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Traditional and geometric morphometrics detect morphological variation of lower pharyngeal jaw in *Coris julis* (Teleostei, Labridae)

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Abstract

In the present study, variation in the morphology of the lower pharyngeal element between two Sicilian populations of the rainbow wrasse *Coris julis* has been explored by the means of traditional morphometrics for size and geometric morphometrics for shape. Despite of close geographical distance and probable high genetic flow between the populations, statistically significant differences have been found both for size and shape. In fact, one population shows a larger lower pharyngeal element that has a larger central tooth. Compared to the other population, this population has also medially enlarged lower pharyngeal jaws with a more pronounced convexity of the medial-posterior margin. The results are discussed in the light of a possible more pronounced durophagy of this population.

Keywords: *Labridae*, *pharyngeal jaws*, *geometric morphometrics*

Introduction

The pharyngeal jaw apparatus of perciform fishes is a well-developed system that functions in sophisticated prey processing behaviours, complementing the functions of the oral jaw apparatus (Wainwright 2005). The pharyngeal jaw apparatus has been studied in many Teleostei (Sibbing 1982; Tigano et al. 1999; Vandewalle et al. 2002). Among Teleostei, labroid fishes share a derived condition of the pharyngeal jaw apparatus that, besides other peculiarities, is characterized by the fusion of the lower pharyngeal elements into a single structure (Wainwright 2005). Morphological variation in the lower pharyngeal jaw has been widely explored in cichlids at the interspecific (Barel et al. 1977; Smits et al. 1996) and intraspecific levels (Meyer 1990a,b; Huysseune 1994; Smits et al. 1996, 1997). At the intraspecific level two morphs of lower pharyngeal jaws, papilliform or nonhypertrophied and molariform or hypertrophied, have been described in relation to a soft or a more durophagous diet respectively. In general terms durophagous forms possess enlarged pharyngeal jaw bones that are able to resist the higher pressures, produced by enlarged muscles, needed to crush hard preys (Wainwright 2005). In studies on the lower pharyngeal element, tooth size has been previously found to be larger in the most durophagous forms (Hoogerhoud 1984; Meyer 1990b; Smits et al. 1997). Many studies also describe an increase in size of the lower pharyngeal element in the most durophagous forms, be it in certain specific measurements like width (Barel et al. 1977; Hoogerhoud 1984) or as a general enlargement (Liem & Kaufman 1984) or hypertrophication (Smits et al. 1997). A more pronounced convexity of the posterior margin of the element has also been described in the most durophagous forms (Smits et al. 1997). Such an excurvation is consistent with the hypothesis (Smits et al. 1997) that food items are crushed on the caudo-medial part of the element. The occurrence of the papilliform or molariform morph has been generally interpreted as a result of phenotypic plasticity (Meyer 1990a,b) although Kornfield & Taylor (1983) believed that the two morphs in *Cichlasoma minckley*i are determined genetically. Morphology of the lower pharyngeal jaw element has been studied both using a descriptive approach (Barel et al. 1975; Smits et al. 1997; Herler et al. 2006) and using traditional morphometrics (Hoogerhoud 1984; Meyer 1990b; Smits et al. 1996). Geometric morphometric methods have shown a growing success throughout the 1990's (Adams et al. 2004) and are also particularly powerful in the analysis of patterns of variation below the species level (Loy 1996). Landmark-based methods are nowadays widely used but they have the important limitation that in certain structures a sufficient number of landmarks may not be available or important shape features may be lost using only landmarks (Rohlf 1990; Adams et al. 2004); in such cases the use of outline-based methods is imperative. The "sliding semilandmarks" approach proposed by Bookstein (1997) has the advantage of putting outline data in the same analytical framework as landmark data (Zelditch et al. 2004). This method consists of digitizing a number of points, called semilandmarks, along the outline of interest, which are not homologous but retain positional correspondence. In addition to being scaled, translated and rotated as in usual Procrustes superimposition, semilandmarks are allowed to slid along a vector that approximates the outline curve in order to minimize differences due to variation in the positions of the semilandmarks along the curves. After this step, the semilandmarks are treated in subsequent analyses as if they were landmarks. Despite the fact that labrids have been considered marine counterparts of cichlids (Westneat et al. 2005), studies on the morphological variation of lower pharyngeal jaws in this group have been scarce and focused essentially at the interspecific level (Koblmüller et al. 2003). *Coris julis* (Linnaeus) is a labrid species that is widely spread in the Mediterranean Sea and along part of the European and African Atlantic coasts and it represents the only Mediterranean species of its genus (Tortonese 1970; Quignard & Pras 1986). The species feeds mainly on mollusks, echinoderms and crustaceans (Tortonese 1970; Quignard & Pras 1986; De Pirro et al. 1999; Guidetti 2004). *C. julis* has also recently generated interest as bioindicator species (Chiea et al. 2002; Bonacci et al. 2003; Bonacci et al. 2007; Ferrito et al. 2008a,b; Tomasello et al. 2008). Despite variation in color among different sites (Michel et al. 1987; Laurent & Lejeune 1988; Arigoni et al. 2002) and the view of *C. julis* as a species that "presents conspicuous morphological variation between populations" (Aurelle et al. 2003), the intraspecific morphometric variation in this species is still largely understudied. The aim of the present study is to compare the morphology of the lower pharyngeal jaw element between two geographically close Sicilian populations of *C. julis* (Linnaeus), thus exploring for trophically-related morphological variation. For this purpose, traditional and geometric morphometric techniques were used to study variation in size and shape, respectively, to study variation in size, and to study variation in shape. Two geographically close sampling sites were chosen to avoid differences in the pharyngeal element between populations to be caused by genetic differences maintained by geographic distance, which at such a small scale seems very unlikely. The two sites also show very different environmental conditions, one of them being subjected to industrial pollution which has probably led to degraded macrobenthic associations (Russo 1982). In such an experimental design, any difference in the pharyngeal element size or shape could be explained as environmental-driven. The use of traditional methods allows a comparison with previous findings in the literature, while higher statistical power and better depiction of the results are guaranteed by the use of geometric morphometric techniques.

Materials and methods

Specimen collection and preparation

For the morphometric analyses of the present study a total of 92 specimens of *C. julis* from two Eastern Sicilian populations has been used. Half of them were caught in the Augusta harbor (37°11’49” N 15°14’07” E) while the remaining 46 were caught near Riposto, in Torre Archirafi (37°43’30” N 15°13’00” E). The specimens were then stained with the technique described by Dingerkus and Uhler (1977) and their standard length (SL) recorded. After staining, the lower pharyngeal jaw (Figure 1) was collected from each specimen and examined using a Wild microscope. Measurements of traits of the lower pharyngeal jaw were taken using the microscope micrometer. The lower pharyngeal element was also photographed using a digital camera. All specimens were included in the traditional analysis, only specimens which provided optimal pictures were used in the geometric morphometric analysis. A preliminary survey of stomach content was also carried out on additional specimens caught at the studied sites

(Augusta n=21; Riposto n=28) and at a third site (Baia del Silenzio-Brucoli; 37°17'N 15°08'E; n=17), close to Augusta but out of the industrially polluted area. Despite the fact that hard stomach content is crushed finely by pharyngeal jaws, thus limiting prey identification, the recognizable portion of the content was identified at the lowest possible taxonomic level.

Meristic counts and traditional morphometrics

The number of teeth both on the rostral zone and for the entire dentigerous portion of the pharyngeal bone was recorded for each specimen. The measurements taken on the lower pharyngeal jaw are listed in Table I and shown in Figure 2; the nomenclature of the various portions follows Barel et al. (1975, 1977). Both the meristic counts and the standard length of the specimens were compared between the populations by the means of a Mann-Whitney U test. For the statistical analysis of the morphometric measurements, a preliminary test of common slopes was performed on the regression of each log-transformed measurement on logSL. The comparison of regression slopes was carried out with GraphPad Prism version 5.01 for Windows (GraphPad Software, San Diego, USA). If the slopes being compared were not statistically different, then all the measurements for that variable were included in the analysis that consisted of a MANCOVA/ANCOVA of log-transformed measurements using logSL as covariate (thus removing allometric variation). Comparison of slopes aside, all the statistical analyses were performed using the STATISTICA (StatSoft Inc.) software package.

Sliding semilandmarks

Two grids were drawn on the images of the pharyngeal elements of each of 58 specimens (29 for each population) using the "comb" option of the MakeFan6 program of the IMP software package (Sheets 2002). The first grid was drawn to span the posterior portion of the pharyngeal element, the second to span the rostral zone. Using the software tpsDig (Rohlf 2006), 34 points were digitized at most of the intersections between the lines and the external contour of the pharyngeal element, following the scheme depicted in Figure 3. Points 1 and 18 were used as fixed landmarks, while the remaining points were treated as sliding semilandmarks. Semilandmarks were slid and configurations aligned with tpsRelw (Rohlf 2007a) using five iterations and setting as sliding criterion the minimization of the squared Procrustes distance; this criterion was chosen because it removes all the tangential variation along outlines whereas choosing the criterion of minimizing bending energy some of the tangential variation is retained (Perez et al. 2006). Symmetrized half-configurations were computed with the software SAGE (Márquez 2007). A preliminary test for common slopes between the two populations for the regression of shape variables on logSL was performed using tpsRegr (Rohlf 2007b), following the procedure presented in the program help files. The allometric component was then removed using the software Standard6 of the IMP package to regress the shape variables on logSL, and the residuals were summed to the predicted shape at the minimum observed SL. Using the software tpsRelw, the dataset of standardized half-configurations was then used for the calculation of the weight matrix, a matrix of shape variables that comprises both the uniform and non-uniform shape components and that was analyzed with standard multivariate statistic methods (Bookstein 1991). The statistical comparison of the two populations was then carried out using NTSYSpc (Rohlf 2007c) by the means of a CVA using the weight matrix as a data matrix. Statistical analyses were performed on symmetrized half-configurations and not on whole configurations, because if whole configurations were used the very high correlations between members of each pair of symmetric semilandmarks would potentially lead to the within-group covariance matrix being nearly singular and statistical analyses, such as CVA, that require within-group covariance matrix inversion would not be possible (Corti et al. 2001; Klingenberg et al. 2002). For visualization purposes the size-standardized half-configurations were regressed on the canonical scores using tpsRegr (Rohlf 2007b), a procedure previously used for landmark data (Loy et al. 1996; Cavalcanti 2004; Kaliontzopoulou et al. 2007). The estimated extreme shapes of such regressions were then back-reflected using the program LMEdit of the IMP package and the two resulting extreme shapes were visualized using the "wireframe graph" option of the software MorphoJ (Klingenberg 2011). The same analysis has been performed using elliptic Fourier descriptors with the same results that, therefore, will not be presented here.

Results

The preliminary survey of stomach content showed that the frequency of individuals with hard stomach content was lower in Riposto than at the other two sites. While Molluscs and Crustaceans were common at all sites, the stomach content of specimens caught within the Augusta harbour was dominated by Gastropods while at the other two sites a more diverse and balanced content was found. The Molluscs recognizable morphotypes were also different among all sites. In stomach content of specimens from the Riposto area were also found parts of Anellids and Echinoderms, which were, however, less common than Molluscs and Crustaceans.

Meristic counts and traditional morphometrics

There was no statistically significant difference between the two populations in regards to meristic counts and SL (Table II). The univariate tests for common slopes performed for the regression of the log-transformed measurements on logSL showed no statistical difference ($p>0.05$) between the slopes estimated using observations from a single population and the slopes estimated using observations from both populations so all variables were used in the traditional morphometric analyses. The multivariate analysis showed highly significant statistical differences between the two populations (Wilk's Lambda, d.f. = 8, p=0.000007). Descriptive statistics and significance level of between-sites comparisons for the meristic counts and morphometric measurements are provided in Table II.

Sliding semilandmarks

The test for common slopes of the regression of shape variables on logSL was not statistically significant so the regression approach to removal of the allometric component of shape variation was used. In the CVA 67.3% of the cases were correctly classificated (cross-validated classification) and the multivariate tests showed significant statistical difference between groups (Wilk's Lambda, d.f. = 57, p=0.0043). The shape variation corresponding to the direction of the canonical vector is shown, as back-reflected entire shape, in Figure 4.

Discussion

In the present study the morphological variation in size and shape of the lower pharyngeal jaws in two geographically close populations of *C. julis* has been studied by the means of geometric morphometric techniques and univariate comparisons of linear measurements. The most relevant result of the present study is that both approaches have shown, despite the small geographic distance (about 58 Km), strong statistical support against the null hypothesis of no differences between the populations. The traditional morphometric analyses performed (Table II) show in general a marked difference between the two populations in the size of the measured traits (but there is no significant difference in SL). The means of the measurements for each population (Table II) indicate that the Augusta population has a larger lower pharyngeal element and the size of the studied tooth is larger. The geometric morphometric approach detects a significant difference in the shape of the lower pharyngeal element between the populations consisting in an enlargement and a more pronounced caudal convexity of the central portion of the lower pharyngeal element in the Augusta population. These morphological traits of the Augusta population might be explained based on previously published work (Barel et al. 1977; Hoogerhoud 1984; Liem & Kaufman 1984; Meyer 1990b; Smits et al. 1997) as changes towards a more durophagous form. The differences assessed in various previous studies (Meyer 1990b; Huysseune et al. 1994) are between clearly distinguishable pharyngeal morphs (namely a "papilliform" and a "molariform" morph) in polymorphic species that show very few intermediate morphs (Meyer 1989). However, in the populations of *C. julis* studied in the present work there is not a sharp discontinuity between extremely different "morphs" but a number of anatomically localized, yet statistically significant, differences in shape (of the lower pharyngeal element) and a generalized difference in size (both in the lower pharyngeal element and its central tooth) between populations so that at one sampling site (Augusta) the pharyngeal element possess more durophagous traits. The preliminary surveys of stomach content carried out for this study, which show that in Augusta *C. julis* feeds mainly on Gastropods, and previous literature suggest that the morphological differences between sites might be due to the differences between the two sites in the diversity of the macrobenthic invertebrates that can be preyed upon by *C. julis.* In fact, the Augusta harbor presents intense industrial pollution (Sciacca & Fallico 1978; Magazzù et al. 1995) whereas the Riposto area has no significant industrial pollution. As a probable consequence of such pollution in the Augusta harbor it has been documented a degraded situation where the molluscs comprise 85.55% of the macrobenthic community (Russo 1982). The malacological association itself has been described as degraded with only 73 species, among which *Corbula gibba* (Olivi, 1792) constitutes 78% of the association. On the opposite, an extremely diverse macrobenthic association has been found in localities close to Riposto (Cantone et al. 2003; Russo et al. 2004).

Another possible cause of the difference in hardness of the ingested food at two geographically close locations is the different substrate composition of the Augusta area (with a calcareous substrate) and the Riposto area (which presents a volcanic rocky substrate). In fact, water parameters have been found to influence calcification in molluscs and other marine organisms (Bevelander & Benzer 1948; Gazeau et al. 2007). Interestingly enough, Vizzini and Mazzola (2009) have documented in *C. julis* a certain degree of variation in the trophic level index among geographically close sampling localities. This species has planktonic eggs (Quignard & Pras 1986) so, given the small geographical distance, it is unlikely that the differences found in the present study are due to a low genetic flow between the two populations. Therefore, given that phenotypic plasticity has been recognized in cichlids' lower pharyngeal jaws element in response to different trophic resources (that is to different levels of hardness of food) (Huysseune et al. 1994), it seems reasonable to hypothesize that the morphological variation between the two populations studied in the present paper may be explained as the variation of phenotypically plastic traits (size and shape of *C. julis* lower pharyngeal jaw) in response to the different trophic resources found at the two collection sites. An alternative hypothesis to explain the morphological differences assessed in the present work would be a differential mortality of different phenotypes in the two sites. Juveniles with a wide range of genetically-determined phenotypic traits after the larval phase would settle in different sites and then the different environments would select individuals belonging to one or the other side of the morphological spectrum, thus leading to the gradual shift of each population towards a different direction in the morphological space. Meyer (1989) hypothesized a similar mechanism for the two morphs of *Amphilophus citrinellus* (Gunther), where the relative abundance of hard and soft prey determine the relative abundance of the two morphs. However, while Meyer (1987) hypothesized for *A. citrinellus* that the temporal fluctuations in the relative abundance of hard and soft prey would perpetuate the coexistence of both morphs preventing the competitive exclusion of either one, for *C. julis* the dispersion during the larval phase would seem sufficient and more likely if ever a differential-mortality mechanism was responsible for the observed differences between populations. Both the hypotheses of phenotypic plasticity and of environmentally-induced differential mortality, however, will need specifically designed studies, such as common-garden experiments, to be properly tested.

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Tables

Table I. Abbreviations for the traditional morphometric measurements.

WBT	Width of the biggest tooth
LBT	Length of the biggest tooth
PDW	Width of the dentigerous area
PDL	Length of the dentigerous area
PDR	Length of the rostral dentigerous area
LPW	Width of the pharyngeal element (comprising the horns)
LPL	Length of the pharyngeal element
LPR	Length of the rostral part of the pharyngeal element

Table II. Mean, standard deviation (S.D.) and significance of comparisons for meristic counts and morphometric measurements; all measurements in mm.

Variable	Augusta		Riposto		p-level
	Mean	S.D.	Mean	S.D.	
SL	101.15	11.60	98.13	17.07	0.086
Total number of teeth	41.39	5.57	40.98	5.96	0.79
Number of teeth on the rostral portion	13.39	2.43	13.35	2.79	0.58
WBT	1.17	0.21	0.97	0.28	0.000005
LBT	1.25	0.25	1.02	0.30	0.000001
PDW	5.11	0.67	4.64	0.86	0.00001
PDL	4.69	0.65	4.29	0.78	0.011
PDR	2.73	0.37	2.51	0.51	0.029
LPW	7.29	1.14	6.65	1.35	0.0093
LPL	5.38	0.92	4.75	1.09	0.0002
LPR	3.38	0.69	3.03	0.84	0.02131

Figure captions

Figure 1. Picture of a *Coris julis* lower pharyngeal jaw stained with alizarin red.

Figure 2. Schematic representation of the morphometric traits measured. In grey the dentigerous area. Abbreviations are given in Table I.

Figure 3. Schematic representation of the two grids and the position of the digitized semilandmarks.

Figure 4. Shape variation corresponding to the difference between the two populations. The grey dashed line (open circles) represents the predicted shape in the direction of the Augusta population, the black line/circles represent the average shape between the two shapes predicted by the CVA analysis.

Figure 1

Figure 2

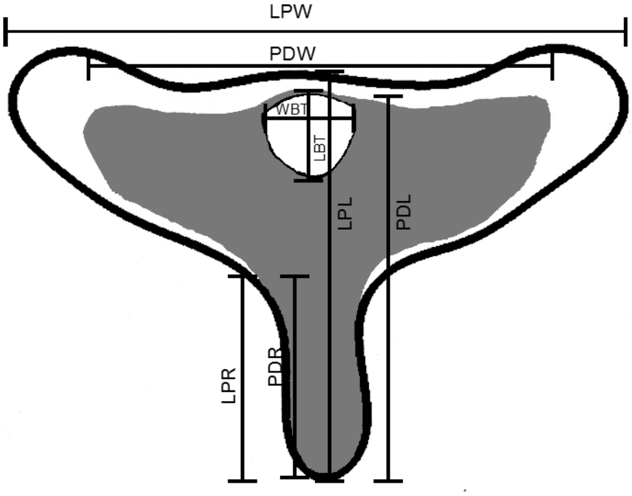

Figure 3

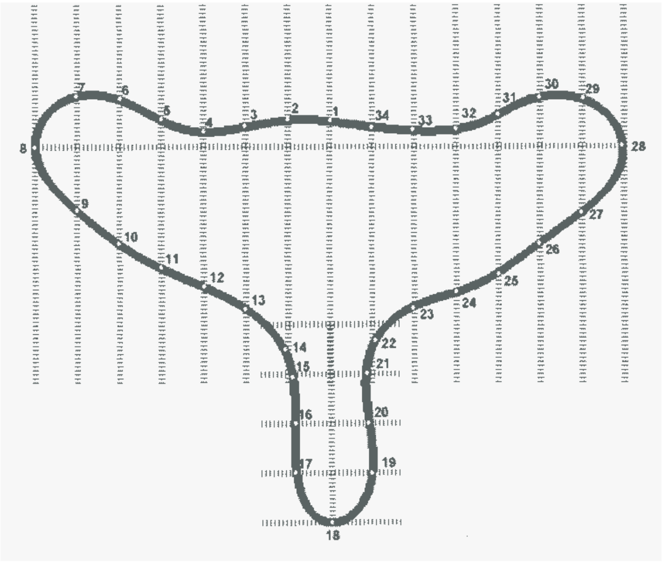

Figure 4