

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

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14 **Abstract**

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

25

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

27

28 **Introduction**

29 The pharyngeal jaw apparatus of perciform fishes is a well-developed system that
30 functions in sophisticated prey processing behaviours, complementing the functions of
31 the oral jaw apparatus (Wainwright 2005). The pharyngeal jaw apparatus has been
32 studied in many Teleostei (Sibbing 1982; Tigano et al. 1999; Vandewalle et al. 2002).
33 Among Teleostei, labroid fishes share a derived condition of the pharyngeal jaw
34 apparatus that, besides other peculiarities, is characterized by the fusion of the lower
35 pharyngeal elements into a single structure (Wainwright 2005). Morphological variation
36 in the lower pharyngeal jaw has been widely explored in cichlids at the interspecific
37 (Barel et al. 1977; Smits et al. 1996) and intraspecific levels (Meyer 1990a,b;
38 Huyseune 1994; Smits et al. 1996, 1997). At the intraspecific level two morphs of
39 lower pharyngeal jaws, papilliform or nonhypertrophied and molariform or

40 hypertrophied, have been described in relation to a soft or a more durophagous diet
41 respectively. In general terms durophagous forms possess enlarged pharyngeal jaw
42 bones that are able to resist the higher pressures, produced by enlarged muscles, needed
43 to crush hard preys (Wainwright 2005). In studies on the lower pharyngeal element,
44 tooth size has been previously found to be larger in the most durophagous forms
45 (Hoogerhoud 1984; Meyer 1990b; Smits et al. 1997). Many studies also describe an
46 increase in size of the lower pharyngeal element in the most durophagous forms, be it in
47 certain specific measurements like width (Barel et al. 1977; Hoogerhoud 1984) or as a
48 general enlargement (Liem & Kaufman 1984) or hypertrophication (Smits et al. 1997).
49 A more pronounced convexity of the posterior margin of the element has also been
50 described in the most durophagous forms (Smits et al. 1997). Such an excurvation is
51 consistent with the hypothesis (Smits et al. 1997) that food items are crushed on the
52 caudo-medial part of the element. The occurrence of the papilliform or molariform
53 morph has been generally interpreted as a result of phenotypic plasticity (Meyer
54 1990a,b) although Kornfield & Taylor (1983) believed that the two morphs in
55 *Cichlasoma minckleyi* are determined genetically. Morphology of the lower pharyngeal
56 jaw element has been studied both using a descriptive approach (Barel et al. 1975; Smits
57 et al. 1997; Herler et al. 2006) and using traditional morphometrics (Hoogerhoud 1984;
58 Meyer 1990b; Smits et al. 1996). Geometric morphometric methods have shown a
59 growing success throughout the 1990's (Adams et al. 2004) and are also particularly
60 powerful in the analysis of patterns of variation below the species level (Loy 1996).
61 Landmark-based methods are nowadays widely used but they have the important
62 limitation that in certain structures a sufficient number of landmarks may not be
63 available or important shape features may be lost using only landmarks (Rohlf 1990;
64 Adams et al. 2004); in such cases the use of outline-based methods is imperative. The
65 "sliding semilandmarks" approach proposed by Bookstein (1997) has the advantage of

66 putting outline data in the same analytical framework as landmark data (Zelditch et al.
67 2004). This method consists of digitizing a number of points, called semilandmarks,
68 along the outline of interest, which are not homologous but retain positional
69 correspondence. In addition to being scaled, translated and rotated as in usual Procrustes
70 superimposition, semilandmarks are allowed to slid along a vector that approximates the
71 outline curve in order to minimize differences due to variation in the positions of the
72 semilandmarks along the curves. After this step, the semilandmarks are treated in
73 subsequent analyses as if they were landmarks. Despite the fact that labrids have been
74 considered marine counterparts of cichlids (Westneat et al. 2005), studies on the
75 morphological variation of lower pharyngeal jaws in this group have been scarce and
76 focused essentially at the interspecific level (Koblmüller et al. 2003). *Coris julis*
77 (Linnaeus) is a labrid species that is widely spread in the Mediterranean Sea and along
78 part of the European and African Atlantic coasts and it represents the only
79 Mediterranean species of its genus (Tortonese 1970; Quignard & Pras 1986). The
80 species feeds mainly on mollusks, echinoderms and crustaceans (Tortonese 1970;
81 Quignard & Pras 1986; De Pirro et al. 1999; Guidetti 2004). *C. julis* has also recently
82 generated interest as bioindicator species (Chiea et al. 2002; Bonacci et al. 2003;
83 Bonacci et al. 2007; Ferrito et al. 2008a,b; Tomasello et al. 2008). Despite variation in
84 color among different sites (Michel et al. 1987; Laurent & Lejeune 1988; Arigoni et al.
85 2002) and the view of *C. julis* as a species that “presents conspicuous morphological
86 variation between populations” (Aurelle et al. 2003), the intraspecific morphometric
87 variation in this species is still largely understudied. The aim of the present study is to
88 compare the morphology of the lower pharyngeal jaw element between two
89 geographically close Sicilian populations of *C. julis* (Linnaeus), thus exploring for
90 trophically-related morphological variation. For this purpose, traditional and geometric
91 morphometric techniques were used to study variation in size and shape, respectively, to

92 study variation in size, and to study variation in shape. Two geographically close
93 sampling sites were chosen to avoid differences in the pharyngeal element between
94 populations to be caused by genetic differences maintained by geographic distance,
95 which at such a small scale seems very unlikely. The two sites also show very different
96 environmental conditions, one of them being subjected to industrial pollution which has
97 probably led to degraded macrobenthic associations (Russo 1982). In such an
98 experimental design, any difference in the pharyngeal element size or shape could be
99 explained as environmental-driven. The use of traditional methods allows a comparison
100 with previous findings in the literature, while higher statistical power and better
101 depiction of the results are guaranteed by the use of geometric morphometric
102 techniques.

103

104 **Materials and methods**

105 *Specimen collection and preparation*

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

118 (Augusta n=21; Riposto n=28) and at a third site (Baia del Silenzio-Brucoli; 37°17'N
119 15°08'E; n=17), close to Augusta but out of the industrially polluted area. Despite the
120 fact that hard stomach content is crushed finely by pharyngeal jaws, thus limiting prey
121 identification, the recognizable portion of the content was identified at the lowest
122 possible taxonomic level.

123

124 *Meristic counts and traditional morphometrics*

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

139

140 *Sliding semilandmarks*

141 Two grids were drawn on the images of the pharyngeal elements of each of 58
142 specimens (29 for each population) using the “comb” option of the MakeFan6 program
143 of the IMP software package (Sheets 2002). The first grid was drawn to span the

144 posterior portion of the pharyngeal element, the second to span the rostral zone. Using
145 the software tpsDig (Rohlf 2006), 34 points were digitized at most of the intersections
146 between the lines and the external contour of the pharyngeal element, following the
147 scheme depicted in Figure 3. Points 1 and 18 were used as fixed landmarks, while the
148 remaining points were treated as sliding semilandmarks. Semilandmarks were slid and
149 configurations aligned with tpsRelw (Rohlf 2007a) using five iterations and setting as
150 sliding criterion the minimization of the squared Procrustes distance; this criterion was
151 chosen because it removes all the tangential variation along outlines whereas choosing
152 the criterion of minimizing bending energy some of the tangential variation is retained
153 (Perez et al. 2006). Symmetrized half-configurations were computed with the software
154 SAGE (Márquez 2007). A preliminary test for common slopes between the two
155 populations for the regression of shape variables on logSL was performed using tpsRegr
156 (Rohlf 2007b), following the procedure presented in the program help files. The
157 allometric component was then removed using the software Standard6 of the IMP
158 package to regress the shape variables on logSL, and the residuals were summed to the
159 predicted shape at the minimum observed SL. Using the software tpsRelw, the dataset
160 of standardized half-configurations was then used for the calculation of the weight
161 matrix, a matrix of shape variables that comprises both the uniform and non-uniform
162 shape components and that was analyzed with standard multivariate statistic methods
163 (Bookstein 1991). The statistical comparison of the two populations was then carried
164 out using NTSYSpc (Rohlf 2007c) by the means of a CVA using the weight matrix as a
165 data matrix. Statistical analyses were performed on symmetrized half-configurations
166 and not on whole configurations, because if whole configurations were used the very
167 high correlations between members of each pair of symmetric semilandmarks would
168 potentially lead to the within-group covariance matrix being nearly singular and
169 statistical analyses, such as CVA, that require within-group covariance matrix inversion

170 would not be possible (Corti et al. 2001; Klingenberg et al. 2002). For visualization
171 purposes the size-standardized half-configurations were regressed on the canonical
172 scores using tpsRegr (Rohlf 2007b), a procedure previously used for landmark data
173 (Loy et al. 1996; Cavalcanti 2004; Kaliontzopoulou et al. 2007). The estimated extreme
174 shapes of such regressions were then back-reflected using the program LMEdit of the
175 IMP package and the two resulting extreme shapes were visualized using the
176 "wireframe graph" option of the software MorphoJ (Klingenberg 2011). The same
177 analysis has been performed using elliptic Fourier descriptors with the same results that,
178 therefore, will not be presented here.

179

180 **Results**

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

189

190 *Meristic counts and traditional morphometrics*

191 There was no statistically significant difference between the two populations in regards
192 to meristic counts and SL (Table II). The univariate tests for common slopes performed
193 for the regression of the log-transformed measurements on logSL showed no statistical
194 difference ($p > 0.05$) between the slopes estimated using observations from a single
195 population and the slopes estimated using observations from both populations so all

196 variables were used in the traditional morphometric analyses. The multivariate analysis
197 showed highly significant statistical differences between the two populations (Wilk's
198 Lambda, d.f. = 8, $p=0.000007$). Descriptive statistics and significance level of between-
199 sites comparisons for the meristic counts and morphometric measurements are provided
200 in Table II.

201

202 *Sliding semilandmarks*

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

210

211 **Discussion**

212 In the present study the morphological variation in size and shape of the lower
213 pharyngeal jaws in two geographically close populations of *C. julis* has been studied by
214 the means of geometric morphometric techniques and univariate comparisons of linear
215 measurements. The most relevant result of the present study is that both approaches
216 have shown, despite the small geographic distance (about 58 Km), strong statistical
217 support against the null hypothesis of no differences between the populations. The
218 traditional morphometric analyses performed (Table II) show in general a marked
219 difference between the two populations in the size of the measured traits (but there is no
220 significant difference in SL). The means of the measurements for each population
221 (Table II) indicate that the Augusta population has a larger lower pharyngeal element

222 and the size of the studied tooth is larger. The geometric morphometric approach detects
223 a significant difference in the shape of the lower pharyngeal element between the
224 populations consisting in an enlargement and a more pronounced caudal convexity of
225 the central portion of the lower pharyngeal element in the Augusta population. These
226 morphological traits of the Augusta population might be explained based on previously
227 published work (Barel et al. 1977; Hoogerhoud 1984; Liem & Kaufman 1984; Meyer
228 1990b; Smits et al. 1997) as changes towards a more durophagous form. The differences
229 assessed in various previous studies (Meyer 1990b; Huysseune et al. 1994) are between
230 clearly distinguishable pharyngeal morphs (namely a “papilliform” and a “molariform”
231 morph) in polymorphic species that show very few intermediate morphs (Meyer 1989).
232 However, in the populations of *C. julis* studied in the present work there is not a sharp
233 discontinuity between extremely different “morphs” but a number of anatomically
234 localized, yet statistically significant, differences in shape (of the lower pharyngeal
235 element) and a generalized difference in size (both in the lower pharyngeal element and
236 its central tooth) between populations so that at one sampling site (Augusta) the
237 pharyngeal element possess more durophagous traits. The preliminary surveys of
238 stomach content carried out for this study, which show that in Augusta *C. julis* feeds
239 mainly on Gastropods, and previous literature suggest that the morphological
240 differences between sites might be due to the differences between the two sites in the
241 diversity of the macrobenthic invertebrates that can be preyed upon by *C. julis*. In fact,
242 the Augusta harbor presents intense industrial pollution (Sciacca & Fallico 1978;
243 Magazzù et al. 1995) whereas the Riposto area has no significant industrial pollution.
244 As a probable consequence of such pollution in the Augusta harbor it has been
245 documented a degraded situation where the molluscs comprise 85.55% of the
246 macrobenthic community (Russo 1982). The malacological association itself has been
247 described as degraded with only 73 species, among which *Corbula gibba* (Olivi, 1792)

248 constitutes 78% of the association. On the opposite, an extremely diverse macrobenthic
249 association has been found in localities close to Riposto (Cantone et al. 2003; Russo et
250 al. 2004).

251 Another possible cause of the difference in hardness of the ingested food at two
252 geographically close locations is the different substrate composition of the Augusta area
253 (with a calcareous substrate) and the Riposto area (which presents a volcanic rocky
254 substrate). In fact, water parameters have been found to influence calcification in
255 molluscs and other marine organisms (Bevelander & Benzer 1948; Gazeau et al. 2007).
256 Interestingly enough, Vizzini and Mazzola (2009) have documented in *C. julis* a certain
257 degree of variation in the trophic level index among geographically close sampling
258 localities. This species has planktonic eggs (Quignard & Pras 1986) so, given the small
259 geographical distance, it is unlikely that the differences found in the present study are
260 due to a low genetic flow between the two populations. Therefore, given that
261 phenotypic plasticity has been recognized in cichlids' lower pharyngeal jaws element in
262 response to different trophic resources (that is to different levels of hardness of food)
263 (Huyseune et al. 1994), it seems reasonable to hypothesize that the morphological
264 variation between the two populations studied in the present paper may be explained as
265 the variation of phenotypically plastic traits (size and shape of *C. julis* lower pharyngeal
266 jaw) in response to the different trophic resources found at the two collection sites. An
267 alternative hypothesis to explain the morphological differences assessed in the present
268 work would be a differential mortality of different phenotypes in the two sites. Juveniles
269 with a wide range of genetically-determined phenotypic traits after the larval phase
270 would settle in different sites and then the different environments would select
271 individuals belonging to one or the other side of the morphological spectrum, thus
272 leading to the gradual shift of each population towards a different direction in the
273 morphological space. Meyer (1989) hypothesized a similar mechanism for the two

274 morphs of *Amphilophus citrinellus* (Gunther), where the relative abundance of hard and
275 soft prey determine the relative abundance of the two morphs. However, while Meyer
276 (1987) hypothesized for *A. citrinellus* that the temporal fluctuations in the relative
277 abundance of hard and soft prey would perpetuate the coexistence of both morphs
278 preventing the competitive exclusion of either one, for *C. julis* the dispersion during the
279 larval phase would seem sufficient and more likely if ever a differential-mortality
280 mechanism was responsible for the observed differences between populations. Both the
281 hypotheses of phenotypic plasticity and of environmentally-induced differential
282 mortality, however, will need specifically designed studies, such as common-garden
283 experiments, to be properly tested.

284

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288

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459 **Tables**

460

461 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

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476 Table II. Mean, standard deviation (S.D.) and significance of comparisons for meristic
477 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

478

479 **Figure captions**

480

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

482

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

485

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

488

489 Figure 4. Shape variation corresponding to the difference between the two populations.

490 The grey dashed line (open circles) represents the predicted shape in the direction of the

491 Augusta population, the black line/circles represent the average shape between the two

492 shapes predicted by the CVA analysis.

Figure 1



Figure 2

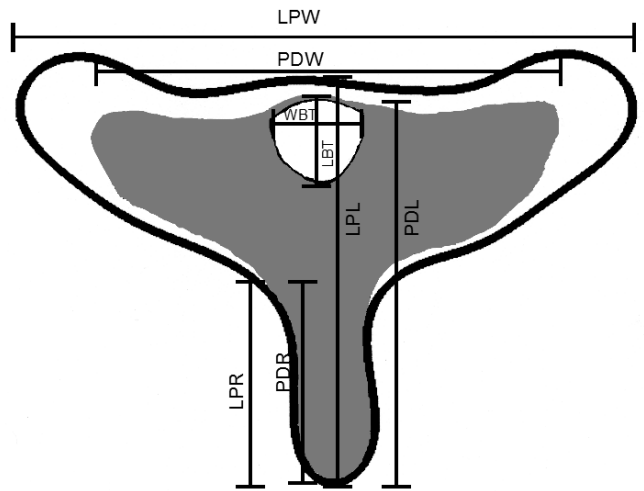


Figure 3

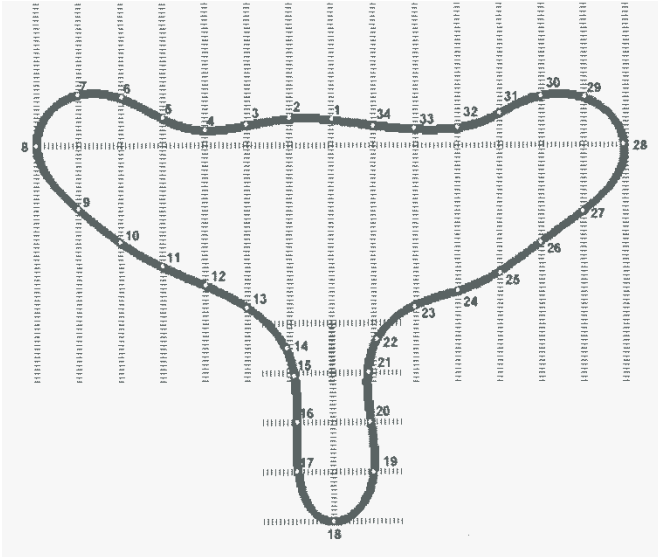


Figure 4

