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Symbiosis, hybridization and speciation in Mediterranean octocorals (Octocorallia, Eunicellidae)

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ABSTRACT

Understanding how species can form and remain isolated in the marine environment still stimulates active researches. Here we study the differentiation and the possibility of hybridization among three temperate octocorals: *Eunicella cavolini*, *E. singularis* and *E. verrucosa*. Morphologically intermediate individuals have been observed between them. Among these three species, *E. singularis* is the only one described in mutualistic symbiosis with photosynthetic Symbiodiniaceae. The symbiosis between Symbiodiniaceae and scleractinian corals is well studied, especially in the context of the response to anthropogenic climate change. Nevertheless, the potential role of symbiotic interactions in speciation processes remains unknown in cnidaria. We tested here the possibility of hybridization between symbiotic and non-symbiotic *Eunicella* species. Through multivariate analyses and hybrid detection, we prove the existence of on-going gene flow between *E. singularis* and *E. cavolini*, with the observation of F1 and F2 hybrids, and backcrosses. Demographic inferences indicate a scenario of secondary contact between these two species. Despite current gene flow, these two species appear genetically well differentiated. Our data also suggest an intermediate abundance of Symbiodiniaceae in the hybrids of the two parental populations. We discuss the evolution of the Symbiodiniaceae / cnidarian symbiosis in the light of our results.

Keywords: speciation, hybridization, symbiosis, transcriptome, RAD sequencing, octocoral

1 **Introduction**

2

3 As corner stones of evolutionary biology, species and speciation still raise a wealth of
4 questions fuelled by the technological and conceptual advancements in genomics. Genomic
5 data allow testing hypotheses about species boundaries and origins. Named species are
6 indeed hypotheses, built on available data, that can be rejected or validated through the
7 integration of additional data and / or the use of additional criteria based on evolutionary
8 concepts (Pante *et al.*, 2015b). Sound species delimitations are useful, among others, to
9 better estimate species range and biodiversity patterns (Muir *et al.*, 2022; Coelho *et al.*,
10 2023), to avoid biases in studies of connectivity (Pante *et al.*, 2015b), and of adaptive
11 abilities (Brenner-Raffalli *et al.*, 2022). However, proposing sound species delimitation can be
12 problematic because different delimitation criteria may bring contradictory conclusions about
13 species boundaries (the Grey Zone of de Queiroz, 2007). This grey zone corresponds to
14 puzzling cases such as the absence of gene flow among morphologically undifferentiated
15 sets of organisms (i.e. cryptic species, Cahill *et al.*, 2024), or conversely, the detection of
16 gene flow among sets of organisms recognized, based on morphological distinctiveness, as
17 distinct species (Leroy *et al.*, 2020). Evolutionary inferences, based on genomic data, allow
18 testing scenarios of speciation and current gene flow: this provides a better understanding
19 on the origin and persistence of species at the light of genomic divergence (Roux *et al.*,
20 2016; De Jode *et al.*, 2023).

21 In the marine realm, the question of speciation is considered as particularly confusing.
22 Notably, how new species can originate from populations with large effective size associated
23 to high level of gene flow is still abundantly debated in the literature (e.g. Palumbi, 1992;
24 Mayr, 2001; Faria *et al.*, 2021). Difficulties in sampling and rearing organisms also hamper
25 experiments to test reproductive isolation (Faria *et al.*, 2021). Important progresses in
26 methodologies now allow to better understand spatial patterns of genetic structure in marine
27 organisms, for example through the study of oceanographic connectivity (Reynes *et al.*,
28 2021), clines in allele frequencies (Gagnaire *et al.*, 2015), and hybrid zones (Bierne *et al.*,
29 2003).

30 In this context, the role of symbiotic interactions in reproductive isolation remains poorly
31 investigated. There are various examples of the involvement of microbial species in
32 reproductive isolation, especially in insects (Brucker and Bordenstein, 2012). For marine
33 species, microbial communities have been mainly explored in light of adaptative evolution
34 (Rosenberg and Zilber-Rosenberg, 2018). Shallow water scleractinian corals (hexacorals)
35 are usually associated with various species of photosynthetic zooxanthellae, in the family
36 Symbiodiniaceae (Cairns, 2007; LaJeunesse *et al.*, 2018). Changes in associated
37 Symbiodiniaceae can impact the thermotolerance of the coral holobiont, and the possibility

38 of adaptation facing climate change (Berkelmans and van Oppen, 2006; van Oppen &
39 Medina, 2020). Inferences from the phylogeny of Anthozoans (hexacorals and octacorals)
40 have shown multiple acquisitions of the symbiotic state throughout evolution (Cairns, 2007;
41 Campoy *et al.*, 2020, Mc Fadden *et al.*, 2021). The symbiotic interactions between
42 Anthozoans and Symbiodiniaceae provide important mutualistic benefits especially from a
43 nutritional point of view (Furla *et al.*, 2005). These interactions require specific adaptations
44 for the animal host, as for example protection against oxygen produced by photosynthesis
45 (Furla *et al.*, 2005). Therefore, one can hypothesize that in hybrids such adaptations could
46 be modified and a breakdown of symbiosis could occur, leading to reduced fitness. The
47 association with Symbiodiniaceae can range from mutualism to parasitism (Sachs and
48 Wilcox, 2006; Lesser *et al.*, 2013; see also Matz, 2024), and a change in the genomic
49 background in hybrid hosts could modify the nature of symbiosis as well. The presence of
50 Symbiodiniaceae could then be involved in genetic incompatibilities with the host genome,
51 as previously observed with bacterial species (Bordenstein, 2003; Brucker and Bordenstein,
52 2012). All these observations raise the question of the potential role of Symbiodiniaceae in
53 speciation and reproductive isolation in Anthozoans. This topic has been poorly explored up
54 to now. In *Plexaura* octacorals, two incompletely isolated species have been shown to
55 present different populations of Symbiodiniaceae, questioning their role in species
56 boundaries (Pelosi *et al.*, 2020).

57 Here we explore the robustness of species limits between named species of the gorgonian
58 genus *Eunicella* (Octocorallia, Eunicellidae) documented as displaying different symbiotic
59 relationships. In shallow conditions (above 50 m depth), three *Eunicella* species are mainly
60 present in the Mediterranean Sea: *Eunicella cavolini* (Koch, 1887), *E. singularis* (Esper,
61 1971), and *E. verrucosa* (Pallas, 1766). These three species have partially overlapping
62 ranges, and they can be observed in sympatry in the area of Marseille (France). *Eunicella*
63 *singularis* hosts Symbiodiniaceae corresponding to the *Philozoon* genus (Forcioli *et al.*,
64 2011; LaJeunesse *et al.*, 2018, 2022; Porro, 2019), whereas the two other gorgonian species
65 are devoided of these symbionts (Carpine and Grasshoff, 1975). The Symbiodiniaceae
66 contribute to the carbon metabolism of *E. singularis*, but a non-symbiotic *aphyta* morph has
67 already been observed (Gori *et al.*, 2012). The lack of variability in mitochondrial DNA does
68 not allow to distinguish these three species (Calderón *et al.*, 2006), and a study using two
69 nuclear introns suggested the possibility of hybridization between *E. cavolini* and
70 *E. singularis* (Aurelle *et al.*, 2017). Moreover, demographic inferences based on a large
71 number of nuclear loci in *E. cavolini* and *E. verrucosa* indicated the possibility of current
72 gene flow between these two species (Roux *et al.*, 2016). However, these data are
73 incomplete because neither individuals identified as *E. singularis*, nor individuals that are
74 morphologically difficult to attribute to a named species (potential hybrids) have been

75 analysed. Here, we will go further on these topics with the following objectives: i) estimate
76 the genomic differentiation among these three species and test for species limits, ii) test
77 whether symbionts are present or absent in the hybrids, to look for a possible breakdown in
78 symbiosis, and iii) infer scenarios of speciation. Studying the history of speciation is useful to
79 infer how divergence happened, and to test the possibility of current and past gene flow.
80 Analysing the hybrid status on morphologically intermediate individuals allows to further test
81 if hybridization is still on-going. We used restriction sites associated DNA sequencing (RAD-
82 sequencing; Baird *et al.*, 2008) to test species limits and hybridization. We complementary
83 used transcriptome data for demographic inferences, for the analysis of putative hybrids, and
84 to test for the presence of Symbiodiniaceae. The results will be useful to better understand
85 the evolution of these species in different environments and particularly the possible impact
86 of hybridization in adaptation to changing environment.

87

88 **Material and methods**

89

90 **Species distribution**

91

92 *Eunicella verrucosa* is present both in the Eastern Atlantic Ocean and the Mediterranean
93 Sea (Carpine and Grasshoff, 1975). In the Atlantic, *E. verrucosa* can be found from Ireland
94 and West coasts of Britain, to Angola (Grasshoff, 1992; Readman and Hiscock, 2017).
95 *Eunicella verrucosa* has been observed in the North Western Mediterranean Sea, in
96 Sardinia (Canessa *et al.*, 2022), and in the Adriatic and Aegean Seas (Chimienti, 2020). In
97 the Mediterranean Sea, *E. verrucosa* can be observed from shallow conditions (20-40 m) up
98 to 200 m depth (Sartoretto and Francour, 2011; Fourt and Goujard, 2012; Chimienti, 2020).

99 *Eunicella singularis* and *E. cavolini* are only present in the Mediterranean Sea. *Eunicella*
100 *cavolini* can be observed in the Western Mediterranean, Adriatic and Aegean Seas, from 5 to
101 200 m depth (Sini *et al.*, 2015; Carugati *et al.*, 2022). *Eunicella singularis* can be found in the
102 Western Mediterranean and Adriatic Seas, and, less frequently, in the Eastern
103 Mediterranean (Gori *et al.*, 2012). It is usually observed up to 40 m depth. *Eunicella*
104 *singularis* is the only Mediterranean octocoral known to harbour Symbiodiniaceae (but see
105 Bonacolta *et al.*, 2024). These Symbiodiniaceae belong to the temperate clade A (Forcioli *et*
106 *al.*, 2011; Casado-Amezúa *et al.*, 2016), now corresponding to the *Philozoon* genus
107 (LaJeunesse *et al.*, 2018, 2022). Deep occurrences (up to 70 m) of *E. singularis* have been
108 mentioned, and assigned to the *aphyta* morph, without Symbiodiniaceae (Gori *et al.*, 2012).
109 In the area of Marseille, these three species can be observed in sympatry and at the same
110 depth (Sartoretto and Francour, 2011).

111

112 **Sampling**

113

114 The sampling for RAD sequencing included 25 specimens identified as *E. cavolini*, 23
115 *E. singularis*, seven *E. verrucosa*, and 12 morphologically intermediate individuals (potential
116 hybrids). These latter individuals displayed intermediate colors and branching patterns
117 between *E. cavolini* and *E. singularis* (Figure S1), and they were analysed to test their hybrid
118 status (Aurelle *et al.*, 2017). The specimens have been sampled by scuba diving at different
119 periods in the area of Marseille, where the three species are present in sympatry (Figure S2;
120 Table S1).

121 For transcriptome sequencing, specimens attributed to *E. cavolini*, *E. singularis*, and
122 *E. verrucosa* have been collected in the Mediterranean (for the three species), and in the
123 Atlantic (*E. verrucosa* only; Table S2; Figure 1) in 2016. The final sampling for
124 transcriptomics included five *E. cavolini*, eight *E. singularis*, three *E. verrucosa*, and four
125 potential hybrids.

126

127 Sampling was non-destructive, with authorizations from the local authorities, including
128 Marine Protected Areas.

129

130 **Mitochondrial MutS**

131 To test the genetic proximity of three *Eunicella* species studied here, we built a tree with
132 mitochondrial MutS sequences (McFadden *et al.*, 2011), available in GenBank. The methods
133 and sequences are detailed in supplementary Figure S3, and Table S3.

134

135 **RAD sequencing**

136

137 DNA has been extracted with the Macherey-Nagel NucleoSpin DNA RapidLyse kit. RAD
138 library preparation (with the PstI restriction enzyme) and sequencing (Illumina NovaSeq600
139 with 150 nucleotides paired-end sequencing) have been performed at the MGX platform
140 (CNRS). The MGX platform performed control quality, demultiplexing and removal of PCR
141 duplicates with unique molecule identifiers. Potential contaminants have been removed with
142 kraken2 (Wood *et al.*, 2019; Lu *et al.*, 2022). RAD loci have been assembled with ipyrad
143 (Eaton and Overcast, 2020). We tested four assembly strategies to test the robustness of
144 the results: a *de novo* assembly, with a clustering threshold of 0.85, and assembly on a
145 reference genome, with each of the three available genomes: for *E. cavolini*, *E. singularis*,
146 and *E. verrucosa* (Ledoux *et al.*, in prep).

147 From these datasets, we built four datasets focused on the differentiation between
148 *E. cavolini* and *E. singularis*: we excluded *E. verrucosa* samples and we retained the first

149 percent of the loci with the highest F_{ST} between *E. cavolini* and *E. singularis*. These last
150 datasets will be labelled as “1%” (see characteristics of the different datasets are
151 summarised in Table S4).

152

153 **Transcriptome sequencing and SNPs calling**

154

155 Total RNA has been extracted as in Haguenaer *et al.* (2013). RNAs were sent to the LIGAN
156 genomic platform for sequencing (Lille, France) on four flow cells of Illumina NextSeq 500
157 (2 x 75 bp). The transcriptomes have been assembled with the *de novo* RNA-Seq Assembly
158 Pipeline (DRAP ; Cabau *et al.*, 2017) with Oases (Schulz *et al.*, 2012) and default
159 parameters. We performed an individual assembly, and a meta-assembly to be used as
160 reference. The statistics describing the assembled transcriptomes are given in Table S2. We
161 used the BLAT software (Kent, 2002) and the `blat_parser.pl` script to remove potential
162 Symbiodiniaceae sequences in the obtained transcriptomes, with the transcriptome of the
163 type A1 (Baumgarten *et al.*, 2013) as a reference.

164 We mapped the reads on the meta transcriptome filtered for Symbiodiniaceae sequences
165 with `bwa` option `mem` (Li and Durbin, 2009). The obtained sam files were converted in bam
166 format with `samtools` 1.9 (Li *et al.*, 2009), and sorted with Picard tools (‘Picard Toolkit’, 2019).
167 The SNPs calling has been performed with `reads2snp` 2.0 with default parameters
168 (Tsagkogeorga *et al.*, 2012; Gayral *et al.*, 2013). The obtained dataset, including variable
169 and non variable sites, will thereafter be referred as the “all sites” dataset. We performed
170 separate SNP calls with `reads2snp` for pairwise comparisons among species and without the
171 potential hybrid samples. These three datasets have been used for demographic inferences,
172 and will be referred as “all-CS” for the *E. cavolini* / *E. singularis* comparison, “all-CV” for
173 the *E. cavolini* / *E. verrucosa* comparison, and “all-SV” for the *E. singularis* / *E.*
174 *verrucosa* comparison.

175 For an analysis of genetic differentiation, we filtered the “all sites” vcf file with `vcftools`
176 (Danecek *et al.*, 2011). We retained biallelic sites, without missing data, and separated by at
177 least 1 kb: this is the “polymorphic sites” dataset. As for RAD sequencing, we built a dataset
178 focused on the differentiation between *E. cavolini* and *E. singularis*, retaining the 1% loci with
179 the highest differentiation between *E. cavolini* and *E. singularis* (Table S4).

180

181 **Presence of Symbiodiniaceae**

182

183 We analysed the presence of Symbiodiniaceae in *Eunicella* gorgonians with transcriptome
184 data. First, we counted the number of reads corresponding to the Symbiodiniaceae
185 transcriptome type A1 with Salmon (Patro *et al.*, 2017). Second, we used the percentage of

186 assembled sequences (contigs) in the *Eunicella* transcriptomes corresponding to
187 Symbiodiniaceae following the BLAT analysis. We used a Kruskal-Wallis test in R to test for
188 differences among the four groups of samples (the three *Eunicella* species and the potential
189 hybrids) for each metric. Additionally, we performed a BLAST analysis with the LSU, ITS and
190 psbA sequences of *Philozoon* (LaJeunesse *et al.*, 2022) to try to identify the
191 Symbiodiniaceae genera present in the different samples.

192 As our data pointed to the unexpected presence of Symbiodiniaceae in *E. cavolini* (see
193 Results), we further explored this topic with preliminary data from another experiment
194 dedicated to studying the microbiome of *E. cavolini* and *E. singularis*. This pilot study
195 involved an analysis of microeukaryotic communities through 18S rDNA metabarcoding on
196 two colonies of *E. cavolini*, and one *E. singularis* (Supplementary File S2).

197

198 **Genetic differentiation and analysis of hybrids**

199

200 With RAD sequencing data, we performed the analysis of genetic diversity with the four
201 datasets including all loci. With transcriptomes, we performed the same analyses with the
202 “polymorphic sites” dataset. We used the LEA R package to estimate ancestry coefficients
203 (Frichot *et al.*, 2014; Frichot and François, 2015). We tested K values from 1 to 10, with 10
204 replicates for each K. To analyse the genetic differences among individuals, we performed a
205 Principal Component Analysis (PCA) with the R package adegenet (Jombart, 2008). The
206 pairwise F_{ST} (Weir and Cockerham, 1984) estimated among species were computed with the
207 R package Genepop (Rousset, 2008; Rousset *et al.*, 2020), after conversion of the vcf file
208 with PGDSpider (Lischer & Excoffier, 2012). The distribution of F_{ST} among loci was obtained
209 with vcftools.

210 The hybrid status (e.g. first generation hybrids) of morphologically intermediate individuals
211 was analysed with the NewHybrids software (Anderson and Thompson, 2002). We used the
212 genepopedit R package to prepare the input file from genepop format (Stanley *et al.*, 2017).
213 Following the results of the LEA and PCA analyses, we compared *E. cavolini*, *E. singularis*
214 and potential hybrids. The NewHybrids analysis has difficulties to converge with a high
215 number of loci compared to the number of individuals
216 (<https://github.com/erigande/newhybrids/issues/5>). We therefore used the different “1%
217 SNP” datasets of RAD sequencing and transcriptome datasets (i.e. the most differentiated
218 loci) for the NewHybrids analysis. As a prior, we used individuals with the lowest levels of
219 admixture in LEA as potential parental individuals. For the RAD datasets, this corresponded
220 to ten individuals of each species as priors. For transcriptome sequencing this corresponded
221 to three individuals for *E. cavolini*, and six individuals for *E. singularis*. Each NewHybrids
222 analysis was repeated ten times to test the robustness of the results.

223

224 **Scenarios of speciation**

225

226 We tested scenarios of speciation with the Demographic Inferences with Linked Selection
227 (DILS) pipeline (Csilléry, *et al.*, 2012; Pudlo *et al.*, 2016; Fraïsse *et al.*, 2021) on
228 transcriptome data only. Note that with the high number of loci recovered with
229 transcriptomes, the numbers of specimens used here are adequate for robust inferences
230 (Roux *et al.*, 2016). The DILS pipeline allows the analysis of two species scenarios only: we
231 therefore performed separate analyses for the three two-species comparisons, with the “all-
232 CS”, “all-CV”, and “all-SV” pairwise datasets. We did not include the potential hybrids in the
233 analysis, which would have required the consideration of a separate population. The tested
234 scenarios are presented in Figure S4 (see Fraïsse *et al.*, 2021 for details). Briefly, DILS
235 allows testing scenario with current migration (i.e. gene flow), such as isolation / migration or
236 secondary contact, versus scenarios of current isolation (no gene flow), such as complete or
237 ancestral migration (gene flow among ancestral populations).

238 We used the same priors for all analyses, with different numbers of sequences per gene and
239 per sample according to the dataset (Table S5). For all pairwise comparisons, we performed
240 two DILS analyses: one with constant population sizes, and one with variable population
241 sizes.

242

243 **Results**

244

245 **Mitochondrial MutS**

246

247 The mitochondrial MutS sequences available in GenBank confirmed the proximity of the
248 three *Eunicella* species analysed here: all sequences were identical for these three species,
249 as well as for three other sequences deposited in GenBank as unidentified *Eunicella* (Figure
250 S3). The closest species to this group was *Eunicella racemosa*. All other *Eunicella* MutS
251 sequences (*E. tricornata* and *E. albicans*) grouped separately with *Complexum monodi*, but
252 with low bootstrap support.

253

254 **Presence of Symbiodiniaceae**

255

256 The transcriptomes showed low numbers of reads counts aligning on the Symbiodiniaceae
257 transcriptome (1868 to 58406 reads; Table S6). The proportion of contigs corresponding to
258 Symbiodiniaceae with BLAT was also very low (between 0.00276 and 0.03686; Table S6).
259 Significant differences were observed among species in both cases (Kruskal-Wallis test, $p =$

260 0.047 for reads counts, and $p = 0.002$ for the proportions of contigs). The pairwise Wilcoxon-
261 Test showed significant differences only for the comparisons of proportions of contigs
262 involving *E. singularis*, which was higher than in other species (Table S7; Figure 2). The
263 mean values of reads counts and contigs for the Symbiodiniaceae in the hybrids were lower
264 than in *E. singularis* and *E. cavolini* but higher than in *E. verrucosa*, although pairwise tests
265 were not significant.

266 The BLAST analysis with the LSU, ITS and psbA sequences of *Philozoon* only retrieved
267 corresponding sequences in the transcriptomes of *E. singularis*. Regarding the pilot study of
268 18S rDNA metabarcoding, a diversity of 92 Operational Taxonomic Units (OTUs)
269 corresponding to Symbiodiniaceae in the Silva database was observed in *E. singularis*, with
270 a single OTU largely dominant in abundance (Supplementary file S2). The same OTU was
271 also observed in *E. cavolini* with a low abundance of reads, but still representing 99% of all
272 12 to 13 Symbiodiniaceae OTUs detected in the two analysed colonies. A BLAST search in
273 GenBank identified a subset of Symbiodiniaceae sequences related to this OTU.
274 Phylogenetic inference based on these data indicated that this OTU was related to clade A of
275 the Symbiodiniaceae.

276

277 **Genetic differentiation and analysis of hybrids**

278 With RAD sequencing we obtained between 12 952 and 29 061 SNPs for the assembly on
279 *E. cavolini* and *E. verrucosa* genomes respectively (Table S4). The F_{ST} estimates from RAD
280 sequencing were highest for the comparisons between *E. verrucosa* and all other samples
281 (F_{ST} between 0.51 and 0.66 depending on dataset; Table S8). The F_{ST} between *E. cavolini*
282 and *E. singularis* was lower (F_{ST} between 0.29 and 0.38), and the lowest F_{ST} values were
283 observed for hybrids compared to these two species (F_{ST} between 0.09 and 0.13). The
284 cross-entropy analysis using LEA with RAD sequencing showed a minimum at $K = 3$ for the
285 four datasets (results not shown). The barplots of coancestry coefficients were very similar
286 for the four datasets, with a separation of the three species, and an admixture between
287 *E. cavolini* and *E. singularis* for the morphologically intermediate individuals (Figure S5). The
288 PCAs on RAD sequencing were very similar for all datasets, with a separation between
289 *E. verrucosa* and all other samples on the first axis (Figure S6). The second axis separated
290 *E. cavolini* and *E. singularis*, with the potential hybrids in intermediate position between
291 them. Projections on axes 3 and 4 resulted mainly in the separations of *E. verrucosa*
292 samples from each other.

293 With transcriptomes, we obtained 31 369 SNPs for the “polymorphic sites” dataset. With this
294 dataset, the highest F_{ST} values were observed for the comparisons between *E. verrucosa*
295 and all other samples ($F_{ST} > 0.43$; Table S9). The F_{ST} between *E. cavolini* and *E. singularis*
296 was much lower (0.21), and the lowest F_{ST} values were observed for hybrids compared to

297 these two species (F_{ST} around 0.07 in both cases). These differences corresponded to
298 different distributions of F_{ST} over SNPs (Figure S7). For the 1% SNPs with the highest F_{ST}
299 estimates, 52 SNPs were shared by both comparisons involving *E. cavolini* (i.e. *E. cavolini*
300 vs *E. singularis* and *E. cavolini* vs *E. verrucosa*), 116 top 1% SNPs were shared by both
301 comparisons involving *E. singularis*, and 1042 top 1% SNPs were shared by both
302 comparisons involving *E. verrucosa*.

303 The cross-entropy analysis using LEA with transcriptomes indicated a best clustering
304 solution corresponding to $K = 2$ or $K = 3$ clusters (Figure S8). At $K = 2$, the first distinction
305 was observed between *E. verrucosa* and all other samples (Figure 3). The $K = 3$ analysis
306 further separated *E. cavolini* and *E. singularis*, with morphologically intermediate individuals
307 admixed between these two species. Conversely the individuals representative of *E. cavolini*
308 and *E. singularis* presented low levels of admixture, apart from the *E. cavolini* of the site in
309 Algeria (code ANB), and, at a small level, two *E. singularis* individuals from Banyuls (BAN).
310 At $K = 4$, the two *E. cavolini* individuals from Algeria separated from other *E. cavolini* from
311 the northern part of the Mediterranean.

312 As with RAD sequencing, the PCA on transcriptome SNPs separated *E. verrucosa* from
313 other samples on the first axis (Figure 4). The second axis separated *E. cavolini* and
314 *E. singularis*, with the potential hybrids in intermediate position between them. The third axis
315 separated the *E. cavolini* samples from Algeria (ANB site) from all other samples (Figure
316 S6).

317 The NewHybrids analysis on RAD sequencing indicated that all morphologically intermediate
318 individuals, except one, appeared as hybrids: first generation (F1), second generation (F2),
319 or backcrosses with *E. singularis* or *E. cavolini* (Table 1). These samples also appeared
320 admixed on the basis of LEA (Figure S5). One individual identified as a potential hybrid *in*
321 *situ*, was inferred as a parental *E. singularis*. For four individuals, the hybrid status varied
322 according to the dataset: F2 or backcross with *E. cavolini* in two cases, F1 or F2 in two
323 cases. Potential parental individuals not included in the priors were well inferred as parental
324 with NewHybrids. The NewHybrids analysis with transcriptomes indicated that the
325 morphologically intermediate individuals were hybrids with a probability of one in all ten
326 iterations of the analysis. One individual was a F1 hybrid, another one was F2 hybrid, and
327 the two other ones corresponded to backcrosses with *E. singularis* (Figure 3; Table 1). In the
328 same analysis, the *E. cavolini* and *E. singularis* individuals not included as priors for parental
329 species (see Figure 3 for the individuals used as priors), were indeed inferred as parental
330 with a probability of one, including the *E. cavolini* individual from Algeria (ANB).

331

332 **Scenarios of speciation**

333

334 The average pairwise net divergence estimated from DILS was 0.0018 between *E. cavolini*
335 and *E. singularis*, and around 0.007 for the two comparisons with *E. verrucosa* (Table S9,
336 https://zenodo.org/records/12532817/files/results_DILS_suppl_file.ods?download=1). The
337 DILS analysis indicated the existence of current gene flow between *E. cavolini* and
338 *E. singularis* with high probability, both with constant and variable population sizes ($p = 0.87$
339 and 0.88 respectively; Table 2). This possibility of gene flow corresponded to a scenario of
340 secondary contact. Conversely, a model of current isolation was inferred for the comparisons
341 between *E. verrucosa* and each of the two other species, with a probability $p \geq 0.87$: in these
342 two cases, the inferred scenario included a period of ancestral migration, though with
343 moderate support (p between 0.61 and 0.69). A genomic heterogeneity in effective size (i.e.
344 variations among loci) was inferred with strong support ($p \geq 0.99$) for all analyses. In the
345 case of current gene flow (between *E. cavolini* and *E. singularis*), a genomic heterogeneity in
346 migration rates was inferred ($p \geq 0.82$). We repeated the DILS analysis without including the
347 two divergent samples of *E. cavolini* from Algeria: this led to similar results, with inference of
348 secondary contact for the comparison with *E. singularis*, and ancestral migration for the
349 comparison with *E. verrucosa* (results not shown). For parameters inferences, we used the
350 complete datasets, with all *E. cavolini* samples. The inferred parameters for the different
351 scenarios are presented in Supplementary Table S9. We will first present the results
352 obtained for the constant population sizes models. The divergence time between *E. cavolini*
353 and *E. singularis* (median 403 273 generations) was much lower than between *E. cavolini*
354 and *E. verrucosa* (median 1 054 488 generations), and between *E. singularis* and
355 *E. verrucosa* (median 899 098 generations). For the comparison between *E. cavolini* and
356 *E. singularis*, the time of secondary contact was estimated after around 85% of time spent in
357 isolation since divergence. Following secondary contact, the gene flow was similar in both
358 directions for these two species. The duration of ancestral migration roughly corresponded to
359 6% and 8% of the total time since divergence for the comparison between *E. cavolini* and
360 *E. verrucosa*, and for the comparison between *E. singularis* and *E. verrucosa*, respectively.
361 For these last two cases, the gene flow (forward in time) during ancestral migration was
362 higher towards *E. verrucosa* than in the opposite direction. The estimated effective sizes
363 were of similar order for *E. cavolini* and *E. verrucosa*. Similar results were obtained for the
364 models including variations in effective size, except for the estimate of current gene flow
365 between *E. cavolini* and *E. singularis*: with variable population size, gene flow from
366 *E. singularis* to *E. cavolini* was higher than in the opposite direction.

367

368

369 **Discussion**

370

371 The three named *Eunicella* species studied here have been previously described with
372 differences in colony morphology, sclerites shape, and in the presence of photosynthetic
373 Symbiodiniaceae (Carpine and Grasshoff, 1975). Our results demonstrate a continuum
374 between *E. cavolini* and *E. singularis*, with morphologically intermediate individuals, on-going
375 gene flow, and hybrids characterised by a reduced frequency of Symbiodiniaceae compared
376 to *E. singularis*. On the other hand, *E. verrucosa* appears genetically isolated from these two
377 species. We will discuss here the differences observed among markers, the outcome of
378 hybridization, the speciation scenarios, and what can be learnt on the evolution of symbiosis.
379

380 **Discordances between molecular markers**

381

382 As previously observed (Aurelle *et al.*, 2017), mitochondrial DNA did not allow to discriminate
383 the three species due to the usually slow evolution of mitochondrial DNA in octocorals
384 (McFadden *et al.*, 2011; Muthye *et al.*, 2022). The use of transcriptome sequences first
385 confirmed the closer proximity between *E. cavolini* and *E. singularis* than with *E. verrucosa*.
386 This had been previously suggested with two intron sequences, but with incomplete lineage
387 sorting (Aurelle *et al.*, 2017). The Mediterranean *Eunicella* then add a new example of the
388 lack of power of mitochondrial DNA to discriminate genetically differentiated octocoral
389 species, as demonstrated in other genera (Erickson *et al.*, 2021; Pante *et al.*, 2015a). The
390 slow rate of evolution of mitochondrial DNA in octocorals has been linked to the presence of
391 the mitochondrial locus MutS, an homolog of a bacterial gene involved in DNA repair.
392 However, there are contradictory examples showing that the presence of this locus is not the
393 only factor explaining the slow evolution of mitochondrial DNA in octocorals (Muthye *et al.*,
394 2022). More generally, as hybridization can lead to the sharing of mitochondrial DNA among
395 species, the use of multiple independent nuclear loci is required for species discrimination in
396 such cases.

397

398 **Incomplete reproductive isolation among two named species**

399

400 Inferences of genetic ancestry and hybrid status confirmed that morphologically intermediate
401 individuals are indeed hybrids between *E. singularis* and *E. cavolini*, with the identification of
402 F1, F2 and backcrosses with both parental lineages: first generation hybrids can then be
403 fertile. The fact that gene flow indeed goes further than the hybrid levels is confirmed by the
404 DILS analysis, which did not include hybrid individuals. Reproductive isolation is therefore at
405 least partial between these lineages. The ease to find hybrids in the area studied here, as
406 well as similar observations in other sites (S. Sartoretto, pers. com.) indicate that
407 hybridization is not rare on an evolutionary scale. Similarly, transcriptome sequencing has

408 led to infer hybridization among *Plexaura* species on the basis of a small number of samples
409 (Pelosi *et al.*, 2022).

410 The alternation of populations with and without hybrids would point to a mosaic hybrid zone
411 (Bierne *et al.*, 2003), where hybrids could form in different areas and from different parental
412 populations. As, or because, hybridization between *E. cavolini* and *E. singularis* had not
413 been reported before, the presence of hybrids has probably been overlooked up to now. This
414 may be the consequence of previously focusing on colonies with “typical” morphologies. The
415 frequency of hybridization therefore remains to be studied.

416 Our results allow discussing the evolution of genomic divergence among these species. The
417 persistence of genomic differentiation between these lineages in sympatry, despite current
418 gene flow, indicates that intrinsic (i.e. genomic incompatibilities) or extrinsic (e.g. ecology)
419 factors can maintain partial isolation. Difference or overlap in the timing of reproduction
420 should also be considered in contributing to pre-mating isolation (Pelosi *et al.*, 2022). A
421 better characterization of the ecological range of parental and hybrid populations would be
422 useful to test if local adaptation is involved in their distribution. Intrinsic factors such as
423 genetic incompatibilities, potentially coupled with differences in adaptation to local
424 environments, can be present as well (Bierne *et al.*, 2011). A genome wide analysis of
425 differentiation is required to investigate whether divergence between *E. cavolini* and
426 *E. singularis*, is homogeneous along the genome (as suggested by the DILS analysis which
427 inferred a homogeneity of gene flow), or whether genomic islands of differentiation exist
428 (Peñalba *et al.*, 2024). We could then better understand to what stage of divergence the
429 *E. cavolini* / *E. singularis* split corresponds: from intra-specific polymorphism to species
430 separated by semipermeable barriers to gene flow.

431 One interesting question in this context is whether changes in selection regimes induced by
432 human activities can change the outcome of hybridization (Ålund *et al.*, 2023). For example,
433 Mediterranean octocorals are impacted by mortality events linked with climate change (Sini
434 *et al.*, 2015; Estaque *et al.*, 2023), and the impact of these events could be different for
435 hybrids and parental individuals. In scleractinian corals, interspecific hybridization has been
436 reported to enhance the survival under elevated temperature conditions (Chan *et al.*, 2018) .
437 Regarding *E. verrucosa*, the more ancient divergence corresponded to much more loci with
438 high F_{ST} . Among the list of the most highly differentiated loci, more overlap was also
439 observed for the two comparisons involving *E. verrucosa* than for the other pairwise
440 comparisons: this may indicate that few genomic areas of potential incompatibilities with
441 *E. verrucosa* are involved in the divergence between *E. cavolini* and *E. singularis*.

442

443 **Scenarios of speciations**

444

445 The scenarios of speciations inferred with DILS supported the current isolation (no gene
446 flow) of *E. verrucosa* with the two other species with high posterior probability. Conversely
447 current gene flow was strongly supported versus isolation between *E. cavolini* and
448 *E. singularis*. The posterior probabilities for ancestral migration (for *E. verrucosa* versus the
449 two other species), and secondary contact (*E. cavolini* and *E. singularis*), were lower than for
450 inferences on current gene flow. These scenarios were indeed the best ones among those
451 tested here but they might not provide the best possible representation of the evolutionary
452 history. Other models of evolution could be tested for better inferences, for example by
453 including the three species and hybrids, or gene flow from unsampled taxa (Tricou *et al.*,
454 2022). The current isolation of *E. verrucosa* from *E. cavolini* is also at odds with previous
455 results which showed the possibility of current gene flow between these two species despite
456 an important divergence (Roux *et al.*, 2016). It will be useful to explore the reasons for the
457 discrepancy between this last study and the present one, which are both based on
458 transcriptome datasets but obtained from different samples and sequencing platforms.

459 *Eunicella verrucosa* is currently widely distributed in the North Eastern Atlantic Ocean, and
460 less frequent in the Mediterranean Sea, whereas both other species are only present in the
461 Mediterranean Sea. The Atlantic / Mediterranean Sea transition does not seem to act as a
462 phylogeographic barrier for *E. verrucosa* (Macleod *et al.*, 2024). We can propose a scenario
463 where the split between *E. verrucosa* and both other species occurred in allopatry between
464 the Atlantic Ocean and the Mediterranean Sea, followed by the colonization of the
465 Mediterranean Sea by *E. verrucosa*. The generation time remains unknown for the *Eunicella*
466 species, and previous studies have shown important variation in the age at first reproduction
467 in gorgonians, from 2 to 13 years (see references in Munro, 2004). If we use a generation
468 time of two years for *Eunicella* species, with a median estimate of divergence time around
469 900 000 generations for *E. verrucosa* / *E. singularis* and 1 000 000 for *E. verrucosa* /
470 *E. cavolini*, and based on a mutation rate set at 3.10^{-9} , this would indicate a divergence at
471 least around 2 000 000 years (2 Ma). The divergence time between *E. cavolini* and
472 *E. singularis* would be 2.5 times more recent, around 800 000 years, with a median time of
473 secondary contact around 60 000 generations, corresponding to 15% of the time spent since
474 divergence. It is difficult to infer past distributions of *E. singularis* and *E. cavolini*, but one can
475 note that even if they are currently found in sympatry in different areas, their range do not
476 completely overlap. For example *E. cavolini* is nearly absent at the West of the Rhone
477 estuary on the French coast, whereas *E. singularis* is present there. The ecological range of
478 *E. singularis* and *E. cavolini* is also not completely overlapping, as *E. cavolini* can be
479 observed deeper than *E. singularis* (Gori *et al.*, 2012; Carugati *et al.*, 2022). Therefore one
480 can envision an historical separation of these two species either geographically or
481 ecologically, followed by a secondary contact where gene flow took place. In any case,

482 additional information on generation time, mutation rate and past demographic fluctuations
483 are required to be more precise on the history of these species.

484

485 **Evolution of symbiosis**

486

487 As previously discussed, we clearly demonstrated here the possibility of gene flow between
488 symbiotic (hosting Symbiodiniaceae) and non-symbiotic octocorals. Symbiodiniaceae could
489 nevertheless be involved in genetic incompatibilities with the genome of some cnidarian
490 hosts, but this would require additional analysis of symbiotic status in hybrids. The methods
491 used here did not aim at a precise quantification of Symbiodiniaceae, and one can note the
492 low levels of sequences corresponding to these symbionts, even in *E. singularis*, which may
493 be due to difficulties in extracting the RNA of the symbionts (but see Guzman *et al.*, 2018;
494 Rivera-García *et al.*, 2019). Despite these limits we observed, as expected, a higher
495 Symbiodiniaceae concentration in *E. singularis* than in *E. cavolini* and *E. verrucosa*.
496 Interestingly, the hybrids showed a lower frequency of Symbiodiniaceae than *E. singularis*,
497 and possibly than *E. cavolini*, though this last result remains to be confirmed. In
498 *E. singularis*, the transmission of Symbiodiniaceae seems to occur both vertically, through
499 ovules, and horizontally, from the environment (Forcioli *et al.*, 2011). Both transmission
500 modes did not restore the levels of Symbiodiniaceae in the hybrids to those of *E. singularis*.
501 This suggests a breakdown of or a failure to establish symbiosis for hybrid genotypes, which
502 may impact the fitness of hybrids and consequently the possibility of introgression. The
503 *aphyta* type of *E. singularis* observed in deep conditions indicates a plasticity of symbiotic
504 status apart from hybridization. Nevertheless, here the hybrids were sampled in shallow
505 conditions (10-20 m depth) which underlines the role of hybridization in reducing the extent
506 of symbiosis. More precise estimates of Symbiodiniaceae abundance, and of physiological
507 parameters such as photosynthetic and respiration rates (Ezzat *et al.*, 2013). would help
508 understanding the role of symbionts in hybrids fitness. It would also be interesting to study if
509 the Symbiodiniaceae of the different samples belong to the same population (Pelosi *et al.*,
510 2022).

511 Our results also question the evolution and significance of octocoral / Symbiodiniaceae
512 symbiosis. In scleractinians, the transition between symbiotic and non-symbiotic states
513 happened repeatedly, but mostly in the direction of the acquisition of symbiosis, with very
514 low rates of reversal (Campoy *et al.*, 2020). This could indicate that investing in such
515 mutualistic interactions for the cnidarian would lead to increasingly relying on autotrophy for
516 energetic supply, making reversal to heterotrophy difficult. In octocorals, an evolutionary
517 versatility in symbiotic state seems possible, as in various families and genera, both
518 symbiotic and non-symbiotic species are present (Van Oppen *et al.*, 2005). In the

519 Mediterranean Sea, all octocoral species are non-symbiotic, except for *E. singularis* (but see
520 Bonacolta et al. 2024). The most parsimonious scenario here would be an acquisition of
521 symbiosis in *E. singularis* during or following its divergence from *E. cavolini*. The symbiotic
522 status of *E. singularis* nevertheless could be facultative as previously mentioned for the
523 *aphyta* type (Gori et al., 2012). Additionally, experimental physiological studies have
524 demonstrated the nutritional plasticity of *E. singularis* which is able to use either heterotrophy
525 or autotrophy for its metabolism (Ezzat et al., 2013). Nevertheless, in natural conditions,
526 autotrophy seems to provide an important contribution to the metabolism of *E. singularis*,
527 and the collapse of photosynthetic capacities in too warm conditions can contribute to
528 mortality events in this species (Coma et al., 2015).

529 The question of symbiosis could be reversed as well: why are Symbiodiniaceae not more
530 abundant in *E. cavolini*? This species can be observed in shallow conditions (less than 10 m
531 depth) where there is enough light for photosynthesis, and in syntopy with *E. singularis*. The
532 availability of preys or particulate organic matter may provide enough energy to *E. cavolini* in
533 its habitat, but this species may have never engaged in mutualistic interaction with
534 Symbiodiniaceae. Interestingly we observed a low rate of sequences related to
535 Symbiodiniaceae in the transcriptomes of *E. cavolini* (and even lower, but not null in
536 *E. verrucosa*). This could either correspond to a signal from free living Symbiodiniaceae, or
537 to rare, transient, associations with the cnidarian. In addition, a Symbiodiniaceae OTU that is
538 common to *E. singularis* and *E. cavolini* was identified among the microeukaryotes
539 associated with the two species: this OTU is related to strains observed in symbiosis with
540 *E. singularis* and other Mediterranean cnidarians. Molecular markers also allowed to
541 evidence the presence of Symbiodiniaceae in species previously supposed to be asymbiotic,
542 as in the Mediterranean octocoral *Paramuricea clavata*, and in several Hawaiian
543 antipatharian species (Wagner et al., 2011; Bonacolta et al., 2024). These results, and our
544 observations in *Eunicella* species, obviously underline the dynamic nature of interactions
545 between Symbiodiniaceae and cnidarians: the establishment of symbiosis may be preceded
546 by more or less stable, and more or less mutualistic interactions. The development of
547 effective symbiosis, with stable relationships, and higher abundance of symbiont, would
548 require specific adaptation from both partners. We can see here that even if on a macro-
549 evolutionary scale the acquisition of symbiosis is much more frequent than its loss, on a
550 micro-evolutionary scale the gene flow between the *Eunicella* species considered here has
551 not led to the full development of symbiosis in *E. cavolini*.

552

553 **Conclusions and perspectives**

554

555 We demonstrated the lack of genetic isolation between octocorals with contrasted levels of
556 mutualistic interaction with Symbiodiniaceae. Understanding the evolution and adaptation of
557 these species in heterogeneous environments should then consider the possible impact of
558 introgression. We also show that symbiosis is more flexible than previously envisioned in
559 octocorals. For these species it will be useful to estimate the frequency and spatial extent of
560 hybrid zones: does it correlate with particular environments with a coupling between
561 endogenous and exogenous barriers to gene flow (Bierne *et al.*, 2011)? Characterizing the
562 genomic landscape of introgression would help to look for the effects of introgression on
563 adaptation or symbiosis for example. Indeed, even low levels of interspecific gene flow can
564 have important consequences on the evolution of species (Arnold *et al.*, 1999). Finally,
565 various cases of hybridization have been demonstrated in symbiotic anthozoans (e.g.
566 Combosch and Vollmer, 2015; Pelosi *et al.*, 2022): it would then be interesting to study the
567 dynamics of symbiosis in these cases, especially when different Symbiodiniaceae strains are
568 involved.

569

570

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572

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604

605

606 **Data availability**

607 The transcriptome raw sequences are available in Genbank under BioProject ID
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609 PRJNA1122331.

610 The scripts used in this study, the vcf files from RAD sequencing, and the detailed results of
611 the DILS analysis are available at <https://doi.org/10.5281/zenodo.14007931>

612

613 **Conflict of interest disclosure**

614 The authors declare that they have no conflict of interest in relation to the content of the
615 article

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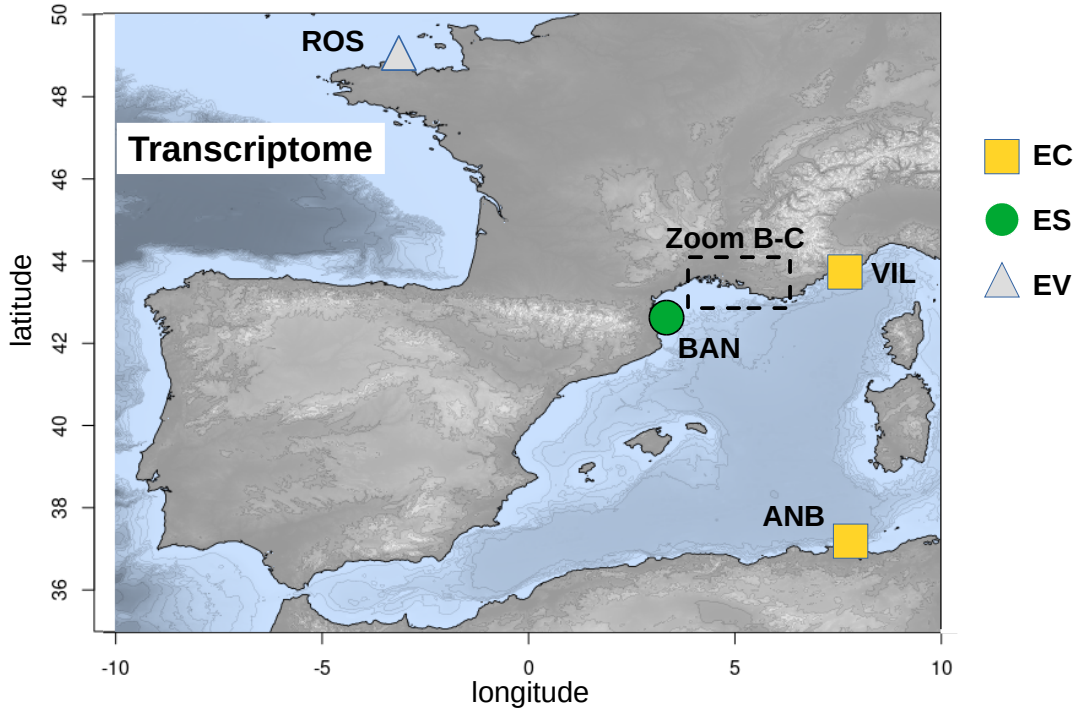
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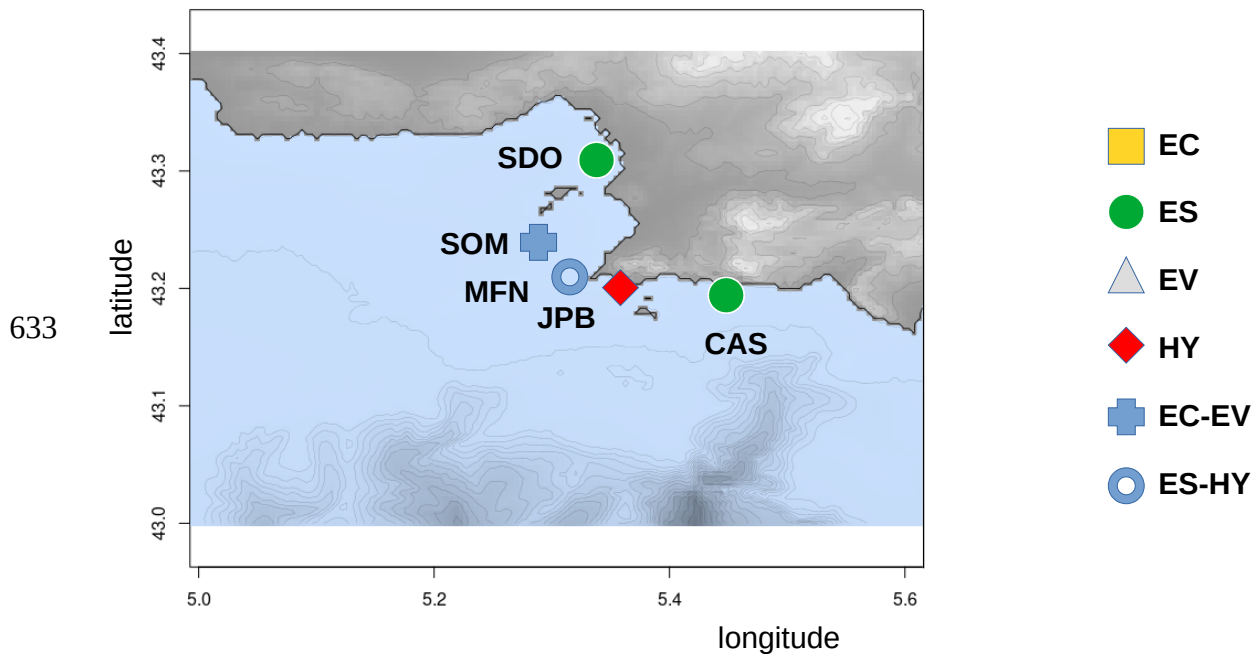
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624 **Figure 1:** map of sampling sites for transcriptomes: A) general view, B) zoom on the area of
 625 Marseille. The symbols correspond to different samples: EC *E. cavolini*, ES *E. singularis*, EV
 626 *E. verrucosa*, HY potential hybrids. The three letters correspond to the codes of the
 627 sampling. The maps have been produced with the marmap R package (Pante & Simon-
 628 Bouhet, 2013) and following the tutorial of Krueger-Hadfield (2015).

629 **A)**



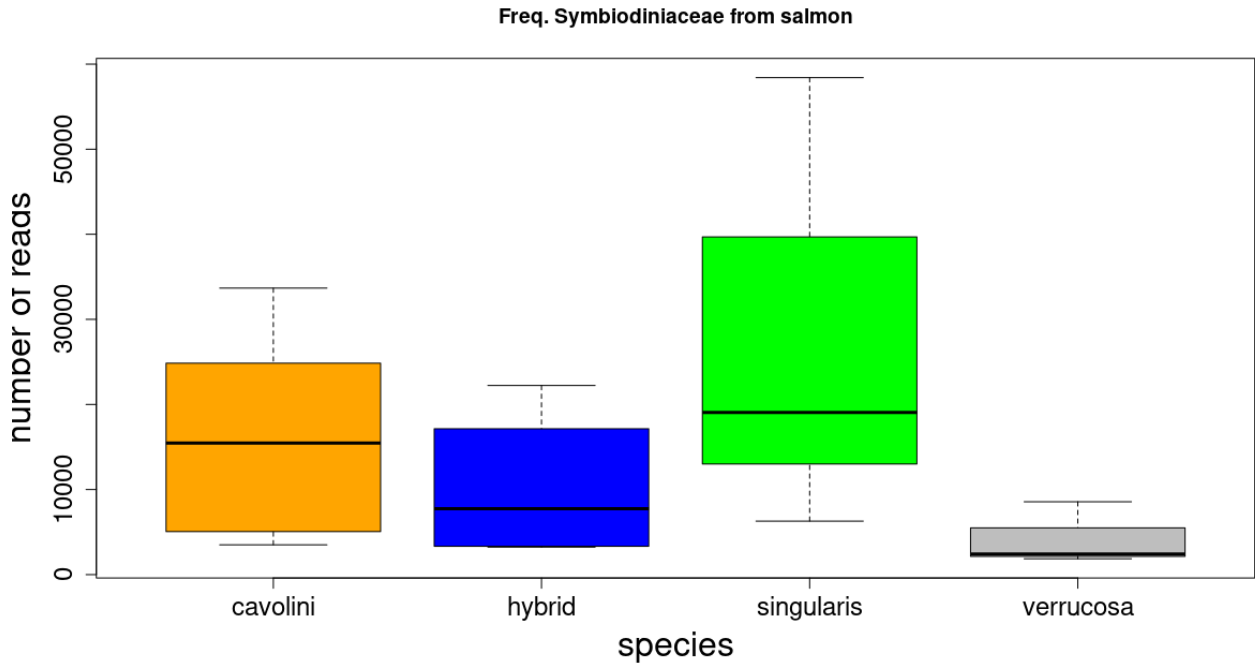
630
 631 **B)**
 632



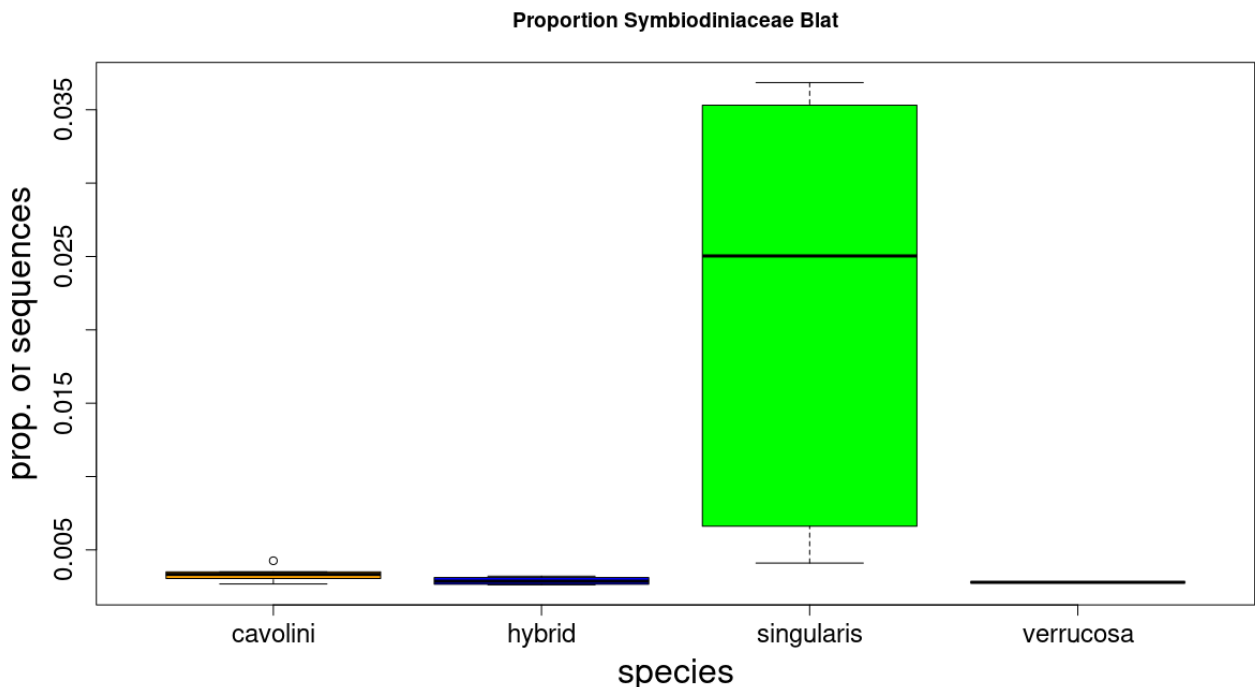
634

635 **Figure 2:** distribution of the frequency of Symbiodiniaceae sequences in the individual
636 transcriptomes according to the species based A) on the number of reads estimated with
637 Salmon, and B) on the proportion of assembled sequences (contigs) with the BLAT
638 analyses.

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640 **A)** Read counts with Salmon; mean values per group: *E. cavolini*: 16508; hybrids: 10238;
641 *E. singularis*: 26023; *E. verrucosa*: 4285. Kruskal-Wallis test of the differences among
642 groups: chi-squared = 7.9467, df = 3, p-value = 0.047.

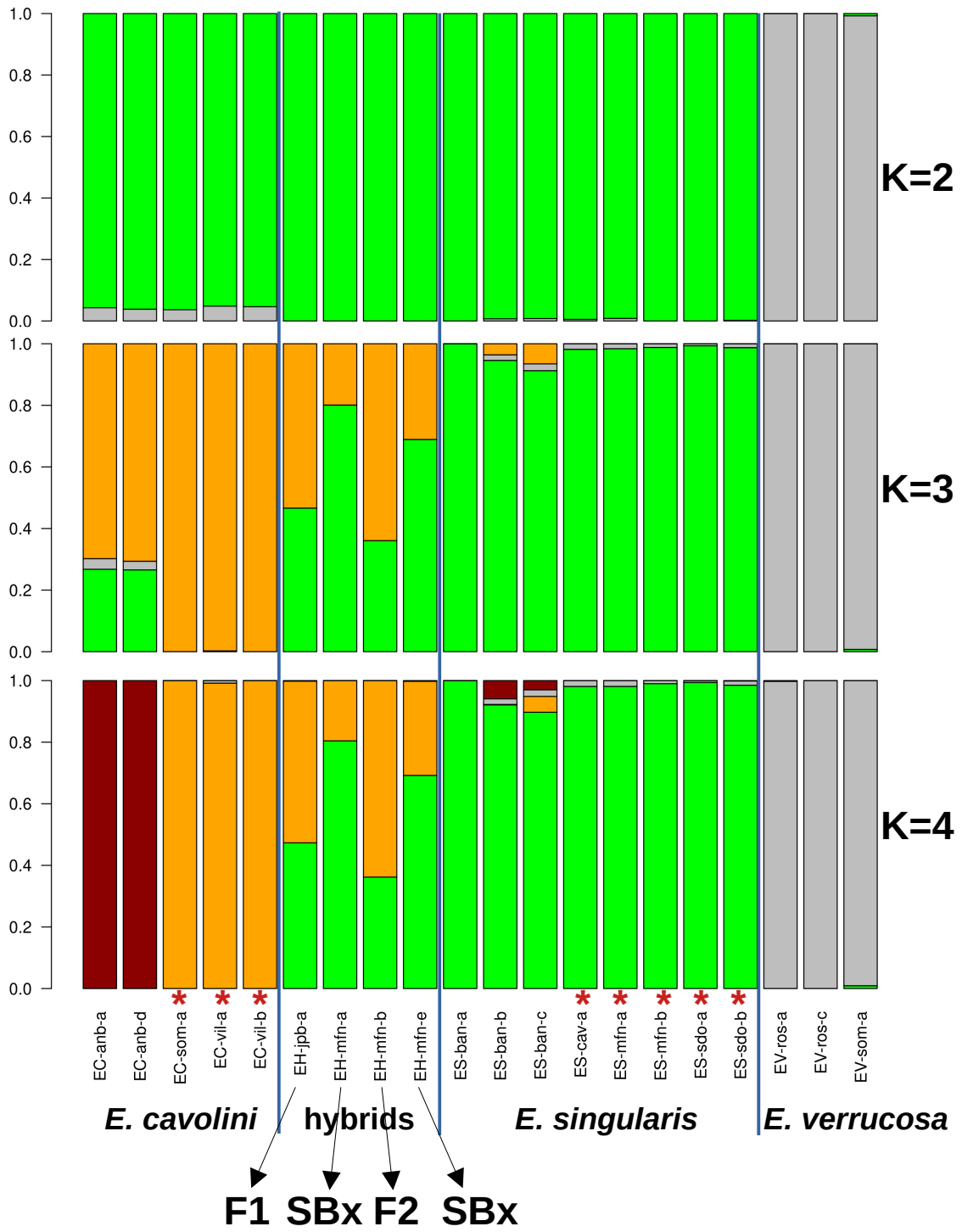


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645 **B)** Assembled sequences with BLAT; mean values per group: *E. cavolini*: 0.0034; hybrids:
646 0.0029; *E. singularis*: 0.0219; *E. verrucosa*: 0.0028. Kruskal-Wallis test of the differences
647 among groups: chi-squared = 14.352, df = 3, p-value = 0.002.



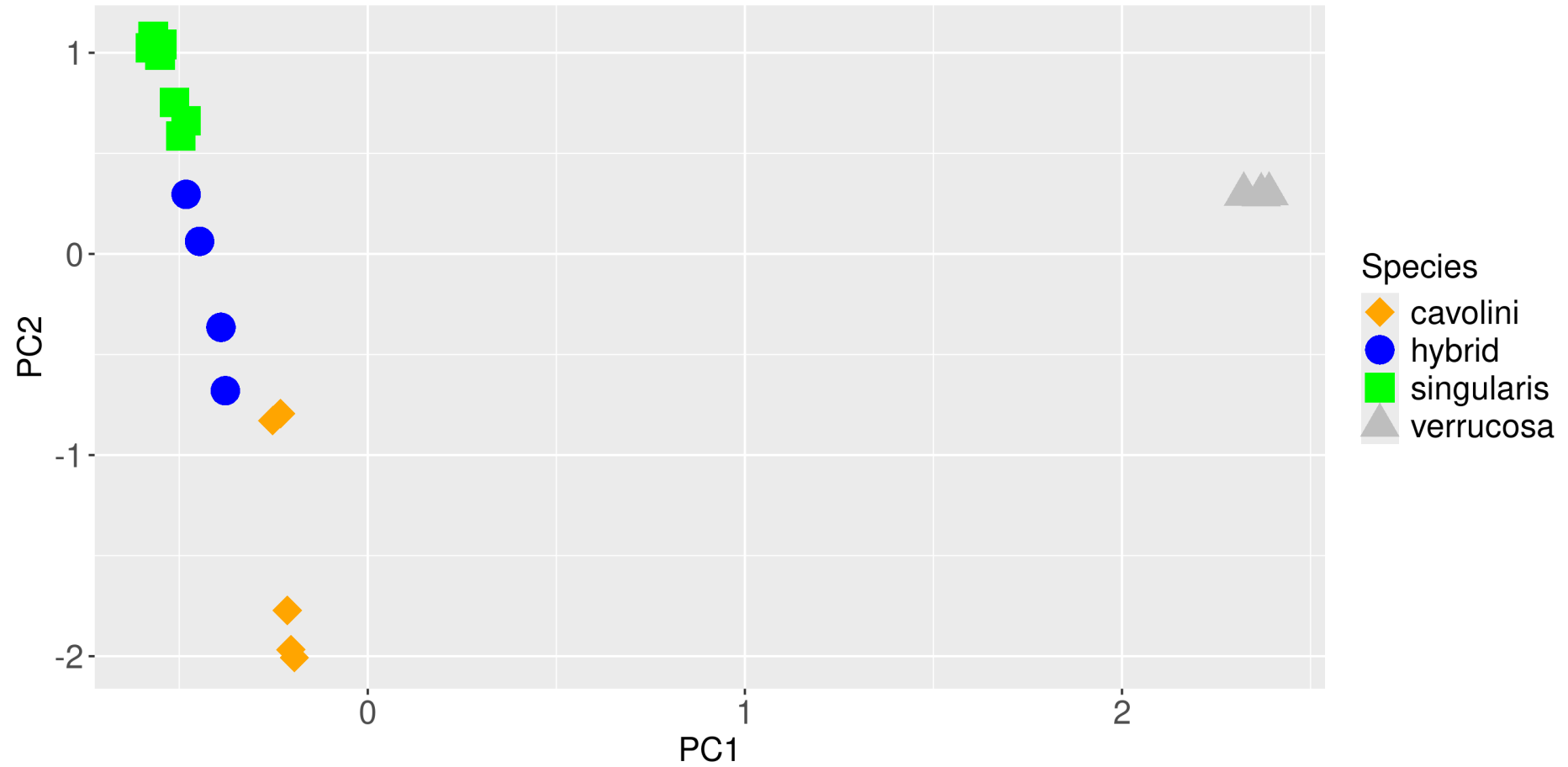
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649 **Figure 3:** barplots of coancestry coefficients inferred with the LEA R package. The analysis
650 is based on the “polymorphic sites” transcriptome dataset. The red asterisks indicate the
651 individuals used as prior for parental status in the NewHybrids analysis. The results of the
652 NewHybrids analysis are indicated below the hybrid individuals: F1, 1st generation; F2, 2nd
653 generation; Sbx, backcross with *E. singularis*. The coancestry analysis is based on 31 369
654 SNPs, whereas the NewHybrids analysis is based on 326 SNPs showing high differentiation
655 between *E. cavolini* and *E. singularis*.
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Figure 4: principal Component Analysis based on the “polymorphic SNPs” transcriptome dataset; axis 1 represents 33.2% of the variance, axis 2 represents 13% of the variance



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666 **Table 1** : inference of hybrid status with NewHybrids for transcriptome and RAD sequencing.
667 For transcriptomes, all probabilities were at 1 for the inferred status and for the ten
668 replicates. For RAD sequencing, the results are given for the four datasets (different
669 assembly strategies). If no probability is mentioned for RAD sequencing, the hybrid status
670 was supported by a probability higher than 0.999 over the ten replicates. In the other cases,
671 the numbers indicate the minimal probability threshold over the ten replicates for this status
672 (and the status was coherent over the ten replicates as well, with slight variations in
673 probability). NA indicates an individual which was removed during the filtering of SNPs
674 because of too many missing data. The lines highlighted in grey indicate the cases where
675 different status were inferred depending on the dataset. Bx-ES and Bx-EC indicate
676 backcrosses with *E. singularis* and *E. cavolini* respectively; ES indicates parental
677 *E. singularis*.
678

Individual - RAD sequencing	de novo	ref. <i>E. cavolini</i>	ref. <i>E. singularis</i>	ref. <i>E. verrucosa</i>
EC-X-MFNB	F2	F2	F2	F2
EC-X-MFNC	F2	NA	Bx-EC	NA
EC-X-MFND	Bx-ES	Bx-ES	Bx-ES	Bx-ES
EC-X-MFNE	Bx-EC	Bx-EC	Bx-EC	Bx-EC
EC-X-MFNF	Bx-EC	F2 > 0.95	Bx-EC > 0.92	F2
EC-X-MFNG	F2	F2	F2	F2
EC-X-MFNH	Bx-ES	Bx-ES	Bx-ES > 0.67	Bx-ES > 0.98
EC-X-MFNI	F1	F1	F1	F2
EC-X-MFNL	F1	F1 > 0.99	F1	F2
ES-X-MFNA	Bx-ES	Bx-ES	Bx-ES	Bx-ES
ES-X-MFNJ	F2	F2 > 0.96	F2	F2
ES-X-MFNK	ES	ES	ES	ES
Individual - transcriptome				
EH-JPB-a	F1			
EH-MFN-a	Bx-ES			
EH-MFN-b	F2			
EH-MFN-e	Bx-ES			

679

680 **Table 2:** results of demographic inferences with DILS with transcriptome data. The columns indicate the species comparison, the model choice for
681 population size (constant vs. variable), and the results of inferences: current (on-going) gene flow (migration vs isolation); if current migration is
682 inferred DILS compares isolation / migration (IM) with secondary contact (SC); if no current migration is inferred, the comparison is between strict
683 isolation (SI) and ancestral migration (AM); the last columns give the results of the tests of homogeneity or heterogeneity among loci for inferences in
684 effective size (N-homo vs N-hetero), and gene flow (M-homo vs M-hetero). The probability of each scenario is given in the same case. Homogeneity
685 and heterogeneity indicate no variation or variation among loci respectively.
686

Comparison	Population size	Current gene flow	IM / SC	SI / AM	N-hetero / N-homo	M-hetero / M-homo
<i>cavolini / singularis</i>	constant	Migration; 0.87	SC; 0.79	-	N-hetero; 0.99	M-homo; 0.82
<i>cavolini / singularis</i>	variable	Migration; 0.88	SC; 0.77	-	N-hetero; 1	M-homo; 0.87
<i>cavolini / verrucosa</i>	constant	Isolation; 0.90	-	AM; 0.65	N-hetero; 1	-
<i>cavolini / verrucosa</i>	variable	Isolation; 0.89	-	AM; 0.69	N-hetero; 1	-
<i>singularis / verrucosa</i>	constant	Isolation; 0.87	-	AM; 0.61	N-hetero; 1	-
<i>singularis / verrucosa</i>	variable	Isolation; 0.87	-	AM; 0.61	N-hetero; 1	-

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