



HAL
open science

Subtle limits to connectivity revealed by outlier loci within two divergent metapopulations of the deep-sea hydrothermal gastropod *Ifremeria nautilei*

Adrien Tran Y. Lu, Stephanie Ruault, Claire Daguin-Thiébaud, Jade Castel, Nicolas Bierne, Thomas Broquet, Patrick Wincker, Aude Perdereau, Sophie Arnaud-Haond, Pierre-Alexandre Gagnaire, et al.

► To cite this version:

Adrien Tran Y. Lu, Stephanie Ruault, Claire Daguin-Thiébaud, Jade Castel, Nicolas Bierne, et al.. Subtle limits to connectivity revealed by outlier loci within two divergent metapopulations of the deep-sea hydrothermal gastropod *Ifremeria nautilei*. *Molecular Ecology*, 2022, 31 (10), pp.2796-2813. 10.1111/mec.16430 . hal-03669635

HAL Id: hal-03669635

<https://hal.umontpellier.fr/hal-03669635v1>

Submitted on 28 Oct 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Subtle limits to connectivity revealed by outlier loci within two divergent**
2 **metapopulations of the deep-sea hydrothermal gastropod *Ifremeria nautiliei***

3
4 **Adrien Tran Lu Y^{1*}, Stéphanie Ruault², Claire Daguin-Thiébaud², Jade Castel², Nicolas**
5 **Bierne¹, Thomas Broquet², Patrick Wincker⁵, Aude Perdereau⁵, Sophie Arnaud-Haond³,**
6 **Pierre-Alexandre Gagnaire¹, Didier Jollivet², Stéphane Hourdez⁴, François Bonhomme¹**

7 1:ISEM, Institut des Sciences de l'Evolution, Univ Montpellier, CNRS, EPHE, IRD, Montpellier, France,

8 2: Sorbonne Université, CNRS, UMR 7144, 'Dynamique de la Diversité Marine' (DyDiv) Lab, Station biologique de Roscoff,
9 Place G. Teissier, 29680 Roscoff, France

10 3: MARBEC, Marine Biodiversity Exploitation and Conservation, Univ Montpellier, CNRS, IFREMER, IRD, Sète, France

11 4: Sorbonne Université, CNRS, UMR 8222, Laboratoire d'Ecogéochimie des Environnements Benthiques, Observatoire
12 Océanologique de Banyuls, Avenue Pierre Fabre, 66650, Banyuls-sur-Mer, France

13 5: Génomique Métabolique, Génomscope, Institut de Biologie François Jacob, CEA, CNRS, Université Évry, Université Paris-
14 Saclay, Évry, France

15

16 *Corresponding author: adrien.tran-lu-y@umontpellier.fr

17

18 **Keywords:**

19 **Genetic connectivity, Demographic inference, ddRAD-seq, Hydrothermal vents,**
20 **Western Pacific, Outlier detection**

21

22

23

24

25

26

27

28 **Abstract.** Hydrothermal vents form archipelagos of ephemeral deep-sea habitats that raise
29 interesting questions about the evolution and dynamics of the associated endemic fauna,
30 constantly subject to extinction-recolonization processes. These metal-rich environments are
31 coveted for the mineral resources they harbor, thus raising recent conservation concerns. The
32 evolutionary fate and demographic resilience of hydrothermal species strongly depend on the
33 degree of connectivity among and within their fragmented metapopulations. In the deep sea,
34 however, assessing connectivity is difficult and usually requires indirect genetic approaches.
35 Improved detection of fine-scale genetic connectivity is now possible based on genome-wide
36 screening for genetic differentiation.

37 Here, we explored population connectivity in the hydrothermal vent snail *Ifremeria nautilei*
38 across its species range encompassing five distinct back-arc basins in the Southwest Pacific.
39 The global analysis, based on 10 570 single nucleotide polymorphism (SNP) markers derived
40 from double digest restriction-site associated DNA sequencing (ddRAD-seq), depicted two
41 semi-isolated and homogeneous genetic clusters. Demo-genetic modeling suggests that these
42 two groups began to diverge about 70 000 generations ago, but continue to exhibit weak and
43 slightly asymmetrical gene flow. Furthermore, a careful analysis of outlier loci showed subtle
44 limitations to connectivity between neighboring basins within both groups. This finding
45 indicates that migration is not strong enough to totally counterbalance drift or local selection,
46 hence questioning the potential for demographic resilience at this latter geographical scale.
47 These results illustrate the potential of large genomic datasets to understand fine-scale
48 connectivity patterns in hydrothermal vents and the deep sea.

49

50

Introduction

51

52 Understanding the connectivity of populations is a central issue for evolutionary ecology,
53 conservation and management (Cayuela et al., 2018). Direct approaches such as population
54 monitoring or mark-recapture experiments are rarely applicable in marine environments,
55 because many marine species have large population sizes and high dispersal capabilities due to
56 their minute pelagic propagules. These characteristics are likely to reduce the ability of
57 population genetics to assess population connectivity at local and regional scales, except in
58 situations where there is sufficient genetic differentiation or where a large fraction of the
59 population can be sampled (Jones et al., 2005; Pinsky et al., 2010). Deep-sea hydrothermal
60 ecosystems have attracted much attention since their discovery in the late 1970s (Dover et al.,
61 2001; Tunnicliffe et al., 1998) due to the oasis-like distribution of these unique and chaotic
62 habitats harboring rich and endemic fauna. Hydrothermal environments are mostly found in
63 tectonically active areas, such as mid-oceanic ridges, where neighboring vents are often
64 separated by tens of meters to hundreds of kilometers, resulting in an almost linear, but
65 fragmented and unstable distribution of vent communities (Chevaldonné et al., 1997;
66 Hannington et al., 2011; Jollivet et al., 1999; Vrijenhoek, 2010).

67 Studies of slow (*e.g.* Mid-Atlantic Ridge) and fast (*e.g.* East Pacific Rise) spreading ridges have
68 shown that number of species are able to maintain gene flow over thousands of kilometers
69 (Breusing et al., 2016; Craddock et al., 1995; Hurtado et al., 2004; Jollivet et al., 1995; Teixeira
70 et al., 2011, 2012; Won et al., 2003; Yahagi et al., 2019). Many vent invertebrates possess long-
71 range planktonic larvae that can rapidly (re)colonize newly formed sites (Mullineaux et al.,
72 2010). This high larval dispersal capacity leads to local colonization processes following a
73 stepping-stone mechanism of exchanges, or to the formation of more complex metapopulation

74 dynamics where local extinctions and migration may vary greatly according to the geotectonic
75 context of the venting sites (Audzijonyte & Vrijenhoek, 2010; Jollivet et al., 1999; Vrijenhoek,
76 1997, 2010). Population connectivity can, however, be severely hampered by physical barriers
77 to larval dispersal such as transform faults, diverging ocean currents or microplates (Johnson et
78 al., 2006; Plouviez et al., 2009; Plouviez, Schultz, et al., 2013).

79 The hydrothermal ecosystems found in the Southwest Pacific are mainly associated with the
80 formation of back-arc basins (BABs). BABs result from complex subduction processes between
81 several plates, leading to a discontinuous and nonlinear distribution of venting sites. Hence, the
82 question arises as to the degree of connectivity between populations inhabiting these BABs,
83 noticeably to address the issue of their resilience with respect to deep-sea mining projects
84 (Gena, 2013; Niner et al., 2018). In this context, only a few studies to date have focused on
85 understanding the general patterns of spatial genetic connectivity in ecologically vulnerable
86 hydrothermal species. For instance, Thaler et al. (2011) showed that the gastropod *Ifremeria*
87 *nautiliei* is genetically differentiated between the Manus and North-Fiji/Lau basins. Similar
88 results have been reported in other species, such as the limpet *Lepetodrilus schrolli* (Plouviez
89 et al., 2019), the shrimp *Rimicaris variabilis* and the squat lobster *Munidopsis lauensis* (Thaler
90 et al., 2014). Moreover, this latter species is characterized by additional intra-basin structuring.
91 In contrast, the limpet *Shinkailepas tollmani* does not show any differentiation at any of these
92 scales (Yahagi et al., 2020). However, due to the use of only a limited number of markers, none
93 of these studies have reached the resolution necessary for the fine-scale assessment of
94 connectivity in these species.

95 During the last decade, the development of next-generation sequencing (NGS) and associated
96 techniques have increased the quantity and accessibility of population genomic data,
97 particularly in non-model species. Analyzing these large datasets with thousands of markers

98 along the entire genome using demo-genetic inference methods helps reveal the complex
99 demographic histories of species (Excoffier et al., 2013; Feutry et al., 2020; Gutenkunst et al.,
100 2009; Rougeux et al., 2017; Tine et al., 2014). NGS datasets also give access to unprecedented
101 statistical power to detect non-neutral genetic variation (outlier loci) that can potentially provide
102 finer scale spatial information on connectivity, dispersal and possibly local adaptation
103 (Gagnaire et al., 2015; Milano et al., 2014; Wyngaarden et al., 2017). This information can
104 potentially help distinguish situations of genetic connectivity—whereby local populations are
105 demographically independent (*i.e.* mainly replenished by local propagules) but long-range gene
106 flow mediated by a small number of propagules is sufficient to ensure the circulation of genetic
107 variation among them—from situations of demographic connectivity where a substantial
108 fraction of local population size is made up of immigrants (Lowe & Allendorf, 2010). The
109 consequences of this difference in population connectivity in terms of resilience to local
110 extinction or habitat destruction are quite obvious, with prompt recolonization being expected
111 only in the second situation.

112 The aim of the present study was to elucidate fine-scale population structure and connectivity
113 using high-throughput double-digest restriction-site associated DNA (ddRAD) sequencing on
114 *Ifremeria nautilei*, whose known distribution covers the Southwest Pacific, from the Manus
115 BAB in Papua New Guinea to the Lau BAB off the Tonga Islands. This hydrothermal gastropod
116 of family Provannidae harbors chemoautotrophic symbiotic bacteria in its gills to produce
117 organic matter and forms dense aggregations around diffuse fluid venting at temperatures lower
118 than 15°C (Borowski et al., 2002; Windoffer & Giere, 1997). The species is gonochoric with a
119 nearly equal sex ratio and females brood their progeny in a metapodial pouch until the
120 lecithotrophic embryos (several thousands of similar size) reach a specific and unique pre-
121 veliger stage known as Warèn's larva (Reynolds et al., 2010; Warèn & Bouchet, 1993) . The

122 gastropod reproduces via internal fertilization leading to a patchwork of brooding and
123 nonbrooding females throughout the year due to asynchronous spawning. Brooding is a
124 reproductive trait that usually limits the dispersal ability of species; however, the lifespan of *I.*
125 *nautiliei* pelagic larvae is not known, nor is the maximum distance veligers can travel prior to
126 settlement on a new venting site. Constituting a large portion of the biomass and harboring
127 other species such as *S. tollmanni* and *L. schrolli*, *I. nautiliei* is a keystone species important for
128 these deep-sea ecosystems. Furthermore, it is classified as endangered by the IUCN
129 (<https://www.iucnredlist.org/species/145380421/145380604>) along with some other
130 hydrothermal species. Therefore, studying the connectivity patterns of *I. nautiliei* is a flagship
131 endeavor to assess the potential impact of deep-sea mining on this keystone species and its
132 associated fauna.

133 For this study, extensive sampling was carried out on 29 sites in 17 hydrothermal vent fields
134 across five basins distributed over 5000 km in the Southwest Pacific.

135

136 **Materials and Methods**

137 *Sample collection and DNA extraction*

138 A hierarchical sampling plan was deployed on board the French oceanographic vessel RV
139 *L'Atalante* during the ChubacArc 2019 oceanographic cruise using the remotely operated
140 underwater vehicle (ROV) *Victor 6000*. A total of 684 individuals were collected in the
141 Southwestern Pacific Ocean from 29 sampling sites distributed among 17 vent localities or
142 hydrothermal fields across four BABs and one volcanic submarine area (Futuna), spanning the
143 entire known geographical distribution range of *I. nautiliei* (Figure 1, SI Table S1). This

144 sampling includes samples from the newly discovered active site La Scala in the Woodlark
145 basin (Boulart et al., in press)

146 In addition, 27 individuals were added from collections of previous oceanographic cruises, with
147 22 samples obtained by S. Hourdez during the Lau basin 2009 oceanographic cruise with few
148 at the now-extinct Kilo Moana site and 5 samples obtained during the Manus basin 2009 cruise
149 kindly provided by C. L. Van Dover (SI Table 1). Altogether, a total of 456 unique samples
150 were used and analyzed in this study, of which 362 remained after filtering the sequence dataset
151 (SI Table 2).

152 Once on board, the snails were dissected and various tissues were preserved in EtOH or frozen
153 at -80°C. Genomic DNA was extracted from fresh foot tissue to limit DNA contamination by
154 symbionts hosted in the gills. A fraction of the tissue samples was stored in 90% EtOH for
155 backup. Samples from C. L. Van Dover's collection were preserved in 90% EtOH, and those
156 from S. Hourdez were kept frozen at -80°C. Genomic DNA was extracted using the NucleoSpin
157 Tissue kit according to the manufacturer's protocol (Macherey-Nagel); some samples were
158 extracted using a standard phenol-chloroform method.

159 *Preparation and sequencing of ddRAD libraries.*

160 DNA extracts were visualized on 0.8% agarose gels and each concentration was standardized
161 to between 10 and 50 ng.µl⁻¹ after a fluorometric quantification with the QuantiFluor dsDNA
162 system (Promega). Individual double-digest restriction-site associated DNA (ddRAD) *Pst*I-
163 *Mse*I libraries were prepared following (Brelford et al., 2016) after modifications detailed in
164 (Thiébaud et al., 2021). Five pooled libraries were prepared with a combination of four to eight
165 Illumina indexes and 24 barcodes per index, multiplexing a total of 486 samples, including 27
166 pairs of replicates for quality control, representing 456 individuals. The sequencing effort was

167 sized to produce on average 3×10^6 read pairs per individual. Each genomic pool was sequenced
168 on one lane of a HiSeq4000 Illumina sequencer (paired end, 150 bp) at the Genoscope
169 sequencing facility (Centre National de Séquençage, Evry, France).

170 *De novo ddRAD-tag assembly, SNP calling and filtering*

171 Fastqc (V.0.11.9) was used only for quality control of raw reads, no filters were applied on
172 them. Individual reads were demultiplexed using the “Processradtag” pipeline in Stacks
173 (V.2.52) (Rochette et al., 2019). Due to the lack of a reference genome for *I. nautiliei*, reads
174 were assembled *de novo* using each Stacks module one by one (ustacks, cstacks, sstacks,
175 tsv2bam, gstacks and populations). To identify the most appropriate assembly parameters, we
176 followed previously published recommendations (Mastretta-Yanes et al., 2015; Paris et al.,
177 2017) (See SI and SI Figures 1-5 for details). Briefly, we used the genotyping error rate between
178 replicates, the number of variants (SNP), polymorphic loci (ddRAD-tags) and nucleotide
179 diversity (π estimated in Stacks) as a function of the assembly parameters (m and $M = n$)
180 determined with a subset of individuals covering all basins and localities ($n = 84$). The selected
181 parameters were as follows: $m = 4$ (the minimum number of reads to assemble a stack), $M = 8$
182 (the maximum number of mismatches between putative alleles within individuals), $n = 8$ (the
183 maximum number of mismatches between putative loci within the catalog of individuals) and
184 $R = 0.8$ (the minimum percentage of individuals sharing a locus across all populations in the
185 “populations” module).

186 After *de novo* assembly, several filters were applied using VCFtools (V.0.1.16) (Danecek et al.,
187 2011) to reduce missing data and to account for potential paralogs (see SI Table 3). Briefly, we
188 removed 1 of each of the 27 replicated individuals with the highest value of missing data. Then,
189 we excluded SNPs with heterozygosity > 0.6 , SNP and only individuals with less than 10%

190 missing data were kept. Variants with a mean coverage higher than 80X were excluded. Using
191 VCFtools, we excluded loci with a minor allele frequency (MAF) lower than 5% (alternative
192 allele), followed by those that deviated significantly from Hardy-Weinberg equilibrium (p -
193 value ≤ 0.05). We then kept only one randomly chosen SNP per ddRAD-tag to avoid short
194 distance linkage disequilibrium between SNPs. PGDSpider (V.2.1.1.5) (Lischer & Excoffier,
195 2012) was used to convert the final VCF into the formats required for subsequent analyses.

196 *Population structure and diversity*

197 Principal component analysis (PCA) was first performed on the final dataset with the R package
198 SNPrelate (V.1.24.0) (Zheng et al., 2012) . Pairwise fixation indices (F_{ST}) were calculated in
199 Arlequin (V.3.5.2.2) (Excoffier & Lischer, 2010). AMOVA (Excoffier et al., 1992) was
200 performed with 10 000 permutations of genotypes between populations by considering
201 hierarchical geographic structure of localities within basins (See SI for parameters). Co-
202 ancestry analyses were performed through ADMIXTURE (V.1.3.0) (Alexander & Lange,
203 2011) with 10 independent runs for $K = 1$ to 5. The best K value was selected by using the cross-
204 validation error as recommended by the authors. Runs of ADMIXTURE were grouped using
205 CLUMPAK (Kopelman et al., 2015), graphical visualizations of the results were plotted using
206 library ggplot2 (V.3.3.3) in R (V.4.0.1). TreeMix (V.1.13) (Pickrell & Pritchard, 2012) was
207 performed with 10 independent runs with migration events ranging from 0 to 5. The optimal
208 number was selected according to the log-likelihood of each model. F_3 admixture tests (Reich
209 et al., 2009) were done using the THREEPOP programs implemented in TreeMix (V.1.13)
210 package with default values.

211 To detect potential kinship, SNPrelate was used to compute identity-by-state between pairs of
212 individuals. We used this approach to (1) minimize the risk of labeling error/exchange during

213 the process of library construction and sequencing and (2) infer the level of kinship structure
214 between non-replicated individuals because the existence of undetected underlying kinship
215 structure can distort the population structure estimated by the gene genealogies.

216 Observed heterozygosity (H_o), expected heterozygosity (H_e), heterozygote deficiency (F_{IS}),
217 nucleotide diversity (π) and raw nucleotide divergence (D_{xy}) were calculated with the
218 population module of Stacks using all sites from all ddRAD-tags in the final dataset.

219

220 *Demo-genetic history of the species*

221 The demographic history of the species targeting past and present gene flow between
222 metapopulation clusters was inferred using a modified version of $\partial a \partial i$ (V.2.1) (Diffusion
223 Approximations for Demographic Inference; Gutenkunst et al., 2009), with a dual annealing
224 optimization function. This software simulates the joint allele frequency spectrum (JAFS) of
225 two (or more) interacting populations according to different demo-genetic scenarios. Here, we
226 considered 28 distinct scenarios built according to the population models used in Rougeux et
227 al. (2017) with very few modifications, detailed below.

228 Basically, all these models derive from four basic models representing strict isolation (SI),
229 isolation with migration (IM), ancient migration (AM), and secondary contact (SC). Each of
230 them consists of an ancestral population of N_{anc} size that splits into two sister populations of
231 effective size N_1 and N_2 during time T_s for the (SI) and T_{sm} for the (IM) model, $T_{am}+T_s$ for
232 the (AM) model and T_s+T_{sc} for the (SC) model, where T_s is the time spent since the split of
233 the two populations without migration, T_{sm} , the time spent since the split of the two populations
234 with migration, T_{am} , the duration of the ancient migration period after the split of the ancestral
235 population and before the emergence of strict isolation (T_s) and T_{sc} the duration of a secondary

236 contact after a period T_s of strict isolation. Directional migration between populations is
237 allowed at rates m_{12} and m_{21} from population 2 to population 1 and vice versa.

238 Further complexity was introduced as in Rougeux et al. (2017), by adding several processes
239 occurring after the split, such as population expansion or contraction (G), the effect of linked
240 selection reducing the effective population size of loci over a certain fraction of the genome
241 ($2N$) and the effect of semipermeable genetic barriers (*i.e.* partial reproductive isolation)
242 reducing the effective migration rate of loci over a certain fraction of the genome ($2m$).
243 Furthermore, to dissociate the effect of the effective population size (genetic drift) and
244 migration (gene flow), we only allowed the growth parameters (G) to vary during the migration
245 phase of each model. Graphical representation of the four basic models and the three more
246 complex models are displayed in SI Figure 7.

247 For the input dataset, we considered the two metapopulations defined by the global analyses
248 (see Results), which corresponded to Manus/Woodlark and North-Fiji/Futuna/Lau. We used
249 the folded joint allele frequency spectrum (folded JAFS), because no external group was
250 available to identify the allelic ancestral states. All models were fitted independently of the
251 dataset using dual-annealing optimization and run 10 times independently each to check
252 convergence. Model comparisons were based on the Akaike information criterion (AIC). Using
253 the best selected models, we then converted demographic parameters into biological units. In
254 the absence of precise information on mutation rate and generation time for this species, we
255 used 10^{-8} as the mutation rate per site per generation. This widely used value falls within the
256 range proposed by (Lynch, 2010), although admittedly the real value may be much larger or
257 much smaller, as recalled in the Discussion. Parameters estimated using $\partial a \partial i$ are scaled from
258 the ancestral effective population size (N_{anc}), which was estimated using the following formula:

259
$$N_{\text{anc}} = \frac{\theta}{(4 \times \mu \times L)}$$

260 where L represents the total length of the DNA sequence used in $\partial\text{a}\partial\text{i}$:

261
$$L = \frac{z \times y \times 275}{x}$$

262 where z represents the number of SNPs used, y the number of RAD-tags of 275 bp, and x the
263 initial number of SNPs ($z = 17\,365$, $y = 17\,365$ and $x = 250\,502$, $L = 331\,032$).

264 Estimated times were calculated in units of $2 \times N_{\text{anc}}$ generations and the migration parameters
265 (m_{12} and m_{21}) were divided by $2 \times N_{\text{anc}}$ to obtain the number of migrants in each population per
266 generation. The standard deviations were estimated using the Fisher information matrix (FIM)
267 method implemented in $\partial\text{a}\partial\text{i}$.

268 *Outlier loci and detection of fine-scale structure*

269 To test whether fine-scale genetic structure exists within each genetic cluster defined as a result
270 of the global analyses described in the preceding paragraph (see Results for details), we used
271 several genome-scanning methods to identify candidate outlier SNPs (*i.e.* loci showing higher
272 or lower levels of differentiation than expected under assumed neutrality). Such loci may be
273 informative about fine-scale population structure and connectivity patterns (Gagnaire et al.,
274 2015). Four different outlier detection approaches were used. The rationale behind this multiple
275 testing is that these methods operate with somewhat different underlying assumptions or test
276 statistics and are known to have varying discriminatory power depending on the situations to
277 which they are applied (Villemereuil et al., 2014). Outlier loci were selected according to
278 statistical thresholds (p -value ≤ 0.05 and 0.01) in each software package, while checking that
279 candidate loci outnumbered the number of loci expected to fall outside the distribution by
280 chance only (false positives). Furthermore, to focus on the relevant scale and avoid the detection

281 of false positives due strong geographic structuring, these programs were run independently on
282 each Manus/Woodlark and North-Fiji/Futuna/Lau metapopulation previously defined in the
283 global analyses, while considering populations either by basin or by locality within these
284 groups.

285 Four methods were used. (1) BayeScan (V.2.0) (Foll & Gaggiotti, 2008) detects potential outlier
286 loci by using differences in allele frequencies under a simple island model in a Bayesian
287 framework. Five independent runs were performed with the default settings. (2) PCAdapt
288 (V.4.3.3) (Luu et al., 2017) uses the correlation of SNPs with the first principal components of
289 the PCA to detect outliers by computing a Mahalanobis distance between their z-score on each
290 PC. (3) Arlequin (V.3.5.2.2) (Excoffier & Lischer, 2010) detects outlier SNPs under a non-
291 hierarchical finite island model integrating F_{ST} and heterozygosity through 20 000 coalescence
292 simulations of the neutral distribution with 100 demes each. (4) The core model of Baypass
293 (V.2.1) (Gautier, 2015) based on a hierarchical Bayesian model in which loci that are more
294 differentiated than expected under a non-equilibrium drift model are identified through the
295 distribution of a statistic similar to F_{ST} corrected to account for demographic history. Baypass
296 was run five times independently with default settings under the core model.

297 PCA, ADMIXTURE and F_3 tests were then performed on the various outlier subsets to explore
298 the information they convey.

299 In addition, outlier loci identified at the threshold ($p \leq 0.05$) were first blasted (BLASTN, E-
300 value threshold: 10^{-5}) against the *Alviniconcha boucheti* transcriptome, which was previously
301 assembled using rnaSPAdes (V.3.13.1) (Bankevich et al., 2012) (cf. Castel et al., in prep).
302 Transcript hits with a size greater than 300 bp were subsequently blasted (BLASTX, E-value

303 threshold: 10^{-5}) against the NCBI UniProtKB/Swiss-Prot database using the software Geneious
304 Prime® 2021.2.2.

305

306

Results

307 *De novo assembly and data filtering*

308 *De novo* assembly resulted in a dataset of 38 608 ddRAD-tags with a mean coverage of 14X
309 for 486 samples. The mean genotyping error rate was 0.48% and the maximum value was 1.06%
310 from all pairs of replicates. These ddRAD-tags contained 649 106 SNPs. Following SNP
311 filtering, the final dataset resulted in a VCF file containing 362 individuals with 10 570 unlinked
312 bi-allelic variants with an individual mean coverage of 17.7X and a maximum of 10% of
313 missing data per individual and variant.

314 *Population structure analyses considering the global dataset*

315 A PCA was performed to explore the level of population structure over the five western Pacific
316 BABs (Figure 2, A). This analysis showed a very clear geographical separation with two distinct
317 clusters, one corresponding to the Manus and Woodlark basins and the other to the North-Fiji,
318 Futuna and Lau basins. The first component (PC1) explained 26.03% of the total variance; the
319 second one carried only 0.03% of the total variance (Figure 2 and SI Figure 8). This pattern was
320 consistent with the AMOVA results (Table 1), which also showed a strong and significant
321 genetic differentiation between Manus/Woodlark and North-Fiji/Futuna/Lau, but no
322 differentiation among basins and localities within these two groups (between Manus/Woodlark
323 and North-Fiji/Futuna/Lau, $F_{ST} = 0.387$, p -value < 0.001 , inter-basins within Manus/Woodlark
324 and North-Fiji/Futuna/Lau, $F_{CT} = -0.050$, NS). In addition, the between-basins pairwise F_{ST}

325 (Table 2) were only significant between Manus/Woodlark and North-Fiji/Futuna/Lau pairs. No
326 significant pairwise F_{ST} values were observed between localities within either of the two groups
327 Manus/Woodlark and North-Fiji/Futuna/Lau (SI Table 3).

328 This finding is also consistent with the ADMIXTURE (Figure 2 B & SI Figures 9–10)
329 clustering results and strongly supports the same two distinct clusters ($K = 2$) with very few
330 individuals showing very low percentages of mixed ancestry (from 0.1% to 3%). The identity-
331 by-state distribution (SI Figure 11) did not show evidence of any internal structure due to
332 kinship.

333 TreeMix analyses showed an optimal number of two migration events, whereas additional
334 events did not improve the likelihood (SI Figure 12 A). Displaying the first migration event
335 showed a very low migration weight from Manus/Woodlark towards North-Fiji (SI Figure 12
336 B), and adding the second migration event indicated a very slight differentiation between
337 Woodlark and Manus (SI Figure 12 C). The F_3 statistics showed a significant admixture signal,
338 with source populations from each genetic cluster only when North-Fiji was chosen as the focal
339 population (SI Figure 13).

340 The estimated genetic diversity of the populations considering all DNA positions of the 10 570
341 ddRAD-tags was bimodal, with slightly higher nucleotide diversity in Manus/Woodlark ($\pi =$
342 0.00572) compared with North-Fiji/Futuna/Lau ($\pi = 0.00535$), regardless of the populations
343 being considered by geographic basin or by genetic cluster (SI Figure 14). The raw nucleotide
344 divergence (D_{xy}) between the two genetic clusters was estimated to be 0.0136.

345 Hence, the analyses of the complete SNP dataset of *I. nautiliei* indicate the co-occurrence of two
346 quasi-panmictic metapopulations, one associated with the Manus/Woodlark basins and the
347 other with the North-Fiji/Futuna/Lau basins, on either side of the Solomon Islands/New

348 Hebrides arc. Thus, these two metapopulations are both sufficiently homogeneous and
349 differentiated from each other to be analyzed using $\partial a \partial i$ demo-genetic inference, which aims at
350 summarizing the global genome-wide history of divergence/contact between them over a long
351 period of time.

352 *Inference of demographic history and gene flow*

353 *1. Model comparisons*

354 The folded JAFS in Figure 3 (A) shows how allele frequencies are shared between the
355 Manus/Woodlark and North-Fiji/Futuna/Lau metapopulations. The $\partial a \partial i$ framework can fit
356 population models on the observed dataset and compares them based on their AIC values
357 (Figure 4).

358 Among the four simplest models (SI, AM, IM and SC), SC was significantly the best fitting
359 model. Increasing complexity by adding the parameters G, 2m, 2N independently improved the
360 AIC values regardless of the basic model used. However, capturing the effect of linked selection
361 (2N) explained the data much better than models with population growth (G) and heterogeneous
362 gene flow (2m) only (Figure 4).

363 Conversely, the combination of these parameters led to only a slight improvement in the AIC
364 values. Nevertheless, models including the effect of linked selection (2N) were still better than
365 the other models (Figure 4).

366 Hence, considering all models together, the best ones were those that took all parameters (2N,
367 2m, G) into consideration, followed by models with only 2N+G (Figure 4). Moreover, for the
368 2N+2m and 2N+2m+G models, the proportion 1-P of the genome that evolves under restricted
369 migration in 2m models amounted to 0.52–0.56 for the best AIC simulation among all runs,
370 meaning that the proportion of the genome that evolves under a reduced effective migration

371 rate (barrier loci) may be quite substantial. With the increasing number of population
372 parameters, the secondary contact hypothesis was no longer the best evolutionary scenario
373 explaining our genetic dataset: the models IM+2N+2m+G, SC+2N+2m+G, AM+2N+2m+G
374 and AM+2N+G, IM+2N+G, SC+2N+G showed very similar AICs ($\Delta_{AIC} \leq 10$, Figure 4).

375 *2. Inferences of model parameter values*

376 According to the best models based on AIC, the two metapopulations may have diverged due
377 to early (AM), late (SC) or constant (IM) gene flow and it is difficult to distinguish among these
378 three possibilities. However, these models have some interesting features in common. First,
379 although the standard deviations (SDs) are rather large, the effective population sizes of the two
380 derived populations estimated since the split (N_1 & N_2) indicate a demographic expansion (b_1
381 and $b_2 > 1$), regardless of the model, including a temporal change in population size (G).
382 Second, the local effect of selection at linked sites seems to affect a very large proportion of
383 loci ($Q = 0.99$) with a small value of *hrf* (Hill-Robertson factor = ~ 0.02) (Table 3). Third, the
384 number of contemporary migrants (estimated by $(N_1 * b_1 * m_{12})/2$ and $(N_2 * b_2 * m_{21})/2$) shows
385 asymmetrical, but weak flow between the two metapopulations, slightly higher from North-
386 Fiji/Futuna/Lau to Manus/Woodlark (4.2–4.6) than in the opposite direction (2.9–3.2). Fourth,
387 nearly half of loci show a restricted migration rate ($0.52 \leq 1-P \leq 0.56$). Fifth, the total estimated
388 divergence time expressed as the number of generations are very similar, regardless of the
389 model (Tsm, Tam+Ts, Ts+Tsc) and estimated to be between 66 951 and 70 295 generations.

390 *Outlier loci and detection of fine-scale structure*

391 Despite the absence of geographic structuring within each metapopulation depicted in the
392 global analysis, several outlier loci were identified in each metapopulation at the thresholds of
393 $p \leq 0.05$ and $p \leq 0.01$ (SI Table 4).

394 BayeScan identified much fewer candidate outliers than expected by chance only, and hence
395 was not considered further. PCAdapt was largely out of its working range because it searches
396 for loci that exceed the possible differentiation level captured by the very first principal
397 component as opposed to the second-order axes. However, all axes, except axis 1 which
398 separates the two geographic metapopulations, belong to this second category due to the lack
399 of internal structure. Hence, all the second-order axes primarily captured noise, and they were
400 unable to reveal additional structuring (SI Figure 15,16). Therefore, Baypass and Arlequin were
401 the only two methods considered further. To increase the probability of considering true
402 positives only, we only kept loci identified in both approaches (predicted by the intersection
403 depicted in the Venn diagram in Figure 5 at the thresholds $p \leq 0.05$ and $p \leq 0.01$ in SI Figure
404 17). Only 458 and 223 outlier loci were shared between the methods Baypass and Arlequin in
405 Manus/Woodlark and North-Fiji/Futuna/Lau at the threshold of $p \leq 0.05$ respectively.

406 PCA based on these different sets of outlier loci helped to visualize their contribution to the
407 internal heterogeneity of each metapopulations (Figure 5 and SI Figure 18). Interestingly,
408 although outliers were defined within each metapopulation, the inter-metapopulation
409 differentiation was retrieved in all cases. Nevertheless, a clear signal of differentiation was
410 highlighted within both regions. Individuals from the Manus and Woodlark basins showed clear
411 genetic differentiation with no overlap on PC2 (Figure 6 A). Individuals from North-Fiji were
412 slightly pulled towards Manus/Woodlark individuals based on PC1 (Figure 6 B), but they were
413 also shifted on PC2 when outliers were considered at the threshold of $p \leq 0.05$.

414 The ADMIXTURE analyses based on the outlier SNPs datasets with the threshold of $p \leq 0.05$
415 displayed optimal K values at $K=2$. With North-Fiji/Futuna/Lau outliers, North-Fiji displayed
416 an admixture proportion from Manus/Woodlark ranging between 5 and 15% (Figure 6 C). For
417 Manus/Woodlark outliers, we also found an admixture proportion from North-Fiji/Futuna/Lau

418 in Woodlark (Figure 6 D). However, very similar values of cross-validation errors were
419 obtained with $K=3$ (SI Figure 19,20,21), North-Fiji and Woodlark each being individualized as
420 the third cluster in their respective runs.

421 The F_3 statistics calculated based on outliers only did not provide any additional information
422 (SI Figure 22).

423 When blasted onto the *A. boucheti* transcriptome, 30% of outlier loci identified at the threshold
424 of $p \leq 0.05$ matched with the coding sequences of transcribed regions. This number was greater
425 than expected by chance from randomly picked ddRAD loci along the *I. nautili* genome. Half
426 of these 30% of outlier loci (129 transcripts) had annotations on the protein database. Among
427 these annotations, many involved genes that encode for DNA/RNA replication and repair
428 enzymes, transmembrane carriers and synapse/microtubule biosynthesis, but also genes
429 involved in the exocytosis/endocytosis regulation, and more especially the GTPase regulation
430 pathway (SI Table 7). In addition, two genes involved in spermatogenesis were also detected.

431

432

433

434

Discussion

435 Previous work by Thaler et al. (2011) using microsatellites and mitochondrial *cox1* sequences
436 demonstrated that the southwestern Pacific deep-sea hydrothermal vent gastropod *Ifremeria*.
437 *nautili* is genetically structured into two distinct populations from Manus and North-Fh-
438 Fiji/Lau BABs. Our study extends these previous results to a finer scale, owing to our larger,
439 nested sampling design that includes the newly discovered La Scala vent field in the Woodlark

440 basin (Boulart et al., in press), the Futuna volcanic arc (Konn et al., 2016) and the newly
441 discovered northernmost Mangatolo site at the entrance of the Lau basin. Using a 10 570 SNP
442 genome-wide dataset—unprecedented for a hydrothermal species—, we confirm that *I. nautili*
443 is structured into two loosely connected metapopulations corresponding to two BAB
444 ensembles. These ensembles display an almost complete internal genetic homogeneity;
445 however, our analyses of outlier loci nevertheless revealed fine-scale differentiation among
446 basins within each metapopulation. We discuss below the possible implications of these results
447 in terms of larval dispersal and demographic connectivity and ultimately their consequences on
448 the resilience of hydrothermal communities.

449 *Long-term gene flow and history of differentiation*

450 One metapopulation comprises the Manus and Woodlark basins (*i.e.* the Manus/Woodlark
451 BAB) west of the Salomon/New Hebrides arc, whereas the other extends east of it with the
452 North-Fiji basin, Futuna volcanic arc and Lau basin (*i.e.* the North-Fiji/Futuna/Lau BAB). The
453 genetic divergence between the two metapopulations is relatively strong (average $F_{ST} = 0.387$,
454 $p \leq 0.001$, $D_{xy} = 0.0136$), but each of these two ensembles appears to be panmictic ($F_{CS} = -0.05$,
455 NS, SI Table 3). The demo-genetic inferences gleaned from $\partial a \partial i$ suggest that the two
456 metapopulations diverged with only a brief period of isolation (T_s was found to vary between
457 0.001 and 0.443 in the AM and SC models), although the existence of constant gene flow (IM)
458 could not be formally excluded. The incorporation of several other demographic parameters
459 ($2N$, $2m$, G) produced a clear improvement in model fit. Considering each parameter
460 independently, the effect of linked selection ($2N$) had a much greater influence on AIC than the
461 other two parameters ($2m$ and G), suggesting that a non-negligible proportion of loci may be
462 influenced by linked selection. For the best models ($2N+2m+G$), this proportion approaches Q
463 $= 0.99$ (which seems to be unrealistic), whereas only half of the markers appear to be under the

464 influence of heterogeneous migration ($0.52 \leq 1-P \leq 0.56$). Nevertheless, these models are very
465 close to the $2N+G$ models ($\Delta_{AIC} \leq 10$), which estimate a proportion of loci under linked selection
466 ($0.56 \leq Q \leq 0.58$) and do not take into account the effect of heterogeneous migration.
467 Disentangling these two effects is thus difficult and suggests that there are many genomic
468 regions, possibly with lower recombination rates, where background selection and possibly
469 selective sweeps have accelerated the rate of lineage sorting during divergence (Rougeux et al.,
470 2017). This strong bimodality between two classes of loci affected or not by linked selection is
471 also captured by the distribution of F_{ST} , which shows a clear trough and then a peak around
472 0.15–0.2 (SI Figure 23). However, this bimodality reduces the ability to distinguish between
473 the isolation-with-migration, the secondary contact, or the ancient migration scenarios in the
474 more complex models (IM+2N+2m+G, AM+2N+2m+G and SC+2N+2m+G).
475 Considering an average DNA mutation rate of 10^{-8} /site/generation, we estimated the time for
476 the onset of divergence between the two metapopulations to be 60 000–70 000 generations (but
477 admittedly this could as well be 10 times greater if the mutation rate is 10 times smaller). The
478 generation time of *I. nautiliei* is still unknown. Nevertheless, most hydrothermal species display
479 an *r*-strategy suggesting short generation times (1-2 years) as an adaptation to the unstable and
480 ephemeral nature of their habitat (Tyler & Young, 1999). Hence, we can suppose that the two
481 populations started to diverge between 60 and 140 thousand ago (*kya*) for a mutation rate per
482 site and per generation of 10^{-8} and 10 times more with a mutation rate of 10^{-9} . However, these
483 estimates correspond to discrete non-overlapping generations and the reproduction of older
484 cohorts may increase the equivalent generation time and, as a result, the divergence estimates.
485 These values may be tentatively compared with estimates from the *coxI* sequences in Boulart
486 et al. (in press) (net divergence 0.615% estimated on all sites). This latter value would amount
487 to ~0.550 million years ago (*mya*) considering the widely used divergence rate of 1.4%/million

488 years (*myr*) for mitochondrial DNA (Knowlton & Weigt, 1998), but can reach 1.2 *mya* ,
489 depending on the average mitochondrial substitution rate considered for vent species (0.2-
490 0.3%/myr (Chevaldonné et al., 2002; Breusing et al., 2020; Castel et al. in prep.) . Although
491 these estimates are notoriously highly variable and error-prone (see for instance Breusing et al.,
492 2020), divergence time could range between 0.5 and 1 *mya*. This estimate is in rough agreement
493 with Martinez & Taylor, (1996) who showed that the center of the Manus BAB started to spread
494 quite recently (~ 0.78 *mya*), suggesting that hydrothermal vents within the spreading center may
495 be younger than this estimate. Thus, it cannot be excluded that the divergence history of *I.*
496 *nautili* is relatively recent and not linked to the formation of BABs, but instead to regional
497 modifications of surface and deep-sea currents during previous glacial maxima in relation to
498 the extension of the Antarctic ice sheet which culminated around 0.126 *mya* (Barrows et al.,
499 2011; Joy et al., 2014).

500 In addition to these divergence time estimates, the models allowed us to quantify the existence
501 of an ongoing bidirectional and asymmetrical gene flow, with migration from North-
502 Fiji/Futuna/Lau to Manus/Woodlark being higher than in the opposite direction. Despite this
503 slight asymmetry, a genetic influence of the Manus/Woodlark metapopulation was detected in
504 North-Fiji, which shows foreign alleles coming from the former rather than from the
505 Lau/Futuna populations (also observed at mtDNA *coxI* gene in Thaler et al., 2011 and Boulart
506 et al., (in press), but not the other way around (*i.e.* influence of North-Fiji/Futuna/Lau on
507 Woodlark, but see below). This result is consistent with the geography of the region, because
508 North-Fiji and Woodlark are the closest BABs between the two metapopulations. Connectivity
509 through larval dispersal between these two BAB ensembles has been tested by Mitarai et al.
510 (2016) who simulated larval dispersal through entrainment of particles by oceanic currents
511 prevailing at depths of 1000 m in the western Pacific. That study inferred a weak stepping-stone

512 connection through a long planktonic larval duration (PLD of 170 days), provided that active
513 hydrothermal sites in the Solomon and New Hebrides/Vanuatu arcs act as a relay. Such fields
514 are known to exist, mostly associated with seamounts such as Nifonea, Tinakula or Stanton
515 along the New Hebrides/Vanuatu arc (McConachy et al., 2005; Schmidt et al., 2017). The larval
516 dispersal model developed by Mitarai et al. (2016) suggests a scenario where dispersal is mainly
517 oriented from east to west: a situation also depicted in this region by Yearsley & Sigwart (2011)
518 for a non-hydrothermal species at several depths (800 and 1400 m) and with various PLD
519 lengths (27–151 days). However, when looking at surface countercurrents between
520 Manus/Woodlark and North-Fiji/Futuna/Lau, Ganachaud et al. (2014) found surface currents
521 oriented mainly from west to east through Solomon Islands and Vanuatu waters.

522

523 Cases of asymmetrical bidirectional gene flow between two metapopulations have also been
524 found in two other hydrothermal gastropod species occurring sympatrically with *I. nautiliei*, *L.*
525 *schrolli* (Plouviez et al., 2019) and *A. boucheti* (Breusing et al., 2021). But, in contrast to *I.*
526 *nautiliei*, the predominant gene flow is oriented eastward from Manus to Lau, ($m_{M \rightarrow L} = 0.625$,
527 $m_{L \rightarrow M} = 0.1725$ for *L. schrolli* and $m_{M \rightarrow L} = 12$, $m_{L \rightarrow M} = 2.6$ for *A. boucheti*).

528 Similarly to *I. nautiliei*, *L. schrolli* is considered to possess lecithotrophic larvae (Berg, 1985;
529 Craddock, Lutz, & Vrijenhoek, 1997; Tyler et al., 2008)). As for *A. boucheti*, its larval stage
530 remains unknown, although both its morphology (Warèn & Bouchet, 1993) and the eDNA
531 detection of *Alviniconcha* larvae close to the surface suggest planktonotrophy (Sommer et al.,
532 2017). Provided that our estimates reflect ongoing migration, we hypothesize that *I. nautiliei*
533 larvae may be influenced by deep as well as surface currents, which could explain bidirectional
534 gene flow, one direction being slightly stronger than the other. This asymmetry suggests vertical

535 migration of larvae. However, further investigations including oceanographic modeling and
536 laboratory experiments are needed to address this hypothesis. For example, larvae of the
537 hydrothermal gastropod *Shinkailepas myojinensis* (Yahagi et al., 2017) are able to migrate
538 through the water column, and there is evidence of hydrothermal species' larvae in near-surface
539 waters (Arellano et al., 2014, Sommer et al., 2017). Nevertheless, although many unknowns
540 remain, our results indicate that *I. nautili* has a complex dispersal strategy and pattern.

541 *Fine-scale population structure and connectivity*

542 The high homogeneity of the two clearly distinct *I. nautili* metapopulations necessarily entails
543 that the intra-metapopulation migration (*i.e.* inter-localities within each BAB and inter-BABs
544 within each metapopulation) is strong or extremely recent. Moreover, no kinship-related
545 structure was detected in the SNP dataset, indicating that there is either no self-recruitment even
546 though females brood their larvae to the trochophoran stage, or that population sizes are so large
547 that the probability of detecting potential kin is too small (Table 4). Consequently, genetic
548 connectivity within each metapopulation appears to be high, with evenly distributed
549 polymorphisms among sampled sites despite the patchy distribution of hydrothermal vents and
550 the inter-site distances, which may vary from hundreds of meters to more than a thousand
551 kilometers within each metapopulation. This genetic connectivity therefore suggests that *I.*
552 *nautili* larvae are able to disperse within the range of each metapopulation after spawning.

553 The question is now whether this genetic homogeneity of each metapopulation arises from
554 demographic connectivity (*i.e.* recruitment at one site being strongly influenced by the
555 exportation of propagules from other sites) or is due to sporadic/rare larval exchanges able to
556 counterbalance very attenuated genetic drift due to large local population sizes. The mechanism
557 behind the observed genetic homogeneity has strong implications in terms of conservation

558 biology, because demographic connectivity can play a crucial role in the resilience of
559 populations faced with local extinction potentially exacerbated by deep-sea mining. The global
560 analysis relying on a panel of primarily neutral markers indicates no differentiation at the
561 metapopulation scale, but—as advocated by Gagnaire et al., (2015)— a few loci markers
562 potentially undergoing direct, or indirect selective pressures may locally harbor distinct allele
563 frequency in the recipient population. This pattern can be explained by local selection for
564 foreign alleles that are better adapted or less loaded by deleterious mutations than resident ones,
565 or by resolving intrinsic asymmetrical incompatibilities between divergent genomes (Simon et
566 al., 2021) creating local soft sweeps through linked selection. These processes result in
567 enhanced local introgression of certain marker loci, a common pattern observed in blue mussels
568 (Fraïsse et al., 2016) or European sea bass (Robinet et al., 2020), for example. These markers
569 will appear as F_{ST} outliers that may indicate recent dispersal events.

570 Our outlier analyses indeed suggest introgression of some loci. In Figure 6 B and SI Figure 18
571 B, individuals from the North-Fiji basin seem to be closer to Manus/Woodlark than Lau/Futuna
572 on PC1, which may correspond to introgression in some of the outlier loci. An introgression
573 pattern was confirmed by the F_3 tests performed with the North-Fiji basin as the focal
574 populations (significant negative value of the F_3 statistic, SI Figure 13) and the ADMIXTURE
575 analyses (Figure 6 C). These results indicate that some alleles found at high frequency in North-
576 Fiji individuals are the consequence of long-range migration from Manus/Woodlark.
577 Interestingly, with Manus/Woodlark outliers, although not visually detectable on the PCA
578 (Figure 6 A and 18 A), Woodlark individuals exhibit some level of admixture from the North-
579 Fiji/Futuna/Lau metapopulation (Figure 6 D). This low-level admixture corroborates our
580 inference of ongoing bidirectional gene flow. However, it is not yet clear as to why its impact
581 appears stronger in populations of the North-Fiji basin, against the predominant direction

582 according to our $\partial a \partial i$ inferences. Although we are unable to determine the precise mechanisms
583 behind these frequency changes, these alleles have not diffused from North-Fiji to Lau/Futuna,
584 indicating a subtle—but real—limitation in connectivity between the former and the latter. The
585 same reasoning applies for the traces of admixture detected in Woodlark that appear to have
586 not diffused to Manus.

587 Another kind of differentiation depicted by outlier loci seems to be explained by intra-
588 metapopulation divergence. The question arises as to the origin of these slight divergences on
589 a PC axis orthogonal to the main inter-metapopulation divergence, which does not necessarily
590 proceed from gene flow between differentiated populations as described above. Allele
591 frequency differences for outlier loci between Manus and Woodlark are detectable on PC2
592 (Figure 6 A, SI Figure 18 A, ADMIXTURE $K = 3$, SI Figure 20). The same question applies
593 to the eastern North-Fiji/Futuna/Lau metapopulation, with differences between North-Fiji and
594 Futuna/Lau (Figure 6 B, ADMIXTURE $K = 3$, SI Figure 21). This pattern can be due to any
595 combination of drift and/or selection. Local selection may result from major differences in
596 depth or vent fluid composition. The fact that the fraction of outliers mapping on transcribed
597 regions is greater than by chance and targets a few metabolic/regulatory pathways suggests their
598 possible involvement in local adaptation to depth or different fluid chemistry, but this remains
599 to be investigated (Jennings et al., 2013; Liu et al., 2021). In the absence of high demographic
600 connectivity required to ensure the interdependency of local populations, this local
601 differentiation can remain detectable for several generations before being shuffled among all
602 metapopulation demes.

603 These subtle limitations in connectivity between basins can be associated with abyssal plains,
604 which may limit gene flow in a disconnected ridge system such as that found at the regional
605 scale of these BABs. Physical barriers in other parts of the world, such as transform faults and

606 microplates, have already been shown to greatly impede the effective migration of deep-sea
607 vent species at a much more restricted spatial scale (Johnson et al., 2006; Plouviez et al., 2009;
608 Plouviez, et al., 2013). However, regarding the populations of the Futuna volcanic arc and Lau
609 basin, our in-depth scrutiny of outliers did not reveal any sign of genetic differentiation. Hence,
610 the hypothesis of demographic correlation between these two regions cannot be rejected,
611 although we cannot infer with certitude the directionality of the exchanges.

612 **Conclusions**

613 Overall, our analyses revealed a clear genetic differentiation of *Ifremeria nautilei* populations
614 between the Manus/Woodlark and the North-Fiji/Futuna/Lau BABs, with very high gene flow
615 within each of these two metapopulations as well as higher genetic diversity in
616 Manus/Woodlark. Despite an in-depth scrutiny of genome-wide genetic variation, no
617 geographic substructure was detected between or within localities sampled within each
618 individual ridge system. This genetic connectivity probably indicates high local
619 (re)colonization capacity for this hydrothermal vent species due to the ephemeral nature of
620 active sites in this region, at least at the scale of a given back-arc basin.

621 However, our outlier analyses revealed that this genetic connectivity does not necessarily
622 equate with demographic connectivity at the larger inter-basin intra-metapopulation scale. The
623 specific investigation of outlier loci illustrates how a few loci in a large genome-wide dataset
624 can carry useful information about actual barriers to dispersal in high gene flow species. Deep-
625 sea mining holds the potential to exacerbate dispersal barriers and limit population resilience,
626 because if a large proportion of the vent habitat is destroyed locally, population rescue from
627 other basins will be restricted.

628 Furthermore, our demographic simulations indicated a long period of divergence during the
629 Quaternary period (several tens of thousands of generations) associated with restricted long-
630 range gene flow over a large fraction of the genome. Although the effects of linked selection
631 and reduced migration (barrier loci) are not clearly distinguishable, our results suggest that the
632 effect of the latter is less pronounced. This interpretation agrees with the fact that the global
633 divergence among the two metapopulations is still quite low (net nuclear nucleotide divergence,
634 0.81%). This divergence perhaps reflects the very beginning of an ongoing speciation process,
635 where a handful of barrier loci may already exist and at the same time overall genetic
636 differentiation is not hampered by weak contemporary and asymmetrical gene flow between
637 metapopulations.

638

639

640

641

642

643

644

645

646

647

648

649

650

651

652

653 **Acknowledgments**

654 We are deeply grateful to the R/V *L'Atalante* crew as well as the ROV *Victor 6000* crew during
655 the ChuBacArc 2019 cruise without whom nothing would have been possible. Many thanks
656 also to Cindy L. Van Dover for sharing some Manus 2008 samples and to John Parianos and
657 Paul Lahari from Nautilus Minerals group for sharing information on their Solwara mining
658 prospects in the Manus basin. W. We also thank Gwenn Tanguy for advice and, and access to
659 the Biogenouest Genomer Platform at the Station Biologique de Roscoff. Data were stored and
660 analyzed at the Biogenouest AbiMS Bioinformatics platform, which provided data storage and
661 computing resources, as well as at the Montpellier Bioinformatics Biodiversity platform
662 (LabEx CeMEB). We also thank Khalid Belkhir and Christelle Fraïsse for their debugged
663 version of *ada*. We are most grateful to four anonymous reviewers whose detailed and
664 insightful comments significantly improved the quality and clarity of the manuscript.

665 Ship time was supported by the French Oceanographic Fleet program (CHUBACARC cruise
666 doi [10.17600/18001111](https://doi.org/10.17600/18001111) to D. J. and S. H.). Travel expenses of ChuBacArc participants and logistic
667 expenses were funded by CNRS-Institut Ecologie et Environnement. Lab work and A. T. L. Y.
668 PhD fellowship were funded through the Agence Nationale de la Recherche ANR
669 'CERBERUS' (contract number ANR-17-CE02-0003 to S. H.). Sequencing was integrated in
670 the eDNabyss project (contract AP2016-228 to S. A. H.) funded by France Génomique (ANR-
671 10-INBS-09) and Genoscope-CEA.

672

673

674

675

References

- 676 Alexander, D. H., & Lange, K. (2011). Enhancements to the ADMIXTURE algorithm for individual
677 ancestry estimation. *BMC Bioinformatics*, *12*(1), 246. [https://doi.org/10.1186/1471-2105-12-](https://doi.org/10.1186/1471-2105-12-246)
678 246
- 679 Arellano, S. M., Van Gaest, A. L., Johnson, S. B., Vrijenhoek, R. C., & Young, C. M. (2014). Larvae
680 from deep-sea methane seeps disperse in surface waters. *Proceedings of the Royal Society B:*
681 *Biological Sciences*, *281*(1786), 20133276. <https://doi.org/10.1098/rspb.2013.3276>
- 682 Audzijonyte, A., & Vrijenhoek, R. C. (2010). When gaps really are gaps : Statistical phylogeography
683 of hydrothermal vent invertebrates. *Evolution*, *64*(8), 2369-2384.
684 <https://doi.org/10.1111/j.1558-5646.2010.00987.x>
- 685 Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., Lesin, V. M.,
686 Nikolenko, S. I., Pham, S., Prjibelski, A. D., Pyshkin, A. V., Sirotkin, A. V., Vyahhi, N.,
687 Tesler, G., Alekseyev, M. A., & Pevzner, P. A. (2012). SPAdes : A new genome assembly
688 algorithm and its applications to single-cell sequencing. *Journal of Computational Biology*,
689 *19*(5), 455-477. <https://doi.org/10.1089/cmb.2012.0021>
- 690 Barrows, T. T., Hope, G. S., Prentice, M. L., Fifield, L. K., & Tims, S. G. (2011). Late Pleistocene
691 glaciation of the Mt Giluwe volcano, Papua New Guinea. *Quaternary Science Reviews*, *30*(19-
692 20), 2676-2689. <https://doi.org/10.1016/j.quascirev.2011.05.022>
- 693 Berg, C. J. (1985). Reproductive strategies of mollusks from abyssal hydrothermal vent communities.
694 *Bulletin of The Biological Society of Washington*, 185-197.
- 695 Boulart, C., Rouxel, O., Scalabrin, C., Le Meur, P., Pelleter, E., Poitrimol, C., Thiébaud, E., Matabos,
696 M., Castel, J., Tran Lu Y, A., Michel, L.N., Cathalot, C., Cheron, S., Boissier, A., Germain, Y.,
697 Arnaud-Haond, S., Bonhomme, F., Broquet, T., Cuff-Gauchard, V., Le Layec, V.,
698 L'Haridon, S, Mary, J., Le Port, A-S., Tasiemski, A., Kuama, D.C., Hourdez, S., Jollivet, D.
699 (*in press*). Active hydrothermal vents in the Woodlark Basin may act as dispersing centres for
700 hydrothermal fauna *Communications Earth & Environment*.
- 701 Borowski, C., Giere, O., Krieger, J., Amann, R., & Dubilier, N. (2002). New aspects of the symbiosis
702 in the provannid snail *Ifremeria nautilei* from the North-Fiji back arc basin. *Cahiers de*
703 *Biologie Marine*, *5*.
704 [https://www.researchgate.net/publication/27262835_New_aspects_of_the_symbiosis_in_the_](https://www.researchgate.net/publication/27262835_New_aspects_of_the_symbiosis_in_the_provannid_snail>Ifremeria_nautilei_from_the_North_Fiji_Back_Arc_Basin)
705 [provannid_snail>Ifremeria_nautilei_from_the_North_Fiji_Back_Arc_Basin](https://www.researchgate.net/publication/27262835_New_aspects_of_the_symbiosis_in_the_provannid_snail>Ifremeria_nautilei_from_the_North_Fiji_Back_Arc_Basin)
- 706 Brelsford, A., Dufresnes, C., & Perrin, N. (2016). High-density sex-specific linkage maps of a
707 European tree frog (*Hyla arborea*) identify the sex chromosome without information on
708 offspring sex. *Heredity*, *116*(2), 177-181. <https://doi.org/10.1038/hdy.2015.83>
- 709 Breusing, C., Biastoch, A., Drews, A., Metaxas, A., Jollivet, D., Vrijenhoek, R. C., Bayer, T.,
710 Melzner, F., Sayavedra, L., Petersen, J. M., Dubilier, N., Schilhabel, M. B., Rosenstiel, P., &
711 Reusch, T. B. H. (2016). Biophysical and population genetic models predict the presence of
712 “phantom” stepping stones connecting Mid-Atlantic Ridge vent ecosystems. *Current Biology*,
713 *26*(17), 2257-2267. <https://doi.org/10.1016/j.cub.2016.06.062>
- 714 Breusing, C., Johnson, S. B., Tunnicliffe, V., Clague, D. A., Vrijenhoek, R. C., & Beinart, R. A.
715 (2020). Allopatric and sympatric drivers of speciation in alviniconcha hydrothermal vent
716 snails. *Molecular Biology and Evolution*, *37*(12), 3469-3484.
717 <https://doi.org/10.1093/molbev/msaa177>
- 718 Castel, J., Hourdez, S., Pradillon, F., Daguin-Thiébaud, C., Ballenghien, M., Ruault, S., Corre, E., Tran
719 Lu Y, A., Mary, J., Comtet, T., Gagnaire, P-A., Bonhomme, F., Bierne, N., Breusing, C.,
720 Broquet, T., & Jollivet D. (2022): A story of divergence and inter-specific exchanges in deep-

721 sea hydrothermal vent gastropods *Alviniconcha*. *Genes*. Manuscript
722 submitted for publication.

723 Cayuela, H., Rougemont, Q., Prunier, J. G., Moore, J.-S., Clobert, J., Besnard, A., & Bernatchez, L.
724 (2018). Demographic and genetic approaches to study dispersal in wild animal populations : A
725 methodological review. *Molecular Ecology*, 27(20), 3976-4010.
726 <https://doi.org/10.1111/mec.14848>

727 Chevaldonné, P., Jollivet, D., Desbruyères, D., Lutz, R., & Vrijenhoek, R. (2002). Sister-species of
728 eastern Pacific hydrothermal vent worms (Ampharetidae, Alvinellidae, Vestimentifera)
729 provide new mitochondrial COI clock calibration. *CBM - Cahiers de Biologie Marine*, 43(3-
730 4), 367-370. <https://archimer.ifremer.fr/doc/00000/895/>

731 Chevaldonné, P., Jollivet, D., Vangriesheim, A., & Desbruyères, D. (1997). Hydrothermal-vent
732 alvinellid polychaete dispersal in the eastern Pacific. 1. Influence of vent site distribution,
733 bottom currents, and biological patterns. *Limnology and Oceanography*, 42(1), 67-80.
734 <https://doi.org/10.4319/lo.1997.42.1.0067>

735 Craddock, C., Hoeh, W. R., Gustafson, R. G., Lutz, R. A., Hashimoto, J., & Vrijenhoek, R. J. (1995).
736 Evolutionary relationships among deep-sea mytilids (*Bivalvia* : Mytilidae) from hydrothermal
737 vents and cold-water methane/sulfide seeps. *Marine Biology*, 121(3), 477-485.
738 <https://doi.org/10.1007/BF00349456>

739 Craddock, C., Lutz, R. A., & Vrijenhoek, R. C. (1997). Patterns of dispersal and larval development of
740 archaeogastropod limpets at hydrothermal vents in the eastern Pacific. *Journal of*
741 *Experimental Marine Biology and Ecology*, 210(1), 37-51. [https://doi.org/10.1016/S0022-](https://doi.org/10.1016/S0022-0981(96)02701-3)
742 [0981\(96\)02701-3](https://doi.org/10.1016/S0022-0981(96)02701-3)

743 Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., Handsaker, R. E.,
744 Lunter, G., Marth, G. T., Sherry, S. T., McVean, G., Durbin, R., & 1000 Genomes Project
745 Analysis Group. (2011). The variant call format and VCFtools. *Bioinformatics*, 27(15), 2156-
746 2158. <https://doi.org/10.1093/bioinformatics/btr330>

747 Dover, C. L. V., Humphris, S. E., Fornari, D., Cavanaugh, C. M., Collier, R., Goffredi, S. K.,
748 Hashimoto, J., Lilley, M. D., Reysenbach, A. L., Shank, T. M., Damm, K. L. V., Banta, A.,
749 Gallant, R. M., Götz, D., Green, D., Hall, J., Harmer, T. L., Hurtado, L. A., Johnson, P., ...
750 Vrijenhoek, R. C. (2001). Biogeography and Ecological Setting of Indian Ocean
751 Hydrothermal Vents. *Science*, 294(5543), 818-823. <https://doi.org/10.1126/science.1064574>

752 Excoffier, L., Dupanloup, I., Huerta-Sánchez, E., Sousa, V. C., & Foll, M. (2013). Robust
753 demographic inference from genomic and SNP data. *PLOS Genetics*, 9(10), e1003905.
754 <https://doi.org/10.1371/journal.pgen.1003905>

755 Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5 : A new series of programs to perform
756 population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10(3),
757 564-567. <https://doi.org/10.1111/j.1755-0998.2010.02847.x>

758 Excoffier, L., Smouse, P. E., & Quattro, J. M. (1992). Analysis of molecular variance inferred from
759 metric distances among DNA haplotypes : Application to human mitochondrial DNA
760 restriction data. *Genetics*, 131(2), 479-491. <https://www.genetics.org/content/131/2/479>

761 Feutry, P., Devloo-Delva, F., Y, A. T. L., Mona, S., Gunasekera, R. M., Johnson, G., Pillans, R. D.,
762 Jaccoud, D., Kilian, A., Morgan, D. L., Saunders, T., Bax, N. J., & Kyne, P. M. (2020). One
763 panel to rule them all : DArTcap genotyping for population structure, historical demography,
764 and kinship analyses, and its application to a threatened shark. *Molecular Ecology Resources*,
765 20(6), 1470-1485. <https://doi.org/10.1111/1755-0998.13204>

766 Foll, M., & Gaggiotti, O. (2008). A genome-scan method to identify selected loci appropriate for both
767 dominant and codominant markers: A Bayesian Perspective. *Genetics*, 180(2), 977-993.
768 <https://doi.org/10.1534/genetics.108.092221>

769 Fraïsse, C., Belkhir, K., Welch, J. J., & Bierne, N. (2016). Local interspecies introgression is the main
770 cause of extreme levels of intraspecific differentiation in mussels. *Molecular Ecology*, 25(1),
771 269-286. <https://doi.org/10.1111/mec.13299>

772 Gagnaire, P.-A., Broquet, T., Aurelle, D., Viard, F., Souissi, A., Bonhomme, F., Arnaud-Haond, S., &
773 Bierne, N. (2015). Using neutral, selected, and hitchhiker loci to assess connectivity of marine

774 populations in the genomic era. *Evolutionary Applications*, 8(8), 769--786.
775 <https://doi.org/10.1111/eva.12288>

776 Ganachaud, A., Cravatte, S., Melet, A., Schiller, A., Holbrook, N. J., Sloyan, B. M., Widlansky, M. J.,
777 Bowen, M., Verron, J., Wiles, P., Ridgway, K., Sutton, P., Sprintall, J., Steinberg, C.,
778 Brassington, G., Cai, W., Davis, R., Gasparin, F., Gourdeau, L., ..., & Send, U. (2014). The
779 Southwest Pacific Ocean circulation and climate experiment (SPICE). *Journal of Geophysical*
780 *Research: Oceans*, 119(11), 7660--7686. <https://doi.org/10.1002/2013JC009678>

781 Gautier, M. (2015). Genome-wide scan for adaptive divergence and association with population-
782 specific covariates. *Genetics*, 201(4), 1555--1579.
783 <https://doi.org/10.1534/genetics.115.181453>

784 Gena, K. (2013). Deep sea mining of submarine hydrothermal deposits and its possible environmental
785 impact in Manus basin, Papua New Guinea. *Procedia Earth and Planetary Science*, 6, 226-
786 233. <https://doi.org/10.1016/j.proeps.2013.01.031>

787 Gutenkunst, R. N., Hernandez, R. D., Williamson, S. H., & Bustamante, C. D. (2009). Inferring the
788 joint demographic history of multiple populations from multidimensional SNP frequency data.
789 *PLOS Genetics*, 5(10), e1000695. <https://doi.org/10.1371/journal.pgen.1000695>

790 Hannington, M., Jamieson, J., Monecke, T., Petersen, S., & Beaulieu, S. (2011). The abundance of
791 seafloor massive sulfide deposits. *Geology*, 39(12), 1155--1158.
792 <https://doi.org/10.1130/G32468.1>

793 Hurtado, L. A., Lutz, R. A., & Vrijenhoek, R. C. (2004). Distinct patterns of genetic differentiation
794 among annelids of eastern Pacific hydrothermal vents. *Molecular Ecology*, 13(9), 2603--2615.
795 <https://doi.org/10.1111/j.1365-294X.2004.02287.x>

796 Jennings, R. M., Etter, R. J., & Ficarra, L. (2013). Population differentiation and species formation in
797 the deep sea: The potential role of environmental gradients and depth. *PLOS ONE*, 8(10),
798 e77594. <https://doi.org/10.1371/journal.pone.0077594>

799 Johnson, S. B., Young, C. R., Jones, W. J., Warén, A., & Vrijenhoek, R. C. (2006). Migration,
800 isolation, and speciation of hydrothermal vent limpets (gastropoda; lepetodrilidae) across the
801 Blanco transform fault. *The Biological Bulletin*, 210(2), 140-157.
802 <https://doi.org/10.2307/4134603>

803 Jollivet, D., Chevaldonné, P., & Planque, B. (1999). Hydrothermal-vent alvinellid polychaete dispersal
804 in the Eastern Pacific. 2. A metapopulation model based on habitat shifts. *Evolution*, 53(4),
805 1128--1142. <https://doi.org/10.1111/j.1558-5646.1999.tb04527.x>

806 Jollivet, D., Desbruyères, D., Bonhomme, F., & Moraga, D. (1995). Genetic differentiation of deep-
807 sea hydrothermal vent alvinellid populations (Annelida : Polychaeta) along the East Pacific
808 Rise. *Heredity*, 74(4), 376--391. <https://doi.org/10.1038/hdy.1995.56>

809 Jones, G. P., Planes, S., & Thorrold, S. R. (2005). Coral reef fish larvae settle close to home. *Current*
810 *Biology*, 15(14), 1314--1318. <https://doi.org/10.1016/j.cub.2005.06.061>

811 Joy, K., Fink, D., Storey, B., & Atkins, C. (2014). A 2 million year glacial chronology of the
812 Hatherton Glacier, Antarctica and implications for the size of the East Antarctic Ice Sheet at
813 the Last Glacial Maximum. *Quaternary Science Reviews*, 83, 46--57.
814 <https://doi.org/10.1016/j.quascirev.2013.10.028>

815 Knowlton, N., & Weigt, L. A. (1998). New dates and new rates for divergence across the Isthmus of
816 Panama. *Proceedings of the Royal Society of London. Series B: Biological Sciences*,
817 265(1412), 2257--2263. <https://doi.org/10.1098/rspb.1998.0568>

818 Konn, C., Fourné, E., Jean-Baptiste, P., Donval, J. P., Guyader, V., Birot, D., Alix, A. S., Gaillot, A.,
819 Perez, F., Dapoigny, A., Pelleter, E., Resing, J. A., Charlou, J. L., & Fouquet, Y. (2016).
820 Extensive hydrothermal activity revealed by multi-tracer survey in the Wallis and Futuna
821 region (SW Pacific). *Deep Sea Research Part I: Oceanographic Research Papers*, 116, 127--
822 144. <https://doi.org/10.1016/j.dsr.2016.07.012>

823 Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., & Mayrose, I. (2015). Clumpak : A
824 program for identifying clustering modes and packaging population structure inferences across
825 K. *Molecular Ecology Resources*, 15(5), 1179--1191. <https://doi.org/10.1111/1755-0998.12387>

- 827 Lischer, H. E. L., & Excoffier, L. (2012). PGDSpider : An automated data conversion tool for
828 connecting population genetics and genomics programs. *Bioinformatics*, 28(2), 298--299.
829 <https://doi.org/10.1093/bioinformatics/btr642>
- 830 Liu, R., Liu, J., & Zhang, H. (2021). Positive selection analysis reveals the deep-sea adaptation of a
831 hadal sea cucumber (*Paelopatides* sp.) to the Mariana Trench. *Journal of Oceanology and*
832 *Limnology*, 39(1), 266--281. <https://doi.org/10.1007/s00343-020-0241-0>
- 833 Lowe, W. H., & Allendorf, F. W. (2010). What can genetics tell us about population connectivity?
834 *Molecular Ecology*, 19(15), 3038--3051. <https://doi.org/10.1111/j.1365-294X.2010.04688.x>
- 835 Luu, K., Bazin, E., & Blum, M. G. B. (2017). pcadapt : An R package to perform genome scans for
836 selection based on principal component analysis. *Molecular Ecology Resources*, 17(1), 67--77.
837 <https://doi.org/10.1111/1755-0998.12592>
- 838 Lynch, M. (2010). Evolution of the mutation rate. *Trends in Genetics*, 26(8), 345--352.
839 <https://doi.org/10.1016/j.tig.2010.05.003>
- 840 Martinez, F., & Taylor, B. (1996). Backarc spreading, rifting, and microplate rotation, between
841 transform faults in the Manus Basin. *Marine Geophysical Researches*, 18(2--4), 203--224.
842 <https://doi.org/10.1007/BF00286078>
- 843 Mastretta-Yanes, A., Arrigo, N., Alvarez, N., Jorgensen, T. H., Piñero, D., & Emerson, B. C. (2015).
844 Restriction site-associated DNA sequencing, genotyping error estimation and de novo
845 assembly optimization for population genetic inference. *Molecular Ecology Resources*, 15(1),
846 28--41. <https://doi.org/10.1111/1755-0998.12291>
- 847 McConachy, T. F., Arculus, R. J., Yeats, C. J., Binns, R. A., Barriga, F. J. A. S., McInnes, B. I. A.,
848 Sestak, S., Sharpe, R., Rakau, B., & Tevi, T. (2005). New hydrothermal activity and alkalic
849 volcanism in the backarc Coriolis Troughs, Vanuatu. *Geology*, 33(1), 61--64.
850 <https://doi.org/10.1130/G20870.1>
- 851 Milano, I., Babbucci, M., Cariani, A., Atanassova, M., Bekkevold, D., Carvalho, G. R., Espiñeira, M.,
852 Fiorentino, F., Garofalo, G., Geffen, A. J., Hansen, Jakob. H., Helyar, S. J., Nielsen, E. E.,
853 Ogden, R., Patarnello, T., Stagioni, M., Consortium, F., Tinti, F., & Bargelloni, L. (2014).
854 Outlier SNP markers reveal fine-scale genetic structuring across European hake populations
855 (*Merluccius merluccius*). *Molecular Ecology*, 23(1), 118--135.
856 <https://doi.org/10.1111/mec.12568>
- 857 Mitarai, S., Watanabe, H., Nakajima, Y., Shchepetkin, A. F., & McWilliams, J. C. (2016). Quantifying
858 dispersal from hydrothermal vent fields in the western Pacific Ocean. *Proceedings of the*
859 *National Academy of Sciences*, 113(11), 2976--2981.
860 <https://doi.org/10.1073/pnas.1518395113>
- 861 Mullineaux, L. S., Adams, D. K., Mills, S. W., & Beaulieu, S. E. (2010). Larvae from afar colonize
862 deep-sea hydrothermal vents after a catastrophic eruption. *Proceedings of the National*
863 *Academy of Sciences*, 107(17), 7829--7834. <https://doi.org/10.1073/pnas.0913187107>
- 864 Niner, H. J., Ardron, J. A., Escobar, E. G., Gianni, M., Jaekel, A., Jones, D. O. B., Levin, L. A.,
865 Smith, C. R., Thiele, T., Turner, P. J., Van Dover, C. L., Watling, L., & Gjerde, K. M. (2018).
866 Deep-Sea Mining With No Net Loss of Biodiversity—An Impossible Aim. *Frontiers in*
867 *Marine Science*, 5. <https://www.frontiersin.org/article/10.3389/fmars.2018.00053>
- 868 Paris, J. R., Stevens, J. R., & Catchen, J. M. (2017). Lost in parameter space : A road map for stacks.
869 *Methods in Ecology and Evolution*, 8(10), 1360--1373. <https://doi.org/10.1111/2041-210X.12775>
- 870
- 871 Pickrell, J. K., & Pritchard, J. K. (2012). Inference of population splits and mixtures from genome-
872 wide allele frequency data. *PLOS Genetics*, 8(11), e1002967.
873 <https://doi.org/10.1371/journal.pgen.1002967>
- 874 Pinsky, M. L., Jr, H. R. M., & Palumbi, S. R. (2010). Using isolation by distance and effective density
875 to estimate dispersal scales in anemonefish. *Evolution*, 64(9), 2688--2700.
876 <https://doi.org/10.1111/j.1558-5646.2010.01003.x>
- 877 Plouviez, S., Faure, B., Guen, D. L., Lallier, F. H., Bierne, N., & Jollivet, D. (2013). A new barrier to
878 dispersal trapped old genetic clines that escaped the easter microplate tension zone of the

879 Pacific vent mussels. *PLOS ONE*, 8(12), e81555.
880 <https://doi.org/10.1371/journal.pone.0081555>

881 Plouviez, S., LaBella, A. L., Weisrock, D. W., Meijenfildt, F. A. B., von Ball, B., Neigel, J. E., &
882 Dover, C. L. V. (2019). Amplicon sequencing of 42 nuclear loci supports directional gene
883 flow between South Pacific populations of a hydrothermal vent limpet. *Ecology and*
884 *Evolution*, 9(11), 6568--6580. <https://doi.org/10.1002/ece3.5235>

885 Plouviez, S., Schultz, T. F., McGinnis, G., Minshall, H., Rudder, M., & Van Dover, C. L. (2013).
886 Genetic diversity of hydrothermal-vent barnacles in Manus Basin. *Deep Sea Research Part I:*
887 *Oceanographic Research Papers*, 82, 73--79. <https://doi.org/10.1016/j.dsr.2013.08.004>

888 Plouviez, S., Shank, T. M., Faure, B., Daguin-Thiébaud, C., Viard, F., Lallier, F. H., & Jollivet, D.
889 (2009). Comparative phylogeography among hydrothermal vent species along the East Pacific
890 Rise reveals vicariant processes and population expansion in the South. *Molecular Ecology*,
891 18(18), 3903--3917. <https://doi.org/10.1111/j.1365-294X.2009.04325.x>

892 Reich, D., Thangaraj, K., Patterson, N., Price, A. L., & Singh, L. (2009). Reconstructing Indian
893 population history. *Nature*, 461(7263), 489--494. <https://doi.org/10.1038/nature08365>

894 Reynolds, K. C., Watanabe, H., Strong, E. E., Sasaki, T., Uematsu, K., Miyake, H., Kojima, S.,
895 Suzuki, Y., Fujikura, K., Kim, S., & Young, C. M. (2010). New molluscan larval form :
896 brooding and development in a hydrothermal vent gastropod, *Ifremeria nautilei*
897 (Provannidae). *The Biological Bulletin*, 219(1), 7--11. <https://doi.org/10.1086/BBLv219n1p7>

898 Robinet, T., Roussel, V., Cheze, K., & Gagnaire, P.-A. (2020). Spatial gradients of introgressed
899 ancestry reveal cryptic connectivity patterns in a high gene flow marine fish. *Molecular*
900 *Ecology*, 29(20), 3857--3871. <https://doi.org/10.1111/mec.15611>

901 Rochette, N. C., Rivera-Colón, A. G., & Catchen, J. M. (2019). Stacks 2 : Analytical methods for
902 paired-end sequencing improve RADseq-based population genomics. *Molecular Ecology*,
903 28(21), 4737--4754. <https://doi.org/10.1111/mec.15253>

904 Rougeux, C., Bernatchez, L., & Gagnaire, P.-A. (2017). Modeling the multiple facets of speciation-
905 with-gene-flow toward inferring the divergence history of lake whitefish species pairs
906 (*Coregonus clupeaformis*). *Genome Biology and Evolution*, 9(8), 2057--2074.
907 <https://doi.org/10.1093/gbe/evx150>

908 Schmidt, K., Garbe-Schönberg, D., Hannington, M. D., Anderson, M. O., Bühring, B., Haase, K.,
909 Haruel, C., Lupton, J., & Koschinsky, A. (2017). Boiling vapour-type fluids from the Nifonea
910 vent field (New Hebrides Back-Arc, Vanuatu, SW Pacific) : Geochemistry of an early-stage,
911 post-eruptive hydrothermal system. *Geochimica et Cosmochimica Acta*, 207, 185--209.
912 <https://doi.org/10.1016/j.gca.2017.03.016>

913 Simon, A., Fraïsse, C., Ayari, T. E., Liautard-Haag, C., Strelkov, P., Welch, J. J., & Bierne, N. (2021).
914 How do species barriers decay? Concordance and local introgression in mosaic hybrid zones
915 of mussels. *Journal of Evolutionary Biology*, 34(1), 208--223.
916 <https://doi.org/10.1111/jeb.13709>

917 Sommer, S. A., Van Woudenberg, L., Lenz, P. H., Cepeda, G., & Goetze, E. (2017). Vertical gradients
918 in species richness and community composition across the twilight zone in the North Pacific
919 Subtropical Gyre. *Molecular Ecology*, 26(21), 6136--6156. <https://doi.org/10.1111/mec.14286>

920 Teixeira, S., Cambon-Bonavita, M.-A., Serrão, E. A., Desbruyères, D., & Arnaud-Haond, S. (2011).
921 Recent population expansion and connectivity in the hydrothermal shrimp *Rimicaris exoculata*
922 along the Mid-Atlantic Ridge. *Journal of Biogeography*, 38(3), 564--574.
923 <https://doi.org/10.1111/j.1365-2699.2010.02408.x>

924 Teixeira, S., Serrão, E. A., & Arnaud-Haond, S. (2012). Panmixia in a fragmented and unstable
925 environment: The hydrothermal shrimp *Rimicaris exoculata* disperses extensively along the
926 Mid-Atlantic Ridge. *PLOS ONE*, 7(6), e38521. <https://doi.org/10.1371/journal.pone.0038521>

927 Thaler, A. D., Plouviez, S., Saleu, W., Alei, F., Jacobson, A., Boyle, E. A., Schultz, T. F., Carlsson, J.,
928 & Dover, C. L. V. (2014). Comparative population structure of two deep-sea hydrothermal-
929 vent-associated decapods (*Chorocaris sp. 2* and *Munidopsis lauensis*) from Southwestern
930 Pacific Back-Arc basins. *PLOS ONE*, 9(7), e101345.
931 <https://doi.org/10.1371/journal.pone.0101345>

- 932 Thaler, A. D., Zelnio, K., Saleu, W., Schultz, T. F., Carlsson, J., Cunningham, C., Vrijenhoek, R. C.,
933 & Van Dover, C. L. (2011). The spatial scale of genetic subdivision in populations of
934 *Ifremeria nautilei*, a hydrothermal-vent gastropod from the southwest Pacific. *BMC*
935 *Evolutionary Biology*, 11(1), 372. <https://doi.org/10.1186/1471-2148-11-372>
- 936 Thiébaud, C. D., Ruault, S., Roby, C., Broquet, T., Viard, F., & Brelsford, A. (2021, septembre 21).
937 *Construction of individual ddRAD libraries*. Protocols.io.
938 <https://www.protocols.io/view/construction-of-individual-ddrad-libraries-bv4tn8wn>
- 939 Tine, M., Kuhl, H., Gagnaire, P.-A., Louro, B., Desmarais, E., Martins, R. S. T., Hecht, J., Knaust, F.,
940 Belkhir, K., Klages, S., Dieterich, R., Stueber, K., Piferrer, F., Guinand, B., Bierne, N.,
941 Volckaert, F. A. M., Bargelloni, L., Power, D. M., Bonhomme, F., ..., & Reinhardt, R. (2014).
942 European sea bass genome and its variation provide insights into adaptation to euryhalinity
943 and speciation. *Nature Communications*, 5(1), 5770. <https://doi.org/10.1038/ncomms6770>
- 944 Tunnicliffe, V., McArthur, A. G., & McHugh, D. (1998). A biogeographical perspective of the deep-
945 sea hydrothermal vent fauna. Dans J. H. S. Blaxter, A. J. Southward, & P. A. Tyler (Éds.),
946 *Advances in Marine Biology* (Vol. 34, p. 353--442). Academic Press.
947 [https://doi.org/10.1016/S0065-2881\(08\)60213-8](https://doi.org/10.1016/S0065-2881(08)60213-8)
- 948 Tyler, P. A., Pendlebury, S., Mills, S. W., Mullineaux, L., Eckelbarger, K. J., Baker, M., & Young, C.
949 M. (2008). Reproduction of gastropods from vents on the East Pacific Rise and the Mid-
950 Atlantic Ridge. *Journal of Shellfish Research*, 27(1), 107--118. [https://doi.org/10.2983/0730-8000\(2008\)27\[107:ROGFVO\]2.0.CO;2](https://doi.org/10.2983/0730-8000(2008)27[107:ROGFVO]2.0.CO;2)
- 951 Tyler, P. A., & Young, C. M. (1999). Reproduction and dispersal at vents and cold seeps. *Journal of*
952 *the Marine Biological Association of the United Kingdom*, 79(2), 193--208.
953 <https://doi.org/10.1017/S0025315499000235>
- 954 Villemereuil, P. de, Frichot, É., Bazin, É., François, O., & Gaggiotti, O. E. (2014). Genome scan
955 methods against more complex models : When and how much should we trust them?
956 *Molecular Ecology*, 23(8), 2006--2019. <https://doi.org/10.1111/mec.12705>
- 957 Vrijenhoek, R. C. (1997). Gene flow and genetic diversity in naturally fragmented metapopulations of
958 deep-sea hydrothermal vent animals. *Journal of Heredity*, 88(4), 285--293.
959 <https://doi.org/10.1093/oxfordjournals.jhered.a023106>
- 960 Vrijenhoek, R. C. (2010). Genetic diversity and connectivity of deep-sea hydrothermal vent
961 metapopulations. *Molecular Ecology*, 19(20), 4391--4411. <https://doi.org/10.1111/j.1365-294X.2010.04789.x>
- 962 Warén, A., & Bouchet, P. (1993). New records, species, genera, and a new family of gastropods from
963 hydrothermal vents and hydrocarbon seeps. *Zoologica Scripta*, 22(1), 1--90.
964 <https://doi.org/10.1111/j.1463-6409.1993.tb00342.x>
- 965 Windoffer, R., & Giere, O. (1997). Symbiosis of the hydrothermal vent gastropod *Ifremeria nautilei*
966 (provannidae) with endobacteria-structural analyses and ecological considerations. *The*
967 *Biological Bulletin*, 193(3), 381-392. <https://doi.org/10.2307/1542940>
- 968 Won, Y., Hallam, S. J., O'Mullan, G. D., & Vrijenhoek, R. C. (2003). Cytonuclear disequilibrium in a
969 hybrid zone involving deep-sea hydrothermal vent mussels of the genus *Bathymodiolus*.
970 *Molecular Ecology*, 12(11), 3185-3190. <https://doi.org/10.1046/j.1365-294X.2003.01974.x>
- 971 Wyngaarden, M. V., Snelgrove, P. V. R., DiBacco, C., Hamilton, L. C., Rodríguez-Ezpeleta, N.,
972 Jeffery, N. W., Stanley, R. R. E., & Bradbury, I. R. (2017). Identifying patterns of dispersal,
973 connectivity and selection in the sea scallop, *Placopecten magellanicus*, using RADseq-
974 derived SNPs. *Evolutionary Applications*, 10(1), 102-117. <https://doi.org/10.1111/eva.12432>
- 975 Yahagi, T., Fukumori, H., Warén, A., & Kano, Y. (2019). Population connectivity of hydrothermal-
976 vent limpets along the northern Mid-Atlantic Ridge (Gastropoda : Neritimorpha:
977 Phenacolepadidae). *Journal of the Marine Biological Association of the United Kingdom*,
978 99(1), 179-185. <https://doi.org/10.1017/S0025315417001898>
- 979 Yahagi, T., Thaler, A. D., Dover, C. L. V., & Kano, Y. (2020). Population connectivity of the
980 hydrothermal-vent limpet *Shinkailepas tollmanni* in the Southwest Pacific (Gastropoda :
981 Neritimorpha: Phenacolepadidae). *PLOS ONE*, 15(9), e0239784.
982 <https://doi.org/10.1371/journal.pone.0239784>

985 Yahagi, T., Watanabe, H. K., Kojima, S., & Kano, Y. (2017). Do larvae from deep-sea hydrothermal
986 vents disperse in surface waters? *Ecology*, 98(6), 1524-1534. <https://doi.org/10.1002/ecy.1800>
987 Yearsley, J. M., & Sigwart, J. D. (2011). Larval transport modeling of deep-sea invertebrates can aid
988 the search for undiscovered populations. *PLOS ONE*, 6(8), e23063.
989 <https://doi.org/10.1371/journal.pone.0023063>
990 Zheng, X., Levine, D., Shen, J., Gogarten, S. M., Laurie, C., & Weir, B. S. (2012). A high-
991 performance computing toolset for relatedness and principal component analysis of SNP data.
992 *Bioinformatics*, 28(24), 3326-3328. <https://doi.org/10.1093/bioinformatics/bts606>
993

994

995 **Data Accessibility**

996 Individual fastq files are available at the European Nucleotide Archive (study accession
997 number PRJEB47533). SNP data (VCF) and associated metadata are available at Dryad :
998 <https://doi.org/10.5061/dryad.ffbg79cwq> (preview [https://datadryad.org/stash/share/WfF-](https://datadryad.org/stash/share/WfF-hiYEO6nnKdwjG_E78-dA67mmAgnJ4i4Q6iL9-JU)
999 [hiYEO6nnKdwjG_E78-dA67mmAgnJ4i4Q6iL9-JU](https://datadryad.org/stash/share/WfF-hiYEO6nnKdwjG_E78-dA67mmAgnJ4i4Q6iL9-JU)) Scripts (R, $\partial a \partial i$) are available in a
1000 public Github repository (<https://github.com/Atranluy/Scripts-Ifremeria>).

1001 **Author's contribution:**

1002 D. J. and S. H. designed the CHUBACARC and CERBERUS projects, F. B. supervised the
1003 genetic work. A. T. L. Y., S. R., C. D. T., J. C., P. W. and A. P. performed laboratory work. A.
1004 T. L. Y. performed bioinformatics statistical analyses with the contribution of F. B., D. J., P. A.
1005 G., N. B. and T. B. A. T. L. Y., F. B. wrote the manuscript with feedback of T. B., D. J., P. A.
1006 G., N. B., S. A. H. and C. D. T. All authors approved the manuscript.

1007

1008

1009

1010

1011

1012

1013

1014

1015

1016

1017

Tables

1018 *Table 1: Analysis of molecular variance (AMOVA) on the final dataset with 10 000 permutations (***: $p < 0.001$, **: $p < 0.01$*
 1019 **, $p < 0.05$)*

Manus/Woodlark vs. North-Fiji/Futuna/Lau	0.38773***	F_{ST}
Basins in M/W and NF/F/L	-0.05	F_{CT}
Localities within Basins	-0.00011	F_{SC}
Individuals within Localities	-0.05084	F_{IS}

1020 *M/W: Manus/Woodlark, NF/F/L: North-Fiji/Futuna/Lau*

1021

1022 *Table 2: Pairwise (between basins) F_{ST} matrix on the final dataset with 10 000 permutations after Bonferroni correction*
 1023 *(***: $p < 0.001$, **: $p < 0.01$, * $p < 0.05$).*

	Lau	Futuna	North-Fiji	Manus	Woodlark
Lau	0.00000				
Futuna	-0.00040	0.00000			
North-Fiji	0.00029*	-0.00004	0.00000		
Manus	0.38350***	0.38275***	0.37651***	0.00000	
Woodlark	0.39986***	0.39656***	0.38647***	-0.00016	0.00000

1024

1025

1026

1027

1028

1029

1030

1031 *Table 3: Parameters estimated from $\partial a \partial i$ for the three best models (IM2N2mG, SC2N2mG and AM2N2mG*) with their*
 1032 *standard deviations (SD) estimated using a Fisher information matrix. (*isolation with migration (IM), secondary contact*
 1033 *(SC), ancient migration (AM) and with parameters describing effective population size (2N), migration rate (2m) and*
 1034 *population growth (between basins))*

Parameter	IM+2N+2m+G	SD	SC+2N+2m+G	SD	AM+2N+2m+G	SD
N₁ (NF/F/L)	0.435	0.187	0.913	0.573	0.390	0.127
N₂ (M/W)	0.411	0.157	0.840	0.573	0.356	0.119
b1	30.410	13.176	16.947	8.127	34.367	12.399
b2	25.097	8.683	13.714	8.451	29.288	10.523
hrf	0.023	0.006	0.021	0.006	0.022	0.006
Ts			0.443	0.527	0.001	0.024
Tsm/Tsc/Tam	1.631	0.280	1.470	0.379	1.681	0.335
m12	0.444	0.147	0.422	0.145	0.461	0.137
m21	0.825	0.192	0.810	0.261	0.825	0.198
me12	0.038	0.020	0.038	0.024	0.039	0.018
me21	0.283	0.063	0.270	0.088	0.300	0.070
P	0.483	0.103	0.439	0.093	0.471	0.095
Q	0.990	0.136	0.990	0.185	0.990	0.136
Theta	271.772	31.55	243.283	64.91	273.222	32.988

1035 *N* represents the population size of each population; **b**, the population growth factor; **hrf**, the Hill-Robertson factor; **Ts**, the
 1036 *time of strict divergence*; **Tm/Tsc/Tam**, the time of divergence with migration; **m12**, represents the unrestricted migration
 1037 *rate from the population 2 towards population 1*; **me12**, the restricted migration rate (e.g. barrier loci) from population 2
 1038 *towards population 1*; **Q**, the proportion of loci that are under the effect of linked selection (i.e. Hill-Robertson effect); **P**, the
 1039 *proportion of loci that have unconstrained migration*; M/W, Manus/Woodlark; NF/F/L, North-Fiji/Futuna/Lau

1040

1041

1042

1043 Table 4: Estimates of the effective number of migrants (N_m) exchanged per generation between metapopulations, total time of
 1044 divergence since the population split and effective population size (N_e) for three demographic models (isolation with
 1045 migration (IM), ancient migration (AM), and secondary contact (SC).

	$N_{m2 \rightarrow 1}$ (M/W \rightarrow NF/F/L)	$N_{m1 \rightarrow 2}$ (NF/F/L \rightarrow M/W)	T (in generations)	N_1 (NF/F/L)	N_2 (M/W)
IM+2N+2m+G	2.935	4.255	66 951	271 506	211 708
SC+2N+2m+G	3.265	4.665	70 295	284 279	211 653
AM+2N+2m+G	3.09	4.305	69 380	276 561	215 142

1046 Metapopulation M/W, Manus/Woodlark; metapopulation NF/F/L, North-Fiji/Futuna/Lau.

1047

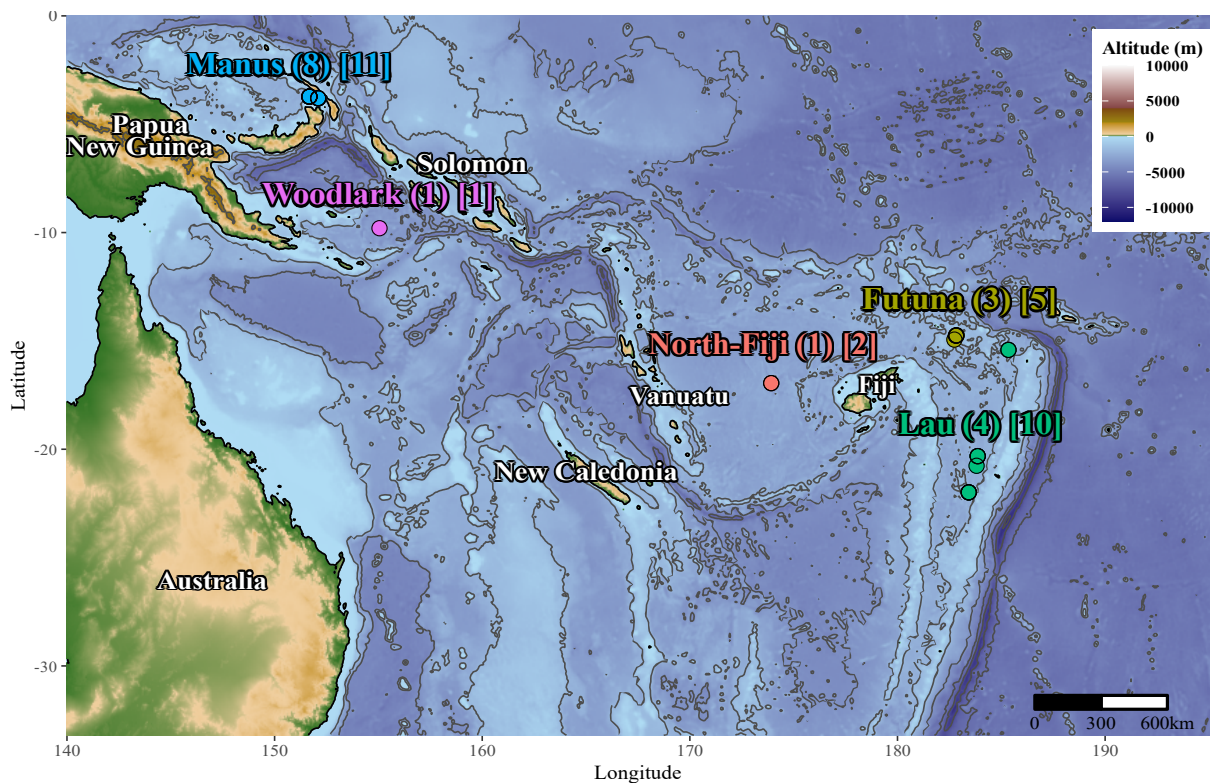
1048

1049

1050

1051

Figures

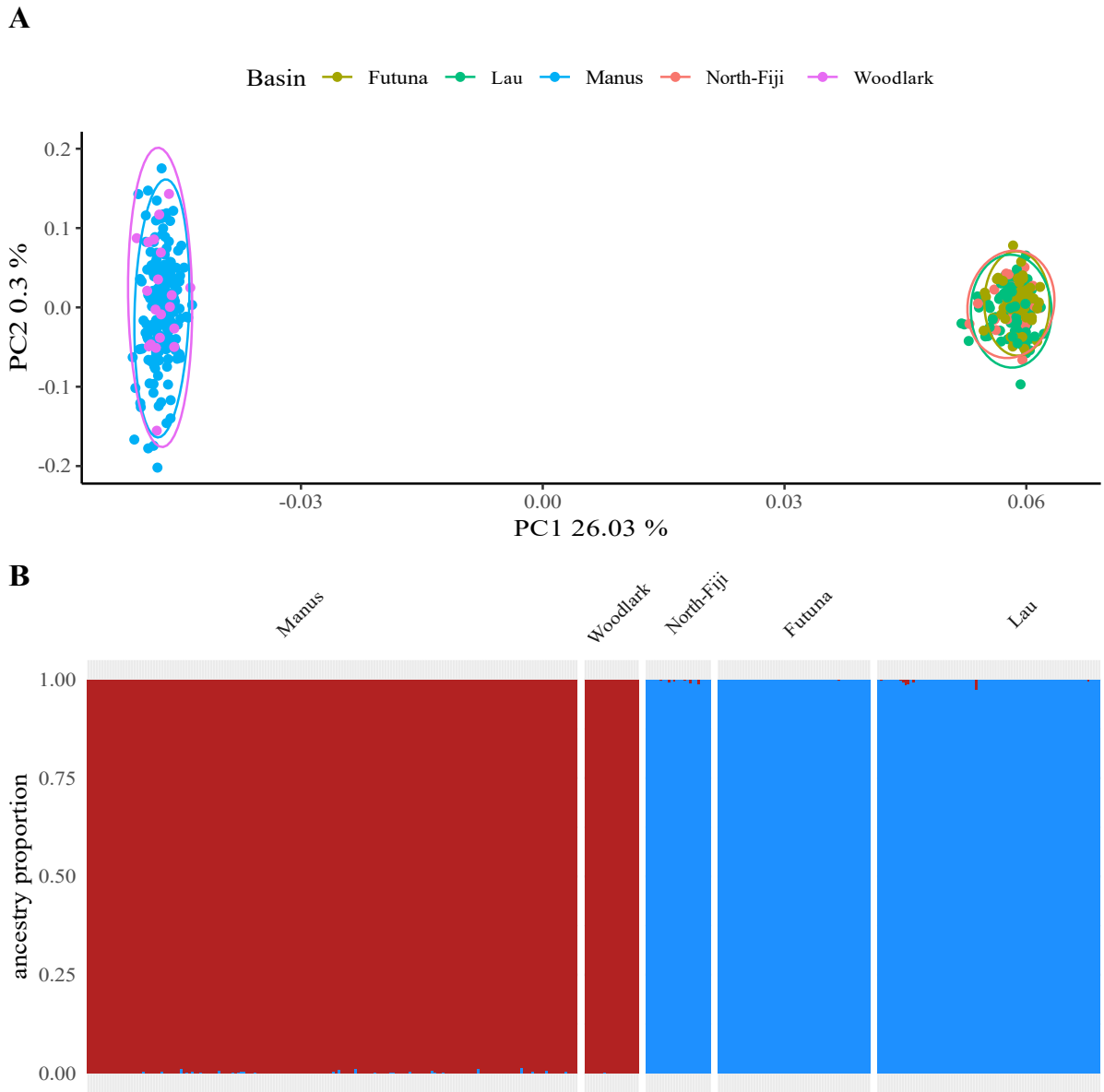


1052

1053 Figure 1: Colors: Back-arc basins. Sampling map of *Ifremeria nauteili* in the Southwestern Pacific Ocean. The number of
 1054 localities is given in parentheses and the total number of sampled sites in brackets.

1055

1056



1057

1058

1059

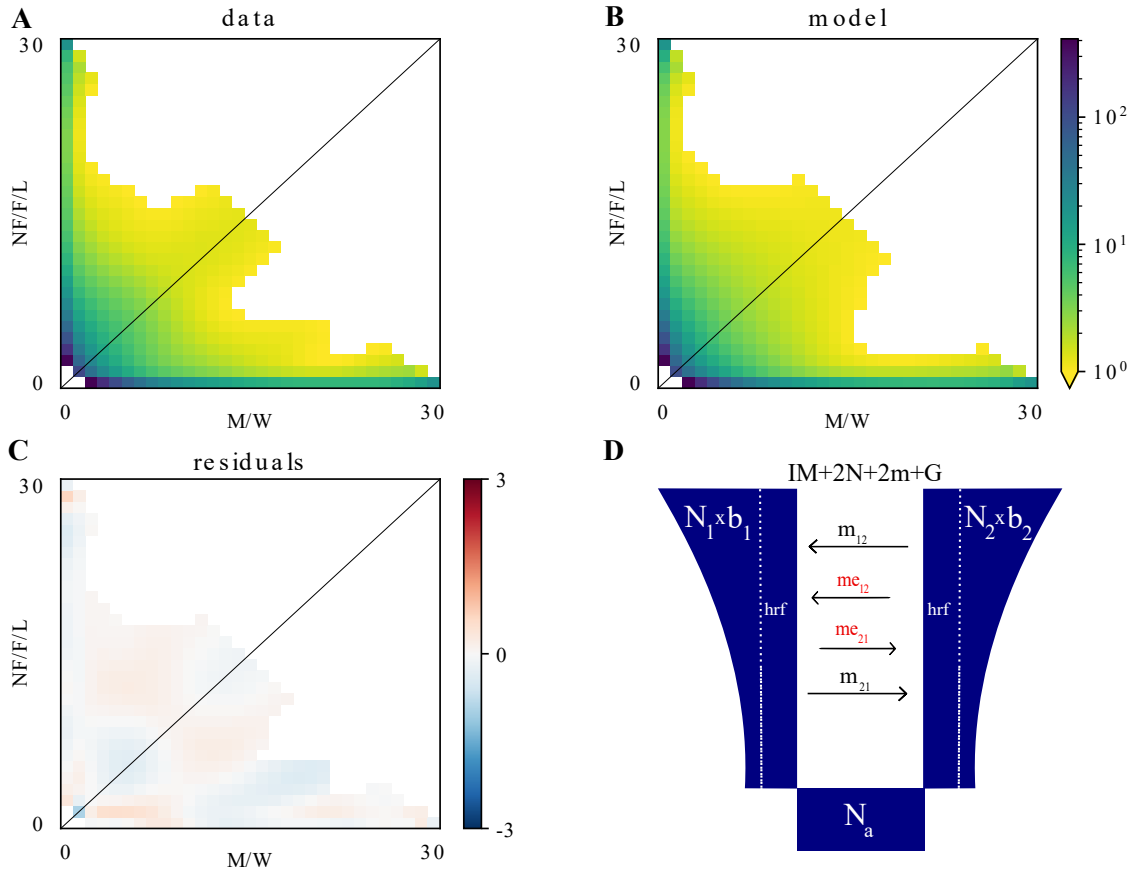
1060

1061

Figure 2: (A) Principal component analysis plot of 362 *Ifremeria nautili* individuals from five hydrothermal basins scored at 10 570 SNPs, open circles represent the multivariate normal distribution of each group (basins) at 95%. (B) ADMIXTURE plot for each individual with their ancestry proportions obtained on the final dataset for the best K (K=2). Individuals are grouped according to their basin of origin.

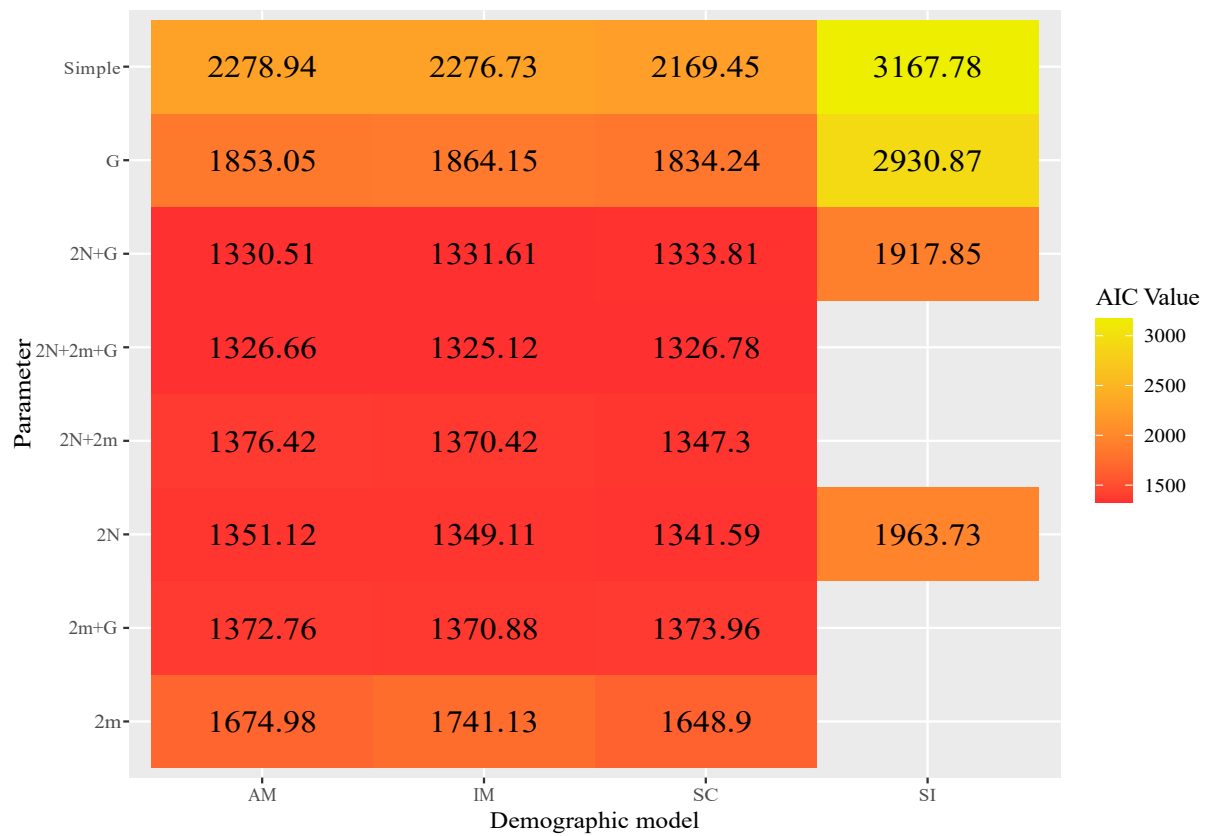
1062

1063
1064



1065
1066
1067
1068
1069
1070
1071
1072
1073
1074
1075
1076

Figure 3: (A) Joint allele frequency spectrum (JAFS) between the Manus/Woodlark (M/W) and North Fiji/Futuna/Lau (NF/F/L) basin systems. (B) Simulated JAFS under the IM2N2mG model (see Figure 4), the log scale indicates the density of SNPs in each frequency class. (C) Residuals of the fit of the simulated model on the data. (D) Representation of the fitted model. (N represents population size; b , population growth factor; hrf , the Hill-Robertson factor, which simulates linked selection; m , unrestricted migration rate; me , restricted migration rate, which simulates barrier loci)

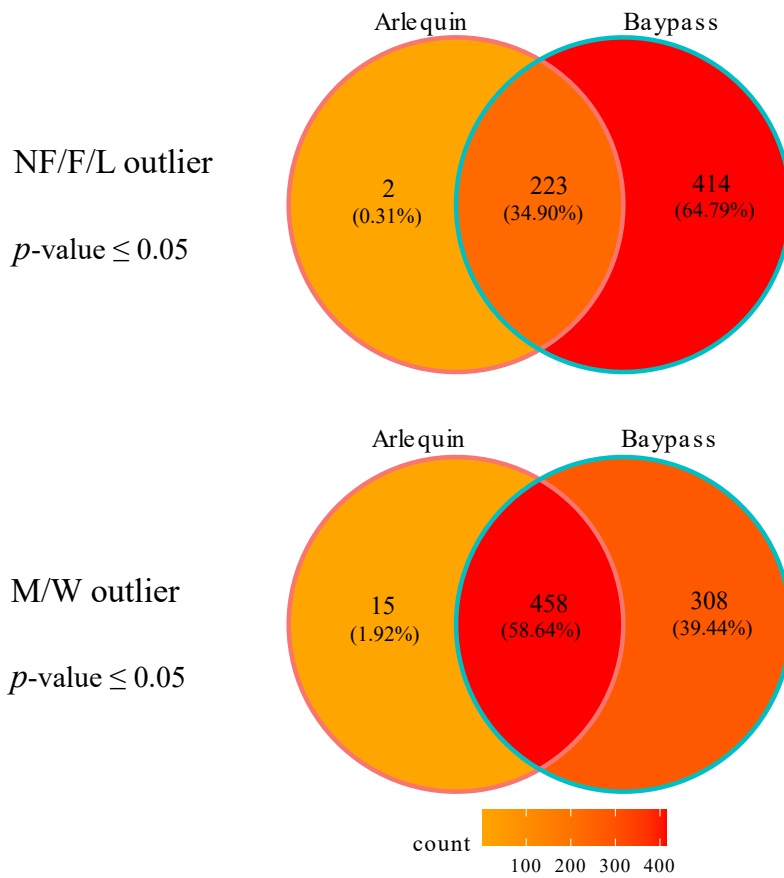


1077

1078 *Figure 4: Heat-map of the best Akaike information criterion (AIC) value for each parameter combination (population*
 1079 *expansion or contraction (G), effect of linked selection (2N) and heterogeneous migration (2m)) and demographic model*
 1080 *(strict isolation (SI), isolation with migration (IM), ancient migration (AM), and secondary contact (SC)).*

1081

1082



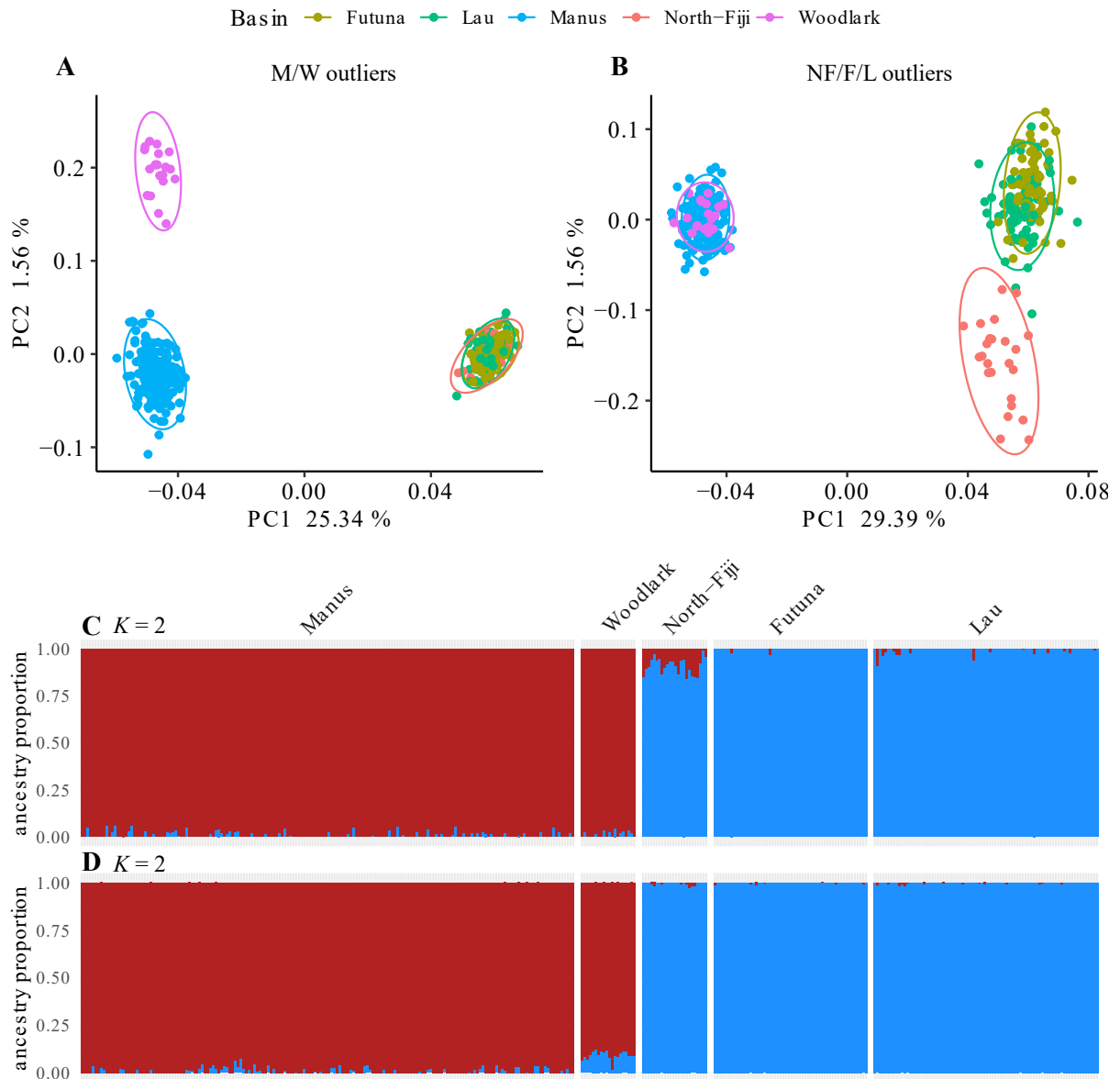
1083

1084

1085

1086

Figure 5: Venn diagram of shared outlier loci identified in Arlequin and Baypass with a p -value less than or equal to 0.05 within each metapopulation (Manus/Woodlark, M/W and North Fiji/Futuna/Lau, NF/F/L) independently.



1088

1089 *Figure 6: Principal component analysis on all individuals with the outlier loci found in both Arlequin and Baypass at a 0.05*
 1090 *p-value threshold. (A) Outlier loci detected in Manus/Woodlark (M/W). (B) Outlier loci detected in North-Fiji/Futuna/Lau*
 1091 *(NF/F/L). Plot of ancestry proportion inferred with Admixture at $K = 2$ on all individual at the 0.05 p-value threshold, for*
 1092 *(C) North-Fiji/Futuna/Lau outliers and (D) for Manus/Woodlark outliers.*

1093

1094

1095

1096

1097