## **1** Atlantic-Mediterranean and within-Mediterranean molecular variation

## in *Coris julis* (L. 1758) (Teleostei, Labridae)

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17 Abstract Sequence variation of the mitochondrial control region was studied in the Mediterranean rainbow wrasse

18 (Coris julis), a species with pronounced pelagic larval phase inhabiting the Mediterranean Sea and the adjacent

19 coastal eastern Atlantic Ocean. A total of 309 specimens from 19 sampling sites was analysed with the aim of

- 20 elucidating patterns of molecular variation between the Atlantic and the Mediterranean as well as within the
- 21 Mediteranean Sea. Phylogeographic analyses revealed a pronounced structuring into a Mediterranean and an
- 22 Atlantic group. Samples from a site at the Moroccan Mediterranean coast in the Alboran Sea showed intermediate
- 23 frequencies of "Mediterranean" and "Atlantic" haplotypes. We recognized a departure from molecular neutrality
- 24 and a star-like genealogy for samples from the Mediterranean Sea, which we propose to have happened due to a
- 25 recent demographic expansion. The results are discussed in the light of previous studies on molecular variation in
- 26 fish species between the Atlantic and the Mediterranean and within the Mediterranean.

## 28 Introduction

29 Molecular variation among marine fish populations has been described in diverse patterns 30 ranging from a panmictic model to a genetic structuring with separate local populations. 31 Genetic variation in marine fish has been traditionally considered smaller than variation in 32 freshwater and anadromous species due to the presumed lack or paucity of barriers to gene 33 flow. Many early studies (Ward et al. 1994) reported results much more consistent with a 34 panmictic model. However, phenomena such as larval retention due to currents (Stepien et al. 35 2001; Palumbi 2003) or larval behaviour (Palumbi 1994) can lead, even in species with a 36 pronounced planktonic larval phase, to a significant genetic divergence of populations. For 37 example, Gerlach et al. (2007) showed that some planktonic larvae are capable of 38 distinguishing "olfactory signatures" of their natal reefs, thus opening the possibility for 39 homing behaviour and the formation of retention zones. In some cases, distinct geographical 40 lineages have been detected for species with high dispersal capacity even in complete absence 41 of apparent oceanographic barriers to gene flow (Burford 2009; Bergek and Bjorklund 2009). 42 Furthermore, species with similar ecological traits do not always show consistent 43 phylogeographic patterns: Bargelloni et al. (2003) studied five sparid species sharing similar 44 biological features and found a strong phylogeographic break between the Atlantic Ocean and 45 the Mediterranean Sea in three species, but no evidence of such a break in others. 46 This Mediterranean-Atlantic transition has been the subject of a variety of studies on both 47 vertebrates and invertebrates (reviewed by Patarnello et al. 2007). No general relationship 48 between either dispersal ability or life history traits, and patterns of partial or complete genetic 49 isolation between Atlantic and Mediterranean populations could be found (Patarnello et al. 50 2007). The authors also noticed that some of the species which exhibit Atlantic-Mediterranean 51 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 ofGibraltar (which separates the Mediterranean Sea from the Atlantic Ocean).

54 Within the Mediterranean, a pronounced genetic structuring in fishes was detected in 55 comparisons between populations from the Adriatic Sea and the remaining Mediterranean. For 56 example, a divergence of the Adriatic population from other Mediterranean populations has 57 been noticed in the sand goby (*Pomatoschistus minutus*; Gysels et al. 2004), in the red mullet 58 (Mullus barbatus; Maggio et al. 2009) and in European sprat (Sprattus sprattus; Debes et al. 59 2008). In the latter study (Debes et al. 2008), individuals from the Adriatic and the Tyrrhenian 60 Sea were highly differentiated at mitochondrial DNA which was explained as the result of 61 postglacial warming and the subsequent inability of this boreal, cold adapted species to 62 maintain gene flow at its southernmost distribution limit under present physical oceanographic 63 conditions.

64 Previous studies on *Thalassoma pavo*, a wrasse species of tropical origin, found no evidence of 65 geographic structure between the Atlantic and the Mediterranean Sea, instead, it was suggested 66 that there could be a phylogeographic break in the Aegean area between the eastern and the 67 western Mediterranean (Costagliola et al. 2004; Domingues et al. 2008). A divergence between 68 eastern and western Mediterranean populations has also been noticed both in other fish species 69 (Thunnus thynnus; Carlsson et al. 2004; Pomatoschistus marmoratus; Mejri et al. in 70 press) and other marine organisms (*Cerastoderma glaucum*; Nikula and Väinölä 2003; 71 Posidonia oceanica; Arnaud-Haond et al. 2007; Serra et al. 2010; Patella rustica; Sà-Pinto et 72 al. 2010). However, a study on two species of the genus Scomber highlighted a divergence 73 between eastern and western Mediterranean populations for one of them, but no evidence of 74 such a divergence in the other species (Zardoya et al. 2004).

75 The Mediterranean rainbow wrasse (*Coris julis*, L. 1758) is a small-sized labrid fish that is

videly spread in the Mediterranean Sea and along the adjacent European and African Atlantic

77 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).

84 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)

87 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

89 Aurelle et al. (2003) based on microsatellite markers. The study by Guillemaud and colleagues

90 (2000), although based on an extremely low sample size (only seven *C. julis* specimens),

91 showed a divergence between sequences of specimens of Atlantic and Mediterranean origin.

92 The study by Aurelle et al. (2003) revealed the Atlantic-Mediterranean transition as a

93 phylogeographic break but no further genetic differentiation on each side of the Strait of

94 Gibraltar, even between geographically distantly separated sites like continental Portugal and

95 the offshore situated Azores Islands. The authors hypothesized that genetic differentiation

96 within the Mediterranean Sea might be more pronounced than detected, as they acknowledged

97 a limited power of their study due to a low sample size and therefore considered their results on

98 the Mediterranean Sea as preliminary (Aurelle et al. 2003).

99 The present study aims at contributing to clarify the role of the Atlantic-Mediterrranean

100 transition (Strait of Gibraltar versus Almerian-Oran front) as a phylogeographic barrier by

101 testing its relevance for a small demersal wrasse species and additionally testing for a genetic

102 structuring of the rainbow wrasse within the Mediterranean Sea.

## 104 Materials and methods

A total of 309 specimens of Coris julis from 19 Mediterranean and Atlantic sampling sites (Fig 105 106 1, Table 1), sampled with fish traps, nets, fishing rods and hand lines, was analysed. Individual 107 white muscle tissue samples were collected from the right side of each fish and preserved in 108 95% ethanol. 109 Total DNA was extracted using a commercial silica-based spin column kit (GenElute 110 Mammalian Genomic DNA Miniprep Kit, Sigma Aldrich). We amplified a mitochondrial DNA 111 fragment of about 490 bp (base pairs) including part of the tRNA threonine gene, the tRNA proline gene and the 5' portion of the control region via PCR. For the PCR we used modified 112 113 universal primers THR2m1 (5'-AGAGCGCCGGTCTTGTAAAC-3') and TDKDm2 (5'-114 CTGAAGTAGGAACCAAATGCCAGGAA-3') derived, respectively, from L15926 (Kocher 115 et al 1989) and TDKD (Slade et al 1994). PCR amplification reactions were obtained for a total 116 volume of 50 µl in1X buffer, 1 mM MgCl<sub>2</sub>, 0.2 mM of each dNTP, 0.2 µM of each primer, 117 1.25 units of Taq DNA polymerase (Invitrogen) and 2 µl of extracted DNA. The PCR cycling 118 profile comprised an initial denaturation step of 4 minutes at 95°C, 30 cycles of denaturation 119 (94°C for 30 seconds), annealing (56°C for 1 minute) and extension (72°C for 1 minute), and a final extension step of 72°C for 10 minutes; the ramp was 2°C/s for all the steps. Obtained 120 121 amplified fragments were sequenced using an external sequencing service (Secugen S.L., 122 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 123 124 was deposited in GenBank (accession numbers HQ917534-HQ917613). 125 The best nucleotide substitution model was estimated with ModelTest (Posada 2008) using AIC 126 (An Information Criterion; Akaike 1974) as information criterion. The selected model, TrN+I 127 (Tamura and Nei 1993) was then used to correct genetic distances whenever possible.

#### 128 Analysis of genetic structure

129 To investigate the population genetic structure in rainbow wrasse, Analysis of Molecular

- 130 Variance (AMOVA, Excoffier et al. 1992) and computation of F<sub>st</sub> pairwise values between
- 131 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
- 133 AMOVA was performed on the Tamura and Nei (1993) distance matrix using various
- 134 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
- 136 permutations). The R script provided by Fitzpatrick (2009) was used to ensure that it was
- 137 possible to obtain a P-level smaller than 0.05 with the selected groupings.

138 Nonmetric multidimensional scaling analysis (Kruskal 1964a,b) was also performed, using the

139 software NTSYSpc 2.2 (Rohlf 2007), on the matrix of pairwise  $F_{st}$  between sampling sites both

- 140 using all samples and using only the samples from the Mediterranean (with the exception of the
- 141 Moroccan one). Ordinations of sampling sites along the first two dimension of the space
- 142 obtained by multidimensional scaling were then plotted against each other.

#### 143 Neutrality tests

- 144 Departures from neutrality of molecular evolution were tested for different geographic
- 145 groupings by computing the value of Ramos-Onsins and Rozas R<sub>2</sub> statistic (Ramos-Onsins and
- 146 Rozas 2002) and testing its significance with the procedure based on coalescent simulations
- 147 (1000 simulated samples) with DNAsp 5.0 (Librado and Rozas 2009). The software Arlequin
- 148 was used to compute Fu's F<sub>s</sub> (Fu 1997) values and to test their deviation from neutrality
- 149 expectations by the means of the coalescent algorithm implemented in the program (10,000
- 150 simulated samples). The choice of such tests has been made in the light of previous studies on
- 151 the power of various neutrality tests (Fu 1997; Ramos-Onsins and Rozas 2002). In fact, we
- decided to use both Ramos-Onsins and Rozas R<sub>2</sub> and Fu's F<sub>s</sub> because they have been shown to

153 be more powerful than a number of alternatives, albeit the sample size defines which of the two

154 is more powerful (Ramos-Onsins and Rozas 2002).

#### 155 Relationships among haplotypes

156 The relationships among haplotypes were described using two network methods: median-

157 joining (Bandelt et al. 1999) and neighbor-net (Bryant and Moulton 2004). The median-joining

158 network was constructed using the software Network (Fluxus Technology Ltd), the neighbor-

159 net network (Bryant and Moulton 2004) based on the uncorrected p-distance was constructed

160 using the software SplitsTree4 (Huson and Bryant 2006). Additionally, we obtained a bayesian

161 phylogenetic tree with the software MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) under a

162 model that corresponds to the GTR model of substitution with a proportion of invariable sites.

163 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
- 165 tree after discarding the first 25% of generations.

#### 166 **Spatial patterns**

167 The software NTSYSpc 2.2 (Rohlf 2007) was used to obtain plots of pairwise genetic distances

168 (TrN-corrected distances among group means) versus pairwise geographic distances, to

169 calculate correlations between matrices measuring genetic differences and matrices measuring

170 geographical distances, and to test their significance by the means of a Mantel test (Mantel

171 1967) with 1000 permutations. Geographical distances were computed both as "direct"

172 distances and as shortest waterway distances (obtained using the software Google Earth) among

- 173 sampling locations. Both geographical distances in kilometres and their logarithmic
- 174 transformations were used. Plots of pairwise genetic versus pairwise geographic distances were
- 175 chosen because, in case of significant correlation, they can give a hint on the underlying

176 process (Guillot et al. 2009).

177 Spatial autocorrelation analysis, as implemented in the software Alleles in Space (Miller 2005) 178 was also performed using 12 distance classes. To test if the method of choice of distance 179 classes (equal distances and unequal sample sizes or unequal distances and equal sample sizes) 180 or their number had an effect on the analysis, the analysis was repeated using both methods 181 and 5, 8, 10, 12 distance classes. 182 The Israeli and Cretan samples were excluded from the computation of correlations and from 183 the spatial autocorrelation analysis due to their low sample size. 184 The software Barrier (Manni et al. 2004), which computes a Delaunay triangulation on a 185 Voronoi tessellation and then uses Monmonier's (1973) maximum difference algorithm in 186 order to identify barriers, was used to identify the position of possible genetic barriers among 187 sampling sites based on the TrN-corrected distances among sites. Two runs of the software 188 were performed; the first one computing only one barrier, the second computing two barriers. 189 Finally, a landscape shape interpolation of genetic distances, which interpolates observed 190 genetic distances across the landscape and shows them graphically as heights (Z axis) in a 191 graph where the base (values along X and Y axes) represents the geographic space, was 192 obtained using the software Alleles in Space (Miller 2005). 193

#### 194 **Results**

#### 195 Analysis of genetic structure

196 By using grouping schemes A (Atlantic versus Mediterranean) and B (Atlantic samples +

197 Morocco versus the remaining Mediterranean samples) most of the molecular variation was

198 explained by the among-groups variance component, which is statistically significant (Table 2).

199 In particular, using scheme A, 94.9% of molecular variance was explained by the among-

200 groups term, while using scheme B this term explained 83.16% of the observed variation. On

201 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.

205 The pairwise comparison between sampling sites (Table 3) shows that the Portuguese sample 206 was significantly different from all other samples except the one from the Cantabrian Sea 207 which, in turn, showed a similar pattern. Also, the Moroccan sample is significantly different 208 from many other samples. Nonmetric multidimensional scaling required 10 dimensions to get 209 an almost perfect (sensu Kruskal 1964a) stress value (0.00026) when analysing only 210 Mediterranean sites (with the exception of Morocco), while 2 dimensions were sufficient to 211 achieve zero stress when using also Moroccan and Atlantic samples. Looking at the patterns in 212 the first two dimensions obtained by nonmetric multidimensional scaling analyses, in the 213 analyses with both Mediterranean and Atlantic samples (Fig 2) most of sampling sites are so 214 close together in the two-dimensional space obtained by the analysis that only three points are 215 distinguishable in the graph: one point for all the Mediterranean samples (except the Moroccan 216 one), a second point for the Moroccan sample and a third point for all the Atlantic samples. On 217 the opposite, the analysis performed only on Mediterranean samples (excluding the Moroccan 218 one, Fig 3) show in the first two dimensions a pattern in which geographic proximity does not 219 necessarily reflect proximity in the multivariate space.

#### 220 Neutrality tests

- 221 Results of the analyses of Ramos-Onsins and Rozas R<sub>2</sub> in geographic groupings of samples are
- 222 provided in Table 4. Mediterranean samples showed a significant departure from neutrality.
- 223 Analyses using Fu's F<sub>s</sub> gave the same results (not shown).

#### 224 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 wereprivate).

230 The neighbour-net network is depicted in Fig 4 (the median-joining network and the Bayesian 231 tree provided similar results and both are not shown). It can be noticed that the "Atlantic" sub-232 network (the smallest of the two sub-networks) comprised haplotypes 68, 69, 72 and 76. Of 233 them, haplotype 68 was shared among the Portuguese, Moroccan and Cantabrian Sea samples, 234 the other three haplotypes were private of Atlantic (69 and 72) or Moroccan (76) samples. 235 Moreover, all haplotypes found in the two Atlantic samples (68, 69 and 72) grouped 236 exclusively into the "Atlantic" sub-network. Haplotypes found in the Moroccan sample 237 grouped both in the "Atlantic" sub-network and in the "Mediterranean" sub-network which, in 238 turn, comprised only Mediterranean haplotypes and showed a "star-like" topology. 239 Spatial patterns 240 The correlation between "direct" geographic distances and genetic distances was R=0.6 using 241 raw distances, R=0.5 using log-transformed distances and significant in both cases 242 (respectively p=0.004 and p=0.003). The correlations between shortest waterway distances and 243 genetic distances were R=0.7 using raw geographic distances and R=0.57 using their 244 logarithmic transformations, and higher than expected by chance alone (P<0.001 and P=0.002, 245 respectively). A plot of pairwise genetic distances versus pairwise geographic distances 246 (shortest waterway, raw) is shown in Fig 5. 247 The spatial autocorrelation analysis using 12 distance classes (unequal distances, equal sample 248 size) was overall significant (P<0.001). When analysing each distance class, all the classes 249 from the 990-1126 Km class to the largest class showed a significant upper tail (within-class 250 genetic distances higher than expected by chance). Using a different method of choice or a 251 different number of distance classes did not appreciably affect the outcome of the analysis. 252 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.

257 Looking at the interpolation plot (Fig 7), it can be noticed that the genetic diversity appears 258 higher in the Western portion (Atlantic Ocean and Western Mediterranean) of the sampled area, 259 and lower in the Eastern portion (Central-Eastern Mediterranean). The exact values (data not 260 shown) along the X and Y axes for the highest peaks on the Z axis of the plot show that the 261 highest genetic divergence is found along a line that separates the two Atlantic samples from all 262 the others..Not surprisingly, this line corresponds mostly to the Iberian Peninsula, but also to 263 the Strait of Gibraltar. Other high (but less pronounced) peaks were located along the 264 separation between the Moroccan sample and the rest of the Mediterranean, corresponding to 265 the Almeria-Oran front.

#### 266 **Discussion**

267 This study highlights the patterns of molecular differentiation in *Coris julis* in the

268 Mediterranean Sea and the Atlantic Ocean.

A single haplotype (haplotype 2) was shared among all the Mediterranean sampling sites

270 (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

273 of the analyses of population differentiation, on the whole, can be interpreted as an absence of

274 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

276 Atlantic Ocean, with the Moroccan sample being intermediate.

277 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

279 Mediterranean samples do. However, results of neutrality tests on the Atlantic and Moroccan 280 samples should be considered cautiously as the low sample size of those samples might have 281 lead, even with powerful tests as the ones used, to a lack of statistical power. As causes of 282 departure from neutrality for the Mediterranean Sea sample we cannot dismiss background 283 selection and genetic hitchhiking. However, an event of recent demographic and range 284 expansion represents a likely hypothesis. Such a hypothesis would also be supported by Ray et 285 al.'s (2003) suggestion that, in the presence of high exchange of migrants among neighbouring 286 demes, a large spatial expansion can lead to signatures similar to those arising from a pure 287 demographic expansion. Given that C. julis is regarded as thermophilic and it has been known 288 to expand his geographical range in response to water warming during very recent historical 289 times (Piron et al. 2007; Lipej et al. 2009), it is conceivable that this species could have also 290 been subject to changes in its range as a consequence of strong changes in climate in more 291 distant times. In particular, it can be hypothesised that due to the drop in water temperatures 292 during the late Pleistocene Mediterranean populations were isolated at thermally favoured 293 locations. A subsequent rise in temperature then caused a demographic and spatial expansion in 294 the Mediterranean leading to the observed deviation from neutrality. Interestingly enough, 295 Patarnello et al. (2007) found signatures of a population expansion in Mediterranean in 5 out of 296 the 7 fish species for which they carried out separate analyses for Atlantic and Mediterranean 297 samples; 4 out of the 5 species that showed signs of expansion also showed a significant Fu's 298 F<sub>s</sub> statistic. The relationships among haplotypes represented in the network, as well as in the 299 Bayesian tree, showed two sub-networks separated by a long distance. One of the two sub-300 networks comprised Mediterranean haplotypes and showed a "star-like" topology, the other 301 essentially Atlantic haplotypes. Both the "star-like" genealogy and the excess of rare mutations 302 (that can be found in the "Mediterranean" sub-network) have been considered to arise as a 303 consequence of population growth (Slatkin and Hudson 1991; Harpending and Rogers 2000). A 304 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.

307 Moreover, the "intermediate" position of the Moroccan sample, with haplotypes belonging to

308 both the Atlantic and the Mediterranean sub-networks, mirrors the results of analyses of genetic

309 differentiation.

310 The results of the Mantel tests show a significant correlation between genetic and geographic

311 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

314 geographic distance (Fig 5) suggests that the significant correlation between genetic and

315 geographic distance found by the Mantel test might be the product of the particular sampling

316 scheme used in this study. In fact, the discontinuity among groups of distances observed in the

317 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

319 differentiation among Atlantic, Moroccan and Mediterranean samples.

320 Overall, the plot of genetic versus geographic distance, the computation of barriers with the

321 Monmonier algorithm and the landscape shape interpolation plot, , all confirm the results of the

322 analyses of genetic differentiation and show a separation of the Atlantic samples and to a lower

323 extent also of the Moroccan sample. The general result of an overall Atlantic-Mediterranean

324 differentiation confirms previous findings based on a very limited sampling of Mediterranean

325 sites and a different set of genetic markers (Guillemaud et al. 2000; Aurelle et al. 2003).

326 However, the concurrent presence of Atlantic and Mediterranean haplotypes in the Alboran Sea

327 is a new finding for the species. Interestingly, most of the species with Atlantic-Mediterranean

328 differentiation show steep changes of allele frequencies associated either with the Almeria-

- 329 Oran 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

331 Strait of Gibraltar and the currents within the Alboran Sea which create the Almeria-Oran 332 Front. The Alboran Sea might then be partially genetically isolated both from the Atlantic 333 Ocean and the rest of the Mediterranean Sea, with marked genetic differentiation between the 334 two basins and a limited, possibly current, gene flow between the two via the Alboran Sea. In 335 this respect, the Alboran Sea might, therefore, represent a still partially isolated zone of 336 secondary contact between two previously separate C. julis lineages. Interestingly enough, 337 Laurent and Lejeune (1988) have noticed in the Alboran Sea and in French Mediterranean 338 waters the presence of secondary rainbow wrasse individuals exhibiting the "Atlantic" 339 secondary colour pattern. In this context it is also worth noting that Lemaire et al. (2005) have 340 found intermediate frequencies of Atlantic and Mediterranean haplotypes of the sea bass 341 (Dicentrarchus labrax) in Alboran Sea samples, thus hypothesising that the Almeria-Oran front 342 had been crossed by Mediterranean migrants. The northern African coast has been widely 343 neglected in studies on phylogeography in the Mediterranean. While this study represents an 344 exception it will be important to sample more localities to confirm that Atlantic haplotypes are 345 gradually replaced by Mediterranean haplotypes as the distance from Gibraltar or the 346 Almeria/Oran fronts increases. Moreover, our results highlight the importance of samples from 347 the Alboran Sea in phylogeographic studies in fish because intermediate frequencies of 348 Mediterranean and Atlantic haplotypes in the Alboran Sea might be more common than 349 expected. 350 Within the rest of the Mediterranean, the present study reports no significant differentiation 351 among samples or areas. This is in contrast with previous findings on Thalassoma pavo, 352 another labrid species with similar biological features. Here, no differentiation between 353 Atlantic and Mediterranean samples was detected but a weak differentiation was found between 354 western and eastern Mediterranean in terms of a genetic discontinuity at the Peloponnesus

355 (Costagliola et al. 2004; Domingues et al. 2008).

356 Regarding the differentiation within the Atlantic, Aurelle et al. (2003) reported for C. julis no 357 genetic differentiation between samples from the Azores Islands and most of the Atlantic 358 continental samples, despite a distance of more than 1,800 km between the archipelago and the 359 continent. The absence of genetic differentiation noticed by Aurelle et al. (2003) within the 360 Atlantic and the absence of genetic differentiation within the Mediterranean noticed in the 361 present study can be explained by the high dispersal potential of the species during the 362 planktonic larval phase. In fact, given the length of the larval phase, it is quite possible that 363 certain sampling localities of the present study exchange migrants directly at each generation. 364 For example, on the basis of previous data on water currents in the Adriatic Sea (Poulain 2001) 365 and on larval phase duration (Gordoa et al. 2000; Raventòs and Macpherson 2001), and assuming conservatively a water velocity of 5 cm  $s^{-1}$  and a larval phase duration of 21 days, 366 367 larvae could travel as far as 864 Km, which is considerably more than the approximate distance 368 between the Split and Lecce (Porto Cesareo) samples (about 540 Km). While this computation 369 constitutes an oversimplification that does not take into account larval retention, pelagic egg 370 phase, larval mobility and presence of unsampled localities, it still points out that there is a high 371 potential of within-basin migrant exchange of larvae. The results of the present study disagree 372 with the supposition by Aurelle et al. (2003) that genetic differentiation in the Mediterranean 373 Sea might have been more pronounced than within the Atlantic Ocean, while still confirming 374 the authors' finding of an Atlantic-Mediterranean differentiation. However, despite a larger 375 sample size in our study compared to Aurelle et al (2003), the confirmation of the unexpected 376 absence of a genetic structure among Mediterranean samples through the present study might 377 still be limited by the sometimes lower resolution of population structure found in our marker 378 type used (e.g. Shaw et al 1999). Nevertheless, many studies indicate the opposite; with a more 379 pronounced population structure being detected in mitochondrial DNA opposed to 380 microsatellites (Hoarau et al. 2003, see also Hefti-Gautschi et al. 2009 and references therein). 381 Discrepancies between mitochondrial and nuclear markers have also been found to arise as a

382 consequence of a number of factors such as different mutation rates or effective population

383 sizes and, while usually regarded as neutral, they can both behave as non-neutral (Ballard and

- 384 Whitlock 2004; Nielsen et al. 2006; Larsson et al. 2007; Zink and Barrowclough 2008; Galtier
- 385 et al. 2009).
- 386 Furthermore, the present study suggests that the species might have undergone a recent
- 387 population expansion within the Mediterranean, and not in the Atlantic. Accordingly, while the
- 388 lack of a recent population expansion in the Atlantic should be confirmed by studies on
- 389 mitochondrial control region employing a better sampling of Atlantic locations, this study
- 390 supports the hypothesis that a population expansion has taken place after a contraction phase
- 391 when parts of the Mediterranean area constituted warmer *refugia* isolated from the Atlantic.
- 392 This would oppose the hypothesis proposed by Aurelle *et al.* (2003) that about 1–2 million
- 393 years ago, *C. julis* colonized the temperate north-eastern Atlantic from the Mediterranean Sea
- 394 via the Strait of Gibraltar.

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398

### 399 **References**

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## 551 Figure legends

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

553

554 Fig 2 Plot of the first two dimensions obtained from nonparametric multidimensional scaling of

555 pairwise F<sub>st</sub> among both Mediterranean and Atlantic sampling sites.

556

- 557 Fig 3 Plot of the first two dimensions obtained from nonparametric multidimensional scaling of
- 558 pairwise F<sub>st</sub> among Mediterranean sampling sites (excluding the sample from Morocco).

559

- 560 **Fig 4** Neighbor-net network depicting the relationships among haplotypes. The "Atlantic"
- 561 (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
- 563 from the Atlantic Ocean and Morocco, the "Mediterranean" sub-network only haplotypes from
- 564 Mediterranean.

565

**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.

570

571 **Fig 6** Barriers to gene flow suggested by the Monmier algorithm. The left side of the map

572 corresponds to the eastern side of geographic space. The barrier labelled with "a", which

573 separates the two most eastern sampling sites (Atlantic sites) from the others, was found when

574 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.
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Sampling site	Abbreviation	n	Coordinates	Year of sampling
Shahaf Island, Israel	IS	2	33°04'N 35°05'E	2004
Gavdos, Greece	GA	14	34°49'N 24°06'E	2001
Chania, Crete Island, Greece	CR	2	35°30'N 23°59'E	2001
Porto Cesareo-Lecce, Italy	LE	24	40°14'N 17°52'E	2007 and 2009
Split, Croatia	SP	26	43°28'N 16°24'E	2007
Augusta, Sicily, Italy	AU	23	37°11'N 15°14'E	2007
Riposto, Sicily, Italy	RI	24	37°43'N 15°13'E	2007
Valun, Cres Island, Croatia	CI	12	44°54'N 14°21'E	1999
Naples, Italy	NA	25	40°46'N 14°12'E	2007
Rovinj, Croatia	RO	6	45°04'N 13°37'E	1999
Mazara del Vallo, Sicily, Italy	MA	25	37°38'N 12°35'E	2007
Pantelleria, Italy	PN	12	36°50'N 11°59'E	2008
Calvi, Corsica, France	CA	22	42°34'N 08°43'E	1996 and 1998
Oristano, Sardinia, Italy	OR	23	39°48'N 8°31'E	2007
Tossa de Mar, Spain	ТМ	8	41°43'N 02°56'E	1999
Mallorca, Spain	ML	33	39°31'N 2°39'E	2000 and 2007
Cantabrian Sea, Spain	CS	4	43°28'N 03°41'W	1999
Al Hoceima, Morocco	MO	15	35°14'N 03°59'W	2001
Portugal	PT	9	37°04'N 08°17'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: A= Mediterranean (I) vs Atlantic (II), B=Atlantic + Morocco (II) vs Mediterranean (I), C=Eastern Mediterranean (III) vs Central Mediterranean (I) vs Western Mediterranean (II), D=Eastern Mediterranean (II) vs the remaining Mediterranean samples except Morocco (I)

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

#### Results

	Fixa	tion ind	ices	Р	ercent variance expl	Significance levels					
Grouping scheme	Fsc	Fst	Fct	Among groups	Among populations within groups	Within populations	Fsc	Fst	Fct		
А	0.114	0.955	0.949	94.9	0.58	4.52	< 0.001	< 0.001	< 0.01		
В	0.364	0.893	0.832	83.16	6.14	10.7	< 0.001	< 0.001	< 0.01		
С	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	Tossa de Mar	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	-

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

Group of samples	Ramos-Onsins & Rozas R <sub>2</sub>								
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	Tossa de Mar	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
Hap10	-	-	_	0.042	_	_	_	-	_	-	-	_	_	-	-	-	_	_	-	0.003
Han21	-	-	_	0.042	_	_	_	-	_	-	-	_	_	-	-	-	_	_	-	0.000
Han22	-	0 071	-	0.042	-	-	-	-	-	-	0 040	-	-		-	-	-	-	-	0.000
Han23	_	-	_	0.042	0 039	_	_	_	_	_	-	_	_	_	_	0 030	_	_	_	0.010
Hap23		_	-	0.042	0.033		0 0/2	_		0 333	_	0.083	_	_	_	0.050	-	0.067	-	0.010
Hap24		-	-	_	0.039	-	0.042	-	-	0.333	-	0.005	-	-		0.001	-	0.007	-	0.020
Hap26	-	-	-	-	-	-	0.042	-	-	-	-	-	-	-	-	0.030	-	-	-	0.000
Hap20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.030	-	-	-	0.003
Hap27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.030	-	-	-	0.003
Hap28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.030	-	-	-	0.003
Hap29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.030	-	-	-	0.003
Hap30	-	-	-	-	-	-	-	-	-	-	-	0.092	-	-	-	0.030	-	-	-	0.003
нарзі	-	-	-	-	-	-	-	-	-	-	-	0.083	-	-	-	0.030	-	-	-	0.006
нарза	-	-	-	-	-	-	-	-	-	-	-	-	0.046	-	-	0.030	-	-	-	0.006
Нарзз	-	-	-	-	-	-	-	-	-	-	0.040	-	-	-	-	-	-	-	-	0.003
Нар34	-	-	-	-	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	-	-	-	-	-	-	-	-	0.040	-	-	-	-	-	-	-	-	-	-	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











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