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Morphology of the karyologically identified spined loach *Cobitis taenia* (Teleostei, Cobitidae) from a diploid population

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A b s t r a c t . The variability of morphological characters was studied in a population of spined loach from Klawój Lake karyologically identified as a pure diploid *C. taenia* (2n = 48). 24 metric features were used to calculate correlation coefficients and linear regressions to determine the relationships between metric features with total length. As regards 11 meristic features, females had more lateral and predorsal spots than males. Statistically significant differences between males and females were also found in mean values of 15 metric indices. Age variability in the shape of the *lamina circularis* of males was revealed.

Key words: diploids, *Cobitis taenia,* morphology, osteology, taxonomy

Introduction

Chromosomal studies of several *Cobitis* populations in Europe (e.g., V a s i l ' e v et al. 1989, B o r o ń 1999, R á b et al. 2000) have identified distinct bisexual *Cobitis* fish species and polyploid hybrid forms. The *Cobitis* species and their different hybrids are very similar morphologically. Since V a s i l ' e v et al. (1989), apart from morphological features, the number of chromosome 2n = 48 are used to identify the spined loach *C. taenia*. Among 18 karyologically identified *Cobitis* populations distributed in Poland, only three contained exclusively *C. taenia* individuals (B o r o ń et al. 2006).

The occurrence of *Cobitis* species in mixed populations has raised questions regarding the results of papers with only morphological identification of the studied material. Most data on morphology and biology of samples of *C. taenia* not genetically identified seems to be related to different *Cobitis* species and/or individuals of different ploidy levels (V a s i l ' e v a 1984, N a l b a n t 1993, B o r o ń 1994, E c o n o m i d i s & N a l b a n t 1996). Only a few reports on morphological features of *C. taenia* were based on specimens identified karyologically, at least in part (V a s i l ' e v et al. 1989, K r z y k a w s k i et al. 2000, K o t u s z 2000, 2001).

The aim of this paper was to describe the diversity of morphological features of the karyologically identified spined loach *C. taenia* from an exclusively diploid population.

Material and Methods

A total of 208 *C. taenia* individuals: 133 females, 53 males and 22 juveniles were collected between May to September 2001 and April to September 2002 from a diploid population in Klawój Lake (Sajna, Guber and Łyna River drainages; Baltic Sea basin), located in northern Poland (52°50'N; 20°55'E). The area of the lake is 29.6 ha, the maximum and mean depth is 17.3 m and 7.1 m respectively.

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All the specimens were identified as possessing 2n = 48 chromosomes (V a s i l'e v et al. 1989, B o r o ń 1999). Sex was determined by gonad examination (J u c h n o & B o r o ń 2006) and the presence of the *lamina circularis* in males. Some quantitative features, such as pigmentation pattern, followed Gambetta's, and the shape of suborbital spine, *lamina circularis* and black spot at the base of the caudal fin were observed (N a l b a n t 1993).

The following were counted to characterized the meristic features: number of rays in the fins, number of gill rakers (*sp.br.*) and pharyngeal teeth (*PhF*), vertebrae number (*Vert.*), number of large spots on body sides (*n. macul. lateral.)* and spots on the dorsal part; in front of the dorsal fin (*n. macul. praeD.*) and behind this fin (*n. macul. postD.*).

The following 24 features selected according to V a s i l'e v a (1984) and B o r o ń (1994) were measured (with 0.1 mm accuracy): standard (*Sl*), total (*Tl*) and head (*lc*) lengths; preorbital (*prO*), interorbital (*iO*) and postorbital (*poO*) distances; eye diameter (*O*); head depth (*hc*); length of barbel (*lb*); predorsal (*pD*) and postdorsal (*poD*) distances; maximum (*H*) and minimum (*h*) body depths; preanal (*pA*), *P-V*, and *V-A* distances; length of: caudal peduncle (*lpc*), caudal (*lC*), dorsal (*lD*), anal (*lA*), pectoral (*lP*) and ventral (*lV*) fins; height of dorsal (*hD*) and anal (*hA*) fins.

Some selected bony elements (V a s i l'e v et al. 1989) of 25 females and 6 males were measured after clearing and staining (D i n g e r k u s & U h l e r 1977) using a stereoscope microscope NIKON (800) and Multiscan software. Eighteen features were measured: neurocranium; width at the level of sphenotics (*sSph*) and pterotics (*sPt*), length of the middle occipital (If_1) and lateral occipital (If_2) foramens, length of neurocranium (*LN*); depth of notch on the suborbital spike (*Isorb*) and length of suborbital spike (*Lsorb*); first (h_1 *Mx*) and second depth (h_2 *Mx*) of maxilla; length (*LPm*) and depth (*hPm*) of premaxilla; length (*LD*), depth (*hD*) and distance to the end of a process (*rD*) of dentale; length (*LH*), width (*wH*) and length of front part (*lH*) of hyomandibulare; length (*Lop*) and depth (*hOp*) of

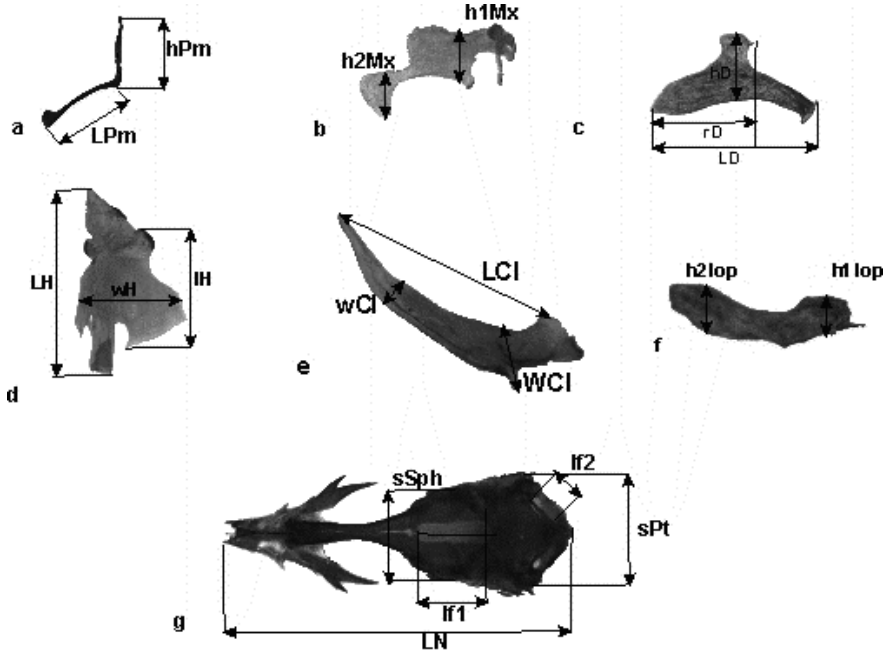

Fig. 1. Selected bones of female from Klawój Lake; a) praemaxillare, b) maxillare, c) dentale, d) hyomandibulare, e) cleithrum, f) interoperculum, g) neurocranium (8–20x).

operculum; length (*Lpop*), distance to a process (*rPop*) and width (*wPop*) of praeoperculum; first (h_1 *Iop*) and second depth (h_2 *Iop*) of interoperculum and also length (*LCl*), maximum width of lower (*WCl*) and of upper part (*wCl*) of cleithrum (Fig. 1).

Values of the features related to body and head were presented as percentages of the standard (*Sl*) and head (*lc*) lengths respectively. Most values of the features related to the neurocranium were presented as percentages of neurocranium base length (*LN*). Significance of differences in mean values between females, males and juveniles were determined with the analysis of variance (ANOVA) or Student's t-test. Browna-Forsythe's test for equal variances and the multiple Tukey's test for a posteriori comparisons were used.

Absolute values were used to calculate correlation coefficients and linear regression to determine the relationships between the features of external morphology with total length (*Tl*). The linear regression formula y = a + bx was used, where y – feature, x – *Tl*, b – regression coefficient, a – free equation factor. In order to determine the differences between the sexes, F statistics was used, verifying the hypothesis H_0: $b_M=b_F$. In cases when the hypothesis H_0 was accepted, the same statistics was used to test the free equation factors (a). In the case of statistically significant differences, the regression equations were given separately for females and males. To determine the relationships between morphological features and *Tl*, the hypothesis (H_0: a = 0) was verified using the Student's t-test.

Osteological features under study were analyzed separately, as to date they have only been presented by V a s i l' e v et al. (1989).

To compare the present results with the literature data, the hypothesis H_0 on the equality of the mean values was verified whenever possible (with given the samples size and standard deviation of these data), using the Student's test. All analyses were performed using STATISTICA-Pl 6.0 package.

Results

Q u a n t i t a t i v e a n d m e r i s t i c f e a t u r e s

Lateral spots were well developed and consisted of 10 to 19 large, oval or square shaped spots (Table 1). At the base of caudal fin all investigated fishes had one clearly visible black spot in the shape of a comma, situated slantwise. In all specimens, the suborbital spine was bifurcated with a well developed but short and broad latero-median process (Fig. 1). Males older than three years had a more rounded edge of the *lamina circularis* than younger males, which had a straighter or more concave edge (Fig. 2).

Females had more *n. macul. lateral.* and *n. macu. praeD.* spots than males. Both groups had more lateral spots than juveniles. Moreover females had more spots in front of the dorsal fin than juveniles. Thus, the following formulae: D (II) III 6-7(8); A (II) III 5(4, 6); P I 6 (5, 7); V I 5-6; C I 14 I; *Sp. branch.* 10-11 (9, 12); *Os. PhF* 10-12 (8, 9, 13); *Vert.* 43 (41, 45); *n. macul. lateral.* 14-15 (11, 18, 19) females /13 (17, 18) males; *n. macul. praeD.* 9, 10 (7,

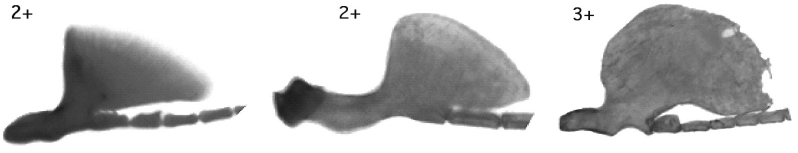

Fig. 2. Shape of the *lamina circularis* of *C. taenia* males (45–57 mm Sl) (20x).

14, 15) females / 9 (7, 10) males; *n. macul. postD*. 8, 9 (6, 10-11) can be used to describe the meristic features of mature *C. taenia* from a diploid population (Table 1).

Metric features

The relationships between body measures and total length were statistically significant, correlation coefficients were positive and significant at the level 0.05, and their values ranged from 0.8450 to 0.9968. Eight of 13 features common for the two sexes increased proportionally with fish total length: *lpc*, *lD*, *lA*, *P-V*, *V-A*, *prO*, *poO* and *lb* (Table 2). Relative values of these features did not change with fish size. The free factor in the regression equations did not differ significantly from zero (H_0: a = 0; p>0.05).

Other features related to fish size similarly in males and females: *lc*, *poD*, *pA*, *O* and *hc* did not increase proportionate to fish length (H_0: a = 0; p<0.05). Distances (*poD*) and (*pA*) increased more rapidly than *Tl* (Table 2). Three features of the head: *lc*, *hc* and *O* increased less rapidly than *Tl*.

Spined loach females differed significantly from males with respect to nine features: *pD*, *H*, *h*, *lC*, *hD*, *hA*, *lP*, *lV* and *iO* (Table 2). Two features, *H* and *hA,* increased proportionally to *Tl*, whereas *pD* increased less rapidly than *Tl* of males and females. In females, *h* and *lC* increased proportionate to *Tl*. The following features: *hD*, *lP*, *lV* and *iO* increased less rapidly than *Tl* of *C. taenia* females. The last four features, different in males and females, increased proportionally to *Tl* in the case of males (Table 2).

Relative values of metric features of spined loach are given in Table 3. At the same body length, females were characterized by higher mean values of *prO*, *hc*, *P-V* and *V-A*. The relative values of all other features different in both sexes related to head length (*lc*): *O* and *iO*, and those related to body length (*Sl*): *Tl*, *lc*, *h*, *lC*, *hD*, *lA*, *hA*, *lP* and *lV* were higher in males.

Females were characterized by higher means of relative values of *prO*, *H*, *h*, and *lD*, and lower relative values of *O*, *iO*, *lc*, *pD*, *H*, *h*, *lC*, *lD* than juveniles (Table 3). Males characterized by higher values of *lP*, *lV*, *lA*, *lD, hD*, *hA*, *H*, *h* related to the standard length and by lower relative values of *pD*, *O* and *lc* than juveniles (Table 3).

Table 1. Data on meristic features of *C. taenia* from the Klawój lake; sample size (n), females (F), males (M), juveniles (juv.), mean ($\bar{x}$), standard deviation (S.D.).

Sex	n	*Db*			*Ab*			*Cb*			*Pb*			*Vb*		
		range	$\bar{x}$	S.D.	range	$\bar{x}$	S.D.	range	$\bar{x}$	S.D.	range	$\bar{x}$	S.D.	range	$\bar{x}$	S.D.
F	133	6–8	7.0	0.42	4–6	5.3	0.52	12–15	13.9	0.44		6.2	0.71		5.5	0.52
M	53	6–7	6.9	0.36	4–6	5.0	0.48	13–14	13.9	0.23	5–7	6.1	0.62	5–6	5.3	0.57
juv.	22	6–7	6.8	0.40	4–5	5.2	0.43	13–14	13.9	0.47		6.1	0.50		5.4	0.50

	Sp. br.				*PhF*				*Vert.*			
	n	range	$\bar{x}$	S.D.	n	range	$\bar{x}$	S.D.	n	range	$\bar{x}$	S.D.
F	106	9–12	10.6	1.08	133	8–13	10.8	1.41	25	41–45	42.9	1.23
M	50	9–12	10.5	0.89	53	8–13	10.7	1.77	3	42–43	42.7	0.58
juv	20	9–12	9.9	1.07	22	8–12	10.0	1.04	–	–	–	–

	n. macul.lateral.				*n. macul. praeD.*				*n. macul. postD.*			
	n	range	$\bar{x}$	S.D.	n	range	$\bar{x}$	S.D.	n	range	$\bar{x}$	S.D.
F	133	11–19	14.8	1.55	133	7–15	9.7	1.44	133	6–11	8.7	1.31
M	53	11–18	14.1	1.64	53	7–10	8.3	0.99	53	7–10	8.9	0.88
juv	22	10–16	13.2	1.63	22	7–11	8.7	1.17	22	6–11	8.9	1.32

Table 2. Linear regression equation for the relationships between metric features (y) and total length *Tl* (x) of *C. taenia* males (M) and females (F). * Ranges were calculated taking extreme values of total fish length *Tl* and calculating the respective features from the regression equations. The results are given as the percentage of *Tl*.

Features (y)	Sex	Regression equation y = a + bx	H_0: a = 0	Range* (%)
lc	M, F	y = 1.472 + 0.144x	p<0.05	17.2–15.8
pD	M	y = 1.786 + 0.405x	p<0.05	45.2–44.0
	F	y = 1.265 + 0.422x	p<0.05	44.2–43.4
poD	M, F	y = -1.078 + 0.380x	p<0.05	35.9–37.0
H	M	y = -1.882 + 0.151x	p>0.05	11.5–12.8
	F	y = -1.618 + 0.121x	p>0.05	9.4–10.6
pA	M, F	y = -2.139 + 0.653x	p<0.05	61.1–63.3
h	M	y = 1.016 + 0.093x	p<0.05	11.3–10.5
	F	y = -0.267 + 0.087x	p>0.05	8.3–8.4
lpc	M, F	y = -0.697 + 0.152x	p>0.05	13.9–14.5
lC	M	y = 0.615 + 0.120x	p<0.05	7.4–12.7
	F	y = -0.043 + 0.123x	p>0.05	12.2–12.3
lD	M, F	y = 0.090 + 0.076x	p>0.05	7.8–7.7
hD	M	y = -0.033 + 0.126x	p>0.05	12.6–12.6
	F	y = 0.519 + 0.109x	p<0.05	11.8–11.4
lA	M, F	y = -0.128 + 0.060x	p>0.05	5.8–5.9
hA	M	y = -0.734 + 0.102x	p>0.05	8.7–9.3
	F	y = -0.052 + 0.084x	p>0.05	8.3–8.4
lP	M	y = -0.886 + 0.150x	p>0.05	13.3–13.9
	F	y = 1.174 + 0.089x	p<0.05	10.8–10.0
lV	M	y = -0.612 + 0.111x	p>0.05	9.9–10.4
	F	y = 0.625 + 0.082x	p<0.05	9.2–8.8
P-V	M, F	y = -1.752 + 0.287x	p>0.05	15.3–27.0
V-A	M, F	y = -2.692 + 0.251x	p>0.05	19.9–22.5
prO	M, F	y = 0.282 + 0.049x	p>0.05	5.4–5.2
O	M, F	y = 0.760 + 0.015x	p>0.05	3.0–2.2
poO	M, F	y = 0.410 + 0.080x	p>0.05	8.8–8.4
hc	M, F	y = 0.457 + 0.082x	p<0.05	9.1–8.6
iO	M	y = -0.117 + 0.021x	p>0.05	1.9–2.0
	F	y = 0.058 + 0.017x	p<0.05	1.8–1.7
lb	M, F	y = 1.114 + 0.018x	p<0.05	4.0–2.9

Osteological metric features

Several (*sSph*, If_2, *hPm*, *WCl*, h_1/h_2 *Iop*.) of the relative values of osteological elements had larger means in males. Whereas females were characterized by higher means of *lH*, *rD* and h_1/h_2 *Mx* (Table 4).

Discussion

The Canestrini's scale has been described as variable in shape (Vasil'ev et al. 1989, Vasil'eva 1984, 2000, Kotusz 2000, 2001). This bone plate was clearly visible in males over 40 mm *Tl* (Robotham 1981). Observation of the sample from Klawój Lake

Table 3. Relative values of metric features of *C. taenia* from the Klawój Lake; mean ($\bar{x}$), standard deviation (S.D.)

Feature	Females (n = 133)			Males (n = 53)			Juveniles (n = 22)		
	Range	$\bar{x}$	S.D.	Range	$\bar{x}$	S.D.	Range	$\bar{x}$	S.D
Sl, mm	53.3–89.2	76.7	8.48	45.0–72.5	57.4	5.76	31.0–56.1	42.2	7.77
				in % of body length *Sl*					
Tl	108.2–115.9	113.9	1.11	112.9-117.8	114.9	0.94	109.4-116.8	114.3	1.61
lc	14.4–20.1	17.9	0.71	16.7–20.6	18.8	0.86	17.7–20.3	18.9	0.73
pD	47.7–58.0	49.7	1.38	47.6–53.6	49.7	1.23	47.7–60.2	51.3	2.69
poD	32.4–43.8	41.2	1.38	36.5–43.6	41.2	1.50	36.6–43.9	40.9	1.80
H	11.8–16.6	13.9	1.01	12.1–15.9	14.0	0.86	11.6–15.2	13.2	0.96
pA	60.7–84.0	73.2	2.33	69.3–74.4	72.6	1.24	65.7–77.7	73.8	2.54
h	7.2–10.1	8.6	0.58	7.4–10.7	8.9	0.73	5.9–8.9	7.8	0.72
lpc	13.4–18.2	16.2	1.44	12.5–18.5	16.1	1.27	12.2–18.4	15.8	1.54
lC	9.3–15.9	13.9	0.91	12.9–17.8	14.9	0.94	13.5–16.8	14.8	0.96
lD	7.7–14.4	9.1	0.78	7.4–11.2	9.1	0.91	5.8–10.1	8.2	1.01
hD	10.0–15.3	13.1	0.86	12.4–16.7	14.4	0.97	11.3–14.7	12.9	0.91
lA	4.2–8.8	6.6	0.54	5.1–8.5	6.9	0.73	3.6–7.8	6.1	0.84
hA	7.7–10.9	9.4	0.67	7.8–12.9	10.4	0.95	6.4–10.3	9.1	0.93
lP	10.2–13.5	11.7	0.65	12.3–19.8	15.7	1.61	10.1–13.5	11.8	1.01
lV	8.4–10.7	10.2	0.67	9.8–14.1	11.7	0.91	8.2–11.7	9.9	0.89
P-V	27.4–37.2	31.0	1.51	27.1–33.2	30.4	1.58	28.3–34.2	30.5	1.57
V-A	22.3–27.8	25.8	3.36	22.1–29.2	24.7	1.34	22.3–26.9	24.6	1.58
lc, mm	10.0–15.9	13.7	1.50	8.0-12.5	10.8	0.97	5.9–10.7	8.3	1.43
				in % of head length *lc*					
prO	29.7–40.6	34.3	1.81	28.4–36.7	33.4	1.90	29.4–37.1	32.9	2.20
O	12.1–18.4	15.2	0.99	13.4–20.0	16.3	1.36	14.8–19.4	17.5	1.29
poO	43.7–59.4	50.7	2.07	45.0–56.4	50.3	2.46	45.1–53.5	49.7	2.44
hc	50.2–64.5	55.2	2.26	48.6–61.3	54.1	3.05	49.2–60.3	55.2	2.72
iO	8.8–16.4	11.3	1.15	7.6–15.6	11.9	1.58	8.8–18.3	12.7	2.44
lb	8.2–14.5	11.2	1.20	8.1–15.3	11.3	1.49	7.6–17.5	11.6	2.39

indicated that its hatchet-like shape was attained by males aged at least three years. Thus, variation in the shape of the *lamina circularis* seemed to be related to the age of males.

The spined loach *C. taenia* was first described by Linnaeus on the basis of material collected by Artedi, likely in Mälare Lake in Sweden. The fin formula, given by N a l b a n t (1993) for the Swedish population, is exceptionally important for the taxonomy of this species: D II (III) 7, A II 5, V I 6 - I 6, P I 8 – I 8, C 7 + 7n, which is very similar to the results of K o t u s z (2000, 2001). The specimens under study had one and two branched rays less respectively in the ventral and pectoral fins. This may be caused by counting samples without staining or by studying only few specimens by N a l b a n t (1993). The obtained results supported that the meristic features are characterized by rather low intrapopulational variability (N a l b a n t 1993).

Metric features are more variable. Differences in body proportions between sexes seem to reflect the influence of size variability, since females have a larger mean body length than males. So far, the described differences between males and females of *C. taenia* (karyologically verified) were the length of pectoral fins (V a s i l' e v et al. 1989) and body

Table 4. Data on metric features of bony elements of *C. taenia* females and males; n–sample size, $\bar{x}$–mean, S.D. – standard deviation, Student's t-test, $p<0.05$.

Feature	Females (n = 25)			Males (n = 6)		
	Range	$\bar{x}$	S.D.	Range	$\bar{x}$	S.D.
Sl (mm)	57.3–89.2	75.8	8.39	45.0–60.7	51.2	5.84
LN	7.5–13.1	10.4	1.40	6.8–10.1	8.2	1.10
			in % *LN*			
sSph	22.4–37.5	29.6	2.87	26.6–39.7	32.2	1.26
sPt	32.2–53.6	42.4	2.30	34.6–51.6	41.8	1.87
If_1	14.6–24.3	19.2	3.10	17.8–26.5	21.5	1.99
If_2	6.2–10.3	8.1	2.38	8.1–12.1	9.8	1.05
			in % of analyzed bone length			
rPop	22.1–36.8	29.1	0.74	23.7–35.4	28.7	0.61
wPop	9.8–14.9	12.9	0.62	11.4–17.0	13.8	0.76
wH	49.8–64.8	59.0	0.95	48.5–72.3	58.6	0.92
lH	51.6–78.0	65.2	0.84	51.9–76.0	62.8	1.52
hPm	67.6–112.7	89.1	1.62	76.4–110.0	92.9	3.00
rD	53.3–88.8	70.2	1.10	56.9–85.0	68.9	1.30
hD	37.7–62.8	49.6	0.63	39.6–59.2	48.1	0.73
ISorb	11.3–18.8	14.9	0.50	12.5–18.7	15.2	0.65
w Cl	10.5–17.5	13.9	0.47	10.8–16.1	13.1	0.58
W Cl	26.1–38.2	31.7	0.77	28.1–41.6	34.0	0.63
hOp	38.9–64.8	51.2	0.60	42.7–61.8	51,7	0,43
h_1/h_2 *Mx*	65.5–109.6	86.3	1.98	71.2–98.1	84.1	2.18
h_1/h_2 *Iop*	73.8–123.0	91.2	1.95	82.0–119.4	99.2	2.21

length (R o b o t h a m 1981). Statistically significant differences of linear correlation coefficients between *C. taenia* males and females were found in interorbital (*iO*) and predorsal (*pD*) distances; maximum (*H*) and minimum (*h*) body depth; length of caudal (*lC*), pectoral (*lP*) and ventral (*lV*) fins; height of dorsal (*hD*) and anal (*hA*) fins. Females and males from Głębokie Lake differed in means of the relative values of length of paired and caudal fins, height of dorsal and anal fins, and additionally V-A distance (K r z y k a w s k i et al. 2000).

The comparison between *C. taenia* females from Klawój and Głębokie Lakes showed significant differences in most characters except *lc*, *V-A* and *O*. The only feature which did not differ between females from both populations was *pD*. Males from those three compared populations differed in most analysed parameters. Specimens from Klawój Lake did not differ from those in the Moscow River in *pD*, *H* and *h* as well as *lD* whilst from those in Głębokie Lake in *Tl*, *lA* and *V-A*.

The mean values of bone measurements of females and males from Klawój Lake were similar to those from the Moscow River (V a s i l' e v at al. 1989). The best differentiating feature for both sexes in those two populations was the ratio of h_1/h_2 *Iop*.

The detailed morphological features presented in this study concern the spined loach *C. taenia* (identified as possessing 2n = 48 chromosomes) species protected in Europe, characterized by a decreasing distribution area of exclusively diploid populations. These data are of great importance for taxonomic studies in genus *Cobitis*, particular for the comparison of close related species.

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