Biometrical Features of Intergeneric Hybrid between *Leuciscus cephalus* (L.) and *Chalcalburnus chalcoides* (G.) (Osteichthyes-Cyprinidae) Distributed in Lake Tödürge (Sivas-Turkey)

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Abstract

A total of 159 (57 *Leuciscus cephalus*, 47 *Chalcalburnus chalcoides*, 55 hybrid) specimens were collected between April 1994 and November 1997 from Lake Tödürge. The identification of hybrid fish depends on the intermediacy of the suspected hybrid specimens in characteristics that serve to discriminate the parental species. For this reason, the morphometric and meristic characteristics, which are present an intermediate position in the hybrid were determined. Among the morphometric (X7: standard length/height of caudal peduncle, X19: standard length/length of anal fin, X21: standard length/length of pectoral fin, X24: standard length/ventral anal distance, X38: length of upper jaw/length of lower jaw) and meristic (X41: count of soft rays in anal, X43: count of soft rays in ventral, X49: transversal scales (upper part), X51: pharyngeal teeth) characteristics were the most effective characteristics in discrimination of both parental species and the hybrids. Two linear discriminant functions that explain 100% of the total variability of the morphometric and meristic features of hybrid and both its parents were obtained. On the basis of statistical analysis, an intergeneric hybridization was determined to occur between chub (*L. cephalus*) and Danube bleak (*C. chalcoides*) in Lake Tödürge (Sivas-Turkey), and chub and Danube bleak were really the parental species of this hybrid. Statistical analyses were also showed that there was no back-crossing with one of the parental species and therefore all hybrids were F_1.

Key words: Chub, Danube bleak, intergeneric hybridization, Lake Tödürge, Turkey.

Introduction

Cyprinidae has an immense richness in both species and form, with a worldwide distribution in inland waters. Hybrids are more frequent in inland waters than in the sea (Economidis and Sinis, 1988). Naturally occurring hybridization is a widespread phenomenon among freshwater fish. Most of these hybrids are the result of interference by men's activities such as reservoir building, modifications of rivers and introduction of exotic species. They can also occur due to change of climatic conditions, an overlap of the breeding sites and reproduction time of the species (Hubbs, 1955; Crivelli and Dupont, 1987; Economidis and Sinis, 1988; Unver *et al.*, 2005).

As reported in the world hybrid list (Schwartz, 1972), interspecific and intergeneric fertilization between cyprinid species commonly occur throughout the entire palearctic region. Nevertheless, in this list, there is no record about any hybrids of freshwater fishes in Turkey.

The interest of ichthyologists on hybrids has increased over time. Hybrids in European cyprinids are well-known (Schwartz, 1972). According to Hubbs (1955), Cyprinidae contains the largest number of hybrid combinations. Between *Leuciscus* and *Chalcalburnus* the highest hybridization frequencies have been recorded (Unver *et al.,* 2005; Economidis and Sinis, 1988; Schwartz, 1972; Berg, 1949). Hybrid studies have often worked with low sample numbers, ranging between 5-23 (Wheeler, 1978; Wheeler and Easton, 1978; Bianco, 1982; Blachuta and Witkowski, 1984; Bianco, 1988; Mir *et al.*, 1988).

Morphological character analysis, hybrid index, and discriminant analysis are usually used to identify the parental species of fish hybrids (Ross and Cavender, 1981). Confidence in the identification is enhanced by the demonstration of intermediacy in numerous characters. Therefore the purpose of this study was to determine intermediate morphometric and meristic characters of the natural cyprinid hybrid between the leuciscine species, chub, *L. cephalus*, and Danube bleak, *C. chalcoides*, by using two statistical methods (analysis of variance and canonical discriminant analysis).

Materials and Methods

The Study Area

Lake Tödürge, having a karstic origin, is situated at the Kızılırmak Basin in Central Anatolia. Tödürge has a surface area of 350 h. and an altitude of 1,295 m. The average and maximum depth of the lake is 2 m. and 28 m., respectively. The lake is mainly fed by Acısu Stream, rainfall, and karstic ground water (Değirmenci *et al.,* 1995). As the lake has both inlet and outlet, it is a limnologically open-lake. Drainage canal discharges lake water into Kızılırmak around Yarhisar village and it is situated in large agricultural

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areas (Figure 1).

Lake Tödürge, inhabiting *Cyprinus carpio, Leuciscus cephalus, Chalcalburnus chalcoides, Chondrostoma nasus, Capoeta capoeta, Capoeta tinca*, is a typical cyprinid lake, as most of lakes in Turkey (Unver, 1998). Fishery has been carried out by cooperatives for around 20 years.

Sample Collection and Analysis

Specimens of *L. cephalus* (57 specimens) and *C. chalcoides* (47 specimens) and their hybrids (55 specimens) were collected from Lake Tödürge in Sivas, between April 1994-November 1997. Samples were caught using gill nets with mesh sizes of 15, 18, 20, 24 and 32 mm.

Major morphological characteristics of the hybrid specimens and parental species were described. In addition, 39 morphometric and 14 meristic characters were examined for each specimen. Measurements were taken to the nearest 0.1 mm with caliper. The morphometric characters were expressed as percentages of standard length and head length. For each metric and meristic features the mean, standard deviation (S.D.) and coefficient of variation (C.V.=S.D./Mean*100 which is a measure of dispersion) were calculated. For the statistical analysis, 53 different (X1-X39 are morphometric and X40-X53 are meristic) characteristics were examined. Two statistical methods, Analysis of Variance and Canonical Discriminant Analysis, were used to show the main differences between the hybrid and parental species. In the first stage of the statistical analyses, the comparison of the means of the variables from three different samples (H: hybrid, LC: *L. cephalus*, CC: *C. chalcoides*) were considered, and ANOVA-test (One-Way Analysis of Variance or Scheffe-test) was then applied to variables (Johnson and Wichern, 2002). In the second stage, the Canonical Discriminant Analysis was applied to 39 (X1-X39) morphometric and 13 (X40-X51, X53) meristic characteristics (variable X52 was ignored due to only 9 valid observations), by using the stepwise variable selection method (for obtaining reduced/unsaturated model) (Manly, 1986).

Results

The specimens of the hybrid had a medium sized body; maximum total length is 243 mm. The body depth and width of the hybrid were greater than both *L. cephalus* and *C. chalcoides.* Their dorsal was blue-green and darker, the sides and ventral were white-silvery. The body was thick and flattened laterally. The snout was partly rounded and the mouth was in sub-dorsal situation. The free margin of dorsal fin was straight, scarcely convex. The caudal fin was more forked than in *L. cephalus*; the tip of lobes was slightly rounded. The caudal peduncle was long and stout form. The free margin of anal fin was convex. There was an abdominal keel, which was scaleless between ventral and anal fins. The colour of pectoral, pelvic, anal, dorsal, and caudal fins was orange or reddish. There were black dots on the posterior of the scales. The scale of hybrid resembled the scale of *L. cephalus* (Figure 2).

The number of branched rays of anal fin, the scale number in transversal line (upper) and of vertebrae was intermediate in the hybrids. The pharyngeal teeth formula showed great variation from 2.5-5.3 to 3.5-5.3 (two specimens), 3.6-5.3, 4.5-5.3 and 2.6 (one specimen). The shape of pharyngeal teeth and arch differed from those of the parental species. The teeth had slightly hooked tips and the posterior four or five had a crenelate edge only on one side. As the pharyngeal teeth, the number and shape of rakers on the first gill arch of the hybrid had shown also great variation. The rakers were short, thick and knoblike, some of them were forked.

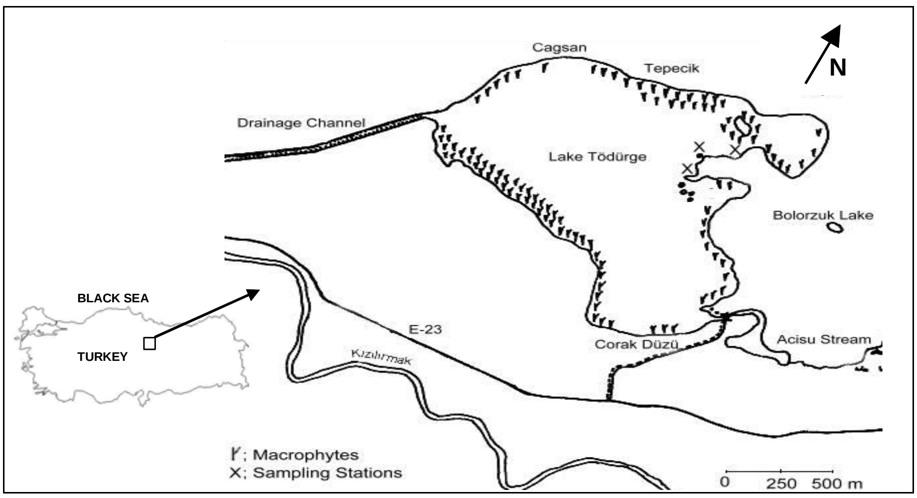

Figure 1. Lake Tödürge and the sampling sites.

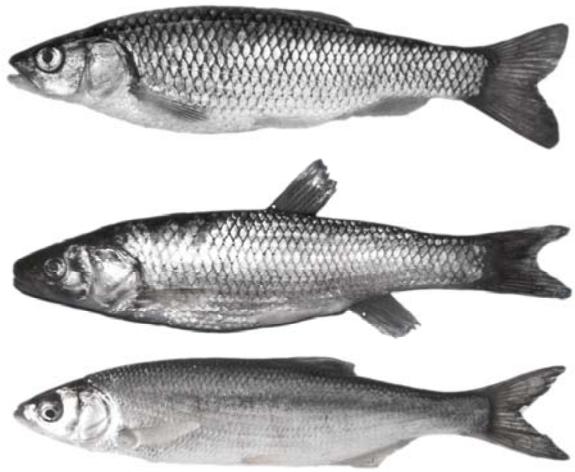

Figure 2. The Hybrid (in the centre) and its putative parents, *Leuciscus cephalus* (above) and *Chalcalburnus chalcoides* (below).

Some of the characters (X8, X14, X22, X40, X44-X47, and X52) were excluded from the analysis. Results of the ANOVA of the morphometric and meristic characters were presented in Table 1.

In the second stage, the canonical discriminant analysis was applied to morphometric characteristics (X1-X39) and two significant discriminant functions were calculated. Function 1 explained 93.2% of the variance, and function 2 explained only 6.8% of the variance. The standardized and unstandardized coefficients of the chosen variables (X7, X17, X19, X21, X22, X24, X27, X29, X33, and X38), and total effects of these characteristics on the calculated functions were given in Table 2.

From the unstandardized coefficients given above, the values of functions at group centroids of three samples (groups) were calculated and given in Table 3. Canonical Discriminant Function scores that were calculated from the same equations (unstandardized functions' coefficients) were given in Figure 3.

According to extracted two discriminant functions and their group centroids, the correctly classified rate of the analysis was 100%.

In the second step, the canonical discriminant analysis was also applied to meristic characteristics (X40-X51, X53) and two significant discriminant functions were derived again. Function 1 explained the 99.1% of the variance; function 2 explained only 0.9% of the variance.

The standardized and unstandardized coefficients of the chosen variables (X41, X43, X49, X50 and X51) and total effects of these characteristics on the derived functions were given in Table 4.

From the unstandardized coefficients given above, the values of functions at group centroids of three samples (groups) were calculated and given in Table 5.

Canonical discriminant functions scores calculated from the same equations (unstandardized functions' coefficients) were given in Figure 4.

Discussion

In nature, hybridization is generally not a very extensive phenomenon, it occurs accidentally when breeding sites and reproductive periods overlap (Hubbs, 1955; Stoumboudi *et al.,* 1992). Although fish species are genetically completely isolated from each other, in teleost fish and especially in cyprinids, hybridization seems very common. Most of the hybrids are inter-generic, such as *Alburnus* x *Rutilus* (Crivelli and Dupont, 1987; Blachuta and Witkowski, 1984), *Alburnus* x *Leuciscus* (Wheeler, 1978; Bianco, 1982; Witkowski and Blachuta, 1980), *Alburnus* x *Blicca* and *Alburnus* x *Abramis* (Blachuta and Witkowski, 1984), *Barbus* x *Capoeta* (Stoumboudi *et al*., 1992; Mir *et al*., 1988), *Leuciscus* x *Chalcalburnus* (Economidis and Sinis, 1988; Berg, 1949), *Leuciscus* x *Rutilus* (Wheeler and Easton, 1978), *Rutilus* x *Abramis* (Witkowski and Blachuta, 1980), and they may be either extremely fecund or completely sterile (Crivelli and Dupont, 1987). It was considered that hybridization between *L. cephalus* and *C. chalcoides* occurred due to overlapping spawning sites and reproduction periods. *L. cephalus* is rheophilous and *C. chalcoides* is limnophilous. However, both species live and feed in Lake Tödürge. They migrate only to reproduce from the lake to Acısu Stream. The spawning migration of both species begins in May and continues until the end of June (Unver, 1998). Spawning sites are small areas with clear running shallow water and gravel bottom, because both species are lithophilous (Economidis and Sinis, 1988). Because of the limited spawning sites, the possibility of gamete-mixing of the two species is considerably high. By reason of spawning of two parental species at the same time and place, it is a fact that the hybrids have occurred in Lake Tödürge. According to Ross and Cavender (1981), most hybridization occurring in such habitats is probably a result of accidental union of gametes from different species spawning in close proximity. However, in order to understand clearly occurring of hybridization mechanisms, spawning behaviour of both parental species in Lake Tödürge should be strictly examined.

Recognition of the hybrids is not always straightforward. According to Wheeler (1978), cyprinids are capable of complex interbreeding between parents and offspring, resulting in a large array of morphological characteristics that are

Table 1. List of the variables and summary statistics LC_{50} values (mg/L) with their fiducial limits

Variables	Symbols	Descriptions	Mean	S.D.	C.V.	Sig.
X1	Sl/lc	Standard length/Head length	4.15	0.349	8.4	0.000
X2	sl/hc	Standard length/Head height	6.11	0.472	7.7	0.000
X3	sl/lac	Standard length/Head width	8.09	1.082	13.4	0.000
X4	sl/H	Standard length/Maximum body height	4.26	0.315	7.4	0.008
X5	sl/hb	Standard length/Body height	7.45	0.751	10.1	0.000
X6	sl/lpc	Standard length/Length of caudal peduncle	6.46	0.595	9.2	0.000
X7	sl/hcp	Standard length/ Heigth of caudal peduncle	10.16	1.192	11.7	0.000
X8	sl/oh	Standard length/Orbital height	18.53	3.820	20.6	0.068
X9	sl/prO	Standard length/Preorbital length	17.54	3.103	17.7	0.000
X10	sl/poO	Standard length/Postorbital length	8.14	1.008	12.4	0.000
X11	sl/io	Standard length/Interorbital distance	11.95	2.030	17.0	0.000
X12	sl/lmx	Standard length/Length of upper jaw	13.44	2.427	18.1	0.000
X13	sl/lmd	Standard length/ Length of lower jaw	13.52	2.974	22.0	0.000
X14	sl/pD	Standard length/Predorsal length	1.83	0.053	2.9	0.114
X15	sl/poD	Standard length/Postdorsal length	3.04	0.180	5.9	0.001
X16	sl/hD	Standard length/Height of dorsal fin	0.76	0.101	13.3	0.000
X17	sl/lD	Standard length/Length of dorsal fin	1.16	0.140	12.0	0.000
X18	sl/hA	Standard length/Height of anal fin	0.92	0.089	9.6	0.000
X19	sl/lA	Standard length/Length of anal fin	1.02	0.313	30.7	0.000
X20	sl/lC	Standard length/Length of caudal fin	3.91	0.264	6.7	0.000
X21	sl/lP	Standard length/Length of pectoral fin	5.73	0.391	6.8	0.000
X22	sl/lV	Standard length/Length of ventral fin	7.12	0.391	5.5	0.419
X23	sl/P-V	Standard length/Pectoventral distance	3.66	0.230	6.3	0.012
X24	sl/V-M	Standard length/Ventral anal distance	4.94	0.381	7.7	0.000
X25	sl/PV	Standard length/Preventral distance	1.94	0.092	4.8	0.000
X26	sl/PA	Standard length/Preanal distance	1.39	0.055	4.0	0.000
X27	lc/hc	Head length/Head height	1.48	0.072	4.9	0.003
X28	lc/lac	Head length/Head width	1.94	0.138	7.1	0.000
X29	lc/oh	Head length/Orbital height	4.46	0.798	17.9	0.000
X30	lc/prO	Head length/Preorbital length	4.21	0.489	11.8	0.004
X31	lc/poO	Head length/ Postorbital length	1.96	0.114	5.8	0.000
X32	lc/io	Head length/Interorbital distance	2.87	0.295	10.3	0.000
X33	lc/lmx	Head length/Length of upper jaw	3.22	0.379	11.7	0.000
X34	lc/lmd	Head length/Length of lower jaw	3.30	0.846	25.7	0.000
X35	Hc/lac	Head height/Head width	1.32	0.098	7.4	0.000
X36	H/hb	Maximum body height/Body height	1.76	0.147	8.3	0.000
X37	lpc/hcp	L. of caudal peduncle/Heigth of caudal peduncle	1.58	0.165	10.5	0.000
X38	lmx/lmd	Length of upper jaw/Length of lower jaw	1.04	0.305	29.3	0.000
X39	pD/poD	Predorsal length/Postdorsal length	1.66	0.107	6.4	0.000
X40	Db	Count of soft rays in D	8.00	0.236	2.9	0.069
X41	Ab	Count of soft rays in A	10.70	2.357	22.0	0.000
X42	Pb	Count of soft rays in P	14.60	0.804	5.5	0.000
X43	Vb	Count of soft rays in V	8.26	0.579	7.0	0.000
X44	Du	Count of hard rays in D	3.01	0.166	5.5	0.355
X45	Au	Count of hard rays in A	2.99	0.083	2.8	0.355
X46	Pu	Count of hard rays in P	1.00	-	-	-
X47	Vu	Count of hard rays in V	1.99	0.083	4.2	0.423
X48	L.lat	Ligne lateral	52.72	11.764	22.3	0.000
X49	Upper part	Transversal scales (upper part)	8.93	1.689	18.9	0.000
X50	Lower part	Transversal scales (lower part)	3.75	0.676	18.0	0.000
X51	Ft	Pharyngeal teeth	17.32	6.904	39.8	0.000
X52	Gr	Gill rakers on the first arch	42.22	0.441	1.0	1.000
X53	vertebra	Count of vertebra	40.37	1.749	4.3	0.000

S.D. :Standard deviation, C.V. : Coefficient of variation, Sig. : Significancy.

Table 2. Canonical Discriminant Functions' coefficients (Variables: X7, X17, X19, X21, X22, X24, X27, X29, X33, X38)

	Standardized Functions			Unstandardized Functions	
Variables	1	2	Total Effect (%)	1	2
X7	-0.655	0.490	15.83	-0.930	0.695
X17	-0.212	0.334	5.35	-2.782	4.391
X19	1.090	-0.075	25.41	14.157	-0.972
X21	0.415	-0.720	10.56	1.408	-2.446
X22	-0.195	0.631	5.33	-0.574	1.854
X24	0.386	0.152	9.15	1.140	0.448
X27	-0.132	-0.349	3.51	-1.885	-4.973
X29	-0.161	-0.966	4.97	-0.235	-1.413
X33	0.248	0.758	6.72	0.933	2.851
X38	0.519	0.868	13.17	4.734	7.918
Constant	-	-	100.00	-15.754	-16.685

Table 3. Functions at group centroids

	Functions	
Groups	1	2
CC	-6.779	1.466
H	-2.187	-2.561
LC	7.252	0.599

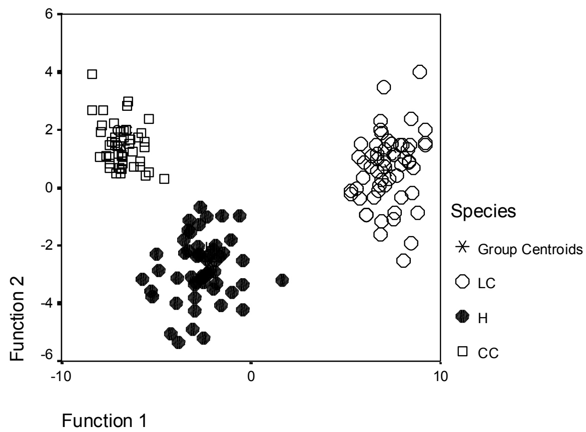

Figure 3. Canonical Discriminant Function scores of the observations (*; group centroids, o; LC, ●; H, □; CC).

Table 4. Canonical Discriminant Functions' coefficients (Variables:X41, X43, X49, X50, X51)

Variables	Standardized Functions			Unstandardized Functions	
	1	2	Total Effect (%)	1	2
X41	0.665	-0.173	21.90	1.214	0.317
X43	0.569	0.400	18.84	1.169	0.822
X49	0.697	0.638	23.12	2.608	2.387
X50	-0.180	0.661	6.15	-0.552	2,023
X51	0.906	-0.589	29.99	1.196	-0.778
Constant	-		100.00	-63.823	-26.807

Table 5. Functions at group centroids

	Functions	
Groups	1	2
H	-3.019	1.855
LC	-18.510	-1.485
CC	20.282	-0.889

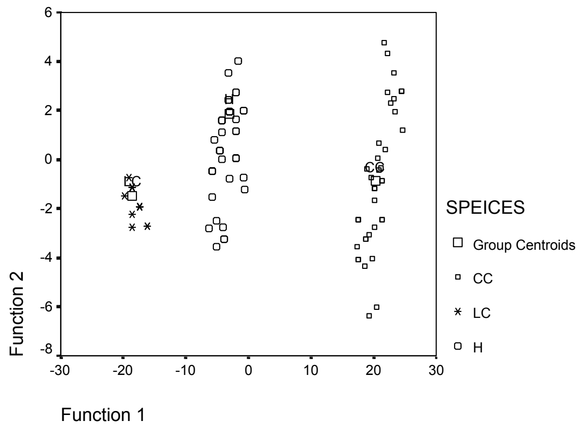

Figure 4. Canonical Discriminant Function scores of the observations (□: group centroids, ⁎: LC, o: H, □: CC).

continuous between those of the parental species. In such cases, positive identification of the hybrid may be difficult (Stoumboudi *et al*., 1992). Although the hybrid externally resembled *L. cephalus*, many morphological, metric and meristic features were intermediate between *L. cephalus* and *C. chalcoides* (Figure 2). Body colour, pigmentation of peritoneum, mouth form, shape of anal, dorsal and caudal fins, head length, length and depth of caudal peduncle, branched rays of anal fin, scale number in transversal line (upper) and vertebrae number, pharyngeal teeth and arch form, number and shape of gill rakers were significantly different from the parental species.

The discriminant analysis based on almost all the morphometric and meristic characteristics, established very clearly the identity of both parental species of this hybrid. Approximately 159 observations (individuals) were considered and for statistical investigation, 53 different characteristics (39 morphometric and 14 meristic) were taken into account. In the first stage of the statistical analyses, from the comparison of the group means of the variables, it was found that except X8, X14, X22, X40, X44-X47, and X53 the differences between the three group means of 44 variables were statistically significant ($P \leq 0.05$). In the second stage, for distinguishing the observations of three groups (samples), the canonical discriminant functions analysis was applied both to 39 metric and 14 meristic variables (due to parsimony rule, to decrease the number of the variables of functions, the stepwise discriminant functions analysis was applied).

In the discriminant analysis, individuals of the hybrid were dispersed tightly around the centre of gravity (Figure 3 and 4), which may indicate that no back-crossing with either of the parental species could be identified. Thus, all the hybrids may belong to F_1 generation. No F_2 hybrid could be detected in the discriminant analysis. Similarly, Crivelli and Dupont (1987) have stated that back-crossing was not observed in *Alburnus alburnus* x *Rutilus rubilio* hybrids when the discriminant analysis was performed and therefore all hybrids were F_1. As a result, in the first step of the discriminant analysis, 10 metric characteristics X7, X17, X19, X21, X22, X24, X27, X29, X33, and X38 were chosen. Among these, standard length/heigth of caudal peduncle (X7), standard length/length of anal fin (X19), standard length/length of pectoral fin (X21), standard length/ventral anal distance (X24), and length of upper jaw/length of lower jaw (X38) were the most effective characteristics in discrimination of both parental species and the hybrids.

In the second step of the discriminant analysis, 5 meristic variables (X41, X43, X49, X50, and X51) were chosen as significant characteristics. Some of these characteristics [count of soft rays in anal and ventral fin (X41, X43), transversal scales number-upper part (X49) and count of pharyngeal teeth (X51)] were the most effective ones in discriminating the hybrid and both parental species. According to Crivelli and Dupont (1987), two axes explain 99.9% of the total variability of the hybrid and of both of its parental species. The most important contributors to axis 1 were dorsal and anal base length, eye diameter; on axis 2, they were the smallest body depth, head length, caudal fin length and eye diameter (Figure 4). Therefore, the discriminant analysis clearly indicated that *L. cephalus* and *C. chalcoides* were really the parental species of this hybrid in Lake Tödürge. It also showed that, among all the hybrid specimens found, there was no back-crossing with one either of the parental species and that all hybrids were F_1 generation.

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