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K. Iliadou [a]; M. J. Anderson [b]
[a] Biology Department, Section of Animal Biology, University of Patras. Patras. Greece
[b] School of Biological Sciences and Centre for Research on Ecological Impacts of Coastal Cities, Marine Ecology Laboratories A11, University of Sydney. Australia

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Morphometric comparative analysis of pharyngeal bones of the genus *Scardinius* (Pisces: Cyprinidae) in Greece

K. ILIADOU† and M. J. ANDERSON‡

†*Biology Department, Section of Animal Biology, University of Patras, 26500 Patras, Greece*
‡*School of Biological Sciences and Centre for Research on Ecological Impacts of Coastal Cities, Marine Ecology Laboratories A11, University of Sydney, NSW 2006, Australia*

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Morphometric data have long been used in the classification of different fish species. This paper presents the first morphometric study of the pharyngeal bones and teeth of three species of freshwater fish of the genus *Scardinius* from Greece. Two of the species, *S. graecus* Stephanidis, 1937 and *S. acarnanicus* Stephanidis, 1939, are endemic to Greece and one, *S. erythrophthalmus* (Linnaeus, 1758), is widely distributed in Europe as well. Morphometric data were collected from individual fish with regard to pharyngeal bone and teeth measurements and external body measurements. Discriminant function analysis was used, showing that the pharyngeal bone measurements could be used to successfully discriminate different species with identical tooth type and tooth formulae within the same genus. The results of separate discriminant function analyses on (1) pharyngeal bones and their teeth, versus (2) external body measurements are discussed.

KEYWORDS: Endemic Greek cyprinid fish, *Scardinius*, pharyngeal bones, morphometrics, comparative analysis.

Introduction

Morphological research of the skeleton of fishes has been systematically useful and many such studies have been made of geographically separated populations of fish species. There are also various reports which refer to or describe the pharyngeal bones and their teeth of fishes, but none of these studies has been devoted to the cyprinid fishes of Greece. Some previous studies have described the development of pharyngeal teeth (Weisel, 1967; Nakajima, 1984, 1987, 1990; Nakajima and Yue, 1989), while others have presented detailed analyses of pharyngeal teeth replacement (Evans and Deubler, 1955; Schwartz and Dutcher, 1962; Nakajima, 1979). External features of the pharyngeal teeth, their number, arrangement and tooth formulae have also been investigated as criteria for the classification of cyprinids (Eastman and Underhill, 1973; Suzuki and Hibiya, 1985; Thompson and Iliadou, 1990; Gould and Kaya, 1991).

A few papers (Chu, 1935; Vasarhelyi, 1958; Horoszewicz, 1960) have given a thorough comparative morphological description of the pharyngeal bones and their teeth of many species of the family Cyprinidae. The information given is not enough, however, to distinguish different species within the same genus, especially when species have identical tooth type and tooth formulae. As underlined by Horoszewicz (1960), the lack of differences in the shape of pharyngeal bones between closely related species, e.g. of the same genus, suggests the need for other head bones to be used as well.

The present paper describes the first morphometric comparative analysis of the pharyngeal bones and their teeth. It examines and compares these structures from three species of freshwater fish of the genus *Scardinius* Bonaparte, 1837 from Greece. In particular, it was of interest to determine whether measurements of pharyngeal bones alone could be used to discriminate among the species, and if so, which measurements of these bones were the best identifiers of fish species.

The cyprinid genus *Scardinius* is widely distributed in western, central and eastern Europe as well as in western Asia from Asia Minor to the Aral Sea (Berg, 1949; Ladiges and Vogt, 1979). Members of the genus are found in the north and central regions of Greece but not in the south (Peloponnesus).

The genus *Scardinius* in Greece is represented by three species: the European species *Scardinius erythrophthalmus* (Linnaeus, 1758), and two endemic species, *Scardinius graecus* Stephanidis, 1937 and *Scardinius acarnanicus* Stephanidis, 1939 (Economidis, 1991). The populations of these species are distributed in three biogeographical areas and are genetically isolated by land barriers. Populations of *S. erythrophthalmus* occur in Thrace, Macedonia and Thessaly. Populations of *S. graecus* exist only in Boeotia in the eastern-central part of Greece, whereas populations of *S. acarnanicus* live in Etoloacarnania in the western-central part of Greece (figure 1).

A recent study of the morphometrics and meristics of external body features of these species indicated that the two endemic species, *S. graecus* and *S. acarnanicus*, were close to each other, while both of them were more distantly related to the European species, *S. erythrophthalmus* (Iliadou *et al.*, 1996). The use of measurements of pharyngeal bones and teeth for the classification of fishes has never been done before. We wished to examine the degree to which the species could be classified by the morphometric data of the pharyngeal bones and their teeth. It was also of interest to determine the extent to which the taxonomic relationships obtained with external body measurements corresponded to novel results obtained from measurements of pharyngeal bones. For this reason, the body measurements were analysed again, using the same method applied to pharyngeal bones, for comparison.

Materials and methods

Specimens and measurements

All collected material came from the same three samples used in previous work (Iliadou *et al.*, 1996). These samples were obtained from three different sites in Greece from 1986 to 1989. The first sample of 81 fish of *S. erythrophthalmus* (mean SL = 145·66 mm ± 1·34 SE) was caught in Lake Koronia in northern Greece (Macedonia). The second sample of 191 specimens of *S. graecus* (mean SL = 144·61 mm ± 1·64 SE) was taken from Lake Yliki in eastern-central Greece (Boeotia) and the third sample of 89 individuals of *S. acarnanicus* (mean SL = 189·88 mm ± 2·73 SE) was fished from Lake Trichonis in western-central Greece (Etoloacarnania) (see table 1 in Iliadou *et al.*, 1996). Some fish were excluded from analyses due to missing data for certain characters (Data Analysis).

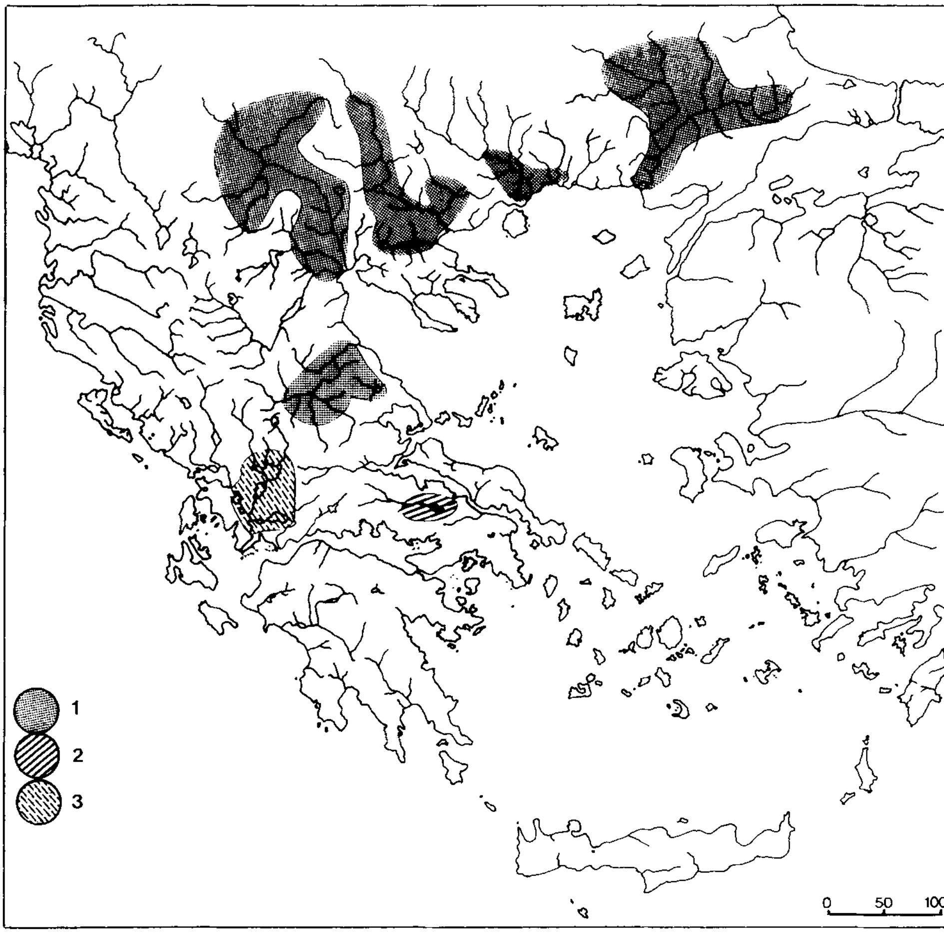

FIG. 1. Distribution of the three species of the genus *Scardinius* in Greece: (1) *Scardinius erythrophthalmus*; (2) *S. graecus*; (3) *S. acarnanicus*.

Study specimens of the three samples were not fixed in formalin but were brought to the laboratory frozen for measurement and immediate dissection. All fish, except juveniles, were measured and weighed. A total of 42 morphometric and meristic characters were measured or counted. For more complete details and analyses of all of these characters for each species, see Iliadou *et al.* (1996). Only the morphometric linear measurements (not meristic data or weights) were used in the present analyses and compared to the data on the pharyngeal teeth. There was a total of 23 such variables of body measurements (including the standard length of each fish) used here (Results).

The pair of pharyngeal bones (arches) of each fish was dissected from the head as a unit, together with their muscles and mucous membranes. They were submerged in cold, clean water for 15 days at room temperature in small containers, then rinsed under running cold water until all soft tissue was removed, and left to dry. This treatment provided perfect cleaning of the pharyngeal arches around and between the teeth, without any damage to these delicate structures.

The pharyngeal teeth in all species of the genus *Scardinius* in Greece are laterally flattened, serrated and arranged in two rows on each pharyngeal arch (figure 2). The major (internal) row of each arch normally has five teeth while the minor (external) row has three teeth. The tooth positions are numbered from anterior to posterior in the row and the tooth count is given by the pharyngeal tooth formula: 3·5–5·3. Rarely, some fish had tooth counts that differed from this formula (e.g. 2·5–5·3, etc.). No specimen with a missing tooth or a tooth-count formula differing from 3·5–5·3 was included in analyses.

For measurements of the pharyngeal arches, the position of the bones used by Horoszewicz (1960), was accepted. A horizontal line and two vertical lines (perpendicular to the horizontal) were drawn on a white piece of paper. The arches for

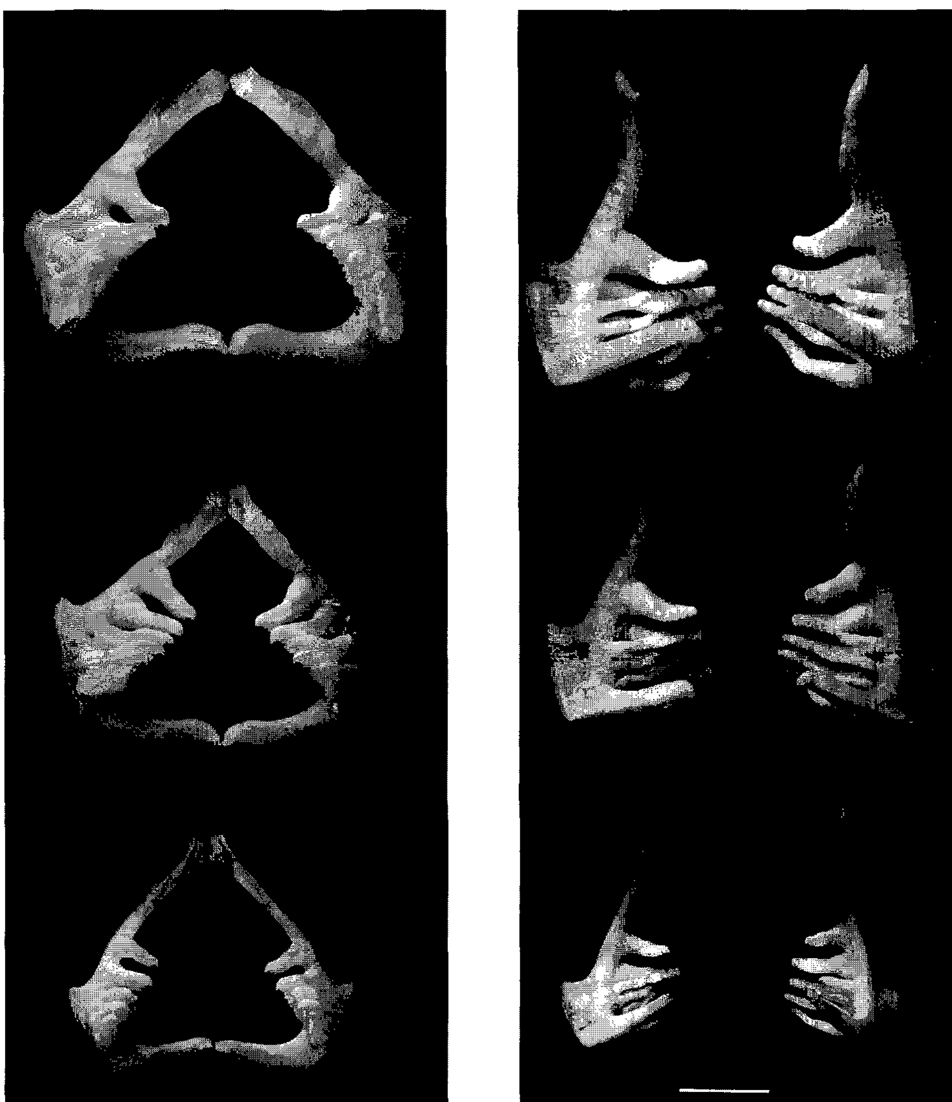

FIG. 2. Examples of pharyngeal bones from each of the three species of *Scardinius* examined in the present study, from top to bottom: *S. acarnanicus*; *Scardinius erythrophthalmus* and *S. graecus*. Scale bar indicates 5 mm.

each of the left and right sides of the fish were placed with the tips of their posterior limbs (terminology follows that of Hubbs *et al.*, 1974) touching the horizontal line, where it crossed the vertical, and the tips of the anterior limbs touching the vertical lines, as shown in figure 3. Note that the arches were inverted when placed in this orientation, thus the arch on the right hand side of the picture shown in figure 3 corresponds to the arch originally from the left side of the fish.

After placing the pharyngeal bones from each specimen of each species onto a separate sheet of paper, the following method proposed here for the first time, was used to take measurements. For each specimen, the specific points corresponding to the location of the tips and apices of the pharyngeal arches on each side were marked on the paper with a sharp needle. These points were labelled as shown in figures 3, 4. The points for either arch on each side were then connected with one another by straight lines on the page, each of which was measured (figures 3, 4, left side: lines KJ, KG, JG, JL and LG). The line KJ is the length of the anterior limb; line KG is the length of the posterior limb; line JG is the distance from the tip of the anterior limb to the tip of the posterior limb; line JL is the distance from the tip of the anterior limb to the posterior apice and LG is the distance from the tip of the posterior limb to the posterior apice. Lines were also drawn and measured between the points at each of the two apices of each arch to a point providing a perpendicular to the vertical lines (figures 3, 4, left side: lines KI and LH). The perpendiculars (AE, LH) are not part of the initial horizontal line (figure 3), which was used only for the placement of pharyngeal bones exactly opposite each other. The distance from the tip of the posterior limb to the point at the perpendicular was also measured (figures 3, 4, left side: line HG). Measurements of these three extra lines (figures 3, 4, left side: KI, LH and HG) were not included as variables in the analysis but allowed the two angles corresponding to the angles of the apices for each arch to be calculated (figures 3, 4, left side ∠JKG and ∠JLG). All of

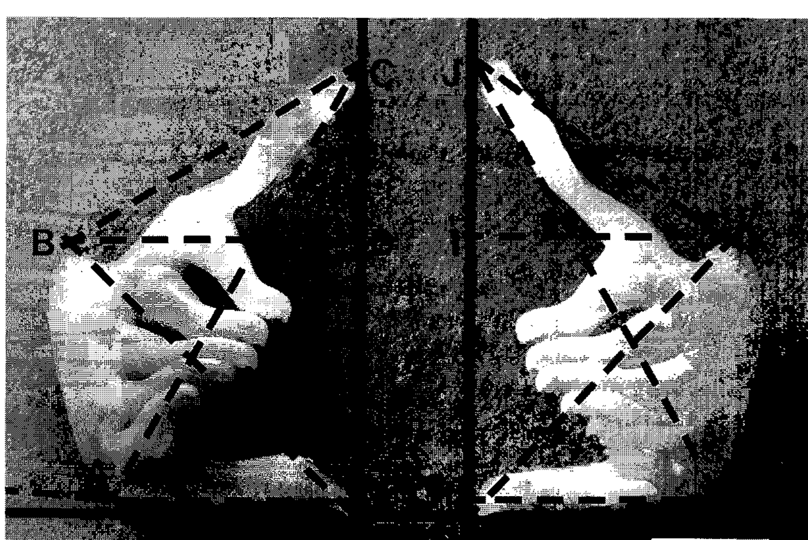

FIG. 3. Orientation of pharyngeal bones on the grid used for taking particular measurements. NB: the bones have been inverted for analysis, meaning the pharyngeal bone from the left side of the fish is on the right-hand side of the photograph. Scale bar indicates 5 mm.

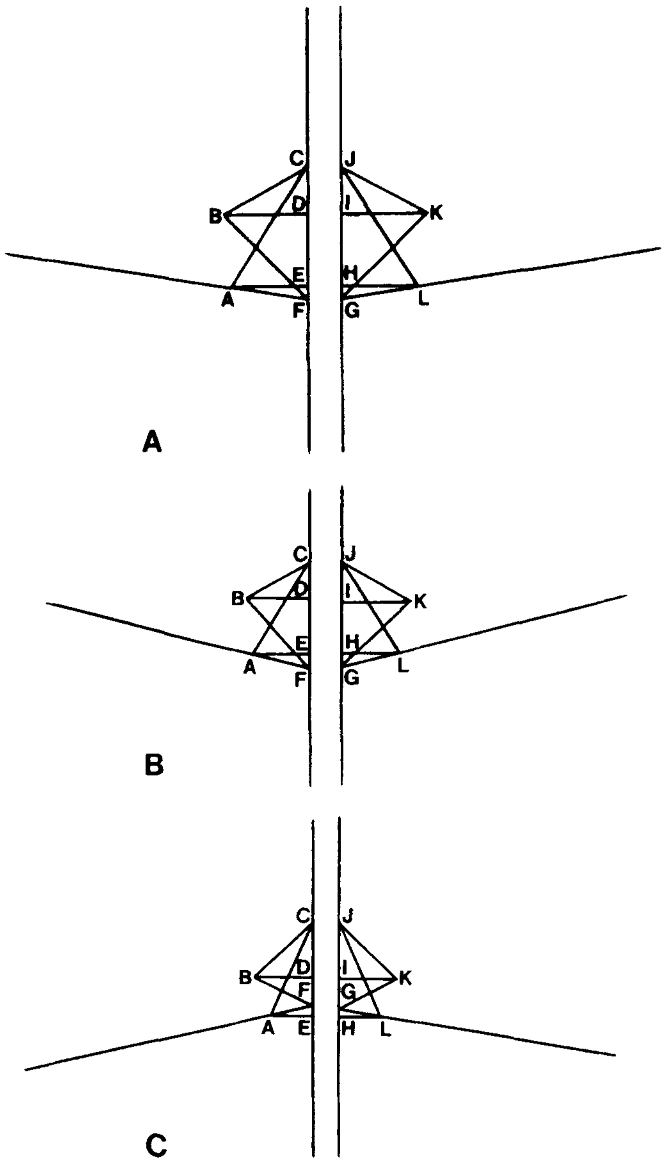

FIG. 4. Diagrams showing how points corresponding to apices and tips of pharyngeal arches were named and connected with straight lines for purposes of taking morphometric measurements for analysis for each of: (a) *S. acarnanicus*; (b) *Scardinius erythrophthalmus*; and (c) *S. graecus*. Note how the tips (F, G) of the posterior limbs of arches of *S. graecus* are located above the perpendiculars (AE, LH) to the vertical lines. Scale is actual size.

these angles were $<180°$ and thus (as transformed to values between 0 and 2π radians) could be included as quantitative variables in the analysis (Underwood and Chapman, 1985).

It should be noted that the points «F» and «G» were always the tips of the posterior limbs of the pharyngeal arches on the vertical lines, while the points «C» and «J» the tips of the anterior limbs. For *S. erythrophthalmus* and *S. acarnanicus*, the points «F» and «G» were almost always below the points «E» and «H» on the vertical lines (89 and 81% respectively). For *S. graecus*, in contrast, «F» and «G» were almost always above «E» and «H» (90%) (figure 4). Sometimes, for any species, the points «F» and «G» fell exactly on the points «E» and «H».

The morphometric variables for the arches used in the analyses included the five straight-line distances (lengths) indicated above and in figures 3, 4 (measured with calipers to 0·01 mm), the two angles calculated for the pharyngeal arches, and two other measurements: the length of bone occupied by the teeth (the dentigerous zone) and the height of the highest tooth. Consistent differences between the right and left pharyngeal arches can occur, thus, all the variables were analysed separately for each of the right and left sides of fish.

Data analysis

The analysis of morphometric characters can be influenced by the size of the individual fish from which measurements were taken and any allometric relationship between lengths of particular characters and the standard length of the fish. For this reason, the standard length of the fish was included in the analysis of (1) the pharyngeal bone measurements; and (2) the external body measurements. Due to differences in scale of the measurements (particularly between the standard length and the pharyngeal bone measurements), all of the data (except for the measurements of angles of arches) were first transformed to natural logarithms (Haddon and Willis, 1995). Individual variables (transformed to natural logarithms) were analysed for differences among the three species by analysis of covariance (ANCOVA), where ln (SL) was treated as a covariable. For each variable, where the slopes of the regression lines for the different species did not differ significantly, the test for differences among the intercepts was done. When the test among intercepts showed significant differences among the three species, *a posteriori* pair-wise tests were done using least squares. One-way analysis of variance (ANOVA) was done for the two variables which corresponded to angles of the arch, which were expressed as radians.

Multivariate analyses of the ability of characters to discriminate the three species were done using discriminant function analysis (DFA, Blackith and Reyment, 1971). DFAs were done separately for each of (1) the pharyngeal bone measurements; and (2) the body measurements. In order to compare the results, measurements from the same individual fish were analysed in each case. Of the fish collected, there were 88 *S. acarnanicus*, 77 *S. erythrophthalmus* and 52 *S. graecus* for which both body measurements and pharyngeal bone measurements had been taken. Measurements from these 217 fish were used for analyses. Due to missing measurements for certain characters, a subset number of these fish was used in each analysis such that no values were missing for any character included therein, but this did not affect the sample sizes greatly (Results). Due to differences in sample size, Type III sums of squares were used for all analyses (Searle, 1987; Shaw and Mitchell-Olds, 1993).

The taxonomic relationships, based on the two different kinds of morphometric data, were assessed by examining the squared Mahalanobis distances between the group centroids. They were also examined by graphical representation of the first two canonical variables obtained using DFA, and by projection of the frequencies of points from different groups onto the first and second canonical variables. Step-wise discriminant function analyses were used to identify the smallest subset of the total number of variables, for each of the pharyngeal bone measurements and the body measurements, which would still discriminate as successfully among the three species as the full set of variables. We did not use the traditional approach of using ratios of measurements against some indicator of body size, such as standard length, as was done in previous work (Iliadou *et al.*, 1996). Instead, the standard length (transformed to natural logarithms, as were other linear measurements) was always included as a variable in all DFAs (Blackith and Reyment, 1971; Haddon and Willis, 1995).

Results

Pharyngeal bone measurements

All of the linear measurements obtained from pharyngeal bones showed significant linear relationships with standard length ($P<0{\cdot}001$ for all). The results obtained from the left and right pharyngeal arches of the fish were similar for all analyses and the right arches had greater incidences of missing values, so the results presented here are for the left arches only. The measurement of length JG (the distance between the tips of the anterior and posterior limbs) had significant differences in the slope of the regression with standard length for different species (table 1). As was the case for the body measurements (see below), for all of the variables where the regression slopes were not significantly different, the intercept values for the different species were significantly different (table 1).

In general, the pharyngeal bone measurements were characterized by being proportionally bigger for *S. acarnanicus* than for the European species *S. erythrophthalmus*, which in turn were, on average, bigger than for the other endemic Greek species, *S. graecus* (table 1). Particularly, the pharyngeal bones of

Table 1. Summary of results of analyses of covariance and analyses of variance for data on measurements and angles of pharyngeal bones (left side only) for three species of fish: *Scardinius erythrophthalmus* (SE) ($n=70$), *S. graecus* (SG) ($n=50$) and *S. acarnanicus* (SA) ($n=82$). All linear measurements were transformed to natural logarithms before analysis. The covariable used for ANCOVAS was ln(standard length). Tests of homogeneity of slopes among the three species were done first, followed by tests of the intercepts where slopes did not differ significantly. Results of pair-wise comparisons using least squares means analysis under the full model are also shown for variables where intercepts were found to be significantly different among species and for significant ANOVAS of angles. The names of distances and angles used as variables are described in the Methods and are shown in figure 4.

Variable (ln)	Slope (1, 198 df)	Intercept (2, 198 df)	Results of least squares comparisons
KJ	$F=1{\cdot}06$, $P>0{\cdot}349$	$F=727{\cdot}80$, $P<0{\cdot}001$	SA = SG > SE
KG	$F=0{\cdot}95$, $P>0{\cdot}387$	$F=467{\cdot}66$, $P<0{\cdot}001$	SA = SE > SG
JG	$F=8{\cdot}63$, $P<0{\cdot}001$	—	—
JL	$F=2{\cdot}36$, $P>0{\cdot}097$	$F=124{\cdot}37$, $P<0{\cdot}001$	SA > SE = SG
LG	$F=1{\cdot}57$, $P>0{\cdot}211$	$F=309{\cdot}42$, $P<0{\cdot}001$	SA > SE > SG
Length of bone occupied by teeth	$F=1{\cdot}10$, $P>0{\cdot}334$	$F=300{\cdot}96$, $P<0{\cdot}001$	SA > SE > SG
Height of highest tooth	$F=0{\cdot}27$, $P>0{\cdot}765$	$F=305{\cdot}62$, $P<0{\cdot}001$	SA > SE > SG
Angle (radians)	ANOVA results		
∠JKG	$F_{(2199)}=16{\cdot}93$, $P<0{\cdot}001$		SA = SG > SE
∠JLG	$F_{(2199)}=45{\cdot}87$, $P<0{\cdot}001$		SE > SA > SG

S. graecus differed from those of the other two species by the proportionally smaller length of their posterior limb, smaller distance between tip of posterior limb and posterior apice, smaller length of dentigerous zone and smaller height of highest tooth. The pharyngeal bones of *S. erythrophthalmus* were distinguished from those of the other two species by the proportionally smaller length of their anterior limb, while the pharyngeal bones of *S. acarnanicus* differed from those of the other two species by the proportionally bigger distance between the tip of the anterior limb and the posterior apice. In addition, the angles of the pharyngeal arches were significantly different for the different species: ∠JKG was smaller for *S. erythrophthalmus* than for the two endemic Greek species; ∠JLG was, on the other hand, generally smallest for *S. graecus* (table 1). This latter result confirms observations (Materials and methods) that the tips of the posterior limbs of the pharyngeal arches were usually above the perpendicular lines for *S. graecus*, whereas they were usually below the perpendicular lines for either of the other two species (figure 2, 4).

Discriminant function analysis of the nine variables measured from pharyngeal bones plus standard length was also very effective in distinguishing the three species (Wilks' $\lambda = 0{\cdot}02051$, $F_{(20,\ 380)} = 113{\cdot}66$, $P < 0{\cdot}001$). The raw and standardized coefficients for the nine variables of the pharyngeal bone measurements and angles plus standard length for each of the discriminant functions (canonical variables) are shown in table 2. These discriminant functions identified the membership (classification) of individual fish in the data to one of the three species with a success rate of 99·5%, only very slightly less than the success of discriminant functions obtained from the body measurements (see below). The single misclassified fish was an outlier in the analysis and may have been caused by measurement error.

In contrast to the results obtained from the body measurements (see below), the squared Mahalanobis distances indicated that the shortest distance was between

Table 2. Values of raw and standardized coefficients for the discriminant function analysis of nine individual variables from measurements of pharyngeal bones (left side only) and standard length of three species of fish: *Scardinius erythrophthalmus* ($n=70$), *S. graecus* ($n=50$) and *S. acarnanicus* ($n=82$). All variables except angles were transformed to natural logarithms before analysis. The names of distances and angles used as variables are described in the Methods and are shown in figure 4.

	Raw coefficients		Standardized coefficients	
Variable (ln)	Canonical variable 1	Canonical variable 2	Canonical variable 1	Canonical variable 2
Standard length	19·532	−4·020	1·864	−0·384
KJ	10·457	−0·864	1·128	−0·093
KG	−9·886	−10·838	−1·196	−1·312
JG	−0·654	0·677	−0·074	0·076
JL	−3·573	23·196	−0·385	2·497
LG	−2·333	1·596	−0·291	0·199
Length of bone occupied by teeth	−6·513	−3·535	−0·787	−0·427
Height of highest tooth	−5·346	0·072	−0·724	0·010
∠JKG (rad)	2·437	1·965	0·239	0·193
∠JLG (rad)	−0·774	0·217	−0·058	0·016
Constant	−62·760	−14·945	—	—

S. acarnanicus and *S. erythrophthalmus* (19·68), while the distance was much greater for the comparison between *S. graecus* and either of *S. acarnanicus* (63·65) or *S. erythrophthalmus* (50·53). These results were supported by the analysis of the frequencies of canonical scores on the first canonical axis (figure 5). The first discriminant function (canonical variable) serves largely to discriminate between *S. graecus* and the other two species (figure 5). The second canonical variable then discriminates between *S. acarnanicus* and *S. erythrophthalmus*.

There were only three variables from the pharyngeal bone data plus standard length which were required to discriminate among the three groups (species) with a success rate of 99·1% (figure 6, Wilks' $\lambda=0{\cdot}03103$, $F_{(8392)}=229{\cdot}17$, $P<0{\cdot}001$). These variables were: standard length (required for the analysis) and lengths KJ (length of anterior limb which was the smallest for *S. erythrophthalmus*), KG (length of posterior limb which was the smallest for *S. graecus*) and JL (distance between tip of anterior limb and posterior apice which was the greatest for *S. acarnanicus*). Their raw and standardized coefficients for the discriminant functions are shown in table 3. As was found for the analysis with all nine variables plus standard length, the squared Mahalanobis distance was smallest between *S. acarnanicus* and *S. erythrophthalmus* (18·93), and much greater between either of these two species and *S. graecus* (44·17 and 33·11, respectively).

Body measurements

All of the body measurements (ln-transformed) included in analyses showed significant linear relationships with standard length ($P<0{\cdot}001$ for all). The variables of head depth, diameter of eye, interorbital width, maximum and minimum body depth, and the lengths of the dorsal and anal fin bases each showed significant differences in the slope of the regression with standard length for different species (table 4). For all of the variables where the regression slopes were not significantly different, the intercept values for the different species were significantly different (table 4).

The species *S. graecus* differed from the other two species, in general, by having a proportionally greater preorbital and post-orbital distance, a greater head length, greater post-dorsal distance and greater length of caudal peduncle (table 4). By contrast, the European species, *S. erythrophthalmus*, differed from the other two species generally by having a proportionally greater height of dorsal and anal fin, greater length of pectoral and ventral (pelvic) fin and greater total length (table 4). *S. acarnanicus* was distinguished from the others by having, on average, a proportionally greater distance between ventral and anal fins and greater preventral and preanal distances (table 4).

Discriminant function analysis showed that the species could be distinguished very effectively by the 23 variables of body measurements (Wilks' $\lambda=0{\cdot}00184$, $F_{(46\ 380)}=184{\cdot}09$, $P<0{\cdot}001$). The raw and standardized coefficients for the 23 variables of body measurements for each of the discriminant functions (canonical variables) are shown in table 5. Once so derived, the discriminant functions obtained from the body measurements were able to identify the membership (classification) of individual fish in the data to one of the three species with a success rate of 100%.

The squared Mahalanobis distance, calculated between each pair of groups (species), was found to be lowest between the two endemic species, *S. graecus* and *S. acarnanicus* (107·0), which were each more distant from the European species

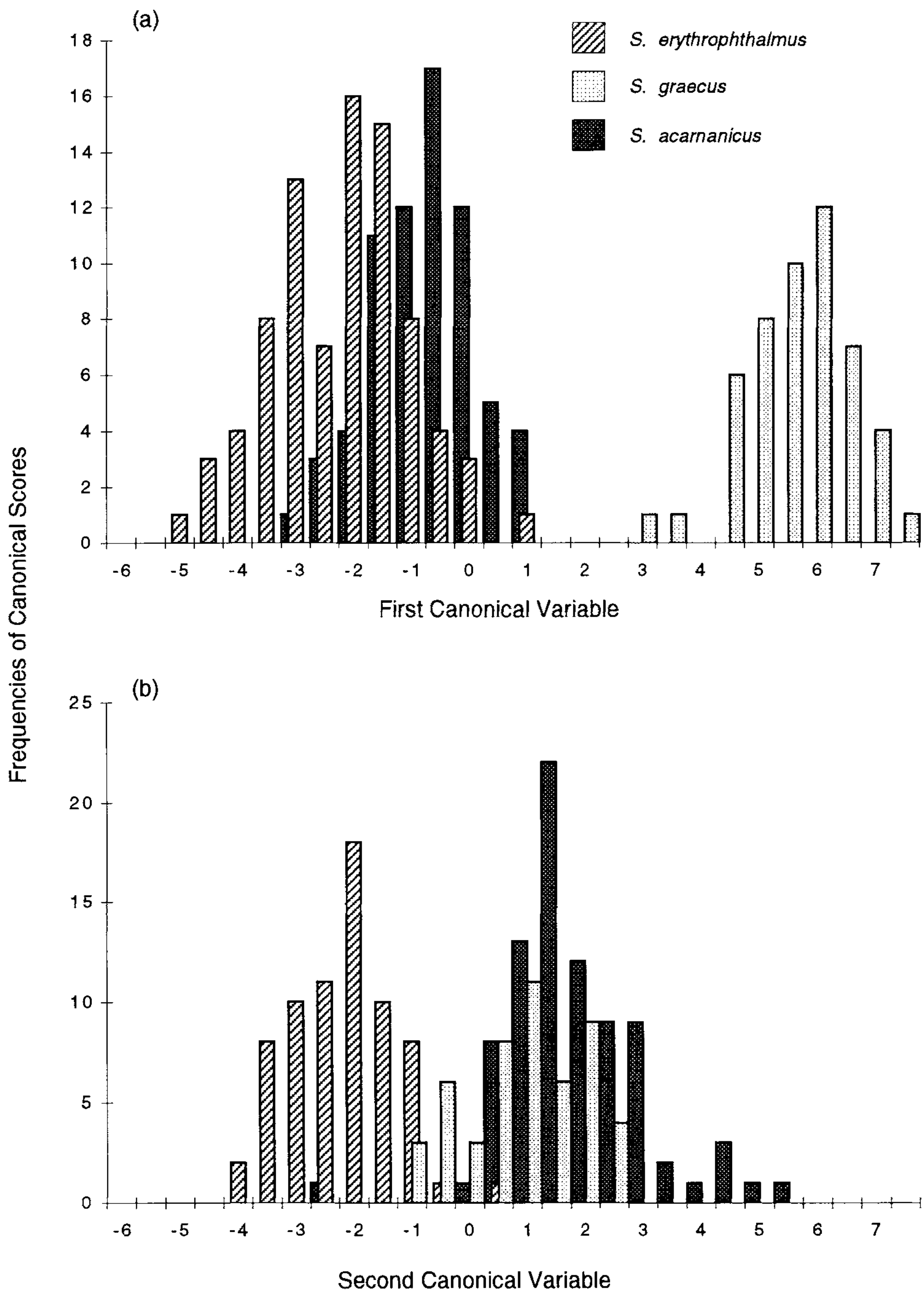

FIG. 5. Frequency histograms for canonical scores for three different species of fish on each of: (a) the first canonical variable; and (b) the second canonical variable after discriminant function analysis of morphometric data on pharyngeal bones.

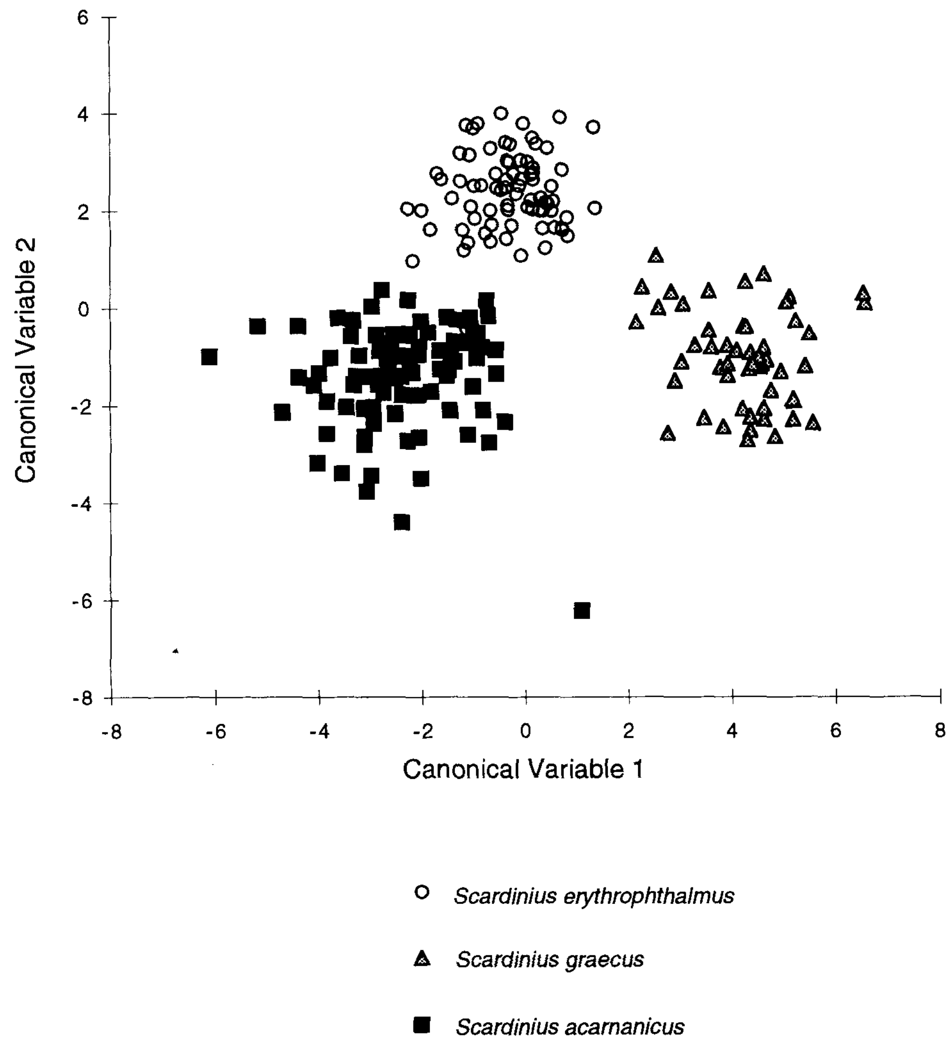

FIG. 6. Plot of canonical discriminant factor scores for the fish of the three species of the genus *Scardinius* using three measurements from the pharyngeal bones as variables plus the standard length of the fish, as listed in table 3.

S. erythrophthalmus (241·9 and 132·4 respectively). This result was also seen in the frequency histogram of the canonical scores for the different species on the canonical variables from the discriminant function analysis. The first canonical variable primarily separates the two endemic Greek species from the European species of *Scardinius*, while the second variable primarily separates the two endemic Greek species from one another (figure 7).

Similar results regarding the relative proximity of species were obtained in a discriminant function analysis of a subset of only five of the body measurement variables (figure 8, Wilks' $\lambda = 0{\cdot}00985$, $F_{(10\ 420)} = 381{\cdot}21$, $P < 0{\cdot}001$). These five variables from all of the body measurements yielded discriminant functions capable of 100% success in classification of fish to their correct species. These variables were: standard length (required for the analysis), interorbital width, length of anal

Table 3. Values of raw and standardized coefficients for the discriminant function analysis of three individual variables from measurements of pharyngeal bones (left side only) and standard length of three species of fish: *Scardinius erythrophthalmus* ($n = 77$), *S. graecus* ($n = 52$) and *S. acarnanicus* ($n = 87$). All variables were transformed to natural logarithms before analysis. The names of distances and angles used as variables are described in the Methods and are shown in figure 4.

	Raw coefficients		Standardized coefficients	
Variable (ln)	Canonical variable 1	Canonical variable 2	Canonical variable 1	Canonical variable 2
Standard length	16·025	1·814	1·529	0·173
KJ	11·020	− 0·067	1·189	−0·007
KG	−15·224	13·259	−1·842	1·605
JL	−12·370	−21·445	−1·332	−2·309
Constant	−36·733	16·923	—	—

fin base, height of anal fin and length of ventral fin. The raw and standardized coefficients for these variables in the discriminant functions are shown in table 6. Once again, the squared Mahalanobis distance was lowest between the two endemic species, *S. graecus* and *S. acarnanicus* (27·0), which were each more distant from the European species *S. erythrophthalmus* (92·4 and 87·7 respectively).

Discussion

The present study confirms once again the existence of three different species of the genus *Scardinius* in Greece and establishes that the species are clearly discriminated not only on the basis of their external features (as also reported by Iliadou *et al.*, 1996), but on the basis of their pharyngeal bone features as well. It was found that a subset of only four of 22 body measurement variables and a subset of only three variables from nine pharyngeal bone measurements (each in addition to the standard length of the fish) are enough to obtain 100% and 99·1% success, respectively, in classification of fish to their correct species using DFA. The identification of these species using the pharyngeal bones can be achieved, in practice, using the following equations (table 3):

$$CV1 = -36{\cdot}733 + 16{\cdot}025\ln(SL) + 11{\cdot}020\ln(KJ) - 15{\cdot}224\ln(KG) - 12{\cdot}370\ln(JL)$$

$$CV2 = 16{\cdot}923 + 1{\cdot}814\ln(SL) - 0{\cdot}067\ln(KJ) + 13{\cdot}259\ln(KG) - 21{\cdot}445\ln(JL)$$

where JL, KG, and KJ are particular measurements (figures 3, 4). Calculating the values for CV1 and CV2 and using figure 6, it is easy to find out to which species some particular pharyngeal bone belongs.

In addition, the new results clearly show that, according to body measurements, the two endemic Greek fish species are remarkably inter-related, whereas each is more distant from the European species. These results are in agreement with those presented by Iliadou *et al.* (1996), in spite of these two studies using completely different methodologies. In each case, similar morphological features were identified which differ from one species to another.

Moreover, the present study revealed also that the nature of the taxonomic relationships between these three species as suggested by their external body measurements is different to that derived from the pharyngeal bone and teeth

Table 4. Summary of results of analyses of covariance for data on external body measurements for three species of fish: *Scardinius erythrophthalmus* (SE) ($n=77$), *S. graecus* (SG) ($n=52$) and *S. acarnanicus* (SA) ($n=86$). All data were transformed to natural logarithms before analysis. The covariable used for ANCOVAS in each case was ln(standard length). Tests of homogeneity of slopes among the three species were done first, followed by tests of the intercepts where slopes did not differ significantly. Results of pair-wise comparisons using least squares means analysis under the full model are also shown for variables where intercepts were found to be significantly different among species.

Variable (ln)	Slope (1211 df)	Intercept (2211 df)	Results of least squares comparisons
Total length	$F=0{\cdot}58$, $P>0{\cdot}562$	$F=10{\cdot}13$, $P<0{\cdot}001$	SE > SG > SA
Preorbital distance	$F=0{\cdot}10$, $P>0{\cdot}906$	$F=111{\cdot}79$, $P<0{\cdot}001$	SG > SA > SE
Post-orbital distance	$F=0{\cdot}95$, $P>0{\cdot}388$	$F=270{\cdot}87$, $P<0{\cdot}001$	SG > SA > SE
Head depth	$F=6{\cdot}95$, $P<0{\cdot}002$	—	—
Diameter of the eye	$F=6{\cdot}51$, $P<0{\cdot}002$	—	—
Interorbital width	$F=4{\cdot}02$, $P<0{\cdot}020$	—	—
Head length	$F=1{\cdot}01$, $P>0{\cdot}366$	$F=306{\cdot}88$, $P<0{\cdot}001$	SG > SA > SE
Max. body depth	$F=8{\cdot}63$, $P<0{\cdot}001$	—	—
Min. body depth	$F=3{\cdot}05$, $P<0{\cdot}050$	—	—
Predorsal distance	$F=0{\cdot}13$, $P>0{\cdot}881$	$F=4{\cdot}93$, $P<0{\cdot}001$	SE > SG, but SE = SA and SA = SG
Post-dorsal distance	$F=1{\cdot}47$, $P>0{\cdot}233$	$F=82{\cdot}60$, $P<0{\cdot}001$	SG > SE = SA
Caudal peduncle length	$F=0{\cdot}13$, $P>0{\cdot}881$	$F=115{\cdot}23$, $P<0{\cdot}001$	SG > SA = SE
Length of dorsal fin base	$F=3{\cdot}84$, $P<0{\cdot}030$	—	—
Height of dorsal fin	$F=1{\cdot}04$, $P>0{\cdot}357$	$F=175{\cdot}45$, $P<0{\cdot}001$	SE > SA = SG
Length of anal fin base	$F=4{\cdot}43$, $P<0{\cdot}020$	—	—
Height of anal fin	$F=0{\cdot}47$, $P>0{\cdot}624$	$F=166{\cdot}07$, $P<0{\cdot}001$	SE > SA = SG
Length of pectoral fin	$F=2{\cdot}39$, $P>0{\cdot}094$	$F=94{\cdot}18$, $P<0{\cdot}001$	SE > SA > SG
Length of ventral fin	$F=0{\cdot}57$, $P>0{\cdot}567$	$F=302{\cdot}56$, $P<0{\cdot}001$	SE > SA > SG
Distance beween pectoral and ventral fins	$F=0{\cdot}53$, $P>0{\cdot}588$	$F=43{\cdot}50$, $P<0{\cdot}001$	SA = SE > SG
Distance between ventral and anal fins	$F=0{\cdot}55$, $P>0{\cdot}580$	$F=196{\cdot}56$, $P<0{\cdot}001$	SA > SE > SG
Preventral distance	$F=0{\cdot}04$, $P>0{\cdot}958$	$F=45{\cdot}23$, $P<0{\cdot}001$	SA > SG = SE
Preanal distance	$F=0{\cdot}66$, $P>0{\cdot}518$	$F=81{\cdot}40$, $P<0{\cdot}001$	SA > SE > SG

Table 5. Values of raw and standardized coefficients for the discriminant function analysis of 23 individual variables from external body measurements of three species of fish: *Scardinius erythrophthalmus* ($n=77$), *S. graecus* ($n=52$) and *S. acarnanicus* ($n=86$). All variables were transformed to natural logarithms before analysis.

	Raw coefficients		Standardized coefficients	
Variable (ln)	Canonical variable 1	Canonical variable 2	Canonical variable 1	Canonical variable 2
Total length	3·726	−24·452	0·341	−2·239
Standard length	−12·131	29·993	−1·158	2·863
Preorbital distance	−3·421	1·418	−0·369	0·153
Post-orbital distance	3·001	3·421	0·182	0·208
Head depth	−5·691	−5·266	−0·601	−0·556
Diameter of the eye	5·870	10·486	0·622	1·111
Interorbital width	17·402	−8·266	1·998	−0·949
Head length	−19·176	−5·486	−1·747	−0·500
Max. body depth	7·069	5·665	0·837	0·671
Min. body depth	0·850	−0·900	0·100	−0·106
Predorsal distance	−1·041	−6·281	−0·105	−0·633
Post-dorsal distance	−0·661	−7·316	−0·064	−0·715
Caudal peduncle length	−2·477	−2·433	−0·236	−0·232
Length of dorsal fin base	4·215	1·939	0·479	0·220
Height of dorsal fin	1·211	−3·827	0·105	−0·333
Length of anal fin base	1·024	−10·102	0·108	−1·063
Height of anal fin	1·788	−8·996	0·159	−0·802
Length of pectoral fin	−1·325	6·989	−0·121	0·640
Length of ventral fin	6·342	13·535	0·550	1·174
Distance between pectoral and ventral fins	0·921	−0·579	0·105	−0·066
Distance between ventral and anal fins	0·629	5·508	0·079	0·696
Preventral distance	−1·274	5·542	−0·125	0·544
Preanal distance	−9·374	1·446	−0·963	0·149
Constant	56·957	−20·261	—	—

measurements. With these measurements, one of the endemic species, *S. graecus*, is clearly uniquely distinguishable from the other two, *S. acarnanicus* and *S. erythrophthalmus*, which together form a separate group. Consequently, the two endemic Greek species are close to each other with their external body features and, at the same time, are distant from one another regarding their pharyngeal bones and teeth.

Here, we can only speculate that the cause of these differences is correlated with environmental peculiarities of the area inhabited by particular species and with the degree of its isolation. In this case, the occurrence of consistent and significant differences may be genetically controlled. According to Iliadou *et al.* (1996), the two endemic Greek species, *S. graecus* and *S. acarnanicus*, were probably derived from a common stock and their morphological differentiation was proportional to the time and the living conditions of their isolation. The species *S. graecus* appears to be the oldest one, since its existence is extremely restricted to a very small region which remained isolated since at least the upper Tertiary and which was not affected by glaciation (Economidis and Banarescu,

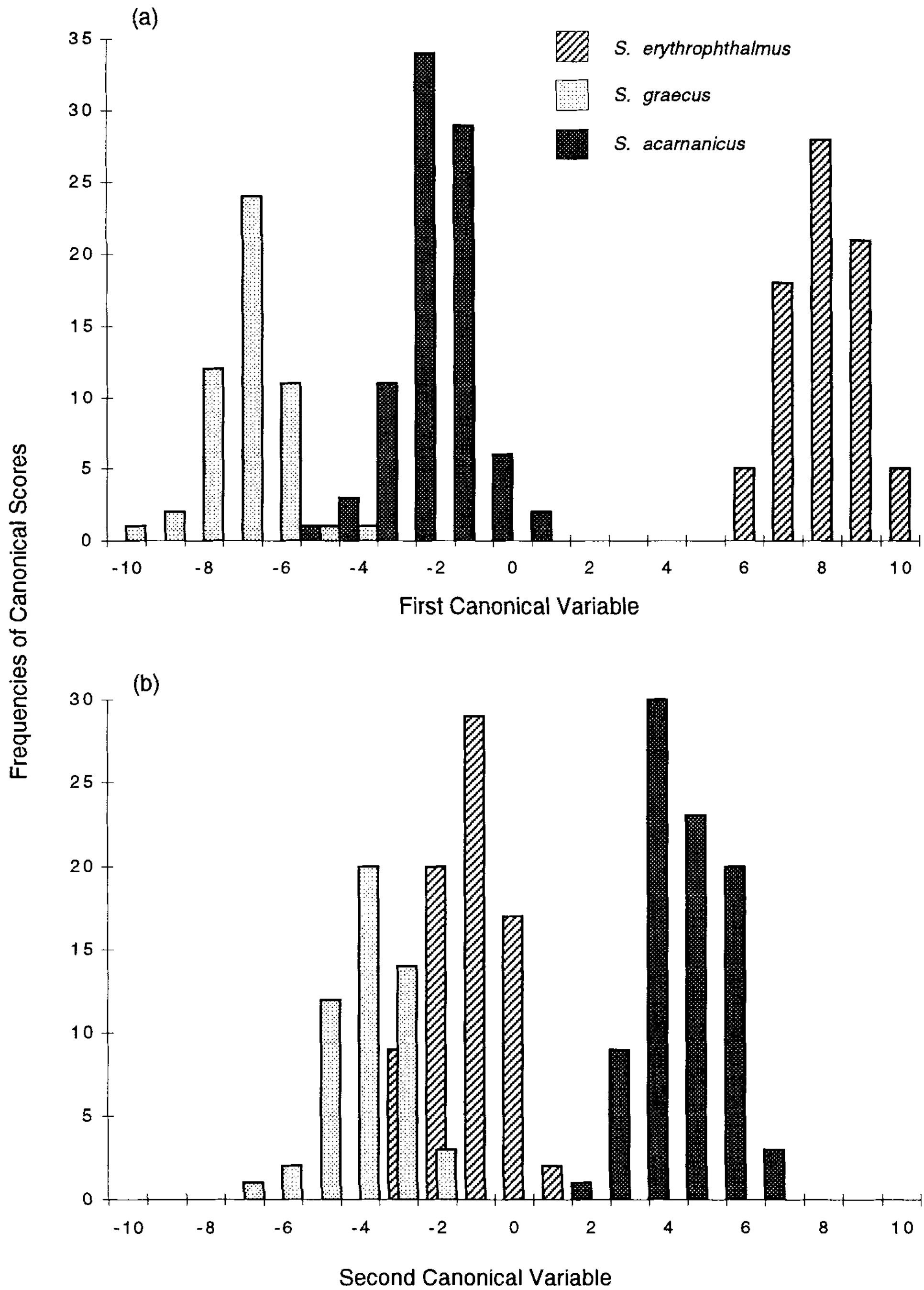

FIG. 7. Frequency histograms for canonical scores for three different species of fish on each of: (a) the first canonical variable; and (b) the second canonical variable after discriminant function analysis of morphometric data on external body measurements.

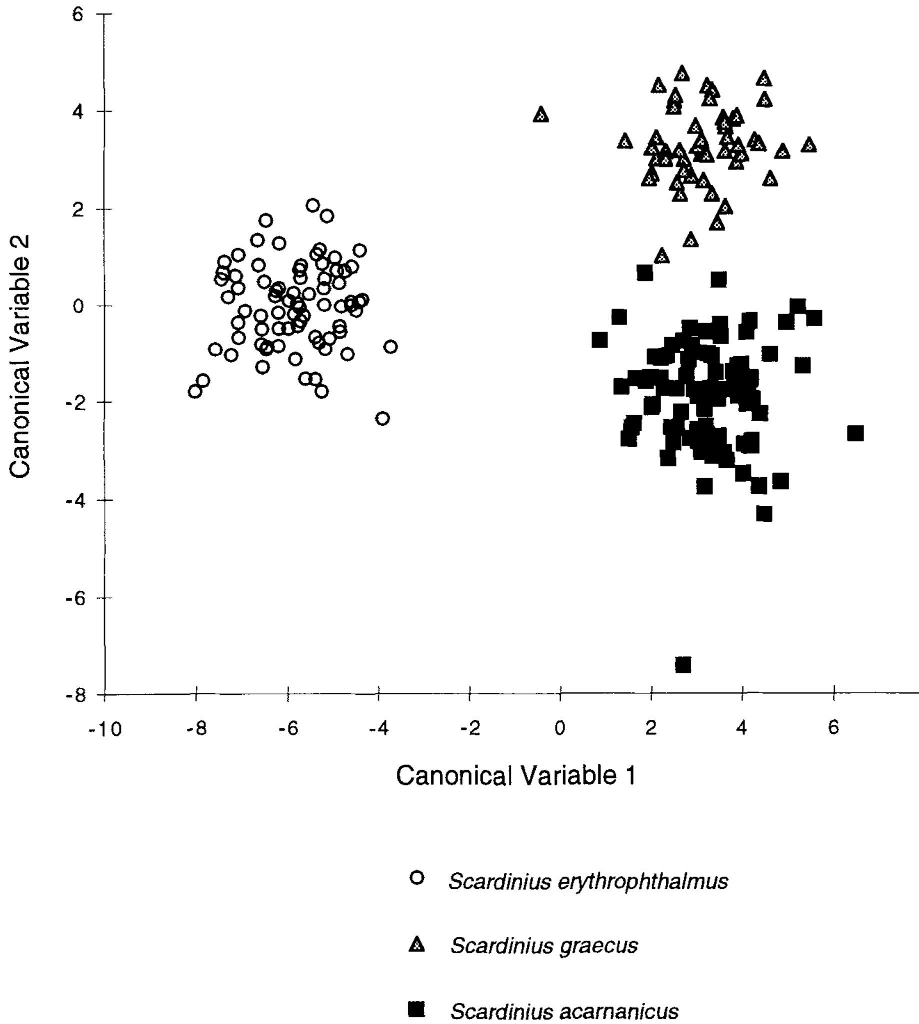

FIG. 8. Plot of canonical discriminant factor scores for the fish of the three species of the genus *Scardinius* using five external body measurement variables, as shown in table 6.

1991). The species, *S. erythrophthalmus*, continuously distributed from central Europe to central Greece, is the most recent one.

Therefore, concerning the structure (proportions) of pharyngeal bones, the evolutionary trend was, apparently, toward feeding adaptations. It appears that specialization led to an increase in the size, thickness and weight of pharyngeal bones only of two species, *S. acarnanicus* and *S. erythrophthalmus*. Indeed, the results of the present study indicate that these two species are closely related regarding these bones. In addition, as the adults of both species have adapted to feeding on higher aquatic plants and macro-algae (Iliadou, 1991), the maintenance of strong, massive pharyngeal bones by selection pressure may have proved, therefore, useful, even if *S. erythrophthalmus* is characterized as omnivorous, while *S. acarnanicus* is a purely herbivorous fish. Whereas, the species *S. graecus*, having relatively delicate pharyngeal bones but with sharp serrated teeth, as in the other two species, seems to remain

Table 6. Values of raw and standardized coefficients for the discriminant function analysis of five individual variables from external body measurements of three species of fish: *Scardinius erythrophthalmus* ($n=77$), *S. graecus* ($n=52$) and *S. acarnanicus* ($n=88$). All variables were transformed to natural logarithms before analysis.

	Raw coefficients		Standardized coefficients	
Variable (ln)	Canonical variable 1	Canonical variable 2	Canonical variable 1	Canonical variable 2
Standard length	37·776	10·700	3·621	1·026
Interorbital width	−18·898	−8·038	−2·179	−0·927
Length of anal fin base	−9·858	6·620	−1·039	0·698
Height of anal fin	−6·077	9·072	0·543	0·810
Length of ventral fin	−0·230	−24·098	−0·020	−2·091
Constant	−94·864	−2·246	—	—

adapted to feeding on a, more or less, soft food such as soft algae, aquatic and terrestrial insects and their larvae (Iliadou, unpublished).

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