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Geographic morphological variation within and between colour phases in *Coris julis* (L. 1758), a protogynous marine fish species

C. Fruciano*, C. Tigano, V. Ferrito
Dipartimento di Biologia, University of Catania, Catania, Italy

Running head: Geographic variation in a protogynous fish

* *Corresponding author:* Dipartimento di Biologia - University of Catania, via Androne 81, 95124 Catania (Italy)
Tel.: +390957306025 fax: +39095327990
e-mail: c.fruciano@unict.it

Abstract

1
2 The possible differences between sexes in patterns of morphological variation in
3 geographic space have been explored only in gonochorist freshwater species. We
4 explored patterns of body shape variation in geographic space in a marine sequential
5 hermaphrodite species, *Coris julis* (L. 1758), analyzing variation both within and between
6 colour phases, through the use of geometric morphometrics and spatially-explicit statistical
7 analyses. We also tested for the association of body shape with two environmental
8 variables, temperature and chlorophyll a concentration, obtained from time-series of
9 satellite-derived data. Both colour phases showed a significant morphological variation in
10 geographic space and patterns of variation divergent between phases. In fact, while the
11 morphological variation was qualitatively similar, individuals in the initial colour phase
12 showed a more marked variation than individuals in the terminal phase. Body shape
13 showed a weak but significant correlation with environmental variables which was more
14 pronounced in primary specimens.

15

16 Keywords: geometric morphometrics – spatial analysis – body shape – geographic variation –
17 ecomorphology – sexual dimorphism

18

19 Introduction

20 Phenotypic variation in geographic space can be the consequence of, in addition to other
21 evolutionary factors such as gene flow or genetic drift, variation in environmental variables
22 which, in turn, can influence phenotypic traits both directly and indirectly through trait
23 correlations and interactions with other environmental factors (Lande & Arnold, 1983;
24 DeWitt & Langerhans, 2003;
25 Langerhans, Chapman & Dewitt, 2007). Moreover, the effect of environmental variables on
26 phenotypes can lead to divergent patterns among populations which may or may not be
27 parallel between sexes. That is, given that the majority of the genome is shared between
28 males and females, sexes might show similar patterns of population divergence, but,
29 conversely, differences between sexes (such as in morphology or behaviour) may interact
30 with environmental gradients to produce divergent patterns of phenotypic variation
31 between sexes in geographic space (Hendry *et al.*, 2006).

32 Body shape variation in fish can reflect ecological and behavioural differences both within
33 and between species, so shape is of particular interest in ecological and evolutionary
34 research (Burns, Di Nardo & Hood, 2009). In particular, intraspecific variation in fish body
35 shape has been related to various factors such as water flow (Langerhans, 2008), feeding
36 habits (Ruehl & DeWitt, 2005), habitat use (Svanbäck & Eklöv, 2003,2004), predation
37 pressure (Gomes & Monteiro, 2008), temperature (Beacham, 1990; Loy *et al.*, 1996) and
38 diet (Wimberger, 1992). Morphometric analyses have been useful in highlighting patterns
39 of stock differentiation in geographic space (Cadrin & Silva, 2005; Cadrin, 2010; Cadrin *et al.*
40 *et al.*, 2010), even in cases where stock differentiation was undetectable by genetic studies
41 (Levi *et al.*, 2004; Salini *et al.*, 2004). In particular, in the Mediterranean Sea, the
42 morphometric analysis of body shape in *Trachurus trachurus* (L. 1758) has highlighted a
43 structuring in three geographic groups (Murta, Pinto & Abaunza, 2008), a result which is in

44 agreement with analyses using other approaches (Abaunza *et al.*, 2008). However,
45 analyses of body shape variation at the Mediterranean scale are rare as most of studies
46 on body shape focus on a smaller spatial scale.

47 One of the most studied factors affecting body shape in fish is temperature, which typically
48 results in deeper bodies at higher temperatures and more elongated bodies at lower
49 temperatures (Loy *et al.*, 1996; Marcil, Swain & Hutchings, 2006). A recent study on *Danio*
50 *rerio* (Hamilton, 1822) has also evidenced how rearing temperature during the larval phase
51 can have consequences on adult body shape (Georga & Koumoundouros, 2010). While
52 many studies have focused on the plastic response of a single genotype to temperature
53 (phenotypic plasticity), Kavanagh *et al.* (2010) have shown that temperature can induce in
54 *Thymallus thymallus* (L. 1758) genetically-based phenotypic divergence even under
55 conditions of low genetic variation and ongoing gene flow. Temperature can also be
56 correlated with trophic resources: sampling sites with temporally persistent above-average
57 chlorophyll a concentration and below average sea surface temperature have been
58 considered productivity hotspots and have been found to be potentially associated with
59 fisheries resources (Valavanis *et al.*, 2004). Chlorophyll a variability is also associated with
60 feeding intensity in sardine (Garrido *et al.*, 2008). Despite the fact that time series of sea
61 temperature and chlorophyll a data are now easy to obtain as satellite-derived data, such
62 data have not been widely used to study patterns of morphological variation in the field.

63 While patterns of geographic variation in body shape have been studied in both marine
64 and freshwater species, the possible differences between sexes in such patterns have
65 been explored, to the best of authors' knowledge, only in gonochorist freshwater species
66 (Hendry *et al.*, 2006; Langerhans & Makowicz, 2009). In fact, while various studies on the
67 morphometric variation in hermaphrodite fish species exist (Vidalis, Markakis &
68 Tsimenides, 1997; Moran, Burton & Caputi, 1999; Palma & Andrade, 2002,2004), they do
69 not use advanced techniques such as the combination of geometric morphometrics and

70 spatial analysis and they do not test for differences between sexual phases in patterns of
71 geographic variation.

72 The Labridae family is the third largest family of marine fish comprising species which
73 occur worldwide in tropical, subtropical and temperate seas (Parenti & Randall, 2000). The
74 phenomenon
75 of sexual inversion, which is often associated to a change in colour, is very common in
76 labrids (Warner & Robertson, 1978). *Coris julis* (L. 1758) is a small protogynous diandric
77 labrid fish, which is common and almost continuously distributed in most of the
78 Mediterranean Sea. The species, can also be found in the eastern Atlantic European and
79 North African coasts, where it can live in sympatry with its sister species *C. atlantica*
80 (Gunther, 1862) (Parenti & Randall, 2000). *C. julis* is mainly found in rocky coastal areas
81 and *Posidonia oceanica* (L.) Delile, 1813 beds and it is known to have a burrowing
82 behaviour on sandy bottoms at night (Tortonese, 1970)..The species is known to ingest
83 gastropods, crustaceans, bivalves, small fish, polychaetes, echinoderms and benthic algae
84 (Tortonese, 1970; Pinnegar & Polunin, 2000; Kabasakal, 2001) and is considered
85 omnivorous with a preference for animal material (Karachle & Stergiou, 2008). Moreover,
86 Vizzini & Mazzola (2009) have documented for the species a certain degree of variation in
87 the trophic level index among geographically close sampling localities. Guidetti &
88 D'Ambrosio (2004), studying the distribution patterns of *C. julis* and *Thalassoma pavo* (L.
89 1758), another labrid species with similar trophic traits, hypothesized that *T. pavo*, being
90 more markedly thermophilic, outcompetes *C. julis* in shallower waters. *C. julis* juveniles
91 have been repeatedly reported to act as facultative cleaners (Van Tassell, Brito & Bortone,
92 1994; Zander & Nieder, 1997; Zander & Sötje, 2002; Fischer *et al.*, 2007), a behaviour that
93 may be correlated to the presence of a lateral dark stripe (Arnal, Verneau & Desdevises,
94 2006). *C. julis* exhibits two colorations which are so different in morphology that they were
95 originarily described as separate species. The initial phase coloration (also called "primary

96 livery”) is mainly exhibited by females and consists in a brown upper (dorsal) part of the
97 body and a white ventral part; along with these main features there other sometimes less
98 evident traits like a black and a yellow stripe along flanks. The terminal phase coloration
99 (also known as “secondary livery”) is typical of males and is more colourful with a dorsal
100 portion which is typically green (but also brown or bluish), a ventral white portion and an
101 orange wavy band along flanks with a black elongated mark extending from about the
102 pectoral fin to mid of the body. Initial and terminal colorations are, however, quite variable
103 (Tortonese, 1970; Michel, Lejeune & Voss, 1987; Arigoni *et al.*, 2002). Protogynous sex
104 change is typically accompanied by colour change from the primary to the secondary
105 coloration through a “transitional livery” phase with intermediate colour traits which is
106 relatively short in duration (Bacci & Razzauti, 1957; Bentivegna & Cirino, 1984).
107 Behavioural observations (Lejeune, 1982; Bentivegna & Cirino, 1984) have shown that
108 individuals with primary liveries are sedentary, individuals with secondary liveries are
109 territorial and engage in agonistic behaviours when they meet each other, especially
110 during the reproductive season. Social factors (male/female ratio) have been also shown
111 to induce sexual inversion in the species which lacks of a critical size for sex inversion
112 (Bentivegna & Cirino, 1984).
113 Despite the variation in coloration within livery and among sampling sites (Michel *et al.*,
114 1987; Laurent & Lejeune, 1988; Arigoni *et al.*, 2002) and the view of *C. julis* as a species
115 that “presents conspicuous morphological variation between populations” (Aurelle *et al.*,
116 2003), its intraspecific morphometric variation is still largely understudied. The variability in
117 osteological trophic traits in two geographically close populations of the species has been
118 recently assessed (Fruciano, Tigano & Ferrito, *in press a*) but, to the best of authors'
119 knowledge, the scientific literature is lacking of any other morphometric study on the
120 intraspecific morphological variability of the species. Recent work on the species (Fruciano
121 *et al.*, *in press b*) has shown the absence of neutral genetic structuring within the

122 Mediterranean Sea which has been suggested to be the consequence of the high
123 dispersal capabilities of the species, which are, in turn, probably correlated to the long
124 planktonic larval phase (Gordoa, Moli & Raventos, 2000; Raventòs & Macpherson, 2001).
125 The aims of the present study are to test if, despite the absence of neutral genetic
126 structuring, *C. julis* morphology varies in geographic space at the Mediterranean scale and
127 if primary and secondary individuals show different patterns of variation, and also to test
128 for the association with environmental parameters. We expect a significant variation of
129 shape in geographic space for both colour phases with a deeper body at sites with higher
130 temperatures. We also expect that, as already observed in gonochorist freshwater
131 species, patterns of variation in primary and secondary individuals are different because
132 the two colour phases are subjected to different pressures.

133 To test these expectations, a geometric morphometric analysis of body shape variation
134 among nine Mediterranean sampling sites has been performed using, in addition to other
135 methods, spatially explicit analytical tools such as bearing analysis (Falsetti & Sokal, 1993)
136 and trend surface analysis
137 (Krumbein, 1959; Sneath, 1967; Gittins, 1968; Watson, 1971). While Mantel tests of the
138 correlation between morphometric and geographic distances have been used widely in
139 morphometric studies, the use of other spatially-explicit methods with geometric
140 morphometric data is still in its infancy, despite of its advantages. In fact, bearing analysis,
141 which detects the direction in geographic space of greatest correlation of morphometric
142 and geographic distances, has never been used with morphometric data. Similarly, trend
143 surface analysis, which allows to model the variation of one or more dependent variables
144 in geographic space, has been used with geometric morphometric data only recently
145 (Cardini, Jansson & Elton, 2007; Cardini & Elton, 2009; Cardini *et al.*, 2010).

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Materials and methods

148 For the present study a total of 223 *C. julis* specimens from 9 different mediterranean
149 populations (Fig. 1; Table 1) was used. Shortly after collection, fish were preserved in 95%
150 ethanol and brought to the laboratory for the acquisition of morphometric data.

Data gathering and dataset preparation

152 Pictures of the left side of each specimen were taken using a digital camera mounted on a
153 copy stand with an experimental design in which every specimen had two presentations
154 (two pictures) and two digitizations of landmarks for each presentation, for a total of 4 sets
155 of coordinates (such a design was deemed appropriate following the results of a
156 preliminary study of measurement error on a subset of specimens). Throughout the data
157 gathering phase, several measures have been taken to minimize, as much as possible,
158 bias and error: the digital camera was distant from the specimens to reduce the effect of
159 parallax (Mullin & Taylor, 2002), fish were kept straight by running a long needle of
160 appropriate length through the right side of the body (Windsor Aguirre, *personal*
161 *communication*), all the steps of the analysis have been performed by the same operator,
162 populations were not photographed and digitized at a single session (e.g. all the
163 specimens of a certain population in a single day) but in “rounds” of subsamples to avoid
164 the effects of a potential bias in the way the operator performed his tasks (Windsor
165 Aguirre, *personal communication*); further details on methodological steps are provided by
166 Fruciano (2009).

167 Twenty points (both landmarks and semilandmarks; Fig. 2) were digitized using the
168 software tpsDig
169 (Rohlf, 2006). The landmark/semilandmark configurations were then subjected to a
170 generalized Procrustes analysis with sliding semilandmarks (Bookstein, 1997) using the
171 software tpsRelw (Rohlf, 2007a) setting ten iterations and the minimization of the squared

172 Procrustes distance as sliding criterion because this criterion removes all the tangential
173 variation along outlines (Perez, Bernal & Gonzalez, 2006).
174 To reduce both directional and non-directional measurement error, the full dataset
175 comprising four landmark configurations for each specimen has been first subjected to the
176 procedure described in Valentin *et al.* (2008), then the resulting coordinates of each
177 specimen (now adjusted for body arching) have been averaged so to obtain a single
178 landmark configuration for each specimen. Average centroid size measures were also
179 computed for each specimen. Given the marked difference in shape between primary and
180 secondary individuals and following the results of a
181 preliminary study, all subsequent analyses were performed separately for primary and
182 secondary
183 specimens. The program Standard6 of the IMP package (Sheets, 2002) was then used to
184 obtain a
185 dataset in which size-related allometric variation had been removed by regression on
186 centroid size. The resulting configurations of points were then subjected to a principal
187 component analysis (PCA)
188 and PCA scores were used in subsequent analyses.

189 *Shape variation among populations*

190 To analyse body shape variation among populations two Multivariate Analyses of Variance
191 (MANOVA; one for primary individuals and the other for secondary individuals) were
192 performed using STATISTICA (StatSoft Inc) on size-adjusted PCA scores. Pairwise
193 differences between populations were also tested using the permutation procedures on
194 Mahalanobis distances implemented in the CVA module of MorphoJ (Klingenberg, 2011)
195 testing the null hypothesis that
196 the difference was zero. Only sampling sites with at least five specimens were subjected to
197 MANOVA.

198 To test for differences between colour phases in the pattern of morphological variation
199 between populations, a MANOVA testing for the interaction of livery and site was carried
200 out with STATISTICA on the samples containing at least five specimens of each colour
201 phase.

202 *Analysis of spatial patterns*

203 To analyse the patterns of shape variation in geographic space, three different spatially-
204 explicit approaches were used: Mantel tests (Mantel, 1967), bearing analysis (Falsetti &
205 Sokal, 1993) and trend surface analysis (Krumbein, 1959; Sneath, 1967; Gittins, 1968;
206 Watson, 1971).

207 The Mantel test (Mantel, 1967) was used to test the null hypothesis that the correlation
208 between pairwise geographic distances and pairwise tangent Procrustes distances
209 between populations was zero. Both geographic distances from geographic coordinates
210 and shortest waterway distances among sampling locations were used. The analysis was
211 carried out both using mean shapes for each livery and each sampling site and using all
212 specimens of a certain colour phase. The test was performed with NTSYSpc 2.2 (Rohlf,
213 2007b) and PASSaGE 2 (Rosenberg & Anderson, 2011) using 10000 permutations.

214 Bearing analysis is a method of determining the direction of greatest correlation between
215 data distance and geographic distance that has been introduced by Falsetti & Sokal (1993)
216 for the use with genetic data. In bearing analysis, each geographic distance between two
217 points is weighted by the squared cosine of the angle between the line connecting the two
218 points and a reference vector. Then the correlation between the matrix of spatial distances
219 weighted in this way and the matrix of data distances (morphometric distances, in this
220 study) is computed and can also be tested using a Mantel test. The same calculations are
221 then repeated changing the direction of the reference vector and the results can be plotted
222 in a graph where the obtained correlation is depicted as function of the reference direction
223 used for weighting. For the present study, bearing analysis has been performed with

224 PASSaGE 2 using pairwise tangent Procrustes distances among individuals as data
225 distances. To visualize morphological variation predicted by bearing analysis, the software
226 tpsRegr (Rohlf, 2007c) was used to regress shape variables on the projection of each
227 locality on the geographic direction of greatest correlation between morphometric and
228 geographic distances and to produce three-times exaggerated thin-plate splines of
229 predicted shapes at each extreme. Bearing analysis has been carried out only on
230 populations with at least five usable specimens.

231 Trend surface analysis is one of the most powerful tools of spatial pattern analysis
232 (Legendre & Legendre, 1998) but it has been introduced in the geometric morphometrics
233 field only recently by Cardini and colleagues (Cardini *et al.*, 2007; Cardini & Elton, 2009;
234 Cardini *et al.*, 2010). Trend surface analysis consists of the application of polynomial
235 regression to spatial data. In practice, it is a regression of one or more response variables
236 (in the case of the present study shape variables) on a polynomial expansion of
237 geographic coordinates (longitude and latitude, usually centred before the actual analysis).
238 Being of interest in the present study the broad scale trend of shape variation, a
239 polynomial of third degree was deemed appropriate following Legendre & Legendre (1998)
240 and the model $X+Y+X^2+XY+Y^2+X^3+Y^3$ (where X represents the mean-centred longitude, Y the
241 mean-centred latitude) was used as full model. Three different approaches to find a model
242 with less parameters were used: the Legendre & Legendre (1998) stepwise method, a
243 modification of the Legendre & Legendre (1998) method and AICc (Hurvich & Tsai, 1989).
244 Using the Legendre & Legendre (1998) method, shape variables (principal component
245 scores) were regressed on all the terms of the full model, eliminating backwards terms not
246 significant at the 5% probability level one at a time. In case more than one variable was
247 non-significant in the regression the one to be removed was chosen based on the F-
248 statistic value (the variable with lowest F-statistic value was removed first). In the modified
249 version of the Legendre & Legendre (1998) method the only difference was that the

250 significance level to determine exclusion was 1% ($p=0.01$) so to reduce further the number
251 of variables in the chosen model. Finally, AICc (Hurvich & Tsai, 1989) is a modified version
252 of the AIC (An Information Criterion; Akaike, 1973), adjusted for small sample sizes. The
253 script provided by Al-Subaihi (2002) was used to select the best model using the AICc
254 criterion. All the regressions were performed using the software STATISTICA. For
255 visualization purposes, predicted values for relative warp scores were computed and back-
256 transformed to xy-coordinates, then a five-times exaggerated thin-plate spline of each
257 predicted shape on the consensus shape was produced.
258 Tangent Procrustes distances between all pairs of predicted shapes were then computed
259 with tpsSmall (Rohlf, 2003) and the correlation between the matrices of distances between
260 predictions in primary and secondary specimens was then tested by the means of a
261 Mantel test.

262 *Association of body shape with ecological variables*

263 Time-series of chlorophyll a concentration (October, 16th, 1987-October, 16th 2007;
264 monthly interval) and sea surface temperature (June, 7th, 2004-January, 5th, 2009; weekly
265 interval) at the sampling locations were obtained from satellite data of the American
266 National Aeronautics and Space Administration (NASA). Given that the chlorophyll
267 concentration data were incomplete, only the sampling sites for which more than 40% of
268 the time series was available were used in analyses using this variable, so discarding
269 specimens from Oristano, Pantelleria and Riposto. Arithmetic mean sea surface
270 temperature and geometric mean chlorophyll a concentration (Supplemental material S1)
271 were used as variables choosing geometric mean for chlorophyll a concentration because
272 chlorophyll a distributions tend to be roughly log-normal and it has been noticed that using
273 geometric mean is a better estimator of central tendency (MacFadyen, 1998).

274 The association of shape with chlorophyll a concentration and sea surface temperature
275 was studied with two-block partial least squares analysis using both ecological variables
276 and also with separate regressions of shape on each ecological variable.
277 Separate regressions of shape variables on chlorophyll a concentration and sea surface
278 temperature were performed using tpsRegr visualizing statistically significant regressions
279 with thin-plate splines of three-times the maximum and minimum observed values relative
280 to reference.
281 A two-block partial least squares analysis using as first block the shape variables and as
282 second block the two ecological variables (standardized to unit variance) was performed
283 with MorphoJ and the significance of the association of morphometric and ecological
284 variables was obtained by the permutational procedure implemented in the program
285 (10000 permutations). Shape variation predicted by partial least squares analysis was
286 visualized with MorphoJ producing “wireframe graphs”.

287

288

Results

289

Shape variation among populations

290 MANOVA rejected the null hypothesis of no difference between population means both in
291 primary (Wilks' Lambda= $F_{234,329.47}=2.029$ $p<0.001$) and secondary (Wilks' Lambda=0.09
292 $F_{273,482.11}=1.752$ $p<0.001$) specimens. The pairwise comparison among sampling sites
293 using primary specimens is
294 always significant ($p<0.001$). Pairwise comparisons using secondary specimens is always
295 significant ($p<0.001$) except in the case of the comparison between Augusta and Lecce
296 samples.
297 The MANOVA testing for the interaction effect of colour phase and sampling site is also
298 highly significant (Wilks' Lambda=0.0001 $F_{320,536.283}=3.551$ $p<0.001$).

299

Analysis of spatial patterns

300 Correlations between shape distances and geographic distances (both as linear distances
301 from coordinates and shortest waterway distances) using average shapes for each
302 sampling site are very low (correlation coefficients ranging from 0.05 to -0.14) and Mantel
303 tests shows that in no case such correlations are statistically significant. On the other
304 hand, correlations are significant when using all specimens separately (that is, the
305 observed Z value is higher than most of the Z values obtained from the permutational
306 procedure; p-value comprised between 0.01 and 0.0004). Also, except in the case of
307 primary specimens and shortest waterway distances (where the correlation is 0.09), the
308 other correlations between morphometric and physical distances using separate
309 specimens are, albeit still low, much higher (range 0.11-0.14) than the ones observed
310 using sampling site means.

311 The bearing analysis of primary individuals showed that the direction of highest significant
312 correlation between geographic and morphometric distances is 30° (correlation 0.1206,
313 permutational p-value 0.002), corresponding to a direction NE-SW. The bearing analysis of
314 secondary individuals suggests that the direction of highest significant correlation is 111°
315 (correlation 0.1535, permutational p-value <0.001), corresponding to a direction NW-SE.
316 However, the plots of correlations obtained using the various reference directions
317 (Supplemental material S2) show that there are significant correlations also along different
318 directions in geographic space.

319 The regression of shape variables on projections of sampling localities on the direction of
320 highest correlation explains only 3.74% of variance in primary individuals, 5.14% in
321 secondary individuals. Body shape variation along the directions of highest correlation is
322 depicted in Fig. 3.

323 The polynomial expansion of latitude and longitude terms performed, as expected, better
324 than a simple regression on latitude and longitude (which would have explained for

325 primary and secondary specimens respectively 9.73% and 8.11% of variance). Table 2
326 shows the results of trend surface analysis while Fig. 4 and 5 show shapes predicted from
327 TSA at sampling sites using the model selected by AICc (the other models, while
328 accounting for slightly more variance, produced similar patterns and will not be shown).
329 The mean tangent Procrustes distance between shapes predicted by AICc is 0.009 for
330 primary specimens, 0.006 for secondary individuals. The correlation between tangent
331 Procrustes distances among predictions of primary and secondary specimens is high and
332 significant ($r=0.77$, $p=0.011$).

333 *Association of body shape with ecological variables*

334 Results of the partial least squares analysis are provided in Table 3 and show a weak but
335 significant association between shape and environmental variables. Both in primary and
336 secondary specimens temperature has a positive correlation while chlorophyll has a
337 negative correlation with the first pair of singular axes. Shape changes predicted by the
338 association of shape and environmental variables are depicted in Fig. 6.

339 Separate linear regression of primary specimens' body shape on sea surface temperature
340 and chlorophyll a concentration are both significant (respectively $p=0.0028$ and $p=0.002$)
341 and explain respectively 5.33% and 5.6% of variance; Fig. 7 depicts shape changes
342 predicted by these two regressions.

343 Linear regression of secondary specimens' body shape on chlorophyll a concentration was
344 significant using parametric tests ($p=0.037$) but non significant using permutational
345 approaches (0.06) so, given also the extremely low explained variance (1.6%), the
346 regression was deemed non-significant. Regression on sea surface temperature, on the
347 other hand, is highly significant ($p=0.002$) and accounts for 3.9% of variance; Fig. 8 shows
348 shape changes predicted by the regression of body shape on sea surface temperature in
349 secondary individuals.

350

Discussion

351

Looking at the results of the MANOVA analyses, it can be said that overall the populations are differentiated from each other.

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While the results of Mantel tests should be interpreted with caution as this test has been

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shown to be affected by both sample size and inequality of sample sizes (Luo & Fox,

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1996), the fact that correlations between geographic and morphometric distances are

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small suggests that in *C. julis* shape differences in geographic space cannot be explained

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as mere consequence of the distance among sites. The results of bearing analysis show

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that, along certain directions in geographic space, there is a significant correlation between

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geographic and morphometric distances, thus suggesting the existence of clines of shape

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variation. Interestingly, the directions of maximum correlations are different in primary and

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secondary individuals. The results of trend surface analysis suggest a complicated pattern

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of variation in geographic space because the polynomial expansion of latitude and

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longitude terms provides a much better fit to the data (so a simple model describing a

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linear effect along a direction in geographic space is not completely adequate). The fact

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that a simple directional gradient in geographic space is not adequate to model shape

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variation in *C. julis* is also corroborated by the higher levels of explained variance in trend

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surface analysis compared to variance explained by regression of shape on the projection

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of sampling sites on the directions of maximum correlation resulting from bearing

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analyses. While a simple linear effect is not adequate to model shape variation in

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geographic space, looking at the shapes predicted by trend surface analysis at selected

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sites for primary specimens, it is possible to discern a pattern. In fact, it can be noticed that

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the Split population has the most elongated and narrow predicted shape and Naples and

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Lecce have also an elongated and narrow predicted shape (but not to the extent of the

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Split sample); all the more southern samples show in general a deeper body. Similarly,

375 Oristano shows, compared to the Mallorca population, a more elongated shape (still not to
376 the extent of the Split sample). Trying to capture an easily explainable general trend, it
377 could be said that at similar longitudes northern populations show a narrower and more
378 elongated body shape, at similar latitudes eastern populations show a narrower body. It is
379 important to remember, however, that such a “general overview” represents an
380 oversimplification of a more complex, statistically significant, pattern at the studied
381 geographic scale. In secondary individuals, it can be said that the pattern is somewhat
382 similar, but less marked, to the one found in primary individuals with Split having narrower
383 bodies than the other samples. While the inclusion of more sampling sites would probably
384 have allowed for a more precise modelling of morphometric variation in geographic space,
385 it is remarkable that all the methods used agree in detecting a variation in geographic
386 space.

387 The analysis of the association between body shape and environmental variables show a
388 weak but significant correlation. In primary individuals, given the different sign of the
389 coefficient on the first singular axis for chlorophyll a concentration and sea surface
390 temperature, the thin-plate spline visualizing shape changes along the first singular axis
391 suggests that, in areas with higher chlorophyll a concentration and lower temperature,
392 individuals have a narrower, more elongated body. Predicted shapes for the separate
393 regressions on chlorophyll concentration and temperature reveal a similar pattern.

394 Considering that high chlorophyll a concentration and low sea surface temperature are
395 associated to productivity hotspots (Valavanis *et al.*, 2004) and that chlorophyll has been
396 shown to be associated to phytoplankton fraction and feeding intensity in sardine (Garrido
397 *et al.*, 2008), it is possible that body shape of primary individuals of *C. julis* might be
398 influenced by productivity and/or correlated variables.

399 In secondary individuals, while the association between shape and ecological variables is
400 significant, the correlation between temperature and the first singular axis is extremely

401 high while the negative correlation of chlorophyll a concentration with the first singular axis
402 is low. Moreover, considering separate regressions, only the regression on sea surface
403 temperature is significant and the predicted shapes show that individuals inhabiting
404 environments with lower sea surface temperature exhibit a more elongated shape while
405 individuals from habitats with higher sea surface temperature exhibit a deeper body, the
406 same general pattern shown by primary individuals in the regression on sea surface
407 temperature. The variation in body shape observed in both primary and secondary
408 individuals might have functional significance. In fact, a more elongated shape minimizes
409 drag and is predicted to be favoured in case of steady swimming, while a deeper body
410 guarantees higher efficiency in case of unsteady swimming with rapid bouts of activity
411 (Walker, 1997; Langerhans, 2008). Variation in *C. julis* body shape might also be affected
412 by the burrowing behaviour which this species exhibits but such an effect should be tested
413 thoroughly. A more elongated shape in environments with lower sea surface temperature
414 might be caused by a variation in the number of vertebrae, which has been shown to
415 increase with latitude in many fish species (Jordan, 1891; McDowall, 2008) and which can
416 have both genetic and phenotypically plastic components (Yamahira, Lankford & Conover,
417 2006; Yamahira & Nishida, 2009). Temperature has been found to have an effect on body
418 shape in various fish species (Beacham, 1990; Loy *et al.*, 1996; Marcil *et al.* 2006; Georga
419 & Koumoundouros, 2010; Kavanagh *et al.*, 2010). Beacham (1990) in a study on
420 *Oncorhynchus keta* (Walbaum, 1792) found that temperature had a marked effect on juvenile
421 morphometric variation and higher rearing temperatures have been found to determine
422 deeper bodies in *Cyprinus carpio* L. 1758 juveniles (Loy *et al.*, 1996). Similarly, Marcil *et al.*
423 (2006) found in *Gadus morhua* L. 1758 that temperature and food abundance had an effect
424 on body shape: individuals reared at higher temperature or food level had a deeper body
425 and a larger head than those reared at the lower temperature or food supply, with the
426 effect of temperature being more pronounced. Marcil *et al.* (2006), noticing that an

427 increase in body depth has been found to be associated with higher temperature in various
428 fish species, have also suggested that such a response might be general in fish.

429 Wimberger (1992) found that different diets produced different morphologies in two
430 species of the genus *Geophagus* (Teleostei, Cichlidae). In the light of the above-mentioned
431 previous studies on the effect of diet and temperature on body shape, it is possible to
432 hypothesize that in *C. julis* temperature and chlorophyll a concentration have direct and/or
433 indirect effects. In fact, while a direct effect of temperature is easily hypothesizable given
434 that this environmental factor is known to act directly on body shape (Beacham, 1990;
435 Marcil *et al.*, 2006; Georga & Koumoundouros, 2010), it seems more reasonable that the
436 chlorophyll a concentration is associated with some other, presently unknown, variable
437 which causes the variation in shape. The significant effect of chlorophyll a concentration
438 obtained in the present study might also be a mere consequence of its negative correlation
439 with temperature. Moreover, the fact that the association between body shape and the
440 environmental variables studied is weak suggests that there may be other factors affecting
441 the geographic variation of body shape in *C. julis*. Another possible factor affecting body
442 shape variation in geographic space which should be investigated in future studies is the
443 variation of biological communities in geographic space. In particular, *Thalassoma pavo*,
444 being more markedly thermophilic, is believed to outcompete *C. julis* in shallow waters
445 (Guidetti & D'Ambrosio, 2004) and the abundance of *T. pavo* has been shown to decrease
446 at northern sites (Guidetti *et al.*, 2002). It is, therefore, possible that the extent of the
447 competition between *C. julis* and *T. pavo* varies with temperature in geographic space and
448 that this competition drives, at least in part, the morphological variation observed in *C.*
449 *julis*.

450 The present study evidences also interesting differences between primary and secondary
451 individuals both in patterns of variation in geographic space and in the association with
452 environmental variables. This is highlighted by the significant interaction between colour

453 phase and sampling site in MANOVA. Moreover, while the similarity of patterns of
454 geographic variation in primary and secondary individuals is evident from the examination
455 of plots of predicted shapes and the high correlation of morphometric distances between
456 predicted shapes in primary and secondary individuals, the patterns of variation are less
457 marked in secondary specimens (as shown by the smaller average distance between
458 predictions). Moreover, all the chosen models in trend surface analysis account for more
459 variation in primary specimens. This discrepancy might be explained hypothesizing that
460 the secondary specimens are less prone to factors that shape variability at the studied
461 geographical scale.

462 The fact that in primary specimens both separate regressions on ecological variables are
463 significant while in secondary specimens only the regression on sea surface temperature
464 is statistically significant suggests the hypotheses that secondary individuals are either
465 less sensitive to the studied ecological variables or that they are influenced by these
466 environmental variables in a more complex way, not revealed by the analyses performed.
467 It is possible to hypothesize that the difference between primary and secondary individuals
468 in patterns of variation in geographic space and association with ecological variables is a
469 consequence of a difference between liveries in constraints that determine morphological
470 variation. In fact, while primary specimens are gregarious and, probably, mostly interested
471 in obtaining food resources, secondary specimens are known to establish territories and
472 engage in agonistic behaviours with each other (Lejeune, 1982; Bentivegna & Cirino,
473 1984) so body shape in secondary individuals might also be influenced by other factors
474 and be the results of more complex trade-offs.

475

476

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482

483

References

- 484 **Abaunza P, Murta AG, Campbell N, Cimmaruta R, Comesaña AS, Dahle G, García**
485 **Santamaría MT, Gordo LS, Iversen SA, MacKenzie K, Magoulas A, Mattiucci S,**
486 **Molloy J, Nascetti G, Pinto AL, Quinta R, Ramos P, Sanjuan A, Santos AT, Stransky**
487 **C, Zimmermann C. 2008.** Stock identity of horse mackerel (*Trachurus trachurus*) in the
488 Northeast Atlantic and Mediterranean Sea: Integrating the results from different stock
489 identification approaches. *Fisheries Research* **89**: 196-209.
- 490 **Akaike H. 1973.** Information Theory and an Extension of the Maximum Likelihood
491 Principle. In: Perron BN, Csaki F, eds. *2nd International Symposium in Information Theory*.
492 Budapest: Akademiai Kiado, 207-261.
- 493 **Al-Subaihi A. 2002.** Variable Selection in Multivariable Regression Using SAS/IML.
494 *Journal of Statistical Software* **7**.
- 495 **Arigoni S, Francour P, Harmelin-Vivien M, Zaninetti L. 2002.** Adaptive colouration of
496 Mediterranean labrid fishes to the new habitat provided by the introduced tropical alga
497 *Caulerpa taxifolia*. *Journal of Fish Biology* **60**: 1486–1497.
- 498 **Arnal C, Verneau O, Desdevises Y. 2006.** Phylogenetic relationships and evolution of
499 cleaning behaviour in the family Labridae: importance of body colour pattern. *Journal of*
500 *Evolutionary Biology* **19**: 755-763.

- 501 **Aurelle D, Guillemaud T, Afonso P, Morato T, Wirtz P, Santos RS, Cancela ML. 2003.**
502 Genetic study of *Coris julis* (Osteichthyes, Perciformes, Labridae) evolutionary history and
503 dispersal abilities. *Comptes Rendus Biologies* **326**: 771-785.
- 504 **Bacci G, Razzauti A. 1957.** Falso gonocorismo in *Coris julis* (L.). *Rendiconti*
505 *dell'Accademia dei Lincei serie 8* **23**: 181-189.
- 506 **Beacham TD. 1990.** A genetic analysis of meristic and morphometric variation in chum
507 salmon (*Oncorhynchus keta*) at three different temperatures. *Canadian Journal of Zoology*
508 **68**: 225–229.
- 509 **Bentivegna F, Cirino P. 1984.** Sexual inversion in *Coris julis* L. 1758. *Cybium* **8**: 51-61.
- 510 **Bookstein FL. 1997.** Landmark methods for forms without landmarks: localizing group
511 differences in outline shape. *Medical Image Analysis* **1**: 225–243.
- 512 **Burns JG, Di Nardo P, Hood FH. 2009.** The role of predation in variation in body shape in
513 guppies *Poecilia reticulata*: a comparison of field and common garden phenotypes. *Journal*
514 *of Fish Biology* **75**: 1144–1157.
- 515 **Cadrin SX. 2010.** Stock identification of marine populations. In: Elewa AMT, ed.
516 *Morphometrics for Nonmorphometricians*. Springer, 219-231.
- 517 **Cadrin SX, Silva VM. 2005.** Morphometric variation of yellowtail flounder. *ICES Journal of*
518 *Marine Science* **62**: 683-694.
- 519 **Cadrin SX, Bernreuther M, Daníelsdóttir AK, Hjörleifsson E, Johansen T, Kerr L,**
520 **Kristinsson K, Mariani S, Nedreaas K, Pampoulie C, Planque B, Reinert J, Saborido-**
521 **Rey F, Sigurðsson T, Stransky C. 2010.** Population structure of beaked redfish,
522 *Sebastes mentella*: evidence of divergence associated with different habitats. *ICES*
523 *Journal of Marine Science* **67**: 1617-1630.
- 524 **Cardini A, Diniz Filho JAF, Polly PD, Elton S. 2010.** Biogeographic analysis using
525 geometric morphometrics: clines in skull size and shape in a widespread African arboreal
526 monkey. In: Elewa AMT, ed. *Morphometrics for Nonmorphometricians*. Springer, 191-218.

- 527 **Cardini A, Elton S. 2009.** Geographical and taxonomic influences on cranial variation in
528 red colobus monkeys (Primates, Colobinae): introducing a new approach to
529 'morph' monkeys. *Global Ecology and Biogeography* **18**: 248–263.
- 530 **Cardini A, Jansson A-U, Elton S. 2007.** A geometric morphometric approach to the study
531 of ecogeographical and clinal variation in vervet monkeys. *Journal of Biogeography* **34**:
532 1663–1678.
- 533 **DeWitt TJ, Langerhans RB. 2003.** Multiple prey traits, multiple predators: keys to
534 understanding complex community dynamics. *Journal of Sea Research* **49**: 143–155.
- 535 **Escoufier Y. 1973.** Le traitement des variables vectorielles. *Biometrics* **29**: 751–760.
- 536 **Falsetti AB, Sokal RR. 1993.** Genetic structure of human populations in the British Isles.
537 *Annals of Human Biology* **20**: 215-229.
- 538 **Fischer S, Patzner RA, Müller CHG, Winkler HM. 2007.** Studies on the ichthyofauna of
539 the coastal waters of Ibiza (Balearic Islands, Spain). *Rostocker Meeresbiologische*
540 *Beiträge* **18**: S. 30-62.
- 541 **Fruciano C, Tigano C, Ferrito V. in press a.** Traditional and geometric morphometrics
542 detect morphological variation of lower pharyngeal jaw in *Coris julis* (Teleostei, Labridae).
543 *Italian Journal of Zoology*.
- 544 **Fruciano C, Hanel R, Tigano C, Debes PV, Ferrito V. in press b.** Atlantic-Mediterranean
545 and within-Mediterranean molecular variation in *Coris julis* (L. 1758) (Teleostei, Labridae).
546 *Marine Biology*
- 547 **Fruciano C. 2009.** Pattern di variazione morfologica e molecolare in *Coris julis* (L. 1758)
548 (Teleostei, Labridae). Doctoral thesis, University of Catania.
- 549 **Garrido S, Ben-Hamadou R, Oliveira PB, Cunha ME, Chícharo MA, van der Lingen**
550 **CD. 2008.** Diet and feeding intensity of sardine *Sardina pilchardus*: correlation with
551 satellite-derived chlorophyll data. *Marine Ecology Progress Series* **354**: 245-256.

- 552 **Georga I, Koumoundouros G. 2010.** Thermally induced plasticity of body shape in adult
553 zebrafish *Danio rerio* (Hamilton, 1822). *Journal of Morphology* **271**: 1319-1327.
- 554 **Gittins R. 1968.** Trend-Surface Analysis of Ecological Data. *Journal of Ecology* **56**: 845-
555 869.
- 556 **Gomes JL, Monteiro LR. 2008.** Morphological divergence patterns among populations of
557 *Poecilia vivipara* (Teleostei Poeciliidae): test of an ecomorphological paradigm. *Biological*
558 *Journal of the Linnean Society* **93**: 799–812.
- 559 **Gordoa A, Molì B, Raventos N. 2000.** Growth performance of four wrasse species on the
560 north-western Mediterranean coast. *Fisheries Research* **45**: 43–50.
- 561 **Guidetti P, D'Ambrosio P. 2004.** Spatial distribution patterns of *Coris julis* and
562 *Thalassoma pavo* (Pisces, Labridae) along the south-eastern Apulian coast (SE Italy).
563 *Thalassia Salentina* **27**: 81-90.
- 564 **Guidetti P, Bianchi CN, La Mesa G, Modena M, Morri C, Sara G, Vacchi M. 2002.**
565 Abundance and size structure of *Thalassoma pavo* (Pisces: Labridae) in the western
566 Mediterranean Sea: variability at different spatial scales. *Journal of the Marine Biological*
567 *Association of the UK* **82**: 495-500.
- 568 **Hendry AP, Kelly ML, Kinnison MT, Reznick DN. 2006.** Parallel evolution of the sexes?
569 Effects of predation and habitat features on the size and shape of wild guppies. *Journal of*
570 *Evolutionary Biology* **19**: 741-754.
- 571 **Hurvich CM, Tsai CL. 1989.** Regression and Time Series Model Selection in Small
572 Samples *Biometrika* **76**: 297-307.
- 573 **Jordan DS. 1891.** Relations of temperature to vertebrae among fishes. *Proceedings of the*
574 *United States National Museum* **14**: 107–120.
- 575 **Kabasakal H. 2001.** Description of the feeding morphology and the food habits of four
576 sympatric labrids (Perciformes Labridae) from south-eastern Aegean Sea, Turkey.
577 *Netherlands Journal of Zoology* **51**: 439-455.

- 578 **Karachle PK, Stergiou KI. 2008.** The effect of season and sex on trophic levels of marine
579 fishes. *Journal of Fish Biology* 72: 1463-1487.
- 580 **Kavanagh KD, Haugen TO, Gregersen F, Jernvall J, Vøllestad LA. 2010.**
581 Contemporary temperature-driven divergence in a Nordic freshwater fish under conditions
582 commonly thought to hinder adaptation. *BMC Evolutionary Biology* 10: 350.
- 583 **Klingenberg CP. 2011.** MorphoJ: an integrated software package for geometric
584 morphometrics. *Molecular Ecology Resources*, advance online publication.
- 585 **Krumbein WC. 1959.** Trend surface analysis of contour-type maps with irregular control-
586 point spacing. *Journal of Geophysical Research* 64: 823-834.
- 587 **Lande R, Arnold SJ. 1983.** The measurement of selection on correlated characters.
588 *Evolution* 37: 1210–1226.
- 589 **Langerhans RB. 2008.** Predictability of phenotypic differentiation across flow regimes in
590 fishes. *Integrative and Comparative Biology* 48: 750–768.
- 591 **Langerhans RB, Chapman LJ, Dewitt TJ. 2007.** Complex phenotype–environment
592 associations revealed in an East African cyprinid. *Journal of Evolutionary Biology* 20:
593 1171-1181.
- 594 **Langerhans RB, Makowicz AM. 2009.** Shared and unique features of morphological
595 differentiation between predator regimes in *Gambusia caymanensis*. *Journal of*
596 *Evolutionary Biology* 22: 2231-2242.
- 597 **Langerhans RB. 2008.** Predictability of phenotypic differentiation across flow regimes in
598 fishes. *Integrative and Comparative Biology* 48: 750-768.
- 599 **Laurent L, Lejeune P. 1988.** Coexistence en Méditerranée de deux livrées terminales
600 différentes chez la girelle *Coris julis* (Pisces, Labridae). *Cybiurn* 12: 91-95.
- 601 **Legendre P, Legendre L. 1998.** *Numerical ecology*. 2nd English edition. Elsevier Science
602 BV, Amsterdam.

- 603 **Lejeune P. 1982.** A propos de quelques Poissons de la Méditerranée. *Revue Française*
604 *d'Aquariologie* **9**: 27-32.
- 605 **Levi D, Patti B, Rizzo P, Lo Brutto S, Parrinello N, Arculeo M. 2004.** Genetic and
606 morphometric variations of Mediterranean hake, *Merluccius merluccius*, in the Strait of
607 Sicily
608 (central Mediterranean): Implications for stock assessment of shared resources. *Italian*
609 *Journal of Zoology* **71**: 165-170.
- 610 **Loy A, Ciccotti E, Ferrucci L, Cataudella S. 1996.** An application of automated feature
611 extraction and geometric morphometrics: temperature-related changes in body form of
612 *Cyprinus carpio* juveniles. *Aquacultural Engineering* **15**: 301-311.
- 613 **Luo J, Fox BJ. 1996.** A review of the Mantel Test in dietary studies: effect of sample size
614 and inequality of sample sizes. *Wildlife Research* **23**: 267-288.
- 615 **MacFadyen A. 1998.** Modeling primary productivity from satellite-derived chlorophyll in the
616 Monterey Bay region. *Intern Papers of The Monterey Bay Aquarium Research Institute*. 19
617 pp.
- 618 **Mantel NA. 1967.** The detection of disease clustering and a generalized regression
619 approach. *Cancer Research* **27**: 209-220.
- 620 **Marcil J, Swain DP, Hutchings AJ. 2006.** Genetic and environmental components of
621 phenotypic variation in body shape among populations of Atlantic cod (*Gadus morhua* L.).
622 *Biological Journal of the Linnean Society* **88**: 351-365.
- 623 **McDowall RM. 2008.** Jordan's and other ecogeographical rules, and the vertebral number
624 in fishes. *Journal of Biogeography* **35**: 501-508.
- 625 **Michel C, Lejeune P, Voss J. 1987.** Biologie et comportement des Labridés européens
626 (Labres, Crénilabres, Rouquiers, Vieilles et Girelles). *Revue française d'Aquariologie* **14**:
627 1-180.

- 628 **Moran M, Burton C, Caputi N. 1999.** Sexual and local variation in head morphology of
629 snapper, *Pagrus auratus*, Sparidae, in the Shark Bay region of Western Australia. *Marine*
630 *and Freshwater Research* **50**: 27-34.
- 631 **Mullin SK, Taylor PJ. 2002.** The effects of parallax on geometric morphometric data.
632 *Computers in Biology and Medicine* **32**: 455–464.
- 633 **Murta AG, Pinto AL, Abaunza P. 2008.** Stock identification of horse mackerel (*Trachurus*
634 *trachurus*) through the analysis of body shape. *Fisheries Research* **89**: 152–158.
- 635 **Palma J, Andrade JP. 2002.** Morphological study of *Diplodus sargus*, *Diplodus puntazzo*,
636 and *Lithognathus mormyrus* (Sparidae) in the Eastern Atlantic and Mediterranean Sea.
637 *Fisheries Research* **57**: 1-8.
- 638 **Palma J, Andrade JP. 2004.** Morphological study of *Pagrus pagrus*, *Pagellus bogaraveo*,
639 and *Dentex dentex* (Sparidae) in the eastern Atlantic and the Mediterranean Sea. *Journal*
640 *of the Marine Biological Association of the UK* **84**: 449-454.
- 641 **Parenti P, Randall JE. 2000.** An annotated checklist of the species of the labroid fish
642 families Labridae and Scaridae. *Ichthyological Bulletin* **68**: 1-97.
- 643 **Perez IS, Bernal V, Gonzalez PN. 2006.** Differences between sliding semilandmark
644 methods in geometric morphometrics, with an application to human craniofacial and dental
645 variation. *Journal of Anatomy* **208**: 769–784.
- 646 **Pinnegar JK, Polunin NV. 2000.** Contributions of stable-isotope data to elucidating food
647 webs of Mediterranean rocky littoral fishes. *Oecologia* **122**: 399-409.
- 648 **Raventòs N, Macpherson E. 2001.** Planktonic larval duration and settlement marks on
649 the otoliths of Mediterranean littoral fishes. *Marine Biology* **138**: 1115-1120.
- 650 **Rohlf FJ. 2003.** tpsSmall, version 1.20. Department of Ecology and Evolution, State
651 University of New York at Stony Brook.
- 652 **Rohlf FJ. 2006.** tpsDig, digitize landmarks and outlines, version 2.10. Department of
653 Ecology and Evolution, State University of New York at Stony Brook.

- 654 **Rohlf FJ. 2007a.** tpsRelw, relative warps analysis, version 1.45. Department of Ecology
655 and Evolution, State University of New York at Stony Brook.
- 656 **Rohlf FJ. 2007b.** NTSYSpc: Numerical Taxonomy System, ver. 2.20. Exeter Publishing,
657 Ltd.: Setauket, NY.
- 658 **Rohlf FJ. 2007c.** tpsRegr, shape regression, version 1.33. Department of Ecology and
659 Evolution, State University of New York at Stony Brook.
- 660 **Rosenberg MS, Anderson CD. 2011.** PASSaGE: Pattern Analysis, Spatial Statistics and
661 Geographic Exegesis. Version 2. *Methods in Ecology and Evolution* 2: in press.
- 662 **Ruehl CB, DeWitt TJ. 2005.** Trophic plasticity and fine-grained resource variation in
663 populations of western mosquitofish, *Gambusia affinis*. *Evolutionary Ecology Research* 7:
664 801–819.
- 665 **Salini JP, Milton DA, Rahman MJ, Hussain MG. 2004.** Allozyme and morphological
666 variation throughout the geographic range of the tropical shad, hilsa *Tenuulosa ilisha*.
667 *Fisheries Research* 66: 53-69.
- 668 **Sheets HD. 2002.** IMP-Integrated Morphometrics Package. Department of Physics,
669 Canisius College, Buffalo, New York.
- 670 **Sneath PHA. 1967.** Trend-surface analysis of transformation grids. *Journal of Zoology*
671 151: 65-122.
- 672 **Svanbäck R, Eklöv P. 2003.** Morphology dependent foraging efficiency in perch: a trade-
673 off for ecological specialization? *Oikos* 102: 273–284.
- 674 **Svanbäck R, Eklöv P. 2004.** Morphology in perch affects habitat specific feeding
675 efficiency. *Functional Ecology* 18: 503-510.
- 676 **Tortonese E. 1970.** *Fauna d'Italia - Osteichthyes - Pesci Ossei*. Ed. Calderini Bologna.
- 677 **Valavanis VD, Kapantagakis A, Katara I, Palialexis A. 2004.** Critical regions: A GIS
678 based model of marine productivity hotspots. *Aquatic Sciences* 66: 139–148.

- 679 **Valentin AE, Penin X, Chanut J-P, Sévigny J-M, Rohlf FJ. 2008.** Arching effect on fish
680 body shape in geometric morphometric studies. *Journal of Fish Biology* **73**: 623-638.
- 681 **Van Tassell JL, Brito A, Bortone SA. 1994.** Cleaning behavior among marine fishes and
682 invertebrates in the Canary Islands. *Cybium* **18**: 117-127.
- 683 **Vidalis K, Markakis G, Tsimenides N. 1997.** Discrimination between populations of
684 picarel (*Spicara smaris* L., 1758) in the Aegean Sea, using multivariate analysis of
685 phenetic characters. *Fisheries Research* **30**: 191-197.
- 686 **Vizzini S, Mazzola A. 2009.** Stable isotopes and trophic positions of littoral fishes from a
687 Mediterranean marine protected area. *Environmental Biology of Fishes* **84**: 13-25.
- 688 **Walker JA. 1997.** Ecological morphology of lacustrine threespine stickleback
689 *Gasterosteus aculeatus* L. (Gasterosteidae) body shape. *Biological Journal of the Linnean*
690 *Society* **61**: 3-50.
- 691 **Warner RR, Robertson DR. 1978.** Sexual patterns in the labroid fishes of the western
692 Caribbean, I: the Wrasses (Labridae). *Smithsonian Contributions to Zoology* **254**: 1-27.
- 693 **Watson GS. 1971.** Trend-Surface Analysis. *Mathematical Geology* **3**: 215-226.
- 694 **Wimberger PH. 1992.** Plasticity of fish body shape. The effects of diet, development,
695 family and age in two species of *Geophagus* (Pisces: Cichlidae). *Biological Journal of the*
696 *Linnean Society* **45**: 197-218.
- 697 **Yamahira K, Lankford TE, Conover DO, 2006.** Intra- and Interspecific Latitudinal
698 Variation in Vertebral Number of *Menidia* spp. (Teleostei: Atherinopsidae). *Copeia*
699 **2006(3)**: 431-436.
- 700 **Yamahira K, Nishida T. 2009.** Latitudinal variation in axial patterning of the medaka
701 (Actinopterygii: Adrianichthyidae): Jordan's rule is substantiated by genetic variation in
702 abdominal vertebral number. *Biological Journal of the Linnean Society* **96**: 856-866.
- 703 **Zander CD, Nieder J. 1997.** Interspecific associations in Mediterranean fishes: Feeding
704 communities, cleaning symbioses and cleaner mimics. *Vie et Milieu* **47**: 203-212.

Fruciano C, Tigano C, Ferrito V. 2011. Geographic morphological variation within and between colour phases in *Coris julis* (L. 1758), a protogynous marine fish. *Biological Journal of the Linnean Society* 104, 148-162.
This is the accepted version of the manuscript. The definitive version is available at <http://onlinelibrary.wiley.com/doi/10.1111/j.1095-8312.2011.01700.x/full>

705 **Zander DC, Sötje I. 2002.** Seasonal and geographical differences in cleaner fish activity in
706 the Mediterranean Sea. *Helgoland Marine Research* **55**: 232-241.

Figure legends

Figure 1. Geographic locations for the samples used in the study. AU=Augusta, SR, Italy; LE=Porto Cesareo, LE, Italy; ML=Badia de Palma, Mallorca, Spain; MA=Mazara del Vallo, TP, Italy; NA=Capo Posillipo, Naples, Italy; OR=Oristano, Italy; PN=Pantelleria, Italy; RI=Riposto, CT, Italy; SP=Split, Croatia

Figure 2. Landmark (filled circles) and semilandmark positions.

Figure 3. Three-times exaggerated thin-plate splines obtained to visualise results of the bearing analysis in primary (a, solid arrow) and secondary (b, dashed arrow). The splines show the predicted shape at three times each extreme versus the average shape for each livery (used as reference)

Figure 4. Predicted shapes for the trend surface analysis of primary specimens at the sampled locations.

Figure 5. Predicted shapes for the trend surface analysis of secondary specimens at the sampled locations.

Figure 6. Shape change predicted by partial least squares analysis. The figure shows shape change associated to an increase in temperature and a decrease in chlorophyll a concentration. The reference (average shape) is shown in light grey dashed line and empty circles.

Figure 7. Three-times exaggerated splines showing shape changes predicted by separate regressions of shape on chlorophyll a and temperature using primary specimens. a=negative chlorophyll extreme, b=positive chlorophyll extreme, c=negative temperature extreme, d=positive temperature extreme; in all splines the average shape of primary individuals has been used as reference.

Figure 8. Three-times exaggerated splines showing shape changes predicted by the regression of shape on sea surface temperature using secondary specimens. a=negative extreme, b=positive extreme.

S2. Bearing analysis: plot of correlations at different directions. Circles indicate significant correlations. While the bearing analysis to find the direction of highest correlation on which the splines are based has been carried out at 1 degree intervals for good resolution, the plot shows correlations only at 5 degree intervals to produce an easier to understand picture.

Tables

Table 1 Sampling data for each population used in the study

Sample	Sampling time	Environment	Coordinates	Livery		Total
				Primary	Secondary	
Split (SP)	March 2007	<i>Posidonia oceanica</i> beds; a few meters of depth	43°28'N 16°24'E	7	10	17
Naples (NA)	February 2007	Gravel bottom; 10-12 meters of depth	40°46'N 14°12'E	4	8	12
Lecce (LE)	May 2007 and May 2009	Patches of rocky bottom and <i>Posidonia oceanica</i> beds; 8-20 meters of depth	40°14'N 17°52'E	3	29	32
Oristano (OR)	April 2007	Rocky bottom with photophilic algae; <i>Posidonia oceanica</i> beds; 5-6 meters of depth	39°48'N 8°31'E	24	2	26
Mallorca (ML)	June 2007	Rocky bottom with photophilic algae; 9-17 meters of depth	39°31'N 2°39'E	21	19	40
Riposto (RI)	January 2007; March 2007; April 2008	Patches of rocky and sandy bottom; 4-8 meters of depth	37°43'N 15°13'E	26	2	28
Mazara del Vallo (MA)	June 2007	<i>Posidonia oceanica</i> beds; 3-5 meters of depth	37°38'N 12°35'E	14	19	33
Augusta (AU)	May 2008 and June 2008	Rocky bottom with few photophilic algae; 4-10 meters of depth	37°11'N 15°14'E	6	17	23
Pantelleria (PN)	October 2008	Rocks with <i>Posidonia oceanica</i> patches close to a sandy area with <i>Posidonia</i> ; 5-15 meters of depth	36°50'N 11°59'E	1	11	12
Total				106	117	223

Table 2 Results of the trend surface analysis on primary and secondary specimens; X=mean-centered latitudinal term; Y=mean-centered longitudinal term

Primary specimens		
Method	Chosen model	Explained Variance
Full model	$X+Y+X^2+XY+Y^2+X^3+Y^3$	22.61%
Legendre & Legendre (1998)	$X+Y+X^2+X^3+Y^3$	20.33%
Modified Legendre & Legendre (1998)	$X+X^2+X^3+Y^3$	15.82%
AICc	$X+Y^3$	12.86%
Secondary specimens		
Method	Chosen model	Explained Variance
Full model	$X+Y+X^2+XY+Y^2+X^3+Y^3$	17.79%
Legendre & Legendre (1998)	$X+Y+X^2+Y^2+X^3+Y^3$	15.4%
Modified Legendre & Legendre (1998)	$X^2+Y^2+Y^3$	11.21%
AICc	X^2+Y^2	9.39%

Table 3 Results of the partial least squares (PLS) analysis in primary and secondary specimens. The RV coefficient (Escoufier, 1973) is a multivariate analogue of the squared correlation.

Dataset	Correlation between blocks		Results for each PLS singular axis					
	RV	p-value	Axis	% of total covariance	Correlation of scores between blocks	p-value	Correlation of environmental variables with each PLS axis	
Primary specimens	0.1877	0.0008	1	84.689	0.702	0.0012	Temperature	0.6948
			2	15.311	0.448	0.0579	Chlorophyll	-0.7192
Secondary specimens	0.1368	<0.0001	1	78.272	0.574	0.0001	Temperature	0.9906
							Chlorophyll	-0.1367
			2	21.728	0.352	0.0027	Temperature	-0.1367
							Chlorophyll	-0.9906

Figure 1

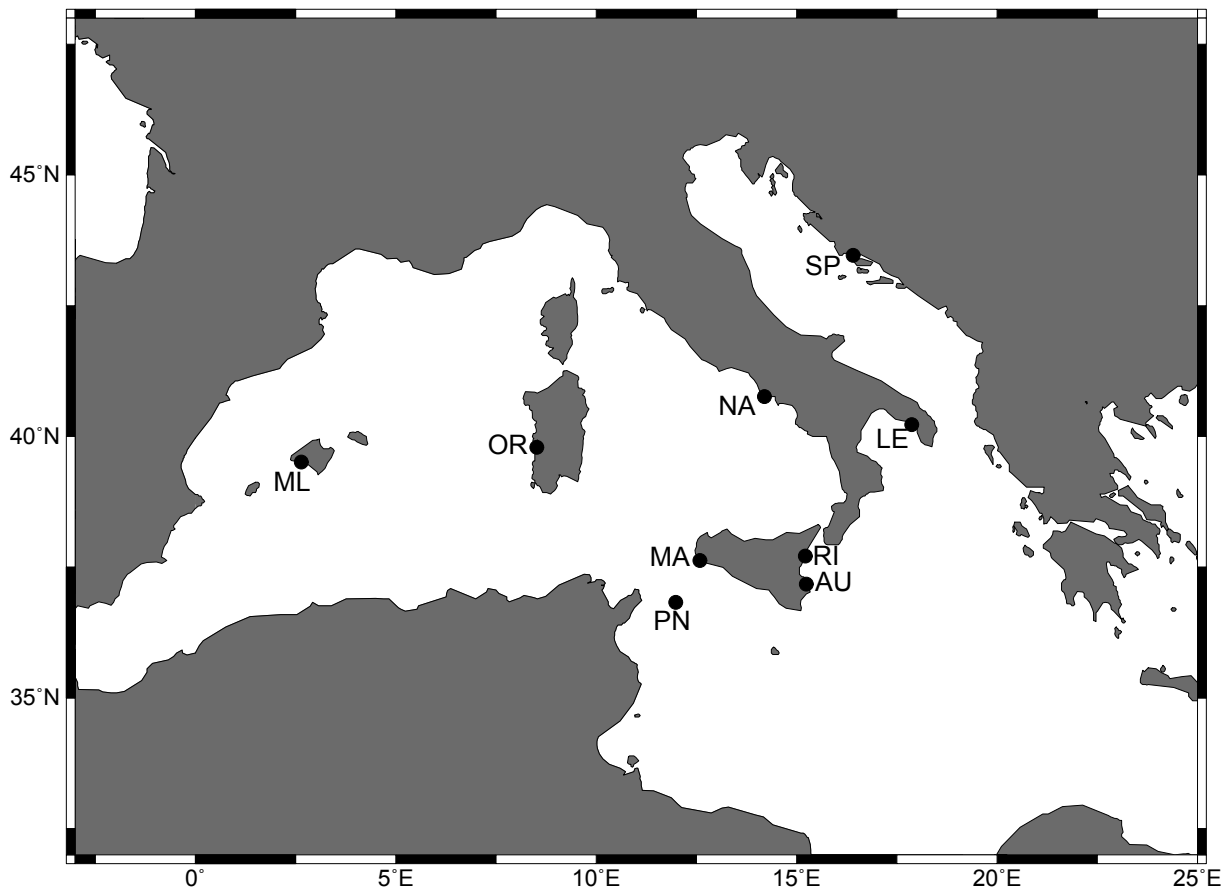


Figure 2

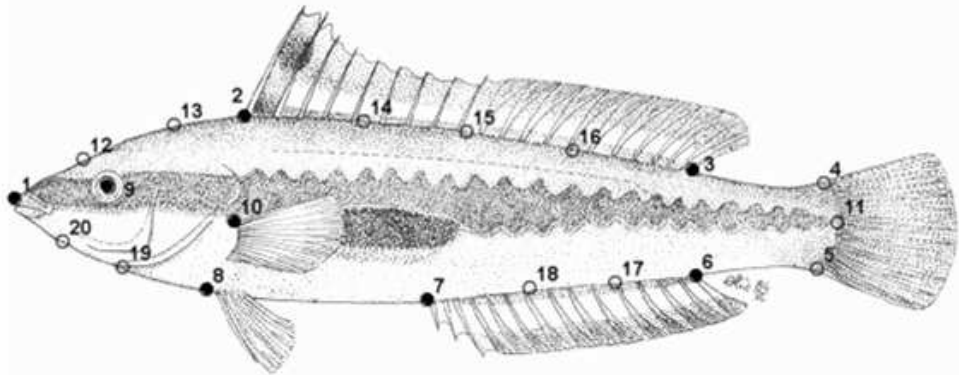


Figure 3

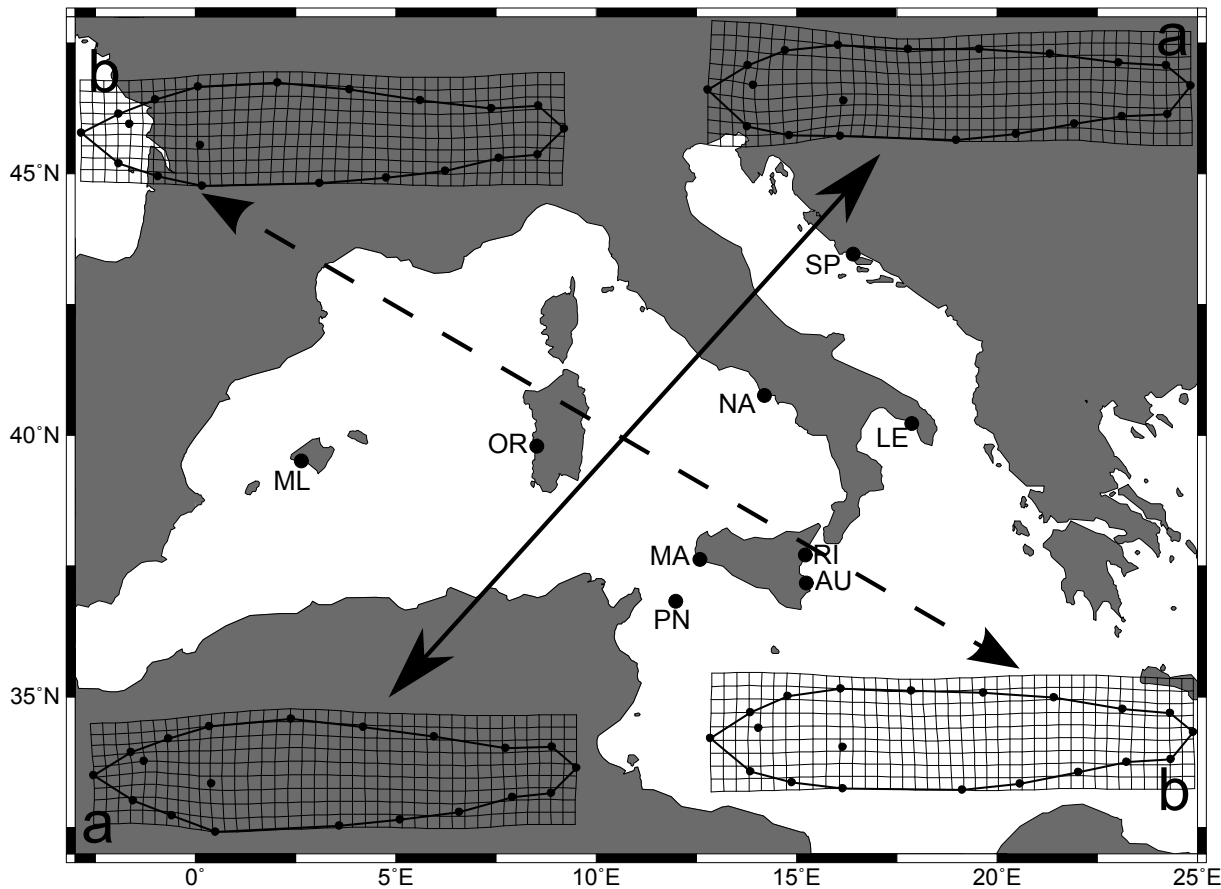
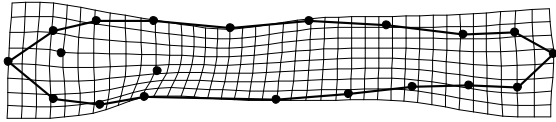
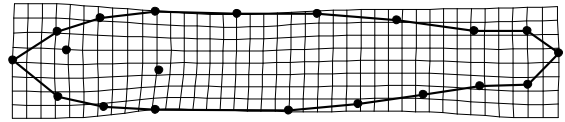


Figure 4

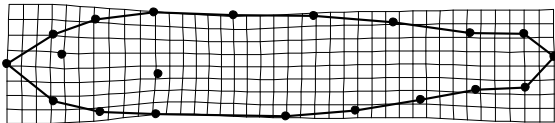
Split



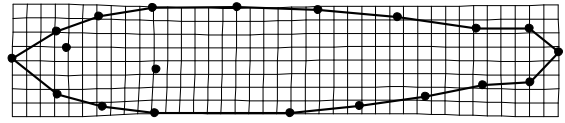
Naples



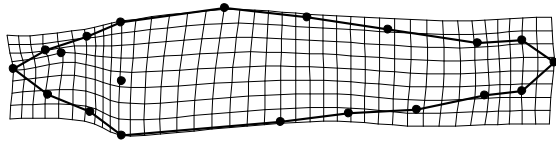
Lecce



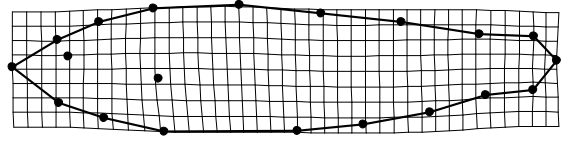
Oristano



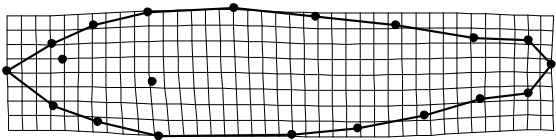
Mallorca



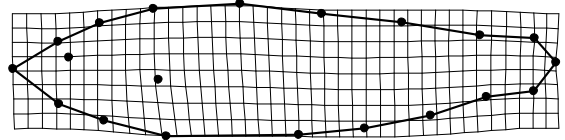
Riposto



Mazara del Vallo



Augusta



Pantelleria

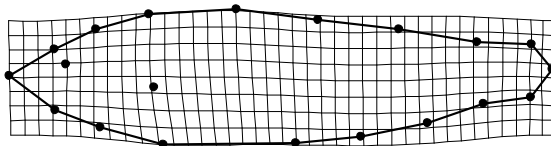
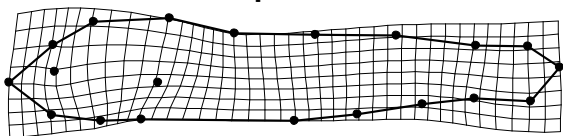
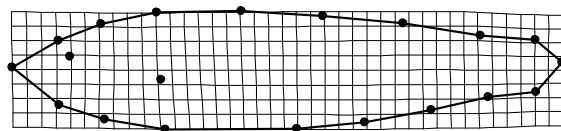


Figure 5

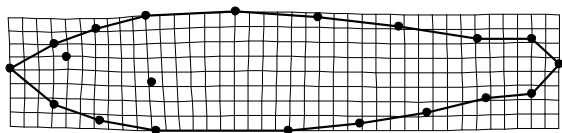
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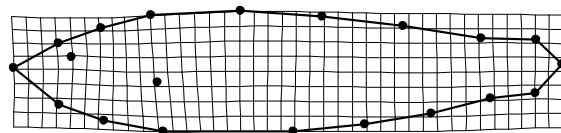
Naples



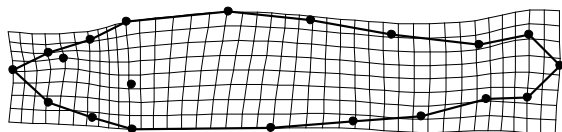
Lecce



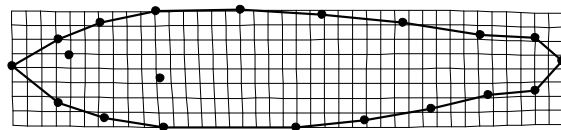
Oristano



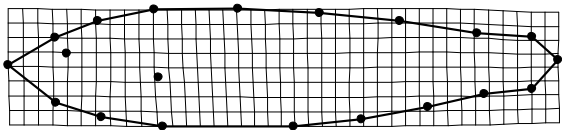
Mallorca



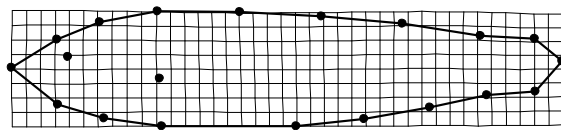
Riposto



Mazara del Vallo



Augusta



Pantelleria

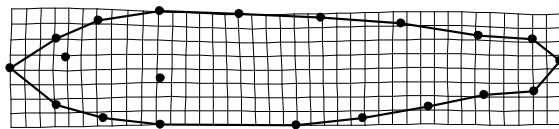
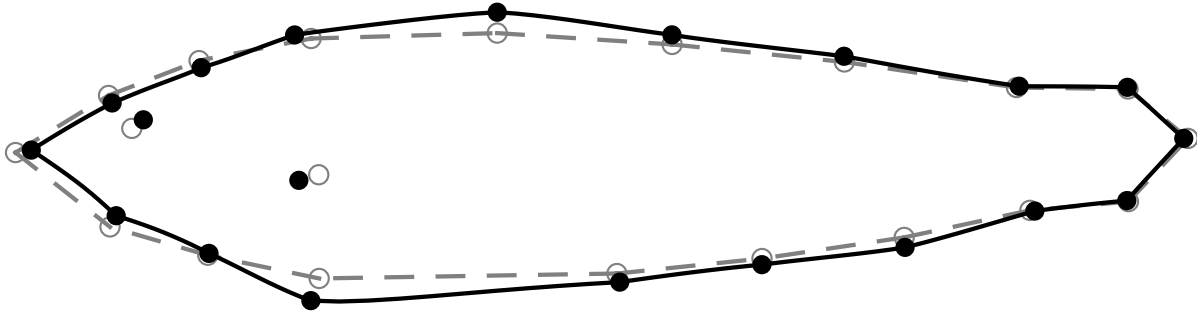


Figure 6

Primary specimens



Secondary specimens

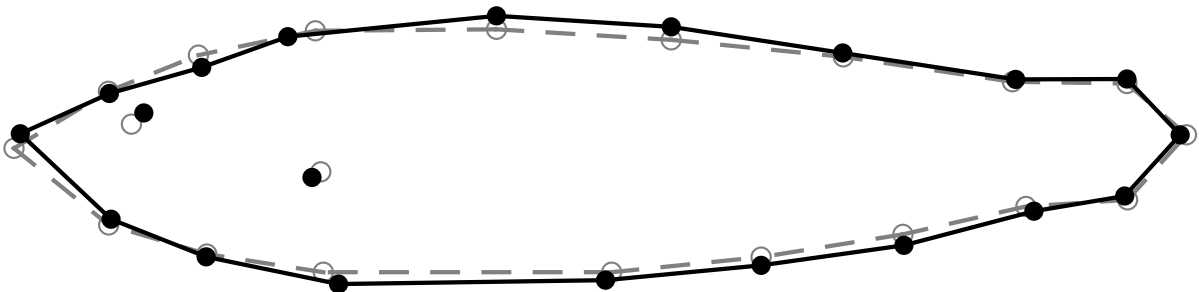
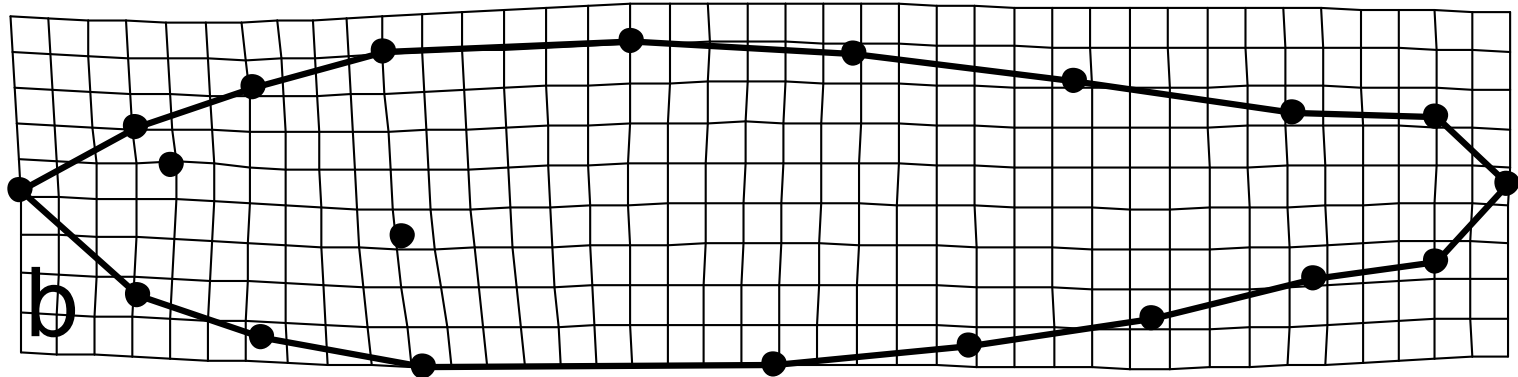
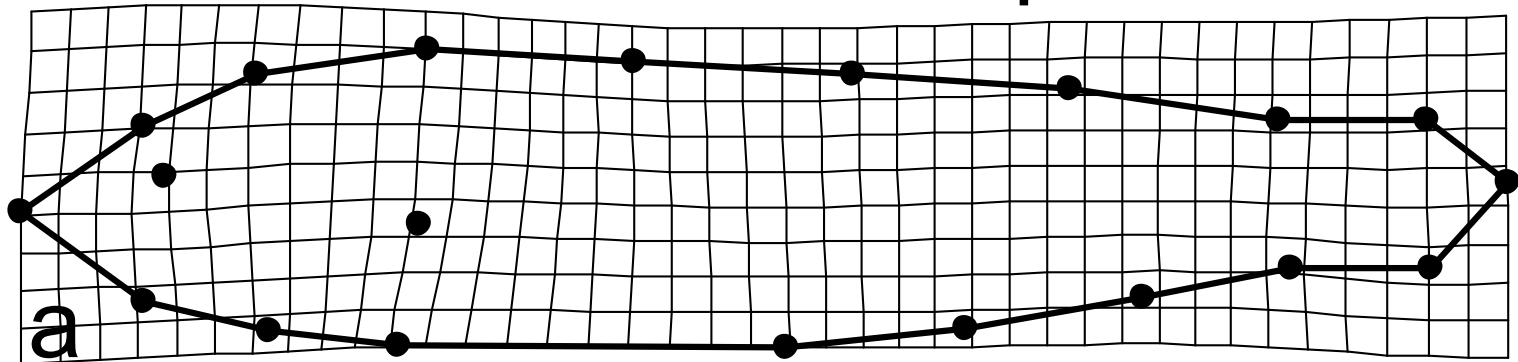


Figure 8

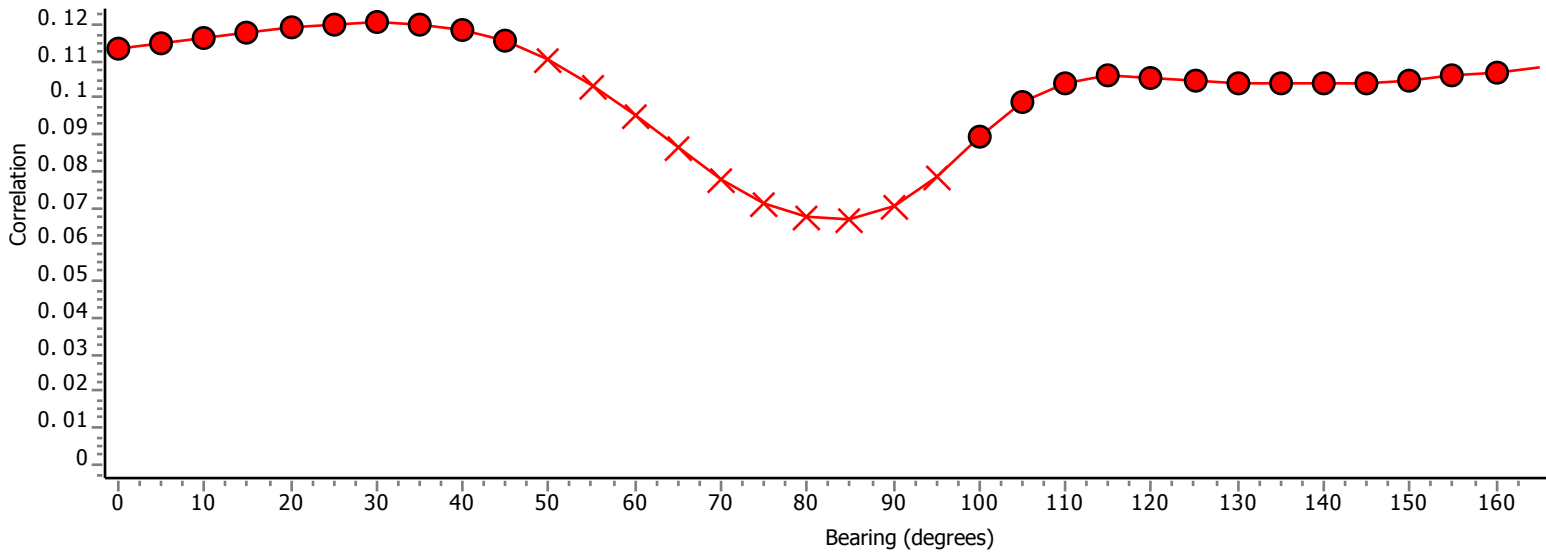
Sea surface temperature



Supplemental material S1: Satellite-derived ecological data.
Sea surface temperature (SST) in Celsius degrees, chlorophyll a concentration in mg/m⁻³

Sampling site	Average SST	Chlorophyll geometric mean
Augusta	20.1825	0.359396216
Lecce	19.38583333	0.379711734
Mazara	19.33291667	0.393831368
Mallorca	19.9975	0.32646652
Naples	19.95833333	0.791435495
Oristano	18.98416667	-
Pantelleria	20.06625	-
Riposto	20.13041667	-
Split	18.41666667	0.501116893

Primary individuals



Secondary individuals

