Chaotic Genetic Patchiness in the Highly Valued Atlantic Stalked Barnacle Pollicipes pollicipes From the Iberian Peninsula: Implications for Fisheries Management

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23   **stock management, connectivity.**

24   **Abstract**

25   The stalked barnacle *Pollicipes pollicipes* inhabits rocky shores from the Atlantic coasts Brittany
26   (France) to Senegal. Because of the culinary traditions of southern Europe, stalked barnacles
27   represent an important target species for local fisheries on the Iberian Peninsula. To manage this
28   fishery sustainably, it is therefore important to assess the dynamics of local populations over the
29   Iberian coast, and how they are interconnected at a wider scale using finely tuned genetic markers. In
30   this work, a new enriched library of GT microsatellites for *P. pollicipes* was prepared and sequenced
31   using Ion TorrentTM Next Gen-Sequencing technology. 1423 adults and juveniles were sampled in
32   15 localities of three geographic regions: southern Portugal, Galicia and Asturias (both in northern

33 Spain). Twenty polymorphic loci arranged in five multiplex PCRs were then tested and validated as
34 new molecular tools to address the spatial and temporal genetic patterns of *P. pollicipes*. Our results
35 revealed high genetic diversity among adults. However, juveniles were genetically more structured
36 than their adult counterparts, which alternatively displayed much more connectivity among the three
37 studied regions. The lack of spatial genetic heterogeneity in adults may be due to the overlapping of
38 several generations of settlers coming from different geographic origins, which mainly depends on
39 the orientation of residual currents along the coast during reproduction. The genetic differentiation of
40 juveniles may indeed be congruent with Iberian Peninsula hydrodynamics, which can produce
41 chaotic genetic patchiness at small temporal scales due to sweepstake reproductive success, collective
42 dispersal and/or self-recruitment. Remarkably, most of the genetic heterogeneity of juveniles found
43 in this work was located in Galicia, which could represent an admixture between distinct
44 metapopulations or an old refuge for most northern populations. To conclude, high genetic variation
45 in *P. pollicipes* can lead to the false impression of population panmixia at the Iberian scale by
46 masking more restricted and current-driven larval exchanges between regions. This possibility should
47 be taken into consideration for further specific management and conservation plans for the species
48 over the Iberian Peninsula.

49 **1   Introduction**

50 The percentage of stocks exploited at biologically unsustainable levels increased from 10% in 1974
51 to 34.2% in 2017 (FAO, 2020) after decades of management strategies based on catch-rate
52 limitations (i.e., the EU Common Fisheries Policy). As an alternative or complementary approach,
53 management practices are increasingly incorporating the spatial allocation of fishing intensity
54 through marine protected areas, marine zoning, or spatial user rights, particularly for sessile or low-
55 motility species (Lorenzen et al., 2010; Rassweiler et al., 2012). Optimization of these processes
56 depends on the accurate estimation of the connectivity among management units, mediated by the
57 dispersal of the planktonic larval stages (Silva et al., 2019). In this regard, a fundamental issue
58 concerns whether the dispersal scales are consistent with the management scales (Ouréns et al.,
59 2015). Although advection by ocean currents should lead to long dispersal distances exceeding the
60 scale of management, there is increasing evidence that long-distance dispersal may be rare on
61 ecological time scales (D'Aloia et al., 2015; Palumbi, 2003; Selkoe et al., 2010). This phenomenon
62 can be explained by a combination of seascape characteristics such as eddies, gyres or upwellings of
63 deep water bodies and specific larval behavior that would favor local retention and reduced dispersal
64 (Morgan et al., 2009, 2018; Barshis et al., 2011; Kough et al., 2013). An additional line of evidence
65 reveals surprising patterns of spatial and temporal genetic structure observed in some marine species
66 at a scale where genetic variation should be efficiently homogenized by gene flow via larval
67 dispersal, collectively coined chaotic genetic patchiness (CGP) (Johnson and Black, 1982;
68 Hedgecock and Pudovkin, 2011; Eldon et al., 2016).

69 The stalked barnacle (*Pollicipes pollicipes*) is a pollicipedomorph cirriped (Chan et al., 2021)
70 inhabiting rocky coasts that are highly exposed to waves in the northeast Atlantic. Its range extends
71 from southwestern England through the coasts of Brittany (France), Spain, Portugal, and West Africa
72 to Dakar (Senegal) (Barnes, 1996; Barnes, 2008; Southward, 2008; Fernandes et al., 2010). In the
73 Iberian Peninsula, it represents a highly valued resource that reaches very high market prices due to
74 an old gastronomic tradition (Molares and Freire, 2003; Jacinto et al., 2011; Rivera et al., 2014).
75 Remains of its consumption have been found in early Holocene archaeological sites, mainly
76 associated with Mesolithic and Neolithic shell-middens on both the Atlantic and Mediterranean
77 coasts (Álvarez-Fernández et al., 2010, 2013; Álvarez-Fernández, 2011). Between 2013 and 2016,
78 the European stalked barnacle fisheries have an annual economic value of EUR 10 million, involving

79 approximately 500 t of landings and 2,100 professional fishers (Aguión et al., 2021). At some
80 localities, the pressure exerted by poachers can be extremely high (more than 60% of their catches),
81 especially in banned areas or periods (Jacinto et al., 2010; Rivera et al., 2014; Ruiz-Díaz et al., 2020).

82 Management of the stalked barnacle fishery in the Iberian Peninsula is highly heterogeneous (Aguión
83 et al., 2021). In Galicia (NW Spain) since 1992, the regional government has developed a co-
84 management system between fishers' guilds ("cofradías") and the fisheries authority through
85 territorial user rights for fishing (TURFs) (Molares and Freire, 2003; Macho et al., 2013), where
86 exclusive right of access are granted to fishing communities (Costello et al., 2010; Rivera et al.,
87 2014). Similarly, in the West coast of Asturias (N Spain), the barnacle fishery has been managed
88 through a co-management system with TURFs since 1994 (Rivera et al., 2014, 2017). Both Galicia
89 and western Asturias present adaptive spatial management with nested scales at regional, local and
90 patch/rock levels; recognized to promote fisheries sustainability (Aguión et al., 2021). However, on
91 the eastern coast of Cape Peñes (eastern Asturias) and Portugal, the fishery is managed at a regional
92 scale through general regulations without management plans (Aguión et al., 2021). In Portugal,
93 however, there are two protected areas subjected to specific regulations for harvesting *P. pollicipes*:
94 the Reserva Natural das Berlengas (RNB) and the Parque Natural do Sudoeste Alentejano e Costa
95 Vicentina (PNSACV) (Sousa et al., 2013; Cruz et al., 2015; Carvalho et al., 2017). The first one
96 (RNB) is subjected to local management, resembling a TURF in many aspects (Aguión et al., 2021).
97 Currently, there is interest and potential for developing co-management systems similar to the one in
98 Galicia and western Asturias in both Portuguese protected areas (Cruz et al., 2015; Sousa et al.,
99 2020). Among different management approaches, TURFs represent the best option for the sustainable
100 management of small-scale sessile fisheries (Gutiérrez et al., 2011; Rivera et al., 2017; Aguión et al.,
101 2021). However, the design of management areas mandates a good understanding of population
102 renewals for which estimates of connectivity are crucial (Aceves-Bueno et al., 2017; Silva et al.,
103 2019). Dispersal, settlement, and subsequent recruitment are decisive processes in the population
104 dynamics of marine invertebrates with planktonic larval stages, allowing the connection between
105 remote populations and leading to meta-populations that are globally viable (Cowen and Sponaugle,
106 2009).

107 *P. pollicipes* larvae go through six planktotrophic *nauplius* stages before turning into a lecithotrophic
108 stage, called *cypris*. According to Molares et al. (1994) and Franco et al. (2016, 2017), the pelagic
109 larval development is finalized after 15 days to one month under optimal conditions in the laboratory,
110 whereas in the natural environment, the total pelagic larval duration is estimated to last two months
111 (Cruz, 2000; Macho et al., 2006). The presence of stalked barnacles on the shore might favor the
112 settlement of *cyprids*, because recruitment is intense on conspecifics (e.g. Cruz et al. (2010);
113 Fernandes et al. (2021)). The dynamics of ocean circulation are recognized as important aspects in
114 shaping connectivity patterns among marine populations (Treml et al., 2008). In this situation,
115 significant effort is required to study population dynamics locally to adequately manage the resource
116 (Molares and Freire, 2003). For *P. pollicipes*, a minimum potential passive migration distance of 600
117 km during the planktonic stage has been suggested (Quinteiro et al., 2007); nevertheless, reanalysis
118 of genetic data and basic biophysical modeling point to modest dispersal distances in the range of
119 tens of kilometers in the Asturian region (Rivera et al., 2013). At a large spatial scale, it has been
120 suggested that *P. pollicipes* displays a metapopulation structure, where disconnected adult
121 populations share a common larval pool (the n-islands model hypothesis) (Molares and Freire, 2003).
122 However, the metapopulation structure has not yet been addressed. Alternatively, species with long
123 larval dispersal potential, such as *P. pollicipes*, may exhibit surprising patterns of spatial and
124 temporal genetic structure. CGP (Johnson and Black, 1982; Hedgecock and Pudovkin, 2011; Eldon et
125 al., 2016) has been consistently reported in marine species that broadcast larvae at a scale where

126   genetic variation should be efficiently homogenized by gene flow via larval dispersal. Eldon et al.
127   (2016) reviewed and discussed how selection, sweepstake reproductive success, collective dispersal,
128   and temporal shifts in local population dynamics may play a crucial role in generating such
129   unexpected patterns. Moreover, Pineda et al. (2006) reported the existence of "recruitment windows"
130   in a close barnacle species (*Semibalanus balanoides*), in which after a recruitment period of
131   approximately 3 months, only recruits able to settle in just a couple of weeks survive after settlement
132   and mature into adults. In spite of its interest for the management of this species, the processes that
133   shape the genetic structure of *P. pollicipes* in the Atlantic Ocean have not been studied.

134   Genetic markers are a powerful tool for fisheries management because they present an array of very
135   useful applications: they can address the correct identification of species, delimit distinct fish stocks
136   (Borrell et al., 2012; Papa et al., 2020), assess relatedness levels within populations (Veliz et al.,
137   2006; Plough et al., 2014), expose population connectivity (Pascual et al., 2017; Muñoz-Ramírez et
138   al., 2020), estimate larval dispersal (Van Wyngaarden et al., 2017) and larval diversity (Chen et al.,
139   2013; Wong et al., 2014; Alshari et al., 2021) or the source-sink dynamics within the population
140   structure (Pineda et al., 2007; Brault et al., 2013; Lindegren et al., 2014). Genetic data, however,
141   integrate information on the past demographic history of populations and are not always easily
142   applicable for the present-days management for marine species with high fecundity and dispersal
143   capabilities (Gagnaire et al., 2015). Estimating some of the population parameters that are crucial for
144   stock management imposes the need to develop numerous highly polymorphic markers. These will
145   help to discriminate between past and present-day processes that shape populations of species with
146   highly effective population sizes (e.g. Hongjamrassilp et al. 2020). Despite the economic relevance of
147   the *P. pollicipes* fishery, only a few articles have been published on the genetics of the stalked
148   barnacles, most of which are based on mitochondrial markers (Quinteiro et al., 2007; Campo et al.,
149   2010; Rivera et al., 2013). According to Quinteiro et al. (2007), the panmixia hypothesis is rejected,
150   and 5 population groups are established: 1) Brittany; 2) Asturias-East; 3) Galicia, Portugal and
151   Morocco; 4) Canary Islands and 5) Cape Verde Islands, with the latter being extremely divergent.
152   The Cape Verde population was later considered a new species (Van Syoc et al., 2010) and described
153   as *Pollicipes caboverdensis* (Fernandes et al., 2010). Campo et al. (2010) revealed genetic differences
154   among populations between Brittany (France) and the rest of the species distribution range, while
155   Rivera et al. (2013) described small-scale, asymmetric connectivity in gooseneck barnacle
156   populations, when reanalyzing data from Campo et al. (2010) for the Cantabrian coast.
157   Microsatellites usually display high levels of genetic variation and can detect subtle genetic
158   differentiation among populations separated by only a few hundred kilometers (Borrell et al., 2012).
159   Moreover, they seem to be very useful to detect parentage/familial structures, when assessing the
160   origin of recruits (St-Onge et al., 2015; Couvray and Coupé, 2018; Dubé et al., 2020). Microsatellite
161   markers have been previously developed and, in some cases, used to infer the population genetic
162   structure for several closely related acorn barnacles, such as *S. balanoides* (Dufresne et al., 1999;
163   Flight et al., 2012); *Chthamalus montagui* (Pannacciulli et al., 2005; Fontani, 2009); *Tetraclita* spp.
164   (Dawson et al., 2010; Chen et al., 2015); *Megabalanus coccopoma* (Reigel et al., 2015); *Chelonibia*
165   *testudinaria* (Ewers-Saucedo et al., 2016, 2017); *Notochthamalus scabrosus* (Barahona et al., 2019)
166   and two stalked barnacle species: *Pollicipes elegans* (Plough and Marko, 2014) and *P. pollicipes*
167   (Fernandes et al., in prep.; Seoane-Miraz et al., 2015). The latter appear to have shown positive
168   results with specific cross-amplifications in the congeners *P. elegans*, *P. polymerus*, and *P.*
169   *caboverdensis*.

170   The aim of the present study was to revisit and test the previously described genetic homogeneity of
171   *P. pollicipes* at the scale of the Iberian Peninsula with highly polymorphic microsatellite markers.
172   The final goal is to provide support for the design of adequate and sustainable fishery management

173 plans, using an in-depth analysis of genetic patterns inferred from a hierarchical geographic sampling
174 of the barnacle populations along the Iberian coastline. However, preliminary tests using published
175 microsatellite markers have provided inconsistent and nonreproducible PCR results in two different
176 and independent genetic labs, necessitating the development of new highly variable genetic markers
177 for the species *P. pollicipes* (this study).

178 ## 2   Materials and Methods

179 ### 2.1   Study area and sampling

180 A total of 1423 individuals from 15 different localities belonging to three Atlantic regions of the
181 Iberian Peninsula covering the most important spots of the barnacle fishery were sampled. These
182 three regions were SW Portugal, Galicia (NW Spain), and W Asturias (N Spain). Thus, a hierarchical
183 sampling of populations was performed in which five distinct localities were sampled within each
184 region (Fig. 1). The five localities belonging to Portugal are Aljezur (AL), Azenha do Mar (AZ),
185 Cabo Sardão (CO), Malhão (MA) and Sines (CS). The five localities belonging to Galicia are Baiona
186 (BA), Cabo Home (CH), Aguiño (AG), Camelle (CA) and A Coruña (AC). The five localities
187 belonging to Asturias are La Cruz (PC), El Cuerno (CU), Las Llanas (LM), La Erbosa (ER) and El
188 Corviru (EC). (Fig. 1; Table 2). Samples were transferred to the laboratory and frozen on the same
189 day of collection until further individualization and labeling.

190 Within each of the targeted localities, one hundred individuals collected in September and October
191 2017 were randomly sampled within two distinct developmental cohorts according to their rostrum-
192 carinal (RC) length (see Fig. 4 in Parada et al. (2013)) (50 adults of commercial size greater than >18
193 mm; 50 juveniles between 2 and 4 mm). As barnacles are usually found in groups of sessile
194 individuals, fixed on primary rocky substrates with small juveniles attached to adult peduncles (Cruz
195 et al., 2010), juveniles were first removed from the adults, avoiding the collection of more than one
196 juvenile by adult and then treated secondarily. Each barnacle was put individually in a tube
197 previously labeled and preserved in absolute ethanol at room temperature. In the laboratory, a small
198 portion of the peduncle muscle was dissected from each individual for genomic DNA extraction. In
199 the case of adults, special care was taken to dissect the tissue from the inner part of the peduncle to
200 avoid possible contamination by attached post-larvae (*cyprids*) and juveniles.

201 To characterize the typical upwelling circulation during the stalked barnacle larval season in
202 summer/autumn 2017 along the coasts of northern and western Iberia, sea surface temperature (SST)
203 along with modelled sea surface currents datasets were retrieved during the peak of meridional
204 Ekman transport at central Portugal on 11-08-2017. Daily 4km SST data were obtained from the all-
205 satellites combined Copernicus´ product
206 (https://cds.climate.copernicus.eu/cdsapp#!/dataset/satellite-sea-surface-
207 temperature?tab=overview). The 5-days averaged meridional and zonal components of the surface
208 currents were obtained from the OSCAR model with a spatial resolution of 0.33°
209 (https://coastwatch.pfeg.noaa.gov/erddap/griddap/jplOscar_LonPM180.html).

210 ### 2.2   Microsatellite markers and multiplex PCR development

211 Genomic DNA from five adult individuals was extracted using the EZNA® Mollusk Kit (Omega Bio-
212 Tek Inc., Norcross, GA, USA). An enriched biotin-labeled CT/GT library for dinucleotides was
213 obtained using the methodology described by Bloor et al. (2001) and Sotelo et al. (2007), where
214 DNA was digested with HaeIII (NEB). Digestions were run in 1.5% agarose gels stained with
215 ethidium bromide. Fragments between 400 and 800 bp were excised from gels and purified using a

QIAquick Gel Extraction Kit (Qiagen). Fragments were ligated to a double-stranded adaptor using ligase (NEB) and enriched by PCR using oligoA. Purified PCR products were denatured and incubated with 200 pmol of 5' biotinylated $(CT)_{12}$ and $(GT)_{12}$ probes (Invitrogen) attached to streptavidin-coated magnetic beads (Streptavidin MagneSphere Paramagnetic Particles, Promega). Hybridization was carried out in 6 SSC for 30 min at 60°C in a thermocycler. Specific fragments were recovered after washing the bead suspension with solutions progressively desalted at 60°C, and subsequently amplified using Oligo A. A DNA library was prepared using an Ion Plus Fragment Library Kit (Thermo Fisher Scientific, Austin, TX, USA) according to the manufacturer's protocol. Next-generation Ion Torrent sequencing of the library was conducted using the Ion Torrent platform on an Ion PGM System (Life Technologies) using Ion PGM 400 sequencing reagents and Ion 318v2 chips following the manufacturer's instructions at the University of Vigo Central Services (CACTI). Quality control procedures and filtering of the resulting reads were afforded using PRINSEQ software (Schmieder and Edwards, 2011). Tag Sequence Check and Sequence Duplication routines were used to trim adapters and eliminate duplicates. Sequences shorter than 100 bp with a mean quality Phred score lower than 20 were removed. Tandem Repeats Finder (Benson, 1999) was used with all the parameters by default for locating and displaying tandem repetitions in DNA sequences. Forward and reverse primers were designed for effective microsatellite amplifications using FastPCR 6.5 software following Kalendar et al.'s (2009) recommendations. Finally, primers were proposed and tested by individual PCR on 30 individuals of *P. pollicipes* from 3 distinct geographic populations: 10 individuals from Baiona (Galicia, Spain), 10 individuals from Los Xatos (Asturias, Spain) and 10 individuals from Toulbroc'h (Brittany, France) (Fig. 1). PCR tests were equally subdivided between different research laboratories with 41 primers tested per laboratory at the University of Vigo, the University of Oviedo and the Roscoff Marine Station. In this way, microsatellite markers were calibrated between geographic regions and the three institutes involved in the project.

Individual PCRs were conducted in a 20 μL total volume with Green GoTaq® Flexi Buffer (1x) (Promega Corporation, Madison, WI, USA), $MgCl_2$ (2.5 mM), dNTPs (0.5 mM), 0.2 μM of each primer, 0.1 U of GoTaq® G2 Flexi Polymerase (Promega Corporation, Madison, WI, USA), and 50 ng of DNA in sterile distilled water. The PCR program included an initial 5 min denaturation step at 95°C, 35 cycles of denaturation at 95°C for 30 s, annealing at 57°C for 30 s and elongation at 72°C for 30 s. PCR products were visualized using electrophoresis on a 2% agarose gel stained with SimplySafe[TM] (EURx, Gdańsk, Poland). Primer pairs without amplification, leading to a multiband pattern or a band size differing from its expected size, were discarded. Twelve microsatellite loci were amplified reliably and arranged in three multiplex PCRs (M1, M2 and M3 with four microsatellite markers per multiplex each) using Multiplex Manager 1.2 software (Holleley and Geerts, 2009) according to the dye colors and expected amplicon sizes. In addition to the twelve microsatellite markers retained with this screening, eight microsatellite markers previously developed in a parallel study (Fernandes et al., in prep) were tested, calibrated, and added in two supplementary multiplexes (M4 and M5) following the previously detailed methodology. This process resulted in a total of 5 multiplex PCRs. Forward primers were labeled using fluorescent dyes: 6-FAM[TM], NED[TM], VIC® and PET® (Applied Biosystems, Foster City, CA, USA) (Table 1). PCR products were sequenced at the Genomer platform of the Roscoff Marine Station and at Servicios Científico-Técnicos of the University of Oviedo. Allele sizes were manually scored using GeneMapper v.4.0 (Applied Biosystems, Foster City, CA, USA).

2.3   Multiplex PCR and microsatellite genotyping

261 As explained above, all adult DNA was extracted with the EZNA® Mollusk Kit (Omega Bio-Tek
262 Inc., Norcross, GA, USA). Juvenile DNA was extracted using the Chelex® 100 (Bio-Rad
263 Laboratories Inc., Hercules, CA, USA) method (Estoup et al., 1996). PCRs were carried out
264 following a unidirectional workflow that started in a pre-PCR room to prepare PCR plates.
265 Amplification by PCR and processing of the subsequent PCR products always took place in a post-
266 PCR area to avoid any possible contamination. M1, M2 and M3 multiplex PCRs were conducted
267 using the QIAGEN Multiplex PCR Kit (QIAGEN Inc., Venlo, The Netherlands) in a final reaction
268 volume of 13 μL with the following components: 1x QIAGEN Multiplex PCR Master Mix, 1x Q-
269 Solution, 50 ng of DNA template and 0.2 to 0.5 μM of each primer (Table 1). PCR conditions
270 consisted of an initial denaturation step at 95°C for 15 min, followed by 40 cycles at 94°C for 30 s,
271 an annealing temperature of 60°C (M1 and M2) or 64°C (M3) for 1:30 min and 72°C for 1 min, with
272 a final extension at 60°C for 30 min. M4 and M5 multiplex PCRs were incorporated and tested in a
273 later stage and they were conducted using the TouchDown PCR technique (Hecker and Roux, 1996).
274 TouchDown PCRs were conducted in a 15 μL total volume with Colorless GoTaq® Flexi Buffer (1x)
275 (Promega Corporation, Madison, WI, USA), MgCl$_2$ (1.5 mM for M4 and 1.16 for M5), dNTPs (0.1
276 mM), 0.06 - 0.13 μM of each primer (Table 1), 0.4 U of GoTaq® G2 Flexi Polymerase (Promega
277 Corporation, Madison, WI, USA), 200 ng/μL bovine serum albumin (BSA), and 5 to 10 ng of DNA
278 in distilled water. The samples were initially heated at 95°C for 5 min, followed by 10 cycles
279 consisting of 95°C for 30 s, 60°C (decreasing incrementally by 0.5°C per cycle) for 40 s, and 72°C
280 for 40 s, followed by 25 cycles at 95°C for 30 s, 55°C for 40 s, and 72°C for 40 s, culminating in a
281 final cycle at 72°C for 10 min. PCR results were checked on a 2% agarose gel. For each multiplex
282 amplification, 2 μL of reaction product (diluted 1/40 with Milli-Q water for M1, M2 and M3) was
283 mixed with 9.5 μL of Hi-Di formamide (Applied Biosystems, Foster City, CA, USA) and 0.50 μL of
284 SM594 molecular weight marker (Mauger et al., 2012). The mixture was heated at 94°C for 5 min,
285 immediately chilled on ice for 2 min, loaded in an ABI Prism® 3130XL automatic sequencer
286 (Applied Biosystems) of 16 capillaries using POP-7 polymer and run at 60°C, 15 kV, 1200 s using
287 the sequencing platform Plateforme Genomer (Station Biologique de Roscoff). To ensure that the
288 allele spread calibration held between the set of samples analyzed, controls were included in each
289 plate to be genotyped as reference genotypes. Each genotype was then scored after analyzing the
290 amplification products with Genemapper 4.0 (Applied Biosystems, Foster City, CA, USA).

291 **2.4   Population genetic analyses**

292 The allele frequencies, number of alleles per locus (k), observed heterozygosity (H$_O$) and unbiased
293 expected heterozygosity (H$_E$) were calculated with GENETIX 4.05 (Belkhir et al., 2004). Moreover,
294 possible genotyping errors and null allele frequency estimation were conducted using MICRO-
295 CHECKER 2.2.3 (Van Oosterhout et al., 2004) and FreeNa (Chapuis and Estoup, 2007) with a
296 number of replicates fixed to 10 000. Moreover, to explore the influence of null alleles on data we
297 assessed F_{IS} and F_{ST} correlation, F_{IS} and the number of missing data (putative null homozygotes)
298 correlation and estimated the StrdErrF_{IS} and StrdErrF_{ST} values following the De Meeûs (2018) and
299 Manangwa et al. (2019) (but see Waples (2018)). The significance of correlations was tested with a
300 unilateral ($\rho > 0$) Spearman's rank correlation test with Rcmdr package (Fox, 2005; Fox, 2007) for R.
301 Furthermore, for each population, the number of private alleles was calculated with GENALEX
302 6.5.03 (Peakall and Smouse, 2012).

303 Possible deviations from expected proportions in Hardy Weinberg's equilibrium and linkage
304 disequilibrium for each locus and population were assessed using FSTAT 2.94 software (Goudet,
305 1995). FSTAT 2.94 software (Goudet, 1995) was used to calculate the allelic richness (A$_R$) and to
306 determine the fixation indices (F-statistics) within and across populations using the method described

307 by Weir and Cockerham (1984). Significance levels of F_{IS} were estimated by permutating alleles
308 between genotypes within samples 2000 times and adjusted following Bonferroni correction (Rice,
309 1989) from all tested juvenile and adult samples. To test self-recruitment, the relatedness between
310 individuals (R_{XY}) was estimated with the "related" package in R (Pew et al., 2015). The relative
311 performance of seven different relatedness estimators was examined (dyadml, lynchli, lynchrd,
312 quellergt, ritland, trioml and wang) through comparison of the observed values to expected values
313 generated from a simulated sample set of 400 individuals of known relatedness (with one hundred
314 individuals from 4 categories: parent-offspring ($R_{XY} = 0.500$), full-sib ($R_{XY} = 0.500$), half-sib ($R_{XY} =$
315 0.250) and unrelated pairs ($R_{XY} = 0.000$)). The results showed that the dyadic likelihood relatedness
316 estimator (dyadml) provided the most consistent estimates through all possible levels of kinship;
317 therefore, it was performed with 500 iterations. The bottleneck hypothesis was tested using the
318 software BOTTLENECK 1.2.02 (Piry et al., 1999) under the two-phased model of mutation (TPM),
319 taking into account 90% single stepwise mutations with a variance of 12.

320 Comparisons between regions and between cohorts (adults and juveniles) were conducted using a
321 two-sided statistical analysis included in the FSTAT software for several statistics (A_R, H_O, H_E, F_{IS},
322 F_{ST}, relatedness (R) and corrected relatedness). In addition, F_{ST} values were estimated using FreeNa,
323 which estimates unbiased F_{ST} following the ENA method (Chapuis and Estoup, 2007). The F_{ST}
324 values and associated p-values between cohorts and within and between regions were also calculated
325 using FSTAT 2.94 (Goudet, 1995) to test for the regional and local structure. To assess the
326 significance levels of F_{ST}, multilocus genotypes were permutated 2000 times between pairs of
327 samples, and the significance threshold was obtained by applying a false discovery rate (FDR) over
328 samples (Benjamini and Hochberg, 1995). Partial Mantel tests to estimate the correlation between
329 genetic and geographical distance were performed with FSTAT 2.94 (Goudet, 1995) using the INA
330 correction method for the chord distance (Cavalli-Sforza and Edwards, 1967) (D_{CSE}) provided by
331 FreeNA (Chapuis and Estoup, 2007) and combining a ln transformation of Haversine geographic
332 distances following Séré et al. (2017) and Rousset's $\theta/(1 - \theta)$ and a log transformation of Haversine
333 geographic distances with 10 000 permutations (Rousset, 1997).

334 The software BayeScan v2.1. (Foll and Gaggiotti, 2008) was used to identify candidate loci deviating
335 from neutral expectations from genetic data using differences in allele frequencies between
336 populations. Twenty pilot runs of 5000 iterations each, followed by an additional burn-in of 50 000
337 iterations and then 5000 samplings with a thinning interval of 10, were conducted. To correct for
338 multiple testing, the program computes q-values based on the posterior probability for each locus.
339 Loci with α-values significantly >0 and q-values < 0.05 were defined as "outliers" –, i.e., loci
340 putatively under directional selection. Loci with α-values significantly <0 were considered putatively
341 under balancing selection. The remaining loci were classified as neutral.

342 An analysis of molecular variance (AMOVA) implemented in Arlequin 3.5.1.3 (Excoffier et al.,
343 2005) to partition genetic variation across nested levels, regions and sites within regions was used.
344 For the AMOVA, the number of different alleles was used as a measure of genetic variation (F_{ST}-like
345 option in Arlequin), and 10 000 permutations were used to test for statistical significance. Moreover,
346 the "adegenet" package in R was used to estimate the genetic differentiation and visualize individual
347 clustering with principal component analysis (DAPC, Jombart, 2008; Jolliffe, 2011) among adults
348 and juveniles from each of the three regions separately and both among adults and among juveniles
349 for all three regions pooled together. A neighbor-joining (NJ) tree based on the pairwise Nei's genetic
350 distance D_A (Nei et al., 1983) for all microsatellites and localities (15 localities; adults and juveniles
351 grouped together) and then adding temporal cohorts as independent samples (i.e.: 15 localities and 2
352 cohorts, 30 samples) was constructed with the software POPTREEW (Takezaki et al., 2014) using 10

353 000 bootstraps and visualized in The Interactive Tree of Life (Letunic and Bork, 2019)
354 (https://itol.embl.de). Finally, STRUCTURE 2.3.4 (Pritchard et al., 2000) was also run to explore the
355 population structure with Bayesian clustering. STRUCTURE was run using the 15 localities and 30
356 samples using admixture (Gilbert et al., 2012; Novembre, 2016) and also using adults and juveniles
357 taken separately from the three regions (Portugal, Galicia and Asturias) in the same conditions to
358 explore putative genetic units. The settings used were an admixture model from K = 1 to K = 30 in
359 20 runs following Evanno et al. (2005) and (Gilbert et al., 2012). Assignment clusters were made
360 with burn-in periods of 20,000 and 200,000 Markov chain Monte Carlo repetitions. The most likely
361 value of K was chosen using the delta K statistic (Evanno et al., 2005) using STRUCTURE
362 HARVESTER software (Earl and VonHoldt, 2012), and visualization and grouping of the individual
363 STRUCTURE runs was performed using CLUMPAK (Kopelman et al., 2015).

364 **3   Results**

365 The typical upwelling circulation during the stalked barnacle larval season in summer/autumn 2017
366 along the coasts of northern and western Iberia, sea surface temperature (SST) along with modelled
367 sea surface currents datasets revealed that the SST patterns showed strong onshore advection of cold
368 waters (13-15°C) on the Galician and Portuguese shelves with upwelling filaments extending further
369 offshore especially at the upwelling centers of Fisterra, A Guarda and Cape da Roca (Fig 1). Slightly
370 onshore cooling indicative of upwelling was also observed along the western Cantabrian coast.
371 Westward and southward currents in the order of few cm/s off the Cantabrian and Atlantic shores,
372 respectively, clearly pointed to upwelling circulation (Fig. 1). These flows are weaker close to the
373 coast probably due to friction with the coastal boundary layer. Off southern/central Portugal in
374 between Cape da Roca and Cape San Vincente, an anticlockwise cyclonic eddy was apparent with
375 strong southward currents (>10 cm/s) along its western side. The dynamic structure of this feature
376 matched SST patterns remarkably well, with a warm core (18°C) surrounded by colder upwelled
377 waters (14°C).

378 The microsatellites markers development process produced libraries with a total amount of 42 860
379 reads showing a mean sequence length of 91.61 ± 103.29 bp (minimum length: 25 bp - maximum
380 length: 517 bp) and a mean GC content of $63.66 \pm 18.90\%$. A total of 10 781 sequences with a mean
381 sequence length of 244.48 ± 97.61 bp, a length range of 418 bp and a mean GC content of $50.30 \pm$
382 5.61%, resulted after quality control procedures. A total of 1140 sequences containing di, tri, tetra
383 and pentanucleotides were selected after locating and displaying tandem repetitions in DNA
384 sequences. Finally, 123 pairs of primers were proposed and tested in three different research
385 laboratories (University of Vigo, University of Oviedo and the Roscoff Marine Station). A new set of
386 twelve microsatellite loci currently arranged into three multiplex PCRs (M1, M2 and M3) was
387 developed for the stalked barnacle *P. pollicipes* in this work (Genbank accession numbers:
388 MW443103-MW443114). Moreover, eight previously developed microsatellite loci by Fernandes et
389 al. (in prep) were also tested and included in another two multiplexes (M4 and M5, Genbank
390 accession numbers: MZ576446-MZ576456). This procedure resulted in a total of 5 multiplex PCRs
391 (Table 1) leading to scorable and reproducible genotypes for all 20 microsatellite loci. None of these
392 loci showed evidence of linkage disequilibrium between alleles (p>0.05). These loci were highly
393 polymorphic and exhibited approximately 15% private alleles (n = 87) only present at one locality
394 (Table 1 and Table 2).

395 The number of alleles per locus (k) varied greatly from 10 to 63 between loci, with an average of
396 29.10, and yielded an average (min-max) allelic richness (A_R) of 12.170 (11.074-12.918) per locality.
397 The observed and expected heterozygosities across loci ranged from $H_O = 0.277$ (M2; OV89) and H_E
398 $= 0.404$ (M2; OV89) to $H_O = 0.905$ (M4; Ppol_09) and $H_E = 0.960$ (M5; Ppol_08), with observed and

399 expected multilocus mean heterozygosities equal to 0.627 (0.561-0.667) and 0.764 (0.746-0.785),
400 respectively (Table 1). All markers and all populations showed significant deviations from Hardy-
401 Weinberg equilibrium (mean F_{IS} = 0.179) due to heterozygote deficiencies (Table 2). When testing
402 these markers for null alleles with MICRO-CHECKER 2.2.3. (Van Oosterhout et al., 2004) and
403 FreeNA (Chapuis and Estoup, 2007), we found that heterozygote deficiency could be due to null
404 alleles for at least 8 highly polymorphic loci: RF12 locus (Brookfield 1 Statistic = 0.110); OV113
405 locus (B = 0.226); OV89 locus (B = 0.092); OV121 locus (B = 0.097); OV103 locus (B = 0.154);
406 RF03 locus (B = 0.224); Ppol_03 locus (B = 0.101) and Ppol_04 locus (B = 0.280) (Table 1). The
407 correlation between F_{IS} and F_{ST} appeared to be significant (Spearman's ρ = 0.606, p-value=0.005).
408 However, F_{IS} and the number of missing data (putative null homozygotes) were not correlated
409 (Spearman's ρ = 0.098, p-value=0.3402) and the standard error for F_{IS} (StrdErrF_{IS} = 0.044) was higher
410 than for F_{ST} (StrdErrF_{ST} = 0.001). The mean overall F_{ST} value for the 20 microsatellites was F_{ST} =
411 0.002 (P = 0.0001), and three loci clearly showed higher F_{ST} values (OV100 (F_{ST} = 0.006), OV89
412 (F_{ST} = 0.011) and RF03 (F_{ST} = 0.010)) (Table 1).

413 The comparative analysis for levels of genetic variation between regions (Portugal (PTL), Galicia
414 (GAL) and Asturias (AST)) revealed no significant differences for expected heterozygosities (HS)
415 (Table 2). Slight differences in genetic diversities were, however, observed depending on the
416 population parameter estimated. Galicia showed the highest values for allelic richness and observed
417 and expected heterozygosity in adults and juvenile populations (Table 2). Portugal showed the
418 highest number of private alleles (mean A_PPTL = 3.3), which was mainly attributable to adults (mean
419 A_P = 4.4) (Table 2). Significant differences in allelic richness were also observed for juveniles
420 (A_RPTL: 11.569; A_RGAL: 12.418; A_RAST: 11.900; p<0.01) and in observed heterozygosity for
421 adults (H$_O$PTL: 0.640; H$_O$GAL: 0.656; H$_O$AST: 0.615; p<0.05) and juveniles (H$_O$PTL: 0.605;
422 H$_O$GAL: 0.648; H$_O$AST: 0.601; p<0.01) (Table 2). This phenomenon was especially obvious in
423 Portuguese samples, where the average number of private alleles decreased by 50% (A_PAD: 4.4 to
424 A_PJV: 2.2) (Table 2). In this later region, significant differences were found between adults and
425 juveniles both in terms of allele richness (A_RAD: 12.318; A_RJV: 11.569; p<0.01) and observed
426 heterozygosity (H$_O$AD: 0.640; H$_O$JV: 0.605; p<0.05) or expected heterozygosity (H$_S$AD: 0.773;
427 H$_S$JV: 0.757; p<0.01) (Table 2). Globally, juveniles were also more related to each other (R$_{XY}$ =
428 0.067) than their adult (R$_{XY}$ = 0.058) counterparts, as indicated by relatedness analyses. Juveniles
429 from Portugal (R$_{XY}$ value = 0.073, p<0.002) and Asturias (R$_{XY}$ = 0.070, p<0.002) were much more
430 related than expected from panmixia. Bottleneck software showed that none of the 30 samples tested
431 (15 localities x 2 cohorts) exhibited a significant excess of predicted heterozygotes under the TPM
432 model and could not be considered to have experienced a recent genetic bottleneck (Table 2). When
433 the bottleneck hypothesis was tested with all juveniles and adults together (15 samples) and at the
434 regional scale (15 samples grouped in 3 regions, for 2 cohorts), the statistics remained non-significant
435 (Table 2).

436 According to the overall F_{ST}, there was no significant genetic differentiation of adults between and
437 within regions (Fig. 2a). Only 20 out of the 75 possible pairwise F_{ST} values between adult samples
438 from different regions (25.3%) showed p-values lower than the 0.05 cutoff value, and these critical
439 values were more often encountered between Galicia and Asturias (12/25=48%) (Fig. 2a). However,
440 no p-values remained significant after FDR correction (Fig. 2a). In contrast, the overall F_{ST} statistics
441 estimated for the juveniles between and within regions indicated notable regional and spatial
442 structuring (Fig. 2b). Pairwise F_{ST} estimated between juvenile samples from Galicia and Portugal
443 (12/25=48% before and 6/25=24% after FDR) and between Galicia and Asturias (13/25=52% before
444 and 6/25=24% after FDR) confirmed this trend and showed clear regional structuring (Fig. 2b).
445 Asturias and Portugal were, however, less differentiated from each other, with fewer significant

446 pairwise F_{ST} values (3/25=12% before and 2/25=8% after FDR) (Fig. 2b). Some spatial structuring
447 within regions was detected for juveniles using pairwise F_{ST} analyses but only in the case of Portugal
448 (2/15=13% before and 1/15=6% after FDR) (Fig. 2b). The pairwise F_{ST} analyses between adults and
449 juveniles within regions revealed that only 8 out of the 75 possible comparisons (11%) had p-values
450 lower than the 0.05 cutoff threshold for Portugal and Asturias (but not in Galicia), which, however,
451 did not remain significant after FDR correction (Fig. 2c, 2d and 2e).

452 The analysis conducted with BayeScan v2.1 for outlier detection resulted in no loci under selection or
453 biased by species admixture and hybridization which have the same expectations in terms of outliers;
454 the twenty loci showed signatures of balanced or purifying selection with negative alpha values. The
455 results of the partial Mantel tests indicated no correlation between genetic and geographic distances,
456 with $R^2 = 1.61$ and p-value=0.1916 for adults and $R^2 = 3.16$ and p-value=0.0698 for juveniles using
457 the INA correction method for D_{CSE}, and $R^2 = 0.06$ and p-value=0.8002 for adults and $R^2 = 0.22$ and
458 p-value=0.6241 for juveniles using the Rousset method. The population structure was therefore
459 closer to an n-island model than a stepping stone model, and the pairwise F_{ST} between adjacent sites
460 often exceeded those obtained between geographically distant locations.

461 The DAPC analyses and the hierarchical analysis of molecular variance (AMOVA) using ϕ_{ST}
462 statistics showed no significant genetic differentiation of adults among and within regions (AD:
463 $\phi_{CT(among)}$=0.00032 p>0.05; $\phi_{SC(within)}$=0.00013 p>0.05) (Fig. 3a). However, a globally significant
464 genetic differentiation for juveniles among and within regions was found (JUV: $\phi_{CT(among)}$=0.00093
465 p<0.05; $\phi_{SC(within)}$=0.00217 p<0.001) (Fig. 3b). The analyses also revealed significant genetic
466 heterogeneity between *P. pollicipes* generations in Portugal ($\phi_{CT(among)}$= 0.00127, p<0.01) and
467 Asturias ($\phi_{CT(among)}$= 0.00120, p<0.01), but not in Galicia (Fig. 3c, 3b and 3e). The neighbor-joining
468 tree using adults and juveniles grouped together by localities clearly separated Galicia with high
469 bootstrapping values (i.e.: 90%), where Camelle and Baiona fall apart from the rest of the Galician
470 localities, after which two other different Portuguese and Asturian clades appeared (Fig. 4a). When
471 all populations (15 localities and 2 cohorts, 30 samples) were analyzed, the neighbor-joining tree
472 again showed Galicia samples falling apart and becoming heterogeneous, whereas the Portuguese and
473 Asturian samples were mixed together, with aggregations showing low bootstrap values (Fig. 4b).
474 The STRUCTURE runs using admixture suggested 3 genetic clusters (Evanno's k = 3, L(K) = -
475 117589.9100) when all populations (30 samples) were analyzed (Fig. 5a). The STRUCTURE results
476 also indicated the co-occurrence of 2 genetic clusters (Evanno's k = 2 L(K) = -60822.8150) for adults
477 (Fig. 5b) and 3 clusters (Evanno's k = 3 L(K) = -56241.9750) for juveniles when run separately (Fig.
478 5c).

Discussion

480 The analyses using twenty new microsatellite loci aimed to define, more accurately, the temporal and
481 spatial evolution of the genetic structure of stalked barnacle *P. pollicipes*. This species is highly
482 appreciated in the Spanish and Portuguese markets, and its management must be based on reliable
483 scientific data. Previous studies have suggested that larval dispersal driven by ocean currents, in
484 particular, the Iberian Poleward Current have played a crucial role in determining the population
485 structure, and two distinct regional configurations have been established using mitochondrial DNA
486 for *P. pollicipes* within its distribution range along the northeastern Atlantic. Quinteiro et al. (2007)
487 suggested that *P. pollicipes* is structured into four genetically differentiated groups: French
488 populations, eastern Asturian populations, Galician-Portuguese populations, and Canarian
489 populations. Conversely, Campo et al. (2010) suggested the presence of only two groups, among
490 which French populations were highlighted as a peculiar and differentiated genetic entity, as a result

491   of a past population fragmentation during Pleistocene glacial/interglacial periods. Regardless, later
492   studies based on estimates of population migration rates have suggested that barnacle population
493   connectivity occurred on a small scale and in an asymmetric manner in the Cantabrian coast (Rivera
494   et al., 2013). Information based on highly variable nuclear molecular markers can provide crucial
495   information on both population connectivity and stock renewal for this species within the Iberian
496   Peninsula. This information is needed for the delimitation of conservation/management units in this
497   fishery and the improvement of the management plans and the performance of TURFs.

498   Genetic diversity contributes to the ability of a species to respond to environmental changes, and
499   highly fecund species that release high numbers of small eggs into the environment (the so-called r-
500   strategists) are much more polymorphic than species that produce a small number of relatively large
501   offspring and provide parental care (called K-strategists) (Ellegren and Galtier, 2016). Recent studies
502   in *S. balanoides* have confirmed that barnacles harbor high levels of genome-wide genetic variation
503   (Nunez et al., 2021). The level of genetic diversity of *P. pollicipes* found in this work was
504   particularly high. We observed higher levels of genetic variation in *P. pollicipes* than in other
505   barnacles of the same genus, such as *P. elegans* (Plough and Marko, 2014). Our results showed that
506   Galicia exhibited the highest values for allelic richness and observed and expected heterozygosity in
507   adult and juvenile populations. Conversely, newly settled cohorts (juvenile) had a lower genetic
508   diversity than adults across all the studied regions, particularly when examining both allelic richness
509   and private alleles.

510   The main principal assumption of the Hardy-Weinberg principle is that the sample comes from a
511   single, randomly mating population where perturbing forces (such as selection, genetic drift,
512   mutation, migration) are absent or balanced (Waples, 2014). All loci and populations showed
513   significant deviations from Hardy-Weinberg equilibrium in this work due to, more or less,
514   pronounced, heterozygote deficiencies. This phenomenon could be the consequence of local
515   admixtures of genetically differentiated populations (Wahlund effect), assortative mating, inbreeding,
516   selection (Palumbi, 2003) and finally null alleles. The presence of null alleles has been reported in
517   the vast majority of previous microsatellite studies in barnacles (Dufresne et al., 1999; Pannacciulli et
518   al., 2005; Plough and Marko, 2014; Reigel et al., 2015; Abreu et al., 2016; Ewers-Saucedo et al.,
519   2016) as well as in other marine invertebrate species such as clams (Borrell et al., 2014; Chiesa et al.,
520   2016; Rico et al., 2017), octopus (Greatorex et al., 2000; De Luca et al., 2016), sea urchins
521   (Mccartney et al., 2004; Carlon and Lippé, 2007), jellyfish (Aglieri et al., 2014) and polychaetes
522   (Jolly et al., 2003, 2009, 2014). The presence of null alleles is an inherent trait of microsatellite loci
523   and is caused by mutations in the primer sequences, leading to the lack of amplification and the
524   dropout of alleles (Selkoe and Toonen, 2006). In addition, an increase of the null allele frequency
525   would be expected with the increase of alleles per locus and previous studies have indicated that the
526   presence of null alleles seems to be particularly common in populations with high effective
527   population sizes (Chapuis and Estoup, 2007). Although the presence of null alleles leads to an
528   overestimation of both F_{ST} and genetic distances in cases of significant population differentiation
529   (Chapuis and Estoup, 2007), our results showed no differences worth considering for both the F_{ST} or
530   F_{ST} ENA values. It has been argued that the conservative approach of discarding loci deviating from
531   Hardy-Weinberg equilibrium expectations could rob us of our most informative markers, weakening
532   our ability to interpret biological phenomena (Dharmarajan et al., 2013). Moreover, De Meeûs (2018)
533   stated that in case of null alleles, F_{IS} and F_{ST} are augmented and a positive correlation is expected
534   between F_{IS} and F_{ST} as is expected a positive correlation between F_{IS} and the number of missing data
535   (putative null homozygotes), and StrdErrF_{IS} being at least twice StrdErrF_{ST}. If such correlations do
536   not exist and if StrdErrF_{IS} > StrdErrF_{ST}, then a Wahlund effect better explains the data (De Meeûs,
537   2018; Manangwa et al., 2019). Waples (2018) had also argued about this and simulated 10% of null

538 alleles suggesting that caution in interpreting F_{IS} x F_{ST} correlations under conditions where null
539 alleles might be common it is indeed necessary and more efforts will be needed for a comprehensive
540 evaluation of this complex topic. In this work, panmixia is rarely met for any locus (Table 1), we
541 found positive F_{IS} x F_{ST} correlations, StrdErrF_{IS} > StrdErrF_{ST} and we did not find positive
542 correlations between F_{IS} and the number of missing data (putative null homozygotes) pointing out to
543 the fact that, even when null alleles are present, other biological factors also play a fundamental role
544 to explain significant heterozygote deficits in our data.

545 Heterozygote deficiencies can as well be the result of local admixtures of genetically differentiated
546 cohorts in populations, or due to sweepstake reproductive effort (Waples, 1998; Hedgecock and
547 Pudovkin, 2011). Growth of individuals in *P. pollicipes* populations is highly heterogeneous (Cruz et
548 al., 2010; Jacinto et al., 2015), so that individuals of similar size may differ greatly in age. Our adult
549 samples likely contained a mixture of cohorts from different reproductive and dispersal events,
550 potentially leading to significant departures from Hardy-Weinberg equilibrium, locally. Genetic
551 heterogeneity of cohorts can potentially blur the genetic signal in adults and may decrease the genetic
552 differences over time, given that the geographic origin of migrants might change throughout the
553 breeding/dispersal seasons depending on prevailing local hydrodynamics during these periods.
554 However, it should be noted that a special care was taken in this work to sample only one cohort of
555 juveniles with a specific size (2-4 mm RC). If the deficiencies of heterozygotes were due the
556 superimposition of cohorts, juveniles should not show such deficiencies. This was clearly not the
557 case here as our results demonstrated that juvenile mean F_{IS} values were higher than those for adults
558 in all the three regions (Table 2). It has been stated that the surf zone and its surrounding nearshore
559 waters are known to act as selective barriers to the onshore transport of many larval invertebrates on
560 the local scale (Porri et al., 2006; Rilov et al., 2008). The permeability of such barrier is modulated
561 by small scale topography that generates retentive oceanographic features like coastal fronts (Pineda
562 1999; Shanks et al. 2003). In fact, the larvae of *P. pollicipes* and other barnacles have been shown to
563 accumulate in great numbers at internal waves and river plume fronts off the Cantabrian coast only at
564 some specific locations (Weidberg et al., 2014; Hofer et al., 2017). In this topic the available
565 evidence are indeed scarce however, genetic data seems to confirm it.

566 *P. pollicipes* has asynchronous broods during the reproductive season which usually occurs from
567 March to September (e.g. Cardoso and Yule, 1995; Cruz and Hawkins, 1998; Pavón, 2003; Macho,
568 2006), where several batches of larvae are produced, and potentially lead to the co-occurrence of
569 different settlement events. Juveniles sampled in this study might however come from one to few
570 settlement events. Despite the possibility of several discrete settlement events, post larval mortality
571 might favor one specific batch of survivors, and in the end, the 2-4 mm RC juveniles might become
572 more related than what would have been expected from the mixing of several reproductive events.
573 Pineda et al. (2006) found that recruitment to the reproductive stage of acorn barnacles (*S.
574 balanoides*) was composed of survivors that settled in a recruitment window. The recruitment
575 window (to reproduction in the case of the Pineda study, to 2-4 mm in our study) might be narrower
576 than the recruitment season. If by some reason these survivors correspond to larvae that are
577 genetically more related, then a pattern of genetic differentiation could occur among recruits. The
578 concept of a "recruitment window" proposed by Pineda et al. (2006) matches quite well with
579 Hedgecock's "sweepstakes-chance matching hypothesis" also known as "sweepstakes reproductive
580 success hypothesis", which is based in part on the observation of reduced genetic variability in
581 young-of-the-year populations relative to adult populations. This reduced genetic variability among
582 recruits suggests that the surviving young of the year are the products of spawning by only a small
583 fraction of the adult population, which, according to Hedgecock's hypothesis, happened to produce
584 their offspring at a place and time that was suitable for survival (Hedgecock, 1994). Moreover,

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585 barnacles rear embryos in bags before hatching and there is also the possibility that the larval release
586 is only efficient for a small proportion of the reproductive adults depending on the local
587 hydrodynamics. In this work, we found evidence indicative of reproductive sweepstakes in adult and
588 juvenile samples. Although globally, the relatedness coefficients estimated for *P. pollicipes* were in
589 the same range as those from other studies previously conducted with barnacles (Veliz et al., 2006;
590 Plough et al., 2014), they were significantly slightly greater in juveniles (i.e.: Asturias, Portugal)
591 compared with adults. Juveniles were significantly more related to each other than expected from
592 random mixing despite their larval entrainment in the water column during the planktonic phase.

593 Unexpected genetic differentiation in marine invertebrates can occur due to three neutral processes:
594 sweepstake reproductive success (Hedgecock, 1994), collective dispersal (Johnson et al., 1993; Li
595 and Hedgecock, 1998) and asynchronous population dynamics (Eldon et al., 2016), but also selective
596 processes during the settlement process. According to the Hedgecock's "sweepstakes-chance
597 matching hypothesis" or selective sweepstakes (Hedgecock, 1994), only a fortunate combination
598 (hence sweepstakes) of reproductive traits and oceanographic conditions would allow an individual
599 to complete the long mobile phase from spawning and fertilization through larval survival to
600 recruitment back to the adult habitat. In a highly fecund species and a locally heterogeneous
601 oceanographic setting, this would involve strong selection favoring just a handful of genotypes at
602 each locality, leading to a local-scale genetic mosaic but a relatively large-scale uniformity. Post-
603 larval settlement selection under different environmental conditions has been argued to create chaotic
604 genetic patchiness in coastal areas of temperate regions over a mosaic of contrasting habitats able to
605 impose a strong differential selective sieve or a target for habitat choice in larvae (Eldon et al., 2016).
606 We detected significant genetic differentiation for juveniles among and within regions (but not for
607 adults), together with significant genetic heterogeneity between *P. pollicipes* generations. However,
608 we did not find evidence of such selective processes for the assayed microsatellites. There seemed to
609 be a genome-wide pattern that was more parsimoniously explained by neutral processes such as
610 sweepstake reproductive success, which may greatly reduce the genetic diversity of a given cohort
611 while provoking unexpected heterozygote deficiencies, as seen previously, by mimicking local
612 bottlenecks (genetic diversity drawn from a small subset of parents). In addition to this phenomenon,
613 genetic differentiation may persist in recruits when dispersal is limited in space, when larvae from
614 different cohorts do not mix completely during dispersal (collective dispersal), or when local
615 conditions may promote self-recruitment (Eldon et al., 2016).

616 The genetic data obtained in this work, after applying dissimilar approaches (F and ϕ_{ST} statistics,
617 Discriminant Principal Component and Bayesian analyses), pointed all out to the existence of
618 significant genetic heterogeneity in the Iberian coasts rejecting previous findings using mitochondrial
619 DNA. The results herein highlighted Galicia as a peculiar genetic entity possibly representing a
620 superimposition of two distinct metapopulations or potentially an old refuge for the most northern
621 populations from France (not sampled in this study). Among Galician northernmost populations,
622 Camelle (CA) and A Coruña (AC) are also the most differentiated from Portugal and Asturias and
623 may have a specific demographic history. The sampled *P. pollicipes* populations are located along
624 the Atlantic Iberian coast, whose hydrodynamic patterns have been well studied. The western
625 peninsular coast (SW Portugal and Galicia) is characterized by a complex current system subject to
626 strong seasonality and mesoscale variability, showing inverse patterns between summer and winter in
627 the upper layers of the shelf and slope. During spring and summer (coinciding with *P. pollicipes*
628 breeding season), northerly winds along the coast are dominant, causing coastal upwelling and
629 producing a southward current on the surface and a northward undercurrent on the slope. In the
630 Cantabrian Sea (Asturias) the surface currents flow generally eastward in winter and early spring and
631 shift westward in late spring and summer following the wind force with intermittent summer

upwelling events west of Cape Peñes (ICES, 2021). Different aspects of the oceanographic circulation in Iberia were reviewed by Relvas et al. (2007). Casabella et al. (2014) divided the upwelling affecting the coasts of Galicia into three regions: Rías Baixas, Fisterra-Bares and Cantabrian. These two locations (CA and AC) would be found in the Fisterra-Bares region, which is the region with a greater intensity of upwelling, although the period favorable for upwelling is longer in the region of Rias Baixas (sampled here i.e., Baiona). Galician juveniles showed clear genetical differences from those of Portugal and Asturias. The main explanation for this distinction is that the Biscay Bay Current, characterized by a wide gyre, can trap larvae, and thus should favor self-recruitment and perhaps local importations from the French and Cantabrian populations. This ocean circulation could also be responsible for the differentiation between juveniles from Asturian and Galicia. Previous studies on adult barnacles have found significant differences between the Asturian and Galician localities (Quinteiro et al., 2007). However, it should be noted that most of the Asturian sites sampled in this study are located to the West of Cape Peñes, while the site sampled by Quinteiro et al. (2007) was located to the East of the same cape, which has been described as a biogeographic barrier (Anadón and Niell, 1980). Rivera et al. (2013) showed that during a year of high upwelling activity (2009), the theoretical *P. pollicipes* recruitment success was 94%, with a recruitment peak predicted 56 km west of the emission point. Consistently, migration rates derived from genetic analyses showed that westward dispersal was much more likely along the Cantabrian coast, which matches the upwelling driven circulation typical of the stalked barnacle larval season in summer/autumn (Fig. 1). Thus, the recurrence of upwelling may not only define the spatial scale and direction of the dispersal process but also the genetic structure of the barnacle metapopulation.

The Western Iberian upwelling system represents an important crossroad between Lusitanian and boreal temperate species (Jolly et al., 2006; Maggs et al., 2008). Upwelling/downwelling wind-driven circulation and tides are recurrent physical processes along the Atlantic Iberian coastlines and are among the most energetic phenomena that can affect near-shore circulation during the spring and summer periods when reproduction occurs and, during the summer and beginning of autumn in the case of recruitment (Queiroga et al., 2007). However, when studying a strong upwelling region in the northeastern Pacific coast, Morgan et al. (2009) observed that the larvae of most invertebrate species remain close to the shore even during strong upwelling, where high local retention and limited connectivity have been evidenced in populations of several species, such as *Petrolisthes cinctipes* (Hameed et al., 2016) or in the red rock lobster *Panulirus interruptus* (Iacchei et al., 2013). Despite this phenomenon, upwelling areas have been pointed out as probable climate change refuges for the distribution of *Fucus guiryi*, other barnacles such as *S. balanoides* and other sessile marine species (Gómez et al., 2007 for a review; Hoarau et al., 2007; Provan and Bennett, 2008; Lourenço et al., 2016; Herrera, 2019). In addition, Campo et al. (2010) suggested the existence of a Pleistocene refuge area off the coast of North Africa and two additional northern glacial refuges for *P. pollicipes*, in the English Channel/Brittany region and in the northwestern Iberian Peninsula.

Previous studies have mentioned that the southern region of Portugal also represents a well-known upwelling area (Lourenço et al., 2016) with a high level of barnacle larval settlement (Queiroga et al., 2007) and recruitment (Aguión et al., in prep). Remarkably, the number of private alleles was significantly higher in adults there when compared with those from Galicia and in Asturias. Portuguese juveniles were however significantly less genetically diversified and more related to each other than expected based on random mating. Moreover, Nolasco et al. (in prep.) show that connectivity matrices integrated over the period of the observations (July 2017 to July 2019) indicate high levels of larval retention. Such retention is probably caused by the recurrent eddies driven by upwelling circulation observed off southern-central Portugal in between Cape Roca and Cape San Vicente (Fig 1; Haynes et al., 1993; Batteen et al., 2000; Sanchez and Relvas, 2003; Peliz et al.

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679   2004). These findings suggest that Portuguese populations are likely to export more migrants than
680   they receive. As Queiroga et al. (2007) hypothesized, regular exchanges of larvae over the distance
681   separating the southern and northern parts of Portugal are unlikely. Conversely, the Portugal Current,
682   which shows a north- or southward direction, depending on the season, could be an important factor
683   in promoting gene flow between our sampling locations in southern Portugal and other, unsampled,
684   *P. pollicipes* southernmost areas such as the Canarian and North African coasts. Nevertheless,
685   microsatellite markers have recently shown a genetic differentiation between European and African
686   *P. pollicipes* populations (Fernandes et al, in prep).

687   The correct management of marine ecosystems relies on understanding the scale and magnitude of
688   connectivity among populations through the identification of adaptive genetic differences (Almany et
689   al., 2009; Aceves-Bueno et al., 2017), because locally adapted populations should be considered
690   poorly-connected, separate management units (Waples, 1998). Our results suggested that *P.*
691   *pollicipes* populations in the Iberian Peninsula possibly exhibit a "chaotic genetic patchiness"
692   structure, which extends from a few kilometers apart to as much as hundreds of kilometers apart.
693   This phenomenon has clear consequences for the sustainable management of resources. Currently, an
694   increasing number of small-scale fisheries have successfully implemented co-managed TURFs; a
695   governance arrangement that enables the collaboration across diverse stakeholders, develops new
696   knowledge and increases the capacity of the system to deal with new drivers (Rivera et al., 2014).
697   However, the design of TURFs does not usually account for the spatial configuration of resources
698   (Aceves-Bueno et al., 2017) due to the multi-species nature of fisheries. This mismatch between
699   management and biological scales can compromise the sustainability of sessile stocks (Ouréns et al.,
700   2015), like barnacles. However, a better understanding of the spatial structure and larval dynamics of
701   the population, permits the redefinition of management units according to population boundaries. In
702   addition to these management measures, it would be interesting to implement networks of protected
703   areas at detailed scales to ensure that propagules are available when and where conditions are
704   favorable for their survival (Larson and Julian, 1999; Ouréns et al., 2015).

705   In conclusion, new molecular markers have been developed in the highly valued species *P. pollicipes*
706   and offer useful tools to provide a better fine-tuning assessment of its population dynamics along the
707   Iberian Peninsula. *P. pollicipes* displays high genetic diversity, which is attributable to large effective
708   population sizes representing a well-connected network of local populations. However, temporal and
709   spatial genetic differentiation of populations over regional scales, on one hand, and a significant
710   reduction in genetic diversity in juveniles, on the other hand, clearly indicate that patterns of
711   exchanges together with seasonal wind-induced upwelling may induce genetic differences between
712   settlers throughout generations. Such patterns of chaotic genetic patchiness are likely due to
713   sweepstake reproductive success with possible collective dispersal or episodic self-recruitment
714   events. Therefore, our *P. pollicipes* genetic dataset suggests that recruitment may be stochastic and
715   highly dependent on climatic conditions with multiple sources of emissions. These phenomena may
716   have strong implications in terms of management plans over the whole Iberian Peninsula with the
717   need to protect a series of putative sources within each region. Future research should combine
718   genetic information at broader spatiotemporal scales with larval dispersal models based on ecological
719   and biological characteristics of *P. pollicipes*. This means, among others, mapping the complete
720   species distribution and tracking the genetic structure of age groups over time and space. It also
721   means applying new sequencing technologies to fully understand the dynamics of larval exchanges
722   and the post-larval settlement of the stalked barnacle but also to better apprehend how environmental
723   variations shape genomic variation in this species.

724   **4   List of non-standard abbreviations**

725   CGP: Chaotic Genetic Patchiness

726   TURF: Territorial Use Rights for Fishing

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9   References

Abreu, N. M. N., Marçal, I., Borges Duarte, A., Bettini Pitombo, F., Vilasboa, A., and Gusmão, J. (2016). Microsatellite markers for barnacle studies: Isolation and characterization of polymorphic microsatellite loci from the invasive barnacle *Megabalanus coccopoma* (Darwin, 1854) and its cross-amplification in the Southern Atlantic endemic species *Megabalanus*. *Biochem. Syst. Ecol.* 66, 224–228. doi:10.1016/j.bse.2016.04.006

764 Aceves-Bueno, E., Cornejo-Donoso, J., Miller, S. J., and Gaines, S. D. (2017). Are territorial use
765   rights in fisheries (TURFs) sufficiently large?. *Mar. Policy. 78*, 189-195.
766   doi:10.1016/j.marpol.2017.01.024

767 Aglieri, G., Papetti, C., Zane, L., Milisenda, G., Boero, F., and Piraino, S. (2014). First evidence of
768   inbreeding, relatedness and chaotic genetic patchiness in the holoplanktonic jellyfish *Pelagia*
769   *noctiluca* (Scyphozoa, Cnidaria). *PLoS ONE* 9, e99647. doi:10.1371/journal.pone.0099647

770 Aguión, A., Ojea, E. García-Flórez, L., Cruz, T., Garmendia J.M., Davoult, D., Queiroga, H., Rivera
771   A., Acuña-Fernández, J.L., Macho, G. (2021). Establishing a governance threshold in small-
772   scale fisheries to achieve sustainability. *Ambio*, 1-14. doi:10.1007/s13280-021-01606-x

773 Almany, G. R., Connolly, S. R., Heath, D. D., Hogan, J. D., Jones, G. P., McCook, L. J., ... and
774   Williamson, D. H. (2009). Connectivity, biodiversity conservation and the design of marine
775   reserve networks for coral reefs. *Coral reefs* 28(2), 339-351. doi:10.1007/s00338-009-0484-x

776 Alshari NFMAH, Ahmad SZ, Azlan A, Lee YH, Azzam G, and Nor SAM. (2021). Metabarcoding of
777   fish larvae in the Merbok River reveals species diversity and distribution along its mangrove
778   environment. *Zool. Stud.* 60:76. doi:10.6620/ZS.2021.60-76

779 Álvarez-Fernández, E. (2011). Humans and marine resource interaction reappraised: Archaeofauna
780   remains during the late Pleistocene and Holocene in Cantabrian Spain. *J. Anthropol.*
781   *Archaeol.* 30, 327–343. doi:10.1016/j.jaa.2011.05.005

782 Álvarez-Fernández, E., Barrera, I., Borja, A., Fernández, M., Iriarte, M., and Arrizabalaga, A. (2013).
783   Biometric analysis of the stalked barnacle *Pollicipes pollicipes*, at a Holocene archaeological
784   site in Jaizkibel (Basque Country, northern Spain). *Holocene* 23, 1373–1380.
785   doi:10.1177/0959683613489584

786 Álvarez-Fernández, E., Ontañón-Peredo, R., and Molares-Vila, J. (2010). Archaeological data on the
787   exploitation of the goose barnacle *Pollicipes pollicipes* (Gmelin, 1790) in Europe. *J.*
788   *Archaeol. Sci.* 37, 402–408. doi:10.1016/j.jas.2009.10.003

789 Anadón, R., and Niell, F. X. (1980). Distribución longitudinal de macrófitos en la costa asturiana (N
790   de España). *Investig. Pesq.* 45, 143–156. http://hdl.handle.net/10261/89117

791 Barahona, M., Broitman, B. R., Faugeron, S., Jaugeon, L., Ospina-Alvarez, A., Véliz, D., et al.
792   (2019). Environmental and demographic factors influence the spatial genetic structure of an
793   intertidal barnacle in central-northern Chile. *Mar. Ecol. Prog. Ser.* 612, 151–165.
794   doi:10.3354/meps12855

795 Barnes, M. (1996). Pedunculate cirripedes of the genus *Pollicipes*. *Oceanogr. Mar. Biol. Annu. Rev.*
796   34, 303–394.

797 Barnes, M. (2008). *Pollicipes pollicipes* A goose barnacle. Available at:
798   https://www.marlin.ac.uk/species/detail/43 [Accessed March 15, 2021].

799 Barshis, D. J., Sotka, E. E., Kelly, R. P., Sivasundar, A., Menge, B. A., Barth, J. A., et al. (2011).
800   Coastal upwelling is linked to temporal genetic variability in the acorn barnacle *Balanus*
801   *glandula*. *Mar. Ecol. Prog. Ser.* 439, 139–150. doi:10.3354/meps09339

802 Batteen, M. L., Martinez, J. R., Bryan, D. W., and Buch, E. J. (2000). A modeling study of the
803   coastal eastern boundary current system off Iberia and Morocco. *J. Geophys. Res-Oceans*,
804   105(C6), 14173-14195. doi:10.1029/2000JC900026

805 Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N., and Bonhomme, F. (2004). *GENETIX 4.05, logiciel*
806   *sous Windows TM pour la génétique des populations*. Laboratoire Génome, Populations,
807   Interactions, CNRS UMR 5000, Université de$\sim$\ldots Available at:
808   https://ci.nii.ac.jp/naid/10030209986/ [Accessed October 18, 2017].

809 Benjamini, Y., and Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and
810   Powerful Approach to Multiple Testing. *J. R. Stat. Soc. Ser. B Methodol.* 57, 289–300.
811   doi:10.1111/j.2517-6161.1995.tb02031.x

812 Benson, G. (1999). Tandem repeats finder: a program to analyze DNA sequences. *Nucleic Acids Res.*
813   27, 573–580. doi:10.1093/nar/27.2.573

814 Bloor, P. A., Barker, F. S., Watts, P. C., Noyes, H. A., and Kemp, S. J. (2001) Microsatellite
815   Libraries by Enrichment, Version 1.0. Animal Genomics Laboratory, School of Biological
816   Sciencies. University of Liverpool, United Kingdom.

817 Borrell, Y. J., Arias-Pérez, A., Freire, R., Antonio Valdés, José Antonio Sánchez, Josefina Méndez,
818   et al. (2014). Microsatellites and multiplex PCRs for assessing aquaculture practices of the
819   grooved carpet shell *Ruditapes decussatus* in Spain. *Aquaculture* 426–427, 49–59.
820   doi:10.1016/j.aquaculture.2014.01.010

821 Borrell, Y. J., Piñera, J. A., Sánchez Prado, J. A., and Blanco, G. (2012). Mitochondrial DNA and
822   microsatellite genetic differentiation in the European anchovy *Engraulis encrasicolus* L.
823   *ICES J. Mar. Sci.* 69, 1357–1371. doi:10.1093/icesjms/fss129

824 Brault, S., Stuart, C. T., Wagstaff, M. C., and Rex, M. A. (2013). Geographic evidence for source-
825   sink dynamics in deep-sea neogastropods of the eastern North Atlantic: An approach using
826   nested analysis. *Glob. Ecol. Biogeogr.* 22, 433–439. doi:10.1111/geb.12005

827 Campo, D., Molares, J., Garcia, L., Fernandez-Rueda, P., Garcia-Gonzalez, C., and Garcia-Vazquez,
828   E. (2010). Phylogeography of the European stalked barnacle (*Pollicipes pollicipes*):
829   Identification of glacial refugia. *Mar. Biol.* 157, 147–156. doi:10.1007/s00227-009-1305-z

830 Cardoso, A. C., and Yule, A. B. (1995). Aspects of the reproductive biology of *Pollicipes pollicipes*
831   (Cirripedia; Lepadomorpha) from the southwest coast of Portugal. *Neth. J. Aquat. Ecol.* 29(3),
832   391-396. doi:10.1007/BF02084238

833 Carlon, D. B., and Lippé, C. (2007). Eleven new microsatellite markers for the tropical sea urchin
834   *Tripneustes gratilla* and cross-amplification in *Tripneustes ventricosa*. *Mol. Ecol. Notes* 7,
835   1002–1004. doi:10.1111/j.1471-8286.2007.01755.x

836 Carvalho, A. N., Vasconcelos, P., Piló, D., Pereira, F., and Gaspar, M. B. (2017). Socio-economic,
837   operational and technical characterization of the harvesting of gooseneck barnacle (*Pollicipes*
838   *pollicipes*) in SW Portugal: Insights towards fishery co-management. *Mar. Policy* 78, 34–44.
839   doi:10.1016/j.marpol.2017.01.008

840 Casabella, N., Lorenzo, M. N., and Taboada, J. J. (2014). Trends of the Galician upwelling in the
841   context of climate change. *J. Sea Res.* 93, 23–27. doi:10.1016/j.seares.2014.01.013.

842 Cavalli-Sforza, L. L., and Edwards, A. W. (1967). Phylogenetic analysis. Models and estimation
843   procedures. *Am. J. Hum. Genet* 19(3 Pt 1), 233. doi:10.1111/j.1558-5646.1967.tb03411.x

844 Chan, B. K. K., Dreyer, N., Glenner, H., Pérez-Losada, M., Ewers-Saucedo, C., Gale AS., Kolbasov,
845   G.A., Crandall., K., and Høeg J.T. (2021) The evolutionary diversity of barnacles and a
846   classification of fossil and living forms. *Zool. J. Linn. Soc* 193(3) 789–846.
847   doi:10.1093/zoolinnean/zlaa160

848 Chapuis, M. P., and Estoup, A. (2007). Microsatellite null alleles and estimation of population
849   differentiation. *Mol. Biol. Evol.* 24, 621–631. doi:10.1093/molbev/msl191

850 Chen, H. N., Hoeg, J. T., and Chan, B. K. K. (2013). Morphometric and molecular identification of
851   individual barnacle cyprids from wild plankton: an approach to detecting fouling and invasive
852   barnacle species. *Biofouling*, 29(2): 133-145. doi:10.1080/08927014.2012.753061

853 Chen, H. N., Chan, B. K., and Tsang, L. M. (2015). Transcriptome derived microsatellite markers of
854   *Tetraclita kuroshioensis* and cross amplification among *Tetraclita* spp. *Biochem. Syst. Ecol.*
855   63, 13-16. doi:10.1016/j.bse.2015.09.015

856 Chiesa, S., Lucentini, L., Freitas, R., Nonnis Marzano, F., Ferrari, C., Filonzi, L., et al. (2016). Null
857   alleles of microsatellites for Manila clam *Ruditapes philippinarum*. *Anim. Genet.* 47, 135–
858   136. doi:10.1111/age.12382

859 Costello, C. J., Lynham, J., Lester, S. E., and Gaines, S. D. (2010). Economic Incentives and Global
860   Fisheries Sustainability. *Annu. Rev. Resour. Econ.* 2, 299–318.
861   doi:10.1146/annurev.resource.012809.103923

862 Couvray, S., and Coupé, S. (2018). Three-year monitoring of genetic diversity reveals a micro-
863   connectivity pattern and local recruitment in the broadcast marine species *Paracentrotus*
864   *lividus*. *Heredity* 120, 110–124. doi:10.1038/s41437-017-0013-6

865 Cowen, R. K., and Sponaugle, S. (2009). Larval Dispersal and Marine Population Connectivity.
866   *Annu. Rev. Mar. Sci.* 1, 443–466. doi:10.1146/annurev.marine.010908.163757

867 Cruz, T., Castro, J. J., and Hawkins, S. J. (2010). Recruitment, growth and population size structure
868   of *Pollicipes pollicipes* in SW Portugal. *J. Exp. Mar. Biol. Ecol.* 392, 200–209.
869   doi:10.1016/j.jembe.2010.04.020

870 Cruz, T. and Hawkins, S. J. (1998). Reproductive cycle of *Pollicipes pollicipes* at Cabo de Sines,
871   south-west coast of Portugal. *J. Mar. Biol. Assoc. UK.* 78(2), 483-496.
872   doi:10.1017/S0025315400041576

873 Cruz, T., Jacinto, D., Sousa, A., Penteado, N., Pereira, D., Fernandes, J. N., et al. (2015). The state of
874   the fishery, conservation and management of the stalked barnacle *Pollicipes pollicipes* in
875   Portugal. *Mar. Environ. Res.* 112, 73–80. doi:10.1016/j.marenvres.2015.10.005

876 Cruz, T. P. G. (2000). Biologia e ecologia do percebe *Pollicipes pollicipes* (Gmelin, 1790), no litoral
877   sudoeste portugês. Available at: http://dspace.uevora.pt/rdpc/handle/10174/11214

878 D'Aloia, C. C., Bogdanowicz, S. M., Francis, R. K., Majoris, J. E., Harrison, R. G., and Buston, P.
879   M. (2015). Patterns, causes, and consequences of marine larval dispersal. *Proc. Natl. Acad.*
880   *Sci. U. S. A.* 112, 13940–13945. doi:10.1073/pnas.1513754112

881 Dawson, M. N., Grosberg, R. K., Stuart, Y. E., and Sanford, E. (2010). Population genetic analysis of
882   a recent range expansion: Mechanisms regulating the poleward range limit in the volcano
883   barnacle *Tetraclita rubescens*. *Mol. Ecol.* 19, 1585–1605. doi:10.1111/j.1365-
884   294X.2010.04588.x

885 De Luca, D., Catanese, G., Procaccini, G., and Fiorito, G. (2016). *Octopus vulgaris* (Cuvier, 1797) in
886   the Mediterranean Sea: Genetic diversity and population structure. *PLoS ONE* 11.
887   doi:10.1371/journal.pone.0149496

888 De Meeûs, T. (2018). Revisiting F_{IS}, F_{ST}, Wahlund effects, and null alleles. *J. Hered.* 109(4), 446-
889   456. doi: 10.1093/jhered/esx106

890 Dharmarajan, G., Beatty, W., Rohodes, O.E. (2013). Heterozygote Deficiencies Caused by a
891   Wahlund Effect: Dispelling Unfounded Expectations. *J. Wildl. Manag.* 77(2), 226–234. doi:
892   10.1002/jwmg.458

893 Dubé, C. E., Boissin, E., Mercière, A., and Planes, S. (2020). Parentage analyses identify local
894   dispersal events and sibling aggregations in a natural population of *Millepora* hydrocorals, a
895   free-spawning marine invertebrate. *Mol. Ecol.* 29, 1508–1522. doi:10.1111/mec.15418

896 Dufresne, F., Parent, M., and Bernatchez, L. (1999). Isolation and characterization of microsatellite
897   markers in the acorn barnacle *Semibalanus balanoides*. *Mol. Ecol.* 8, 1558–1559.
898   doi:10.1046/j.1365-294X.1999.07225.x

899 Earl, D. A., and VonHoldt, B. M. (2012). STRUCTURE HARVESTER: A website and program for
900   visualizing STRUCTURE output and implementing the Evanno method. *Conserv. Genet.*
901   *Resour.* 4, 359–361. doi:10.1007/s12686-011-9548-7

902 Eldon, B., Riquet, F., Yearsley, J., Jollivet, D., and Broquet, T. (2016). Current hypotheses to explain
903   genetic chaos under the sea. *Curr. Zool.* 62, 551–566. doi:10.1093/cz/zow094

904 Ellegren, H., and Galtier, N. (2016). Determinants of genetic diversity. *Nat. Rev. Genet.* 17, 422–433.
905   doi:10.1038/nrg.2016.58

906 Estoup, A., Largiadèr, C. R., Perrot, E., and Chourrout, D. (1996). Rapid one-tube DNA extraction
907   for reliable PCR detection of fish polymorphic markers and transgenes. *Mol. Mar. Biol. Biote*
908   5, 295–298. https://hal.inrae.fr/hal-02697475

909 Evanno, G., Regnaut, S., and Goudet, J. (2005). Detecting the number of clusters of individuals using
910   the software STRUCTURE: A simulation study. *Mol. Ecol.* 14, 2611–2620.
911   doi:10.1111/j.1365-294X.2005.02553.x

912　Ewers-Saucedo, C., Chan, B. K. K., Zardus, J. D., and Wares, J. P. (2017). Parallel patterns of host-
913　　　specific morphology and genetic admixture in sister lineages of a commensal barnacle. *Biol.*
914　　　*Bull.* 232, 171–185. doi:10.1086/693356

915　Ewers-Saucedo, C., Zardus, J. D., and Wares, J. P. (2016). Microsatellite loci discovery from
916　　　nextgeneration sequencing data and loci characterization in the epizoic barnacle *Chelonibia*
917　　　*testudinaria* (Linnaeus, 1758). *PeerJ* 2016, e2019. doi:10.7717/peerj.2019

918　Excoffier, L., Laval, G., and Schneider, S. (2005). Arlequin (version 3.0): An integrated software
919　　　package for population genetics data analysis. *Evol. Bioinforma.* 1, 117693430500100.
920　　　doi:10.1177/117693430500100003

921　FAO (2020). *El estado mundial de la pesca y la acuicultura 2020*. FAO doi:10.4060/ca9229es

922　Fernandes, J. N., Cruz, T., and Van Syoc, R. (2010). *Pollicipes caboverdensis* sp. nov. (Crustacea:
923　　　Cirripedia: Scalpelliformes), an intertidal barnacle from the Cape Verde Islands. *Zootaxa*
924　　　2557, 29–38. doi:10.11646/zootaxa.2557.1.3

925　Flight, P. A., O'Brien, M. A., Schmidt, P. S., and Rand, D. M. (2012). Genetic Structure and the
926　　　North American Postglacial Expansion of the Barnacle, *Semibalanus balanoides*. *J. Hered.*
927　　　103, 153–165. doi:10.1093/jhered/esr083

928　Foll, M., and Gaggiotti, O. (2008). A genome-scan method to identify selected loci appropriate for
929　　　both dominant and codominant markers: A Bayesian perspective. *Genetics* 180, 977–993.
930　　　doi:10.1534/genetics.108.092221

931　Fontani, S. (2009). Genetic biodiversity of the European barnacle *Chthamalus montagui*. (Doctoral
932　　　dissertation, University of Plymouth). http://hdl.handle.net/10026.1/2733

933　Fox, J. (2005). Getting started with the R commander: a basic-statistics graphical user interface to R.
934　　　*J. Stat. Softw.* 14(9), 1-42.

935　Fox, J. (2007). Extending the R commander by "plug in" packages. R News, 7(3), 46–52.

936　Franco, S. C., Aldred, N., Cruz, T., and Clare, A. S. (2017). Effects of culture conditions on larval
937　　　growth and survival of stalked barnacles (*Pollicipes pollicipes*). *Aquac. Res.* 48, 2920–2933.
938　　　doi:10.1111/are.13125

939　Franco, S. C., Aldred, N., Cruz, T., Clare, A. S., and Clare, A. S. (2016). Modulation of gregarious
940　　　settlement of the stalked barnacle, *Pollicipes pollicipes*: a laboratory study. *Sci. Mar.* 80, 217–
941　　　228. doi:10.3989/scimar.04342.01A

942　Gagnaire, P. A., Broquet, T., Aurelle, D., Viard, F., Souissi, A., Bonhomme, F., et al. (2015). Using
943　　　neutral, selected, and hitchhiker loci to assess connectivity of marine populations in the
944　　　genomic era. *Evol. Appl.* 8. doi:10.1111/eva.12288

945　Gilbert, K. J., Andrew, R. L., Bock, D. G., Franklin, M. T., Kane, N. C., Moore, J. S., et al. (2012).
946　　　*Recommendations for utilizing and reporting population genetic analyses: The*
947　　　*reproducibility of genetic clustering using the program structure*. John Wiley & Sons, Ltd
948　　　doi:10.1111/j.1365-294X.2012.05754.x

949 Gómez, A., and Lunt, D. H. (2007). Refugia within refugia: patterns of phylogeographic concordance
950   in the Iberian Peninsula. *Phylogeography of southern European refugia* (pp. 155-188).
951   Springer, Dordrecht. doi:10.1007/1-4020-4904-8_5

952 Goudet, J. (1995). FSTAT (Version 1.2): A Computer Program to Calculate F-statistics. *J. Hered.* 86,
953   485–486. doi:10.1093/oxfordjournals.jhered.a111627

954 Greatorex, E. C., Jones, C. S., Murphy, J., Key, L. N., Emery, A. M., and Boyle, P. R. (2000).
955   Microsatellite markers for investigating population structure in *Octopus vulgaris* (Mollusca:
956   Cephalopoda). *Mol. Ecol.* 9, 641–642. doi:10.1046/j.1365-294x.2000.00882-7.x

957 Gutiérrez, N. L., Hilborn, R., and Defeo, O. (2011). Leadership, social capital and incentives promote
958   successful fisheries. *Nature* 470, 386–389. doi:10.1038/nature09689

959 Hameed, S. O., Wilson White, J., Miller, S. H., Nickols, K. J., and Morgan, S. G. (2016). Inverse
960   approach to estimating larval dispersal reveals limited population connectivity along 700 km
961   of wave-swept open coast. *Proc. R. Soc. B Biol. Sci.* 283, 20160370.
962   doi:10.1098/rspb.2016.0370

963 Haynes, R., Barton, E. D., and Pilling, I. (1993). Development, persistence, and variability of
964   upwelling filaments off the Atlantic coast of the Iberian Peninsula. *J. Geophys. Res-Oceans*,
965   98(C12), 22681-22692. doi:10.1029/93JC02016

966 Hecker, K. H., and Roux, K. H. (1996). High and low annealing temperatures increase both
967   specificity and yield in touchdown and stepdown PCR. *BioTechniques* 20, 478–485.
968   doi:10.2144/19962003478

969 Hedgecock, D. (1994). Does variance in reproductive success limit effective population sizes of
970   marine organisms. *Genetics and evolution of aquatic organisms*, 122, 122-134.

971 Hedgecock, D., and Pudovkin, A. I. (2011). Sweepstakes reproductive success in highly fecund
972   marine fish and shellfish: a review and commentary. Bulletin of Marine Science, 87(4), 971-
973   1002. doi:10.5343/bms.2010.1051

974 Herrera, M. (2019). The effects of climate change on reproduction and recruitment success of the
975   acorn barnacle *Semibalanus balanoides* at its southernmost European distribution limit
976   (Galicia, Spain). (Doctoral dissertation, Universidade de Vigo).
977   http://hdl.handle.net/11093/1370

978 Hoarau, G., Coyer, J. A., Veldsink, J. H., Stam, W. T., and Olsen, J. L. (2007). Glacial refugia and
979   recolonization pathways in the brown seaweed *Fucus serratus*. *Mol. Ecol.* 16(17), 3606-3616.
980   doi:10.1111/j.1365-294X.2007.03408.x

981 Höfer, J., Muñiz, C., Weidberg, N., García-Flórez, L., and Acuña, J. L. (2017). High densities of
982   stalked barnacle larvae (*Pollicipes pollicipes*) inside a river plume. *J. Plankton Res.* 39(2),
983   316-329. doi:10.1093/plankt/fbw093

984 Holleley, C. E., and Geerts, P. G. (2009). Multiplex Manager 1.0: A cross-platform computer
985   program that plans and optimizes multiplex PCR. *BioTechniques* 46, 511–517.
986   doi:10.2144/000113156

987 Hongjamrassilp, W., Murase, A., Miki, R., and Hastings, P. A. (2020). Journey to the West: Trans-
988   Pacific Historical Biogeography of Fringehead Blennies in the Genus *Neoclinus* (Teleostei:
989   Blenniiformes). *Zool. Stud.* 59, e9. doi:10.6620/ZS.2020.59-09

990 Iacchei, M., Ben-Horin, T., Selkoe, K. A., Bird, C. E., García-Rodríguez, F. J., and Toonen, R. J.
991   (2013). Combined analyses of kinship and FST suggest potential drivers of chaotic genetic
992   patchiness in high gene-flow populations. *Mol. Ecol.* 22, 3476–3494. doi:10.1111/mec.12341

993 ICES (2021). Bay of Biscay and the Iberian Coast ecoregion – Ecosystem overview. In Report of the
994 ICES Advisory Committee, 2021. ICES Advice 2021, Section 6.1. doi:10.17895/ices.advice.9436

995 Jacinto, D., Cruz, T., Silva, T., and Castro, J. J. (2010). Stalked barnacle (Pollicipes pollicipes)
996   harvesting in the Berlengas Nature Reserve, Portugal: Temporal variation and validation of
997   logbook data. *ICES J. Mar. Sci.* 67, 19–25. doi:10.1093/icesjms/fsp226

998 Jacinto, D., Cruz, T., Silva, T., and Castro, J. J. (2011). Gestión de la explotación de percebe
999   (Pollicipes pollicipes) en la Reserva Natural de Berlengas (Portugal): Evaluación del tope de
1000   capturas y talla mínima. *Sci. Mar.* 75, 439–445. doi:10.3989/scimar.2011.75n3439

1001 Jacinto, D., Penteado, N., Pereira, D., Sousa, A., and Cruz, T. (2015). Growth rate variation of the
1002   stalked barnacle Pollicipes pollicipes (Crustacea: Cirripedia) using calcein as a chemical
1003   marker. *Sci. Mar.* 79(1), 117-123. doi:10.3989/scimar.04135.08B

1004 Johnson, M. S., and Black, R. (1982). Chaotic genetic patchiness in an intertidal limpet, *Siphonaria*
1005   sp. *Mar. Biol.* 70, 157–164. doi:10.1007/BF00397680

1006 Johnson, M. S., Holborn, K., and Black, R. (1993). Fine-scale patchiness and genetic heterogeneity
1007   of recruits of the corallivorous gastropod *Drupella cornus*. *Mar. Biol.* 117, 91–96.
1008   doi:10.1007/BF00346429

1009 Jolliffe, I. (2011). *Principal component analysis*. Berlin, Heidelberg: Springer Berlin Heidelberg.
1010   doi:10.1007/978-3-642-04898-2

1011 Jolly, M., Viard, F., Weinmayr, G., Gentil, F., Thiébaut, E., and Jollivet, D. (2003). Does the genetic
1012   structure of *Pectinaria koreni* (Polychaeta: Pectinariidae) conform to a source–sink
1013   metapopulation model at the scale of the Baie de Seine?. *Helgoland Mar. Res.* 56(4), 238-
1014   246. doi:10.1007/s10152-002-0123-1

1015 Jolly, M. T., Viard, F., Gentil, F., Thiébaut, É., and Jollivet, D. (2006). Comparative phylogeography
1016   of two coastal polychaete tubeworms in the Northeast Atlantic supports shared history and
1017   vicariant events. *Mol. Ecol.* 15(7), 1841-1855. doi:10.1111/j.1365-294X.2006.02910.x

1018 Jolly, M. T., Guyard, P., Ellien, C., Gentil, F., Viard, F., Thiébaut, E., and Jollivet, D. (2009).
1019   Population genetics and hydrodynamic modeling of larval dispersal dissociate contemporary
1020   patterns of connectivity from historical expansion into European shelf seas in the polychaete
1021   *Pectinaria koreni. Limnol. Oceanogr.* 54(6), 2089-2106. doi: 10.4319/lo.2009.54.6.2089

1022 Jolly, M. T., Thiébaut, E., Guyard, P., Gentil, F., and Jollivet, D. (2014). Meso-scale hydrodynamic
1023   and reproductive asynchrony affects the source–sink metapopulation structure of the coastal
1024   polychaete *Pectinaria koreni*. *Mar. Biol.* 161(2), 367-382. doi:10.1007/s00227-013-2342-1

1025 Jombart, T. (2008). adegenet: A R package for the multivariate analysis of genetic markers.
1026   *Bioinformatics* 24, 1403–1405. doi:10.1093/bioinformatics/btn129.

1027 Kalendar, R., Lee, D., and Schulman, A. H. (2009). FastPCR software for PCR primer and probe
1028   design and repeat search. *Genes, Genomes and Genomics*, 3(1), 1-14.

1029 Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., and Mayrose, I. (2015). Clumpak: a
1030   program for identifying clustering modes and packaging population structure inferences
1031   across K. *Mol. Ecol. Resour.* 15(5), 1179-1191. doi:10.1111/1755-0998.12387

1032 Kough, A. S., Paris, C. B., and Butler IV, M. J. (2013). Larval Connectivity and the International
1033   Management of Fisheries. *PLoS ONE* 8. doi:10.1371/journal.pone.0064970

1034 Larson, R. J., and Julian, R. M. (1999). Spatial and temporal genetic patchiness in marine populations
1035   and their implications for fisheries management. *Cal. Coop. Ocean. Fish.* 94–99. Available
1036   at: http://calcofi.org/publications/calcofireports/v40/Vol_40_Larson___Julian.pdf

1037 Letunic, I., and Bork, P. (2019). Interactive Tree of Life (iTOL) v4: recent updates and new
1038   developments. *Nucleic Acids Res.* 47(W1), W256-W259. doi: 10.1093/nar/gkz239

1039 Li, G., and Hedgecock, D. (1998). Genetic heterogeneity, detected by PCR-SSCP, among samples of
1040   larval pacific oysters (*Crassostrea gigas*) supports the hypothesis of large variance in
1041   reproductive success. *Can. J. Fish. Aquat. Sci.* 55, 1025–1033. doi:10.1139/f97-312

1042 Lindegren, M., Andersen, K. H., Casini, M., and Neuenfeldt, S. (2014). A metacommunity
1043   perspective on source-sink dynamics and management: The Baltic Sea as a case study. *Ecol.*
1044   *Appl.* 24, 1820–1832. doi:10.1890/13-0566.1

1045 Lorenzen, K., Steneck, R. S., Warner, R. R., Parma, M., Coleman, F. C., and Leber, K. M. (2010).
1046   The spatial dimensions of fisheries: Putting it all in place. *Bull. Mar. Sci.* 86, 169–177.

1047 Lourenço, C. R., Zardi, G. I., McQuaid, C. D., Serrão, E. A., Pearson, G. A., Jacinto, R., et al. (2016).
1048   Upwelling areas as climate change refugia for the distribution and genetic diversity of a
1049   marine macroalga. *J. Biogeogr.* 43, 1595–1607. doi:10.1111/jbi.12744

1050 Macho, G. (2006). Ecología reproductiva y larvaria del percebe y otros cirrípedos en Galicia.
1051   (Doctoral dissertation, Universidade de Vigo).
1052   https://books.google.es/books?id=sVb9ygEACAAJ

1053 Macho, G., Naya, I., Freire, J., Villasante, S., and Molares, J. (2013). The key role of the barefoot
1054   fisheries advisors in the Co-managed TURF system of Galicia (NW Spain). *Ambio* 42, 1057–
1055   1069. doi:10.1007/s13280-013-0460-0

1056 Maggs, C. A., Castilho, R., Foltz, D., Henzler, C., Jolly, M. T., Kelly, J., ... and Wares, J. (2008).
1057   Evaluating signatures of glacial refugia for North Atlantic benthic marine taxa. *Ecology*,
1058   89(sp11), S108-S122. doi:10.1890/08-0257.1

1059 Manangwa, O., De Meeûs, T., Grébaut, P., Ségard, A., Byamungu, M., and Ravel, S. (2019).
1060   Detecting Wahlund effects together with amplification problems: Cryptic species, null alleles

1061   and short allele dominance in *Glossina pallidipes* populations from Tanzania. *Mol. Ecol.*
1062   *Resour.* 19(3), 757-772. doi: 10.1111/1755-0998.12989

1063   Mauger, S., Couceiro L, and Valero M (2012). A simple and cost-effective method to synthesize an
1064   internal size standard amenable to use with a 5-dye system. *Prime Res. Biotgechnology* 2,
1065   2315–5299

1066   Mccartney, M. A., Brayer, K., and Levitan, D. R. (2004). Polymorphic microsatellite loci from the
1067   red urchin, *Strongylocentrotus franciscanus*, with comments on heterozygote deficit. *Mol.*
1068   *Ecol. Notes* 4, 226–228. doi:10.1111/j.1471-8286.2004.00624.x

1069   Molares, J., and Freire, J. (2003). Development and perspectives for community-based management
1070   of the goose barnacle (*Pollicipes pollicipes*) fisheries in Galicia (NW Spain). *Fish. Res.* 65,
1071   485–492. doi:10.1016/j.fishres.2003.09.034

1072   Molares, J., Tilves, F., and Pascual, C. (1994). Larval development of the pedunculate barnacle
1073   *Pollicipes cornucopia* (Cirripedia: Scalpellomorpha) reared in the laboratory. *Mar. Biol.* 120,
1074   261–264. doi:10.1007/BF00349686

1075   Morgan, S. G., Fisher, J. L., Miller, S. H., McAfee, S. T., and Largier, J. L. (2009). Nearshore larval
1076   retention in a region of strong upwelling and recruitment limitation. *Ecology* 90, 3489–3502.
1077   doi:10.1890/08-1550.1

1078   Morgan, S. G., Miller, S. H., Robart, M. J., and Largier, J. L. (2018). Nearshore larval retention and
1079   cross-shelf migration of benthic crustaceans at an upwelling center. *Front. Mar. Sci.* 5, 161.
1080   doi:10.3389/fmars.2018.00161

1081   Muñoz-Ramírez, C. P., Barnes, D. K. A., Cárdenas, L., Meredith, M. P., Morley, S. A., Roman-
1082   Gonzalez, A., et al. (2020). Gene flow in the Antarctic bivalve *Aequiyoldia eightsii* (Jay,
1083   1839) suggests a role for the Antarctic Peninsula Coastal Current in larval dispersal: Gene
1084   flow patterns in A. eightsii. *R. Soc. Open Sci.* 7, 200603. doi:10.1098/rsos.200603

1085   Nei, M., and Takezaki, N. (1983). Estimation of genetic distances and phylogenetic trees from DNA
1086   analysis. Proc 5th World Cong Genet Appl Livstock Prod, 21(21), 405-412.

1087   Novembre, J. (2016). Pritchard, Stephens, and Donnelly on Population Structure. *Genetics* 204, 391–
1088   393. doi.org/10.1534/genetics.116.195164

1089   Nunez, J. C. B., Rong, S., Damian-Serrano, A., Burley, J. T., Elyanow, R. G., Ferranti, D. A., et al.
1090   (2021). Ecological Load and Balancing Selection in Circumboreal Barnacles. *Mol. Biol. Evol.*
1091   38, 676–685. doi:10.1093/molbev/msaa227

1092   Ouréns, R., Naya, I., and Freire, J. (2015). Mismatch between biological, exploitation, and
1093   governance scales and ineffective management of sea urchin (*Paracentrotus lividus*) fisheries
1094   in Galicia. *Mar. Policy.* 51, 13-20. doi:10.1016/j.marpol.2014.07.015

1095   Palumbi, S. R. (2003). Population genetics, demographic connectivity, and the design of marine
1096   reserves. *Ecol. Appl.* 13, 146–15. doi:10.1890/1051-0761(2003)013[0146:pgdcat]2.0.co;2.

Pannacciulli, F. G., Piyapattanakorn, S., Bishop, J. D. D., Hawkins, S. J., and Maclean, N. (2005). Isolation of highly polymorphic microsatellite markers from the intertidal barnacle *Chthamalus montagui* Southward. *Mol. Ecol. Notes* 5, 641–643. doi:10.1111/j.1471-8286.2005.01027.x

Papa, Y., Oosting, T., Valenza-Troubat, N., Wellenreuther, M., and Ritchie, P. A. (2020). *Genetic stock structure of New Zealand fish and the use of genomics in fisheries management: an overview and outlook*. Taylor and Francis Asia Pacific doi:10.1080/03014223.2020.1788612

Parada, J. M. M., Iglesias, E., Outeiral, R., and Molares, J. (2013). Diameter of the base of the capitulum as a biometric variable of the goose barnacle *Pollicipes pollicipes* (Gmelin, 1789) (Cirripedia, Lepadomorpha). *Crustaceana* 86, 1527–1538. doi:10.1163/15685403-00003251

Pascual, M., Rives, B., Schunter, C., and MaCpherson, E. (2017). Impact of life history traits on gene flow: A multispecies systematic review across oceanographic barriers in the Mediterranean Sea. *PLoS ONE* 12, e0176419. doi:10.1371/journal.pone.0176419

Pavón, M. C. (2003). Biología y variables poblacionales del percebe, *Pollicipes pollicipes* (Gmelin, 1790) en Asturias. (Doctoral dissertation, Universidad de Oviedo). http://hdl.handle.net/10651/16203

Peakall, R., and Smouse, P. E. (2012). GenALEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* 28, 2537–2539. doi:10.1093/bioinformatics/bts460

Peliz, A., Santos, A. M. P., Oliveira, P. B., & Dubert, J. (2004). Extreme cross-shelf transport induced by eddy interactions southwest of Iberia in winter 2001. *Geophys. Res. Lett.* 31(8). doi:10.1029/2004GL019618

Pew, J., Muir, P. H., Wang, J., and Frasier, T. R. (2015). related: An R package for analysing pairwise relatedness from codominant molecular markers. *Mol. Ecol. Resour.* 15, 557–561. doi:10.1111/1755-0998.12323

Pineda, J. (1999). Circulation and larval distribution in internal tidal bore warm fronts. *Limnol. Oceanogr.* 44(6), 1400-1414. doi: 10.4319/lo.1999.44.6.1400

Pineda, J., Hare, J. A., and Sponaugle, S. (2007). Larval transport and dispersal in the coastal ocean and consequences for population connectivity. *Oceanography* 20, 22–39. doi:10.5670/oceanog.2007.27

Pineda, J., Starczak, V., and Stueckle, T. A. (2006). Timing of successful settlement: Demonstration of a recruitment window in the barnacle *Semibalanus balanoides*. *Mar. Ecol. Prog. Ser.* 320, 233–237. doi:10.3354/meps320233

Piry, S., Luikart, G., and Cornuet, J. M. M. (1999). Bottleneck: A Computer Program for Detecting Recent Reductions in the Effective Population Size Using allele Frequency Data. *J. Hered.* 90, 502–503. doi:10.1002/anie.198209122

Plough, L. V. L., Moran, A., Marko, P. P., Andersson, M., Jennions, M., Petrie, M., et al. (2014). Density drives polyandry and relatedness influences paternal success in the Pacific gooseneck barnacle, *Pollicipes elegans*. *BMC Evol. Biol.* 14, 81. doi:10.1186/1471-2148-14-81

Plough, L. V., and Marko, P. B. (2014). Characterization of microsatellite loci and repeat density in the gooseneck barnacle, *Pollicipes elegans*, using next generation sequencing. *J. Hered.* 105, 136–142. doi:10.1093/jhered/est064

Porri, F., McQuaid, C. D., and Radloff, S. (2006) Spatio-temporal variability of larval abundance and settlement of *Perna perna*: differential delivery of mussels. *Mar. Ecol. Prog. Ser.* 315:141–150. doi:10.3354/meps315141

Pritchard, J. K., Stephens, M., and Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* 155, 945–959. doi:10.1093/genetics/155.2.945

Provan, J., and Bennett, K. D. (2008). Phylogeographic insights into cryptic glacial refugia. *Trends Ecol. Evol.* 23(10), 564-571. doi:10.1016/j.tree.2008.06.010

Queiroga, H., Cruz, T., dos Santos, A., Dubert, J., González-Gordillo, J. I., Paula, J., et al. (2007). Oceanographic and behavioural processes affecting invertebrate larval dispersal and supply in the western Iberia upwelling ecosystem. *Prog. Oceanogr.* 74, 174–191. doi:10.1016/j.pocean.2007.04.007

Quinteiro, J., Rodríguez-Castro, J., and Rey-Méndez, M. (2007). Population genetic structure of the stalked barnacle *Pollicipes pollicipes* (Gmelin, 1789) in the northeastern Atlantic: Influence of coastal currents and mesoscale hydrographic structures. *Mar. Biol.* 153, 47–60. doi:10.1007/s00227-007-0783-0

Rassweiler, A., Costello, C., and Siegel, D. A. (2012). Marine protected areas and the value of spatially optimized fishery management. *P. Natl. Acad. Sci. USA.* 109(29), 11884-11889. doi:10.1073/pnas.1116193109

Reigel, A. M., Harrison, J. S., and Gleason, D. F. (2015). Tetranucleotide microsatellites for the barnacle *Megabalanus coccopoma* (Darwin, 1854). *Biochem. Syst. Ecol.* 62, 159–163. doi:10.1016/j.bse.2015.08.010

Relvas, P., Barton, E. D., Dubert, J., Oliveira, P. B., Peliz, A., Da Silva, J. C. B., and Santos, A. M. P. (2007). Physical oceanography of the western Iberia ecosystem: latest views and challenges. *Prog. Oceanogr.* 74(2-3), 149-173. doi:10.1016/j.pocean.2007.04.021

Rice, W. R. (1989). Analyzing Tables of Statistical Tests. *Evolution* 43, 223. doi:10.2307/2409177

Rico, C., Cuesta, J. A., Drake, P., Macpherson, E., Bernatchez, L., and Marie, A. D. (2017). Null alleles are ubiquitous at microsatellite loci in the Wedge Clam (*Donax trunculus*). *PeerJ* 5, e3188. doi:10.7717/peerj.3188

Rilov, G., Dudas, S. E., Menge, B. A., Grantham, B. A., Lubchenco, J., and Schiel, D. R. (2008). The surf zone: a semi-permeable barrier to onshore recruitment of invertebrate larvae?. *J. Exp. Mar. Biol. Ecol.* 361(2), 59-74. doi:10.1016/j.jembe.2008.04.008

Rivera, A., Gelcich, S., García-Flórez, L., and Acuña, J. L. (2017). Trends, drivers, and lessons from a long-term data series of the Asturian (northern Spain) gooseneck barnacle territorial use rights system. *Bull. Mar. Sci.* 93, 35–51. doi:10.5343/bms.2015.1080

Rivera, A., Gelcich, S., García-Florez, L., Alcázar, J. L., Acuña, J. L. (2014). Co-management in Europe: Insights from the gooseneck barnacle fishery in Asturias, Spain. *Mar. Policy* 50, 300–308. doi:10.1016/j.marpol.2014.07.011

Rivera, A., Weidberg, N., Pardiñas, A. F., González-Gil, R., García-Flórez, L., and Acuña, J. L. (2013). Role of upwelling on larval dispersal and productivity of gooseneck barnacle populations in the Cantabrian sea: Management implications. *PLoS ONE* 8, e78482. doi:10.1371/journal.pone.0078482

Rousset, F. (1997). Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics*, 145(4), 1219-1228

Ruiz-Díaz, R., Liu, X., Aguión, A., Macho, G., deCastro, M., Gómez-Gesteira, M., and Ojea, E. (2020). Social-ecological vulnerability to climate change in small-scale fisheries managed under spatial property rights systems. *Mar. Policy. 121*, 104192. doi:10.1016/j.marpol.2020.104192

Sánchez, R. F., and Relvas, P. (2003). Spring–summer climatological circulation in the upper layer in the region of Cape St. Vincent, Southwest Portugal. *ICES J. Mar. Sci.* 60(6), 1232-1250. doi:10.1016/S1054-3139(03)00137-1

Schmieder, R., and Edwards, R. (2011). Quality control and preprocessing of metagenomic datasets. *Bioinformatics* 27, 863–864. doi:10.1093/bioinformatics/btr026

Selkoe, K. A., and Toonen, R. J. (2006). Microsatellites for ecologists: A practical guide to using and evaluating microsatellite markers. *Ecol. Lett.* 9, 615–629. doi:10.1111/j.1461-0248.2006.00889.x

Selkoe, K. A., Watson, J. R., White, C., Horin, T. B., Iacchei, M., Mitarai, S., et al. (2010). Taking the chaos out of genetic patchiness: Seascape genetics reveals ecological and oceanographic drivers of genetic patterns in three temperate reef species. *Mol. Ecol.* 19, 3708–3726. doi:10.1111/j.1365-294X.2010.04658.x

Seoane-Miraz, D., Martínez-Lage, A., and González-Tizón, A. M. (2015). Characterization of 15 polymorphic microsatellite loci in gooseneck barnacle *Pollicipes pollicipes* (Gmelin, 1789), and cross-amplification in other Pollicipes species. *Conserv. Genet. Resour.* 7, 591–593. doi:10.1007/s12686-015-0436-4

Séré, M., Thévenon, S., Belem, A. M. G., and De Meeûs, T. (2017). Comparison of different genetic distances to test isolation by distance between populations. *Heredity* 119, 55–63. doi:10.1038/hdy.2017.26

Shanks, A. L., McCulloch, A., and Miller, J. (2003). Topographically generated fronts, very nearshore oceanography and the distribution of larval invertebrates and holoplankters. *J. Plankton Res.* 25(10), 1251-1277. doi:10.1093/plankt/fbg090

1208 Silva, C. N. S., MacDonald, H. S., Hadfield, M. G., Cryer, M., and Gardner, J. P. A. (2019). Ocean
1209   currents predict fine-scale genetic structure and source-sink dynamics in a marine invertebrate
1210   coastal fishery. *ICES J. Mar. Sci.* 76, 1007–1018. doi:10.1093/icesjms/fsy201

1211 Sotelo, G., Morán, P., and Posada, D. (2007). Identification and characterization of microsatellite loci
1212   in the spiny spider crab *Maja brachydactyla*. *Conserv. Genet.* 8(1), 245-247.
1213   doi:10.1007/s10592-006-9141-x

1214 Sousa, A., Jacinto, D., Penteado, N., Martins, P., Fernandes, J., Silva, T., et al. (2013). Patterns of
1215   distribution and abundance of the stalked barnacle (*Pollicipes pollicipes*) in the central and
1216   southwest coast of continental Portugal. *J. Sea Res.* 83, 187–194.
1217   doi:10.1016/j.seares.2013.04.005

1218 Sousa, A., Jacinto, D., Penteado, N., Pereira, D., Silva, T., Castro, J. J., et al. (2020). Temporal
1219   variation of the fishers' perception about the stalked barnacle (*Pollicipes pollicipes*) fishery at
1220   the Berlengas Nature Reserve (Portugal). *Reg. Stud. Mar. Sci.* 38, 101378.
1221   doi:10.1016/j.rsma.2020.101378

1222 St-Onge, P., Tremblay, R., and Sévigny, J. M. (2015). Tracking larvae with molecular markers
1223   reveals high relatedness and early seasonal recruitment success in a partially spawning marine
1224   bivalve. *Oecologia* 178, 733–746. doi:10.1007/s00442-015-3245-2

1225 Takezaki, N., Nei, M., and Tamura, K. (2014). POPTREEW: web version of POPTREE for
1226   constructing population trees from allele frequency data and computing some other quantities.
1227   *Mol. Biol. Evol.* 31(6), 1622-1624. doi:10.1093/molbev/msu093

1228 Treml, E. A., Halpin, P. N., Urban, D. L., and Pratson, L. F. (2008). Modeling population
1229   connectivity by ocean currents, a graph-theoretic approach for marine conservation. *Landsc.*
1230   *Ecol.* 23, 19–36. doi:10.1007/s10980-007-9138-y

1231 Van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M., and Shipley, P. (2004). MICRO-
1232   CHECKER: Software for identifying and correcting genotyping errors in microsatellite data.
1233   *Mol. Ecol. Notes* 4, 535–538. doi:10.1111/j.1471-8286.2004.00684.x

1234 Van Wyngaarden, M., Snelgrove, P. V. R., DiBacco, C., Hamilton, L. C., Rodríguez-Ezpeleta, N.,
1235   Jeffery, N. W., et al. (2017). Identifying patterns of dispersal, connectivity and selection in
1236   the sea scallop, *Placopecten magellanicus*, using RADseq-derived SNPs. *Evol. Appl.* 10,
1237   102–117. doi:10.1111/eva.12432

1238 Veliz, D., Duchesne, P., Bourget, E., and Bernatchez, L. (2006). Genetic evidence for kin
1239   aggregation in the intertidal acorn barnacle (*Semibalanus balanoides*). *Mol. Ecol.* 15, 4193–
1240   4202. doi:10.1111/j.1365-294X.2006.03078.x

1241 Waples, R. S. (1998). Separating the wheat from the chaff: Patterns of genetic differentiation in high
1242   gene flow species. *J. Hered.* 89(5), 438–450. doi:10.1093/jhered/89.5.438

1243 Waples, R. S. (2014). Testing for Hardy–Weinberg Proportions: Have We Lost the Plot? *J. Hered.*
1244   106(1), 1–19. doi:10.1093/jhered/esu062

1245   Waples, R. S. (2018). Null Alleles and F_{IS} x F_{ST} Correlations. *J. Hered.* 457–461.
1246   doi:10.1093/jhered/esy013

1247   Weidberg, N., Lobón, C., López, E., Flórez, L. G., Rueda, M. D. P. F., Largier, J. L., and Acuña, J. L.
1248   (2014). Effect of nearshore surface slicks on meroplankton distribution: role of larval
1249   behaviour. *Mar. Ecol. Prog. Ser.* 506, 15-30. doi:10.3354/meps10777

1250   Weir, B. S., and Cockerham, C. C. (1984). Estimating F-Statistics for the Analysis of Population
1251   Structure. *Evolution* 38, 1358. doi:10.2307/2408641

1252   Wong, J. Y., Chen, H.N., Chan, B. K. K., Tan, I. K. P., and Chong, V. C. (2014). A combined
1253   morphological and molecular approach in identifying barnacle cyprids from the Matang
1254   Mangrove Forest Reserve in Malaysia: essentials for larval ecology studies. *Raffles B. Zool.*
1255   62, 317-329. doi:10.5281/zenodo.5756768

Tables

1257   Table 1. Overall microsatellites information based on multiplex PCRs typifying *P. pollicipes* populations coming from 15 different localities of the Iberian Peninsula.

M	Locus/ Genbank Accesion number	Dye	Repeat motif	C_F	T_A	Primer sequence (5'-3')	ASR	N	k	A_R (n=26)	H_O	H_E	F_{IS} (p-value)	F_{ST} (p-value)	F_{ST} ENA	F_{IT}	B
M1	**RF12**	6-FAM	CGCA	0.4	60ºC	F: ATTGGATACCCCGTCTAGCTGA	131 – 221	1397	30	12.929	0.685	0.889	0.231	0.003	0.002	0.234	0.110*
	MW443104					R: GTGCTAAGCTCGCCTTATCA							(0.0001)	(0.0001)			
	OV100	VIC	AC	0.2		F: AACGATCCACAAGCATGCAACACG	177 – 323	1408	59	23.881	0.862	0.954	0.096	0.006	0.005	0.101	0.047
	MW443110					R: CATAATTGCAAAATTAAGCCGGTG							(0.0001)	(0.0001)			
	RF17	NED	CGTG	0.2		F: GGCGTTGGTCACCACTGA	135 – 239	1393	12	3.877	0.582	0.602	0.032	0.001	0.001	0.033	0.013
	MW443105					R: AGTTAATCTGCGTGTCCAGGAT							(0.0544)	(0.0652)			
	OV113	PET	GT	0.4		F: GTGGACTACATGTCCCACTGC	107 – 245	1396	62	23.202	0.505	0.944	0.466	0.002	0.002	0.467	0.226*
	MW443112					R: GATTCCTCTGCAACTCAGCGAT							(0.0001)	(0.1068)			
M2	**VG49**	6-FAM	TGAG	0.4	60ºC	F: AGGTAATCGTCTGATAGTCAGCTCGC	331 - 439	1389	37	15.707	0.893	0.921	0.030	0.000	0.000	0.029	0.013
	MW443106					R: TGTGGACACGCATGTGTGCTGGC							(0.0001)	(0.8637)			
	OV89	VIC	CA	0.2		F: CACCTTTTGTGCTCCCAATGGA	127 – 185	1416	13	5.358	0.277	0.404	0.312	0.011	0.013	0.320	0.092*
	MW443109					R: GACTAACACCAGCTGTCCGT							(0.0001)	(0.1251)			
	VG55	NED	CA	0.2		F: GCAACTATCAGCGCTTGACCAT	161 – 209	1419	18	8.512	0.557	0.600	0.070	0.003	0.001	0.073	0.027
	MW443107					R: AGGGGAATCCTAATACCGTCGT							(0.0001)	(0.3934)			
	OV122	PET	CACG	0.2		F: GACGCCATATAGCCTCAGCA	111 - 169	1417	25	12.072	0.713	0.773	0.077	-0.001	0.000	0.077	0.033
	MW443114					R: GTCAAAAAGTGTTGCCCACGAA							(0.0001)	(0.2484)			
M3	**OV121**	6-FAM	TG	0.2	64ºC	F: GATCCGGTCCTGTCAGACAC	95 – 155	1405	29	13.489	0.704	0.888	0.209	0.003	0.003	0.211	0.097*
	MW443113					R: TGCTATCACTTGGCACCGTC							(0.0001)	(0.0115)			
	OV81	VIC	GA	0.2		F: GGCTGTGGAGCATTAGACGT	341 - 423	1356	42	20.294	0.850	0.945	0.096	0.002	0.002	0.097	0.047
	MW443108					R: CCAATGTGGTAGCATCGTTACC							(0.0001)	(0.2331)			

M	Locus	Dye	Motif	C_F	T_A	Primer	ASR	N	k	AR	H_O	H_E	F_{IS}	F_{ST}	F_{ST} ENA	F_{IT}	B
	OV103	NED	ATGT	0.5		F: CACGTGTGCCGCATTTGTA	199 - 296	1340	19	7.749	0.308	0.544	0.437	0.000	0.004	0.437	0.154 *
	MW443111					R: GGCAGAAATAGCCACGCTC						(0.0001)	(0.0151)				
	RF03	PET	TG	0.2		F: TCTTGATTGTGGCACCCATGTT	207 – 367	1260	63	12.882	0.392	0.792	0.494	0.010	0.006	0.500	0.224 *
	MW443103					R: GGACTAACTCGTCCTGCACC						(0.0001)	(0.0148)				
M4	**Ppol_01**	6-FAM	CTGT	0.06	60°C/55°C	F: GTGGGTCTTCCCTGTCAAAC	210 – 254	1356	11	3.801	0.602	0.598	-0.012	-0.003	-0.002	-0.015	-0.006
	MZ576446					R: GATCGTATCAGCACGAAGCTC						(0.7583)	(0.9429)				
	Ppol_03	NED	CACG	0.06		F: GTTGTGTATCCCAGGCTTGC	86 – 142	1381	16	8.185	0.446	0.607	0.270	0.000	0.001	0.270	0.101 *
	MZ576448					R: GATATTTGGCAGCCATAGCC						(0.0001)	(0.5606)				
	Ppol_05	PET	GCGT	0.06		F: CGCGCACGTGTGTATTTAAC	166 – 194	1372	11	5.690	0.559	0.559	0.002	-0.001	-0.001	0.002	0.000
	MZ576450					R: ATCTTCGCGGTTGCTGAC						(0.4446)	(0.8485)				
	Ppol_09	VIC	TAG	0.06		F: CAAAACACCGTATGACGTTCAC	146 – 247	1344	34	21.147	0.905	0.947	0.042	-0.001	-0.001	0.041	0.020
	MZ576453					R: ACCCGTACTACTGCTTTTACCG						(0.0001)	(0.6503)				
M5	**Ppol_08**	NED	CGCA	0.1	60°C/55°C	F: TTCCTGACCGTTAAGCTTGC	156 – 276	1366	51	24.716	0.898	0.960	0.063	0.000	0.000	0.063	0.030
	MZ576452					R: AACTGCACCACCAATTCTCC						(0.0001)	(0.6328)				
	Ppol_02	6-FAM	GTCT	0.1		F: CGTTGCATTCCTATGCCTATC	176 – 232	1371	16	9.136	0.761	0.793	0.034	-0.001	-0.001	0.033	0.014
	MZ576447					R: CGCTGACCGACAAGGTTAC						(0.0041)	(0.1719)				
	Ppol_04	PET	CACG	0.13		F: TGCACAAATCAAGATGCACAG	102 – 178	1165	24	13.196	0.366	0.893	0.594	0.001	0.003	0.594	0.280 *
	MZ576449					R: TCTCTCCAGCCGTCCTTG						(0.0001)	(0.1494)				
	Ppol_07	VIC	(TAC)₉(TGC)(TAC)₇	0.06		F: CCACTCACGACATTACACCAC	104 – 155	1382	10	5.815	0.683	0.673	-0.016	0.000	0.000	-0.017	-0.007
	MZ576451					R: GAGCATCGGCTTTCAGGAC						(0.8298)	(0.2741)				
	Average							1366.650	29.100	12.582	0.627	0.764	0.176	0.002	0.002	0.178	0.076

1258 M: Multiplex. C_F: PCR final concentration. T_A: Annealing Temperature. ASR: Allele size range in bp. N: Sample sizes. k: number of alleles per locus. AR: Allelic richness for the minimum possible number of

1259 diploid individuals per sample (n=26) H_O: Observed Heterozygosity. H_E: Expected Heterozygosity. Weir and Cockerham (1984) F statistics: F_{IS} (* p<0.05 evaluated using 10000 permutations in FSTAT

1260 software) F_{ST}, F_{ST} ENA (excluding null alleles following Chapuis and Estoup, 2007) and F_{IT}. B: Brookfield 1 statistic for null allele's inferences using the Microchecker software (*q>0.05).

1261   Table 2. Genetic variability of *P. pollicipes* populations (adults vs juveniles) coming from 15 distinct localities along the Atlantic Iberian Peninsula coastline.
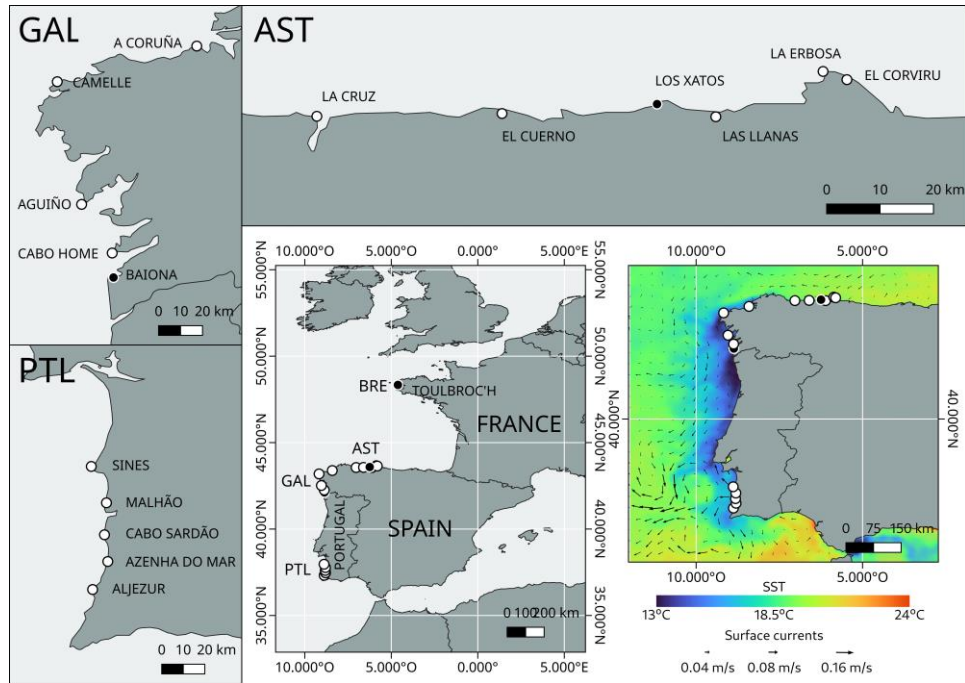
Country	Region	Locality	Coordinates	Sampling date	Life Stage	Code	N	N_A	A_P	A_R	H_O	H_E	F_{IS}	R_{XY}	TPM p
Portugal	SW Portugal	Aljezur	37.32141, -8.879	5-9/10/2017	Adult (AD)	PTL_AL_AD	48	14.800	1	12.455	0.631	0.764	0.176*	0.045	0.959
Portugal	SW Portugal	Azenha do Mar	37.46747, -8.79988	11/10/2017	Adult (AD)	PTL_AZ_AD	48	13.800	4	11.964	0.624	0.762	0.184*	0.048	0.861
Portugal	SW Portugal	Cabo Sardão	37.6068, -8.81716	21/09/2017	Adult (AD)	PTL_CO_AD	45	14.650	6	12.152	0.627	0.770	0.186*	0.058	0.983
Portugal	SW Portugal	Malhão	37.77324, -8.8068	6/10/2017	Adult (AD)	PTL_MA_AD	48	15.000	8	12.514	0.667	0.783	0.150*	0.043	0.968
Portugal	SW Portugal	Sines	37.96286, -8.88591	23/09/2017	Adult (AD)	PTL_CS_AD	48	14.800	3	12.508	0.650	0.779	0.168*	0.045	0.980
						Average PTL_AD	*47.400*	*14.610*	*4.4*	*12.318*	*0.640*	*0.772*	*0.173*	*0.057*	*0.999*
Portugal	SW Portugal	Aljezur		5-9/10/2017	Juvenile (JV)	PTL_AL_JV	45	12.350	1	11.228	0.602	0.750	0.201*	0.085	0.982
Portugal	SW Portugal	Azenha do Mar		11/10/2017	Juvenile (JV)	PTL_AZ_JV	48	13.750	3	11.868	0.617	0.772	0.203*	0.055	0.995
Portugal	SW Portugal	Cabo Sardão		21/09/2017	Juvenile (JV)	PTL_CO_JV	46	13.450	2	11.513	0.606	0.749	0.193*	0.055	0.980
Portugal	SW Portugal	Malhão		6/10/2017	Juvenile (JV)	PTL_MA_JV	46	13.600	3	11.776	0.596	0.747	0.205*	0.058	0.980
Portugal	SW Portugal	Sines		23/09/2017	Juvenile (JV)	PTL_CS_JV	48	13.500	2	11.459	0.602	0.756	0.205*	0.070	0.985
						Average PTL_JV	*46.600*	*13.330*	*2.2*	*11.569*	*0.605*	*0.755*	*0.201*	*0.073**	*0.999*
						Average PTL	**47.000**	**13.970**	**3.3**	**11.944**	**0.622**	**0.763**	**0.187***		**1.000**

Country	Region	Location	Coordinates	Date	Age class	Code	N								
Spain	Galicia	Baiona	42.11847, -8.86672	09/10/2017	Adult (AD)	GAL_BA_AD	48	14.700	1	12.273	0.663	0.776	0.147*	0.049	0.988
Spain	Galicia	Cabo Home	42.25244, -8.87372	05/10/2017	Adult (AD)	GAL_CH_AD	48	14.600	1	12.253	0.655	0.765	0.145*	0.047	0.997
Spain	Galicia	Aguiño	42.51861, -9.04111	09/10/2017	Adult (AD)	GAL_AG_AD	48	15.700	5	12.874	0.662	0.764	0.136*	0.042	0.998
Spain	Galicia	Camelle	43.19, -9.1743	09/10/2017	Adult (AD)	GAL_CA_AD	48	14.850	4	12.433	0.635	0.758	0.165*	0.049	0.993
Spain	Galicia	A Coruña	43.38502, -8.41133	06/10/2017	Adult (AD)	GAL_AC_AD	48	15.650	5	12.918	0.666	0.776	0.143*	0.042	0.959
						Average GAL_AD	*48.000*	*15.100*	*3.2*	*12.550*	*0.656*	*0.768*	*0.147*￼*	*0.055*	*0.999*
Spain	Galicia	Baiona		09/10/2017	Juvenile (JV)	GAL_BA_JV	48	14.250	1	12.025	0.628	0.753	0.146*	0.051	0.996
Spain	Galicia	Cabo Home		05/10/2017	Juvenile (JV)	GAL_CH_JV	48	14.950	1	12.383	0.659	0.770	0.168*	0.046	0.983
Spain	Galicia	Aguiño		09/10/2017	Juvenile (JV)	GAL_AG_JV	42	14.850	3	12.669	0.647	0.759	0.149*	0.043	0.990
Spain	Galicia	Camelle		09/10/2017	Juvenile (JV)	GAL_CA_JV	44	13.700	0	12.372	0.643	0.785	0.183*	0.059	0.938
Spain	Galicia	A Coruña		06/10/2017	Juvenile (JV)	GAL_AC_JV	48	15.350	5	12.640	0.663	0.768	0.137*	0.046	0.997
						Average GAL_JV	*46.000*	*14.620*	*2*	*12.418*	*0.648*	*0.767*	*0.157*￼*	*0.058*	*0.999*
						Average GAL	**47.000**	**14.860**	**2.6**	**12.484**	**0.652**	**0.767**	**0.152***		**0.999**
Spain	Asturias	La Cruz	43.55691, -7.02893	20/09/2017	Adult (AD)	AST_PC_AD	50	15.050	7	12.511	0.607	0.754	0.197*	0.043	0.987
Spain	Asturias	El Cuerno	43.56585, -6.60318	19/09/2017	Adult (AD)	AST_CU_AD	48	15.250	4	12.568	0.610	0.757	0.196*	0.047	0.998

							N	N_A	A_p	A_R	H_O	H_E	F_{IS}	R_{XY}	TPM p
Spain	Asturias	Las Llanas	43.56212, -6.10582	20/09/2017	Adult (AD)	AST_LM_AD	49	14.500	2	12.103	0.625	0.757	0.176*	0.050	0.955
Spain	Asturias	La Erbosa	43.6631, -5.86407	20/09/2017	Adult (AD)	AST_ER_AD	50	14.900	4	12.319	0.620	0.760	0.186*	0.047	0.968
Spain	Asturias	El Corviru	43.64414, -5.80895	20/09/2017	Adult (AD)	AST_EC_AD	44	13.500	1	11.834	0.614	0.762	0.196*	0.056	0.971
						Average AST_AD	*48.200*	*14.640*	*3*	*12.267*	*0.615*	*0.758*	*0.190**	*0.059*	*0.999*
Spain	Asturias	La Cruz		20/09/2017	Juvenile (JV)	AST_PC_JV	47	14.750	3	12.505	0.606	0.768	0.213*	0.055	0.993
Spain	Asturias	El Cuerno		19/09/2017	Juvenile (JV)	AST_CU_JV	50	14.750	3	12.067	0.616	0.760	0.191*	0.053	0.995
Spain	Asturias	Las Llanas		20/09/2017	Juvenile (JV)	AST_LM_JV	50	14.200	3	11.804	0.561	0.753	0.257*	0.060	0.995
Spain	Asturias	La Erbosa		20/09/2017	Juvenile (JV)	AST_ER_JV	50	14.450	3	12.052	0.611	0.752	0.190*	0.053	0.998
Spain	Asturias	El Corviru		20/09/2017	Juvenile (JV)	AST_EC_JV	45	12.150	1	11.074	0.613	0.752	0.186*	0.088	0.978
						Average AST_JV	*48.400*	*14.060*	*2.6*	*11.900*	*0.601*	*0.757*	*0.207**	*0.070***	*0.999*
						Average AST	**48.300**	**14.350**	**2.8**	**12.084**	**0.608**	**0.76**	**0.199***		**0.999**
						Average Iberian Peninsula	**47.433**	**14.393**	**2.9**	**12.170**	**0.627**	**0.76**	**0.179***		

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Figures

1267 **Figure 1.** Study regions of the Iberian Peninsula for genetic analyses of *P. pollicipes* using
1268 microsatellites: Galicia (GAL); Asturias (AST) and SW Portugal (PTL). A total of 15 localities were
1269 sampled (white dots) for population genetic analyses. Three localities from the Bay of Biscay
1270 (including one from the French Brittany (BRE)) were initially sampled for microsatellites developing
1271 procedures (black dots). Also depicted is the upwelling circulation characteristic of the summer along
1272 the Atlantic coast of the Iberian Peninsula.

1273
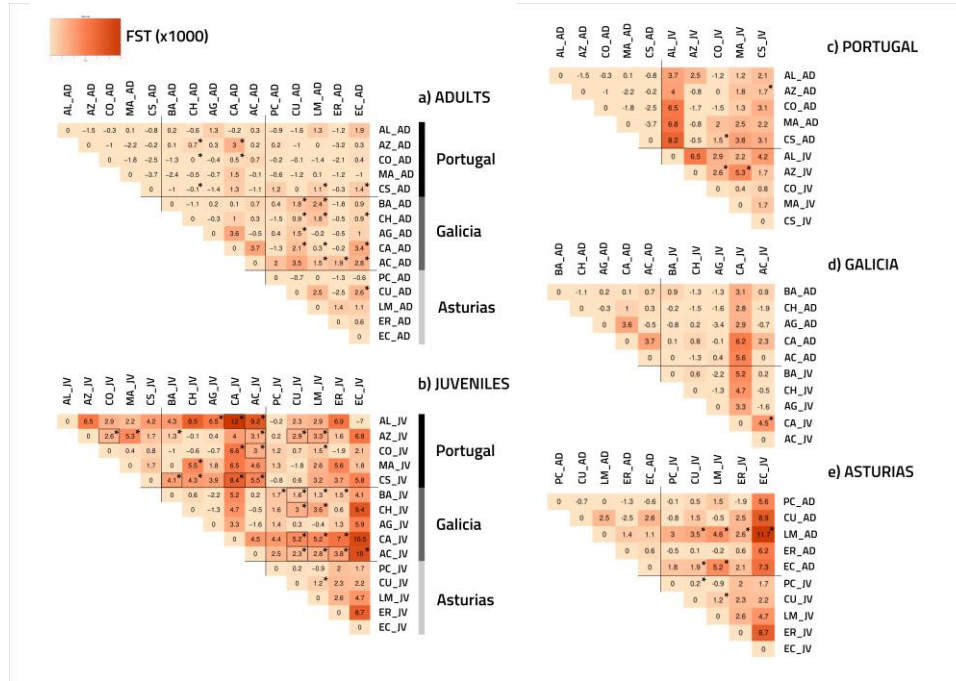
Figure 2. F_{ST} heatmaps (based on Weir and Cockerham (1984)) following genetic analyses of *P.*
pollicipes using microsatellites along the Iberian Peninsula. The darker the color, the higher the F_{ST}
value. Asterisks (*) indicate significant p-values (p<0.05) while significant values after a FDR
correction (Benjamini and Hochberg, 1995) are highlighted by black rectangles: (a) adults (AD) and
(b) juvenile (JV) analyses among regions. (c) Portugal, (d) Galicia, and (e) Asturias analyses between
the two developmental stages (adults vs juvenile) within each of the three regions.
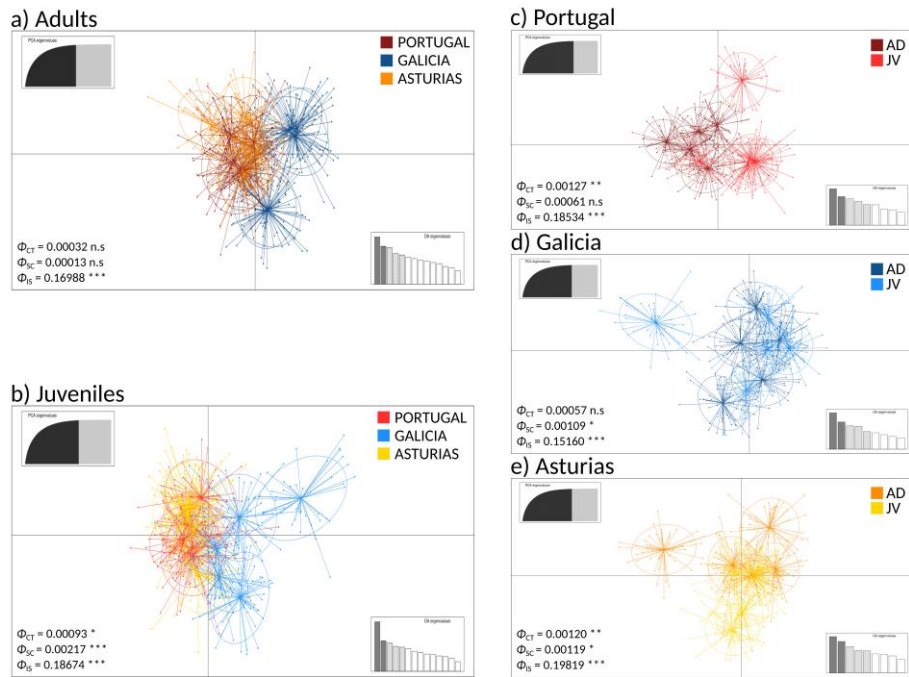
Figure 3. Genetic clustering using Principal Components Analysis (PCA) of *P. pollicipes* populations using microsatellites along the Iberian Peninsula. In each case the Φ statistics are shown together with their p-values after AMOVA analyses (Φ_{CT}: Among groups, Φ_{SC}: Among populations within groups, Φ_{IS}: Among individuals within populations): (a) adults (AD) and (b) juvenile (JV) analyses among regions. (c) Portugal, (d) Galicia, and (e) Asturias analyses between the two developmental stages (adults vs juvenile) within each of the three regions. *P<0.05, ** P<0.01, *** P<0.001.
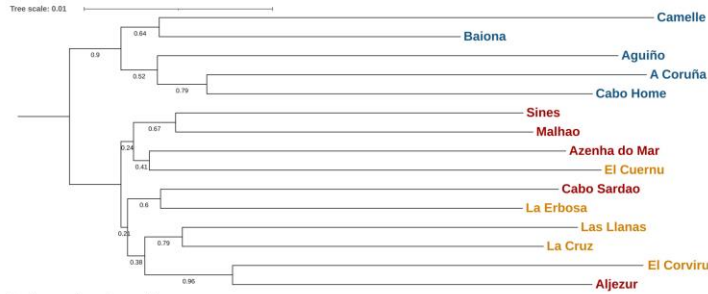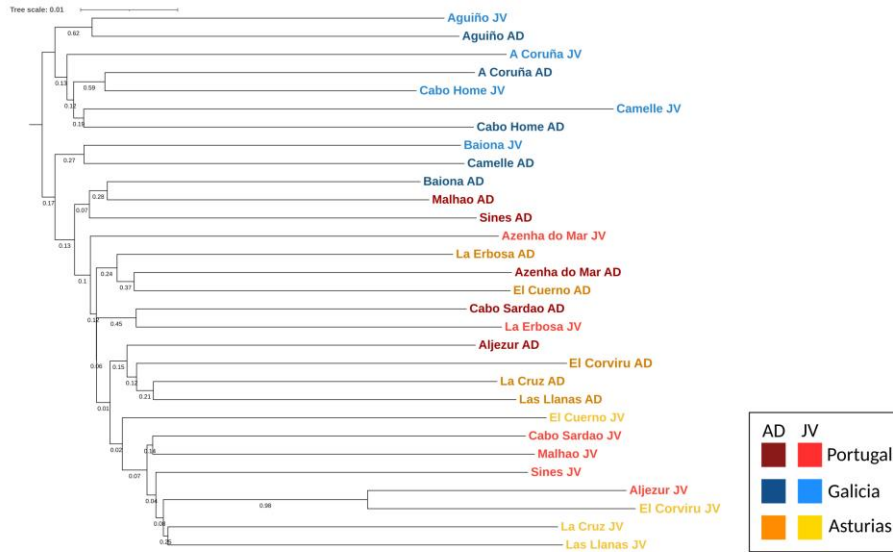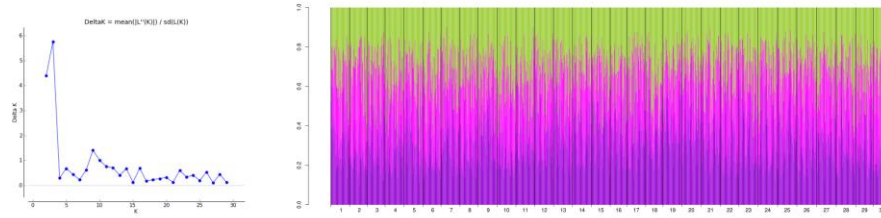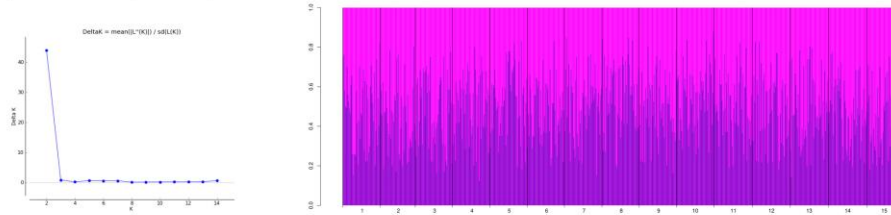
Figure 4. Neighbor Joining trees using DA distance (Nei et al. 1983) of *P. pollicipes* populations using microsatellites along the Iberian Peninsula. (a) Global analysis for 15 localities (adults and juveniles mixed together), (b) global analysis in 30 samples (localities+ developmental stages, i.e.: adults and juveniles).
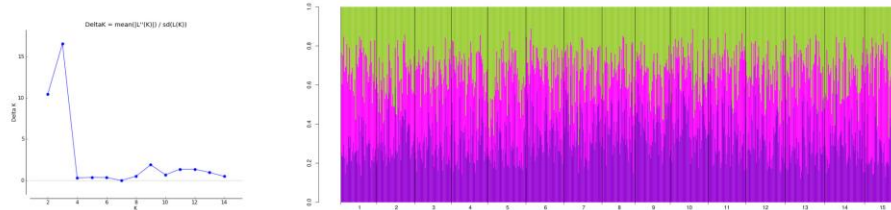
a) Global (K = 3)

b) Adults (K = 2)

c) Juveniles (K = 3)

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1299 **Figure 5.** Structure bar-plot showing the assignment probabilities for each genotyped individual
1300 under admixture model. Global (a), adults (b) and juveniles (c) analysis. Each bar corresponds with
1301 one individual.

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