

***Chondrostoma polylepis* Steindachner × *Rutilus lemmingii* (Steindachner) (Osteichthyes, Cyprinidae), a new natural hybrid from the Duero River basin, Spain**

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Hybrids of the Iberian nase, *Chondrostoma polylepis*, and the pardilla roach, *Rutilus lemmingii*, were found in the River Yeltes, Salamanca, Spain. Morphological multivariate (PCA) and univariate (ANOVA) analyses of 10 *C. polylepis*, 33 *R. lemmingii* and 15 hybrids have shown that the hybrids are intermediate in shape between the parent species, with a stronger resemblance to the nase. A brief discussion of the potential causes of the hybridization is presented.

Key words: *Chondrostoma polylepis*; *Rutilus lemmingii*; hybrids; Spain.

I. INTRODUCTION

Natural hybridization among European cyprinids is a common phenomenon. Recent studies of occurrences in southern European peninsulas have greatly increased the number of hybrids known. In the Iberian Peninsula a considerable number of hybrids, the Leuciscinae, have been described recently by Collares-Pereira (1983), Collares-Pereira & Coelho (1983), Coelho (1987) and Elvira (1987b).

A previously unrecognized hybrid was discovered when sampling fishes in the River Yeltes, Salamanca, Spain. This hybrid, *Chondrostoma polylepis* Steindachner, 1865 × *Rutilus lemmingii* (Steindachner, 1866), is another example of those recently described from the Iberian Peninsula involving endemic species: *R. lemmingii* × '*R. alburnoides* complex', *C. polylepis* × '*R. alburnoides* complex', *C. polylepis* × *R. lusitanicus*, *C. polylepis* × *R. macrolepidotus* (Collares-Pereira, 1983), *C. polylepis* × *R. arcaisii* (Collares-Pereira, 1983; Collares-Pereira & Coelho, 1983; Elvira, 1987b) and *C. polylepis* × *C. toxostoma* (Elvira, 1987b).

The present study deals with the morphological description of the hybrid, its distinction and similarities with the parent species, and includes a brief discussion about the factors affecting this kind of hybridization.

II. MATERIALS AND METHODS

Hybrids and parental species were collected by electrofishing in the River Yeltes (Duero River basin) at Pedraza de Yeltes, Salamanca, Spain. The River Yeltes is the main tributary of the River Huebra, a left-side affluent to the River Duero (NW Spain) near the border with Portugal. Biotic and abiotic features of the Duero fluvial system were described by García

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de Jalón & González del Tánago (1986), while the fish fauna was reviewed by Lobón-Cerviá *et al.* (1989).

A sample from 24 October 1987 consisted of 15 specimens of Iberian nase, *C. polylepis* (32–129 mm S.L.); 121 specimens of pardilla roach, *R. lemmingii* (20–89 mm S.L.), and 15 specimens (67–106 mm S.L.) suspected to be hybrids between these species. Fishes were fixed in formaldehyde solution and later preserved in 70% alcohol. They were all deposited in the fish collection of the National Museum of Natural Sciences, Madrid (leg. J. C. Velasco). In addition, specimens of red roach, *R. arcasii* (Steindachner, 1866) (one ex.); Iberian chub, *Leuciscus carolitertii* Doadrio, 1988, and Iberian barbel *Barbus bocagei* Steindachner, 1865, were collected. The fish fauna of the river also includes the eel, *Anguilla anguilla* (L., 1758).

Age was determined in the hybrid and both putative parents by scale inspection. Sex of the hybrids was not investigated due to the small number of specimens.

Several morphological characters were analysed: colour pattern and shape of the body; scale morphology and presence of a pelvic axillary process; mouth shape and development of a horny layer on the lower lip; morphology of the pharyngeal bone and the pharyngeal teeth, and outlines of the caudal, dorsal and anal fins.

A total of 19 variables were studied. Ten were morphometric features: standard length (S.L.), head length (H.L.), snout to dorsal origin length (S.D.L.), snout to pelvic origin length (S.P.V.L.), body depth (B.D.), caudal peduncle depth (C.P.D.), head height (H.H.), mouth width (M.W.), heat width (H.W.) and interorbital width (I.O.). Nine meristic features were also analysed, these were: lateral line scales (L.L.S.), transverse line scales (T.S.), branched rays of dorsal fin (D), branched rays of pectoral fin (P.t.), branched rays of pelvic fin (P.v.), branched rays of anal fin (A), branched rays of caudal fin (C), number of gill rakers on the external row of the first arch (G.R.) and number of pharyngeal teeth (P.h.T. = sum of both sides). More thorough descriptions of the variables and measurements procedures can be found in Elvira (1980, 1987a,b).

The relative position of the hybrid and both parents on a morphometric multi-dimensional space was assessed through a Principal Components Analysis (PCA) (Smith, 1973; Neff & Smith, 1979) performed on the above listed variables (P.h.T. excepted as all specimens had 5 + 5). We also ran this analysis on both the morphometric features and the meristic features separately. The morphometric variables were log-transformed and the calculations repeated, but, since the results were almost identical, we only present here results obtained from the untransformed data. This also applies to the following section.

We carried out a 1-way analysis of variance (ANOVA) for the 10 morphometric variables (including S.L.), this revealed significant between-group differences ($P < 0.05$) for all of the variables. Since all the variables showed strong positive correlations with S.L. ($P < 0.05$) we ran a 2-way ANOVA using S.L. as the covariate for the nine remaining variables, and identified those showing a higher *F*-ratio against the residual than in the previous 1-way ANOVA. This only happened for B.D., C.P.D. and H.H., which were transformed to S.L. percentages and compared between groups with a non-parametric Kruskal–Wallis ANOVA and a non-parametric Tukey multiple comparisons test (Zar, 1984); since the statistical properties of ratios made the use of parametric statistics inadequate (Atchley *et al.*, 1976; Sokal & Rohlf, 1981). The same two non-parametric procedures were employed to analyse the meristic traits, since previous inspections of the data showed that the assumptions for a parametric ANOVA were incompletely met.

The hybrid index (Hubbs & Kuronuma, 1942; Hubbs *et al.*, 1943) is a widely employed tool in the study of hybrid fishes. Smith (1973) and Neff & Smith (1979) listed the disadvantages of the hybrid index, and discussed the advantages of PCA in hybrid analysis. We estimated the hybrid index value, for every morphometric feature, only in order to facilitate comparisons with existing literature.

III. RESULTS

Age classes found in the sample of Iberian nase and pardilla roach range from 0+ to 4+. Ages of hybrids were 2+ ($n = 3$, 67–71 mm S.L.) and 3+ ($n = 12$, 77–106 mm S.L.), hybridization therefore took place in at least two successive years.

MORPHOLOGY

The appearance of the parent species and their hybrid are shown in Fig. 1. The body of the Iberian nase is long and slender compared to that of the pardilla roach and the hybrid which are shorter and wider (see also Morphometric Features). The nase is dark above the lateral line and whitish below. The roach is darkly spotted both above and below the lateral line (the Spanish name 'pardilla' comes from 'pardo' = brown, dark). The hybrids present an intermediate colouration closer to that of the nase.

Scale morphology is a good proof of hybrid origin. Scales of nase, subquad-rangular in shape, bear radii only on the anterior and posterior fields. This fact was established for all the species of the genus *Chondrostoma* studied by Elvira (1987b). Scales of roach, which are almost ellipsoidal, show radii through the entire scale surface. Hybrids present an intermediate morphology of the scales, as is usual in other cyprinid hybrids (Witkowski & Blachuta, 1980; Soric, 1986; Mir *et al.*, 1988). In the present case hybrid scales usually have the shape of those of the nase, but some radii are also present on the lateral fields. Another scale feature studied was the presence of a pelvic axillary process. It is found in the nase (as in other species of *Chondrostoma*) and the hybrids, but it is lacking in the roach.

The inferior mouth and the presence of a well-developed horny layer on the lower lip is characteristic of all *Chondrostoma* species (Elvira, 1987a,b) (Fig. 1). In the Iberian nase the horny layer is straight and thick. The mouth is arched and subterminal in the roach without a horny layer. The hybrids possess a slightly arched and inferior mouth, with a thin but obvious horny layer which is always present (Fig. 1).

No differences were found in pharyngeal bone and teeth morphology between nase, roach or the hybrids.

The caudal fin is deeply forked in nase and slightly forked in roach, while the fork is intermediate in hybrids (Fig. 1). Outlines of dorsal and anal fins are concave in nase, always convex in roach, the hybrids are intermediate.

MORPHOMETRY

Each one of the three PCAs extracted two components, CI and CII. Variable loadings on them are shown in Table I. When using all the variables CI (63% of total variance) accounts for overall size variation and CII (18%) reflects differences in meristic counts and body depth. Most group separation is achieved by these traits (Fig. 2). Hybrids are distributed into two groups, probably because no intermediacy is possible for A, D and P.V.

Using only the metric characters CI (93% of variance) was again a size component, while CII (4%) expressed differences in body depth and mouth width, also accounting for most inter-group discrimination in the plot of fish component scores (not shown). In the case of the meristic traits CI explained 63% and CII 13% of the total variance. CI represented the general variation in meristic counts (especially L.I.S. and D) and fish groups separated along this component. CII only accounted for the very few individuals whose caudal fin ray count was not 17.

Hybrid values for B.D., C.P.D. and H.H. (expressed as percentages of S.L.) were intermediate between those of both parents, the roach values being the highest in three cases (Fig. 3). These overall between-groups differences were significant on

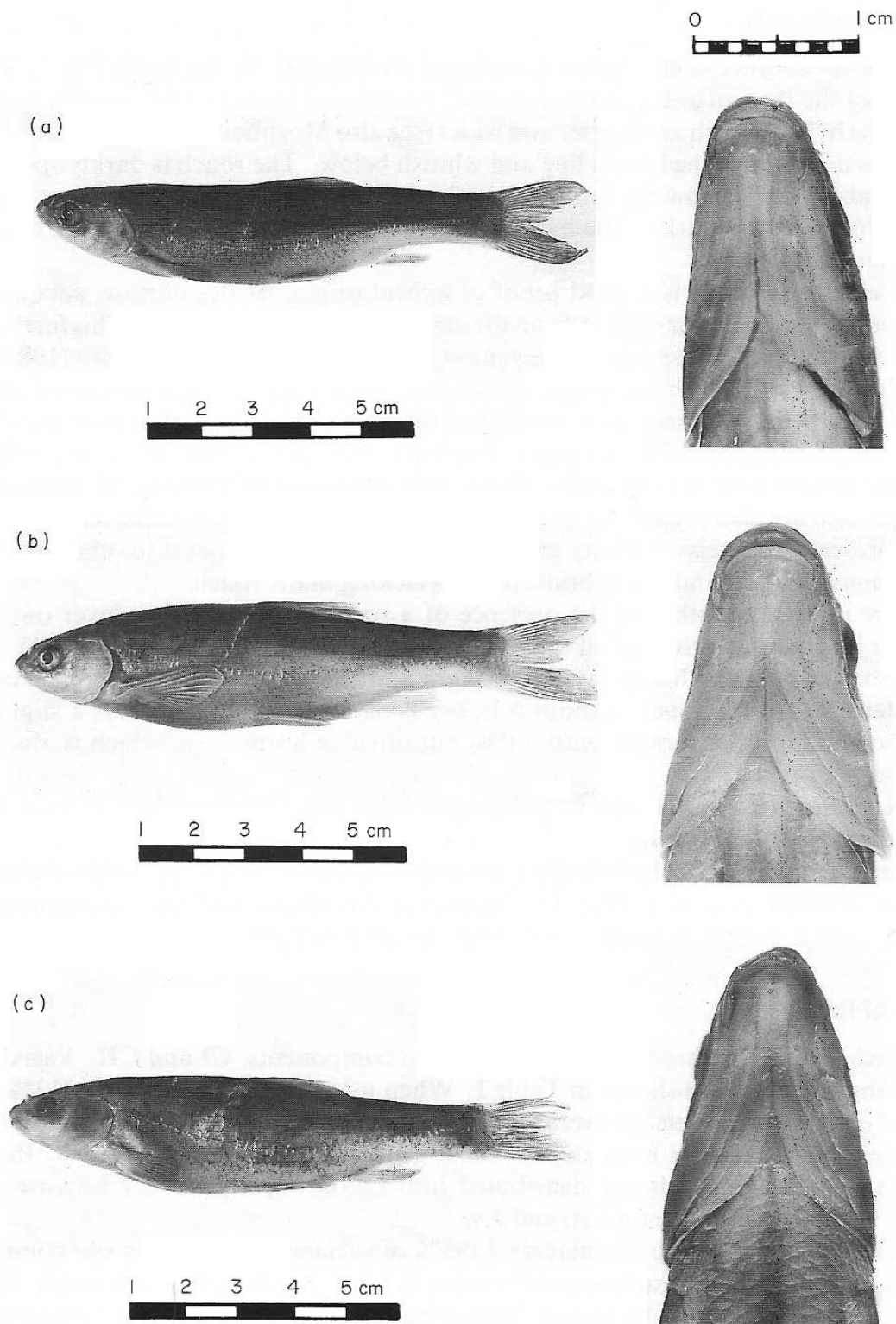


FIG. 1. Lateral views (left) and ventral views of the head (right) of (a) Iberian nase, *Chondrostoma polylepis*; (b) hybrid and (c) pardilla roach, *Rutilus lemmingii*.

three occasions (Kruskal–Wallis test, $P < 0.05$). However, the multiple comparisons test showed that each group was significantly different from the other two for B.D. ($P < 0.05$), while comparisons between hybrid and roach for C.P.D., and between hybrid and nase for H.H. were not statistically significant ($P \geq 0.05$).

TABLE I. Variable loadings on the first two components extracted by the PCAs carried out on the total variable set (PCAt), on the morphometric variables only (PCAmt) and on the meristic variables only (PCAmr)

Character	PCAt		PCAmt		PCAmr	
	CI	CII	CI	CII	CI	CII
S.L.	0.284	-0.145	0.325	-0.063	—	—
H.L.	0.286	-0.113	0.321	-0.138	—	—
S.D.L.	0.284	-0.123	0.321	-0.115	—	—
S.P.V.L.	0.288	-0.092	0.320	-0.163	—	—
B.D.	0.218	-0.339	0.289	0.713	—	—
C.P.D.	0.265	-0.213	0.317	0.256	—	—
H.H.	0.264	-0.238	0.320	0.250	—	—
M.W.	0.284	-0.017	0.305	-0.472	—	—
H.W.	0.275	-0.182	0.322	0.057	—	—
I.O.	0.289	-0.070	0.318	0.275	—	—
L.I.S.	0.204	-0.351	—	—	0.415	-0.032
T.S.	0.143	-0.253	—	—	0.301	-0.320
D	0.204	-0.331	—	—	0.408	0.143
P.t.	0.198	-0.283	—	—	0.376	0.024
P.v.	0.169	-0.367	—	—	0.388	0.028
A	0.185	-0.307	—	—	0.372	0.116
C	0.006	-0.098	—	—	0.041	0.919
G.R.	0.201	-0.267	—	—	0.371	-0.131

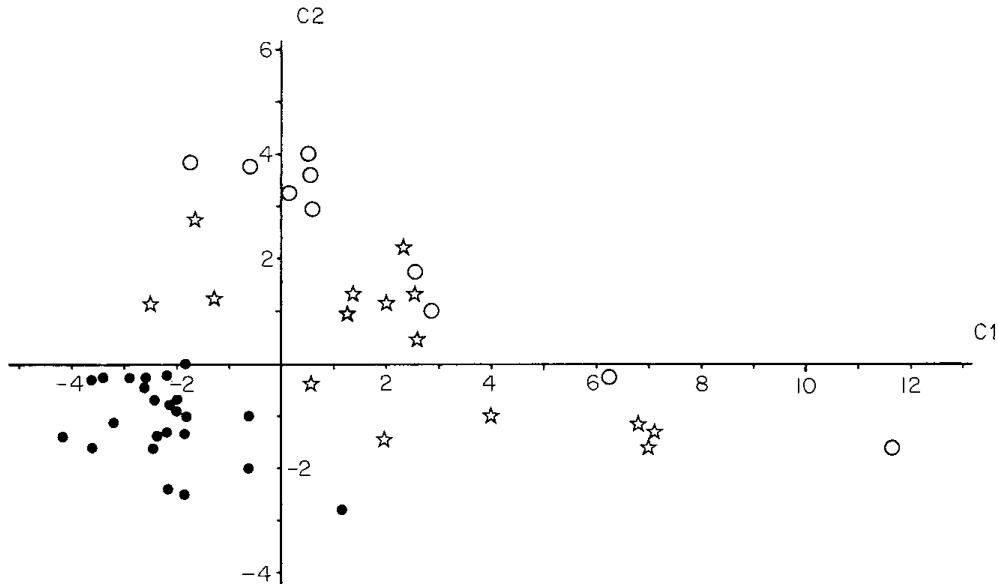


FIG. 2. Plot of the scores of each fish for the first two components extracted by the PCA run on the total variable set. Hybrids are indicated by open star, nase by \circ and roach by \bullet .

The distribution of values for L.I.S., T.S. and G.R. is shown in Fig. 3, and the frequency distribution of D, A, P.t. and P.v. can be seen in Fig. 4. All meristic features, except C, showed significant between-groups differences (Kruskal-Wallis test, $P < 0.05$). When subject to the non-parametric analogue of the Tukey

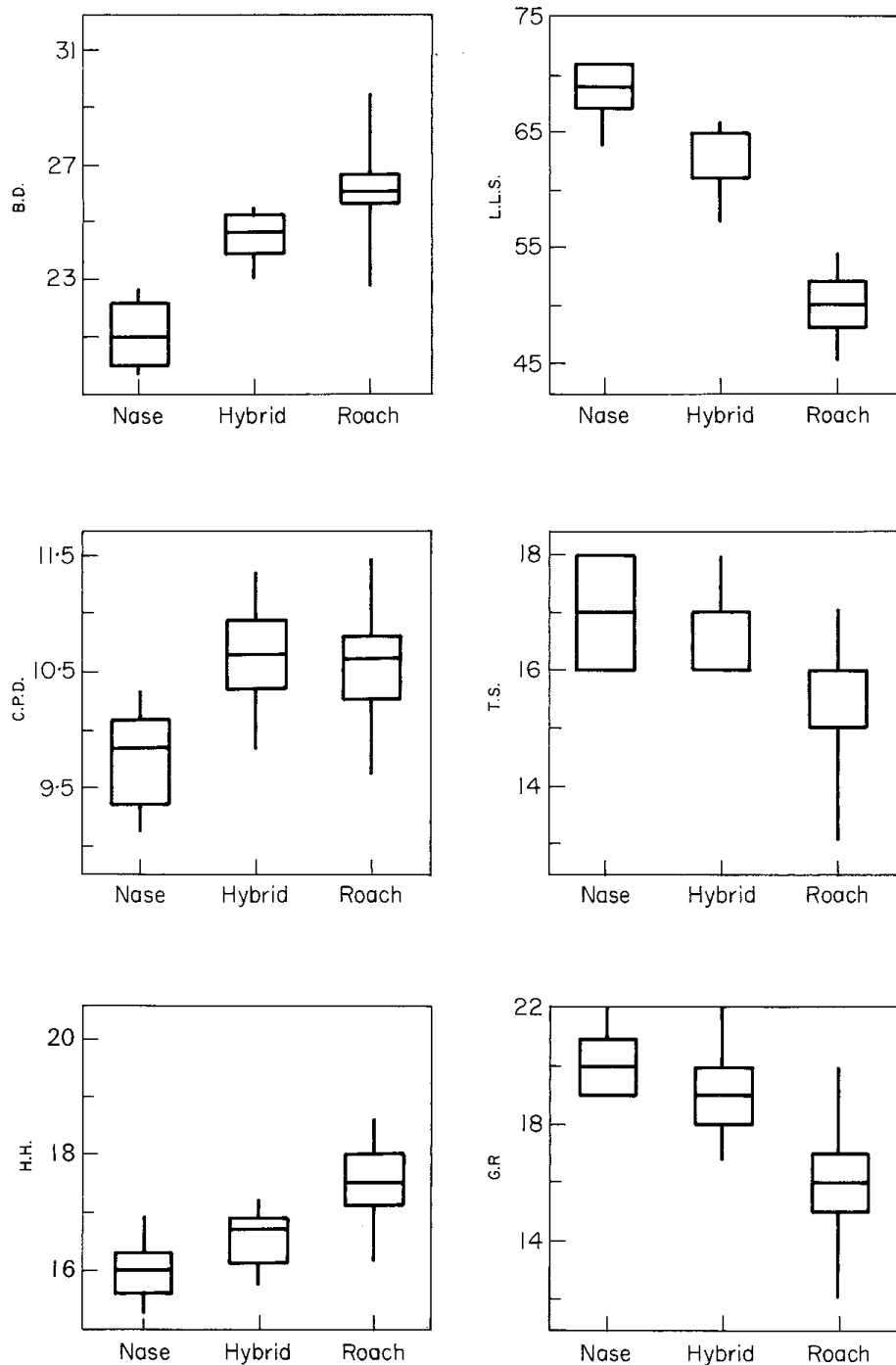


FIG. 3. Range and quartiles of body depth (B.D.), caudal peduncle depth (C.P.D.), head height (H.H.), lateral line scales (L.L.S.), transverse line scales (T.S.) and number of gill rakers (G.R.) for the nase, hybrid and roach. The three morphometric variables are expressed as a percentage of the standard length. Each symbol extends for the whole range of the values of a variable. The upper and lower limits of the central box are the upper and lower quartile respectively, and the horizontal line inside the box marks the position of the median.

multiple comparisons test, L.L.S., P.V. and A showed each group to be significantly separated from the other two ($P < 0.05$), while in the cases of T.S., D, P.t. and G.R., nase-hybrid difference was statistically non-significant ($P \geq 0.05$). It seems that hybrids are intermediate in shape while meristically closer to the nase.

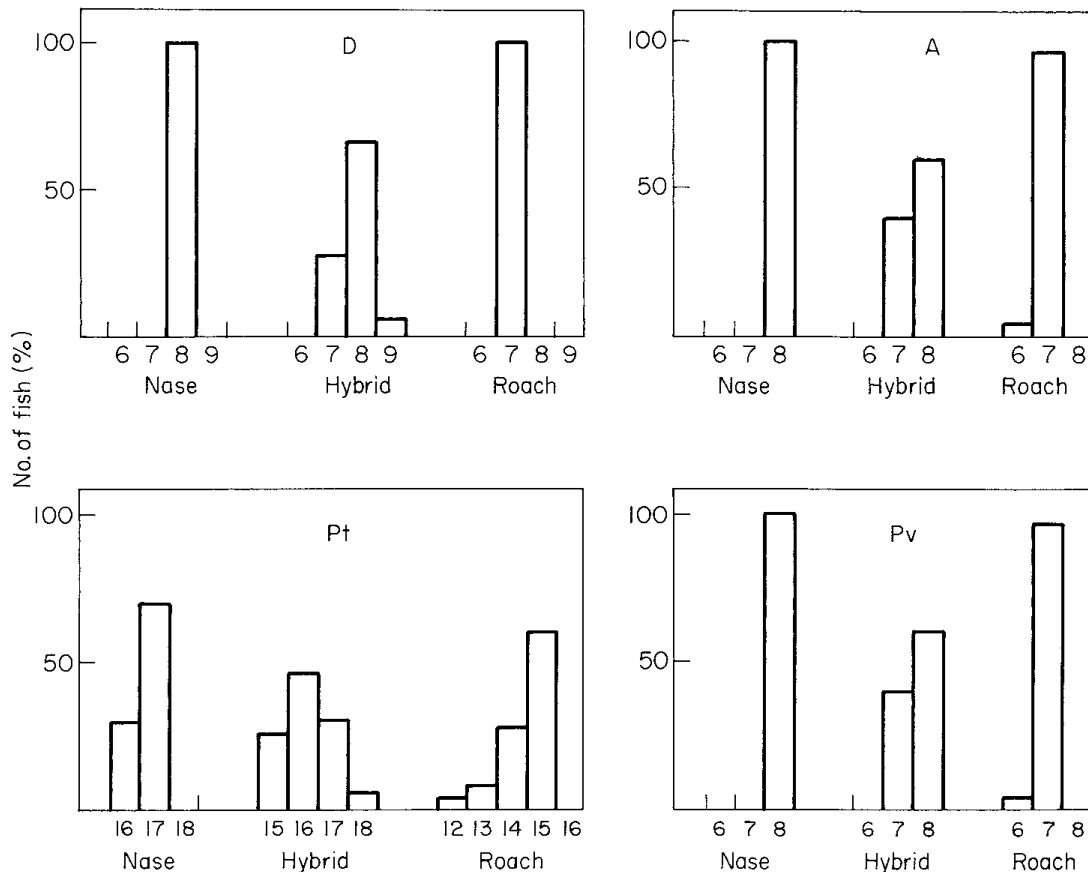


FIG. 4. Frequency distribution of the number of branched rays of the dorsal fin (D), anal fin (A), pectoral fin (P.t.) and pelvic fin (P.v.) for the nase, hybrid and roach.

According to the hybrid index (Table II), hybrids are intermediate but closer to the roach for B.D. and C.P.D., and closer to the nase for H.H. Meanwhile, hybrid indexes of the meristic variables (L.I.S., T.S., D., P.t., P.v., A and G.R.) are intermediate, with the hybrid closer to the nase (Table II).

IV. DISCUSSION

Since hybrids of two age classes (2+ and 3+) exist, we infer that hybridization between *C. polylepis* and *R. lemmingii* can be considered to be regular. Statistical analysis, however, shows no evidence for any introgressive hybridization.

The parent species are native and common in the River Yeltes. Their spawning requirements and reproductive periods are similar. Balon (1975) included them in different but related ecological reproductive groups: Lithophils (*C. polylepis*) and Phyto-lithophils (*R. lemmingii*). However, original data about their reproductive guilds are still unknown. The reproduction of the parents has never been studied in the River Yeltes, but their spawning seasons doubtless overlap. Velasco *et al.* (1990) studied the *R. lemmingii* population in the neighbouring River Huebra, and found that spawning mainly occurs in May (lasting from April to June). Details of reproduction of *C. polylepis* are known from three populations in the Tajo River basin (Lobón-Cerviá & Elvira, 1981; Lobón-Cerviá, 1982; Granado-Lorencio &

TABLE II. Hybrid indexes of morphometric (% of S.L.) and meristic features. Sample sizes: nase ($n = 10$), hybrid ($n = 15$) and roach ($n = 33$, morphometric variables, and $n = 25$, meristic variables)

Character	Nase		Hybrid		Roach		Hybrid index
	$\bar{x} \pm \text{S.D.}$	Coefficient of variation	$\bar{x} \pm \text{S.D.}$	Coefficient of variation	$\bar{x} \pm \text{S.D.}$	Coefficient of variation	
H.L.	22.7 ± 0.91	4.0	22.1 ± 0.65	3.0	22.3 ± 0.75	3.3	141
S.D.L.	52.1 ± 1.31	2.5	52.7 ± 1.32	2.5	51.7 ± 1.50	2.9	-188
S.P.V.L.	52.0 ± 1.08	2.1	51.0 ± 1.58	3.1	49.6 ± 2.51	5.1	38
B.D.	21.2 ± 0.35	5.3	24.5 ± 0.72	2.9	26.1 ± 1.30	5.0	68
C.P.D.	9.7 ± 0.44	4.5	10.5 ± 0.43	4.1	10.6 ± 0.45	4.3	98
H.H.	16.1 ± 0.53	3.3	16.6 ± 0.46	2.8	17.5 ± 0.63	3.6	35
M.W.	6.5 ± 0.31	4.9	6.0 ± 0.54	8.9	5.2 ± 0.44	8.5	38
H.W.	12.9 ± 0.28	2.1	12.8 ± 0.59	4.6	13.1 ± 0.64	4.9	-7
I.O.	9.7 ± 0.41	4.3	9.3 ± 0.26	2.8	9.2 ± 0.42	4.6	90
L.I.S.	68.6 ± 2.37	3.4	61.9 ± 2.67	4.3	49.8 ± 2.23	4.5	36
T.S.	17.0 ± 0.82	4.8	16.9 ± 0.70	4.2	15.7 ± 0.98	6.2	5
D	8.0 ± 0.00	0.0	7.8 ± 0.56	7.2	7.0 ± 0.00	0.0	20
P.t.	16.7 ± 0.48	2.9	16.1 ± 0.88	5.5	14.4 ± 0.82	5.7	28
P.v.	8.0 ± 0.00	0.0	7.6 ± 0.51	6.7	6.8 ± 0.37	5.5	34
A	8.0 ± 0.00	0.0	7.6 ± 0.51	6.7	6.9 ± 0.20	2.9	38
C	17.0 ± 0.47	2.8	17.1 ± 0.26	1.5	17.0 ± 0.29	1.7	—
G.R.	20.0 ± 1.05	5.3	19.1 ± 1.36	7.1	16.3 ± 1.60	9.8	25

García-Novo, 1986). In every case spawning takes place mainly in May but extends from April to June.

Hybridization is also possible since the parents have a close chromosome complement and the same diploid number ($2n = 50$) (Collares-Pereira, 1983, 1985; Elvira, 1987b).

The morphology of the hybrids is clearly intermediate between those of the parents. External shape seems to be closer to the nase in some characters (colour pattern, presence of a pelvic axillary process and a horny layer on the lower lip) and intermediate in others (scale shape, distal profiles of the unpaired fins). Morphometric variables are also intermediate, while meristic values are closer to the nase. In conclusion, hybrids show a greater resemblance to the nase than to the pardilla roach. Collares-Pereira & Coelho (1983) described natural hybrids between two related species (*C. polylepis* × *R. arcasii*); these however resembled *R. arcasii*.

The causes of hybridization in wild populations of fishes originate from different sources. Human activities produce disturbed environments and changes of fish habitats (see e.g. Witkowski & Blachuta, 1980; Soric, 1986; Bianco, 1988), this together with the introduction of non-native related species (Bianco, 1987) may cause hybridization. In the case of the River Yeltes population the lowering of water levels due to extensive agricultural activity and the severe droughts which have occurred in recent years may be factors in the generation of hybrids. Reductions in the number of available breeding sites may force different species to spawn in close proximity.

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