

1 **Implications of population connectivity studies for the design of marine protected areas in**
2 **the deep-sea: an example of a demosponge from the Clarion-Clipperton Zone**

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4 Sergi Taboada^{1,2,*}, Ana Riesgo¹, Helena Wiklund¹, Gordon L.J. Paterson¹, Vasiliki Koutsouveli¹,
5 Nadia Santodomingo¹, Andrew C. Dale³, Craig R. Smith⁴, Daniel O.B. Jones⁵, Thomas G.
6 Dahlgren^{6,7,8} and Adrian G. Glover¹

7

8 ¹*Life Sciences Department, The Natural History Museum, Cromwell Road, London, UK*

9 ²*Departamento de Ciencias de la Vida, Ecología y Ciencias Ambientales, Universidad de Alcalá, Alcalá de Henares,*
10 *Spain*

11 ³*The Scottish Association for Marine Science, Oban, UK*

12 ⁴*Department of Oceanography, University of Hawaii, Honolulu, Hawaii*

13 ⁵*National Oceanography Centre, University of Southampton Waterfront Campus, Southampton, United Kingdom*

14 ⁶*NORCE, Uni Research, Bergen, Norway*

15 ⁷*Department of Marine Sciences, University of Gothenburg, Sweden*

16 ⁸*Gothenburg Global Biodiversity Centre, University of Gothenburg, Sweden*

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18 *Corresponding author: sergiotab@gmail.com

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28 phylogeography; oceanographic modelling

29

30 **Abstract** The abyssal demosponge *Plenaster craigi* inhabits the Clarion-Clipperton Zone (CCZ)
31 in the north-east Pacific, a region with abundant seafloor polymetallic nodules with potential
32 mining interest. Since *P. craigi* is a very abundant encrusting sponge on nodules, understanding
33 its genetic diversity and connectivity could provide important insights into extinction risks and
34 design of marine protected areas. Our main aim was to assess the effectiveness of the Area of
35 Particular Environmental Interest 6 (APEI-6) as a potential genetic reservoir for three adjacent
36 mining exploration contract areas (UK-1A, UK-1B and OMS-1A). As in many other sponges,
37 *COI* showed extremely low variability even for samples ~900 km apart. Conversely, the 168
38 individuals of *P. craigi*, genotyped for 11 microsatellite markers, provided strong genetic
39 structure at large geographical scales not explained by isolation by distance. Interestingly, we
40 detected molecular affinities between samples from APEI-6 and UK-1A, despite being separated
41 ~800 km. Although our migration analysis inferred very little progeny dispersal of individuals
42 between areas, the major differentiation of OMS-1A from the other areas might be explained by
43 the occurrence of predominantly northeasterly transport predicted by the HYCOM hydrodynamic
44 model. Our study suggests that although APEI-6 does serve a conservation role, with species
45 connectivity to the exploration areas, it is on its own inadequate as a propagule source for *P.*
46 *craigi* for the entire eastern portion of the CCZ. Our new data suggest that an APEI located to the
47 east and/or the south of the UK-1, OMS-1, BGR, TOML and NORI areas would be highly
48 valuable.

49 **Introduction**

50 The Clarion-Clipperton Zone (CCZ), a vast area located in the equatorial NE Pacific,
51 encompasses a broad range of habitats, including abyssal hills, seamounts, fracture zones, and
52 extensive abyssal plains, as well as strong gradients in export flux (Wedding *et al.* 2013). Abyssal
53 plains of the region can contain high concentrations of polymetallic nodules (potato-sized
54 concretions of manganese, iron, cobalt, copper and nickel), with sediments around nodules
55 typically consisting of a mixture of well oxygenated siliceous oozes and deep-sea clays (Mewes
56 *et al.* 2014). Importantly, these polymetallic nodules provide a great abundance of hard substrate
57 for sessile organisms and appear to support faunal communities distinct from nearby abyssal soft
58 sediments (Mullineaux 1987; Thiel *et al.* 1993; Smith & Demopoulos 2003; Veillette *et al.* 2007;
59 Amon *et al.* 2016; Vanreusel *et al.* 2016). In fact, epifaunal densities are significantly higher in
60 areas with dense nodule coverage, with some major taxa such as alcyonacean and antipatharian
61 corals being virtually absent from nodule-free areas (Vanreusel *et al.* 2016).

62 The nodule-rich CCZ represents the most important area for deep-sea mining exploration
63 worldwide (an actual exploration area of ca. 6 million km²; Lodge *et al.* 2014), with mining
64 operations potentially to be initiated by 2025 (Smith & Demopoulos 2003; Glover & Smith 2003;
65 Smith *et al.* 2008a). Small-scale impact experiments conducted so far in the CCZ suggest that the
66 local environmental impacts of nodule mining will be substantial (Borowski & Thiel 1998; Thiel
67 *et al.* 2001; Smith *et al.* 2008b; Miljutin *et al.* 2011; Jones *et al.* 2017), and will directly affect
68 abyssal epifauna (Amon *et al.* 2016; Vanreusel *et al.* 2016). Importantly, mining may not only
69 impact areas where nodules are removed, but will also disturb adjacent areas through re-
70 deposition from sediment plumes, potentially impacting larger seafloor areas than those directly
71 affected by nodule removal (Oebius *et al.* 2001; Smith *et al.* 2008a). The long-term effects of this
72 sediment re-deposition is not understood. These issues were central to the establishment by the
73 International Seabed Authority (ISA) of a network of representative protected areas, termed Areas
74 of Particular Environmental Interest (APEIs), across the CCZ, where exploration and mining
75 activities are prohibited (Wedding *et al.* 2013). The CCZ is characterized by gradients in
76 environmental conditions (e.g., surface-productivity and export flux, depth, and sediment

77 characteristics; ISA 2010) along an east-west and also a north-south axis, leading to marked
78 variation in nodule size and coverage, but also changes in faunal composition along these
79 gradients (Glover *et al.* 2002; Smith *et al.* 2008a; Wedding *et al.* 2013). The APEI network was
80 designed accordingly, preserving the gradients of faunal distribution reflecting the biogeography
81 and connectivity of marine benthic fauna across the region (Wedding *et al.* 2013).

82 To maximise protection of biodiversity over broad areas, like the CCZ, an understanding
83 of biogeography, at both the species and community levels, is crucial (Wedding *et al.* 2013). To
84 achieve this, the evaluation of species' ranges and their levels of population connectivity and
85 turnover are needed (Baco *et al.* 2016). Efforts to determine the population genetic connectivity
86 in deep-sea invertebrates have mainly been focused on chemosynthetic environments (Vrijenhoek
87 2010; Taylor & Roterman 2017). However, as stated by Taylor & Roterman (2017) in their recent
88 review, the ephemeral nature and non-equilibrium conditions characteristic of these particular
89 habitats could limit their comparability to other more common and stable deep-sea habitats.
90 Molecular connectivity of marine invertebrates in non-chemosynthetic deep-sea habitats has
91 barely been assessed and two recent reviews on this topic (Baco *et al.* 2016; Taylor & Roterman
92 2017), concluded that there is a clear need to assess the connectivity of deep-sea organisms from
93 a variety of habitats, life history types, taxonomic groups, and depth zones. This is especially true
94 for studies at abyssal depths and deeper since, to date, there is only one genetic study of species
95 occurring below 5,000 m depth (Ritchie *et al.* 2017). For the CCZ very little information is
96 available on the biogeography and connectivity of fauna inhabiting this region (Glover *et al.* 2002;
97 Paterson *et al.* 2015; Janssen *et al.* 2015). Despite the prominent occurrence of nodules (*i.e.* hard
98 substrate) in this abyssal region, the majority of connectivity studies conducted in the CCZ have
99 focused on selected infaunal taxa (annelids and crustaceans) living in the sediment (e.g. Paterson
100 *et al.* 1998; Glover *et al.* 2002) and with only a few using a molecular approach (Smith *et al.*
101 2008b; Janssen *et al.* 2015).

102 The recently-described abyssal demosponge *Plenaster craigi* Lim & Wiklund, 2017 (Lim
103 *et al.*, 2017) appears to be a good model species to assess the molecular connectivity and to
104 establish biogeographic patterns from local to large spatial scales within the CCZ for a number

105 of reasons (Taboada *et al.* 2017). *Plenaster craigi*, potentially endemic to the central abyssal
106 Pacific, is a remarkably common encrusting element of the nodule fauna, highly (or perhaps
107 totally) dependent on nodules that provide the substrate where adults live attached (Lim *et al.*
108 2017). Thus, populations of this organism will surely be eliminated from the mined areas as
109 nodules will be removed or become buried by sediment plumes. As filter-feeders, these organisms
110 are likely to be vulnerable to sediment plumes generated in the water column after mining, as
111 suggested by studies on shallow-water sponges (Schönberg 2016). Also, although nothing is
112 known about its reproductive traits, *P. craigi* may be like most other sponges and it is assumed to
113 have a limited dispersal phase through lecithotrophic larvae, with most larvae spending short
114 periods of time in the water column— usually less than two weeks (see Maldonado 2006). So this
115 species may have relatively limited dispersal ability compared to species with planktotrophic
116 development.

117 Here we present the first study on the molecular connectivity and dispersal capabilities
118 of an abyssal sessile invertebrate, the sponge *P. craigi*, from four different areas in the eastern
119 CCZ: the APEI-6 area and three sampling sites within adjacent exploration contract areas UK-1
120 [UK-1 Stratum A (UK-1A), UK-1 Stratum B (UK-1B)], and Oceans Minerals Singapore OMS-1
121 Stratum A (OMS-1A) (Fig. 1A). The aim of this study is to assess whether APEI-6 may serve as
122 a genetic reservoir and source of propagules for *P. craigi* if the above-mentioned exploration
123 areas are disturbed by mining in the future. A fragment of the mitochondrial cytochrome *c* oxidase
124 subunit I—*COI*— and 14 microsatellite markers previously isolated and characterized (Taboada *et*
125 *al.* 2017) were used to assess the connectivity of the populations of *P. craigi*. In addition,
126 oceanographic models were applied to investigate their relationship with connectivity patterns
127 observed.

128 **Material and methods**

129 *Sample collection, preservation and sampling design*

130 A total of 180 specimens of the demosponge *P. craigi* were collected from four different areas
131 within the CCZ: APEI-6 and UK-1A, UK-1B and OMS-1A (Table 1). UK-1A and UK-1B
132 correspond to the UK exploration contract area while OMS-1A corresponds to the Oceans
133 Minerals Singapore exploration contract area (Fig. 1). Samples were collected during three
134 different oceanographic cruises: ABYSSLINE AB01 cruise (Oct 3–27, 2013), which studied the
135 UK-1A and UK-1B areas on board the RV *Melville*, ABYSSLINE AB02 cruise (Feb 12–Mar 25,
136 2015) exploring the OMS-1 area on board the RV *Thomas G Thompson*, and MIDAS-JC120
137 cruise (April 15–May 19, 2015) exploring the APEI-6 area on board the RRS *James Cook*. The
138 separate specimens of *P. craigi* were found attached to polymetallic nodules primarily collected
139 using a USNEL type boxcore (0.25 m²), but additionally from multicore, Brenke epibenthic sledge,
140 Agassiz trawl, and a ROV (Table 1). Sample and specimen handling followed the protocol in
141 (Glover *et al.* 2015). Nodules were carefully observed individually under the stereoscope and
142 once the sponges were found they were photographed; sponges were then removed from the
143 nodule with a scalpel or forceps, preserved in either 80–95 % ethanol or RNAlater, and
144 immediately stored at -20°C until DNA extraction.

145 Due to proximity between some of the samples collected in the different sampling sites
146 some of the samples from the different areas were pooled together (*i.e.* APEI-6_Flat1 to APEI-
147 6_Flat8 as APEI-6_Flat; APEI-6_Ridge1 to APEI-6_Ridge6 as APEI-6_Ridge; APEI-6_Trough1
148 to APEI-6_Trough4 as APEI-6_Trough; APEI-6_Deep1, APEI-6_Deep2 and APEI-6_Nodule as
149 APEI-6_Deep-Nodule; UK-1A_BC06 and UK-1A_EB03 as UK-1A_BC06-EB03; UK-
150 1A_BC08 and UK-1A_BC05 as UK-1A_BC08-BC05; UK-1B_BC06 and UK-1B_MC25 as UK-
151 1B_BC06-MC25; UK-1B_BC18 and UK-1B_MC13 as UK-1B_BC18-MC13; UK-1B_EB09
152 and UK-1B_BC04 as UK-1B_EB09-BC04; OMS-1A-EB06, OMS-1A-BC11, and OMS-1A-
153 BC08 as OMS-1A-EB06-BC11-BC08; OMS-1A-BC25, OMS-1A-MC23 and OMS-1A-BC26 as
154 OMS-1A-BC25-MC23-BC26). Original sampling sites collected during oceanographic cruises

155 are found in Table 1 and pooled sampling sites considered in this study are shown in Table 2.
156 Thus, a total of four areas and 30 different populations were identified in our study (Table 1–2).

157

158 *Body size of P. craigi*

159 Prior to DNA extraction, all preserved organisms were photographed in the lab using a Zeiss
160 AxioCam Hrc camera attached to a stereoscope. The maximum length and maximum width of
161 complete individuals were measured using the software AxioVision. These measurements were
162 made to test whether there was any link between body size and (i) apparent cohorts inferred from
163 the molecular analyses or (ii) different ecological variables. Linear correlation analysis between
164 maximum length and maximum width using R (<https://www.r-project.org/>) indicated a moderate
165 adjusted R-squared coefficient ($R^2 = 0.473$) and significant correlation between variables ($p <$
166 0.05); thus we used maximum length as the variable for size-frequency distributions. The
167 correlation between maximum length vs. maximum width was plotted in R. One-way analyses of
168 variance (ANOVAs) were conducted on maximum length using StatPlus vs 6
169 (www.analystsoft.com) (1) using sample areas (APEI-6, UK-1A, UK-1B and OMS-1A) as
170 factors, (2) within APEI 6 using the four different sampling stations as factors (APEI-6_Flat,
171 APEI-6_Ridge, APEI-6_Trough and APEI-6_Deep), and (3) between samples assigned to cluster
172 1 from the APEI-6 and UK-1A areas (see Results below). The Tukey-Kramer post-hoc test
173 implemented in StatPlus was used to identify significant pairwise differences between areas.

174

175 *DNA extraction and genotyping*

176 Genomic DNA was extracted from a portion of tissue (approx. 1 mm^3) of each of the 180
177 individuals collected from the four different areas using the Tissue and Blood Qiagen extraction
178 kit (Qiagen, www.qiagen.com) following the protocol provided by the manufacturer to a final
179 elution of $100 \mu\text{L}$. Prior to genotyping using microsatellites, we amplified and sequenced a
180 fragment of the gene cytochrome *c* oxidase subunit I –*COI*– using the primers PorCOI2fwd and
181 PorCOI2rev (Xavier *et al.* 2010) from a selection of 65 individuals from the four different areas
182 (Supplementary Table S1). This *COI* fragment includes the Erpenbeck’s ‘I3-M11’ fragment

183 (Erpenbeck *et al.* 2006), which has shown to be suitable to address intraspecific variability in
184 other sponges (e.g. López-Legentil & Pawlik 2009; Xavier *et al.* 2010). Each PCR reaction mix
185 contained a 21 µL of Red Taq DNA Polymerase 1.1x MasterMix (VWR), 1 µL (10 µM) of each
186 primer and 2 µL of DNA extraction of each individual. For DNA amplification, the following
187 PCR protocol was used [94 °C/5 min – (94 °C/1 min – 55 °C/1 min – 72 °C 1 min) x 38 cycles –
188 72 °C/5 min]. Sequencing was conducted on an ABI 3730XL DNA Analyser (Applied
189 Biosystems) at the Natural History Museum –NHM– molecular labs using the primers (forward
190 and reverse) mentioned above.

191 Owing to the low intraspecific variability observed in the *COI* fragment (see Results), we
192 genotyped all individuals using the 14 microsatellite loci (1Ple, 2Ple, 3Ple, 4Ple, 5Ple, 6Ple, 8Ple,
193 11Ple, 12Ple, 13Ple, 14Ple, 16Ple, and 19Ple) described by Taboada *et al.* (2017), using the PCR
194 conditions described therein. The sizes of the fluorescently labelled PCR products were estimated
195 using GeneScan 500 LIZ (Applied Biosystems, Foster City, CA, USA) on an Applied Biosystems
196 3130xl DNA analyser at the NHM molecular labs. Allele peaks were checked and edited using
197 Geneious vs 8.1.7 (Kearse *et al.* 2012) before being placed into amplicon size “bins” and exported
198 for analysis. Genotyping failed in 12 individuals (11 from APEI-6 and 1 from UK-1A) and thus
199 results reported below for microsatellite analysis refer to 168 individuals from a total of 30
200 sampling stations (Table 1, 2).

201 In order to test for the occurrence of cryptic species between organisms from cluster 1
202 and the rest of specimens (see Population differentiation in Results section for details about cluster
203 assignment), apart from using the information from the I3-M11 fragment, we sequenced a
204 fragment of 28S rRNA of a random selection of nine individuals assigned to the two different
205 clusters (Supplementary Table S1). Primers used were 28Sa and 28Srd5b (Giribet *et al.* 2002;
206 Schwendinger & Giribet 2005), and DNA amplification followed the PCR protocol [95 °C/5 min
207 – (95 °C/1 min – 55 °C/1 min – 72 °C 1 min) x 38 cycles – 72 °C/10 min]. Sequencing was
208 conducted on an ABI 3730XL DNA Analyser (Applied Biosystems) at the NHM molecular labs
209 using the primers mentioned above. Additionally, we performed a spicule analysis of a selection

210 of four individuals of these sequenced specimens to inspect for morphological differences (see
211 Spicule Analysis section below).

212

213 *Genetic diversity in P. craigi populations*

214 Tests for linkage disequilibrium were performed using Genepop through probability tests for each
215 pair of loci in each population with the level of significance determined by the following Markov
216 chain parameters: 5,000 dememorization steps, 1,000 batches and 5,000 iterations per batch.
217 Significance was adjusted by a false discovery rate method (Benjamini & Yekutieli 2001). Since
218 three of the microsatellites appeared to be in linkage disequilibrium (see Results), all results refer
219 to a total of 11 microsatellites.

220 Number of alleles (N_a), number of private alleles (P_a), estimations for the observed (H_o)
221 and expected (H_e) heterozygosity, and the fixation index (F_{IS}), commonly used as an inbreeding
222 coefficient, were performed using GenAlEx 6.5 (Peakall & Smouse 2006, 2012). Genetic (gene)
223 diversity was calculated with GENODIVE vs 2.0b23 (Meirmans & Van Tienderen 2004)
224 although for comparative purposes with other studies we will use H_e as a measure for genetic
225 diversity. We used Genepop web version 4.2 (Raymond & Rousset 1995; Rousset 2008) to obtain
226 values for departure from Hardy–Weinberg equilibrium (HWE) by locus and population
227 (sampling site) using a probability test with level of significance determined by the following
228 Markov chain parameters: 5,000 dememorization steps, 1,000 batches and 5,000 iterations per
229 batch. Significance was adjusted by a false discovery rate method (Benjamini & Yekutieli 2001).
230 These descriptors for the genetic diversity were calculated for the different sample sites separately
231 and grouping samples into the four different areas. Additionally, these descriptors were also
232 computed for the different areas considering samples of cluster 1 and cluster 2 separately.

233

234 *Population differentiation in P. craigi*

235 In order to test for population differentiation in *P. craigi* we used *COI* sequences from a selected
236 number of individuals (65) from the four different areas (Supplementary Table S1) and genotyped
237 11 microsatellites for a total of 168 individuals (Table 1). *COI* overlapping sequence fragments

238 were assembled into consensus sequences using Geneious vs. 8.1.7, and aligned using Q-INS-I
239 option of MAFFT (Kato *et al.* 2002). The *COI* alignment was used to construct an un-rooted
240 haplotype network with the program PopART (<http://popart.otago.ac.nz>) using the TCS network
241 option (Clement *et al.* 2000).

242 Fragments of *28S* sequenced to test for the occurrence of cryptic species were assembled
243 and aligned as described above for *COI*.

244 To examine evidence of clonality, multilocus genotypes of the 168 individuals studied
245 here were compared in GenAlEx 6.5 (Peakall & Smouse 2006, 2012) using the
246 ‘Multilocus/Matches’ function, which outputs a list of pairwise comparisons and the number of
247 differing locus genotypes ignoring missing data. After confirming the absence of clones and also
248 the lack of evidence of cryptic speciation (see Results), we performed four different methods to
249 assess population structure and differentiation in the 168 individuals of *P. craigi* using the 11
250 microsatellites: two of these methods used a clustering approach (STRUCTURE and the
251 discriminant analysis of principal components –DAPC–) and the other two were based on
252 distances (F_{ST} estimations and the analysis of the molecular variance –AMOVA–).

253

254 *Clustering methods.* Samples were assigned to genetically homogenous populations (K) inferred
255 using a Bayesian clustering algorithm without prior geographical information with the program
256 STRUCTURE 2.3.4 (Pritchard *et al.* 2000). An admixture model was used with correlated allele
257 frequencies and 150,000 MCMC iterations (burn-in of 50,000), repeated 10 times for each value
258 of K from 1 to 30. The most likely value of K was determined using Evanno’s *ad hoc* ΔK statistic
259 (Evanno *et al.* 2005) calculated and plotted using Structure Harvester web v0.6.94 (Earl &
260 vonHoldt 2012). The 10 replicates of optimal K were aligned using the *FullSearch* algorithm in
261 the software package CLUMPP v1.1.2 (Jakobsson & Rosenberg 2007), then visualized using
262 DISTRUCT v1.1 (Rosenberg 2004). STRUCTURE was also run, using the same specifications
263 mentioned above, considering members of cluster 1 and cluster 2 separately.

264 We also performed Discriminant Analysis of Principal Components –(– with the *adegenet*
265 package (Jombart 2008) implemented in R. DAPC defines clusters using the clustering algorithm

266 k-means on transformed data with principal component analysis. The algorithm k-means is then
267 run sequentially with increasing values of k, and different clustering solutions are compared using
268 the Bayesian Information Criterion. The number of principal components giving rise to the model
269 with the highest predictive capacity were inferred with the cross-validation optimisation
270 procedure using 100 replicates and the default parameters. The optimal cluster solution should
271 correspond then to the lowest value of root mean squared error. We applied the DAPC analysis
272 for: (i) the complete matrix of all samples grouped in the four different regions (APEI-6, UK-1A,
273 UK-1B and OMS-1A) and in the 30 different sites; (ii) the complete matrix of all samples grouped
274 by the cluster 1, the APEI-6 and UK-1A without samples assigned to cluster 1, UK-1B and OMS-
275 1A; (iii) APEI-6, UK-1A and UK-1B after removing from the analysis samples from cluster 1
276 and all samples from OMS-1A; (iv) samples of APEI-6 and UK-1A belonging to cluster 1; and
277 (v) cluster 2 samples, which included samples of the four different regions (APEI-6, UK-1A, UK-
278 1B and OMS-1A) without members of cluster 1.

279 *Distance methods.* Population differentiation was estimated with the F_{ST} statistic between
280 pairwise sampling sites using an infinite allele model in Arlequin vs 3.0 (Excoffier *et al.* 2005).
281 Significance of F_{ST} values was evaluated by performing 20,000 permutations and corrected based
282 on the false discovery rate method (Benjamini & Yekutieli 2001). Pairwise F_{ST} values grouping
283 all samples by area were also estimated using the same specifications mentioned above. MICRO-
284 CHECKER 2.2.3 (Van Oosterhout *et al.* 2004) was used to detect the presence of null alleles,
285 error scoring owing to stuttering or large allele dropout and error. As the presence of null alleles
286 in well-differentiated populations is known to yield an overestimation of population
287 differentiation (Chapuis & Estoup 2006), we repeated our analysis excluding loci suggesting
288 presence of null alleles. In all cases, the corrections only affected the second or third decimal
289 place in the F_{ST} value for the pairwise comparisons between areas (not affecting significance of
290 values) and consequently the effect of presence of null alleles was disregarded. Significance of
291 F_{ST} values was also calculated for the different areas considering samples of cluster 1 and cluster
292 2 separately.

293 An Analysis of Molecular Variance (AMOVA) was used to determine the hierarchical
294 distribution of genetic variation. To run this analysis, we grouped the sites in the different areas
295 (APEI-6, UK-1A, UK-1B and OMS-1A). The significance of the AMOVAs was calculated with
296 20,000 permutations of the original data in the program Arlequin. Additionally, AMOVA was
297 also calculated for the different areas considering samples of cluster 1 and cluster 2 separately.

298

299 *Spicule analysis*

300 We checked spicule composition of three individuals of cluster 1 (APEI-6_Flat.16, UK-1A.7,
301 UK-1A.8) and one from cluster 2 (APEI-6_Flat.14). A small piece of tissue of the different
302 specimens was first digested in nitric acid using a hotplate and subsequently washed twice with
303 distilled water. Spicules were cleaned in absolute ethanol, mounted on a stub and coated with
304 gold/palladium. Images of spheroyasters were taken using a Zeiss Ultra Plus field emission
305 scanning electron microscope at the NHM Imaging and Analytical Centre (IAC).

306

307 *Dispersal patterns in P. craigi*

308 *Isolation by distance (IBD)*. A Mantel test (100,000 permutations) was performed in GENODIVE
309 to test IBD using the whole data set of 168 individuals from the 30 populations and also using
310 only a subset of individuals not including individuals from cluster 1 (see Population
311 differentiation in Results). Geographical distances between sites were estimated using
312 GENODIVE using the coordinates for every site. These distances were log-transformed and
313 correlated to Slatkin's linearized pairwise F_{ST} estimates ($F_{ST}/1-F_{ST}$).

314

315 *Detection of last-generation migrants*. We performed a population assignment analysis
316 calculating the likelihood ratio thresholds for the populations grouped in the four areas (APEI-6,
317 UK-1A, UK-1B and OMS-1A) based on the Monte Carlo test with an α of 0.002 and 1000
318 replicated data sets using GENODIVE. This method assigns or excludes reference populations as
319 possible origins of individuals on the basis of multilocus genotypes. Genetic assignment methods
320 allow inferring where individuals originated, providing estimates of real-time dispersal through

321 the detection of immigrant individuals. The detection of last-generation migrants was performed
322 in GENODIVE using a random 0.005 frequency (estimated to outperform tests) in 4,000
323 permutations. This test provides the likelihood of an individual belonging to a given population.

324

325 *Migration patterns among areas.* Effective population size (expressed as $\Theta=4N_e\mu$) and migration
326 (M) were estimated with a Bayesian approach as implemented in LAMARC vs 2.1.10 (Kuhner
327 2006). Following suggestions by Kuhner (2006) we randomly reduced sample sizes for each area
328 to 15 in order to increase run efficiency. Default values were used for effective population size
329 and migration parameters. We performed Bayesian analyses with five replicates with 10 initial
330 chains of 5,000 MCMC each, burn-in period of 1,000, and two final chains of 100,000 MCMC
331 each with a burn-in period of 1,000. Three simultaneous heating searches (1, 1.1, and 2) were
332 performed per replicate. LAMARC infers approximate credibility intervals (CIs) around most
333 probable estimates (MPE) for each parameter. Parameter conversion was verified by examining
334 stationarity in parameter trends over the length of the chains and Effective Sample Sizes (ESS)
335 parameter using TRACER vs 1.6 (<http://beast.bio.ed.ac.uk/Tracer>). We interpreted ESS values >
336 250 as an indication that sampled trees were not correlated and thus represent independent
337 simulations. Number of immigrants per generation per area was calculated using Θ and M (ΘM).
338 LAMARC analyses were also run for members of cluster 1, selecting randomly 15 individuals
339 from each area (APEI-6 and UK-1A).

340 Directionality of recent migration patterns was obtained with the *diveRsity* package in R
341 (<https://diversityinlife.weebly.com/>), which uses the method described in Sundqvist *et al.* (2016)
342 to plot the pairwise relative migration levels between populations from microsatellite allele
343 frequency data. The sampling sites were pooled into the four different areas (APEI-6, UK-1A,
344 UK-1B, and OMS-1A). We used the statistic Nm (*i.e.* the effective number of migrants), a more
345 generally suitable measure of migration (Sundqvist *et al.* 2016), with a bootstrap of 10,000.
346 However, since the method is still in experimental stages, results should be interpreted with
347 caution. Additionally, migration patterns using *diveRsity* were also calculated for the different
348 areas considering samples of cluster 1 and cluster 2 separately.

349

350 *Detection of genetic breaks and correlation with geographical discontinuities.* The occurrence of
351 possible barriers determining the genetic structure of *P. craigi* populations was evaluated using
352 the software BARRIER v2.2 (Guerard & Manni 2004). This program links a matrix of
353 geographical coordinates with their corresponding distance matrix (F_{ST}), and applies the Frier's
354 maximum distance algorithm to identify a desired number of 'barriers' to gene flow among sites
355 (*i.e.* zones where genetic differences between pairs of sites are the largest). This was done using
356 the whole data set of 168 individuals from the 30 populations and also using only a subset of
357 individuals not including individuals from cluster 1 (see Population differentiation in Results).

358

359 *Modelling of larval dispersal by currents*

360 The passive transport of larvae was simulated within a 9-year (Oct 2008–Sept 2017) record of
361 daily velocity fields from the GOFS 3.0 1/12° global analysis of the HYCOM hydrodynamic
362 model (Chassignet *et al.* 2007). A fourth order Runge-Kutta advective scheme was used with a
363 1-day timestep and a random horizontal diffusive component (diffusion coefficient $k_x=1 \text{ m}^2\text{s}^{-1}$)
364 representing unresolved scales of motion. In the absence of detailed understanding of larval
365 behaviour and vertical positioning, transport was simulated within the horizontal model layer at
366 3,500 m depth, representing the lower water column at a level that is largely unobstructed by
367 topography. Modelled currents at this depth are weak (the mean instantaneous current speed in a
368 box encompassing the sample sites is 2.2 cm s^{-1} , and the mean residual flow speed, the mean of
369 the underlying mean flow, is 0.5 cm s^{-1} ; Aleynik *et al.* 2017). Larval connectivity between sites
370 X and Y (the four different areas in our study) was assessed by continuously releasing 'particles'
371 (1,000 per day) from site X throughout the first 4 years of the record, and determining the
372 probability that they pass within a 25 km radius of site Y at any time within the following 5 years.
373 Advection over this timescale should not be interpreted as representing the dispersal of a single
374 larval generation, but as representing the cumulative dispersal of multiple generations, albeit
375 represented as a single continuous pathway, so there is an implicit assumption that suitable
376 benthic habitat exists along the pathway.

377 The reproductive effort of adults (determining timing and number of larvae in the water
378 column), and the larval development and behaviour, determine how larvae interact with currents
379 and ultimately influence the timing, distance and trajectory of larvae among habitats (Hilario *et*
380 *al.* 2015). Sympatric shallow-water sponges may substantially differ in their timing for sexual
381 reproduction and these differences appear to be related to changes in seawater temperature
382 (Riesgo & Maldonado 2008). To our knowledge, the only work studying seasonality in relation
383 to sexual reproduction in deep-sea sponges was conducted in the North Atlantic and concluded
384 that the reproduction of *Radiella sol* Schmidt, 1970 was not asynchronous and remained at a
385 constant low level, while *Thenaea abyssorum* Koltun, 1964 showed highly synchronized
386 gametogenesis and was linked to seasonal pulses of particulate organic carbon (Witte 1996). Since
387 the flux of phytoplankton to deep-sea waters in the equatorial Pacific appears to be quasi-
388 continuous, albeit with phytodetrital pulses (Smith *et al.* 1996), we assumed that, similarly to *R.*
389 *sol* and in the absence of any reproductive data in *P. craigi*, the sponge studied here may have a
390 constant low level of reproduction throughout the year.

391

392 *Evaluating bottleneck events & population decline*

393 We tested for recent effective population size reductions (bottlenecks) based on allele data
394 frequencies using the software BOTTLENECK vs. 1.2.02 (Cornuet & Luikart 1996). This
395 software assumes that “populations that have gone through a recent reduction of their effective
396 population size show a reduction of the allelic diversity and heterozygosity, even though the allele
397 frequencies are reduced faster than the heterozygosity” (Cornuet & Luikart 1996). The statistical
398 analyses using a "sign test" (Cornuet & Luikart 1996) and a "Wilcoxon sign-rank test" (Luikart
399 & Cornuet 1998) can be applied when more than 5 (but less than 20) loci are included, and we
400 selected only the two most extreme models of mutation: infinite allele model (IAM) and the
401 stepwise mutation model (SMM).

402 **Results**

403 *I3-M11, 28S and spicule analyses*

404 Grouping of samples in cluster 1 (see Population differentiation section below for details about
405 assignation to individuals to clusters), with specimens showing high molecular affinities despite
406 being several 100's km apart, made us suspect about the occurrence of cryptic species in our
407 samples. Thus, a combination of molecular (*28S* and *COI* sequences) and morphological
408 (spicules) analyses were conducted in a selection of individuals to detect the occurrence of cryptic
409 species within our samples.

410 Although we found variability in the *COI*I3-M11 partition in the 65 individuals analyzed,
411 these differences were not congruent with the two main clusters (cluster 1 and cluster 2) detected
412 in our population genetic analysis using microsatellites (see below). Thus, we could not assign
413 any haplotype to any putative cryptic species. Similarly, the fragment of 421 bp of *28S* from a
414 total of nine individuals (four from cluster 1, including two samples from APEI-6 and two samples
415 from UK-1A; five from cluster 2, including two samples from APEI-6, two from UK-1A and one
416 from OMS-1A; Supplementary Table S1) showed no differences at all.

417 For the spicule analysis we focused on the comparison of spheroxyasters since these
418 spicules were the only ones displaying some morphological variability. We observed no
419 significant differences among spicules coming from organisms in cluster 1 and cluster 2, either
420 in their size or in the number of rays per spicule (Supplementary Material Figure 1). The number
421 of rays did not differ significantly between individuals of the two clusters, ranging from 14–30
422 for organisms from cluster 1 and 16–26 for organisms from cluster 2. However, there were slight
423 differences in the ratio number of rays with spines between the two clusters; in cluster 1 the
424 average was 89 % of rays with spines, whilst in cluster 2 it was 51 %. The size and features
425 observed in spheroxyasters analysed here match those described in the original description of *P.*
426 *craigi*, with a range of 11.2–13.1–15.4 μm for organisms in cluster 1 and 12.1–13.7–15.5 μm for
427 cluster 2 (Lim *et al.* 2017). Importantly, the specimens used for the original description of the
428 species by Lim *et al.* (2017) were collected in the OMS-1A area.

429

430 *Body size of P. craigi*

431 Correlation between maximum length and maximum width in *P. craigi* individuals measured here
432 is shown in Supplementary Figure S2. Mean maximum length varied significantly between the
433 different areas, with maximum lengths ranging from 3624 ± 1265 μm (mean \pm S.D.) in APEI-6 to
434 5133 ± 1776 μm in UK-1B (Fig. 2, Table 3, Supplementary Table S2). Tukey-Kramer post-hoc
435 tests identified significant differences between APEI-6 samples and UK-1A and UK-1B samples
436 (the former being significantly smaller than the two latter), and also between OMS-1A and UK-
437 1B samples (the former being significantly smaller than the latter) (Figure 2, Table 3). Significant
438 differences were also found between individuals assigned to cluster 1 from APEI-6 and UK-1A
439 areas (Table 3), although no significant differences were found between Cluster 1 vs Non-Cluster
440 1 samples from the two different areas. No significant differences were detected for any pairwise
441 comparison from the four different sampling stations within APEI-6 (APEI-6_Flat, APEI-
442 6_Ridge, APEI-6_Trough, and APEI-6_Deep).

443

444 *Genetic diversity in P. craigi populations*

445 Three of the 14 loci (4Ple, 6Ple, and 8Ple) used in our analysis showed significant linkage
446 disequilibrium (LD) in pairwise comparisons with other loci. After removing these three loci from
447 the analysis, none of the pairwise comparisons showed significant LD. Thus, all the subsequent
448 analyses describing the genetic diversity and population differentiation in *P. craigi* are based only
449 on 11 loci (1Ple, 3Ple, 11Ple, 13Ple, 12Ple, 14Ple, 16Ple, 5Ple, 19Ple, 10Ple, and 2Ple) out of the
450 14 microsatellites originally characterized by Taboada *et al.* (2017).

451 Genotypic variation, measured by the percentage of differences between multilocus
452 genotypes in pairwise comparisons between the 168 *P. craigi* samples, showed no identical
453 genotypes (*i.e.* absence of clonality). The total number of alleles per population ranged from 12
454 in UK-1B_BC02 to 114 in APEI-6_Flat, with most of the variation resulting from three
455 hypervariable microsatellites (*i.e.* 3Ple, 12Ple, and 16Ple; see Table 2, Supplementary Table S3).
456 The mean number of alleles per population ranged from 1.091 to 10.364 in UK-1B_BC02 and
457 APEI-6_Flat, respectively (Table 2, Supplementary Table S3). When considering the populations

458 grouped within the four main areas (APEI-6, UK-1A, UK-1B and OMS-1A), the total number of
459 alleles ranged from 146 in OMS-1A to 172 in UK-1A, while the mean number of alleles ranged
460 from 13.273 in both UK-1B and OMS-1A to 15.636 in UK-1A (Table 2, Supplementary Table
461 S3). Private alleles were not present in all populations and ranged from 1 (mean number of private
462 alleles = 0.091) in seven populations in the UK-1B and OMS-1A areas (UK-1B_BC17, UK-
463 1B_BC20, UK-1B_BC02, UK-1B_BC03, OMS-1A-EB06-BC11-BC08, OMS-1A-BC12, and
464 OMS-1A-BC10) to 10 (mean number of private alleles = 0.909) in UK-1A_BC12 (Table 2,
465 Supplementary Table S3). Private alleles were present in all areas and ranged from 9 (mean
466 number of private alleles = 0.727) in APEI-6 to 26 (mean number of private alleles = 2.364) in
467 UK-1A (Table 2, Supplementary Table S3). When grouping samples from cluster 1 and cluster
468 2, the total number of alleles was 98 in APEI-6 and 103 in UK-1A for cluster 1, and ranged from
469 111 in APEI-6 to 146 in both UK-1B and OMS-1A for cluster 2 (Supplementary Table S4). The
470 mean number of alleles was 8.909 in APEI-6 and 9.364 in UK-1A for cluster 1, and ranged from
471 10.091 in APEI-6 to 13.273 in both UK-1B and OMS-1A for cluster 2 (Supplementary Table S4).
472 Private alleles were 29 in APEI-6 (mean number of private alleles = 0.704) and 34 in UK-1A
473 (mean number of private alleles = 0.814) for cluster 1, and ranged from 11 (mean number of
474 private alleles = 1.000) in APEI-6 to 33 (mean number of private alleles = 3.000) in OMS-1A for
475 cluster 2 (Supplementary Table S4).

476 Genetic diversity (H_e) values varied widely across populations, ranging from 0.170 in
477 UK-1B_BC02 to 0.705 in APEI-6_Flat, in part due to the relatively low number of individuals
478 present in some populations (Supplementary Table S3). Genetic diversity was less variable across
479 areas and ranged from 0.728 in UK-1B and OMS-1A to 0.791 in UK-1A (Supplementary Table
480 3). Inbreeding coefficient values (F_{IS}) were positive, resulting from heterozygosity deficit, for all
481 the populations in the APEI-6 area and also for the majority of populations in the other three areas
482 indicating non-random mating between individuals (Table 2). A few populations in UK-1A, UK-
483 1B and OMS-1A showed negative F_{IS} values because of an excess of observed heterozygotes
484 (Table 2). When considering the four large areas, F_{IS} values were always positive (Table 2).
485 Several populations in the four different areas showed significant deviation from HWE, and when

486 considering the four areas together HWE deviations were detected in all of them (Table 2). When
487 loci possibly affected by presence of null alleles (3Ple, 10Ple, 11Ple and 19Ple) were removed
488 from the analysis, some of the populations showed no departure from HWE; however, the four
489 different areas still showed a significant departure from HWE (Table 2). When grouping samples
490 from cluster 1 and cluster 2, H_e values were 0.677 in APEI-6 and 0.712 in UK-1A for cluster 1,
491 and ranged from 0.712 in UK-1A to 0.728 in both UK-1B and OMS-1A for cluster 2
492 (Supplementary Table S4). F_{IS} values were always positive and HWE deviations were detected
493 for all the areas in cluster 1 and cluster 2 (Supplementary Table S4).

494

495 *Population differentiation in P. craigi*

496 *Mitochondrial markers.* A fragment of 526 bp of *COI* was analysed for 65 individuals of *P. craigi*
497 occurring in the four different areas (Supplementary Table S1). Only two haplotypes were
498 inferred in the haplotype network: H1 was the most common haplotype (accounting for 97 % of
499 the total number of individuals) and occurred in individuals from the four areas (Figure 3); and
500 H2, differing only in one mutational step from H1, was only present in two individuals from the
501 UK-1A, namely UK-1A.4 and UK-1A.23 (Figure 3).

502

503 *Microsatellites.* The optimal number of populations for the whole data set obtained by the
504 program STRUCTURE recovered two genetically homogeneous groups ($k = 2$) followed by three
505 groups ($k = 3$) (Figure 4A). Results for $k = 2$ revealed two populations with no clear pattern of
506 geographic subdivision: (i) cluster 1 (Orange group) included most of the samples in APEI-6_Flat,
507 all the samples in UK-1A_BC08-BC05 and UK-1A_BC03 and a few samples in the stations UK-
508 1A_BC06-EB03 and UK-1A_BC10; and (ii) cluster 2 (Blue group) contained the rest of the
509 samples from APEI-6 and UK-1A, and all the samples from the sites in UK-1B and OMS-1A
510 (Figure 4A). Results for $k = 3$ revealed substructure in cluster 2, with three populations of
511 individuals present in different proportions in the four areas (Figure 4A). When grouping samples
512 from cluster 1 and cluster 2, the optimal number of populations detected was two genetic groups
513 ($k = 2$) for cluster 1, and three ($k = 3$) for cluster 2 (Supplementary Figure S3A-B).

514 DAPC analysis considering all the samples grouped in the four areas showed APEI-6 and
515 UK-1A as the most similar areas, UK-1B being closer to UK-1A, while OMS-1A was the most
516 divergent of the areas (Fig. 4B); a similar picture could be observed when analysing the 30
517 different sites separately (Supplementary Figure S3C). When the samples of the cluster 1 were
518 separated and considered as a separated area, UK-1B and the remaining samples of APEI-6 and
519 UK-1A grouped together, while samples from cluster 1 and OMS-1A appeared as the most
520 divergent ones (Fig. 4C). After removing from the analysis all the samples from cluster 1 and
521 OMS-1A area, DAPC showed differences between APEI-6, UK-1A and UK-1B, with samples
522 from UK-1A and UK-1B more closely related than with APEI-6 (Supplementary Figure S4).
523 When grouping samples from cluster 1, two groups with a significant overlap were detected in
524 samples from APEI-6 and UK-1A (Supplementary Figure S3D). When grouping together samples
525 from cluster 2, DAPC showed that APEI-6 and UK-1B samples were the most similar ones, with
526 UK-1A being closer to UK-1B, and OMS-1A being again the most divergent area (Supplementary
527 Figure S3E).

528 When treating all locations separately, F_{ST} values were significant for: (i) the majority of
529 pairwise comparisons between APEI-6_Flat and UK-1A_BC08-BC05 with the rest of sampling
530 sites; (ii) for UK-1B_BC18-MC13 with UK-1B_BC03 and the majority of OMS-1A sampling
531 sites; (iii) OMS-1A_BC25-MC23-BC26 with three of the four sites of APEI-6 and three sites of
532 UK-1A and UK-1B; and (iv) for five of the pairwise comparisons between APEI-6_Ridge with
533 OMS-1A sites, amongst other comparisons (Supplementary Table S5). However, our F_{ST} values
534 for locations treated separately should be interpreted with caution due to the low number of
535 specimens analysed in some populations. When grouping samples per area, F_{ST} values ranged
536 from 0.00709 between APEI-6 and UK-1A to 0.11132 between APEI-6 and OMS-1A, and were
537 significant between all pairwise comparisons except for the comparison between APEI-6 and UK-
538 1A (Table 4). F_{ST} values based only in the 7 microsatellites not affected by null alleles showed
539 the same significant pairwise comparisons as with the whole set of microsatellites
540 (Supplementary Table S6). When grouping samples from cluster 1, F_{ST} value was 0.011 and not
541 significant between APEI-6 and UK-1A, while when considering samples from cluster 2 all

542 pairwise comparisons resulted significant except for the comparisons between APEI-6 and UK-
543 1B, and between UK-1A and UK-1B (Supplementary Table S7).

544 Population differentiation using AMOVA, found significant differences between the four
545 different areas, and also among populations within areas, among individuals within populations,
546 and among all individuals, with the last representing the greatest source of variation (Table 5).
547 When grouping samples from cluster 1, no significant differences were found between APEI-6
548 and UK-1A (Supplementary Table S8); for cluster 2, significant differences were found between
549 the four different areas, among individuals within areas, and within individuals, with the last one
550 representing the greatest source of variation (Supplementary Table S8).

551

552 *Dispersal patterns in P. craigi*

553 The Mantel tests detected no significant IBD when considering the whole data set of individuals
554 ($p = 0.131$) and still was not significant after removing from the analysis individuals from cluster
555 1 (Orange group) ($p = 0.373$), which indicates that the genetic structure observed might be related
556 to other processes (*e.g.* oceanographic currents, see below).

557 When considering the whole data set of individuals and setting two major barriers *a*
558 *priori*, the barriers or genetic discontinuities appeared, in decreasing order of importance,
559 between: (a) all the samples of the APEI-6 area plus three UK-1A sites (UK-1A_BC06-EB03,
560 UK-1A_BC08-BC05, and UK-1A_BC14) and the rest of sites; and (b) most of the samples from
561 OMS-1A (OMS-1A_BC21, OMS-1A_BC22, OMS-1A_BC09, OMS-1A_BC23, OMS-
562 1A_BC25-MC23-BC26, and OMS-1A_BC10) and the rest of sites (Supplementary Figure S5).
563 When removing cluster 1 from the analysis, the barrier between APEI-6 and UK-1A disappeared,
564 and the three main barriers inferred appeared between (a) most of the OMS-1A sites (OMS-
565 1A_BC10, OMS-1A_BC25-MC23-BC26, OMS-1A_BC23, OMS-1A_BC09, OMS-1A_BC22,
566 and OMS-1A-BC21) and the rest of sites; (b) UK-1B_EB09-BC04 and UK-1B_BC02; and (c)
567 OMS-1A-EB06-BC11-BC08, OMS-1A-BC12, and OMS-1A-BC07 (Supplementary Figure S5).
568 Thus, after removing cluster 1 from the analysis the main barriers appeared to be between OMS-
569 1A and the rest of the sites studied here.

570 The population assignment showed remarkable genetic exchange between areas,
571 especially for APEI-6 and UK-1A with almost 50 % of their individuals inferred to come from
572 UK-1A and UK-1B, and from APEI-6 and UK-1B, respectively (Supplementary Figure S6). In
573 contrast, most of the individuals from UK-1B (73 %) and OMS-1A (97 %) were inferred to result
574 from self-recruitment (Supplementary Figure S6). Only one last generation migrant was detected,
575 an individual from UK-1A_BC14 (one of the southernmost sites in UK-1A; Fig. 1) that was
576 inferred to come from UK-1B. Migration inferred using LAMARC showed no clear pattern of
577 gene flow among the four different areas, with relatively low and similar numbers of immigrants
578 per generation among the different pairwise comparisons; similar results were detected after
579 analysing migration between APEI-6 and UK-1A from cluster 1 (Table 6–7). Migration
580 directionality among areas using *diveR*sity detected significant migration both from OMS-1A and
581 UK-1B to UK-1A (Figure 6F), and no significant migration directionality was detected among
582 any of the areas when analysing samples from cluster 1 and cluster 2 separately..

583

584 *Modelled larval dispersal by currents*

585 Modelled currents at the 3500 m level used for advective dispersal simulations are strongly
586 bathymetrically constrained, so mean flow patterns (Fig. 5) showed considerable spatial
587 complexity. Superimposed on these mean patterns is variability induced in part by the deep
588 penetration of passing eddies and other flow structures higher in the water column (Aleynik *et al.*
589 2017). The dispersal of simulated particles therefore reflects intricate stirring with a weak
590 underlying tendency towards a net movement to the north and east (Fig. 6A–E; Supplementary
591 Video S1). The calculated probability of the transport of larvae between sites (Table 8) revealed
592 stronger connectivity between OMS-1A and UK-1B than between these two sites and UK-1A. In
593 part this reflects greater separation, but also a residual flow to the east along a gentle bathymetric
594 slope around 12.5°N tends to restrict direct transport between UK-1A and these two sites. APEI-
595 6, while considerably less connected to the other three sites as a result of its separation distance,
596 is nearly an order of magnitude more likely to receive larvae from UK-1A than from UK-1B or
597 OMS-1A over a 5-year timescale (potentially representing multiple successive generations).

598 **Discussion**

599 *No evidence of cryptic species*

600 The extremely low variability of *COI* I3-M11 partition in samples ~900 km apart showed by *P.*
601 *craigi* in our study is not surprising for sponges. The commonly used Folmer region of the *COI*
602 gene (Folmer *et al.* 1994) has traditionally showed relatively low genetic variation within sponge
603 species (Worheide *et al.* 2005), explained by slow mitochondrial *COI* sequence evolution in
604 sponges, with very few exceptions (Duran & Rützler 2006; DeBiasse *et al.* 2010), possibly related
605 to the active presence of mitochondrial repair mechanisms (Huang *et al.* 2008). Other
606 mitochondrial partitions such as the Erpenbeck's 'I3-M11' fragment (Erpenbeck *et al.* 2006), has
607 proven to be suitable for population connectivity studies in other sponges (e.g. López-Legentil &
608 Pawlik 2009; Xavier *et al.* 2010), but it provided no resolution in our study (Fig. 3). Importantly,
609 this extremely low *COI* variability was observed for samples included in cluster 1, which grouped
610 samples collected from the APEI-6_Flat and several UK-1A sampling sites, two areas ~800 km
611 apart that showed unexpected gene flow in our analysis (Fig. 4A–B). As for the morphology of
612 the spicules, it is important to note that similar morphological differences in spheroxyasters from
613 the specimens of cluster 1 and cluster 2 were already detected in the specimens used in the original
614 description of *P. craigi* (Lim *et al.* 2017); the specimens analysed by Lim *et al.* (2017) were all
615 collected from OMS-1A (all of them belonging to cluster 2), which indicates that the
616 spheroxyasters of *P. craigi* display a moderate intraspecific variability. Thus, our findings of
617 homogeneity in *COI* and 28S, together with our analysis of spicule spheroxyasters morphology
618 and size (Fig. 3, Supplementary Figure S1), provided no evidence for cryptic species in the
619 samples used in our study and suggest that all the organisms used in our study belong to the same
620 species. However, the possibility of members of cluster 1 being a cryptic species should not be
621 ruled out. For this reason, we decided to run most of the downstream analyses considering
622 members of cluster 1 and cluster 2 separately.

623

624 *Body size in P. craigi*

625 The significant size differences observed in the individuals of *P. craigi* collected from APEI-6
626 and OMS-1A compared to the ones collected in UK-1A and UK-1B (Fig. 2, Table 3) might be
627 attributed to specimens in these areas belonging to different age cohorts or explained by
628 ecological differences in the different areas. Under these premises, three possible scenarios are
629 presented: (i) a relatively more recent colonization of nodules by *P. craigi* in APEI-6 and OMS-
630 1A; (ii) population decimations of the sponge causing bottlenecks in APEI-6 and OMS-1A; and
631 (iii) differences in food availability in the different areas. The first scenario might be plausible in
632 the case of APEI-6, since most water and gene flow is predominantly northwards, and, therefore,
633 the individuals in APEI-6 might be the result of a recent colonization. For OMS-1A, though, this
634 possibility seems less likely since the suggested direction of migration originates mainly from
635 OMS-1A into the rest of the areas. Thus this hypothesis will not explain why individuals in OMS-
636 1A are significantly smaller than the ones in UK-1B (Table 3). On the other hand, all areas
637 presented recent signs of bottleneck events (Supplementary Table S9), and, therefore, bottlenecks
638 could not explain the differences in size observed among areas.

639 Alternatively, it seems that differences in food availability might explain differences
640 observed between APEI-6 and UK-1A and UK-1B specimen sizes. *Plenaster craigi* is a filter-
641 feeding organism relying on suspended particulate organic matter, bacteria and other
642 microorganisms. The CCZ is known to have an overall westward and northward trend of reduced
643 primary productivity in the central Pacific (Smith & Demopoulos 2003; Vanreusel *et al.* 2016),
644 that has been suggested to yield a significant decline in the polychaete abundance when moving
645 from the eastern to the western end (Smith *et al.* 2008b) and also to a decline of epifauna
646 associated with manganese nodules in areas to the north (Vanreusel *et al.* 2016). In our case,
647 smaller specimens of *P. craigi* found in the north (APEI-6) compared to those in the south (UK-
648 1A and UK-1B) could result from differences in overlying primary productivity and export flux
649 (Supplementary Figure S7, data extracted from Lutz *et al.* 2007). However, the reason why
650 samples from OMS-1A (also in the south) were significantly smaller than samples from UK-1B
651 despite being at similar latitudes (and only separated by ca. 75 km) and the little differences they
652 show in POC flux (Supplementary Figure S7) remains unclear.

653

654 *Genetic diversity in P. craigi*

655 Mean expected heterozygosity (H_e), commonly used as a measure of genetic diversity, for all loci
656 across all sites ranged from 0.728–0.791 between the four different areas, with similar values
657 being reported when considering cluster 1 and cluster 2 separately. Such relatively high genetic
658 diversity values could be correlated to either high mutation rates and/or relatively stable
659 population sizes (Kimura 1983). In our case, all populations seemed to have similar effective
660 population sizes and all showed signs of population bottlenecks, and, therefore, we could not
661 confirm whether they were stable populations.

662 The genetic diversity values found in our study are within the range of other studies on
663 marine sponges using microsatellite markers. Comparisons with data available in these studies
664 (Duran *et al.* 2004; Blanquer *et al.* 2009; Blanquer & Uriz 2010; Dailianis *et al.* 2011; Guardiola
665 *et al.* 2012, 2016; Bell *et al.* 2014; Pérez-Portela *et al.* 2015; Giles *et al.* 2015; Chaves-Fonnegra
666 *et al.* 2015; Riesgo *et al.* 2016, under review; Padua *et al.* 2017), revealed that H_e increased as the
667 sampling range covered larger distances (Fig. 7). Our H_e values are especially similar to those in
668 studies covering around 1,000 km, a spatial scale similar to ours.

669 Even though genetic diversity reported here was high for most of the populations and all
670 the areas (also when considering members of cluster 1 and cluster 2 separately), high positive F_{IS}
671 values were also observed, indicating strong levels of inbreeding (*i.e.* non-random mating
672 between individuals) in *P. craigi*. Such inbreeding signatures are also supported by the deviations
673 from HWE observed in most populations of *P. craigi*. Signatures of Hardy Weinberg
674 disequilibrium are often the rule in shallow-water sponges (*e.g.* Duran *et al.* 2004; Dailianis *et al.*
675 2011; Pérez-Portela *et al.* 2015; Giles *et al.* 2015; Chaves-Fonnegra *et al.* 2015; Riesgo *et al.*
676 2016) and also in the deep-water reef-forming sponge *Aphrocallistes vastus* Schulze, 1886,
677 although in this case disequilibrium was observed only at global and regional scales and not
678 within sites (Brown *et al.* 2017). As it has recently been discussed by Riesgo *et al.*, (2016) and
679 other studies, reasons explaining the high levels of homozygosity in sponge populations may
680 include a significant effect of null alleles, high levels of inbreeding, selection against

681 heterozygotes, the Wahlund effect, or a combination of these (Freeland *et al.* 2011). In *P. craigi*,
682 the effect of null alleles should be disregarded since, the four different areas still showed a
683 significant departure from HWE (Table 2), although some of the populations showed no departure
684 from HWE when removing the loci possibly affected by the presence of null alleles (3Ple, 10Ple,
685 11Ple and 19Ple). High F_{IS} values and departure from HWE in *P. craigi* are likely related to the
686 biology of the species, as has already been claimed in other studies on shallow-water sponges
687 (Chaves-Fonnegra *et al.* 2015; Riesgo *et al.* 2016). Very little is known about the reproduction of
688 deep-sea sponges in general (Witte 1996), and nothing about the reproduction of *P. craigi* in
689 particular, but we suggest that one of the main reasons behind the high levels of inbreeding and
690 deficit of heterozygosity might be self-recruitment. This may be a result of limited dispersal of
691 either gametes or larvae in *P. craigi*, supported by the observation that deep-sea currents in this
692 area are weak and dispersal by currents is expected to be small between successive generations.
693 Self-recruitment also been suggested for other marine sessile invertebrates with larvae with low-
694 dispersal abilities, including both shallow-water (e.g. Chaves-Fonnegra *et al.* 2015; Pérez-Portela
695 *et al.* 2016; Riesgo *et al.* 2016) and deep-sea species (Le Goff-Vitry *et al.* 2004). Finally, the
696 Wahlund effect caused by subpopulation structure should not be ruled out as a possible reason
697 explaining low levels of heterozygosity, since it has already been documented for sponges
698 (Chaves-Fonnegra *et al.*, 2015) and cnidarians (Ledoux *et al.* 2010).

699

700 *Population differentiation, connectivity and the effect of oceanic circulation in P. craigi*

701 Our microsatellite dataset provided detailed resolution of the genetic differentiation and
702 connectivity of *P. craigi*. The populations studied here showed marked genetic structure at large
703 geographical scales, as indicated by the significant differences observed between the four
704 different areas in the AMOVA analysis, together with the low but significant F_{ST} values when
705 comparing the four areas in pairwise groupings, except for the comparison between APEI-6 and
706 UK-1A (Table 4–5). In this sense, isolation by distance (IBD) could not explain this pattern of
707 large-scale differentiation and instead two major genetic discontinuities were detected: one

708 separating APEI-6 and some sites of UK-1A from the rest of sites sampled, and another one
709 separating most of the samples from OMS-1A from the rest of areas (Supplementary Figure S5).

710 Baco et al., (2016) recently reviewed the incidence of IBD in deep- and shallow-water
711 marine organisms (no sponges were included in their analysis), and they concluded that scales of
712 dispersal and connectivity in deep-water organisms are comparable to those reported for shallow-
713 water organisms, which would then justify comparing our results with others for shallow-water
714 organisms. In this sense, several shallow-water sponges seem to be substantially affected by
715 oceanographic fronts, ocean depth, and water circulation patterns, showing very little incidence
716 of patterns following the stepping stone gene flow derived from IBD (e.g. Dailianis *et al.* 2011;
717 Chaves-Fonnegra et al., 2015; Riesgo et al., 2016; Padua *et al.* 2017). Interestingly, no IBD was
718 detected for a hadal amphipod species of the genus *Paralicella* occurring in the Pacific, with
719 geological events and topographical barriers most likely responsible for the major isolation
720 observed among their populations (Ritchie *et al.* 2017). In contrast, IBD has also commonly been
721 reported in shallow-water sponges specially in studies comprising large-scale sampling sites (e.g.
722 Duran, Pascual, Estoup, & Turon, 2004; Guardiola, Frotscher, & Uriz, 2016; Wörheide, Epp, &
723 Macis, 2008) or even at smaller scales after removing from the analysis populations occurring in
724 areas separated by well-known oceanographic barriers (Riesgo *et al.* 2016), and has commonly
725 been explained by low dispersal abilities of sponges. There is also a wealth of examples in other
726 shallow-water organisms showing IBD between their populations, even when considering species
727 with presumably high dispersal abilities (e.g. Launey *et al.* 2002; Maier *et al.* 2005; Zulliger *et*
728 *al.* 2009).

729 Testing environmental factors responsible for the genetic structure observed is a major
730 goal in ecological analysis and, at the same time, is one of the major challenges for studies aiming
731 to describe genetic connectivity in the deep sea (Hansen & Hemmer-Hansen 2007; Taylor &
732 Roterman 2017). To our knowledge, the combination of ecological and physical models and
733 population genetics has been attempted for relatively few studies of deep-sea organisms but has
734 usually provided greater insights into the factors ultimately determining connectivity among
735 populations (Jorde *et al.* 2015; Dambach *et al.* 2016). Our use of oceanographic models to

736 estimate larval transport may explain some of the patterns in the large-scale population
737 differentiation and connectivity of *P. craigi*. The major differentiation found for the OMS-1A
738 area (both in STRUCTURE and DAPC analyses) could be explained by the occurrence of currents
739 and eddies mainly running northwards from OMS-1A (Fig. 5–6), thus preventing gene flow into
740 OMS-1A from the other sampled areas, which was also observed in the analysis of the
741 directionality of the gene flow (Figure 6F). A northward net larval transport would connect UK-
742 1B and UK-1A, which was also corroborated by the low (although significant) F_{ST} values found
743 between these two areas and the affinities found in the DAPC analyses.

744 We detected signatures of gene flow within samples from cluster 1 recovered in
745 STRUCTURE, which grouped together samples separated ~800 km apart (*e.g.* APEI-6_Flat and
746 UK-1A_BC08-BC05 and UK-1A_BC03), showing significant pairwise F_{ST} comparisons between
747 this group of samples and the rest of sampling sites (Supplementary Table S5). Although relative
748 migration may not be significant between APEI-6 and UK-1A (Fig. 6F), our particle movement
749 model suggested larval flow mainly from UK-1A to APEI-6, potentially enabling connectivity
750 between these two areas (Figure 6) via stepping-stone populations. Thus, despite being separated
751 by ~800 km, individuals from these two areas assigned to cluster 1 showed closer genetic
752 affinities between them than they did with individuals from nearby sites only 10s km apart. This
753 evidence of population structure on 10-km scales could not be explained by our circulation model,
754 and could be related to cryptic speciation and/or unexplained characteristics of the reproductive
755 biology of *P. craigi* causing limited dispersal under some conditions. On the other hand,
756 connectivity patterns over almost 1,000 km as observed between UK-1A and APEI-6 populations
757 are not unexpected, since gene flow in the deep sea appears generally more extensive horizontally
758 over large distances than vertically (*e.g.* Clague *et al.* 2012; O’Hara *et al.* 2014). However, there
759 is no direct knowledge of the reproductive or larval biology of *P. craigi*, and our current
760 understanding of circulation patterns near the CCZ floor remain limited, requiring caution in the
761 interpretation of our circulation modelling.

762 Our migration analyses showed very little movement of individuals between areas, with
763 less than two immigrants per generation in all cases (Table 7). Although relative migration levels

764 were higher from OMS-1A to the rest of the areas, from APEI-6 and UK-1A, and among UK-1A
765 and UK-1B, in general all migration levels were very low (Table 6). This suggests that sponge
766 recolonization follow large-scale mining disturbance in the UK-1 and OMS contract areas may
767 be slow due to the limitations of larval dispersal. For sponges, low migration levels between
768 populations is not rare, since very few migrants are usually reported among locations (e.g., Riesgo
769 et al., 2016), and this pattern is also shared with other sessile invertebrates (Pérez-Portela et al.
770 2015). By contrast, a deep-sea amphipod species of the genus *Paralicella* displayed a remarkably
771 high and reciprocal Pan-Pacific migration between hadal trench populations (Ritchie *et al.* 2017).
772 Contrasting results for *P. craigi* and the above-mentioned hadal amphipod may be explained by
773 the fact that, as for the majority of deep-sea scavenging amphipods, members of the genus
774 *Paralicella* are obligate necrophages with direct development and active dispersal through
775 swimming by juveniles and adults (Van Dolah & Bird 1980).

776

777 *Importance for conservation*

778 A general consensus exists in that there is currently a very limited understanding of the
779 communities and the species present in the deep-sea regions under the threat of major mining
780 disturbances, which compromises our ability to manage them sustainably (Hilario *et al.* 2015). In
781 the development of a Regional Environmental Management Plan for the CCZ, the need to
782 establish a series of no-mining areas was developed. These areas were termed Areas of Particular
783 Environmental Interest (APEI). APEIs have the important proposed role of protecting vulnerable
784 habitats and their appropriate design is crucial to safeguard the biodiversity and ecosystem
785 function present in the region (Wedding *et al.* 2013). However, to date there has been limited
786 study in the CCZ's APEIs. There is thus an urgent need to fill fundamental science gaps in these
787 particular regions, especially for demographic connectivity of the species in these APEIs, one of
788 the critical parameters to be taken into account in reserve design to avoid irreversible losses after
789 anthropogenic disturbances (e.g. Wright *et al.* 2015).

790 In the light of our results, a critical question to address is: does APEI-6 safeguard
791 biodiversity and ecosystem function represented in nearby mining exploration areas such as UK-

792 1A, UK-1B and OMS-1A? From the *P. craigi* data, which is limited to a single-species from a
793 single functional group, it appears that APEI-6 does serve a conservation role (there is species
794 overlap and connectivity between UK-1A and APEI-6), but on its own may be inadequate,
795 especially as a source of propagules, since OMS-1A exhibits population isolation with respect to
796 the other areas and contributes the most to the exchange of genetic diversity in the region. With
797 regard to the genetic diversity exhibited in each area, UK-1A presented the highest values,
798 therefore, the loss of this particular population could have repercussions on the overall genetic
799 diversity of the species. Without further data on *P. craigi* from other APEIs (e.g. APEI-9 to the
800 south-west of the study region), it is hard to make firm recommendations, but it would appear that
801 an APEI designation to the south and/or west of the UK, OMS, BGR (German), NORI (Nauru)
802 and TOML (Tonga) contract areas would be valuable, potentially supporting gene flow in
803 westerly and northerly directions. It is notable that there are no mining exploration areas in this
804 region, and it is thus likely to be suited to APEI designation.

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817
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References

- 830 Aleynik DA, Inall ME, Dale AC, Vink A (2017) Impact of remotely generated eddies on plume
831 dispersion at abyssal mining sites in the Pacific. *Nature Scientific Reports*, **7**, 16959.
- 832 Amon DJ, Ziegler AF, Dahlgren TG *et al.* (2016) Insights into the abundance and diversity of
833 abyssal megafauna in a polymetallic-nodule region in the eastern Clarion-Clipperton Zone.
834 *Scientific reports*, **6**, 30492.
- 835 Baco AR, Etter RJ, Ribeiro PA *et al.* (2016) A synthesis of genetic connectivity in deep-sea
836 fauna and implications for marine reserve design. *Molecular Ecology*, **25**, 3276–3298.
- 837 Bell JJ, Smith D, Hannan D *et al.* (2014) Resilience to disturbance despite limited dispersal and
838 self-recruitment in tropical barrel sponges: implications for conservation and management
839 (R Pronzato, Ed.). *PLoS ONE*, **9**, e91635.
- 840 Benjamini Y, Yekutieli D (2001) The control of the false discovery rate in multiple testing
841 under dependency. *Annals of Statistics*, **29**, 1165–1188.
- 842 Blanquer A, Uriz MJ (2010a) Population genetics at three spatial scales of a rare sponge living
843 in fragmented habitats. *BMC Evolutionary Biology*, **10**, 13.
- 844 Blanquer A, Uriz MJ (2010b) Population genetics at three spatial scales of a rare sponge living
845 in fragmented habitats. *BMC Evolutionary Biology*, **10**, 13.
- 846 Blanquer A, Uriz MJ, Caujapé-Castells J (2009) Small-scale spatial genetic structure In
847 Scopalina lophyropoda, an encrusting sponge with philopatric larval dispersal and frequent
848 fission and fusion events. *Marine Ecology Progress Series*, **380**, 95–102.
- 849 Borowski C, Thiel H (1998) Deep-sea macrofaunal impacts of a large-scale physical
850 disturbance experiment in the Southeast Pacific. *Deep-Sea Research Part II: Topical
851 Studies in Oceanography*, **45**, 55–81.
- 852 Brown RR, Davis CS, Leys SP (2017) Clones or clans: the genetic structure of a deep-sea
853 sponge, *Aphrocallistes vastus*, in unique sponge reefs of British Columbia, Canada.
854 *Molecular Ecology*, **26**, 1045–1059.
- 855 Cabranes C, Fernandez-Rueda P, Martinez JL (2007) Genetic structure of *Octopus vulgaris*
856 around the Iberian Peninsula and Canary Islands as indicated by microsatellite DNA
857 variation. *ICES Journal of Marine Science*, **65**, 12–16.
- 858 Chapuis M-P, Estoup A (2006) Microsatellite null alleles and estimation of population
859 differentiation. *Molecular Biology and Evolution*, **24**, 621–631.
- 860 Chassignet EP, Hurlburt HE, Smedstad OM *et al.* (2007) The HYCOM (HYbrid Coordinate
861 Ocean Model) data assimilative system. *Journal of Marine Systems*, **65**, 60–83.
- 862 Chaves-Fonnegra A, Feldheim KA, Secord J, Lopez J V. (2015) Population structure and
863 dispersal of the coral-excavating sponge *Cliona delitrix*. *Molecular Ecology*, **24**, 1447–
864 1466.
- 865 Clague GE, Jones WJ, Paduan JB, Clague DA, Vrijenhoek RC (2012) Phylogeography of
866 *Acesta* clams from submarine seamounts and escarpments along the western margin of
867 North America. *Marine Ecology*, **33**, 75–87.
- 868 Clement M, Posada D, Crandall K (2000) TCS: a computer program to estimate gene
869 genealogies. *Molecular ecology*.
- 870 Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent
871 population bottlenecks from allele frequency data. *Genetics*, **144**, 2001–2014.
- 872 Dailianis T, Tsigenopoulos CS, Dounas C, Voultziadou E (2011) Genetic diversity of the
873 imperilled bath sponge *Spongia officinalis* Linnaeus, 1759 across the Mediterranean Sea:
874 Patterns of population differentiation and implications for taxonomy and conservation.
875 *Molecular Ecology*, **20**, 3757–3772.
- 876 Dambach J, Raupach MJ, Leese F, Schwarzer J, Engler JO (2016) Ocean currents determine
877 functional connectivity in an Antarctic deep-sea shrimp. *Marine Ecology*, **37**, 1336–1344.
- 878 DeBiasse MB, Richards VP, Shivji MS (2010) Genetic assessment of connectivity in the
879 common reef sponge, *Callyspongia vaginalis* (Demospongiae: Haplosclerida) reveals high
880 population structure along the Florida reef tract. *Coral Reefs*, **29**, 47–55.
- 881 Van Dolah RF, Bird E (1980) A comparison of reproductive patterns in epifaunal and infaunal
882 gammaridean amphipods. *Estuarine and Coastal Marine Science*, **11**, 593–604.
- 883 Duran S, Pascual M, Estoup A, Turon X (2004) Strong population structure in the marine

- 884 sponge *Crambe crambe* (Poecilosclerida) as revealed by microsatellite markers. *Molecular*
885 *Ecology*, **13**, 511–522.
- 886 Duran S, Rützler K (2006) Ecological speciation in a Caribbean marine sponge. *Molecular*
887 *Phylogenetics and Evolution*, **40**, 292–297.
- 888 Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: A website and program for
889 visualizing STRUCTURE output and implementing the Evanno method. *Conservation*
890 *Genetics Resources*, **4**, 359–361.
- 891 Erpenbeck D, Hooper JNA, Wörheide G (2006) CO1 phylogenies in diploblasts and the
892 “Barcoding of Life” - Are we sequencing a suboptimal partition? *Molecular Ecology*
893 *Notes*, **6**, 550–553.
- 894 Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using
895 the software structure: a simulation study. *Molecular Ecology*, **14**, 2611–2620.
- 896 Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): An integrated software
897 package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**, 47–
898 50.
- 899 Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of
900 mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates.
901 *Molecular Marine Biology and Biotechnology*, **3**, 294–299.
- 902 Freeland JR, Kirk H, Petersen S (2011) *Molecular Ecology* (W-B UK., Ed.).
- 903 Giles EC, Saenz-Agudelo P, Hussey NE, Ravasi T, Berumen ML (2015) Exploring seascape
904 genetics and kinship in the reef sponge *Stylissa carteri* in the Red Sea. *Ecology and*
905 *Evolution*, **5**, 2487–2502.
- 906 Giribet G, Edgecombe GD, Wheeler WC, Babbitt C (2002) Phylogeny and systematic position
907 of Opiliones: a combined analysis of Chelicerate relationships using morphological and
908 molecular Data. *Cladistics*, **18**, 5–70.
- 909 Glover A, Dahlgren T, Wiklund H, Mohrbeck I, Smith C (2015) An end-to-end DNA taxonomy
910 methodology for benthic biodiversity survey in the Clarion-Clipperton Zone, Central
911 Pacific abyss. *Journal of Marine Science and Engineering*, **4**, 2.
- 912 Glover AG, Smith CR (2003) The deep-sea floor ecosystem: current status and prospects of
913 anthropogenic change by the year 2025. *Environmental Conservation*, **30**,
914 S0376892903000225.
- 915 Glover AG, Smith CR, Paterson GLJ *et al.* (2002) Polychaete species diversity in the central
916 Pacific abyss: local and regional patterns, and relationships with productivity. *Marine*
917 *Ecology Progress Series*, **240**, 157–169.
- 918 Le Goff-Vitry MC, Pybus OG, Rogers AD (2004) Genetic structure of the deep-sea coral
919 *Lophelia pertusa* in the northeast Atlantic revealed by microsatellites and internal
920 transcribed spacer sequences. *Molecular Ecology*, **13**, 537–549.
- 921 Guardiola M, Frotscher J, Uriz MJ (2012) Genetic structure and differentiation at a short-time
922 scale of the introduced calcarean sponge *Paraleucilla magna* to the western
923 Mediterranean. *Hydrobiologia*, **687**, 71–84.
- 924 Guardiola M, Frotscher J, Uriz M-J (2016) High genetic diversity, phenotypic plasticity, and
925 invasive potential of a recently introduced calcareous sponge, fast spreading across the
926 Atlanto-Mediterranean basin. *Marine Biology*, **163**, 123.
- 927 Guerard E, Manni F (2004) Manual of the user-Barrier version 2.2. *Mind*, 0–31.
- 928 Hansen M, Hemmer-Hansen J (2007) Landscape genetics goes to sea. *Journal of Biology*, **6**, 6.
- 929 Hilario A, Metaxas A, Gaudron SM *et al.* (2015) Estimating dispersal distance in the deep sea:
930 challenges and applications to marine reserves. *Frontiers in Marine Science*, **2**, 6.
- 931 Huang D, Meier R, Todd PA, Chou LM (2008) Slow mitochondrial COI sequence evolution at
932 the base of the metazoan tree and its implications for DNA barcoding. *Journal of*
933 *Molecular Evolution*, **66**, 167–174.
- 934 Jakobsson M, Rosenberg NA (2007) CLUMPP: A cluster matching and permutation program
935 for dealing with label switching and multimodality in analysis of population structure.
936 *Bioinformatics*, **23**, 1801–1806.
- 937 Janssen A, Kaiser S, Meißner K *et al.* (2015) A reverse taxonomic approach to assess
938 macrofaunal distribution patterns in abyssal Pacific polymetallic nodule fields (J Hewitt,

939 Ed.). *PLOS ONE*, **10**, e0117790.

940 Jombart T (2008) Adegnet: A R package for the multivariate analysis of genetic markers.

941 *Bioinformatics*, **24**, 1403–1405.

942 Jones DOB, Kaiser S, Sweetman AK *et al.* (2017) Biological responses to disturbance from

943 simulated deep-sea polymetallic nodule mining. *PLOS ONE*, **12**, e0171750.

944 Jorde PE, Søvik G, Westgaard J-I *et al.* (2015) Genetically distinct populations of northern

945 shrimp, *Pandalus borealis*, in the North Atlantic: adaptation to different temperatures as an

946 isolation factor. *Molecular Ecology*, **24**, 1742–1757.

947 Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple

948 sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, **30**, 3059–

949 3066.

950 Kearse M, Moir R, Wilson A *et al.* (2012) Geneious Basic: An integrated and extendable

951 desktop software platform for the organization and analysis of sequence data.

952 *Bioinformatics*, **28**, 1647–1649.

953 Kimura M (1983) *The Neutral Theory of Molecular Evolution*.

954 Kuhner MK (2006) LAMARC 2.0: maximum likelihood and Bayesian estimation of population

955 parameters. *Bioinformatics*, **22**, 768–770.

956 Launey S, Ledu C, Boudry P, Bonhomme F, Naciri-Graven Y (2002) Geographic structure in

957 the european flat oyster (*Ostrea edulis* L.) as revealed by microsatellite polymorphism.

958 *Journal of Heredity*, **93**, 331–351.

959 Ledoux J-B, Garrabou J, Bianchimani O *et al.* (2010) Fine-scale genetic structure and

960 inferences on population biology in the threatened Mediterranean red coral, *Corallium*

961 *rubrum*. *Molecular Ecology*, **19**, 4204–4216.

962 Lim S-C, Wiklund H, Glover AG, Dahlgren TG, Koh-Siang T (2017) A new genus and species

963 of abyssal sponge commonly encrusting polymetallic nodules in the Clarion-Clipperton

964 Zone, East Pacific Ocean. *Systematics and Biodiversity*, **15**, 507–519.

965 Lodge M, Johnson D, Le Gurun G *et al.* (2014) Seabed mining: International Seabed Authority

966 environmental management plan for the Clarion-Clipperton Zone. A partnership approach.

967 *Marine Policy*, **49**, 66–72.

968 López-Legentil S, Pawlik JR (2009) Genetic structure of the Caribbean giant barrel sponge

969 *Xestospongia muta* using the I3-M11 partition of COI. *Coral Reefs*, **28**, 157–165.

970 Luikart G, Cornuet JM (1998) Empirical evaluation of a test for identifying recently

971 bottlenecked populations from allele frequency data. *Conservation Biology*, **12**, 228–237.

972 Lutz MJ, Caldeira K, Dunbar RB, Behrenfeld MJ (2007) Seasonal rhythms of net primary

973 production and particulate organic carbon flux to depth describe the efficiency of

974 biological pump in the global ocean. *Journal of Geophysical Research: Oceans*, **112**.

975 Maier E, Tollrian R, Rinkevich B, Nürnberger B (2005) Isolation by distance in the

976 scleractinian coral *Seriatopora hystrix* from the Red Sea. *Marine Biology*, **147**, 1109–

977 1120.

978 Maldonado M (2006) The ecology of the sponge larva. *Canadian Journal of Zoology*, **84**, 175–

979 194.

980 Meirmans PG, Van Tienderen PH (2004) GENOTYPE and GENODIVE: Two programs for the

981 analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes*, **4**, 792–794.

982 Mewes K, Mogollón JM, Picard A *et al.* (2014) Impact of depositional and biogeochemical

983 processes on small scale variations in nodule abundance in the Clarion-Clipperton Fracture

984 Zone. *Deep-Sea Research Part I: Oceanographic Research Papers*, **91**, 125–141.

985 Miljutin DM, Miljutina MA, Arbizu PM, Galéron J (2011) Deep-sea nematode assemblage has

986 not recovered 26 years after experimental mining of polymetallic nodules (Clarion-

987 Clipperton Fracture Zone, Tropical Eastern Pacific). *Deep-Sea Research Part I:*

988 *Oceanographic Research Papers*, **58**, 885–897.

989 Mullineaux LS (1987) Organisms living on manganese nodules and crusts: distribution and

990 abundance at three North Pacific sites. *Deep Sea Research Part A, Oceanographic*

991 *Research Papers*, **34**, 165–184.

992 O'Hara TD, England PR, Guasekera RM, Naughton KM (2014) Limited phylogeographic

993 structure for five bathyal ophiuroids at continental scales. *Deep Sea Research Part I:*

- 994 *Oceanographic Research Papers*, **84**, 18–28.
- 995 Oebius HU, Becker HJ, Rolinski S, Jankowski JA (2001) Parametrization and evaluation of
 996 marine environmental impacts produced by deep-sea manganese nodule mining. *Deep Sea*
 997 *Research Part II: Topical Studies in Oceanography*, **48**, 3453–3467.
- 998 Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER:
 999 Software for identifying and correcting genotyping errors in microsatellite data. *Molecular*
 1000 *Ecology Notes*, **4**, 535–538.
- 1001 Padua A, Cunha HA, Klautau M (2017) Gene flow and differentiation in a native calcareous
 1002 sponge (Porifera) with unknown dispersal phase. *Marine Biodiversity*, 1–11.
- 1003 Paterson GLJ, Menot L, Colaço A *et al.* (2015) Biogeography and connectivity in deep-sea
 1004 habitats with mineral resource potential–gap analysis. *MIDAS Deliverable 4.2*, 45.
- 1005 Paterson GLJ, Wilson GDF, Cosson N, Lamont PA (1998) Hessler and Jumars (1974) revisited:
 1006 abyssal polychaete assemblages from the Atlantic and Pacific. *Deep Sea Research Part II:*
 1007 *Topical Studies in Oceanography*, **45**, 225–251.
- 1008 Peakall R, Smouse PE (2006) GENALEX 6: Genetic analysis in Excel. Population genetic
 1009 software for teaching and research. *Molecular Ecology Notes*, **6**, 288–295.
- 1010 Peakall R, Smouse PE (2012) GenALEX 6.5: Genetic analysis in Excel. Population genetic
 1011 software for teaching and research—an update. *Bioinformatics*, **28**, 2537–2539.
- 1012 Pérez-Portela R, Cerro-Gálvez E, Taboada S *et al.* (2016) Lonely populations in the deep:
 1013 genetic structure of red gorgonians at the heads of submarine canyons in the north-western
 1014 Mediterranean Sea. *Coral Reefs*, **35**.
- 1015 Pérez-Portela R, Noyer C, Becerro MA (2015) Genetic structure and diversity of the endangered
 1016 bath sponge *Spongia lamella*. *Aquatic Conservation: Marine and Freshwater Ecosystems*,
 1017 **25**, 365–379.
- 1018 Polato NR, Concepcion GT, Toonen RJ, Baums IB (2010) Isolation by distance across the
 1019 Hawaiian Archipelago in the reef-building coral *Porites lobata*. *Molecular Ecology*, **19**,
 1020 4661–4677.
- 1021 Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus
 1022 genotype data. *Genetics*, **155**.
- 1023 Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact
 1024 tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.
- 1025 Riesgo A, Maldonado M (2008) Differences in reproductive timing among sponges sharing
 1026 habitat and thermal regime. *Invertebrate Biology*, **127**, 357–367.
- 1027 Riesgo A, Perez-Portela R, Pita L *et al.* (2016) Population structure and connectivity in the
 1028 Mediterranean sponge *Ircinia fasciculata* are affected by mass mortalities and
 1029 hybridization. *Heredity*, **117**, 427–439.
- 1030 Ritchie H, Jamieson AJ, Piertney SB (2017) Population genetic structure of two congeneric
 1031 deep-sea amphipod species from geographically isolated hadal trenches in the Pacific
 1032 Ocean. *Deep-Sea Research Part I: Oceanographic Research Papers*, **119**, 50–57.
- 1033 Rosenberg NA (2004) DISTRUCT: A program for the graphical display of population structure.
 1034 *Molecular Ecology Notes*, **4**, 137–138.
- 1035 Rousset F (2008) GENEPOP'007: A complete re-implementation of the GENEPOP software
 1036 for Windows and Linux. *Molecular Ecology Resources*, **8**, 103–106.
- 1037 Schönberg CHL (2016) Effects of dredging on filter feeder communities , with a focus on
 1038 sponges. Report of Theme 6 - Project 6.1 prepared for the Dredging Science Node. *Report*
 1039 *of Theme 6 - Project 6.1.1 prepared for the Dredging Science Node, Western Australian*
 1040 *Marine Science Institution, Perth, Western Australia. 139 pp., 127 pp.*
- 1041 Schwendinger PJ, Giribet G (2005) The systematics of the south-east Asian genus *Fangensis*
 1042 *Rambla* (Opiliones:Cyphophthalmi:Stylocellidae). *Invertebrate Systematics*, **19**, 297.
- 1043 Smith C, Demopoulos A (2003) The deep pacific ocean floor. *Ecosystems of the World*, **28**,
 1044 179–218.
- 1045 Smith CR, Hoover DJ, Doan SE *et al.* (1996) Phytodetritus at the abyssal seafloor across 10° of
 1046 latitude in the central equatorial Pacific. *Deep Sea Research Part II: Topical Studies in*
 1047 *Oceanography*, **43**, 1309–1338.
- 1048 Smith C, Levin L, Koslow A, Tyler P (2008a) The near future of the deep seafloor ecosystems.

1049 In: *Aquatic ecosystems*: (ed Poulin N), pp. 334–351. Cambridge University Press,
1050 Cambridge, UK.

1051 Smith CR, Paterson G, Lambshead J *et al.* (2008b) Biodiversity, species ranges, and gene flow
1052 in the abyssal Pacific nodule province: predicting and managing the impacts of deep
1053 seabed mining. *Project Report*.

1054 Sundqvist L, Keenan K, Zackrisson M, Prodöhl P, Kleinhans D (2016) Directional genetic
1055 differentiation and relative migration. *Ecology and Evolution*, **6**, 3461–3475.

1056 Taboada S, Kenny NJ, Riesgo A *et al.* (2017) Mitochondrial genome and polymorphic
1057 microsatellite markers from the abyssal sponge *Plenaster craigi* Lim & Wiklund,
1058 2017: tools for understanding the impact of deep-sea mining.

1059 Taylor ML, Roterman CN (2017) Invertebrate population genetics across Earth’s largest habitat:
1060 The deep-sea floor. *Molecular Ecology*.

1061 Thiel H, Schriever G, Ahnert A *et al.* (2001) The large-scale environmental impact experiment
1062 DISCOL—reflection and foresight. *Deep Sea Research Part II: Topical Studies in*
1063 *Oceanography*, **48**, 3869–3882.

1064 Thiel H, Schriever G, Bussau C, Borowski C (1993) Manganese nodule crevice fauna. *Deep-*
1065 *Sea Research Part I*, **40**, 419–423.

1066 Vanreusel A, Hilario A, Ribeiro PA, Menot L, Arbizu PM (2016) Threatened by mining,
1067 polymetallic nodules are required to preserve abyssal epifauna. *Scientific Reports*, **6**,
1068 26808.

1069 Veillette J, Juniper SK, Gooday AJ, Sarrazin J (2007) Influence of surface texture and
1070 microhabitat heterogeneity in structuring nodule faunal communities. *Deep-Sea Research*
1071 *Part I: Oceanographic Research Papers*, **54**, 1936–1943.

1072 Vrijenhoek RC (2010) Genetic diversity and connectivity of deep-sea hydrothermal vent
1073 metapopulations. *Molecular Ecology*, **19**, 4391–4411.

1074 Wedding LM, Friedlander AM, Kittinger JN *et al.* (2013) From principles to practice: a spatial
1075 approach to systematic conservation planning in the deep sea. *Proceedings. Biological*
1076 *sciences*, **280**, 20131684.

1077 Witte U (1996) Seasonal reproduction in deep-sea sponges - triggered by vertical particle flux?
1078 *Marine Biology*, **124**, 571–581.

1079 Wörheide G, Epp LS, Macis L (2008) Deep genetic divergences among Indo-Pacific
1080 populations of the coral reef sponge *Leucetta chagosensis* (Leucettidae): Founder effects,
1081 vicariance, or both? *BMC Evolutionary Biology*, **8**, 24.

1082 Wörheide G, Solé-Cava AM, Hooper JNA (2005) Biodiversity, molecular ecology and
1083 phylogeography of marine sponges: patterns, implications and outlooks. *Integrative and*
1084 *Comparative Biology*, **45**, 377–385.

1085 Wright D, Bishop JM, Matthee CA, von der Heyden S (2015) Genetic isolation by distance
1086 reveals restricted dispersal across a range of life histories: Implications for biodiversity
1087 conservation planning across highly variable marine environments. *Diversity and*
1088 *Distributions*, **21**, 698–710.

1089 Xavier JR, Rachello-Dolmen PG, Parra-Velandia F *et al.* (2010) Molecular evidence of cryptic
1090 speciation in the “cosmopolitan” excavating sponge *Cliona celata* (Porifera, Clionaidae).
1091 *Molecular Phylogenetics and Evolution*, **56**, 13–20.

1092 Zulliger DE, Tanner S, Ruch M, Ribi G (2009) Genetic structure of the high dispersal Atlanto-
1093 Mediterranean sea star *Astropecten aranciacus* revealed by mitochondrial DNA
1094 sequences and microsatellite loci. *Marine Biology*, **156**, 597–610.

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1096 **Data Accessibility Statement**

1097 New *28S* and *COI* sequences were obtained and deposited in the genetic sequence database,
1098 Genbank, under the Accession numbers MH138084 - MH138092 and MH138093 - MH138157,
1099 respectively. Final genotypes are stored in the Dryad Digital Repository
1100 (doi:10.1594/PANGAEA.888006). Additional data and figures may be found in Supporting
1101 information.

1102 **Authors Contributions**

1103 S.T., H.W. C.R.S., D.O.B.J., T.G.D. and A.G.G. designed the study and collected the samples;
1104 G.L.J.P., C.R.S., D.O.B.J., T.G.D. and A.G.G. provided funding for the sampling; A.R., G.L.J.P.,
1105 A.C.D., C.R.S., D.O.B.J., T.G.D. and A.G.G. contributed reagents and analytical tools; S.T.,
1106 A.R., H.W., V.K. and N.S. carried out laboratory analyses; S.T. and A.R. analysed molecular
1107 data; S.T., A.R. and N.S. analysed morphological data; A.C.D. analysed oceanographic models;
1108 S.T. wrote the first draft of the paper and A.R., G.L.J.P, A.C.D., C.R.S., D.O.B.J., T.G.D. and
1109 A.G.G. made major contributions to the writing. All the authors reviewed the final version of the
1110 manuscript.

1111 **Tables**

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1113 **Table 1.** Details of the samples analyzed in the present study at each of the sampling
 1114 areas. ^a *AG* Agassiz trawl, *BC* boxcore, *EBS* epibenthic sledge, *HB* Hydraulic benthic *in*
 1115 *situ* sampler (*Hybis* ROV), *MC* multicore. ^b In brackets the number of specimens
 1116 successfully used for microsatellite analysis

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Cruise/Area	Original sampling station	Gear ^a	N ^b	Depth (m)	Latitude (N)	Longitude (W)
APEI-6						
JC120	APEI-6_Flat1	BC	3 (3)	4156	17°14.448	123°0.3978
JC120	APEI-6_Flat2	BC	4 (4)	4161	17°14.9320	123°1.2820
JC120	APEI-6_Flat3	BC	6 (5)	4153	17°15.019	123°1.7570
JC120	APEI-6_Flat4	BC	2 (2)	4180	17°13.184	123°2.667
JC120	APEI-6_Flat5	BC	4 (3)	4162	17°14.381	123°1.584
JC120	APEI-6_Flat6	MC	2 (2)	4162	17°14.433	123°3.967
JC120	APEI-6_Flat7	MC	1 (1)	4155	17°15.022	123°1.759
JC120	APEI-6_Flat8	AG	2 (0)	4169	17°15.455	123°3.5890
Total Flat			24 (20)			
JC120	APEI-6_Ridge1	BC	3 (2)	4021	17°21.5610	122°54.185
JC120	APEI-6_Ridge2	BC	4 (2)	4045	17°18.843	122°54.047
JC120	APEI-6_Ridge3	BC	3 (2)	4028	17°22.00157	122°53.971888
JC120	APEI-6_Ridge4	BC	1 (1)	4015	17°17.31	122°53.068
JC120	APEI-6_Ridge5	BC	2 (2)	4012	17°19.672	122°53.271
JC120	APEI-6_Ridge6	MC	1 (1)	4012	17°17.30046	122°53.07351
Total Ridge			14 (10)			
JC120	APEI-6_Trough1	BC	1 (1)	4264	17°13.868817	122°48.90019
JC120	APEI-6_Trough2	BC	5 (2)	4231	17°17.77448	122°50.12778
JC120	APEI-6_Trough3	MC	2 (2)	4234	17°17.789	122°50.128
Total Trough			8 (5)			
JC120	APEI-6_Deep1	BC	1 (1)	4297	16°54.7716	122°59.8412
JC120	APEI-6_Deep2	MC	1 (1)	4297	16°54.7770	122°59.8290
JC120	APEI-6_Nodule	HB	1 (1)	4321	16°53.4309	122°50.6078
Total Deep_Nodule			3 (3)			
Total APEI-6			49 (38)			
UK-1A						
AB01	UK-1A_BC03	BC	3 (2)	4171	13°52.900	116°28.000
AB01	UK-1A_BC05	BC	17 (17)	4081	13°47.601	116°42.185
AB01	UK-1A_BC06	BC	1 (1)	4084	13°57.794	116°34.093
AB01	UK-1A_BC08	BC	1 (1)	4076	13°48.700	116°42.600
AB01	UK-1A_BC10	BC	7 (7)	4036	13°45.001	116°30.799
AB01	UK-1A_BC12	BC	8 (8)	4050	13°51.801	116°32.800
AB01	UK-1A_BC14	BC	10 (10)	4160	13°43.597	116°40.200
AB01	UK-1A_EB03	EBS	1 (1)	4130	13°57.437	116°30.101
AB01	UK-1A_EB04	EBS	3 (3)	4128	13°48.254	116°28.196
Total UK-1A			51 (50)			

UK-1B						
AB02	UK-1B_BC01	BC	5 (5)	4127	12°24.977	116°42.891
AB02	UK-1B_BC02	BC	2 (2)	4159	12°22.022	116°31.021
AB02	UK-1B_BC03	BC	5 (5)	4144	12°24.410	116°29.085
AB02	UK-1B_BC04	BC	1 (1)	4160	12°22.259	116°36.819
AB02	UK-1B_BC06	BC	2 (2)	4237	12°34.742	116°41.218
AB02	UK-1B_BC13	BC	2 (2)	4130	12°27.066	116°35.661
AB02	UK-1B_BC15	BC	5 (5)	4196	12°27.107	116°30.736
AB02	UK-1B_BC17	BC	3 (3)	4228	12°34.190	116°32.333
AB02	UK-1B_BC18	BC	10 (10)	4136	12°25.195	116°37.477
AB02	UK-1B_BC20	BC	3 (3)	4258	12°35.813	116°29.614
AB02	UK-1B_EB09	EBS	1 (1)	4460	12°21.62	116°41.99
AB02	UK-1B_MC13	MC	1 (1)	4129	12°27.059	116°35.667
AB02	UK-1B_MC25	MC	1 (1)	4224	12°34.953	116°39.058
Total UK-1B			41 (41)			
OMS-1A						
AB02	OMS-1A_BC07	BC	2 (2)	4183	12°07.066	117°20.621
AB02	OMS-1A_BC08	BC	1 (1)	4114	12°10.868	117°15.659
AB02	OMS-1A_BC09	BC	5 (5)	4070	12°04.914	117°10.691
AB02	OMS-1A_BC10	BC	2 (2)	4144	12°00.567	117°10.687
AB02	OMS-1A_BC11	BC	3 (3)	4090	12°13.0425	117°19.5229
AB02	OMS-1A_BC12	BC	4 (4)	4044	12°08.695	117°19.526
AB02	OMS-1A_BC21	BC	6 (6)	4054	12°08.156	117°12.900
AB02	OMS-1A_BC22	BC	7 (7)	4051	12°05.994	117°11.796
AB02	OMS-1A_BC23	BC	3 (3)	4095	12°03.278	117°15.103
AB02	OMS-1A_BC25	BC	3 (3)	4141	12°00.559	117°22.818
AB02	OMS-1A_BC26	BC	1 (1)	4139	12°01.643	117°19.512
AB02	OMS-1A_EB06	EBS	1 (1)	4137	12°15.05	117°19.23
AB02	OMS-1A_MC23	MC	1 (1)	4148	12°00.554	117°22.821
Total OMS-1A			39 (39)			
GRAND TOTAL			180 (168)			

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Table 2. Descriptors of genetic diversity for all 30 locations and the four areas of *P. craigi* using the data set containing 11 loci and also the dataset using 7 loci after removing the four loci (3Ple, 10Ple, 11Ple and 19Ple) possibly being affected by the presence of null alleles. Some of the sampling stations are the result of pooling the original sampling stations from Table 1. *N* sample size, *Na* mean number of alleles per locus, *Pa* mean number of private alleles, *He* expected heterozygosity, *Ho* observed heterozygosity, *F_{IS}* inbreeding coefficient, *HWE* Significant deviation from Hardy-Weinberg Equilibrium after application of Narum correction ($P < 0.05$). ns=not significant, ** $P < 0.01$, *** $P < 0.001$

Area/Sampling station	N	Na		Pa		Ho		He		F _{IS}		HWE	
		11 loci	7 loci	11 loci	7 loci	11 loci	7 loci	11 loci	7 loci	11 loci	7 loci	11 loci	7 loci
APEI-6													
APEI-6_Ridge	10	6.364	5.857	0.273	0.286	0.570	0.671	0.700	0.654	0.163	-0.040	***	ns
APEI-6_Trough	5	4.727	4.143	0.000	0.000	0.564	0.571	0.658	0.597	0.167	0.084	ns	ns
APEI-6_Flat	20	10.364	9.857	0.273	0.143	0.478	0.554	0.705	0.664	0.304	0.140	***	***
APEI-6_Deep-Nodule	3	3.364	3.000	0.000	0.000	0.485	0.476	0.571	0.484	0.170	0.040	ns	ns
Total APEI-6	38	13.455	12.857	0.727	0.571	0.516	0.583	0.775	0.735	0.342	0.225	***	***
UK-1A													
UK-1A_BC06-EB03	2	2.273	2.429	0.000	0.000	0.545	0.643	0.500	0.554	-0.080	-0.143	ns	ns
UK-1A_BC08-BC05	18	8.000	7.571	0.636	0.571	0.480	0.549	0.697	0.657	0.326	0.193	***	***
UK-1A_BC12	8	6.000	5.286	0.909	1.286	0.365	0.385	0.689	0.628	0.395	0.275	***	***
UK-1A_BC03	2	2.636	2.714	0.000	0.000	0.636	0.714	0.466	0.518	-0.383	-0.400	ns	ns
UK-1A_BC14	10	6.273	6.429	0.545	0.857	0.445	0.500	0.658	0.668	0.305	0.229	***	***
UK-1A_EB04	3	2.818	2.571	0.000	0.000	0.576	0.619	0.500	0.460	-0.183	-0.343	ns	ns
UK-1A_BC10	7	5.727	5.286	0.000	0.000	0.498	0.469	0.684	0.635	0.317	0.337	***	***
Total UK-1A	50	15.636	16.000	2.364	2.857	0.472	0.513	0.791	0.760	0.412	0.340	***	***
UK-1B													
UK-1B_BC06-MC25	3	3.364	3.286	0.000	0.000	0.545	0.619	0.571	0.540	0.056	-0.140	ns	ns
UK-1B_BC17	3	3.091	2.857	0.091	0.143	0.545	0.619	0.540	0.508	-0.031	-0.259	ns	ns
UK-1B_BC20	3	3.455	3.286	0.091	0.143	0.470	0.476	0.612	0.587	0.232	0.174	ns	ns
UK-1B_BC01	5	4.091	4.143	0.182	0.143	0.491	0.571	0.595	0.571	0.186	0.019	**	ns

UK-1B_BC18-MC13	11	6.909	6.571	0.455	0.714	0.415	0.455	0.649	0.576	0.331	0.200	***	***
UK-1B_BC13	2	2.000	2.286	0.000	0.000	0.500	0.643	0.364	0.429	-0.383	-0.489	ns	ns
UK-1B_BC15	5	4.000	3.857	0.182	0.000	0.468	0.521	0.571	0.512	0.147	-0.056	ns	ns
UK-1B_EB09-BC04	2	2.455	2.286	0.000	0.000	0.500	0.571	0.523	0.482	0.093	-0.156	ns	ns
UK-1B_BC02	2	1.091	1.143	0.091	0.000	0.136	0.071	0.170	0.125	0.167	0.333	ns	ns
UK-1B_BC03	5	3.273	2.857	0.091	0.143	0.491	0.543	0.532	0.476	0.101	-0.102	ns	ns
Total UK-1B	41	13.273	13.429	1.364	1.429	0.470	0.517	0.728	0.676	0.328	0.206	***	***
OMS-1A													
OMS-1A_EB06-BC11-BC08	5	4.818	4.857	0.091	0.143	0.491	0.543	0.675	0.649	0.256	0.145	***	ns
OMS-1A_BC12	4	3.636	3.286	0.091	0.143	0.545	0.536	0.597	0.531	0.045	-0.032	ns	ns
OMS-1A_BC07	2	2.909	2.714	0.182	0.143	0.591	0.571	0.568	0.518	-0.013	-0.111	ns	ns
OMS-1A_BC21	6	5.273	5.143	0.000	0.000	0.536	0.629	0.663	0.610	0.211	0.041	***	ns
OMS-1A_BC22	7	5.455	5.571	0.455	0.714	0.409	0.449	0.620	0.582	0.317	0.170	***	ns
OMS-1A_BC09	5	4.273	4.143	0.000	0.000	0.418	0.486	0.636	0.577	0.298	0.119	***	ns
OMS-1A_BC23	3	2.545	2.571	0.182	0.286	0.515	0.524	0.455	0.429	-0.088	-0.197	ns	ns
OMS-1A_BC25-MC23-BC26	5	4.455	5.000	0.182	0.286	0.559	0.621	0.613	0.663	0.061	0.022	ns	ns
OMS-1A_BC10	2	2.364	2.286	0.091	0.143	0.682	0.857	0.511	0.500	-0.345	-0.695	ns	ns
Total OMS-1A	39	13.273	13.857	2.091	3.143	0.502	0.557	0.728	0.690	0.300	0.179	***	***
GRAND TOTAL	168	4.267	14.036	--	--	0.498	0.543	0.576	0.715	0.115	0.179	***	***

1126 **Table 3.** Results of the ANOVA analyses on the maximum length of the individuals of
 1127 *Plenaster craigi* from the four different areas and from individuals assigned to the cluster
 1128 1 from APEI-6 and UK-1A areas. *df* degrees of freedom, *F* F-test statistic, *F crit* F-test
 1129 statistic critical value, *MS* mean square, *N* number of individuals, *S.D.* standard deviation,
 1130 *SS* sum of squares. *significant value
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Summary

Areas	N	Mean (µm)	S.D. (µm)
<i>All areas</i>			
APEI-6	48	3624	1265
UK-1A	35	4641	1529
UK-1B	41	5133	1776
OMS-1A	30	3991	1550
<i>Selected indiv. Cluster 1</i>			
APEI-6	16	3551	1372
UK-1A	13	4409	861

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ANOVA

Source of variation	SS	df	MS	F	<i>p</i> -value	F crit
<i>All areas</i>						
Between Groups	57128751	3	19042917	8.15	0.00005*	2.66
Within Groups	350419781	150	2336132			
Total	407548532	153				
<i>Selected indiv. Cluster 1</i>						
Between Groups	5267595	1	5267595	4.60	0.04107*	4.21
Within Groups	30900318	27	1144456			
Total	36167913	28				

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1135 **Table 4.** F_{ST} values between pairs of areas for *P. craigi* based on 11 microsatellites.
1136 *significant values after applying the false discovery rate.
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Area	APEI-6	UK-1A	UK-1B	OMS-1A
APEI-6	-----			
UK-1A	0.00709	-----		
UK-1B	0.06346*	0.06856*	-----	
OMS-1A	0.11132*	0.10801*	0.07711*	-----

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1139 **Table 5.** Results of the Analysis of Molecular Variance (AMOVA) between the four
1140 different areas (APEI-6, UK-1A, UK-1B and OMS-1A). *significant values
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Source of variation	d.f.	Sum of squares	% variation	Fixation indices	P-value
Among areas	3	38,317	5.09	FCT = 0.05089	0.04665*
Among populations within areas	26	96,045	8.84	FSC = 0.09314	0.00000*
Among individuals within populations	138	281,648	24.9	FIS = 0.28931	0.00000*
Within individuals	168	189	61.17	FIT = 0.38830	0.00000*

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Table 6. Asymmetric migration rates and Theta (Θ) inferred in Lamarc for *P. craigi*, with 95% credibility intervals (CIs) in brackets. Values are given for all pairwise comparisons among areas and also for areas from cluster 1.

		Migration FROM					
		APEI-6	UK-1A	UK-1B	OMS-1A	Θ MPE (95% CI)	
Migration TO	APEI-6	-----	0.029741 (0.0565–100.0634)	0.023417 (0.03075–54.0640)	0.673238 (0.1285–2.9865)	9.754 (1.1513–10.1146)	
	UK-1A	0.060954 (0.0212–99.4550)	-----	0.094908 (0.1889–23.1797)	0.031797 (0.0748–98.6775)	9.982 (0.4315–10.0744)	
	UK-1B	0.037368 (0.02336–0.9680)	0.206768 (0.0835–99.1444)	-----	0.031205 (0.0356–1.7503)	9.938 (0.4435–10.3329)	
	OMS-1A	0.152319 (0.5496–98.7647)	0.140648 (0.0263–99.0018)	0.121937 (0.0282–80.6290)	-----	9.896 (1.2072–10.0198)	
Cluster 1		APEI-6	UK-1A	Θ MPE (95% CI)			
		APEI-6	-----	0.054573 (-0.0827–99.4804)	9.911322 (1.4668–10.0467)		
		UK-1A	0.049394 (0.0539–27.2686)	-----	9.218427 (1.5327–10.031)		

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1148 **Table 7.** Number of immigrants per generation (ΘM) between areas for *P. craigi*.
 1149 Values are given for all pairwise comparisons among areas and also for areas from
 1150 cluster 1.

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		Migration FROM			
		APEI-6	UK-1A	UK-1B	OMS-1A
Migration TO	APEI-6	-----	0.07	0.06	1.63
	UK-1A	0.15	-----	0.24	0.35
	UK-1B	0.09	0.51	-----	0.30
	OMS-1A	0.38	0.08	0.08	-----
Cluster 1		APEI-6	UK-1A		
	APEI-6	-----	0.11		
	UK-1A	0.14	-----		

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1154 **Table 8.** Connectivity matrix between the four sites derived from particle transport
 1155 simulations, representing the probability that a particle transported passively from one
 1156 site passes within a 25 km radius of a second site within a timescale of 5 years.
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		Source site			
		APEI-6	UK-1A	UK-1B	OMS-1A
Receiving site	APEI-6	-----	0.016	0.002	0.002
	UK-1A	0.00015	-----	0.177	0.157
	UK-1B	0.00020	0.157	-----	0.254
	OMS-1A	0.00019	0.076	0.426	-----

1158

1159 **Figure Legends**

1160

1161 **Figure 1.** Map of the study area. **A** Map of the CCZ with the mining exploration areas
1162 and the network of APEI's. Inset showing the approximate position of the four different
1163 areas studied (APEI-6, UK-1A, UK-1B, and OMS-1A) **B** Detail of the APEI-6 identifying
1164 the different sampling stations in the area (Ridge_1-6, Trough_1-3, Flat_1-8, Deep_1-2,
1165 and Nodule). **C** Detail of the UK-1A identifying the different sampling stations in the
1166 area. **D** Detail of the UK-1B identifying the different sampling stations in the area. **E**
1167 Detail of the OMS-1A identifying the different sampling stations in the area.

1168

1169 **Figure 2.** Mean and standard deviation of the maximum length of the individuals of *P.*
1170 *craigi* measured in the different areas.

1171

1172 **Figure 3.** *COI* haplotype network for *P. craigi*. Circles are proportional to the number of
1173 individuals for each haplotype. Colour coding refers to the different areas where samples
1174 were collected.

1175

1176 **Figure 4.** **A** Individual genotype assignment of *P. craigi* to clusters (K) as inferred by
1177 STRUCTURE for all studied sites with $k = 2$ and $k = 3$. In orange the individuals
1178 belonging to cluster 1. **B** DAPC analysis with all samples grouped in the four different
1179 areas. **C** DAPC analysis with all samples grouped in the four different areas treating apart
1180 samples from cluster 1.

1181

1182 **Figure 5.** Mean flow at 3500 m from HYCOM simulations averaged over the 9-year
1183 period used for dispersal simulations, 10/2008 to 9/2017. The underlying image is of the
1184 model bathymetry and red circles show the 25 km radius receiving circles used for APEI-
1185 6, UK-1A, UK-1B and OMS-1A.

1186

1187 **Figure 6.** The distribution of particles after 1, 2, 3, 4 and 5 years (**A–E**) of continuous
1188 release and passive transport from APEI-6, UK-1A, UK-1B and OMS-1A subject to
1189 HYCOM velocities at 3500 m. **F** Migration directionality between the different areas as
1190 inferred by diveRsity. Only relative migration from UK-1B to UK-1A and from OMS-
1191 1A to UK-1A resulted significant.

1192

1193

1194 **Figure 7.** Genetic diversity (*He*) correlation between sponges studied using microsatellite
1195 markers. Sponges were grouped in ranges of distances (<1 km, 10–100 km, 1000 km,
1196 2000 km) in order to make results comparable among species. 1. *Scopalina lophryopoda*
1197 (Blanquer *et al.* 2009); 2. *Paraleucilla magna* (Guardiola *et al.* 2012); 3. *Xestospongia*
1198 sp. (Bell *et al.* 2014); 4. *Xestospongia testudinaria* (Bell *et al.*, 201); 5. *Scopalina*
1199 *lophryopoda* (Blanquer & Uriz 2010b); 6. *Stylissa carteri* (Giles *et al.* 2015); 7. *Ircinia*
1200 *fasciculata* (Riesgo *et al.* 2016); 8. *Plenaster craigi* (this study); 9. *Spongia lamella*
1201 (Pérez-Portela *et al.*, 2015); 10. *Crambe crambe* (Duran *et al.*, 2004); 11. *Clathrina aurea*
1202 (Padua *et al.* 2017); 12. *Spongia officinalis* (Dailianis *et al.* 2011); 13. *Cliona delitrix*
1203 (Chaves-Fonnegra *et al.* 2015); 14. *Petrosia ficiformis* (Riesgo *et al.*, under review); 15.
1204 *Paraleucilla magna* (Guardiola *et al.* 2016).