# Atlantic-Mediterranean and within-Mediterranean molecular variation in Coris julis (L. 1758) (Teleostei, Labridae) 

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#### Abstract

Sequence variation of the mitochondrial control region was studied in the Mediterranean rainbow wrasse (Coris julis), a species with pronounced pelagic larval phase inhabiting the Mediterranean Sea and the adjacent coastal eastern Atlantic Ocean. A total of 309 specimens from 19 sampling sites was analysed with the aim of elucidating patterns of molecular variation between the Atlantic and the Mediterranean as well as within the Mediteranean Sea. Phylogeographic analyses revealed a pronounced structuring into a Mediterranean and an Atlantic group. Samples from a site at the Moroccan Mediterranean coast in the Alboran Sea showed intermediate frequencies of "Mediterranean" and "Atlantic" haplotypes. We recognized a departure from molecular neutrality and a star-like genealogy for samples from the Mediterranean Sea, which we propose to have happened due to a recent demographic expansion. The results are discussed in the light of previous studies on molecular variation in fish species between the Atlantic and the Mediterranean and within the Mediterranean.


## Introduction

Molecular variation among marine fish populations has been described in diverse patterns ranging from a panmictic model to a genetic structuring with separate local populations. Genetic variation in marine fish has been traditionally considered smaller than variation in freshwater and anadromous species due to the presumed lack or paucity of barriers to gene flow. Many early studies (Ward et al. 1994) reported results much more consistent with a panmictic model. However, phenomena such as larval retention due to currents (Stepien et al. 2001; Palumbi 2003) or larval behaviour (Palumbi 1994) can lead, even in species with a pronounced planktonic larval phase, to a significant genetic divergence of populations. For example, Gerlach et al. (2007) showed that some planktonic larvae are capable of distinguishing "olfactory signatures" of their natal reefs, thus opening the possibility for homing behaviour and the formation of retention zones. In some cases, distinct geographical lineages have been detected for species with high dispersal capacity even in complete absence of apparent oceanographic barriers to gene flow (Burford 2009; Bergek and Bjorklund 2009). Furthermore, species with similar ecological traits do not always show consistent phylogeographic patterns: Bargelloni et al. (2003) studied five sparid species sharing similar biological features and found a strong phylogeographic break between the Atlantic Ocean and the Mediterranean Sea in three species, but no evidence of such a break in others. This Mediterranean-Atlantic transition has been the subject of a variety of studies on both vertebrates and invertebrates (reviewed by Patarnello et al. 2007). No general relationship between either dispersal ability or life history traits, and patterns of partial or complete genetic isolation between Atlantic and Mediterranean populations could be found (Patarnello et al. 2007). The authors also noticed that some of the species which exhibit Atlantic-Mediterranean differentiation show steep changes of allele frequencies associated with the Almeria-Oran front
(which separates the Alboran Sea from the rest of Mediterranean) rather than with the Strait of Gibraltar (which separates the Mediterranean Sea from the Atlantic Ocean).

Within the Mediterranean, a pronounced genetic structuring in fishes was detected in comparisons between populations from the Adriatic Sea and the remaining Mediterranean. For example, a divergence of the Adriatic population from other Mediterranean populations has been noticed in the sand goby (Pomatoschistus minutus; Gysels et al. 2004), in the red mullet (Mullus barbatus; Maggio et al. 2009) and in European sprat (Sprattus sprattus; Debes et al. 2008). In the latter study (Debes et al. 2008), individuals from the Adriatic and the Tyrrhenian Sea were highly differentiated at mitochondrial DNA which was explained as the result of postglacial warming and the subsequent inability of this boreal, cold adapted species to maintain gene flow at its southernmost distribution limit under present physical oceanographic conditions.

Previous studies on Thalassoma pavo, a wrasse species of tropical origin, found no evidence of geographic structure between the Atlantic and the Mediterranean Sea, instead, it was suggested that there could be a phylogeographic break in the Aegean area between the eastern and the western Mediterranean (Costagliola et al. 2004; Domingues et al. 2008). A divergence between eastern and western Mediterranean populations has also been noticed both in other fish species (Thunnus thynnus thynnus; Carlsson et al. 2004; Pomatoschistus marmoratus; Mejri et al. in press) and other marine organisms (Cerastoderma glaucum; Nikula and Väinölä 2003;

Posidonia oceanica; Arnaud-Haond et al. 2007; Serra et al. 2010; Patella rustica; Sà-Pinto et al. 2010). However, a study on two species of the genus Scomber highlighted a divergence between eastern and western Mediterranean populations for one of them, but no evidence of such a divergence in the other species (Zardoya et al. 2004).

The Mediterranean rainbow wrasse (Coris julis, L. 1758) is a small-sized labrid fish that is widely spread in the Mediterranean Sea and along the adjacent European and African Atlantic coasts. This species is a diandric protogynous hermaphrodite that exhibits two radically
different colour patterns (liveries). The primary livery is exhibited by juveniles, females, and non-territorial males whereas the secondary livery is exhibited by territorial males only (Bacci and Razzauti 1957; Roede 1966; Lejeune 1982; Bentivegna and Cirino 1984). Among the secondary livery, two different geographic colorations can be found with the Atlantic specimens being different from the specimens typically observed in the Mediterranean (Laurent and Lejeune 1988).

After larval settlement the rainbow wrasse inhabits shallow coastal waters (mainly rocky areas) and shows little migratory ability. Contrasting the rather stationary adult phase, pelagic eggs and a pronounced planktonic larval phase (Gordoa et al. 2000; Raventòs and Macpherson 2001) suggest a potentially high dispersal capacity. Intraspecific molecular variation in C. julis has previously been studied by Guillemaud et al. (2000) based on mitochondrial 12S rDNA and by Aurelle et al. (2003) based on microsatellite markers. The study by Guillemaud and colleagues (2000), although based on an extremely low sample size (only seven C. julis specimens), showed a divergence between sequences of specimens of Atlantic and Mediterranean origin. The study by Aurelle et al. (2003) revealed the Atlantic-Mediterranean transition as a phylogeographic break but no further genetic differentiation on each side of the Strait of Gibraltar, even between geographically distantly separated sites like continental Portugal and the offshore situated Azores Islands. The authors hypothesized that genetic differentiation within the Mediterranean Sea might be more pronounced than detected, as they acknowledged a limited power of their study due to a low sample size and therefore considered their results on the Mediterranean Sea as preliminary (Aurelle et al. 2003).

The present study aims at contributing to clarify the role of the Atlantic-Mediterrranean transition (Strait of Gibraltar versus Almerian-Oran front) as a phylogeographic barrier by testing its relevance for a small demersal wrasse species and additionally testing for a genetic structuring of the rainbow wrasse within the Mediterranean Sea.

## Materials and methods

A total of 309 specimens of Coris julis from 19 Mediterranean and Atlantic sampling sites (Fig 1, Table 1), sampled with fish traps, nets, fishing rods and hand lines, was analysed. Individual white muscle tissue samples were collected from the right side of each fish and preserved in 95\% ethanol.

Total DNA was extracted using a commercial silica-based spin column kit (GenElute Mammalian Genomic DNA Miniprep Kit, Sigma Aldrich). We amplified a mitochondrial DNA fragment of about 490 bp (base pairs) including part of the tRNA threonine gene, the tRNA proline gene and the $5^{\prime}$ portion of the control region via PCR. For the PCR we used modified universal primers THR2m1 (5'-AGAGCGCCGGTCTTGTAAAC-3') and TDKDm2 (5'-CTGAAGTAGGAACCAAATGCCAGGAA-3') derived, respectively, from L15926 (Kocher et al 1989) and TDKD (Slade et al 1994). PCR amplification reactions were obtained for a total volume of $50 \mu \mathrm{in} 1 \mathrm{X}$ buffer, $1 \mathrm{mM} \mathrm{MgCl} 2,0.2 \mathrm{mM}$ of each $\mathrm{dNTP}, 0.2 \mu \mathrm{M}$ of each primer, 1.25 units of Taq DNA polymerase (Invitrogen) and $2 \mu \mathrm{l}$ of extracted DNA. The PCR cycling profile comprised an initial denaturation step of 4 minutes at $95^{\circ} \mathrm{C}, 30$ cycles of denaturation $\left(94^{\circ} \mathrm{C}\right.$ for 30 seconds), annealing ( $56^{\circ} \mathrm{C}$ for 1 minute) and extension $\left(72^{\circ} \mathrm{C}\right.$ for 1 minute), and a final extension step of $72^{\circ} \mathrm{C}$ for 10 minutes; the ramp was $2^{\circ} \mathrm{C} / \mathrm{s}$ for all the steps. Obtained amplified fragments were sequenced using an external sequencing service (Secugen S.L., Madrid, Spain). Only the control region portion (334 bp) was used in sequence analyses. Sequences were visually aligned with the program Bioedit 7.0 (Hall 1999) and sequence data was deposited in GenBank (accession numbers HQ917534-HQ917613).

The best nucleotide substitution model was estimated with ModelTest (Posada 2008) using AIC (An Information Criterion; Akaike 1974) as information criterion. The selected model, $\mathrm{TrN+I}$ (Tamura and Nei 1993) was then used to correct genetic distances whenever possible.

## Analysis of genetic structure

To investigate the population genetic structure in rainbow wrasse, Analysis of Molecular Variance (AMOVA, Excoffier et al. 1992) and computation of $\mathrm{F}_{\text {st }}$ pairwise values between sampling sites (with the exclusion of the samples from Israel and Crete whose sample size was deemed too small) were performed with the software Arlequin 3.1 (Excoffier et al. 2005). The AMOVA was performed on the Tamura and Nei (1993) distance matrix using various geographical groupings of the sampling sites (see caption of Table 2 for a rationale) and assessing significance with the permutational procedure implemented in the software (1000 permutations). The R script provided by Fitzpatrick (2009) was used to ensure that it was possible to obtain a P-level smaller than 0.05 with the selected groupings. Nonmetric multidimensional scaling analysis (Kruskal 1964a,b) was also performed, using the software NTSYSpc 2.2 (Rohlf 2007), on the matrix of pairwise $\mathrm{F}_{\mathrm{st}}$ between sampling sites both using all samples and using only the samples from the Mediterranean (with the exception of the Moroccan one). Ordinations of sampling sites along the first two dimension of the space obtained by multidimensional scaling were then plotted against each other.

## Neutrality tests

Departures from neutrality of molecular evolution were tested for different geographic groupings by computing the value of Ramos-Onsins and Rozas $\mathrm{R}_{2}$ statistic (Ramos-Onsins and Rozas 2002) and testing its significance with the procedure based on coalescent simulations (1000 simulated samples) with DNAsp 5.0 (Librado and Rozas 2009). The software Arlequin was used to compute Fu's $\mathrm{F}_{\mathrm{S}}$ (Fu 1997) values and to test their deviation from neutrality expectations by the means of the coalescent algorithm implemented in the program (10,000 simulated samples). The choice of such tests has been made in the light of previous studies on the power of various neutrality tests (Fu 1997; Ramos-Onsins and Rozas 2002). In fact, we decided to use both Ramos-Onsins and Rozas $\mathrm{R}_{2}$ and Fu's $\mathrm{F}_{\mathrm{S}}$ because they have been shown to
be more powerful than a number of alternatives, albeit the sample size defines which of the two is more powerful (Ramos-Onsins and Rozas 2002).

## Relationships among haplotypes

The relationships among haplotypes were described using two network methods: medianjoining (Bandelt et al. 1999) and neighbor-net (Bryant and Moulton 2004). The median-joining network was constructed using the software Network (Fluxus Technology Ltd), the neighbornet network (Bryant and Moulton 2004) based on the uncorrected p-distance was constructed using the software SplitsTree4 (Huson and Bryant 2006). Additionally, we obtained a bayesian phylogenetic tree with the software MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) under a model that corresponds to the GTR model of substitution with a proportion of invariable sites. For this, we sampled every 1,000 generation from a total of $13,706,000$ generations when the split frequency between runs, with six chains each, was $<0.01$. We obtained the phylogenetic tree after discarding the first $25 \%$ of generations.

## Spatial patterns

The software NTSYSpc 2.2 (Rohlf 2007) was used to obtain plots of pairwise genetic distances (TrN-corrected distances among group means) versus pairwise geographic distances, to calculate correlations between matrices measuring genetic differences and matrices measuring geographical distances, and to test their significance by the means of a Mantel test (Mantel 1967) with 1000 permutations. Geographical distances were computed both as "direct" distances and as shortest waterway distances (obtained using the software Google Earth) among sampling locations. Both geographical distances in kilometres and their logarithmic transformations were used. Plots of pairwise genetic versus pairwise geographic distances were chosen because, in case of significant correlation, they can give a hint on the underlying process (Guillot et al. 2009).

Spatial autocorrelation analysis, as implemented in the software Alleles in Space (Miller 2005) was also performed using 12 distance classes. To test if the method of choice of distance classes (equal distances and unequal sample sizes or unequal distances and equal sample sizes) or their number had an effect on the analysis, the analysis was repeated using both methods and $5,8,10,12$ distance classes.

The Israeli and Cretan samples were excluded from the computation of correlations and from the spatial autocorrelation analysis due to their low sample size.

The software Barrier (Manni et al. 2004), which computes a Delaunay triangulation on a Voronoi tessellation and then uses Monmonier's (1973) maximum difference algorithm in order to identify barriers, was used to identify the position of possible genetic barriers among sampling sites based on the Tr N -corrected distances among sites. Two runs of the software were performed; the first one computing only one barrier, the second computing two barriers. Finally, a landscape shape interpolation of genetic distances, which interpolates observed genetic distances across the landscape and shows them graphically as heights ( Z axis) in a graph where the base (values along X and Y axes) represents the geographic space, was obtained using the software Alleles in Space (Miller 2005).

## Results

## Analysis of genetic structure

By using grouping schemes A (Atlantic versus Mediterranean) and B (Atlantic samples + Morocco versus the remaining Mediterranean samples) most of the molecular variation was explained by the among-groups variance component, which is statistically significant (Table 2 ). In particular, using scheme $\mathrm{A}, 94.9 \%$ of molecular variance was explained by the amonggroups term, while using scheme B this term explained $83.16 \%$ of the observed variation. On the opposite, using schemes C (Eastern Mediterranean versus Central Mediterranean versus

Western Mediterranean) and D (Eastern Mediterranean versus the remaining Mediterranean samples except the Moroccan one) resulted in most of the molecular variation being expressed by the within-population term.

The pairwise comparison between sampling sites (Table 3) shows that the Portuguese sample was significantly different from all other samples except the one from the Cantabrian Sea which, in turn, showed a similar pattern. Also, the Moroccan sample is significantly different from many other samples. Nonmetric multidimensional scaling required 10 dimensions to get an almost perfect (sensu Kruskal 1964a) stress value (0.00026) when analysing only Mediterranean sites (with the exception of Morocco), while 2 dimensions were sufficient to achieve zero stress when using also Moroccan and Atlantic samples. Looking at the patterns in the first two dimensions obtained by nonmetric multidimensional scaling analyses, in the analyses with both Mediterranean and Atlantic samples (Fig 2) most of sampling sites are so close together in the two-dimensional space obtained by the analysis that only three points are distinguishable in the graph: one point for all the Mediterranean samples (except the Moroccan one), a second point for the Moroccan sample and a third point for all the Atlantic samples. On the opposite, the analysis performed only on Mediterranean samples (excluding the Moroccan one, Fig 3) show in the first two dimensions a pattern in which geographic proximity does not necessarily reflect proximity in the multivariate space.

## Neutrality tests

Results of the analyses of Ramos-Onsins and Rozas $\mathrm{R}_{2}$ in geographic groupings of samples are provided in Table 4. Mediterranean samples showed a significant departure from neutrality. Analyses using Fu's $\mathrm{F}_{\mathrm{S}}$ gave the same results (not shown).

## Relationships among haplotypes

A total of 80 haplotypes was recognized (Table 5). Of those haplotypes, the most abundant is haplotype 2 which was present in $53.4 \%$ of all specimens and was shared among all sampling sites except the Israeli, Portuguese and Cantabrian Sea ones. Moreover, a high number of rare
haplotypes could be observed ( 60 haplotypes, $75 \%$ of the total number of haplotypes were private).

The neighbour-net network is depicted in Fig 4 (the median-joining network and the Bayesian tree provided similar results and both are not shown). It can be noticed that the "Atlantic" subnetwork (the smallest of the two sub-networks) comprised haplotypes 68, 69, 72 and 76 . Of them, haplotype 68 was shared among the Portuguese, Moroccan and Cantabrian Sea samples, the other three haplotypes were private of Atlantic (69 and 72) or Moroccan (76) samples. Moreover, all haplotypes found in the two Atlantic samples $(68,69$ and 72$)$ grouped exclusively into the "Atlantic" sub-network. Haplotypes found in the Moroccan sample grouped both in the "Atlantic" sub-network and in the "Mediterranean" sub-network which, in turn, comprised only Mediterranean haplotypes and showed a "star-like" topology.

## Spatial patterns

The correlation between "direct" geographic distances and genetic distances was $\mathrm{R}=0.6$ using raw distances, $\mathrm{R}=0.5$ using log-transformed distances and significant in both cases (respectively $\mathrm{p}=0.004$ and $\mathrm{p}=0.003$ ). The correlations between shortest waterway distances and genetic distances were $\mathrm{R}=0.7$ using raw geographic distances and $\mathrm{R}=0.57$ using their logarithmic transformations, and higher than expected by chance alone $(\mathrm{P}<0.001$ and $\mathrm{P}=0.002$, respectively). A plot of pairwise genetic distances versus pairwise geographic distances (shortest waterway, raw) is shown in Fig 5.

The spatial autocorrelation analysis using 12 distance classes (unequal distances, equal sample size) was overall significant $(\mathrm{P}<0.001)$. When analysing each distance class, all the classes from the 990-1126 Km class to the largest class showed a significant upper tail (within-class genetic distances higher than expected by chance). Using a different method of choice or a different number of distance classes did not appreciably affect the outcome of the analysis. The barriers computed with the Monmonier algorithm and the interpolation plot are depicted in Fig 6 and 7 respectively. The application of the Monmonier algorithm to the matrices of genetic
and geographic distances (Fig 6) indicated two barriers: the first between the two Atlantic samples and the remaining samples, the second between the Moroccan sample and the remaining samples.

Looking at the interpolation plot (Fig 7), it can be noticed that the genetic diversity appears higher in the Western portion (Atlantic Ocean and Western Mediterranean) of the sampled area, and lower in the Eastern portion (Central-Eastern Mediterranean). The exact values (data not shown) along the X and Y axes for the highest peaks on the Z axis of the plot show that the highest genetic divergence is found along a line that separates the two Atlantic samples from all the others..Not surprisingly, this line corresponds mostly to the Iberian Peninsula, but also to the Strait of Gibraltar. Other high (but less pronounced) peaks were located along the separation between the Moroccan sample and the rest of the Mediterranean, corresponding to the Almeria-Oran front.

## Discussion

This study highlights the patterns of molecular differentiation in Coris julis in the
Mediterranean Sea and the Atlantic Ocean.
A single haplotype (haplotype 2) was shared among all the Mediterranean sampling sites (except the Israeli one) and was represented in over half of the specimens. This haplotype was absent in Atlantic sampling sites. The next most common haplotype was represented in only $4.5 \%$ of the specimens and there was a large proportion (75\%) of rare haplotypes. The results of the analyses of population differentiation, on the whole, can be interpreted as an absence of genetic structuring within the Mediterranean Sea (with the exception of the Moroccan sample situated in the Alboran Sea) and a strong differentiation between Mediterranean Sea and Atlantic Ocean, with the Moroccan sample being intermediate.

Looking at the results of the neutrality tests, it can be said that the Atlantic and Moroccan samples do not show signs of departure from neutrality expectations while the pooled

Mediterranean samples do. However, results of neutrality tests on the Atlantic and Moroccan samples should be considered cautiously as the low sample size of those samples might have lead, even with powerful tests as the ones used, to a lack of statistical power. As causes of departure from neutrality for the Mediterranean Sea sample we cannot dismiss background selection and genetic hitchhiking. However, an event of recent demographic and range expansion represents a likely hypothesis. Such a hypothesis would also be supported by Ray et al.'s (2003) suggestion that, in the presence of high exchange of migrants among neighbouring demes, a large spatial expansion can lead to signatures similar to those arising from a pure demographic expansion. Given that $C$. julis is regarded as thermophilic and it has been known to expand his geographical range in response to water warming during very recent historical times (Piron et al. 2007; Lipej et al. 2009), it is conceivable that this species could have also been subject to changes in its range as a consequence of strong changes in climate in more distant times. In particular, it can be hypothesised that due to the drop in water temperatures during the late Pleistocene Mediterranean populations were isolated at thermally favoured locations. A subsequent rise in temperature then caused a demographic and spatial expansion in the Mediterranean leading to the observed deviation from neutrality. Interestingly enough, Patarnello et al. (2007) found signatures of a population expansion in Mediterranean in 5 out of the 7 fish species for which they carried out separate analyses for Atlantic and Mediterranean samples; 4 out of the 5 species that showed signs of expansion also showed a significant Fu's $\mathrm{F}_{\mathrm{S}}$ statistic. The relationships among haplotypes represented in the network, as well as in the Bayesian tree, showed two sub-networks separated by a long distance. One of the two subnetworks comprised Mediterranean haplotypes and showed a "star-like" topology, the other essentially Atlantic haplotypes. Both the "star-like" genealogy and the excess of rare mutations (that can be found in the "Mediterranean" sub-network) have been considered to arise as a consequence of population growth (Slatkin and Hudson 1991; Harpending and Rogers 2000). A star-like genealogy is also expected in the case of a very rapid increase in population size
followed by a period of large and constant population size (Slatkin and Hudson 1991). Such a population growth is supported by the above-mentioned results of the neutrality tests. Moreover, the "intermediate" position of the Moroccan sample, with haplotypes belonging to both the Atlantic and the Mediterranean sub-networks, mirrors the results of analyses of genetic differentiation.

The results of the Mantel tests show a significant correlation between genetic and geographic distance among sampling sites. The spatial autocorrelation analysis shows, in general terms, that at longer distances (starting at about 1000 Km ) the genetic distance among sites is larger than expected by chance alone. The pattern revealed by the plot of pairwise genetic versus geographic distance (Fig 5) suggests that the significant correlation between genetic and geographic distance found by the Mantel test might be the product of the particular sampling scheme used in this study. In fact, the discontinuity among groups of distances observed in the plot, can be interpreted (Guillot et al. 2009) as the effect of the presence of barriers to gene flow, thus dismissing the hypothesis of an isolation by distance model as a cause of genetic differentiation among Atlantic, Moroccan and Mediterranean samples.

Overall, the plot of genetic versus geographic distance, the computation of barriers with the Monmonier algorithm and the landscape shape interpolation plot, , all confirm the results of the analyses of genetic differentiation and show a separation of the Atlantic samples and to a lower extent also of the Moroccan sample. The general result of an overall Atlantic-Mediterranean differentiation confirms previous findings based on a very limited sampling of Mediterranean sites and a different set of genetic markers (Guillemaud et al. 2000; Aurelle et al. 2003). However, the concurrent presence of Atlantic and Mediterranean haplotypes in the Alboran Sea is a new finding for the species. Interestingly, most of the species with Atlantic-Mediterranean differentiation show steep changes of allele frequencies associated either with the AlmeriaOran front or with the Strait of Gibraltar (Patarnello et al. 2007). In the case of C. julis, the intermediate situation of the Moroccan sample could be explained by the presence of both the

Strait of Gibraltar and the currents within the Alboran Sea which create the Almeria-Oran Front. The Alboran Sea might then be partially genetically isolated both from the Atlantic Ocean and the rest of the Mediterranean Sea, with marked genetic differentiation between the two basins and a limited, possibly current, gene flow between the two via the Alboran Sea. In this respect, the Alboran Sea might, therefore, represent a still partially isolated zone of secondary contact between two previously separate $C$. julis lineages. Interestingly enough, Laurent and Lejeune (1988) have noticed in the Alboran Sea and in French Mediterranean waters the presence of secondary rainbow wrasse individuals exhibiting the "Atlantic" secondary colour pattern. In this context it is also worth noting that Lemaire et al. (2005) have found intermediate frequencies of Atlantic and Mediterranean haplotypes of the sea bass (Dicentrarchus labrax) in Alboran Sea samples, thus hypothesising that the Almeria-Oran front had been crossed by Mediterranean migrants. The northern African coast has been widely neglected in studies on phylogeography in the Mediterranean. While this study represents an exception it will be important to sample more localities to confirm that Atlantic haplotypes are gradually replaced by Mediterranean haplotypes as the distance from Gibraltar or the Almeria/Oran fronts increases. Moreover, our results highlight the importance of samples from the Alboran Sea in phylogeographic studies in fish because intermediate frequencies of Mediterranean and Atlantic haplotypes in the Alboran Sea might be more common than expected.

Within the rest of the Mediterranean, the present study reports no significant differentiation among samples or areas. This is in contrast with previous findings on Thalassoma pavo, another labrid species with similar biological features. Here, no differentiation between Atlantic and Mediterranean samples was detected but a weak differentiation was found between western and eastern Mediterranean in terms of a genetic discontinuity at the Peloponnesus (Costagliola et al. 2004; Domingues et al. 2008).

Regarding the differentiation within the Atlantic, Aurelle et al. (2003) reported for C. julis no genetic differentiation between samples from the Azores Islands and most of the Atlantic continental samples, despite a distance of more than $1,800 \mathrm{~km}$ between the archipelago and the continent. The absence of genetic differentiation noticed by Aurelle et al. (2003) within the Atlantic and the absence of genetic differentiation within the Mediterranean noticed in the present study can be explained by the high dispersal potential of the species during the planktonic larval phase. In fact, given the length of the larval phase, it is quite possible that certain sampling localities of the present study exchange migrants directly at each generation. For example, on the basis of previous data on water currents in the Adriatic Sea (Poulain 2001) and on larval phase duration (Gordoa et al. 2000; Raventòs and Macpherson 2001), and assuming conservatively a water velocity of $5 \mathrm{~cm} \mathrm{~s}^{-1}$ and a larval phase duration of 21 days, larvae could travel as far as 864 Km , which is considerably more than the approximate distance between the Split and Lecce (Porto Cesareo) samples (about 540 Km ). While this computation constitutes an oversimplification that does not take into account larval retention, pelagic egg phase, larval mobility and presence of unsampled localities, it still points out that there is a high potential of within-basin migrant exchange of larvae. The results of the present study disagree with the supposition by Aurelle et al. (2003) that genetic differentiation in the Mediterranean Sea might have been more pronounced than within the Atlantic Ocean, while still confirming the authors' finding of an Atlantic-Mediterranean differentiation. However, despite a larger sample size in our study compared to Aurelle et al (2003), the confirmation of the unexpected absence of a genetic structure among Mediterranean samples through the present study might still be limited by the sometimes lower resolution of population structure found in our marker type used (e.g. Shaw et al 1999). Nevertheless, many studies indicate the opposite; with a more pronounced population structure being detected in mitochondrial DNA opposed to microsatellites (Hoarau et al. 2003, see also Hefti-Gautschi et al. 2009 and references therein). Discrepancies between mitochondrial and nuclear markers have also been found to arise as a
consequence of a number of factors such as different mutation rates or effective population sizes and, while usually regarded as neutral, they can both behave as non-neutral (Ballard and Whitlock 2004; Nielsen et al. 2006; Larsson et al. 2007; Zink and Barrowclough 2008; Galtier et al. 2009).

Furthermore, the present study suggests that the species might have undergone a recent population expansion within the Mediterranean, and not in the Atlantic. Accordingly, while the lack of a recent population expansion in the Atlantic should be confirmed by studies on mitochondrial control region employing a better sampling of Atlantic locations, this study supports the hypothesis that a population expansion has taken place after a contraction phase when parts of the Mediterranean area constituted warmer refugia isolated from the Atlantic. This would oppose the hypothesis proposed by Aurelle et al. (2003) that about 1-2 million years ago, C. julis colonized the temperate north-eastern Atlantic from the Mediterranean Sea via the Strait of Gibraltar.

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## Figure legends

Fig 1 Map of sampling sites. Abbreviations used are as in Tab I.

Fig 2 Plot of the first two dimensions obtained from nonparametric multidimensional scaling of pairwise $\mathrm{F}_{\mathrm{st}}$ among both Mediterranean and Atlantic sampling sites.

Fig 3 Plot of the first two dimensions obtained from nonparametric multidimensional scaling of pairwise $\mathrm{F}_{\mathrm{st}}$ among Mediterranean sampling sites (excluding the sample from Morocco).

Fig 4 Neighbor-net network depicting the relationships among haplotypes. The "Atlantic" (dashed line) and "Mediterranean" sub-networks have been disconnected due to the excessive length of the branch between them. The "Atlantic" sub-network comprises only haplotypes from the Atlantic Ocean and Morocco, the "Mediterranean" sub-network only haplotypes from Mediterranean.

Fig 5 Plot of pairwise genetic distances versus pairwise geographic (shortest waterway, in kilometers) distances. The dashed ellipses highlight comparisons involving Atlantic (A) and Moroccan (B) samples, their intersection (C) the comparisons between the Moroccan and the two Atlantic samples.

Fig 6 Barriers to gene flow suggested by the Monmier algorithm. The left side of the map corresponds to the eastern side of geographic space. The barrier labelled with "a", which separates the two most eastern sampling sites (Atlantic sites) from the others, was found when computing both one and two barriers.

576 Fig 7 Landscape shape interpolation of genetic distances in geographic space. The position of 577 sampling locations in the "base" of the graph are approximate.

Table 1: Sampling locations and years.

| Sampling site | Abbreviation | n | Coordinates | Year of sampling |
| :---: | :---: | :---: | :---: | :---: |
| Shahaf Island, Israel | IS | 2 | $33^{\circ} 04^{\prime} \mathrm{N} 35^{\circ} 05^{\prime} \mathrm{E}$ | 2004 |
| Gavdos, Greece | GA | 14 | $34^{\circ} 49^{\prime} \mathrm{N} 24^{\circ} 06^{\prime} \mathrm{E}$ | 2001 |
| Chania, Crete Island, Greece | CR | 2 | $35^{\circ} 30^{\prime} \mathrm{N} 23^{\circ} 59^{\prime} \mathrm{E}$ | 2001 |
| Porto Cesareo-Lecce, Italy | LE | 24 | $40^{\circ} 14^{\prime} \mathrm{N} 17^{\circ} 52^{\prime} \mathrm{E}$ | 2007 and 2009 |
| Split, Croatia | SP | 26 | $43^{\circ} 28^{\prime} \mathrm{N} 16^{\circ} 24^{\prime} \mathrm{E}$ | 2007 |
| Augusta, Sicily, Italy | AU | 23 | $37^{\circ} 11^{\prime} \mathrm{N} 15^{\circ} 14^{\prime} \mathrm{E}$ | 2007 |
| Riposto, Sicily, Italy | RI | 24 | $37^{\circ} 43^{\prime} \mathrm{N} 15^{\circ} 13^{\prime} \mathrm{E}$ | 2007 |
| Valun, Cres Island, Croatia | CI | 12 | $44^{\circ} 54^{\prime} \mathrm{N} 14^{\circ} 21^{\prime} \mathrm{E}$ | 1999 |
| Naples, Italy | NA | 25 | $40^{\circ} 46^{\prime} \mathrm{N} 14^{\circ} 12{ }^{\prime} \mathrm{E}$ | 2007 |
| Rovinj, Croatia | RO | 6 | $45^{\circ} 04^{\prime}$ N $13^{\circ} 37^{\prime} \mathrm{E}$ | 1999 |
| Mazara del Vallo, Sicily, Italy | MA | 25 | $37^{\circ} 38^{\prime} \mathrm{N} 12^{\circ} 35^{\prime} \mathrm{E}$ | 2007 |
| Pantelleria, Italy | PN | 12 | $36^{\circ} 50{ }^{\prime} \mathrm{N} 11^{\circ} 59^{\prime} \mathrm{E}$ | 2008 |
| Calvi, Corsica, France | CA | 22 | $42^{\circ} 34^{\prime} \mathrm{N} 08^{\circ} 43^{\prime} \mathrm{E}$ | 1996 and 1998 |
| Oristano, Sardinia, Italy | OR | 23 | $39^{\circ} 48^{\prime} \mathrm{N} 8^{\circ} 31^{\prime} \mathrm{E}$ | 2007 |
| Tossa de Mar, Spain | TM | 8 | $41^{\circ} 43^{\prime} \mathrm{N} 02^{\circ} 56^{\prime} \mathrm{E}$ | 1999 |
| Mallorca, Spain | ML | 33 | $39^{\circ} 31{ }^{\prime} \mathrm{N} 2^{\circ} 39^{\prime} \mathrm{E}$ | 2000 and 2007 |
| Cantabrian Sea, Spain | CS | 4 | $43^{\circ} 28^{\prime} \mathrm{N} 03^{\circ} 41^{\prime} \mathrm{W}$ | 1999 |
| Al Hoceima, Morocco | MO | 15 | $35^{\circ} 14^{\prime} \mathrm{N} 03^{\circ} 59^{\prime} \mathrm{W}$ | 2001 |
| Portugal | PT | 9 | $37^{\circ} 04^{\prime} \mathrm{N} 08^{\circ} 17^{\prime} \mathrm{W}$ | 1999 |

Table 2: AMOVA grouping schemes and results. In the grouping scheme, letters represent a certain schemes, roman numbers represent the group to which sequences of a certain sampling site were assigned. The schemes are as follows: $\mathrm{A}=$ Mediterranean (I) vs Atlantic (II), $\mathrm{B}=$ Atlantic + Morocco (II) vs Mediterranean (I), $\mathrm{C}=$ Eastern Mediterranean (III) vs Central Mediterranean (I) vs Western Mediterranean (II), $\mathrm{D}=$ Eastern Mediterranean (II) vs the remaining Mediterranean samples except Morocco (I)

| Sampling site | Grouping schemes <br> Abbreviation | A | B | C | D |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Shahaf Island, Israel | IS | I | I | III | II |
| Gavdos, Greece | GA | I | I | III | II |
| Chania, Crete Island, Greece | CR | I | I | III | II |
| Porto Cesareo-Lecce, Italy | LE | I | I | I | I |
| Split, Croatia | SP | I | I | I | I |
| Augusta, Sicily, Italy | AU | I | I | I | I |
| Riposto, Sicily, Italy | RI | I | I | I | I |
| Valun, Cres Island, Croatia | CI | I | I | I | I |
| Naples, Italy | NA | I | I | II | I |
| Rovinj, Croatia | RO | I | I | I | I |
| Mazara del Vallo, Sicily, Italy | MA | I | I | II | I |
| Pantelleria, Italy | PN | I | I | II | I |
| Calvi, Corsica, France | CA | I | I | II | I |
| Oristano, Sardinia, Italy | OR | I | I | II | I |
| Tossa de Mar, Spain | TM | I | I | II | I |
| Mallorca, Spain | ML | I | I | II | I |
| Cantabrian Sea, Spain | CS | II | II | - | - |
| Al Hoceima, Morocco | MO | I | II | II | - |
| Portugal | II | II | - | - |  |


| Grouping scheme | Results |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Fixation indices |  |  | Percent variance explained |  |  | Significance levels |  |  |
|  | Fsc | Fst | Fct | Among groups | Among populations within groups | Within populations | Fsc | Fst | Fct |
| A | 0.114 | 0.955 | 0.949 | 94.9 | 0.58 | 4.52 | <0.001 | <0.001 | <0.01 |
| B | 0.364 | 0.893 | 0.832 | 83.16 | 6.14 | 10.7 | <0.001 | <0.001 | <0.01 |
| C | 0.125 | 0.105 | -0.02 | -2.34 | 12.85 | 89.49 | <0.001 | <0.01 | >0.05 |
| D | 0.007 | 0.014 | 0.007 | 0.77 | 0.68 | 98.55 | >0.05 | >0.05 | <0.05 |

Pairwise Comparison
Table 3: Pairwise comparisons among sampling sites. Below diagonal Fst values, above diagonal p-values.

|  | Gavdos | Lecce | Split | Augusta | Riposto | Cres Island | Naples | Rovinj | Mazara del Vallo | Pantelleria | Calvi | Oristano | $\begin{gathered} \text { Tossa } \\ \text { de Mar } \end{gathered}$ | Mallorca | Cantabrian Sea | Morocco | Portugal |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Gavdos | - | 0,424 | 0,071 | 0,07 | 0,216 | 0,525 | 0,1 | 0,762 | 0,347 | 0,286 | 0,135 | 0,496 | 0,305 | 0,173 | <0.001 | 0,092 | <0.001 |
| Lecce | 0,000 | - | 0,11 | 0,644 | 0,71 | 0,612 | 0,431 | 0,114 | 0,613 | 0,349 | 0,491 | 0,592 | 0,792 | 0,105 | <0.001 | 0,016 | <0.001 |
| Split | 0,028 | 0,011 | - | 0,041 | 0,427 | 0,376 | 0,052 | 0,462 | 0,186 | 0,286 | 0,238 | 0,428 | 0,513 | 0,134 | $<0.001$ | 0,015 | <0.001 |
| Augusta | 0,029 | -0,005 | 0,018 | - | 0,403 | 0,247 | 0,204 | 0,144 | 0,215 | 0,023 | 0,722 | 0,578 | 0,543 | 0,358 | <0.001 | 0,007 | <0.001 |
| Riposto | 0,013 | -0,005 | 0,001 | 0,001 | - | 0,609 | 0,726 | 0,197 | 0,842 | 0,568 | 0,792 | 0,958 | 0,674 | 0,866 | <0.001 | 0,009 | <0.001 |
| Cres Island | -0,008 | -0,007 | 0,003 | 0,011 | -0,008 | - | 0,594 | 0,326 | 0,408 | 0,629 | 0,637 | 0,611 | 0,481 | 0,434 | $<0.001$ | 0,117 | <0.001 |
| Naples | 0,029 | 0,001 | 0,021 | 0,012 | -0,008 | -0,009 | - | 0,077 | 0,829 | 0,247 | 0,634 | 0,807 | 0,487 | 0,195 | <0.001 | 0,009 | <0.001 |
| Rovinj | -0,053 | 0,039 | 0,001 | 0,059 | 0,028 | 0,011 | 0,074 | - | 0,145 | 0,591 | 0,134 | 0,359 | 0,074 | 0,298 | 0,003 | 0,272 | <0.001 |
| Mazara del Vallo | 0,004 | -0,005 | 0,010 | 0,009 | -0,012 | 0,002 | -0,013 | 0,065 | - | 0,09 | 0,617 | 0,887 | 0,419 | 0,394 | <0.001 | 0,008 | <0.001 |
| Pantelleria | 0,012 | 0,004 | 0,011 | 0,042 | -0,006 | -0,013 | 0,009 | -0,021 | 0,030 | - | 0,054 | 0,473 | 0,324 | 0,202 | $<0.001$ | 0,111 | <0.001 |
| Calvi | 0,024 | 0,000 | 0,010 | -0,012 | -0,011 | -0,012 | -0,010 | 0,079 | -0,008 | 0,035 | - | 0,812 | 0,346 | 0,374 | <0.001 | 0,014 | <0.001 |
| Oristano | -0,001 | -0,004 | 0,002 | -0,008 | -0,018 | -0,008 | -0,013 | 0,007 | -0,017 | -0,003 | -0,014 | - | 0,71 | 0,599 | <0.001 | 0,014 | <0.001 |
| Tossa de Mar | 0,019 | -0,015 | -0,007 | -0,014 | -0,009 | -0,001 | -0,004 | 0,076 | -0,001 | 0,010 | -0,001 | -0,020 | - | 0,368 | 0,001 | 0,183 | $<0.001$ |
| Mallorca | 0,017 | 0,013 | 0,012 | 0,003 | -0,014 | 0,001 | 0,007 | 0,015 | 0,002 | 0,018 | 0,002 | -0,005 | 0,003 | - | <0.001 | 0,003 | <0.001 |
| Cantabrian Sea | 0,971 | 0,973 | 0,977 | 0,977 | 0,975 | 0,972 | 0,976 | 0,979 | 0,982 | 0,962 | 0,983 | 0,971 | 0,985 | 0,972 | - | 0,005 | 0,523 |
| Morocco | 0,173 | 0,247 | 0,263 | 0,251 | 0,249 | 0,163 | 0,261 | 0,084 | 0,267 | 0,160 | 0,250 | 0,239 | 0,131 | 0,291 | 0,577 | - | <0.001 |
| Portugal | 0,978 | 0,977 | 0,980 | 0,981 | 0,979 | 0,979 | 0,980 | 0,987 | 0,985 | 0,971 | 0,986 | 0,975 | 0,990 | 0,976 | 0,067 | 0,655 | - |

Pagina 1

Table 4: Test values and significance levels of the neutrality tests performed.

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| Group of samples | Statistic | p-value |
| :---: | :---: | :---: |
| Mediterranean | 0.0176 | $<0.001$ |
| Mediterranean minus Morocco | 0.012 | $<0.001$ |
| Atlantic | 0.1804 | 0.506 |
| All sequences | 0.0409 | 0.04 |

Table 5: Relative frequencies of each haplotype within each population and in the complete dataset. Shared haplotypes in boldface.

| Haplotype | Israel | Gavdos | Crete | Lecce | Split | Augusta | Riposto | Cres Island | Naples | Rovinj | Mazara del Vallo | Pantelleria | Calvi | Oristano | $\begin{gathered} \text { Tossa } \\ \text { de } \\ \text { Mar } \\ \hline \end{gathered}$ | Mallorca | Cantabrian Sea | Morocco | Portugal | Total |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Hap1 | - | - | - | - | - | 0.044 | 0.042 | - | - | - | - | - | - | - | - | 0.091 | - | - | - | 0.016 |
| Hap2 | - | 0.500 | 0.500 | 0.375 | 0.538 | 0.609 | 0.542 | 0.333 | 0.600 | 0.500 | 0.720 | 0.500 | 0.773 | 0.522 | 0.625 | 0.545 | - | 0.600 | - | 0.534 |
| Hap3 | - | - |  | - | - | 0.044 | - | - | - |  | - | - |  | - | - |  | - | - | - | 0.003 |
| Hap4 | - | - | - | - | - | 0.044 | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.003 |
| Hap5 | - | - | - | - | - | 0.044 | - | - | 0.040 | - | - | - | 0.046 | 0.044 | - | - | - | - | - | 0.013 |
| Hap6 | - | - | - | 0.042 | - | 0.044 | - | - | - | - | - | - | - | 0.044 | - | - | - | - | - | 0.010 |
| Hap7 | - | - | - | - | - | 0.044 | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.003 |
| Hap8 | - | - | - | - | - | 0.044 | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.003 |
| Hap9 | - | - | - | - | - | 0.044 | - | - | - | - | - | - | 0.046 | - | - | - | - | - | - | 0.006 |
| Hap10 | - | - | - | 0.042 | - | 0.044 | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.006 |
| Hap11 | 0.500 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.003 |
| Hap12 | 0.500 | - | - | - | 0.039 | - | - | - | - | - | - | - | - | - | 0.125 | - | - | - | - | 0.010 |
| Hap13 | - | 0.071 | - | 0.083 | 0.039 | - | 0.042 | 0.083 | 0.120 | - | 0.040 | - | - | 0.044 | - | 0.030 | - | - | - | 0.039 |
| Hap14 | - | - | - | 0.042 | 0.039 | - | 0.042 | 0.083 | - | - | - | - | - | - | - |  | - | - | - | 0.013 |
| Hap15 | - | - | - | 0.083 | - | - | - |  | - | - | - | - | - | - | - | - | - | - | - | 0.006 |
| Hap16 | - | - | - | 0.042 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.003 |
| Hap17 | - | - | - | 0.042 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.003 |
| Hap18 | - | - | - | 0.042 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.003 |
| Hap19 | - | - | - | 0.042 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.003 |
| Hap20 | - | - | - | 0.042 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.003 |
| Hap21 | - | - | - | 0.042 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.003 |
| Hap22 | - | 0.071 | - | 0.042 | - | - | - | - | - | - | 0.040 | - | - | - | - | - | - | - | - | 0.010 |
| Hap23 | - | - | - | 0.042 | 0.039 | - | - | - | - | - | - | - |  | - | - | 0.030 | - | - | - | 0.010 |
| Hap24 | - | - | - | - | 0.039 | - | 0.042 | - | - | 0.333 | - | 0.083 | - | - | - | 0.061 | - | 0.067 | - | 0.026 |
| Hap25 | - | - | - | - | - | - | 0.042 | - | - | - | - | - | - | - | - | 0.030 | - | - | - | 0.006 |
| Hap26 | - | - |  | - | - | - | - | - | - | - | - | - | - | - | - | 0.030 | - |  | - | 0.003 |
| Hap27 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.030 | - | - | - | 0.003 |
| Hap28 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.030 | - | - | - | 0.003 |
| Hap29 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.030 | - | - | - | 0.003 |
| Hap30 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.030 | - | - | - | 0.003 |
| Hap31 | - | - | - | - | - | - | - | - | - | - | - | 0.083 | - | - | - | 0.030 | - | - | - | 0.006 |
| Hap32 | - | - | - | - | - | - | - | - | - | - | - | - | 0.046 | - | - | 0.030 | - | - | - | 0.006 |
| Hap33 | - | - | - | - | . | - | - | - | - | - | 0.040 | - | - | - | - | - | - | - | - | 0.003 |
| Hap34 | - | - | - | - | 0.039 | - | - | 0.083 | 0.040 | - | 0.040 | - | - | 0.044 | - | - | - | - | - | 0.016 |
| Hap35 | - | - | - | - | - | - | - | - | - | - | 0.040 | - | - | - | - | - | - | - | - | 0.003 |
| Hap36 | - | - | - | - | - | - | - | - | - | - | 0.040 | - | - | - | - | - | - | - | - | 0.003 |
| Hap37 | - | - | - | - | - | - | - | - | \% | - | 0.040 | - | - | - | - | - | - | - | - | 0.003 |
| Hap38 | - | - | - | - | - | - | - | . | 0.040 |  | , | - | - | - | - | - | - | - | - | 0.003 |
| Hap39 | - | - | - | - | - | - | - | 0.083 | 0.040 | - | - | - | - | - | - | - | - | - | - | 0.006 |
| Hap40 | - | - | - | - | - | - | - |  | 0.040 |  | - | - | - | - | - | - | - | - | - | 0.003 |
| Hap41 | - | - | - | - | - | - | - | - | 0.040 | - | - | - | - | - | - | - | - | - | - | 0.003 |
| Hap42 | - | - |  | - | - | - |  | 16 | 0.040 |  | - | - | - | . | - | - | - | . 06 | - | 0.003 |
| Hap43 | - | - | - | - | - | - | - | 0.167 | - | - | - | - | - | 0.044 | - | - | - | 0.067 | - | 0.013 |
| Hap44 |  | - |  | - | - | - | - | - | - | - | - | - | - | 0.044 | - | - | - | - | - | 0.003 |
| Hap45 |  | - |  | - | - |  | - | - | - | - | - | - | - | 0.044 | - | - | - | - | - | 0.003 |
| Hap46 |  |  |  | - | - | - | - | - |  | - | - | - | - | 0.044 | - | - | - | - | - | 0.003 |
| Hap47 |  | - |  | - | - | - | - | - | - | - | - | - | - | 0.044 | - | - | - | - | - | 0.003 |
| Hap48 | - |  |  | - | - |  | - | - | - | - |  | - | - | 0.044 | - | - | - | - | - | 0.003 |
| Hap49 |  | - | - | - | - | - | - | - | - | - | - | - | - | 0.044 | - | - | - | - | - | 0.003 |
| Hap50 | - | - | - | - |  |  | - | - | - | - | - | 0.083 | - | - | - | - | - | - |  | 0.003 |
| Hap51 | - | - | - | - | - | - | - | - | - | - | - | 0.083 | - | - | - | - | - | - | - | 0.003 |
| Hap52 | - | - | - | - | - | - | - | - | - | - | - | 0.083 | - | - | - | - | - | - | - | 0.003 |


| Hap53 | - | - | - | - | - | - | - | - | - | - | - | 0.083 | - | - | - | - | - | - | - | 0.003 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Hap54 | - | - | - | - | - | - | 0.042 | - | - | - | - | - | - | - | - | - | - | - | - | 0.003 |
| Hap55 | - | - | - | - | - | - | 0.042 | - | - | - | - | - | - | - | - | - | - | - | - | 0.003 |
| Hap56 | - | - | - | - | - | - | 0.042 | - | - | - | - | - | - | - | - | - | - | - | - | 0.003 |
| Hap57 | - | - | - | - | - | - | 0.042 | - | - | - | - | - | - | - | - | - | - | - | - | 0.003 |
| Hap58 | - | - | - | - | - | - | 0.042 | - | - | - | - | - | - | - | - | - | - | - | - | 0.003 |
| Hap59 | - | - | - | - | - | - | 0.042 | - | - | - | - | - | - | - | - | - | - | - | - | 0.003 |
| Hap60 | - | - | - | - | 0.039 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.003 |
| Hap61 | - | - | - | - | 0.039 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.003 |
| Hap62 | - | - | - | - | 0.039 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.003 |
| Hap63 | - | - | - | - | 0.039 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.003 |
| Hap64 | - | - | - | - | 0.039 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.003 |
| Hap65 | - | - | - | - | 0.039 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.003 |
| Hap66 | - | - | - | - | - | - | - | - | - | - | - | - | 0.046 | - | - | - | - | - | - | 0.003 |
| Hap67 | - | - | - | - | - | - | - | - | - | - | - | - | 0.046 | - | - | - | - | - | - | 0.003 |
| Hap68 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.750 | 0.200 | 0.889 | 0.045 |
| Hap69 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.250 | - | - | 0.003 |
| Hap70 | - | - | - | - | - | - | - | 0.083 | - | - | - | - | - | - | - | - | - | - | - | 0.003 |
| Hap71 | - | - | - | - | - | - | - | 0.083 | - | - | - | - | - | - | - | - | - | - | - | 0.003 |
| Hap72 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.111 | 0.003 |
| Hap73 | - | 0.143 | - | - | - | - | - | - | - | 0.167 | - | - | - | - | - | - | - | - | - | 0.010 |
| Hap74 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.125 | - | - | - | - | 0.003 |
| Hap75 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.125 | - | - | - | - | 0.003 |
| Hap76 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.067 | - | 0.003 |
| Hap77 | - | 0.071 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.003 |
| Hap78 | - | 0.071 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.003 |
| Hap79 | - | 0.071 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.003 |
| Hap80 | - | - | 0.500 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.003 |

Fig 1


## Fig 2




Dimension I

## Fig 3

| 1.3 | ${ }_{0}^{\text {Rovinj }}$ |  |
| :---: | :---: | :---: |
|  |  |  |
|  |  |  |
| $-0.7 \underset{-0.7}{\square}$ | $0.3$ <br> Dimension I | 1.3 |

Fig 4


Fig 5


Fig 6


Fig 7


