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Research Article

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Use of morphological differences for the identification of two picarel species *Spicara flexuosa* and *Spicara maena* (Pisces: Centracanthidae)

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Abstract

The recognition and identification of the two species of *Spicara* genus (*Spicara flexuosa,* picarel and *Spicara maena,* blotched picarel) is difficult, due to a systematic confusion until now. In this work a number of external morphometric features (ten body ratios) are evaluated for their diagnostic possibilities. According to Principal Component Analysis results, the body ratios head length to standard length, head height to head length and the ratios of two body heights, indicated that these characters were not related to the maturity stage of the species. The discriminant analysis based on the above body ratios, indicated a rather high level of discrimination (83.2%) of the examined samples for two species. The results are discussed, and possibilities for improvement of the identification methodology for the two species are proposed.

Keywords: Centracanthidae, spicara, multivariate analysis, morphometric.

Introduction

Species that belong to the genus *Spicara* occur in shallow rocky and muddy bottoms throughout the Mediterranean and the Black Sea, in the Atlantic from Portugal to Morocco and around the Canary Islands (Froese & Pauly, 2013). These species contribute to fisheries in inshore areas of the Greek seas (Mytilineou & Papaconstantinou, 1991; Stergiou *et al*., 2011), and represent a major proportion of the total catch for coastal fisheries in Croatia (Dulcic *et al*., 2000). On the other hand, they are the common by-catch of coastal fisheries in the Mediterranean (especially those operating bottom trawlers) with low commercial value (Ragonese *et al*., 2004).

The genus *Spicara* has posed numerous identification problems and consequently many different species have been described, leading to a variety of synonyms. This fact was attributed to marked variations in coloration related to the effects of sexual dimorphism (reinforced by the protogynous hermaphroditism sex inversion) and colour changes due to the state of sexual maturity (Pollard & Pichot, 1971).

According to the review of Pollard & Pichot (1971), the old classification distinguished two genera, *Maena* and *Smaris* that subsequently were distinguished into two genera *Maena* and *Spicara* and finally were fused into a single genus *Spicara*, which comprises three species: *Spicara maena* (Linnaeus, 1758), *Spicara smaris* (Linnaeus, 1758) and *Spicara chrysalis* Valenciennes, 1830, which is a synonym of *Spicara flexuosa* Rafinesque, 1810 (Tortonese, 1975).

In literature, there was a systematic confusion about whether *S. maena* and *S. flexuosa* are the same (Tortonese, 1975; Eschmeyer, 2013; Froese & Pauly, 2013) or different species (Vasiliev, 1980; Papakonstantinou, 1988; Golani *et al*., 2006; Vasil'eva, 2007; Imsiridou *et al*., 2011). The above conflict is maintained till today; although karyological studies (Vasiliev, 1980) as well as genetic studies (Chiba *et al*., 2009; Imsiridou *et al*., 2011; Turan, 2011) undoubtedly prove that *S. maena* and *S. flexuosa* are two different species. Assuming that *S. maena* and *S. flexuosa* are two different species, identification is possible using meristic and morphometric characteristics (Tortonese, 1986; Fischer *et al*., 1987; Rizkalla, 1996).

Multivariate analysis, on a set of phenotypic characters, is regarded as a powerful technique for the determination of morphological relationships among different populations of a species (Claytor & MacCrimmon, 1988; Corti & Crosetti, 1996; Vidalis *et al*., 1997) and for investigating taxonomic problems among species (Spain *et al*., 1980; Karakousis *et al*., 1991; Iliadou *et al*., 1996; Katselis *et al*., 2006). It also creates the mathematical foundation for the development and support of new tools. The development of specialized software tools such as

TPS-dig software, version 1.37 (Rohlf, 2003) offers the possibility of taking measurements of an item, on a digital photo. These tools are predominantly used in ichthyology nowadays. Despite the benefits, they have limitations associated with the quality of the extracted information. If a fish photo is to be used for determining the species, the recognition characters should be clearly identifiable and measurable.

The aim of this study is to evaluate the similarity/dissimilarity of *S. flexuosa* and *S. maena* species, using easily distinguishable morphometric characters. A second aim is to develop a mathematical background for the diagnosis of the two species, using the multivariate analysis of morphometric characters.

Materials and Methods

Fish Samples

Specimens were collected by professional fishermen in the Thermaikos Gulf (40° 23' N; 22° 50' E), Northern Aegean Sea. Individuals of *S. maena* were collected with purse seine nets (20 mm mesh-size stretched), in 25-35 m depth on sandy bottom with *Posidonia oceanica* beds, while those of *S. flexuosa* were collected with pots (metal frame lined with a wire mesh, box shaped 80x60x30 cm, mesh size 20 mm). Pilchards were used as bait and the pots were set at 25-35 m depth, on sandy or muddy bottoms, with parts covered with hard substrate. The advantages of these fishing gears (purse seines and traps) are that they capture a wide range of fish length from a specific fish population and area, either encircling shoals of fish or baiting individuals that are seeking shelter or food. In contrast, other fishing gears such as static gill nets and bottom trawls are selective on the range of fish length caught, and sweep a broader area. Individuals of *S. maena* and *S. flexuosa* were randomly collected from March to August 2008, a period which coincides with their spawning season (Tortonese, 1986; Karidas *et al*., 2009, 2011). Specimens were identified according to the characters of the identification keys (Tortonese, 1986; Fischer *et al*., 1987).

Morphometric Analysis

A series of measurements was recorded on each specimen, for six (6) distance characters (Fig. 1). Specifically, Total Length (*TL*) is defined as the distance from the tip of the nose to the longest caudal fin ray, Standard Length (*SL*) as the distance from the tip of the nose to the end of the vertebral column, Head Length (*HL*) as the distance from the tip of the nose to the posterior margin of the opercula, Head Height (*HH*) as the distance from the lower end to the upper end of the head, Maximum Body Height 1 (MBH_1) as the vertical distance from the anterior part of the dorsal fin and ventral part of the body and Maximum Body Height 2 (MBH_2) as the vertical distance from the dorsal fin and ventral part of the body. These characters are easily distinguishable and visible on a fish photo, and some of these have been used to identify the two species (Fischer *et al*., 1987; Lythgoe & Lythgoe, 1992; Golani *et al*., 2006). All measurements were made to the nearest 0.1 cm with electronic vernier caliper; for paired structures only the left structure value was taken.

After measuring, fish were dissected and the gonads were removed and observed for sex identification. Sex and maturity stage of gonads were determined macroscopically according to the scale of Nikolsky (1963), slightly modified to fit the characteristics of the gonad stages of maturity of the picarels (Vidalis, 1994). Total weight of body (W) and weight of gonads (Wg) were measured to the nearest 0.1g, and the Gonadosomatic Index (GSI=100*Wg/W) was calculated.

To minimize any variation resulting from allometric growth, all morphometric measurements were standardized according to Reist (1985): $X'_{i,j} = \overline{\log X_i} - b \cdot (\log TL_j - \overline{\log TL_i})$ where $X'_{i,j}$ is the standardized measurement of the i morphometric character; $\overline{\log X_i}$ is the mean logarithm of i morphometric character measurement; TL_j is the total length of the individual j; $\overline{\log TL}$ is the logarithm of the mean total length of pooled individuals and *b* is the slope of the *logX* against *logTL* plot. Also, the anti-logarithms of standardized measurements and their ratios were estimated. Finally, the *t*-test was used (Zar, 1999) to identify whether there were any statistically significant differences between the species for each character ratio.

Principal component analysis (PCA) was used to test the contribution of the morphometric characters ratios and logarithm of *GSI*, to the configuration of fish shape variance (Hair *et al*., 1998). To elucidate the differentiation of the species, forward stepwise discriminant analysis (*DA*), on the characters based on the generalized Mahalanobis distance, was used, to determine the similarity between groups and the ability of these variables to identify the specimens correctly (Hair *et al*., 1998).

In order to identify any significant differences among the sex per species groups (immature, males and females) on the scores of each extracted canonical vari-

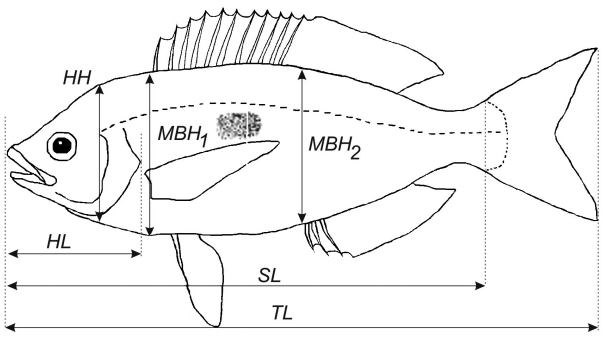

Fig. 1: Measurements that were taken on picarel and blotched picarel individuals. *TL*: total length; *SL*: standard length; *HL*: head length; *HH*: head height; MBH_1: body height in the origin of dorsal fin; MBH_2: maximum body height.

ables (CaV), analysis of variance (ANOVA) was applied. Furthermore, the Tukey HSD test was applied, to check which sex per species differs from each other. Also, to minimize any variation resulting from the maturity stage of individuals, the morphometric ratios that related with the *GSI* were excluded from the discriminant analysis. All statistical analyses were performed with the SPSS PC ver. 10 software package.

Results

Eight hundred and eighty eight (888) individuals were examined for morphometric analysis (9.9-84.3 g total body weight; 9.6-18.1 cm total length). From the above, two hundred and ninety nine (299) individuals of *S. maena* (11.6-18.1 cm total length) and five hundred and eighty nine (589) individuals of *S. flexuosa* (9.6-18.1 cm total length). Summary statistics and *t*-test results are shown in Table 1. Significant differences for all examined ratios of morphometric characters among the species, were shown (*t*-test>5; df= 887; $P<0.05$).

Table 1. Summary statistics for total length (TL), Gonadosomatic index (GSI) and ratios among the morphometric characters, estimated after the Reist (1985) standardization. X: mean value (cm); SD: standard deviation; Min-max: minimum and maximum value (cm); N: number of individuals; IM: immature individuals; M: males and F: females.

	Spicara maena			***Spicara flexuosa***				
	N=299 (IM=0; M=78; F=221)			**N=589 (IM=174; M=269; F=146)**				
Character	**X**	**SD**	**min-max**	**X**	**SD**	**min-max**	**t-test**	***P***
TL	15.37	1.09	11.6-18.1	14.10	1.80	9.6-18.1	13.1	<0.05
GSI	0.08	0.05	0-0.37	0.01	0.02	0-0.24	23.2	<0.05
HL/SL	0.28	0.01	0.25-0.31	0.29	0.01	0.27-0.33	18.4	<0.05
HH/SL	0.24	0.01	0.21-0.29	0.24	0.01	0.19-0.31	5.1	<0.05
MBH_1/SL	0.30	0.02	0.25-0.34	0.27	0.01	0.24-0.36	20.3	<0.05
MBH_2/SL	0.30	0.02	0.26-0.35	0.27	0.01	0.22-0.32	24.8	<0.05
HH/HL	0.86	0.04	0.75-1.02	0.81	0.04	0.67-1.00	17.9	<0.05
MBH_1/HL	1.05	0.06	0.87-1.21	0.93	0.04	0.79-1.11	29.0	<0.05
MBH_2/HL	1.06	0.06	0.93-1.22	0.93	0.04	0.73-1.08	33.0	<0.05
MBH_1/HH	1.22	0.07	0.97-1.45	1.15	0.05	0.90-1.40	16.2	<0.05
MBH_2/HH	1.24	0.07	1.03-1.49	1.15	0.06	0.92-1.34	17.8	<0.05
MBH_2/MBH_1	1.01	0.04	0.93-1.17	1.01	0.04	0.72-1.23	4.5	<0.05

Table 2. Results of principal components analysis (PCA) and factor loadings for each morphometric variable on the four extracted PCA factors (*Fi*), after varimax normalized rotation.

	Factors			
Variance	***F1***	***F2***	***F3***	***F4***
Eigenvalues	5.668	2.416	1.382	1.094
% of Variance	43.25	23.09	15.15	14.52
Cumulative %	43.25	66.34	81.49	96.00
Character				
GSI	0.67	0.22	0.35	0.07
HL/SL	-0.23	0.08	-0.96	-0.05
HH/SL	0.07	0.95	-0.29	-0.08
MBH_1/SL	0.86	0.45	-0.08	-0.23
MBH_2/SL	0.83	0.38	-0.05	0.39
HH/HL	0.24	0.89	0.38	-0.04
MBH_1/HL	0.83	0.34	0.39	-0.16
MBH_2/HL	0.80	0.28	0.40	0.34
MBH_1/HH	0.90	-0.34	0.18	-0.18
MBH_2/HH	0.79	-0.35	0.18	0.46
MBH_2/MBH_1	0.00	-0.09	0.04	0.99

The PCA analysis extracted four factors with eigenvalues>1, explaining 96% of the variance (Table 2). Using a cut-off value of 0.6 for the factor loadings, factor 1 (F1) expressed characters associated with the maximum body height to length ratios and gonadosomatic index, factor 2 (F2) expressed variables associated with the head height to head length and to standard length ratios (*HH/HL* and *HH/SL* respectively). Finally, factor 3 (F3) expressed variables associated with the head length to standard length ratio (*HL/SL*), while factor 4 (F4) expressed variables associated with the maximum body height ratio (MBH_2/MBH_1).

The discriminant analysis (DA) on the *HL/SL*, *HH/SL*, *HH/HL* and MBH_2/MBH_1 presented one canonical variable (*CaV*) which contributed overall to the variance (the expressed characters by factor 1 were excluded, due to their relation to the maturity stage of each fish). The characters of primary importance in distinguishing species were *HL/SL* and *HH/HL*. The discriminant function (*DF*) was:

$$DF = -134.03 - 539.8 \cdot (HH/SL) + 384.4 \cdot (HL/SL) + 175.2 \cdot (HH/HL) + 6.96 \cdot (MBH_2 / MBH_1)$$

The discriminant function identified the membership (classification) of individual fish in the data to one of the two species, with a success rate of 83.19%. The percentage of correctly identified *S. maena* and *S. flexuosa* individuals was 64.5% and 92.7%, respectively (Table 3). The frequency distribution of discriminant function scores for two species is shown in Figure 2. The 77% of *S. flexuosa* individuals were given DF<0, while the 83% of *S. maena* were given DF>0 (Fig. 2). The DF scores differ significantly between sexes per species (ANOVA; df=4.804; F=162; P<0.05). On the other hand, no significant differences appeared among sexes on the same species (Tukey HSD test; P>0.05) (Fig. 3).

Discussion

The morphometric characters (phenetic characters) derive from the composite effect of genotype and environmental factors, and they are under the influence of natural selection (Dobzansky, 1970). It is certain that parameters related to the allometric growth of fish and the timing of the sampling (feeding activity and maturation), could pose some major limitations for the study of morphological relationships between species. This study attempted to minimize variances caused by these parameters through the common sampling period, as well as the transformation of the original measurements (Reist,

Table 3. Results of discriminant analysis classification showing the percentage of specimens classified in each species.

	Predicted species		
Species	***Spicara maena***	***Spicara flexuosa***	**Total individuals**
Spicara maena	64.5	35.5	299
Spicara flexuosa	7.3	92.7	589

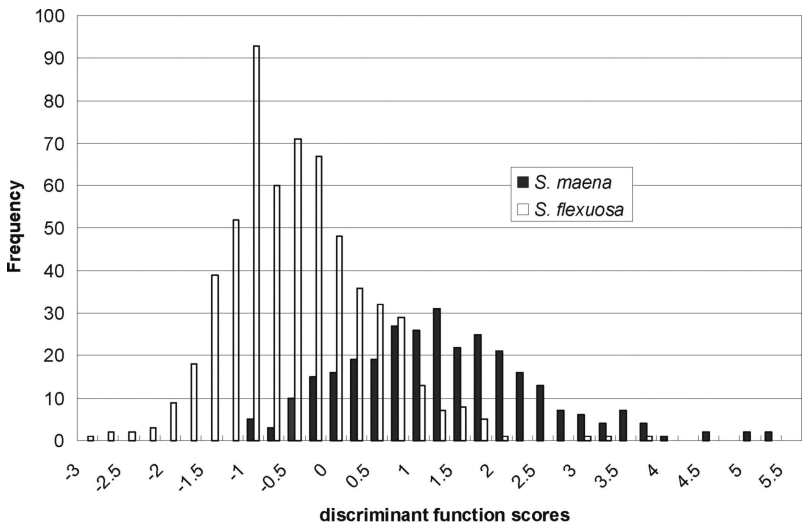

Fig. 2: Frequency distribution of discriminant function scores of the first extracted canonical variable for *S. flexuosa* and *S. maena*.

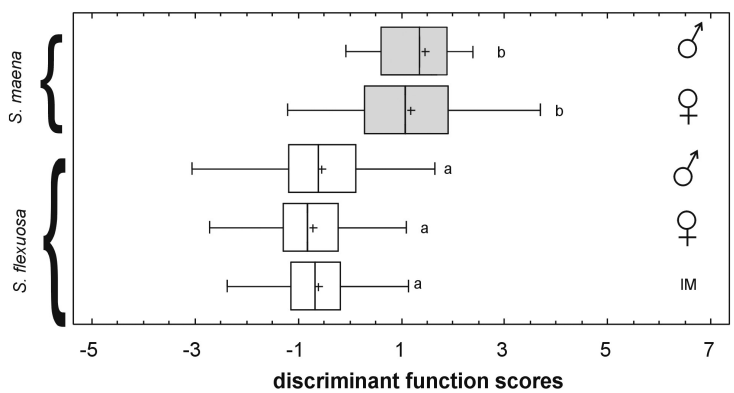

Fig. 3: Box and Whisker plot (min-max, 95% values. Mean, SD) of the discriminant function scores of the first extracted canonical variable for male, female and immature individuals of *S. flexuosa* and *S. maena.* Groups that they do not differ statistically (Tukey HSD test; P>0.05) are indicated with the same letter, a or b.

1985). It also attempted to remove the characters which are related to the maturity stage of fish, from the analysis.

The analysis of the morphological variability presented here, indicated significant differences in external shape between the two species (Table 1). The ratios of body measurements indicated that the individuals of *S. maena* are characterized by shorter/higher head and deeper body than those of *S. flexuosa.* These findings are in agreement with those of other authors (Pollard & Pichot, 1971; Tortonese, 1986; Fischer *et al*., 1987; Lythgoe & Lythgoe, 1992; Miller & Loates, 1997; Golani *et al*., 2006). Some authors have used the relation between head length and maximum body height, to identify the two species. The head length is equal or greater than the body height in *S. flexuosa*, while the head length is shorter than the body height in *S. maena* (Fischer *et al*., 1987; Lythgoe & Lythgoe, 1992; Golani *et al*., 2006). Although our results are confirmed by the above findings, the fact that the ratios of the body height (MBH_1/HL, MBH_2/HL, MBH_1/HH, MBH_2/HH, MBH_1/SL, MBH_2/SL) apart from the ratio of MBH_2/MBH_1, are related to the maturity stage of species (Table 2), decreases the discrimination ability of these characters.

In substitution of these recognizing characters, our results proposed that the ratios of a) head length to standard length (*HL/SL)* as F3, b) head height to head length *(HH/HL)* as F2, c) head height to standard length (*HH/SL*) as F2 and d) the ratio of the two body heights (MBH_2/MBH_1) as F4, show low association (<0.35) to the species maturity stage (*GSI*), since the factor loadings of F2, F3 & F4 are 0.22, 0.35 and 0.07 respectively (Table 2). The discriminant analysis based on these characters indicated a rather high level of species identification. Indeed, 83.2% of the examined specimens could be distinguished and classified correctly in the two species. Moreover, the loadings of discriminant function (linear results of these characters) were not related to the sexual dimorphism of the species (Fig. 3) and could thus constitute a more reliable tool for identification of the two species than the ones used today. Consequently, they can be a reliable tool for the identification of the two species. The sharpness of the characters as well as the high discriminative values (>83%), provide a great possibility to be used for identification of the two species from the new technologies.

The relatively small percentage of non-discrimination (16.8%) of the above characters, can be overcome by more general information about the species such as differences in certain biological aspects i.e. spawning period and sexual behaviour (Tortonese, 1986; Fischer *et al*., 1987; Lythgoe & Lythgoe, 1992; Dulčić *et al*., 2000; length of sex reversal: Lepori, 1960; Relini *et al*., 1999; Dulčić *et al*., 2000; Karakulak *et al*., 2007; Karidas *et al*., 2009*,* 2011), body colour pattern (Lythgoe & Lythgoe, 1992; Louisy, 2002; Karidas *et al*., 2009), and external morphology (Fischer *et al*., 1987; Lythgoe & Lythgoe, 1992; Louisy, 2002; Golani *et al*., 2006). Also, the fact that *S. maena* and *S. flexuosa* are two different species is reinforced by an anatomical character, which is present in *S. maena*: well-developed teeth on the vomer (Tortonese, 1986; Lythgoe & Lythgoe, 1992). Additionally, each species prefers different habitats, with *S. maena* inhabiting sandy - muddy *Posidonia* beds down to about 100 m., while *S. flexuosa* appears on sand or muddy bottoms, with rocky particles down to about 130 m (Tortonese, 1986).

In conclusion, the results from the morphometric analysis revealed discrimination between *S. maena* and *S. flexuosa*, based on four body proportions that derive from five easily identifiable morphological characters (*HH, HL, SL* MBH_1 and MBH_2). The above characters could be used by information technology for developing new species recognition tools. In any case, these findings contribute to the improvement of the identification methods for the two species, based on external features.

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