Natural hybridization of *Barbus bocagei* x *Barbus comizo* (Cyprinidae) in Tagus River basin, central Spain

by

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ABSTRACT. - Hybrids of *Barbus bocagei* and *Barbus comizo* were found in the river Tagus, Madrid province, central Spain. Morphological univariate and multivariate analyses show that hybrids are intermediate in shape between the parent species. The potential causes of the hybridization are discussed.

RÉSUMÉ. - Hybridation naturelle de *Barbus bocagei* x *Barbus comizo* (Cyprinidae) dans le bassin du Tage, Espagne.

Dans cette étude, nous rapportons la présence d'hybrides entre *Barbus bocagei* et *Barbus comizo* dans le fleuve Tage, Espagne. Des analyses uni- et multivariées montrent que les hybrides ont des caractères morphologiques intermédiaires entre les espèces parentales. Nous discutons les raisons possibles de cette hybridation.

Key words. - Cyprinidae - *Barbus bocagei* - *Barbus comizo* - Spain - Tagus River - Hybridization.

Hybridization under natural conditions is a well known common phenomenon in freshwater fishes, in particular between cyprinids (Scribner *et al*., 2001). Two species of Iberian endemic barbels, *Barbus bocagei* Steindachner, 1865 and *Barbus comizo* Steindachner, 1865, live in the Tagus River basin, central Spain. A previously non described hybrid was discovered when sampling fishes in that river. This study deals with the morphological description of the hybrid, its distinction and similarities with the parent species, and a discussion about the likely factors affecting this hybridization.

MATERIAL AND METHODS

Fish sampling was carried out from June 1996 to October 1998 in the River Tagus between the Bolarque Dam and the confluence with the River Jarama. This river section is about 150 km long and its altitude ranges from 480 to 642 m. Sample sites and fish species communities were described by Elvira *et al*. (1998). The examined material was collected by electrofishing and killed by lethal dose of anaesthetic in accordance to the recommended ethical guidelines. Fish were fixed in 70% alcohol and deposited at the Museum of Comparative Morphology and Anatomy of Vertebrates, Universidad Complutense de Madrid.

A sample of 83 specimens was used for the morphological analysis, consisting on 40 specimens of *Barbus bocagei* (167-342 mm in fork length), 21 specimens of *Barbus comizo* (170-250 mm), and 22 specimens suspected to be hybrids between these species (186-257 mm). The identification of the parent species was based on the morphological characters proposed by Almaça and Banarescu (2003a, 2003b), and the key by Banarescu and Bogutskaya (2003a).

A total of nine variables were studied. Eight were morphometric characters: fork length (Fl), preorbital distance (prO), postorbital distance (poO), length of head (lc), interorbital distance (io), depth of dorsal fin = length of the last unbranched ray (hD), length of the ossified section of the last unbranched ray (hDo), and length of the denticulated section of the last unbranched ray (hDd). Measurements were taken to the nearest 0.01 mm using an electronic digital calliper, following Banarescu and Bogutskaya (2003b). Furthermore, a meristic character was considered: the number of denticles of the last unbranched ray of the dorsal fin (ndD). A combination of two variables was also calculated: density of denticles (densd) = number of denticles (ndD) / length of the denticulated section of the last unbranched ray (hDd).

All variables were log-transformed (natural logarithms, Ln) and simple correlations among all characters were performed. Assumptions of normality of distributions and homogeneity of variances were verified through Kolmogorov-Smirnov and Levene's tests, respectively. The significance level for all statistical tests was set at $p = 0.05$.

RESULTS AND DISCUSSION

The seven first variables (Fl, prO, poO, lc, io, hD and hDo) were all significantly correlated among them and con-

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sequently with size; while the other three variables (hDd, ndD and densd) were not significantly correlated with the first seven variables and consequently are not associated with size. Eight log-transformed variables (Fl, prO, poO, lc, io, hD, hDo and densd) fulfil the normality and homogeneity of variance assumptions, while two variables (hDd and ndD) do not. Consequently, parametric tests (ANOVA, ANCOVA and post-hoc Tukey's tests) were performed for the first eight variables set, while non-parametric tests (Kruskal-Wallis and post-hoc Dunn's tests) were done for the other two. ANCOVA (with Fl as the covariate) was performed for the parametric variables associated with size.

The relative position of the hybrids and the parents on a morphometric multidimensional space was assessed through principal component analyses (PCA) performed on the nine above listed variables. In order to study the size-free shape differences between species and hybrids, the variables were also analysed through Burnaby's size correction method (Burnaby Principal Component Analysis, BPCA) using the programme BURNABY PCA (available from N. MacLeod, Natural History Museum, London, http://www.nhm.ac.uk/hosted_sites/paleonet/ftp/ftp.html). In the BPCA approach size and shape components were separated and a multivariate analysis of shape was accomplished eliminating the contribution of size on the second and following principal components (shape components) and restricting the size component to PC I (PC, principal component). This procedure is considered to be the most effective traditional morphometric method for isolating shape from size variation, to successfully differentiate morphologically similar populations and species of fish.

No significant differences were found for size (fork length, Fl) and length of the ossified section of the last unbranched ray (hDo) of three groups (*B. bocagei*, *B. comizo* and hybrids), but overall between-groups differences were significant for the other eight morphological characters analyzed (Tab. I). However, the multiple comparisons tests showed that each group was significantly different from the other two for lc, io and densd; while differences between hybrid and *B. bocagei* for poO and hD, and between hybrid and *B. comizo* for prO, hDd and ndD were not found to be statistically significant (Tab. I). All these results show that hybrids are clearly intermediate in shape between both parental species, being closer to *B. bocagei* in some features while to *B. comizo* in others.

The level of ossification of the last unbranched ray of the dorsal fin (measured as hDo) does not allow to distinct the parental species nor the hybrid. On the other hand, the length of head (lc), the interorbital distance (io) and the density of denticles of the last unbranched ray of the dorsal fin (densd) are characters significantly distinct among both parentals and hybrid. *B. bocagei* has a shorter and broader head than *B. comizo*, while hybrids are intermediate. Likewise, density of denticles is higher in *B. bocagei* than in *B. comizo*, while is intermediate in hybrids.

Preorbital distance (prO) is similar between hybrid and *B. comizo*, and shorter in *B. bocagei*; while length of denticulated section of the last unbranched ray (hDO) and the number of denticles (ndD) are similar between hybrid and *B. comizo* and lower in *B. bocagei*. Finally, postorbital distance (poO) and length of the last unbranched ray (hD) are similar between hybrid and *B. bocagei*, while are respectively higher and lower in *B. comizo*.

Multivariate analysis for morphometric data showed that PC 1 accounted for 89% of the variation and that the most significant weightings were from the length of the denticulated section of the last unbranched ray (hDd) and the number of denticles (ndD) (Tab. II). The size-free shape component BPC 2 was mainly defined by variables prO and hDd, whereas BPC3 was mainly related to variables prO and poO (Tab. II). *B. bocagei* and *B. comizo* were clearly separated when plotting all the individual scores on BPC 2 and

Table I. - Results of statistical tests for total samples of *B. bocagei* (n = 40), *B. comizo* (n = 21) and hybrids (n = 22). Post-hoc comparisons show significant differences among three samples. See text for characters abbreviations. *[Résultat des tests statistiques pour les trois groupes* B. bocagei *(n = 40),* B. comizo *(n = 21) et hybrides (n = 22). Les tests* a posteriori *montrent l'existence de différences significatives entre les trois groupes. Voir le texte pour les abréviations.]*

				B. bocagei	Hybrid	*B. comizo*	Post-hoc
Character	Test	Test value	p value	Mean ± SE	Mean ± SE	Mean ± SE	comparison
Ln Fl	ANOVA	$F_{(2,80)} = 2.99$	p = 0.06	5.42 ± 0.022	5.39 ± 0.029	5.33 ± 0.030	*b* = h = *c*
Ln prO	ANCOVA	$F_{(2,79)} = 7.92$	p < 0.001	2.88 ± 0.017	2.97 ± 0.023	2.99 ± 0.024	*b* < h = *c*
Ln poO	ANCOVA	$F_{(2,79)} = 12.44$	p < 0.001	3.11 ± 0.009	3.14 ± 0.011	3.19 ± 0.012	*b* = h < *c*
Ln lc	ANCOVA	$F_{(2,79)} = 14.17$	p < 0.001	3.87 ± 0.009	3.91 ± 0.012	3.95 ± 0.013	*b* < h < *c*
Ln io	ANCOVA	$F_{(2,79)} = 22.36$	p < 0.001	2.88 ± 0.009	2.82 ± 0.011	2.78 ± 0.012	*b* > h > *c*
Ln hD	ANCOVA	$F_{(2,79)} = 4.39$	p = 0.02	3.59 ± 0.009	3.57 ± 0.011	3.54 ± 0.012	*b* = h > *c*
Ln hDo	ANCOVA	$F_{(2,79)} = 2.47$	p = 0.09	3.34 ± 0.014	3.36 ± 0.018	3.39 ± 0.019	*b* = h = *c*
Ln hDd	Kruskal-Wallis	$H_{(2,N = 83)} = 51.06$	p < 0.001	1.30 ± 0.117	2.55 ± 0.155	2.60 ± 0.162	*b* < h = *c*
Ln ndD	Kruskal-Wallis	$H_{(2,N = 83)} = 37.63$	p < 0.001	1.68 ± 0.151	3.01 ± 0.207	3.10 ± 0.199	*b* < h = *c*
Ln densd	ANOVA	$F_{(2,64)} = 24.29$	p < 0.001	1.09 ± 0.014	1.02 ± 0.014	0.95 ± 0.015	*b* > h > *c*

Table II. - Weights of morphological characters on the first three principal components, relative eigenvalues and percentage of explained variance for total samples of *B. bocagei*, *B. comizo* and hybrids. See text for characters abbreviations. *[Poids des caractères morphologiques sur les trois premiers axes de l'analyse en composantes principales pour les échantillons de* B. bocagei, B. comizo *et hybrides. Voir le texte pour les abréviations.]*

Character	PC 1	BPC 2	BPC 3
Ln Fl	0.046	-0.007	0.344
Ln prO	0.027	-0.045	0.445
Ln poO	0.038	-0.025	0.432
Ln lc	0.030	-0.031	0.395
Ln io	0.073	0.017	0.362
Ln hD	0.049	0.001	0.278
Ln hDo	0.032	-0.020	0.325
Ln hDd	-0.680	-0.035	0.126
Ln ndD	-0.724	0.021	0.095
Eigenvalue	7.976	0.820	0.087
Explained variance	88.6%	9.11%	0.97%

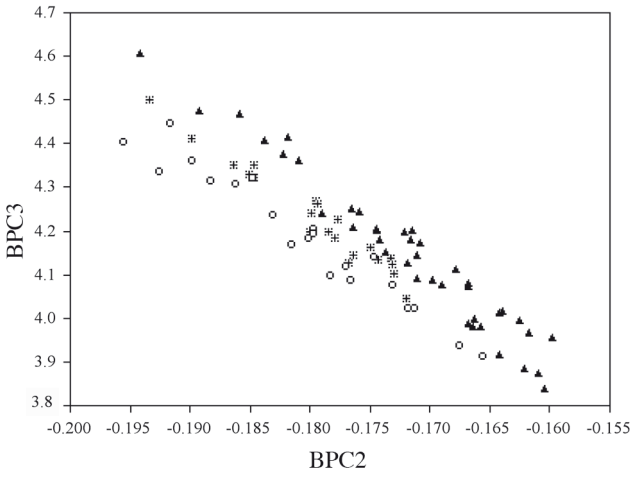

Figure 1. - Multivariate differentiation of samples of *B. bocagei* (triangles), *B. comizo* (open circles) and hybrids (asterisks) according to BPC 2 and BPC 3 in table II. *[Projection des espèces parentales,* B. bocagei *(triangles) et* B. comizo *(cercles), et hybrides (astérisques) sur le plan des axes BPC 2 et BPC 3 (Tab. II).]*

BPC 3 (Fig. 1), whereas hybrids were morphologically intermediate to parent species.

The genus *Barbus* "sensu stricto" in Europe consists of two subgenera: *Barbus* and *Luciobarbus* (Doadrio, 1990; Berrebi and Tsigenopoulos, 2003). Doadrio *et al*. (2002) found that the least amount of divergence in the Iberian species of subgenus *Luciobarbus* occurs between *B. comizo* and *B. bocagei*. Hybridization between *B. comizo* and *B. bocagei* has been postulated by Callejas and Ochando (2000), but after Doadrio *et al*. (2002) it was not based on definite evidence. Hybridization is not rare in *Barbus* and two cases are currently well known, the hybridization between *B. meridionalis* and *B. barbus* in France (Berrebi *et al*., 1993) and *B. meridionalis* and *B. haasi* in Spain (Machordom *et al*., 1990).

The morphology of the hybrids now described is clearly intermediate between those of the parents. Hybrids do not show a greater resemblance to *B. comizo* than to *B. bocagei*. These species live in sympatry in the River Tagus, but their habitat preferences are rather different. *B. bocagei* occupy lotic environments principally within the upper reaches, whereas *B. comizo* is restricted to middle reaches of lentic character. However, dam building in the River Tagus during the second half of the 20th century changed the original fluctuating and lotic condition to a regulated and mostly lentic environment (Elvira *et al*., 1998). Anthropogenic river disturbance currently limits barbel spawning habitat, so that species which may have segregated spatially in the past are now forced to spawn in proximity. The observed interspecific hybridization could be then attributed to accidental cross-fertilization resulting from simultaneous spawning in a shared breeding area. Human influences have historically been invoked as important contributors to hybridization (Scribner *et al*., 2001). However, further genetic and karyological studies are needed to shed light on the origin of the ongoing hybridization and the likely consequences on phylogenetic reconstruction.

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