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Morphometrical comparison of cleithra, opercular and pharyngeal bones of autochthonous Leuciscinae (Cyprinidae) of Spain

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A b s t r a c t . There are fifteen autochthonous species within the subfamily Leuciscinae (Cyprinidae) in Spain, all of them endemic except for two. The diversity, peculiarity and complex taxonomic position of most of these species make them an interesting group to study. In the present work, 456 specimens of 11 species were studied. Cleithra, opercular and pharyngeal bones were extracted from each specimen. These bones were compared and measured morphologically to obtain biometric indices. The biometric differences for each bone and for the three bones together were analysed using these indices by means of Principal Component Analyses. The results show a direct relationship between morphological and biometrical differences and similarities. Besides, this osteological comparison partially agrees with the present taxonomic position of these species, contributing to the difficult systematics and phylogeny of this group.

Key words: cleithra, opercular bone, pharyngeal bone, Principal Component Analysis, Leuciscinae, Spain

Introduction

There are fifteen autochthonous species within the subfamily Leuciscinae (Cyprinidae) in Spain: *Anaecypris hispanica* (Steindachner, 1866), *Chondrostoma arcasii* (Steindachner, 1866), *Chondrostoma arrigonis* (Steindachner, 1866), *Chondrostoma duriense* Coelho, 1985, *Chondrostoma lemmingii* (Steindachner, 1866), *Chondrostoma miegii* Steindachner, 1866, *Chondrostoma polylepis* Steindachner, 1864, *Chondrostoma turiense* Elvira, 1987, *Chondrostoma willkommii* Steindachner, 1866, *Squalius alburnoides* (Steindachner, 1866), *Squalius carolitertii* (Doadrio, 1987), *Squalius cephalus* (Linnaeus, 1758), *Squalius palaciosi* (Doadrio, 1980), *Squalius pyrenaicus* (Günther, 1868) and *Phoxinus phoxinus* (Linnaeus, 1758). All of these species are Iberian endemisms except for *S. cephalus* and *P. phoxinus* (D o a d r i o 2001).

The great diversity of the species present in Spain and their status of endemism, most of them seriously endangered (B l a n c o & G o n z á l e z 1992, D o a d r i o 2001), make the Spanish Leuciscinae an interesting group to study. Many morphological, biometrical, karyological and genetical studies (e.g. C o l l a r e s - P e r e i r a 1985, E l v i r a 1997, Z a r d o y a & D o a d r i o 1998) show the complexity of their taxonomic and phylogenetic positions.

In this work, the morphological and biometrical characteristics of cleithra, opercular and pharyngeal bones were compared among the analysed species. The bones were chosen because of their taxonomic value (C h u 1935, S u z u k i & H i b i y a 1985). They are commonly used in diet determinations of ichthyophagous animals (e.g. P r e n d a et al. 1997) and in paleontological studies (e.g. Y a s u n o 1997).

The aim of this study is to analyse the differences and similarities among these bones within the autochthonous Leuciscinae of the Iberian Peninsula in order to append information to support the studies about the taxonomy of this group.

Material and Methods

In this study 456 specimens from 18 localities were analysed (Table 1, Fig. 1). These belonged to 11 of the 15 species of autochthonous Leuciscinae present in Spain. Among the genus *Chondrostoma* species, *C. arcasii*, *C. lemmingii*, *C. miegii* and *C. duriense* were studied. The latter two species were considered as *C. toxostoma* (Vallot, 1837) and *C. polylepis* Steindachner, 1864 respectively, until E l v i r a (1997) revised the genus. After this revision, it was concluded that six species existed in Spain. *Chondrostoma toxostoma* was divided into *C. arrigonis*, *C. miegii* and *C. turiense*, and *C. polylepis* was divided into *C.*

Table 1. Species and number of specimens (Nº) captured in each sampling point (SP). (1) Skeletons lent by the Museo Nacional de Ciencias Naturales (MNCN, tag 69431–69440 of *Anaecypris hispanica* and 21396, 23167–23171, 23597–23624, 69441–69445 and 69447–69455 of *Squalius palaciosi*).

Species	Nº	River	Basin	Locality	Province	Date	SP
Anaecypris hispanica (1)	8	Gévora	Guadiana	Alburquerque	Badajoz	9 /5/87	1
	1	Quejigares	Guadiana	Fontanosas	Ciudad Real	9 /5/87	2
Chondrostoma arcasii	25	Araquil	Ebro	Ibero	Navarra	5/3/92	7
	9	Arlanzón	Duero	Burgos	Burgos	15/11/93	5
	4	Elorz	Ebro	Otano	Navarra	21/10/93	18
C. duriense	8	Huebra	Duero	Cerralbo	Salamanca	19/8/93	3
	4	Huebra	Duero	Yecla de Yeltes	Salamanca	19/8/93	4
	5	Arlanzón	Duero	Burgos	Burgos	20/7/94	5
	17	Arlanzón	Duero	Burgos	Burgos	15/11/94	5
	12	Arlanza	Duero	S. Pedro de Arlanza	Burgos	29/9/95	6
C. lemmingii	29	David	Tajo	Valencia de Alcántara	Cáceres	15/11/94	15
C. miegii	25	Araquil	Ebro	Ibero	Navarra	24 /2/92	7
	12	Araquil	Ebro	Ibero	Navarra	24/2/92	7
	7	Esca	Ebro	Burgui	Navarra	3/3/92	8
	3	Esca	Ebro	Salvatierra de Esca	Zaragoza	20/6/94	9
	3	Aragón	Ebro	Caparroso	Navarra	2/5/94	10
Squalius alburnoides	56	Gata	Tajo	La Moraleja	Cáceres	14/11/94	12
	11	Huebra	Duero	Yecla de Yeltes	Salamanca	19/8/93	4
S. carolitertii	9	Huebra	Duero	Cerralbo	Salamanca	19/8/93	3
	14	Ausines	Duero	Revillarruz	Burgos	29/9/95	13
S. cephalus	45	Anyet	Muga	Sant Climent Sescebes	Gerona	25/3/94	14
S. palaciosi (1)	15	Jándula	Guadalquivir	Lugar Nuevo	Jaén	4/10/80	11
	29	Jándula	Guadalquivir	Lugar Nuevo	Jaén	13/4/79	11
S. pyrenaicus	44	David	Tajo	Valencia de Alcántara	Cáceres	15/11/94	15
	19	Arrago	Tajo	Cadalso	Cáceres	14/11/94	16
Phoxinus phoxinus	13	Bidasoa		Vera de Bidasoa	Navarra	2/3/92	17
	6	Esca	Ebro	Burgui	Navarra	23/10/93	8
	21	Esca	Ebro	Burgui	Navarra	31/10/93	8
	2	Esca	Ebro	Salvatierra de Esca	Zaragoza	21/7/94	9

Fig. 1. Map of distribution of sampling points. The numbers of sampling points correspond to those listed under SP in Table 1.

duriense, *C. polylepis* and *C. wilkommii*. *Chondrostoma arcasii* and *C. lemmingii* were not included in the genus *Chondrostoma* by Elvira (1997), although nowadays these species are grouped under this genus (Coelho et al. 1997, Zardoya & Doadrio 1998). Therefore, at present they are eight the species of the genus *Chondrostoma* in Spain.

Fish were boiled until flesh was easily removed and the bones were left to air dry. These were labelled (species, number of specimen and sample) and then stored. They are now kept in the Museo de Zoologia of the University of Navarre (Pamplona, Spain).

The skeletons for *A. hispanica* and *S. palaciosi* were provided by the Museo Nacional de Ciencias Naturales (Madrid, Spain).

Only the cleithra, opercular and pharyngeal bones of the right side were used to prevent the bias generated by the possible asymmetric development of each body side (Chu 1935). For each studied piece, the most significant morphological differences were noted. The nomenclature recommended by the World Ichthyoarchaeological Community (Lepiksaar 1983, in: Roselló 1989) was used to describe the bones.

Osseous measurements and biometric indices were obtained (Table 2). The bones were placed as shown in Fig. 2 and viewed using a binocular magnifying glass and Zeiss clear camera. Images were measured using a digitising tablet (Genius HISKETC 1212 model; program IDRISI: Vector Digitalizing Module v.3.03).

Principal Component Analyses (PCA) were performed to detect morphometrical differences among the bones and to determine the contribution of each index to the differentiation of the bones. Analyses were carried out from 1) all indices obtained, 2) averages of the indices distinguishing each bone and 3) averages considering the studied bones altogether. Hotelling's T test was used to test the indices (Hair et al. 1999).

Regression was used to obtain the factor scores for each species from all Principal Component Analyses. Graphics of the distribution of the species were elaborated from these scores for each analysis. The computer program SPSS 9.5 was used.

Table 2. Osteological measurements and biometric indices obtained.

Cleithrum	
Chord lentgh	CLA
External margin length	CLB
Maximum width	CLC
Vertical limb length	CLD
Angle within the limbs	CLF
Indices	
Relative length of external margin	CLB/CLA
Degree of elongation of the cleithrum	CLC/CLA
Relative length of vertical limb	CLD/CLA
Opercular bone	
Articular axis height	OPA
Length of articular process	OPB
Maximum height	OPC
Maximum length	OPD
Posterior angle height	OPE
Distance between superior angle and articulation axis	OPF
Indices	
Relative length of articular process	OPB/OPA
Maximum relative height	OPC/OPA
Degree of elongation of opercular bone	OPD/OPA
Relative height of posterior angle	OPE/OPA
Relative distance between superior angle and articulation axis	OPF/OPA
Pharyngeal bone	
Articulation axis height	AFA
Distance between external angle and articulation axes	AFB
Maximum width of pharyngeal bone	AFC
Dorsal limb length	AFD
Angle within the limbs	AFF
Indices	
Degree of elongation of pharyngeal bone	AFB/AFA
Relative maximum width of pharyngeal bone	AFC/AFA
Relative dorsal limb lenght	AFD/AFA

Results

Cleithrum

The morphological analysis of the cleithra allows the differentiation of the following groups (Fig. 3): 1) *Anaecypris hispanica* and *P. phoxinus*: bones are slender, with the vertical limb sharply inclined backwards. In *P. phoxinus*, the external margin of the horizontal limb is shorter than the internal one and the posterior angle is more elevated, whereas in *A. hispanica* the margins of the horizontal limb have similar lengths; 2) *Chondrostoma duriense*, *C. miegii* and *S. palaciosi*: the posterior angle is marked and the posterior edge of the vertical limb is concave. They present an acute dorsal tip inclined backwards. The articulate crest is well

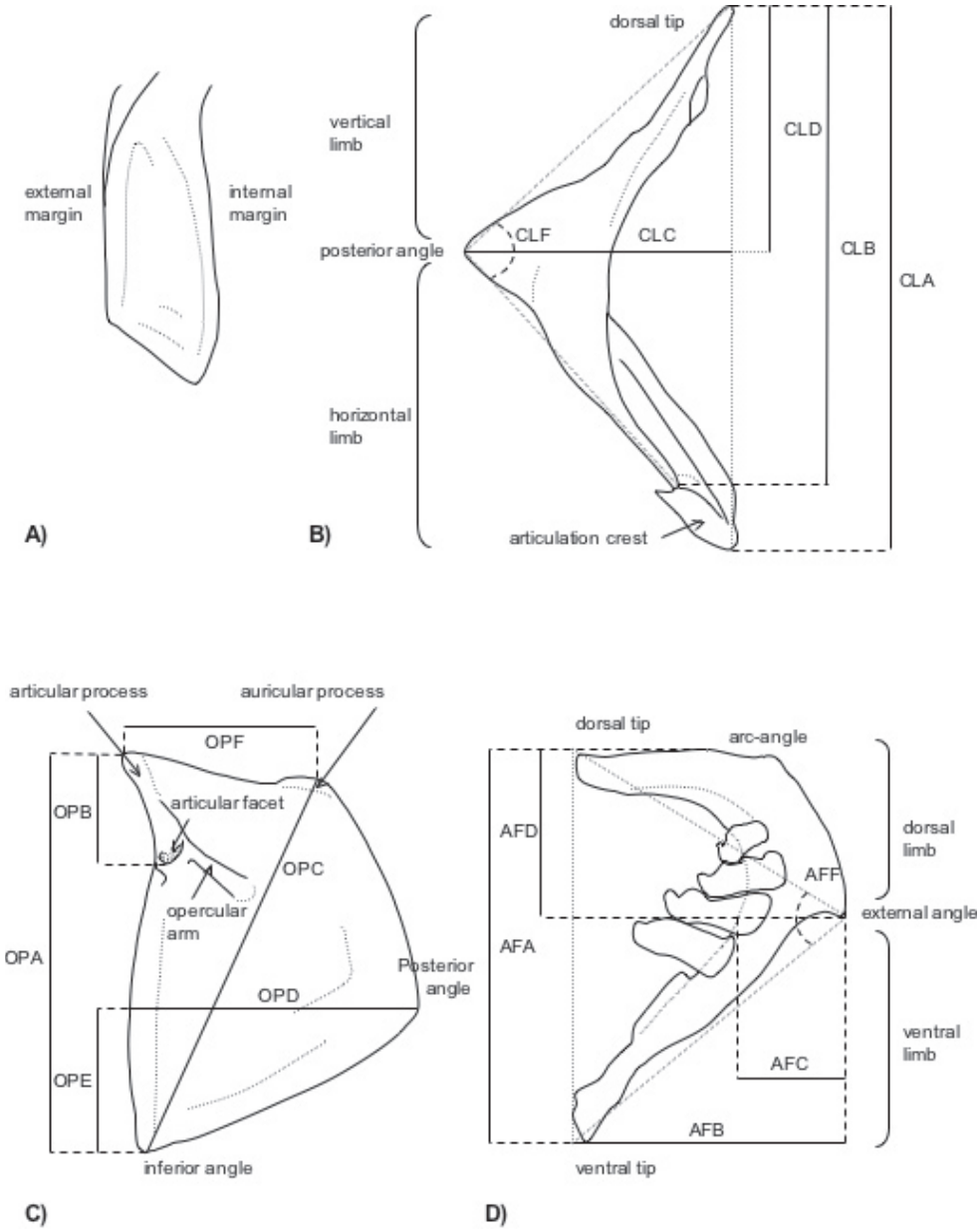

Fig. 2. Measurements taken from each bone. A) Dorsal view of horizontal limb of the cleithrum, B) cleithrum, C) Opercular bone, and D) pharyngeal bone. The abbreviations correspond to those described in Table 2.

developed and hook-shaped; 3) *Chondrostoma arcasii* and *C. lemmingii*: the dorsal tip is very acute and pointed upwards. The vertical limb is very tight and the external margin is shorter than the internal one; 4) *Squalius carolitertii*, *S. cephalus* and *S. pyrenaicus*: the posterior edge of the vertical limb is convex and the posterior angle is not very pronounced. The internal margin of the horizontal limb has an acute tip and the crest of articulation is poorly developed;

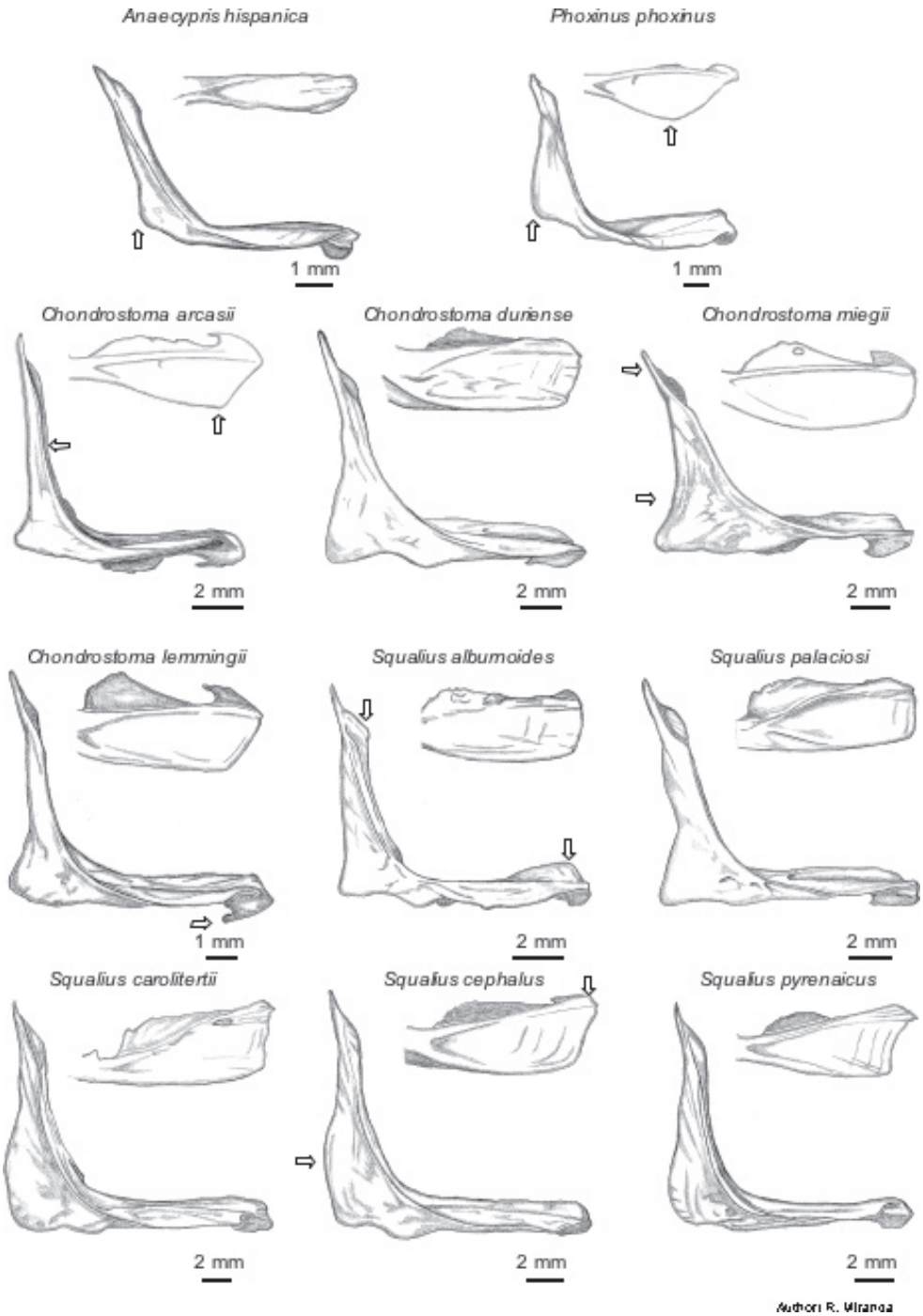

Fig. 3. Dorsal view of the horizontal limb and lateral external view of the right cleithra of the studied species.

5) *Squalius alburnoides*: the posterior edge of the vertical limb is straight and the posterior angle is acute. The crests of the vertical and horizontal limbs are both well developed.

Two components (PC) were extracted from the Factorial Analysis of the averages of the biometric indices (Table 3). These components explain 81.4% of the variance contained in the data. Component I is characterised by the degree of elongation of the bone (CLC/CLA) and the angle within the limbs (CLF). Component II is characterised by the relative length of the external margin (CLB/CLA). Fig. 6A shows *A. hispanica* and *P. phoxinus* (negative factorial scores for factor I) separated from the rest and between them. *Squalius alburnoides* also shows negative values for this factor. The species *C. lemmingii* and *C. arcasii* show the lowest value for factor II, whereas *S. palaciosi* and *C. miegii* show the highest.

Table 3. Principal component (PC) matrix extracted from the averages of the indices for each bone and percentage of the total variance related to each factor (%Variance).

Cleithrum			Opercular bone			Pharyngeal bone		
Index	PC I	PC II	Index	PC I	PC II	Index	PC I	PC II
CLF	-0.957	$4.213x10^{-4}$	OPB/OPA	-0.812	0.458	AFF	-0.947	-0.301
CLC/CLA	0.972	0.055	OPC/OPA	0.946	-0.054	AFB/AFA	0.983	0.145
CLB/CLA	-0.127	0.990	OPD/OPA	0.824	0.053	AFC/AFA	0.967	0.007
CLD/CLA	0.687	0.687	OPE/OPA	0.677	-0.045	AFD/AFA	-0.754	0.656
			OPF/OPA	0.480	0.854			
% Variance	47.26	34.15		58.46	18.95		84.16	13.65

Opercular bone

From the morphological analysis of the opercular bones the following groups were distinguished (Fig. 4): 1) *Anaecypris hispanica*: the posterior angle is in an elevated position and the auricular process is well developed. The articular process and the opercular arm are short and wide; 2) *Phoxinus phoxinus* displays very characteristic opercula, with an acute auricular process and a narrow articular one which is not included in the opercular surface; 3) *Chondrostoma miegii*, *C. arcasii* and *S. palaciosi*: the upper edge is straight and bends towards the ventral area. The auricular process is absent and the opercular arm is usually short and wide. In the case of *S. palaciosi*, some specimens showed a narrow opercular arm, similar to the ones of the following group; 4) genus *Squalius*. The upper edge is convex, close to the articular process. The auricular process is present and the opercular arm is elongated and narrow. In this genus, the opercular bone of *S. alburnoides* displays a great variability, with intermediate characteristics between *Squalius* and the following species; 5) *Chondrostoma arcasii*. The opercular bone presents an extended articulation axis, the auricular process is next to the articulation axis and the posterior angle is rounded. *Chondrostoma lemmingii* has long opercula. The posterior angle is acute and relatively low positioned. These opercular bones are quadrangular-shaped with rectilinear upper and posterior edges.

From the Principal Component Analysis of the averages of the opercular indices, two components have been extracted that explain 77.4% of the total variance (Table 3). Component I is characterised by the maximum relative height (OPC/OPA) and Component II by the relative distance between the superior angle and the articulation axis (OPF/OPA). The scatter plot of the factorial scores (Fig. 6B) shows *A. hispanica*, *C. lemmingii* and *P. phoxinus* separated from the rest (positive values for factors I and factor II). *Chondrostoma miegii* and

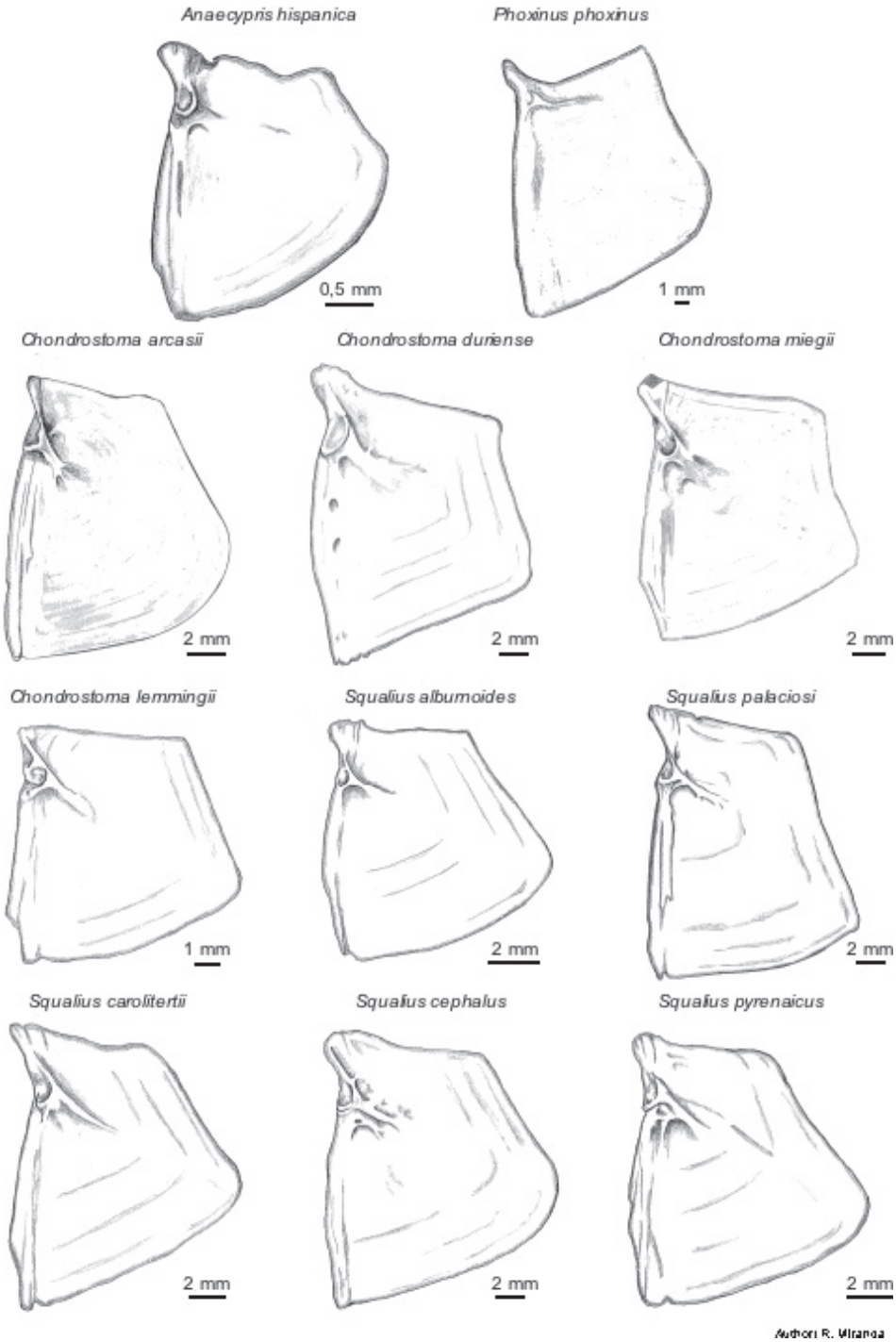

Fig. 4. Internal view of the right opercular bones of the studied species.

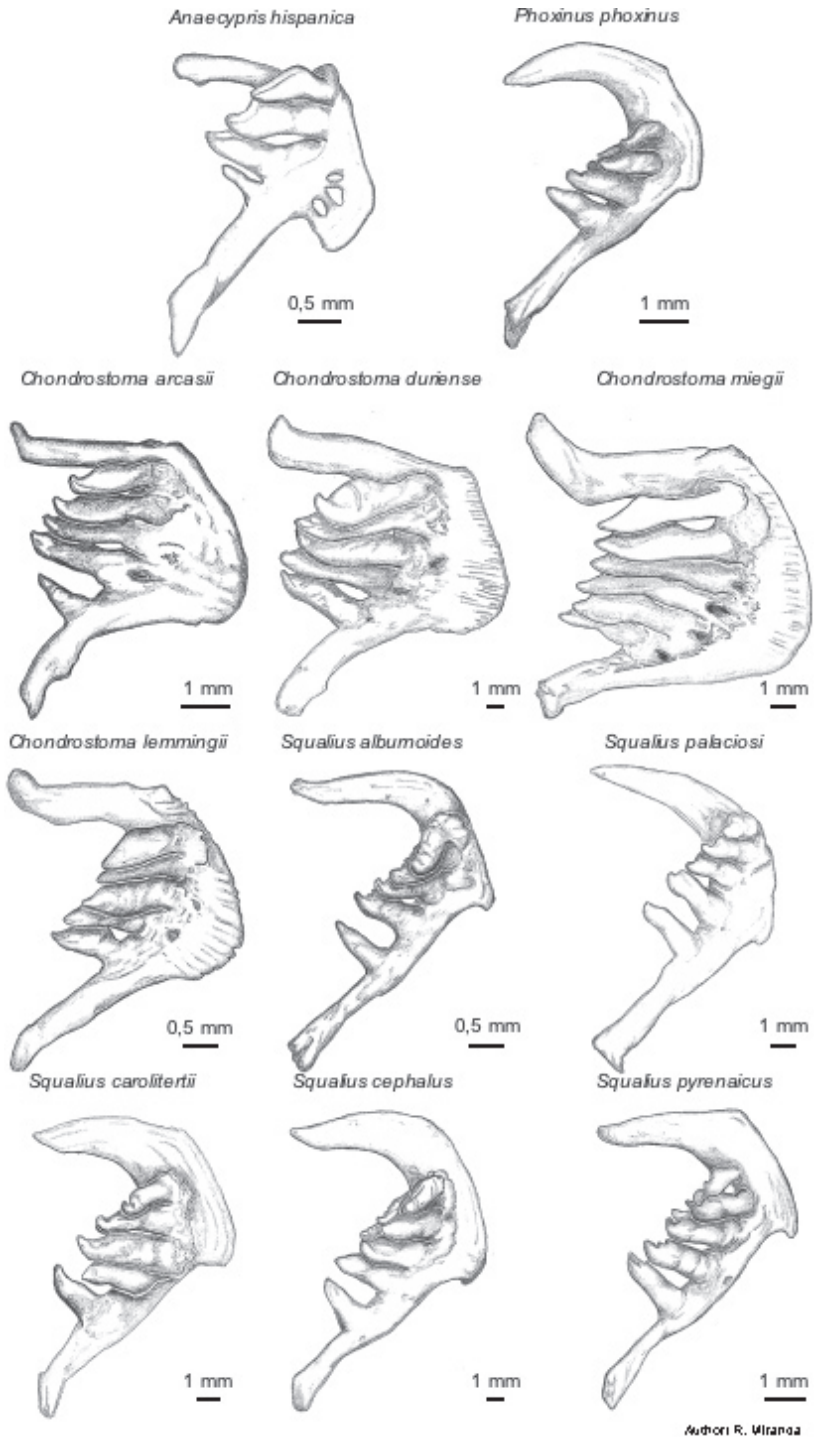

Fig. 5. Internal view of the right pharyngeal bones of the studied species.

C. duriense have negative values for factor I and positives values for factor II. The rest of the species are grouped on the negative values of the axis of factor II.

Pharyngeal bones

Three well-differentiated groups were separated by the morphological characteristics of the arches (Fig. 5): 1) Genus *Chondrostoma*: the limbs are short and the medium area is wide. The dorsal tip is next to the ventral one. The dorsal limb is rectilinear and its tip bends suddenly towards the dorsal area. Teeth are relatively big, dorsoventrally compressed and acute, except for those of *C. arcasii*, which are cylindrical and end in a little hook; 2) Genus *Squalius* and *P. phoxinus*: the arches are sickle-shaped, the dorsal limb is curved and the ventral one is elongated and narrow. The external angle is acute. Teeth are elongated, denticulated and ending in a hook, except for those of *S. palaciosi*, which are very similar to those of the genus *Chondrostoma*; 3) *A. hispanica* shows very peculiar pharyngeal bones. The external angle is well developed and it bends towards the ventral area. Teeth are cylindrical, pointed and have an elongated, rounded and tear-shaped masticatory surface, with a slightly denticulated ventral edge.

The PC extracted from the Factorial Analysis explains 97.8% of the total variance (Table 3). Component I is characterised by the degree of elongation of the pharyngeal bone (AFB/AFA) and the Component II by the relative dorsal limb length (AFD/AFA). The scatter plot of the scores of the species (Fig. 6C) shows *A. hispanica* separated from the other Leuciscinae. The remaining ones are grouped depending on their values for factor I: positive for the species of the genus *Chondrostoma* and negative for the rest.

Principal Component Analysis

Finally, a Principal Component Analysis was carried out for the biometric indices of the three bones together. The factorial values were calculated from biometric indices of all the studied specimens and from the averages of those indices (Table 4).

Table 4. Principal component (PC) matrix extracted from the indices and their averages considering the three bones altogether and percentage of the total variance related to each factor (%Variance).

	Biometric Incides					Averages of Indices			
Index	PC I	PC II	PC III	PC IV	PC V	PC I	PC II	PC III	PC IV
CLF	0.639	0.616	-0.324	0.017	0.152	0.870	0.274	-0.319	-0.086
CLC/CLA	-0.644	-0.622	0.340	0.028	-0.074	-0.862	-0.246	0.381	0.045
CLB/CLA	-0.160	-0.272	0.445	-0.419	0.532	0.274	0.297	0.549	-0.510
CLD/CLA	-0.197	-0.198	0.081	0.695	0.537	-0.439	0.018	0.621	-0.127
OPB/OPA	-0.458	0.228	-0.414	-0.187	0.253	-0.754	-0.033	-0.347	-0.332
OPC/OPA	0.462	0.438	0.620	0.213	0.042	0.737	0.350	0.269	0.396
OPD/OPA	0.286	0.354	0.730	-0.017	-0.221	0.526	0.435	0.288	0.570
OPE/OPA	0.665	0.337	-0.068	0.305	0.159	0.889	-0.081	-0.091	-0.240
OPF/OPA	-0.199	0.658	0.130	-0.471	0.172	0.335	0.850	-0.281	-0.229
AFF	0.842	-0.371	-0.010	-0.151	0.202	0.921	-0.220	0.214	-0.216
AFB/AFA	-0.861	0.392	0.020	0.144	-0.113	-0.912	0.345	-0.156	0.140
AFC/AFA	-0.756	0.412	0.033	0.203	-0.161	-0.813	0.431	-0.076	0.267
AFD/AFA	0.631	-0.328	-0.077	0.011	-0.404	0.540	-0.705	-0.201	0.347
% Variance	33.03	18.17	11.87	8.77	7.79	51.47	16.28	10.86	9.58

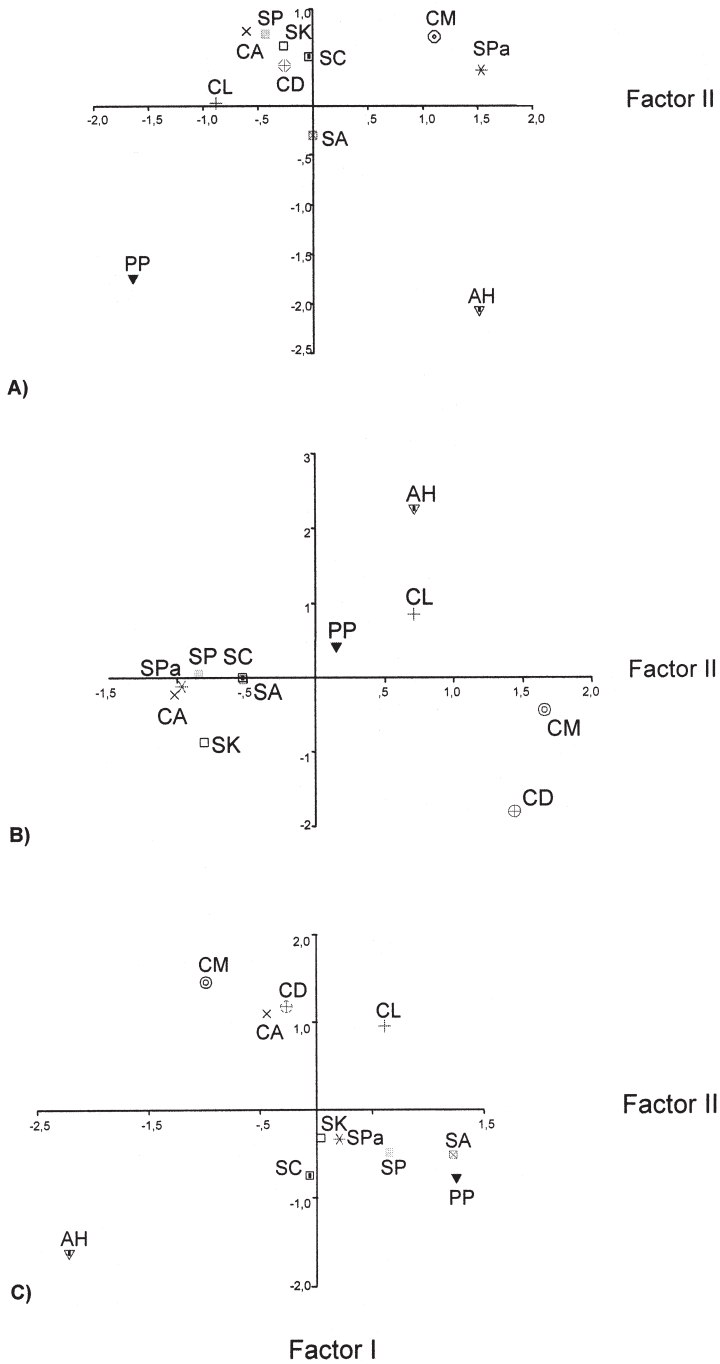

Fig. 6. Scatter plot of the factorial scores obtained from the Principal Component Analysis from the average of the biometric indices of A) the cleithra, B) the opercular bones and C) the pharyngeal bones. AH: *Anaecypris hispanica*, CA: *Chondrostoma arcasii*, CD: *Chondrostoma duriense*, CL: *Chondrostoma lemmingii*, CM: *Chondrostoma miegii*, SA: *Squalius alburnoides*, SK: *Squalius carolitertii*, SC: *Squalius cephalus*, SP: *Squalius pyrenaicus*, SPa: *Squalius palaciosi*, PP: *Phoxinus phoxinus*.

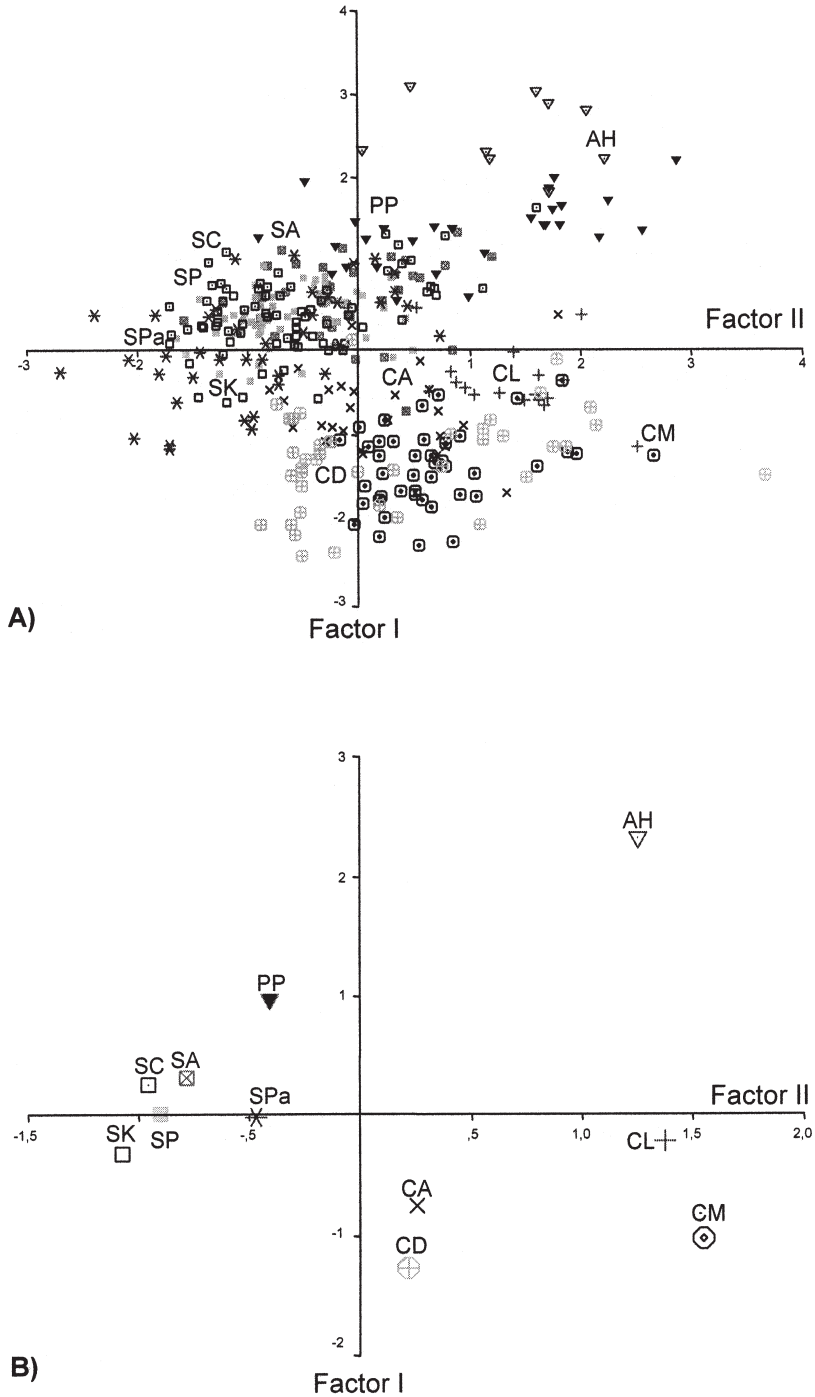

Fig. 7. Scatter plot of the factorial scores obtained from the Principal Component Analyses from the biometric indices (A) and averages (B) considering the bones altogether. The abbreviations correspond to those described in Fig. 6.

From these averages four PC that explain 88.2% of the total variance were extracted. A scatter plot of the scores shows three differentiated groups (Fig. 7A). Firstly, *A. hispanica* is separated from the rest (positive values for both factors). Secondly, the genus *Chondrostoma* (positive values for factor II and negatives for factor I). The rest of species (negative values for factor II) would integrate the last group. *Phoxinus phoxinus* appears separated from this last group.
A scatter plot of factorial scores obtained from the analysis of the biometric indices (Fig. 7B) shows that the species can be grouped in the same way that in the analysis carried out with the averages, although there is a marked overlapping between the values.

For the scores scatter plot, the intermediate distribution of the values of *P. phoxinus* between *A. hispanica* and the genus *Squalius* could be emphasised. A similar phenomenon is observed with the values of *C. arcasii* and *C. lemmingii*, situated between *C. miegii* and *C. duriense* and the genus *Squalius*.

Discussion

Both the morphological and the biometrical analyses permit to group the studied species by the characteristics of their cleithrum, opercular and pharyngeal bones. The groups of species that could be obtained from these two analyses coincide, although there are some exceptions. The achieved results are discussed considering each studied species.

In the case of *A. hispanica*, the analyses show clear differences among its bones and those of the remaining species. The actual taxonomy places this monotypic genus well separated from the rest of the small Leuciscinae and formerly differentiated (Collares-Pereira 1985, Bogutskaya & Collares-Pereira 1997, Zardoya & Doadrio 1998). Bogutskaya & Collares-Pereira (1997) carried out a comparative analysis of morphological and osteological characters between *A. hispanica* and other small cyprinids, concluding that *A. hispanica* has the closest phylogenetic relationships with the Iberian *Chondrostoma*, but with its own wide set of derived characters. This conclusion does not contradict the results of the present work, as the characters that support these relationships do not belong to cleithra, opercula or pharyngeal bones. In the case of the former, Bogutskaya & Collares-Pereira (1997) concluded that the pharyngeal teeth configuration is a unique character and very rarely met in Leuciscinae. Moreover, Zardoya & Doadrio (1998) established that *Anaecypris* constitutes an independent lineage not intimately related to any other Iberian cyprinid.

Phoxinus phoxinus is also differentiated because of the morphology and the biometry of its bones. In the case of the cleithrum and the opercular bone, there are similarities with those of *A. hispanica*, although important differences were observed. Regarding the pharyngeal bones, the morphology is very similar to those of *Squalius*. These results are similar to the ones obtained from the biometric analysis (Fig. 7) that show this species separated from the rest, with intermediate values between the genera *Squalius* and *Anaecypris*.

Phoxinus phoxinus is considered phylogenetically separated from these genera (Almaça 1976, Howes 1991), in the monophyletic Phoxinin lineage within the subfamily Leuciscinae. Hänfling & Brandl (2000) carried out allozymes analyses of European cyprinids and concluded that the genus *Phoxinus* is a sister taxon to the other members of the Leuciscinae-Alburninae lineage. The observed similarities with *A. hispanica* could be due to a convergent evolution of the studied bones in these species (see distribution and biology in Ribeiro et al. 2000, Doadrio 2001).

The genus *Squalius* appears as a well-differentiated group with a high morphological and biometrical similarity among its species. However, *S. alburnoides* is an exception to this. The study of the bones shows similarities between this and the remaining species of the genus, although remarkable and specific differences exist. Both, cleithra and opercular bones of this species are different from the rest. Prenda et al. (1997) did not find differences between several bones of *S. pyrenaicus* and *S. alburnoides* (pharyngeal bones included, but not cleithra or opercular bones). The biometric analysis locates *S. alburnoides* within the *Squalius* group, although for the cleithrum (Fig. 6A) this species is separated from the genus. *Squalius alburnoides* is a polyploid complex with a hybrid origin (Collares-Pereira 1989) and morphometric differences between sexes (Martins et al. 1998). These characteristics could explain the variability found in the studied bones.

The species taxonomic position is controversial. It was formerly described as a species of the genus *Leuciscus*. However, different authors considered that it belonged to different genera until Collares-Pereira et al. (1999) included it again within the genus *Leuciscus*, and subsequently Zardoya & Doadrio (1998) considered within the genus *Squalius*. In the work published by Collares-Pereira et al. (1999), an exhaustive revision of the evolution of the taxonomic position of this species is made. Until this work was published, the species was considered within the genus *Rutilus* according to some authors and within *Tropidophoxinellus* according to others. The present work agrees with the inclusion of this species in the genus *Squalius*. The studied pieces are more similar to the bones of the species of this genus, both biometrically and morphologically.

The studied bones of the species of the genus *Chondrostoma* are clearly different from those of the remaining species. Taxonomic and phylogenetic studies consider this genus well differentiated from the rest (Elvira 1997).

In the case of *C. arcasii* and *C. lemmingii*, the results show similarities with other species of the genus, mainly when considering the pharyngeal bones. Nevertheless, the opercular bones and cleithra of these species have many similarities with those of the genus *Squalius*, with particular characteristics that distinguish them from the remaining species.

Chondrostoma lemmingii was considered by many authors to belong to the genus *Rutilus* (e.g. Blanco & Gonzalez 1992), whereas for others it was included in the genus *Chondrostoma* (e.g. Collares-Pereira 1985). Coelho et al. (1997) compared allozyme characters among the Iberian species of the genera *Chondrostoma* and *Rutilus*, concluding that *C. lemmingii* is more closely related to the Iberian species of the genus *Chondrostoma* than to the species of the genus *Rutilus*. Elvira et al. (1990) described the presence of natural and abundant hybrids between *C. lemmingii* and *C. polylepis*, which could support their phylogenetics proximity. In the case of *C. arcasii*, many authors included it in the genus *Rutilus* (e.g. Collares-Pereira 1985, Blanco & Gonzalez 1992).

According to Almaça (1976) *C. arcasii* and *C. lemmingii* are two recently differentiated species, whose speciation would be carried out at present. Taxonomic revisions place these two species in a new genus, or genera, close to *Chondrostoma* (Casado 1995, in: Elvira 1997). The phylogenetics analyses carried out by Zardoya & Doadrio (1998) place both species in the genus *Chondrostoma*.

These cited studies agree with the results obtained in this work. The factorial analysis considering the three bones altogether (Fig. 7) located *C. lemmingii* and *C. arcasii* in an intermediate position between *Squalius* and *Chondrostoma*, although closer to the species of the latter.

Finally, the study of the bones of *S. palaciosi* is probably the most ambiguous of all. The morphology of the cleithra is very similar to that of *Chondrostoma*. The opercular bones present morphologic characteristics between *Chondrostoma* and *Squalius*, although the biometric analysis locates it within the group *Squalius* (Fig. 6B). The morphology of the pharyngeal bones is similar to that of *Squalius*, although the teeth of the studied specimens have the typical shape of those of *Chondrostoma*. The factorial analysis located the pharyngeal bones of this species very close to the genus *Squalius* (Fig. 6C). In conclusion, the studied bones of this species present intermediate characteristics between the genera *Chondrostoma* and *Squalius*, although the biometric analysis carried out considering the three bones altogether places it close to *Squalius* (Fig. 7).

This species was described as *Iberocypris palaciosi* by D o a d r i o (1980). Attending to its morphology, this fish could be considered close to the genera *Chondrostoma* or *Squalius*. According to C o l l a r e s - P e r e i r a (1989), it could have a hybrid origin between these two genera. Z a r d o y a & D o a d r i o (1998) placed this unisexual species in the genus *Squalius* from the phylogenetics analysis of cytochrome b.

To summarise, the biometric and morphologic analyses of opercula, cleithra and pharyngeal bones contribute to the knowledge of the systematics and phylogeny of these fish species. Recent biochemical or genetic analyses should be complemented with morphological and osteological studies, nowadays forgotten. The application of multivariate statistical techniques to biometric values allows the quantification of the differences and the similarities of the bones between species in a more objective way than the sometimes subtle morphological differences do.

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