

Morphometric differentiation of gudgeon species inhabiting the Carpathian Basin

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Abstract – The aim of this study was to examine morphometric differentiation of gudgeon (*Romanogobio* and *Gobio*) species inhabiting the Carpathian Basin, Hungary, with special regard to two recently described species classified into the *Gobio* genus. For statistical analyses, 23 morphometric and 8 meristic characters were measured on each of the 733 preserved specimens originating from museum collections. The analyses of the standardized morphometric traits revealed low intraspecific variability in all studied species, but varying degrees of interspecific differences. The strongest morphometric differentiation was found in *Romanogobio uranoscopus* (Agassiz, 1828), which can be characterized by the most special environmental needs. The two allopatric *Gobio* species showed the weakest morphometric difference. The meristic characters showed high variability in the *Romanogobio* spp., but did not show significant differences in the two *Gobio* species. The results show that the morphometric and meristic similarity of the studied *Gobio* species is definitely high, which preclude reliable identification based on phenotypic characters if only some specimens are available. It seems that the most usable discriminating feature for these species is the localization of the collection site.

Key words: *Gobio obtusirostris* / *Gobio carpathicus* / *Romanogobio* / meristic and morphometric traits / discriminant analysis

Introduction

The Gobiioninae subfamily forms a distinct taxonomic group within the Cyprinidae family (Kottelat and Freyhof, 2007). This taxon, which consists of about 30 genera and 130 species (Bănărescu, 1992; Bogutskaya and Naseka, 2004; Yang *et al.*, 2006), is widely distributed in Northern Eurasia. Although the highest number of species in the subfamily are present in South-West Asia (Nelson, 2006), a number of gudgeon species also appear in Europe (Kottelat and Freyhof, 2007). Originally all the native European gudgeon species were classified into one genus (*Gobio*). However, due to clear osteologic differences, a subgenus (*Romanogobio*) was raised up to genus level (Naseka, 1996; Naseka and Bogutskaya, 1998; Naseka and Poznjak, 2000; Bogutskaya and Naseka, 2004), which yielded the taxonomic revision of many gudgeon species. Nowak *et al.* (2008a) produced a comprehensive overview of the recent taxonomic status of the European gudgeon species, which can be shortly summarized as follows.

Until the end of the 20th Century, four gudgeon species were known from the Central European Carpathian Basin,

which is surrounded by the Carpathian Mountains, the Alps, the Dinarides and the Balkan Mountains. In recent years from the four Carpathian gudgeon species: European gudgeon [*Gobio gobio* (Linnaeus, 1758)], white-fin gudgeon [*Gobio vladkovi* (Fang, 1943)], sand gudgeon [*Gobio kesslerii* (Dybowski, 1862)] and Danube gudgeon [*Gobio uranoscopus* (Agassiz, 1828)], the latter three were rearranged to the *Romanogobio* genus. Only the sturdier bodied *G. gobio*, characterized by its dispersedly spotted dorsal and caudal fins, remained in the genus *Gobio*. The distribution area of this species covers almost the whole of Europe, and it can be found in highly different habitats (lentic and lotic). Consequently, considerable morphometric differences were found among its populations and many subspecies were described (Bănărescu, 1999). The *G. gobio* was considered as a common species in the Carpathian Basin as well (Harka and Sallai, 2004) and regarded as an indicator fish species of submontane/highland streams in their “natural” state (Erős, 2007; Takács, 2007; Sály *et al.*, 2009).

Some authors (*e.g.*, Vladykov, 1925; Jászfalusi, 1951; Bănărescu, 1954; Berinkey, 1962) noticed notable morphological differentiation of *G. gobio* stocks inhabiting the Tisza drainage system, located at the eastern part of the

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Carpathian Basin. The differences, however, were not considered to provide species level differentiation between the gudgeon stocks found in the eastern and the western part of the Carpathian Basin.

Molecular methods have also resulted in considerable taxonomic changes in the gudgeons of Central Europe. Based on genetic investigations (Freyhof and Naseka, 2005; Mendel *et al.*, 2008), the species *G. gobio* extends widely from the British Isles to the Black Sea coast. However, contrary to the former suppositions, this species is not indigenous in waters of the Carpathian Basin. As a result of this, a recent identification key (Kottelat and Freyhof, 2007), does not mention that the *G. gobio* is from this area. Conversely, two other gudgeon species, the Carpathian gudgeon (*Gobio carpathicus* Vladykov, 1925) and the Danube gudgeon (*Gobio obtusirostris* Cuvier and Valenciennes, 1842) are denoted from the Carpathian Basin. Both species are mentioned as synonyms of *G. gobio* by Bănărescu (1999). The border between the distribution areas of these species is formed by the North–South flow of the Hungarian Danube section (Kottelat and Freyhof, 2007). The *G. carpathicus*, originally described as *G. gobio carpathicus* by Vladykov (1925), is distributed in the drainage system of River Tisza, the largest tributary of the Danube (catchment area: 157 000 km²). Berinkey (1962) agrees with Bănărescu's (1954) opinion that the drainage system of the Upper and Middle Tisza is “terra typica” of this subspecies. Moreover, Jászfalusi (1951) described another subspecies from this drainage, named *G. gobio muresia*. Its distribution area is limited to one of the tributaries of the Lower Tisza drainage system. This subspecies complies with the *G. carpathicus* by Kottelat and Freyhof (2007).

The Danube gudgeon was originally described from a tributary of the Upper Danube. It is indigenous in the inflows of the Upper and Middle Danube, thus should be common in the drainage system of the Transdanubian region in Hungary (Kottelat and Freyhof, 2007). Although the genetic investigations show species level differentiations of the *Gobio* species inhabiting the Eastern and Western regions of the Carpathian Basin, their morphological differences have not been examined in detail so far. Further, the morphological keys given by Kottelat and Freyhof (2007) cannot be really used for distinguishing species inhabiting the Carpathian Basin, because they show complete overlap between the *G. carpathicus* and *G. obtusirostris*. Hungarian museum collections contain many gudgeon specimens that originate from numerous localities throughout the Carpathian Basin (*e.g.*, Sevecsik and Erős, 2008), with a high number of specimens originally identified as *G. gobio*. If it is accepted that *G. gobio* cannot be found in the Carpathian Basin, but there are two other *Gobio* species native in this area, then all the specimens previously identified as *G. gobio* must be assigned into these taxa. According to the map and area descriptions provided by Kottelat and Freyhof (2007), all the *Gobio* specimens originating from the western area of the Carpathian Basin (Transdanubian region) should be identified as *G. obtusirostris*, while all the *Gobio* specimens

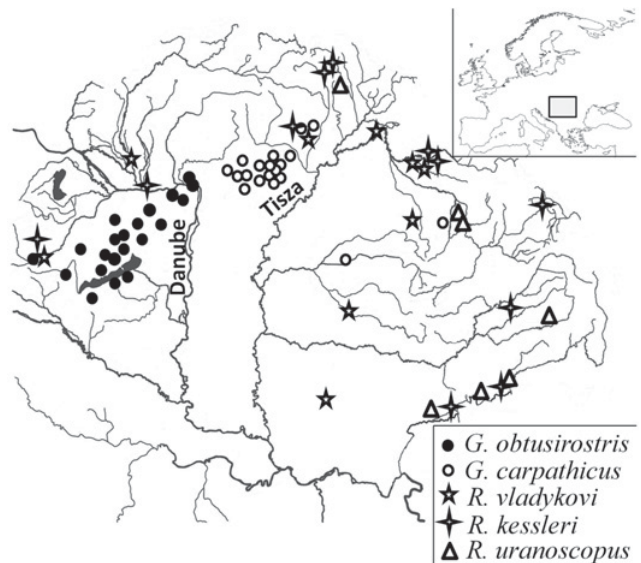


Fig. 1. European localization of the Carpathian Basin (a) and the collection sites in the basin (b).

collected from the Upper- and Middle-Tisza drainage should be identical to *G. carpathicus*. Consequently, to identify the scale of interspecific differentiations, hundreds of preserved specimens are available in museum collections. Consequently, the aim of this study is to identify the extent of morphometric differences between the gudgeon species inhabiting the Carpathian Basin, having special regard to the species classified into the *Gobio* genus using preserved specimens in museum collections.

Materials and methods

Specimens used for the morphometric studies originated from three Hungarian museums (Hungarian Natural History Museum, Budapest; Bakony Museum, Zirc; and Mátra Museum, Gyöngyös). Altogether 733 gudgeon specimens were measured. The number of preserved specimens of each species correlated well with their occurrence in Hungarian waters (personal observation). The highest number of specimens was species previously identified as *G. gobio* ($n = 409$). From the Upper- and Middle-Tisza drainage system, 202 *G. carpathicus* specimens from 19 localities were measured and from the Western part of the basin, 207 *G. obtusirostris* specimens from 21 localities were measured. From the *Romanogobio* genus, specimens of 128 *R. vladykovi* from 9 localities, 135 *R. kesslerii* from 11 localities and 61 *R. uranoscopus* from 7 localities were used in the analyses (Fig. 1). Altogether 23 morphometric characters (Fig. 2) were measured on each specimen with a digital calliper, which were found to be the most important variables in the morphometric identification of gobionins (Bănărescu, 1954, 1999; Berinkey, 1966; Harka, 1986). Standard length (SL) was measured to the end of scale cover.

Preservation causes deformations on the body shape (Shields and Carlson, 1996; Neave *et al.* 2006), but it was

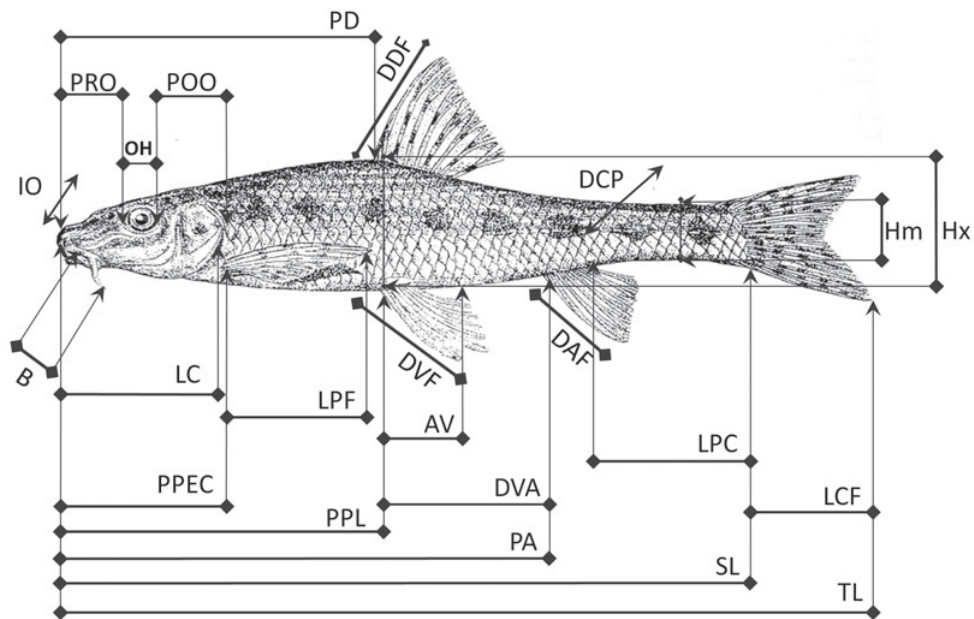


Fig. 2. Morphometric characters measured. Abbreviations: AV, distance between the anal aperture and ventral fin; B, length of barbel; DAF, depth of anal fin; DCP, diameter of caudal peduncle; DDF, depth of dorsal fin; DVA, distance between the ventral and anal fin; DVF, depth of ventral fin; Hx, maximum body depth; IO, interorbital distance; LC, head length; LCF, length of caudal fin; LPC, length of caudal peduncle; LPF, length of pectoral fin; Mm, minimum body depth; OH, eye diameter; PA, preanal distance; PD, predorsal distance; POO, postorbital distance; PPEC, prepectoral distance; PPL, prepelvic distance; PRO, preorbital distance; SL, standard length; TL, total length.

assumed that these deformations affected each specimen uniformly. To minimize conservation bias and ontogenic differences, only the well preserved, adult ($SL > 50$ mm) specimens were used for our study (Bănărescu, 1999). In order to eliminate any size effect in the dataset, the allometric formula of Elliott *et al.* (1995) was used:

$$M_{adj} = M(L_s/L_o)^b$$

where is the original measurement, M_{adj} is the size adjusted measurement, L_o is the SL of fish, L_s is the overall mean of SL for all fish from all samples in each analysis.

Parameter “ b ” was estimated for each character from the observed data as the slope of the regression of $\log M$ on $\log L_o$, using all fish in all groups. The efficiency of size adjustment transformations was assessed by testing the significance of correlations between transformed variables and SL.

To determine the scale of the intraspecific variability, all the standardized data of each specimen were compared with the others classified into the same species using the Bray–Curtis similarity index. Uni- and multivariate analysis of variance (ANOVA) were carried out to test the significance of morphometric differences among the studied species. On the entire standardized dataset principal component analysis (PCA) and discriminant analyses were performed. To test the among species similarities, analysis of similarity (ANOSIM) was used. The between species differences were analysed by ANOVA, Tukey *post hoc* test for each variable. Statistical analyses were performed using SPSS v12.0.1 and PAST 1.95 (Hammer

et al., 2001), graphs were generated using PAST 1.95 and Syn-Tax 2000 (Podani, 2001).

Altogether eight meristic variables were studied. The investigated meristic variables were the number of scales on the lateral line, the number of scales above and below the lateral line, and the number of soft fin rays. The variances of the meristic data were compared using Kruskal–Wallis tests.

Results

For each species, the ranges (min–max) and the means (\pm SD) of SL, and the values of the other characters as a percentage of SL and head length (LC) are shown in Table 1. After the allometric transformation, only total length (TL) showed significant correlation ($P < 0.05$) with the SL. This result indicates that the size effect was removed effectively in the case of the other 21 characters studied.

Considering the entire dataset, the mean intraspecific similarity proved to be high in all cases (*R. uranoscopus*: 0.966 ± 0.01 , *R. kesslerii*: 0.965 ± 0.01 , *R. vladkovi*: 0.962 ± 0.01 , *G. obtusirostris*: 0.962 ± 0.01 , *G. carpathicus*: 0.960 ± 0.01). The univariate ANOVA revealed significant differences between the means of the five gudgeon species for all the 21 standardized morphometric variables ($P < 0.01$). The comparison of the within and among group variations (F -ratios) referred to the maximum (Hx) and minimum (Hm) body height, the post (POO) and interorbital distances (IO) and LC are the most

Table 1. Morphometric characteristic of the analysed gudgeons. The meaning of the abbreviations can be found in the caption of Figure 2.

	Go	Gc	Rv	Rk	Ru
SL (min–max)	53.4–110.8	51.9–111.8	52.7–99.7	51.8–100.2	50.7–99.8
SL (mean \pm SD)	75.91 \pm 15.1	76.26 \pm 14.4	74.30 \pm 17.4	84.16 \pm 17.3	83.37 \pm 14.4
Percentage of SL					
AV	0.11 \pm 0.03	0.11 \pm 0.02	0.13 \pm 0.05	0.11 \pm 0.04	0.09 \pm 0.02
DAF	0.19 \pm 0.04	0.18 \pm 0.04	0.21 \pm 0.06	0.19 \pm 0.04	0.22 \pm 0.04
DCP	0.06 \pm 0.02	0.05 \pm 0.01	0.07 \pm 0.02	0.06 \pm 0.01	0.07 \pm 0.02
DDF	0.18 \pm 0.04	0.17 \pm 0.03	0.20 \pm 0.05	0.18 \pm 0.04	0.21 \pm 0.04
DVA	0.19 \pm 0.04	0.19 \pm 0.04	0.24 \pm 0.07	0.20 \pm 0.05	0.19 \pm 0.04
DVF	0.18 \pm 0.04	0.17 \pm 0.03	0.20 \pm 0.05	0.18 \pm 0.04	0.21 \pm 0.04
Hm	0.11 \pm 0.02	0.10 \pm 0.02	0.11 \pm 0.04	0.08 \pm 0.02	0.08 \pm 0.02
Hx	0.24 \pm 0.06	0.22 \pm 0.04	0.26 \pm 0.08	0.19 \pm 0.05	0.20 \pm 0.04
LC	0.29 \pm 0.06	0.28 \pm 0.06	0.31 \pm 0.09	0.27 \pm 0.06	0.30 \pm 0.06
LCF	0.21 \pm 0.05	0.20 \pm 0.05	0.26 \pm 0.07	0.22 \pm 0.05	0.23 \pm 0.05
LPC	0.24 \pm 0.05	0.24 \pm 0.05	0.29 \pm 0.08	0.27 \pm 0.06	0.28 \pm 0.05
LPF	0.22 \pm 0.05	0.21 \pm 0.05	0.25 \pm 0.06	0.23 \pm 0.05	0.27 \pm 0.06
PA	0.75 \pm 0.16	0.75 \pm 0.15	0.90 \pm 0.27	0.75 \pm 0.16	0.78 \pm 0.15
PD	0.51 \pm 0.11	0.51 \pm 0.10	0.59 \pm 0.17	0.51 \pm 0.11	0.56 \pm 0.10
PPEC	0.28 \pm 0.06	0.27 \pm 0.06	0.31 \pm 0.10	0.26 \pm 0.06	0.28 \pm 0.06
PPL	0.52 \pm 0.11	0.52 \pm 0.11	0.61 \pm 0.18	0.52 \pm 0.11	0.56 \pm 0.11
Percentage of LC					
B	0.29 \pm 0.05	0.25 \pm 0.04	0.33 \pm 0.06	0.34 \pm 0.05	0.38 \pm 0.06
IO	0.26 \pm 0.02	0.26 \pm 0.02	0.26 \pm 0.02	0.24 \pm 0.02	0.24 \pm 0.02
OH	0.23 \pm 0.02	0.23 \pm 0.02	0.26 \pm 0.03	0.23 \pm 0.02	0.20 \pm 0.02
POO	0.44 \pm 0.03	0.45 \pm 0.03	0.41 \pm 0.03	0.42 \pm 0.03	0.40 \pm 0.02
PRO	0.37 \pm 0.03	0.35 \pm 0.04	0.36 \pm 0.03	0.38 \pm 0.03	0.44 \pm 0.03

Table 2. *F*-ratios and among species comparisons of the studied variables.

	<i>F</i> -ratio	Gc	dif	Go	dif	Rv	dif	Rk	dif	Ru
Hm	464.8	A	=	A	>	B	>	C	=	C
Hx	278.2	A	=	A	>	B	>	C	=	C
POO	173.6	A	=	A	>	B	=	B	=	B
IO	117.9	A	=	A	>	B	>	C	<	B
LC	114.6	A	>	B	>	C	=	C	<	D
B	98.2	A	>	B	<	AC	=	C	<	D
OH	94.2	A	=	A	<	B	>	C	>	D
PRO	91.9	A	>	B	>	C	<	B	<	D
PPEC	77.4	A	=	A	>	B	>	C	<	D
LPF	69.7	A	>	B	=	B	<	A	<	C
PD	54.6	A	=	A	>	B	=	B	<	A
DAF	53.8	A	>	B	=	B	=	B	<	C
DVF	44.5	A	>	B	=	B	=	B	<	C
DCP	39.0	A	>	B	<	A	=	A	<	C
LPC	38.2	AB	=	A	>	B	>	C	=	C
PA	35.9	A	=	A	=	A	>	B	=	B
AV	34.8	A	=	A	=	A	>	B	>	C
PPL	30.9	A	=	A	>	B	>	C	<	D
DDF	19.3	A	>	B	<	A	>	C	=	C
DVA	17.8	A	=	AB	=	B	=	AB	>	C
LCF	5.7	A	=	A	<	B	=	AB	=	AB

Variables marked by the same letters do not differ significantly ($P > 0.05$) based on ANOVA *post hoc* comparisons. Abbreviations: dif, difference; Gc, *G. carpathicus* stock from the eastern area of the Carpathian Basin; Go, *G. obtusirostris* stock from the western area of the Carpathian Basin; Rv, *R. vladykovi*; Rk, *R. kesslerii*; Ru, *R. uranoscopus*. The meaning of the abbreviations can be found in the caption of Figure 2.

important variables measured, those were responsible for the global (interspecific) differentiations (Tab. 2). The ANOVA Tukey *post hoc* test (Tab. 2) showed significant between species differences for each variable. The highest

similarity was found between the two *Gobio* species, from the studied 21 characters only 8 showed significant differences. The results of PCA showed considerable differentiations in the case of *R. uranoscopus* and *R. kesslerii*

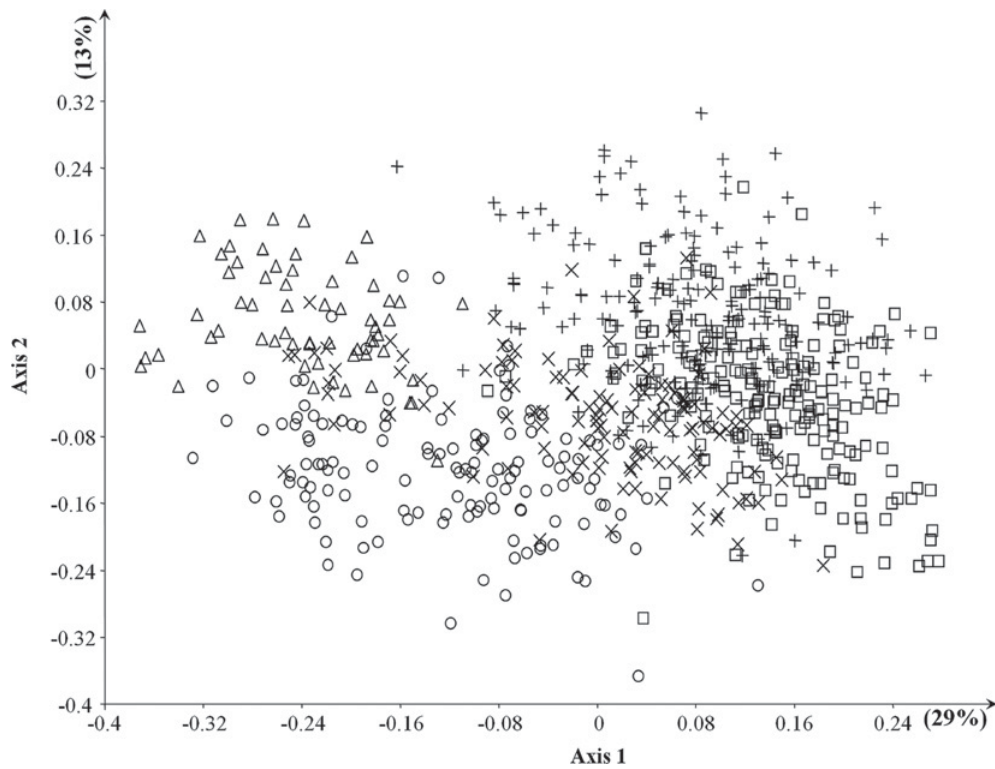


Fig. 3. PCA plot of morphometric data. Δ , *R. uranoscopus*; \circ , *R. kesslerii*; \times , *R. vladkovi*; +, *G. carpathicus* stock from the eastern area of the Carpathian Basin; \square , *G. obtusirostris* stock from the western area of the Carpathian Basin.

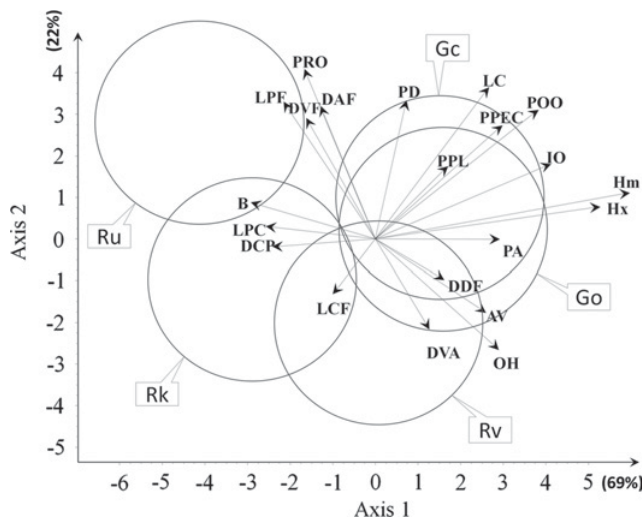


Fig. 4. Plot of the discriminant analysis derived from morphometric data. Abbreviations: Gc: *G. carpathicus* stock from the eastern area of the Carpathian Basin; Go, *G. obtusirostris* stock from the western area of the Carpathian Basin; Rv, *R. vladkovi*; Rk, *R. kesslerii*; Ru, *R. uranoscopus*. The isodensity circles contain the 95% of the specimens classified into a certain group. The meaning of the abbreviations can be found in the caption of Figure 2.

stocks than the others (Fig. 3). The statistical analysis of the entire dataset using ANOSIM showed that all the studied groups differ significantly ($P < 0.01$) from the others.

The discriminant analysis showed remarkable differences between the studied *Romanogobio* species (Fig. 4). Data points belonging to the two *Gobio* species showed almost complete overlap, which is also indicated by the isodensity circles, which contain 95% of the specimens classified into a certain group (Fig. 4). The overall random assignment of individuals into their original group was 84.7% (Tab. 3). The proportion of individuals correctly classified into their original group was highest in the case of *R. kesslerii* (97.3%) and *R. uranoscopus* (95.1%), indicating that these samples are highly different from the others. In the case of *R. vladkovi*, the proportion was lower (86.7%). The two *Gobio* species had the highest proportion of misclassified cases, with their original (preliminary) classification proving the most doubtful (73.3 and 83.6% for *G. carpathicus* and the *G. obtusirostris*, respectively). From the misclassified specimens in these groups 24.3 and 14% were classified into the group of the other *Gobio* species. The study made on the eight meristic variables (Tab. 4) showed significant differences between the *Romanogobio* species (e.g., the number of scales above the lateral line and the number of soft rays), but no significant differences were found between the two *Gobio* species in any cases.

Discussion

Morphometric and meristic traits have been used for a long time for the identification of European gudgeon

Table 3. Comparison of the original and the predicted group memberships given by the discriminant analysis.

Original grouping	Predicted group memberships						
		Gc	Go	Rv	Rk	Ru	
Specimen number	Gc	202	148	49	5	0	0
	Go	207	29	173	5	0	0
	Rv	128	5	7	111	5	0
	Rk	135	0	0	4	131	0
	Ru	61	0	0	0	3	58
Percent (%)	Gc	100	73.3	24.3	2.5	0.0	0.0
	Go	100	14.0	83.6	2.4	0.0	0.0
	Rv	100	3.9	5.5	86.7	3.9	0.0
	Rk	100	0.0	0.0	3.0	97.0	0.0
	Ru	100	0.0	0.0	0.0	4.9	95.1

Gc, *G. carpathicus* stock from the eastern area of the Carpathian Basin; Go, *G. obtusirostris* stock from the western area of the Carpathian Basin; Rv, *R. vladykovi*; Rk, *R. kesslerii*; Ru, *R. uranoscopus*.

Table 4. The average (min–max) values of meristic variables counted on gudgeon specimens.

Meristic variables (N°)		Gc	P	Go	P	Rv	P	Rk	P	Ru
Scales	On the lateral line	40.4 (39–43)	ns	40.0 (38–42)	•	42.6 (40–44)	ns	42.8 (40–44)	ns	42.0 (40–43)
	Above the lateral line	5.3 (5–6)	ns	5.1 (5–6)	ns	4.9 (4–5)	•	4.4 (4–5)	•	5.0 (5–5)
	Below the lateral line	3.6 (3–4)	ns	3.6 (3–4)	•	3.1 (3–4)	ns	3.0 (3–4)	ns	3.0 (3–3)
Soft rays in	Pectoral fin	12.6 (12–13)	ns	12.2 (12–13)	•	11.7 (11–13)	•	13.4 (12–14)	ns	13.0 (13–13)
	Ventral fin	6.6 (6–7)	ns	6.3 (6–7)	ns	6.7 (6–7)	ns	6.8 (6–8)	•	6.1 (6–7)
	Anal fin	5.9 (5–7)	ns	5.8 (5–7)	ns	6.0 (6–6)	ns	6.7 (6–7)	ns	6.3 (6–7)
	Dorsal fin	7.0 (7–7)	ns	7.0 (7–7)	ns	7.0 (7–7)	•	8.0 (8–8)	•	7.0 (7–7)
	Caudal fin	19.4 (18–20)	ns	19.5 (18–21)	ns	19.3 (19–20)	•	18.6 (18–20)	ns	19.0 (19–19)

• = $P < 0.05$, ns = $P > 0.05$; Gc, *G. carpathicus* stock from the eastern area of the Carpathian Basin; Go, *G. obtusirostris* stock from the western area of the Carpathian Basin; Rv, *R. vladykovi*; Rk, *R. kesslerii*; Ru, *R. uranoscopus*.

species (Berg, 1949; Bănărescu, 1954, 1961, 1962, 1999; Vasil'eva *et al.*, 2004, 2005; Kottelat and Persat, 2005; Naseka *et al.*, 2006; Kottelat and Freyhof, 2007; Nowak *et al.*, 2008b, 2009, 2011). But whereas the ranges of the morphometric variables usually overlap, the application of these traits for taxa differentiation is questioned by many authors (*e.g.*, Bănărescu, 1961, 1962, 1999; Koščo, 1997; Mendel *et al.*, 2008). The present study revealed varying degrees of morphometric and meristic differences in the gudgeon species inhabiting the Carpathian Basin. The results show that all these gudgeon species are distinguishable if a proper number of morphometric characters are used. By completing detailed measurements, significant differences in body shape and the meristic characters can be found among the *Romanogobio* species inhabiting the area. The discriminating traits among these three species agree well with data in the literature (Bănărescu, 1999; Kottelat and Freyhof, 2007).

The morphologic differences show high similarity with the habitat preferences of the studied species. The highest interspecific and the lowest intraspecific variation were found in *R. uranoscopus*. This species can be characterized by the most different body shape, caused by its adaption to the environmental circumstances of mountain rivers (*e.g.*, the deepest fusiform body shape, the longest paired fins and the most elongated caudal peduncle, etc.). The other two *Romanogobio* species appear commonly in the same habitats in the upper sections of the Hungarian lowland rivers (Harka and Sallai, 2004). The morphometric traits

of the *R. kesslerii* show transition between the *R. uranoscopus* and the *R. vladykovi* (Fig. 3). Notwithstanding they can be separated from good efficiency using the combination of the morphometric characters measured.

At the same time, remarkable differences were also found in the case of meristic characters, especially for the number of soft rays in the dorsal fins. Among the *Romanogobio* species only the *R. vladykovi* shows major overlap with the *Gobio* species morphologically. Simultaneously, this is the only *Romanogobio* species that commonly appears in the preferred habitat types of the *Gobio* species (Bănărescu, 1964; Harka *et al.*, 2004). The high-level morphologic similarity in the case of the species inhabiting the same habitat types, may suggest that the same kind of adaptation to the environmental circumstances occurred. But it is more likely that interspecific hybridization can occur between these closely related species. And these hybrids may show transitional morphologic traits, or the descendants show both parents' traits at the same time. According to some authors the intraspecific hybridization is plausible in the case of Central European gudgeon species (Bănărescu, 1999; Mendel *et al.*, 2008). The presence of hybrids in the museum collections cannot be ruled out as well. The detection of some hybrid specimens is plausible taking into account the body shape and the presence or absence of the epidermal crests on the dorsal region. But, I think, the identification of the F2, F3, etc. generation hybrids are impossible using just only morphometric methods.

For proving the effect of hybridization on the body shape simultaneous genetic and morphometric measurements would be needed. Unfortunately, the specimens studied were mostly preserved in formalin, thus genetic studies were unaccomplishable in this case.

The highest level of morphometric similarity was detected between the two *Gobio* species. This, however, was not a consequence of hybridization, as there are considerable hydrological distances between their occurrences. In the case of small bodied, benthic fish species that are characterized by shorter generation time, the effect of genetic drift is much more significant than in the case of other species (e.g., migratory species) characterized by other life strategies (Hänfling and Brandl, 1998a, b; Brito and Coelho, 1999). Moreover, the Carpathian Basin was not glaciated during the ice age (Taberlet *et al.*, 1998; Emerson and Hewitt, 2005) so the separation and the restricted gene flow between the eastern and western *Gobio* populations may have existed for a long time. This may cause significant differences in phenotypic traits. In addition, a previous study conducted in the Middle-Tisza drainage showed remarkable genetic differences between the *Gobio* populations within small spatial scales as well (Takács *et al.*, 2008).

Notwithstanding the high *Gobio* specimen number (the comparison of more than 8500 data of 409 specimens), the morphometric differences found less pronounced than in the case of the three *Romanogobio* species. Only 8 of the 21 morphometric traits used for the analysis showed significant differences. It should be noted that more than twice as many specimens were measured in the case of the two *Gobio* species, as in the case of the other three, and these *Gobio* specimens may have originated from collection sites characterized with highly different environmental conditions. As it is proved in many cases (Karakousis *et al.*, 1991; Bourke *et al.*, 1997; Fulton *et al.*, 2001; Brinsmead and Fox, 2002; Turan *et al.*, 2005; Çakmak and Alp, 2010), environmental circumstances can significantly affect the morphologic features. It may be presumed that stocks originating from higher numbers of collection sites show higher morphologic variability. In our case the intraspecific similarities were exactly the same, above 95% in the case of each species. Therefore, the higher specimen number and the higher number of collection sites in the case of *Gobio* species did not affect our results considerably.

Within field conditions, the countable, meristic traits are much more usable for identification than the morphometric features, which need standardization. Results show convincingly that the combination of these traits is well applicable for differentiating the *Romanogobio* species. But in the case of *G. obtusirostris* and *G. carpathicus* the meristic traits used did not show significant detachments, thus these characters are not usable for differentiation.

In summary, considerably higher differences were encountered among the *Romanogobio* species inhabiting the Carpathian Basin than in the case of the two newly described *Gobio* species native in this area. Although the detailed morphometric studies showed significant

differentiation among all the studied species, these results are assignable to the high specimen number. As the extent of similarity between the studied *Gobio* species is high and the meristic traits show no significant differences then their differentiation is difficult if there are only a small number of specimens available. Moreover, it seems that the most usable discriminating feature for these species is the localization of the collection site. Regarding the fact that a genetic study (Mendel *et al.*, 2008) classified these two species into different lineages, it is suggested that these taxa can be handled as sibling species (Bickford *et al.*, 2007). Although molecular biology studies have proved genetic isolation, the segregation has not been apparent morphometrically.

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