorion and length of hatchlings (in mm) in* Cobitis taenia *and* C. bilineata.

Taxon	Number of eggs per spawning act		Spawning acts per female	Mean number of eggs per female	Diameter of yolk	Diameter of chorion	Length of hatchlings
	Mean	Range					
C. taenia	245	89 - 431 ($n = 46$)	14-18	3615	1.14 ± 0.07 ($n = 380$)	2.54 ± 0.22 ($n = 380$)	.03 ± 0.34 ($n = 333$)
C. bilineata	547	212 - 1,650 ($n = 15$)	1-7	2 048	1.21 ± 0.09 ($n = 160$)	2.67 ± 0.3 ($n = 160$)	4.98 ± 0.23 ($n = 74$)

REFERENCES

Bacescu M. C., 1961 - Contributo allo studio dei *Cobitis* dell'Italia settentrionale. Arch. Ocean. Limnol., *7*: 185-189.

Bianco P. G., 1994 - L'Ittiofauna continentale dell'Appenino umbro-marchigano, barriera semipermeabile allo scambio di componenti dell'Italia centrale. Biogeographia, *17*: 427-485.

Boron A., 1992 - Karyotype study of diploid and triploid *Cobitis taenia* (Pisces, Cobitididae) from Vistula river basin. Cytobios, *72*: 201-206.

Cataudella S., Sola L., Accame Muratori R., Capanna E., 1977 - The chromosomes of 11 species of Cyprinidae and one Cobitidae from Italy, with some remarks on the problem of polyploidy in the Cypriniformes. Genetica, *47*: 161-171.

Economidis P. S., Nalbant T. T., 1996 - A study of the loaches of the genera *Cobitis* and *Sabanejewia* (Pisces, Cobitidae) of Greece, with a description of six new taxa. Trav. Mus. natl. Hist. nat. Grigore Antipa, *36*: 295-347.

Koblickaya A. F., 1981 - Identification keys for young of freshwater fishes. Consumer and Food Industry Press, Moscow, 206 pp. (in Russian).

Kottelat M., 1997 - European freshwater fishes. Biologia, *52*, suppl., 5: 1-271.

Lodi E., 1968 - Polimorfismo e livree sessuali di *Cobitis taenia* L. (Osteichthyes, Cobitidae). Boll. Zool., *36*: 157-164.

Lodi E., 1976 - Effect of methyltestosterone on the formation of the Canestrini's organ in female *Cobitis taenia bilineata Canestrini* (Cobitidae, Osteichthyes). Monit. zool. Ital. (N.S.), *10*: 135-140.

Lodi E., Malacarne G., 1991 - Differences in sexual behaviour between two phenotypic stages of spined loach. J. Fish Biol., *38*: 321-323.

Saitoh K., Aizawa H., 1987 - Local differentiation within the striated spined loach (the striata type of *Cobitis taenia* complex). Jpn. J. Ichthyol., *34*: 334-345.

Vasileva E. D., 1984 - Comparative morphological analysis of two spiny loach populations (genus *Cobitis*, Cobitidae) differing from each other in the number of patches at the base of caudal fin. J. Ichthyol., *24*: 44-54.

Vasilev V. P., Vasileva E. D., Osinov A. G., 1989 - Evolution of a diploid-triploid-tetraploid complex in fishes of the genus *Cobitis* (Pisces, Cobitidae). *In*: R. M. Dawley & J. P. Bogart (eds), Evolution and ecology of unisexual vertebrates. New York State Museum Bull., *466*, Albany, pp. 153-169.