MORPHOLOGY OF THE GENUS *GYMNOCEPHALUS* (PISCES) FROM THE LOWER DANUBE RIVER

Cecilia ȘERBAN * and Gabriela GRIGORAȘ **

* "Dunărea de Jos" University, Domnească Street 111, RO-800201 Galați, Romania, cecilia.serban@ugal.ro

** "Răsvan Angheluță" Museum Complex of Natural Sciences, Regiment 11 Siret Street, 6A, RO-800340 Galați, Romania, griggabi@yahoo.com

DOI: 10.2478/trser-2019-0012

KEYWORDS: genus *Gymnocephalus*, phenotypic variability, morphometric, geometrics, lower Danube.

ABSTRACT

The current study completed the information regarding the phenotypic variability in the Danube ruffe (*Gymnocephalus baloni*). We also assessed the phylogenetic relationship of *G. baloni* with the other two species of the genus *Gymnocephalus* from the Lower Danube River.

Ten morphological characters were the most useful together for discriminating between *G. baloni* and *G. cernua* from the Lower Danube River. In addition, we found a more streamlined body shape in *G. baloni* compared with the described holotype, which could be in connection with fish phenotypic response to ecological characteristics of the Lower Danube River.

RESUMÉ: Morphologie du genre *Gymnocephalus* (Pisces) du cours inférieur du Danube.

L'étude actuelle a complété l'information concernant la variabilité phénotypique de la ruffe du Danube (*Gymnocephalus baloni*). On a également évalué la relation phylogénétique de *G. baloni* avec les deux autres espèces du genre *Gymnocephalus* du cours inférieur du Danube.

Dix caractères morphologiques ont été les plus utiles pour différencier *G. baloni* et *G. cernua* du cours inférieur du Danube. De même, on a trouvé une forme de corps plus hydrodynamique chez *G. baloni* par rapport à l'holotype décrit, ce qui pourrait être en rapport avec la réponse phénotypique des poissons aux caractéristiques écologiques du cours inférieur du Danube.

REZUMAT: Morfologia genului *Gymnocephalus* (Pisces) din cursul inferior al Dunării.

Studiul a completat informațiile privind variabilitatea fenotipică a ghiborțului de Dunăre (*Gymnocephalus baloni*). S-a evaluat, de asemenea, relația filogenetică dintre *G. baloni* și celelalte două specii din genul *Gymnocephalus* din cursul inferior al Dunării.

Zece caractere morfologice au fost semnificative pentru discriminarea speciilor *G. baloni* și *G. cernua* din cursul inferior al Dunării. De asemenea, s-a evidențiat forma hidrodinamică mai accentuată a speciei *G. baloni* comparativ cu holotipul, aspect care ar putea fi în legătură cu răspunsul fenotipic al peștilor la caracteristicile ecologice ale fluviului Dunărea în cursul inferior.

INTRODUCTION

The highly diversified and complex Lower Danube River area geoecosystems, generated in time and space by a unique mixture of biotopes and biocoenosis, has formed a highly dynamic and extremely rich fish fauna (e.g. there are 137 fish species only in the Danube Delta) (Antipa, 1909; Bănărescu, 1964; Bănărescu and Bănăduc, 2007; Bănăduc et al., 2016).

In the last century, compared to Antipa (1905, 1909, 1915, 1933, 1934), the Lower Danube-Danube Delta-Black Sea's area habitats heterogeneity and native economic and conservation important fish species diversity and stock abundance have a significantly decreasing trend, and currently, there is no scientific indication that this trend will stop in the near future (Bănăduc et al., 2016). In this present context, it is evident that the regional fish species of conservative and/or economic interest should be studied from a high number of points of view.

Three species of the genus *Gymnocephalus* are widespread in the Lower Danube River basin: the widespread ruffe, *Gymnocephalus cernua* (Linnaeus, 1758), the Danube ruffe, *Gymnocephalus baloni* (Holčík and Hensel, 1974) and the yellow pope, *Gymnocephalus schraetser* (Linnaeus 1758), the last two of these Eurasian fish species being indexed in the Romanian Vertebrate Red List as vulnerable (Bănărescu, 2005) and are protected by Habitats Directive – 92/43/EEC (Bănărescu and Bănăduc, 2007). There is another species of ruffe reported in scientific studies from the Black Sea basin, the Don ruffe, *Gymnocephalus acerina* (Gmelin, 1789) but the information about its presence in the Prut River, a tributary of the Danube River has not been confirmed by some recent ichthyological studies (Davideanu et al., 2008).

The species from the Eurasian genus *Gymnocephalus* date to about 13.4 Mya (Stepien and Haponski, 2015). They show different patterns in body shape, colours and meristic characters as lateral line scale and fin rays counts (Bănărescu, 1964; Holčík and Hensel, 1974; Kottelat and Freyhof, 2007; Stepien and Haponski, 2015).

Gymnocephalus baloni has long been treated as *G. cernua* and just recently described as a specific rank by Holčík and Hensel (1974), the species being poorly known (Bănărescu, 2005; Ratschan, 2012). After its first description, several ichthyological studies reported the first presence of populations in different parts of the Danube River drainage such as the Drava River, Slovenia and Croatia (Povž et al., 1997), and the Mur River, Austria (Ratschan, 2012). A recent study of Tsyba and Kogodiy (2017) notices the presence of the species in another river from Black Sea basin (middle Dniepr/Nistru drainage, Ukraina).

There are several studies from recent years that report *G. baloni* species from different rivers in the Romanian Danube River drainage area: the Danube River – downstream to the town of Brăila, including the Danube Delta arms, the Danube River between Turnu-Severin and Moldova Nouă, as well as the rivers Criș, Someș, Mureș, Ialomița, Argeș, Olt, Vedea, Timiș, Tisovița, and Berzasca (Oțel et al., 1993; Kováč, 1994; Bănărescu and Bănăduc, 2007; Oțel, 2007; Bănăduc, 2009; Telcean and Cupșa, 2009, 2012; Oțel and Năstase, 2010; Györe et al., 2013; Bănăduc et al., 2014, 2016; Nuță et al., 2016; Năstase and Oțel, 2016; Bulat, 2017; Năstase et al., 2017).

However, no publications are known to us to describe the phenotypic variability of populations from the Romanian Danube River basin.

The main aim of our research was to analyse the morphometric features of *G. baloni* from the targeted area and compare them with data published by other authors in recent years, regarding the fish populations from the Danube River basin (Bănărescu, 1964; Holčík and Hensel, 1974; Specziár and Vida, 1995; Kottelat and Freyhof, 2007; Geiger and Schliewen, 2010; Stepien and Haponski, 2015; Tsyba and Kokodiy, 2017).

The intra- and inter-population morphological variations in fish are put in evidence by several authors (Karakousis et al., 1991; Wimberger, 1992; Turan, 1999; Shao et al., 2007). Various references sustain the habitat-associated morphological divergence as a common pattern in fish (Foster et al., 2015).

The morphological characters for reliable discrimination between two of these sympatric species are also analysed in our study. An analysis of phenotypic variation in morphometric or meristic characters is the method most commonly used for fish population delineation (Karakousis et al., 1991; Shao et al., 2007; Samaradivakara et al., 2012). This approach is still commonly used in fish biology, taxonomic studies and assessment of the relationship between various taxa (Turan, 1999), despite the development of molecular techniques (Shao et al., 2007).

Another goal of our research was to analyse the phylogenetic relationship between the sister taxa *G. cernua* and *G. baloni* from the targeted area. Although there is a strong similarity between *G. baloni* and *G. cernua* and a clear discrimination of these two species from *G. schraetser* (with more hydrodynamic shape of the body and also specific colour features), there are still different opinions expressed in the studies of last decades, regarding their phylogenetic relationship (Specziár and Vida, 1995; Geiger and Schliewen, 2010).

Thus, the first taxonomical description of *G. baloni* by Holčík and Hensel (1974) allowed the authors to identify a closer relationship between *G. baloni* and *G. cernua*, placing them in the same subgenus, *Acerina*, while the remaining two elongated species (*G. schraetser* and *G. acerina*) are placed in the subgenus *Gymnocephalus*. Later, Geiger and Schliewen (2010), using genetic techniques, have questioned the phylogenetic position of *G. schraetser* within the genus *Gymnocephalus*, while the new results of Stepien and Haponski (2015), based on molecular data, have suggested a sister – taxa relation between *G. baloni* and *G. schraetser*.

MATERIAL AND METHODS

The morphometric data are from a total of 49 fish specimens in the Lower Danube River area, of them: 12 specimens of the Danube ruffe, *G. baloni*, and 16 specimens of the striped ruffe, *G. schraetser* were sampled near the confluences of the Danube River with the rivers Prut and Siret at Galați City (sea mile 80-76 from Black Sea) while 21 specimens of the widespread ruffe, *G. cernua* were sampled at Brateș fish farm pond (Galați County), which has a Danube River water supply. The random sampling of studied fish specimens occurred in 2015, between June and October. Specimen collection was performed using hook – and – line method.

All collected specimens were fixed in 10% formalin at the site of collection and transported to the laboratory.

A CANON A590 IS camera is used to take digital pictures of the left side of each fish individual.

Bănărescu (1964) and Holčík and Hensel (1974) describe a total of 18 morphometric measurements (Fig. 1) taken. They are as follows:

- SL – standard length (mm);
- BODYDEPTH – maximum body height;
- CAUDPEDDEPTH – minimum height of caudal peduncle;
- PREDORSDIST – predorsal distance;
- CAUDPDLENGTH – length of caudal peduncle;
- PREVENTDIST – pre-ventral distance;
- PREANALDIST – pre-anal distance;
- PVDIST – distance between pectoral and ventral fin insertions;
- VADIST – distance between ventral and anal fin insertions;
- DLENGTH – length of dorsal fin base;
- ALENGTH – length of anal fin base;
- ADEPTH – the distance from the insertion of the anal fin to the end point of the highest ray of anal fin;
- PLENGTH – pectoral fin length;
- VLENTGTH – ventral fin length;
- HEADLENGTH – the distance from the tip of the snout to the ventral base of the largest opercula spine;
- HEADDEPTH – head depth;
- MOUTHLENGTH – preorbital length;
- EYEDIAMETER – eye diameter.

The values express a percentage of the standard length, with the exception of the head depth, mouth length and eye diameter, which count as a percentage of the head length. The mean values of each morphological character, as well as the confidence intervals ($P < 0.05$), were computed using descriptive statistics function with Systat 10.2.

Figure 1: Morphometric characters taken on the ruffe species (picture of *Gymnocephalus baloni*) studied in the Lower Danube River basin.

A multivariate approach, including factor analysis (FA) and discriminant analysis (DA), was applied for the morphometric assessment, using Systat 10.2. For DA, a linear discriminant analysis, using a backward stepwise method, based on Mahalanobis distances (developed by Fisher, 1936, in Engelman, 2005), was performed to establish the most relevant morphometric features that together discriminate the samples of *G. baloni* and *G. cernua* (Engelman, 2005).

MorfoJ 1.05f applies the geometric methods in this study (Klingenberg, 2011) and 16 landmarks are defined and also recorded as two-dimensional coordinates, using TPS Digitize (Rohlf, 2016a), TPS Relative Warps (Rohlf, 2016b), and TPS Utility (Rohlf, 2016c) software. The landmarks were selected to provide a homogeneous frame of the entire shape. The shape information was extracted by Procrustes superimposition, which removes variation in size, position and orientation from data on landmark coordinates (Klingenberg, 2011).

The multivariate statistical analysis of body shape uses coordinates of the superimposed landmarks, and includes principal components analysis (PCA), discriminant analysis (DA), and canonical variate analysis (CVA) in order to address the main question: the current variability of the ruffe species populations from the Lower Danube River basin, around Mm80 area.

The geometric, statistical analysis provides necessary information regarding the fish body shape variation between groups. The interspecific study used the 49 ruffe individuals.

The statistical test is made using the MorphoJ software. After landmark data importing and shape information extracting with Procrustes superimposition (Klingenberg, 2011), several techniques of multivariate tests data were used to analyse the substantiating patterns in the groups and among the variables. The PCA and CVA statistic tools allow the examination of the relationship among variables and cases in a single block of data (Heyman and Noble, 1989).

RESULTS AND DISCUSSION

The morphological characters (Fig. 1) obtained through body measurements of the three ruffe species, as well as the confidence intervals with 95% confidence level, are listed in table 1.

The standard body length of *G. baloni* individuals ranged between 61.72-107.71 mm (minimum – maximum values), the standard body length of *G. cernua* was 87.74-110 mm, and this of *G. schraetser* was 161.99-186.46 mm.

Discrimination between *G. baloni* and its more widespread congener *G. cernua* assessed by the univariate analysis indicated an overlapping of the ranges in some morphometric characters, except the body depth, length of the caudal peduncle, ventral-anal fin distance, length of dorsal base and eye diameter, which is consistent with references (Holčík and Hensel, 1974).

The received results were compared to those published by other authors (Tabs. 2-4), with several studies made in recent years (Bănărescu, 1964; Holčík and Hensel, 1974; Specziár and Vida, 1995; Kottelat and Freyhof, 2007; Geiger and Schliewen, 2010; Stepien and Haponski, 2015; Tsyba and Kokodiy, 2017).

Table 1: Main morphological characters of the three ruffe species *Gymnocephalus baloni*, (n = 12 specimens) *Gymnocephalus cernua* (n = 21 specimens), and *Gymnocephalus schraetser* (n = 16 specimens) studied in the Lower Danube River basin (confidence intervals, $P < 0.05$; % of the standard length except the head depth, mouth length and eye diameter that are expressed as % of head length). Explanations of morphological characters are in the material and methods.

	Morphological character *	Value of morphological character (Confidence interval, in % of standard length, L, which is in mm unit)		
		<i>Gymnocephalus baloni</i>	<i>Gymnocephalus cernua</i>	<i>Gymnocephalus schraetser</i>
1.	SL	(82.4 – 101.8)	(94.3 – 100.5)	161.99 – 186.46
2.	BODYDEPTH	27.6 – 29.1	25.7 – 26.4	22.12 – 23.17
3.	CAUDPEDDEPTH	8.5 – 9.3	8.6 – 9.1	6.78 – 7.16
4.	PREDORSDIST	29.5 – 31.9	29.7 – 30.9	26.81 – 28.66
5.	CAUDDPLENGTH	19.8 – 21.8	21.1 – 22.4	18.94 – 20.82
6.	PREVENTDIST	32.1 – 34.1	33.6 – 34.8	29.08 – 30.79
7.	PREANALDIST	63.8 – 66.7	64.3 – 65.8	66.51 – 68.84
8.	PVDIST	3.5 – 4.5	2.8 – 3.6	3.50 – 4.73
9.	VADIST	31.1 – 34.9	30.6 – 31.8	36.48 – 39.21
10.	DLENGTH	54.1 – 56.8	53.0 – 54.7	55.83 – 57.97
11.	ALENGTH	14.4 – 17.0	13.9 – 15.2	12.06 – 13.60
12.	ADEPTH	19.2 – 23.2	19.5 – 20.8	17.53 – 19.77
13.	PLENGTH	21.4 – 24.0	22.2 – 23.6	17.76 – 18.66
14.	VLENTGTH	21.1 – 22.6	20.6 – 22.2	18.50 – 19.50
15.	HEADLENGTH	30.4 – 32.7	31.5 – 32.6	25.39 – 28.43
16.	HEADDEPTH	75.7 – 79.9	72.3 – 74.8	68.88 – 79.50
17.	MOUThLENGTH	26.4 – 29.8	27.5 – 29.6	37.51 – 41.49
18.	EYEDIAMETER	29.2 – 31.9	27.3 – 29.2	24.46 – 28.73

Our results were consistent with some authors (Tab. 2), however showed differences in some of the morphological features when compared with other scientific researches (the body depth, predorsal distance, caudal peduncle length, preentral distance, and preanal distance).

Table 2: Main morphological characters of *Gymnocephalus baloni* specified by various references (% of the standard length except for the head depth, mouth length and eye diameter). Explanations of morphological characters are in the material and methods.

Morphometric character	Hungarian (Budapest) area of the Danube River (Specziár and Vida, 1995)	Hungarian (Szigetköz) area of the Danube River (Specziár and Vida, 1995)	The Upper Dniepr River, Ukraine (Tsyba and Kokodiy, 2017)	The Middle Dniepr River, Ukraine (Tsyba and Kokodiy, 2017)	Holotype (Holčík and Hensel, 1974)	Paratypes (Holčík and Hensel, 1974)	Others (noted as ZICU by Holčík and Hensel, 1974)	Other material (Holčík and Hensel, 1974)	Holčík and Hensel, 1974	Lake Ammersee, Germany (Geiger and Schliwen, 2010)
STANDLENGTH	78.8	97.5	96.4	93.2	107.3	96.5	112.6	93.7	no data	101.7
BODYDEPTH	29.9	31.2	31.6	31.4	31.6	31.2	35.2	31.0	30.8	32.1
CAUDPEDDEPTH	8.1	8.2	8.4	8.3	8.4	8.7	10.4	8.7	8.6	8.9
PREDORSSDIST	32.4	31.4	37.2	37.1	35.3	33.8	40.0	34.4	34.1	35.4
CAUDPDLENGTH	21.2	20.2	21.0	16.8	18.6	19.7	22.4	19.9	19.8	17.9
PREVENTDIST	36.1	36.7	35.3	36.4	39.4	39.1	41.7	38.8	38.7	37.0
PREANALDIST	66.8	69.1	68.0	69.7	70.3	70.3	77.8	70.2	no data	no data
PVDIST	3.5	4.5	6.0	4.9	12.6	12.3	12.9	12.1	12.0	no data
VADIST	30.7	32.5	32.3	34.7	32.7	34.7	38.6	34.3	no data	no data
DLENGTH	50.6	52.4	56.75	56.9	58.2	56.4	64.8	56.7	56.3	no data
ALENGTH	11.9	10.7	14.8	14.8	13.9	14.4	18.0	14.5	14.4	14.6
ADEPTH	16.7	14.7	17.2	16.6	no data	no data	no data	no data	no data	no data
PLENGTH	21.0	20.4	22.1	21.1	20.1	19.4	23.8	19.8	no data	19.7
VLENGTH	22.4	21.4	21.8	22.9	22.3	21.4	24.8	21.9	no data	20.3
HEADLENGTH	33.0	31.5	31.8	32.2	25.6	30.1	35.1	30.5	no data	29.2
HEADDEPTH	64.2	73.0	80.2	76.1	105.1	85.7	82.1	84.9	no data	84.3
MOUThLENGTH	no data	0.0	32.4	35.2	39.8	33.5	32.2	33.1	no data	35.5
EYEDIAMETER	28.8	28.3	31.4	29.9	33.6	28.6	28.8	29.2	no data	31.8

The body depth of *G. baloni* sampled from the Lower Danube River is smaller than the parameter measured on the holotype (Tab. 2) and smaller than other samples from the Danube River basin showing the specific phenotypic response to lotic habitat and unsteady swimming behaviour of fish. We can mention here the results of Foster et al. (2015), which highlight the general influence of the body shape differences by different habitat types and adaptive phenotypic features in the sympatric species to relative environmental gradients.

Table 3: Main morphological characters of *Gymnocephalus cernua* specified by various references (% of standard length except for the head depth, mouth length and eye diameter). Explanations of morphological characters are in the material and methods.

Morphometric character	<i>G. cernua</i> x <i>G. baloni</i> (Holčík and Hensel, 1974)	Holčík and Hensel, 1974	Syntype (Geiger and Schliewen, 2010)	Brackish Elbe River, Germany (Geiger and Schliewen, 2010)	The Elbe River, Germany (Geiger and Schliewen, 2010)	Lake Ammersee, Germany (Geiger and Schliewen, 2010)	Hungarian (Budapest) area of the Danube River (Spezár and Vida, 1995)	Baltic Sea (Geiger and Schliewen, 2010)	Hungarian (Szeged) area of the Danube River (Spezár and Vida, 1995)	Romanian Danube River basin (Bănărescu, 1964)	The Middle Diepr River, Ukraine (Tsyba, and Kokodiy, 2017)	Tge Upper Diepr River, Ukraine (Tsyba, and Kokodiy, 2017)
STANDLENGTH	no data	no data	101.1	100.3	80.5	98.9	91.9	101.3	84.9	145.0	103.8	93.3
BODYDEPTH	30.2	25.8	35.3	27.0	26.6	27.0	25.8	23.4	27.2	29.5	25.3	27.0
CAUDPEDDEPTH	8.9	8.2	10.1	9.2	9.4	8.8	8.0	7.5	8.2	8.4	7.7	7.8
PREDORSSDIST	34.8	32.7	36.6	38.3	36.2	35.1	30.8	34.2	31.8	34.0	35.2	35.2
CAUDPDLENGTH	20.9	21.6	17.9	20.1	21.2	20.2	22.9	23.3	23.1	21.0	20.1	24.6
PREVENTDIST	36.6	36.4	39.1	36.8	35.0	35.2	35.0	32.2	34.4	no data	33.9	33.3
PREANALDIST	no data	no data	no data	no data	no data	no data	67.2	no data	67.5	no data	67.2	65.3
PVDIST	11.5	9.7	no data	no data	no data	no data	3.2	no data	2.6	no data	4.3	5.9
VADIST	no data	no data	no data	no data	no data	no data	32.3	no data	33.2	no data	34.6	32.1
DLENGTH	5.7	53.8	no data	no data	no data	no data	50.8	no data	50.3	no data	53.5	53.2
ALENGTH	13.4	12.5	15.0	14.7	13.0	12.4	9.9	11.4	9.3	no data	12.3	11.9
ADEPTH	no data	no data	no data	no data	no data	no data	16.7	no data	15.7	no data	14.7	14.8
PLENGTH	no data	no data	21.1	23.6	20.6	20.1	20.5	18.5	22.2	22.0	20.4	20.8
VLENGTH	no data	no data	22.0	22.5	20.4	19.8	21.6	18.6	22.6	21.8	21.3	19.7
HEADLENGTH	no data	no data	30.6	33.2	31.3	29.5	31.8	28.7	31.7	31.3	30.9	30.5
HEADDEPTH	no data	no data	86.3	71.4	72.2	75.9	59.7	69.3	64.0	no data	66.6	71.0
MOUThLENGTH	no data	no data	33.9	36.9	35.6	37.1	0.0	36.6	0.0	32.8	34.0	30.2
EYEDIAMETER	no data	no data	29.9	28.1	31.1	29.6	26.6	31.6	29.6	27.3	27.6	29.7

Table 4: Main morphological characters of *Gymnocephalus schraetser* from the Lower Danube River basin (Bănărescu, 1964). Explanations of morphological characters are in the material and methods.

Morphometric character	Value (% of standard length except the head depth, mouth length and eye diameter)
STANDLENGTH	170.0
BODYDEPTH	21.8
CAUDPEDDEPTH	6.8
PREDORSSDIST	32.8
CAUDPDLENGTH	20.5
PREVENTDIST	no data
PREANALDIST	no data
PVDIST	no data
VADIST	no data
DLENGTH	no data
ALENGTH	no data
ADEPTH	no data
PLENGTH	18.6
VLENGTH	19.8
HEADLENGTH	31.5
HEADDEPTH	no data
MOUThLENGTH	46.0
EYEDIAMETER	22.7

In the same way, as an adaptive answer of fish morphology to water flow regime and other ecological parameters, we recorded smaller values in Danube ruffe body characters than the holotype description for some biometric characters such as predorsal distance, preanal distance, preanal distance. These differences were not recorded by comparing measurements of the fish population sampled from Hungarian Danube River section (Tab. 2) and also to populations in the upper and middle basin of the Dnieper/Nistru River (Tab. 2).

A suggestive image of the morphological variation and relationship among species from the genus *Gymnocephalus* sampled from the Lower Danube River (Mm80), compared with results from the measurements of fish from the various Eurasian rivers obtained by different authors (Tabs. 2-4) was assessed by a hierarchical clustering single linkage method. The dendrogram of figure 2 displays the output of the hierarchical clustering, which represent the trees that put in evidence, the similarity, and dissimilarity between the results.

The cluster based on our results reveals a more advanced degree of morphological similarity of fish with data referring to fish sampled from the upper and middle Dniepr/Nistru Basin (Tabs. 2-4) and the Hungarian stretch of Danube River (Tabs. 2-4). The distances between our results and the clusters of Danube ruffe holotype/paratype (Tab. 2) and widespread ruffe paratype (Tab. 3) are bigger (Fig. 2).

The dendrogram sustains the identified dissimilarity between *G. schraetser* (according to data described by Bănărescu, 1964 on 26 specimens from Danube, Timiș, Bega, Mureș rivers, Romania, Tab. 4) and the other two ruffe species (2.20 Euclidean distance).

Figure 2: Dendrogram of the hierarchical clustering analysis based on morphometric data.

The algorithm used was the single linkage clustering (nearest neighbour) with the Euclidean distance as a similarity measure.

Aco-variance matrix of the Procrustes coordinates of each sample is used to perform the PCA, and the first two components summarised 67.166%, which represent more than the half of total variance.

The scatter plot of PC scores from figure 3 displays the patterns in the relations between observations. PC1 separated *G. schraetser* (blue circle) from the other two studied species of interest while PC2 separated *G. baloni* (red circle) from *G. cernua* (green circle) (Fig. 3).

Figure 3: PC scores and the disposition of specimens in the morphospace of the multivariate analysis. The coordinates of PCA plot are grouped by equal frequency confidence ellipses ($P = 0.9$), which separate *G. schraetser* (blue circle) *G. baloni* (red circle), and *G. cernua* (green circle).

In order to have an optimised representation of the differences among groups, we performed a canonical variate analysis (CVA) that allows a different type of coordination analysis, which improves the separation of the specified groups (species/ecotypes; Klingenberg, 2011).

Figure 4 represents the shape changes associated with the canonical variate in the species from the genus *Gymnocephalus* in the Lower Danube. The scale factor for canonical variate (CV) shape changes is a unit of Mahalanobis distance of within-group shape changes (Klingenberg, 2011). We observed the variation in the pairs of fin insertions, eye diameter, head length and body depth, which is in connection with the elongation degree of body shape.

Figure 4: Transformation grid illustrating the shape variation associated with canonical variates (CV) in the ruffe species from the Lower Danube River. The scale for CV shape changes is in units of Mahalanobis distance.

The CVA generated the matrices of farthest Mahalanobis distances between the groups of *G. baloni* and *G. schraetser* (18.3364) and the smallest distances between *G. baloni* and *G. cernua* (8.7875). The P-values from permutation tests (10.000 permutation rounds) for Mahalanobis distances among the groups were statistically significant ($P < 0.05$).

The CVA chart represents the scatter plot of the canonical variate scores, displaying the species differentiation. The figure 5 allows the description of the relationship among the specimens of the groups by plotting the sample centroids of 90% confidence ellipses of the first two canonical variates CV1 and CV2.

Figure 5: Transformation grid illustrating the shape variation associated with canonical variates (CV) in the ruffe species from the Lower Danube River. The scale for CV shape changes is in units of Mahalanobis distance.

The statistical analysis of morphometric data is for *G. baloni* and *G. cernua*. The objective is in order to point out the phenotypic characters for reliable discrimination between these two species.

The performed factor analysis reveals the first three principal components sum up 59.267%, which means that the first three factors explain more than a half of the variance of all body variables (Fig. 6). Based on the variables with high loadings, it may be seen that PC1 includes the significant, positive contribution of the dorsal fin length, eye diameter, head depth and the negative contribution of the head length, mouth length, preanal, predorsal, preventral and preorbital distances (Fig. 6a). In the same way, PC2 indicates the positive contribution of characters as the pectoral-ventral fin distance, ventral-anal fin distance, and preanal distance, and the negative contribution of the caudal peduncle length (Fig. 6b). PC3 only reveals the positive contribution of the body depth, ventral length, anal fin length, and anal fin depth.

a)

b)

c)

Figure 6a-c: Variables loaded in the factor analysis of the morphometric measurement values of *Gymnocephalus baloni* and *Gymnocephalus cernua* (n = 32); the full names of variables are in material and methods.

We tested the differences between samples with T test. Table 5 notes the significant differences in the main characters of fish from the two groups ($P < 0.05$) as a character “S”.

Table 5: Mean values of variables (%) in *Gymnocephalus baloni* and *Gymnocephalus cernua*. Explanations of morphological characters are in the material and methods. S – significant differences.

No.	Morphological character	<i>G. baloni</i> mean (%)	<i>G. cernua</i> mean (%)
1.	BODYDEPTH ^S	28.327	26.043
2.	CAUDPEDDEPTH	8.945	8.819
3.	PREDORSSDIST	30.691	30.305
4.	CAUDPDLENGTH	20.827	21.767
5.	PREVENTDIST	33.118	34.181
6.	PREANALDIST	65.282	65.076
7.	PVDIST ^S	4.000	3.205
8.	VADIST ^S	32.982	31.210
9.	DLENGTH ^S	55.473	53.833
10.	ALENGTH	15.664	14.538
11.	ADEPTH	21.191	20.171
12.	PLENGTH	22.673	22.914
13.	VLENGTH	21.845	21.362
14.	HEADLENGTH	31.555	32.048
15.	HEADDEPTH ^S	77.800	73.581
16.	MOUThLENGTH	28.118	28.552
17.	EYEDIAMETER ^S	30.518	28.248

Having in view the similarity between *G. baloni* and *G. cernua*, a multivariate test for a morphometric comparison between the collected samples from the Danube River population (sea mile 80 from Black Sea) is used to complete the data regarding the morphological variation in the ruffe species and assess its plasticity.

A linear discriminant analysis using a backward stepwise method allows establishment of the most relevant morphometric features of *G. baloni* and *G. cernua* that together discriminate between the two samples of the fish species.

The between groups F-matrix and analysis of the disparities between the groups and all the variables (Engelman, 2005) reveals the correct classification of the ruffe samples with the farthest distance between *G. baloni* and *G. cernua* (6.754).

The reliability assessment of the discriminant analysis of morphometric characters of fish from the two species, computed by Wilks' lambda statistical index (Engelman, 2005) proved a significant discrimination with an advanced degree of confidence (Wilks' lambda = 0.1514, prob = 0.00008, $P < 0.05$).

The F-to-remove statistics (Engelman, 2005) from table 6 allows us to conclude that 10 of the morphological characters are the most useful together for discriminating among the species (the head features, body depth, the distances between pectoral, ventral and respectively anal fins, and the lengths of fin bases).

Table 6: F-to-remove statistics of the main morphometric characters assessed by the backward stepwise method for discrimination among the ruffe species: *Gymnocephalus baloni* and *Gymnocephalus cernua*. Explanations of morphological characters are in the material and methods.

Variable	F-to-remove	Tolerance
BODYDEPTH	22.69	0.200
PREDORSDIST	6.11	0.219
PREANALDIST	14.09	0.115
PVDIST	18.43	0.255
VADIST	8.27	0.332
ALENGTH	4.15	0.334
PLENGTH	5.56	0.356
VLENGTH	3.65	0.560
HEADLENGTH	6.09	0.057
HEADDEPTH	4.16	0.09

The classification matrix showed a high percentage of classification validation in all cases (100% correct classification).

Jackknife a form of cross-validation using a method of leaving out one case confirms the correct classification of the two sympatric ruffe species. The relative lower percent in the Jackknife panel (73% correct) could be attributed to the high number of predictors and small size of the ruffe samples.

The canonical scores plot generate the graphic with the confidence ellipse ($P < 0.05$) of each group, which is centred on the centroid of each group (Fig. 7). Based on the body measurement values, the groups are distinct.

Figure 7: Diagram of canonical scores (95% confidence level) based on 18 morphological characters (Tab. 1) for *Gymnocephalus cernuus* (blue circle) and *Gymnocephalus baloni* (brown circle) of the samples on which a discriminant analysis was applied (95% confidence ellipses are drawn around group centroids, and the variance proportions represent each canonical function as indicated).

CONCLUSIONS

The current paper completes the information regarding the body plasticity of the Danube ruffe (*Gymnocephalus baloni*) and its relationship with the other two species of the genus *Gymnocephalus* from the Lower Danube River.

We revealed a closer relationship between the population of *Gymnocephalus baloni* from the Lower Danube River and those from the middle stretch of the Danube River and the populations identified in rivers from the eastern part of Europe.

We found a closer phylogenetic relationship between the sister taxa *G. cernua* and *Gymnocephalus baloni* and a distance from *Gymnocephalus schraetser*, results that are consistent with some of previous studies.

Ten morphological characters are the most useful together for discriminating among the ruffe species from the Lower Danube River.

We also found a slightly less hydrodynamic body of *Gymnocephalus baloni* from the Lower Danube River comparing with the description of the holotype, which could be in connection with the phenotypic response of fish to environment.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the following individuals for their support: the anonymous reviewers, Dr. Curtean-Bănăduc A. and Dr. Bănăduc D., the editors of Transylvanian Review of Systematical and Ecological Research for the valuable, critical and helpful comments on the manuscript, and the fishers operating in Lower Danube River for cooperation and providing the fish samples.

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