

Spatial variability in recruitment of benthos near drilling sites at the Iheya North hydrothermal field in the Okinawa Trough

Masako Nakamura^{1*#}, Yuichi Nakajima¹, Hiromi Kayama Watanabe², Takenori Sasaki³, Hiroyuki Yamamoto², and Satoshi Mitarai¹

¹Marine Biophysics Unit, Okinawa Institute of Science and Technology, Onna, Okinawa 904-0412, Japan

²Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Natsushima 2-15, Yokosuka, Kanagawa 237-0061, Japan

³The University Museum, The University of Tokyo, Hongo, Tokyo, 113-0033, Japan

*Corresponding author: Masako Nakamura

email: mnakamura@tsc.u-tokai.ac.jp

tel.: +81-54-334-0411

fax: +81-54-337-0239

[#]Present address: School of Marine Science and Technology, Tokai University, Shimizu, Shizuoka 424-8610, Japan

Key words: Colonization, Population connectivity, Anthropogenic effects, Okinawa Trough

31 **Abstract**

32 Due to increasing anthropogenic impacts on deep-sea hydrothermal vent ecosystems, it is essential to
33 understand population structure and maintenance through larval recruitment and recovery of vent faunas
34 after disturbances. In this study, we quantified vent animal recruitment in the Okinawa Trough, in the
35 western Pacific Ocean. This is the first study to investigate recruitment patterns at a man-made
36 hydrothermal vent. Colonization plates were deployed at three sites. Site 1 manifested new hydrothermal
37 shimmering with small chimneys, white bacterial mats, and some alvinocaridid shrimp that arrived after
38 drilling. Site 2 showed no evidence of newly arrived foundation species after drilling, and Site 3 had pre-
39 existing animal communities in the vicinity of the new vent. Twenty-two months after deployment,
40 colonization plates were retrieved and recruited animals were inventoried. Species composition and
41 abundance differed among sites, but relatively high similarity in species composition was observed at
42 Sites 1 and 3, though not at Site 2. Newly established communities on the plates at Sites 1 and 2 (no pre-
43 existing fauna) showed lower species richness and abundance than at Site 3. Differences in abundance
44 and size-frequency distributions of major recruits on the plates (i.e. *Lepetodrilus nux*, *Bathymodiolus*
45 spp.) suggest the importance of reproductive and early life-history characteristics in spatial variability of
46 recruitment. *Lepetodrilus nux* populations established on the plates at Site 1 showed high genetic
47 connectivity. These results illustrate the importance of localized recruitment, which may have a
48 significant impact on sustainability of vent faunal populations, despite the existence of regional
49 metapopulations.

50

51

52 1. Introduction

53 In deep-sea hydrothermal vent ecosystems, catastrophic eruptions, tectonic disturbances, and
54 cessation of vent fluid discharges alter the geochemical environment and reset succession of benthic
55 faunal communities (e.g. Shank et al., 1998; Mullineaux et al., 2012). Colonization by larvae is therefore
56 critical to recovery of faunal communities and structure of those communities at the nascent stages.
57 Larval dispersal, colonization, and recruitment in hydrothermal vent ecosystems have been studied
58 mainly at eastern (e.g. Kelly et al., 2007; Thomson et al., 2003; Tunnicliffe et al., 1997) and northeastern
59 Pacific vents (e.g. Van Dover et al., 1988; Mullineaux et al., 1998, 2003, 2005, 2010, 2012; Pradillon et
60 al., 2005), and at the Mid-Atlantic Ridge (e.g. Comtet and Desbruyeres, 1998; Cuvelier et al., 2014;
61 Teixeira et al., 2012). These studies found that larvae of certain species have the potential to disperse
62 hundreds of kilometers (Epifanio et al., 1999; Marsh et al., 2001; Teixeira et al., 2012), but in other
63 species, larvae are retained near their natal sites (Thomson et al., 2003), with localized dispersal on a
64 scale of kilometers (Adams and Mullineaux, 2008). Moreover, colonizing species composition and
65 abundance could be affected by larval availability, resident fauna, and local environmental conditions, e.g.
66 vent fluid, temperature, sulfide concentration, pH (Cuvelier et al., 2014; Mullineaux et al., 2012). In spite
67 of advances achieved by these studies in the above areas, western Pacific vents have been poorly studied
68 relative to larval dispersal, colonization, and recruitment. Only one investigation has examined
69 recruitment processes at western Pacific vents (Nakamura et al., 2014). In addition, a recent study
70 demonstrated that distant vent communities in the western Pacific are potentially connected via larval
71 dispersal, due to strong directional currents (Mitarai et al., 2016). With growing scientific and commercial
72 interest in hydrothermal vents (Ramirez-Llodra et al., 2011; Van Dover, 2014), more studies at western
73 Pacific vents are urgently required.

74 The present study was performed after Integrated Ocean Drilling Program (IODP) Expedition 331
75 investigated the Iheya North hydrothermal field in the Okinawa Trough, Japan, to inventory diverse sub-
76 seafloor microbial communities associated with hydrothermal activities (Takai et al., 2012). Expedition
77 331 drilled at five sites and induced the formation of new hydrothermal vents. One of the new vents was
78 created at Site C0014, where hydrothermal fluid discharges had not been previously observed. Several
79 *Calyptogena s.l.* clams were found on the fine-grained, brownish sediment, but most (>90%) were dead
80 (only shells) (Kawagucci et al., 2013). On one of the seven holes at Site C0014, a triangular, gimbaled
81 guide base was mounted with stainless-steel pipe inserted into the hole to a depth of 136.7 m below the
82 seafloor for sampling the fluid. There, high-temperature hydrothermal fluid discharge was recorded for 25
83 months after drilling: the highest recorded temperature was 311°C. Eleven months after the drilling, the
84 seafloor became whitish, probably due to a microbial population in the clay mineral substrate. On the

85 white seafloor, several tiny chimneys and low density of galatheid crabs were observed around the site.
86 Afterward, galatheid crabs became more numerous and alvinocaridid shrimps and some *Paralvinella*
87 polychaetes were observed 16 months after drilling (Nakajima et al, 2015).

88 Post-drilling observations revealed changes in landscape, discharged fluid chemistry, and
89 immigration of megabenthos (Kawagucci et al., 2013; Nakajima et al., 2015). However, no study has ever
90 been made of recruitment at a new vent community. This drilling expedition presented an opportunity to
91 investigate recruitment of vent fauna, community establishment, and succession in western Pacific vents.
92 Therefore, we surveyed recruitment patterns around these artificial hydrothermal vents, comparing them
93 with those occurring at natural hydrothermal vents, to address the question: How do recruitment patterns
94 vary between sites with evidence of newly established venting and communities, with no evidence of
95 either, and with pre-existing communities?

96

97 **2. Materials & Methods**

98 This study was conducted at the Iheya North Field (27°45'-50'N, 126°53'-55'E) in the Okinawa Trough,
99 where IODP Expedition 331 was implemented in September 2010 (Takai et al., 2012) (Fig. 1a).
00 Recruitment surveys were conducted around Hole C0014G at Site C0014, one of drilling sites (Fig. 1b, c).
01 This area was chosen because animal communities were almost nonexistent before drilling. Several
02 *Calyptogena s.l.* clams were found on the fine-grained, brownish sediment, but most (>90%) were dead
03 (only shells). Discharges of hydrothermal fluids and vent endemic fauna were not observed before
04 drilling (Kawagucci et al., 2013; Nakajima et al., 2015); thus, this site was useful for investigating
05 establishment of community structure and succession in a hydrothermal vent community after discharge
06 of hydrothermal fluids commenced, and for observing the effects of anthropogenic disturbances to the
07 deep seafloor. In addition, Hole C0014G was continuously observed for changes in hydrothermal fluid
08 chemistry, landscape, and megabenthos species (Kawagucci et al., 2013; Nakajima et al., 2015).

09 Colonization plates were placed at three sites: a site where new hydrothermal shimmering was
10 occurring and some foundation species arrived after drilling (Site 1, ~0.24 km west-southwest of Hole
11 C0014G); a site with no new visible arrivals of foundation species on the soft substrate (Site 2, ~ 0.13 km
12 east-northeast of Hole C0014G); and a site near Hole C0014G, where vent communities existed in the
13 vicinity of natural hydrothermal fluid discharges before and after drilling (Site 3, ~ 0.47 km west of Hole
14 C0014G) (Fig. 1 and 2, Table 1). Colonization plates were made of Lexan plastic (10 cm x 10 cm x 64
15 mm thick). These were structured as "sandwiches," like those used at the East Pacific Rise (EPR)
16 (Mullineaux et al., 2010, 2012). Each set of colonization plates comprised three sandwiches made of six
17 plastic plates with ~1 cm gaps between plates (Fig. 2). Three sets of colonization plates were deployed at
18 each site during dives 537-538 of the ROV Kaiko-7000II during the KR 12-02 cruise of R/V Kairei,

19 conducted by JAMSTEC (Chief Scientist: Hiroyuki Yamamoto), 16 months after the drilling. Twenty-
20 one months later, two sets of plates at each site (named P1 and P2) were retrieved during dives 1591-1596
21 of the ROV *Hyper-Dolphin* during the NT13-22 cruise of R/V Natsushima, conducted by JAMSTEC
22 (Chief Scientist: Hiroyuki Yamamoto). Each set of plates was gently placed in an individual, sealed
23 collection box to prevent loss of colonizing organisms prior to recovery and to prevent intermixing of
24 samples.

25 All benthic animals on the plates were identified to the lowest taxonomic level possible, based on
26 morphology, and were fixed in 99.5% ethanol. In addition, benthic animals found in the sealed collection
27 boxes were included, since they fell off the plates. Abundances of all taxonomic groups were determined.
28 A diversity index, the Shannon-Weaver index (H'), was calculated using the vegan package for R (ver.
29 3.2.1, R Development Core Team 2014), and similarity of faunal composition among colonization
30 sandwiches was calculated using the Bray-Curtis similarity index after overall transformation of
31 abundance using the fourth root (PRIMER-E ver. 6, Clarke and Gorley, 2006). Results were plotted using
32 non-metric multi-dimensional scaling, with an overlay of similarities estimated by group-average cluster
33 analyses. In addition, abundances were also compared among sites, using univariate ANOVA, with the
34 Tukey HSD post-test, for commonly observed animals at all sites,

35 The most abundant species on colonization plates at Sites 1 and 3, *Lepetodrilus nux*, was analyzed for
36 size distribution and population genetic structure. Shell length of *L. nux*, the longest distance from the
37 shell apex to the anterior edge, was measured according to Nakamura et al. (2014). Size distribution was
38 compared among sets of plates (P1 and P2) and sites, using the Kolmogorov-Smirnov 2 sample test and
39 modal decomposition analysis with the Mclust program, R environment (R Development Core Team,
40 2014). Population genetic structure was compared among cohorts of each plate resulting from modal
41 decomposition analysis between two sites. DNA was extracted from foot tissue of *L. nux* using 10 μ L of
42 ice-cold PCR buffer II (500 mM KCl, 100 mM Tris-HCl, pH 8.3) and 1 μ L of a 10 mg/mL proteinase K
43 solution, with incubation for 3 hr at 55°C. Proteinase K was inactivated by incubating samples 5 min at
44 100 °C. Then 10 μ L of Gene-Releaser were added to each tube as per the Gene-Releaser cycling protocol
45 (Schizas et al. 1997). Each tube was centrifuged 1 min at 13,000 x g. Microsatellite markers developed by
46 Nakajima et al. (2017) were used for population genetic analysis (Table 2). Extracted DNA was amplified
47 using multiplex PCR, adding four primer sets to each PCR tube. Ampli Taq Gold® (Applied Biosystems)
48 was used with 20 μ M fluorescent primers, DNA template, and nuclease-free water for a total reaction
49 volume of 5 μ L (Table 2). Amplification was conducted under the following conditions: 95°C for 9 min
50 followed by 35 cycles at 95°C for 30 sec, 54°C for 30 seconds, 72°C for 1 min, and a final extension of 5
51 min at 72°C. Allelic variations of amplified products were analyzed with a DNA capillary sequencer
52 (3130xl Genetic Analyzer, Applied Biosystems). The GenAEx program (Ver. 6.5) (Peakall and Smouse

2006) was used to calculate the number of alleles, allele frequencies, the number of private alleles, genetic differentiation by means of analysis of molecular variance (AMOVA, Excoffier et al., 1992) and pairwise F_{ST} values. Principal coordinate analysis (PCA) was also performed with GenAlEx.

3. Results

3.1. Diversity of benthic communities on colonization plates

Species composition and abundance in communities established on plates varied among sites (Fig. 3; Table 3), but relatively high similarity was found between Sites 1 and 3 (63.181 - 70.675), relative to Site 2, which was slightly more similar to Site 1 (46.203 - 53.357) than to Site 3 (31.825 - 40.885) (Fig. 4).

Five genera/species were commonly observed at all sites (Polynoidae gen. sp., *Alvinocaris longirostris*, *Bathymacrea* spp., *Provanna subglabra*, *Margarites ryukyuensis*; Table 3). *Bathymacrea* spp. was significantly more abundant at Site 3, while *Provanna subglabra* was more abundant at Site 1 ($p < 0.05$, ANOVA and Tukey HSD test; Table S1). Three species were not found at Site 2 (*Shinkaia crosnieri*, *Bathymodiulus* spp., *Lepetodrilus nux*; Table 3), while neomphalids (Sasaki unpublished) were absent at Site 1. *Paralomis multispina* and *Leptochiton tenuidentatus* were only found at Site 2 (Table 3).

Both the greatest species richness and the largest number of individuals of benthic species were found at Site 3. The top three recruits on colonization plates at Site 3 were *Lepetodrilus nux*, *Bathymodiulus* spp., and *Bathymacrea* spp. (Fig. 3c). *Lepetodrilus nux* was the main recruit at Site 1, despite being absent from Site 2 (Fig. 3a, b). The latter two taxa were rarely encountered at Sites 1 and 2. Moreover, abundances of all benthic taxa were relatively low at Site 2. Polychaetes were relatively more abundant and some gastropods and decapods were observed (Fig. 3b). Since species richness and evenness drive diversity, the Shannon-Weaver index (H') was highest at Site 2 ($H' = 1.01$ at Site 1; 1.75 at Site 2; 1.42 at Site 3).

3.2. Size structure of recruited *Lepetodrilus nux*

Size-frequency distributions of *Lepetodrilus nux* populations settled on colonization plates at two sites in the Iheya North vent field varied between plates at Sites 1 and 3 ($p < 0.001$, Kolmogorov-Smirnov two sample test; Fig. 5). All populations were positively skewed (Table 4) while the populations at Site 1 were slightly skewed compared with those at Site 3. Plate 1 at Site 1 was especially populated with individuals 0.578 - 9.983 mm (shell length) with little skewness (Fig. 5a). However, more than 50 % of the individuals on Plate 2 at Site 1 and Plates 1 and 2 at Site 3 were less than 2 mm (shell length): 66.4 % for Plate 2, Site 1, and 98.7 % and 95.9 % for Plates 1 and 2 Site 3 (Fig. 5b-d).

85 Populations of *Lepetodrilus nux* on colonization plates demonstrated polymodal distributions (Table 4).
86 Based on modal decomposition analysis, numbers of modal components differed between Sites 1 and 3.
87 Two recruitment plates at Site 1 showed 2 - 3 cohorts while those at Site 3 had 8 - 9 cohorts. Modal
88 decomposition analysis showed 7 modal peaks for individuals less than 1 mm at Site 3.
89

90 **3.3 Genetic diversity and population differentiation of recruited *Lepetodrilus nux***

91 The mean number of alleles was 8.25 - 10.50 for eight loci of all size groups at Sites 1 and 3 (average
92 9.21 per size group) (Table 5). Mean observed heterozygosity for all loci was 0.515 - 0.727 for all size
93 groups at these two sites. The mean value for all size groups at those sites was 0.618 ± 0.020 (Standard
94 error, S.E.). Private alleles (PVA) were found for all size groups of two sites (4 - 11 in total). Deviation of
95 heterozygosity from Hardy-Weinberg equilibrium (HWE), shown by F_{IS} , ranged from -0.012 - 0.253 for
96 all size groups at Sites 1 and 3.

97 AMOVA estimated the variance among populations as 0.092 (3%) and within populations as 3.002
98 (97%) (total value: 3.093). There was a significant difference among populations ($p = 0.001$). Values of
99 pairwise population F_{ST} were less than 0.086 (Table 6). Some pairwise F_{ST} for size groups at two sites
00 showed significant differences ($p < 0.05$) (Table 6). In addition, principal coordinates analysis (PCA) did
01 not demonstrate remarkable genetic structure among cohorts at Sites 1 and 3 (Fig S1).
02

03 **4. Discussion**

04 Communities established on colonization plates at Sites 1 and 2, near artificial hydrothermal vent
05 fields, differed in composition and abundance three years after drilling. The major recruits differed:
06 *Lepetodrilus nux* was most abundant at Site 1 and polychaetes predominated at Site 2. Some gastropods,
07 including *Lepetodrilus* spp., and polychaetes have been observed as pioneer species in vent community
08 succession (Juan de Fuca Ridge: Tunnicliffe et al., 1997, East Pacific Rise: Mullineaux et al., 2010, 2012)
09 as these species are likely to mature early and to have continuous reproduction or multiple reproductive
10 periods per year (Tyler and Young, 1999; Tyler et al., 2008). Differences between newly-established sites,
11 Sites 1 and 2, may be related to hydrothermal fluid diffusion and resource limitation (Mullineaux et al.,
12 2003, 2012). Microbial mats resulting from hydrothermal fluid diffusion have been observed and some
13 benthic animals were already present around Site 1 at the time that plates were deployed, while the
14 bacterial mat was relatively poorly developed at Site 2, with no visible animals (Fig. 2). As microbial
15 mats are a likely food source for deposit feeders at vents (Tunnicliffe et al., 1997), they likely impact
16 animal recruitment at vent fields on a small, local scale. At the recovery of the plates, venting activities

17 were also observed at Site 1, but not at Site 2, where microbial mats were more developed compared with
18 the situation at the setting of the plates, but still much less than at Site 1. Therefore, abundance of recruits
19 was relatively low at Site 2, while some vent species, e.g., *Bathyacmaea* spp., were found among the
20 recruits. Moreover, communities on the plates at Site 1 showed similarity to those at Site 3, which existed
21 prior to drilling, but those at Site 2 did not. Recruits at Site 3 demonstrated the highest species richness
22 and abundance among the three sites. *Bathymodiolus* spp. and *Lepetodrilus nux* were especially abundant
23 at Site 3, and adult populations of these species were observed in the vicinity. Differences and similarities
24 in community structure and abundance may reflect environmental characteristics. For example, Site 1
25 changed from no conspicuous hydrothermal activity before drilling (similar to that at Site 2) to evident
26 hydrothermal activity after drilling (similar to Site 3).

27 All species observed at Site 1 were also found at Site 3; however, their relative abundances differed
28 between the two sites. *Lepetodrilus nux* was the most abundant species on the plates at both sites. In
29 contrast, *Bathymodiolus* spp. were much more abundant at Site 3, with very few at Site 1. For *L. nux*, the
30 size-frequency distribution demonstrated that recruits represent multiple cohorts at both sites, however,
31 patterns of size-frequency distribution and numbers of cohorts differed between these sites; Site 3 showed
32 high skewness toward small individuals with 8 - 9 cohorts, consisting mostly of sexually immature
33 individuals (< 2mm, Nakamura et al., 2014), while Site 1 showed less skewness and high variation in size
34 with 2 - 3 cohorts. This indicates intense, continuous recruitment at Site 3, but less frequent recruitment at
35 Site 1. Large numbers of new recruits of *Lepetodrilus nux* at both sites could be due to continuous
36 reproduction at certain periods of the year (Nakamura et al., 2014), resulting in potentially high larval
37 densities in the water column. *Lepetodrilus nux* forms a large metapopulation in the Okinawa Trough
38 (Nakamura et al., 2014). In addition, the present study demonstrated high genetic connectivity among *L.*
39 *nux* colonizing the plates. Therefore, high larval availability in the water makes *L. nux* well suited to
40 colonize new habitats. However, fewer cohorts existed at Site 1 than at Site 3, meaning that recruitment is
41 more stochastic at Site 1. Therefore, even if larval availability of *L. nux* may be relatively high, the
42 presence of a nearby adult population could promote regular and intense recruitment for maintenance of
43 populations, as observed at Site 3, where the size distribution was skewed toward small individuals. In
44 contrast to *L. nux*, *Bathymodiolus* spp. have been observed to reproduce annually (Dixon et al., 2006;
45 Tyler et al., 2007). In addition, synchrony of reproduction among individuals and among sites in a region
46 has been also observed for many *Bathymodiolus* spp. (*B. puteoserpentis*; Le Pennec and Beinger, 1997, *B.*
47 *nov. sp.*; Comtet and Desbruyères, 1998, "*B.*" *childressi*; Eckelbarger and Young, 1999; Tyler et al., 2007,
48 *B. azoricus*; Comtet et al., 1999; Dixon et al., 2006). Assuming that *Bathymodiolus* spp. in the Okinawa
49 Trough have similar reproductive characteristics (reproductive biology of *Bathymodiolus* spp. in the

Okinawa Trough is little known), bathymodiolin larvae could be seasonally abundant, arriving in a given area less frequently than those of *L. nux*. Seasonal reproduction could limit recruits, as observed at Site 1, where adult *Bathymodiolus* were not observed nearby. In contrast, a higher number of recruits was observed at Site 3 under the same conditions. The difference between Sites 1 and 3 was the presence of adults. Site 3 had abundant adult *Bathymodiolus*, suggesting a high potential for self-recruitment at Site 3.

As seen, larvae of these species tend to recruit in proximity to their natal populations, even though they are presumably capable of long-distance dispersal because of long planktonic larval duration resulting from reduced metabolic rates in colder water (Mitarai et al., 2016). At the EPR, hydrodynamics drastically change with distance from the bottom, and this could retain some larvae close to the source (Mullineaux et al., 2005; Adams and Mullineaux 2008). Moreover, long-distance dispersal could be associated with diffusion of larvae, resulting in recruitment to distant communities and also in higher mortality rates during dispersal (Cowen et al., 2000); therefore, self-recruitment due to local retention could play an important role in population maintenance and recovery for deep-sea animals. Local hydrodynamics must be investigated to identify mechanisms of larval retention and to evaluate the magnitudes of local retention and long-distance dispersal.

65

66 **5. Conclusions**

The present study quantified invertebrate recruitment at an artificial hydrothermal vent field composed of seafloor boreholes at the Iheya North Knoll, the first study to examine benthic recruitment at a new vent field. Faunal composition and abundance varied spatially, possibly in relation to microhabitat. Reproductive and larval biology undoubtedly also affect abundance of recruits. Species that reproduce continuously recruit into new habitats more rapidly after environmental disturbances. Our findings indicate that because of these factors, community establishment processes around impacted areas vary, relying on recruitment from remote locations. High recruitment and successive cohorts in the vicinity of existing adult communities suggest that local recruitment is significant, even though many vent fauna species establish metapopulations over large areas. These findings indicate that remote communities are potential sources for repopulation of devastated communities, but that neighboring communities are more important for community sustainability.

78

79 **Acknowledgements**

We are grateful to the ROV operating teams (Kaiko 7000 II and Hyper-Dolphin) and the crews and on board scientists of R/V Kairei and Natsushima for their assistance. We thank Ms. Susan W. Mills and Dr.

82 Stace E. Beaulieu cordially for giving the advices about colonization plates. We thank OIST's technical
83 editor, Dr. Steven D. Aird, for polishing this manuscript.

84 Funding: This research was supported by the Canon Foundation and Grants-in-Aid for Scientific
85 Research No. 24570037 from the Japan Society for the Promotion of Science. We gratefully acknowledge
86 support from the Okinawa Institute of Science and Technology Graduate University to the Marine
87 Biophysics Unit.

88

89 **References**

90 Adams, D.K., Mullineaux, L.S., 2008. Supply of gastropod larvae to hydrothermal vents reflects transport
91 from local larval sources. *Limnol. Oceanogr.* 53, 1945-1955.

92 Clark, K.R., Gorley, R.N., 2006. PRIMER v6: User Manual/Tutorial. PRIMER-E, Plymouth.

93 Comtet, T., Desbruyères, D., 1998. Population structure and recruitment in mytilid bivalves from the
94 Lucky Strike and Menez Gwen hydrothermal vent fields (37°17' N and 37°50' N on the Mid-Atlantic
95 Ridge). *M.E.P.S.* 163, 165-177.

96 Comtet, T., Le Pennec, M., Desbruyères, D., 1999. Evidence of a sexual pause in *Bathymodiolus azoricus*
97 (Bivalvia: Mytilidae) from hydrothermal vents of the Mid-Atlantic Ridge. *J. Mar. Biol. Ass. U.K.* 79,
98 1149-1150.

99 Cowen, R.K., Lwiza, K.M.M., Sponaugle, S., Paris, C.B., Olson, D.B., 2000. Connectivity of marine
00 populations: open or closed? *Science* 287, 857-859.

01 Cuvelier, D., Beesau, J., Ivanenko, V.N., Zeppilli, D., Sarradin, P., Sarrazin, J., 2014. First insights into
02 macro- and meiofaunal colonisation patterns on paired wood/slate substrata at Atlantic deep-sea
03 hydrothermal vents. *Deep-Sea Research Part I* 87, 70-81.

04 Dixon, D.R., Lowe, D.M., Miller, P.I., Villemin, G.R., Colaço, A., Serrão-Santos, R., Dixon, L.R.J.,
05 2006. Evidence of seasonal reproduction in the Atlantic vent mussel *Bathymodiolus azoricus*, and an
06 apparent link with the timing of photosynthetic primary production. *J. Mar. Biol. Ass. U.K.* 86, 1363-
07 1371.

08 Eckelbarger, K.J., Young, C.M., 1999. Ultrastructure of gametogenesis in a chemosynthetic mytilid
09 bivalve (*Bathymodiolus childressi*) from a bathyal, methane seep environment (northern Gulf of Mexico).
10 *Mar. Biol.* 135, 635-646.

11 Epifanio, C.E., Perovich, G., Dittel, A.I., Cary, S.C., 1999. Development and behavior of megalopa larvae
12 and juveniles of the hydrothermal vent crab *Bythograea thermydron*. M.E.P.S. 185, 147-154.

13 Excoffier, L., Smouse, P.E., Quattro, J.M., 1992. Analysis of molecular variance inferred from matrix
14 distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics
15 131, 479-491.

16 Kawagucci, S., Miyazaki, J., Nakajima, R., Nozaki, T., Yakaya, Y., Kato, Y., Shibuya, T., Konno, U.,
17 Nakaguchi, Y., Hatada, K., Hirayama, H., Fujikura, K., Furushima, Y., Yamamoto, H., Watsuji, T.,
18 Ishibashi, J., Takai, K., 2013. Post-drilling changes in fluid discharge pattern, mineral deposition, and
19 fluid chemistry in the Iheya North hydrothermal field, Okinawa Trough. Geochemistry, Geophysics,
20 Geosystems 14, 4774-4790.

21 Kelly, N., Metaxas, A., Butterfield, D., 2007. Spatial and temporal patterns of colonization by deep-sea
22 hydrothermal vent invertebrates on the Juan de Fuca Ridge, NE Pacific. Aquat. Biol. 1, 1-16.

23 Le Pennec, M., Beninger, P.G., 1997. Ultrastructural characteristics of spermatogenesis in three species
24 of deep-sea hydrothermal vent mytilids. Can. J. Zool. 75, 308-316.

25 Marsh, A.G., Mullineaux, L.S., Young, C.M., Manahan, D.T., 2001. Larval dispersal potential of the
26 tubeworm *Riftia pachyptila* at deep-sea hydrothermal vents. Nature 411, 77-80.

27 Mitarai, S., Watanabe, H., Nakajima, Y., Shchepetkin, A.F., McWilliams, J.C., 2016. Quantifying
28 dispersal from hydrothermal vent fields in the western Pacific Ocean. P.N.A.S. 113, 2976-2981.

29 Mullineaux, L.S., Mills, S.W., Goldman, E., 1998. Recruitment variation during a pilot colonization study
30 of hydrothermal vent (9°50'N, East Pacific Rise). Deep-Sea Research II 45, 441-464.

31 Mullineaux, L.S., Peterson, C.H., Micheli, F., Mills, S.W., 2003. Successional mechanism varies along a
32 gradient in hydrothermal fluid flux at deep-sea vents. Ecol. Monogr. 73(4), 523-542.

33 Mullineaux, L.S., Mills, S.W., Sweetman, A.K., Beaudreau, A.H., Metaxas, A., Hunt, H.L., 2005.
34 Vertical, lateral and temporal structure in larval distributions at hydrothermal vents. M.E.P.S. 293, 1-16.

35 Mullineaux, L.S., Adams, D.K., Mills, S.W., Beaulieu, S.E., 2010. Larvae from afar colonize deep-sea
36 hydrothermal vents after a catastrophic eruption. P.N.A.S. 107, 7829-7834.

37 Mullineaux, L.S., Le Bris, N., Mills, S.W., Henri, P., Bayer, S.R., Secrist, R.G., Siu, N., 2012. Detecting
38 the influence of initial pioneers on succession at deep-sea vents. PLoS One 7(12), e50015.

39 Nakajima, R., Yamamoto, H., Kawagucci, S., Takaya, Y., Nozaki, T., Chen, C., Fujikura, K., Miwa, T.,
40 Takai, K., 2015. Post-drilling changes in seabed landscape and megabenthos in a deep-sea hydrothermal
41 system, the Iheya North Field, Okinawa Trough. PLoS ONE 10(4), e0123095.

42 Nakajima, Y., Shinzato, C., Khalturina, M., Nakamura, M., Watanabe, H.K., Nakagawa, S., Satoh, N.,
43 Mitarai, S., 2017. Isolation and characterization of novel polymorphic microsatellite loci for the deep-sea
44 hydrothermal vent limpet, *Lepetodrilus nux*, and the vent-associated squat lobster, *Shinkaia crosnieri*.
45 Marine Biodiversity, 1-8.

46 Nakamura, M., Watanabe, H., Sasaki, T., Ishibashi, J., Fujikura, K., Mitarai, S., 2014. Life history traits
47 of *Lepetodrilus nux* in the Okinawa Trough, based upon gametogenesis, shell size, and genetic variability.
48 M.E.P.S. 505, 119-130.

49 Peakall, R., Smouse, P.E., 2006. GenAlEx6: genetic analysis in Excel. Population genetic software for
50 teaching and research. Mol. Ecol. Notes 6, 288-295.

51 Pradillon, F., Zbinden, M., Mullineaux, L.S., Gaill, F., 2005. Colonisation of newly-opened habitat by a
52 pioneer species, *Alvinella pompejana* (Polychaeta: Alvinellidae), at East Pacific Rise vent sites. M.E.P.S.
53 302, 147-157.

54 Ramirez-Llodra, E., Tyler, P.A., Baker, M.C., Bergstad, O.A., Clark, M.R., Escobar, E., Levin, L.A.,
55 Menot, M., Rowden, A.A., Smith, C.R., Van Dover, C.L., 2011. Man and the last great wilderness:
56 Human impact on the deep sea. PLoS One 6(7), e22588.

57 R Development Core Team, 2014. R: a language and environment for statistical computing. R Foundation
58 for Statistical Computing, VIENNA, www.R-project.org.

59 Schizas, N.V., Street, G.T., Coull, B.C., Chandler, G.T., Quattro, J.M., 1997. An efficient DNA
60 extraction method for small metazoans. Molecular Marine Biology and Biotechnology 6, 381-383.

61 Shank, T.M., Fornari, D.J., Von Damm, K.L., Lilley, M.D., Haymon, R.M., Lutz, R.A., 1998. Temporal
62 and spatial patterns of biological community development at nascent deep-sea hydrothermal vents
63 (9°50'N, East Pacific Rise). Deep-Sea Research Part II 45, 465-515.

64 Takai, K., Motti, M.J., Nielsen, S.H., the Expedition 331 Scientists, 2012. IODP Expedition 331: Strong
65 and expansive seafloor hydrothermal activities in the Okinawa Trough. Scientific Drilling 13, 19-27.

66 Teixeira, S., Serrao, E.A., Arnaud-Haond, S., 2012. Panmixia in a fragmented and unstable environment:
67 the hydrothermal shrimp *Rimicaris exoculata* disperses extensively along the Mid-Atlantic Ridge. PLoS
68 One 7(6), e38521.

69 Thomson, R.E., Mihaly, S.F., Rabinovich, A.B., McDuff, R.E., Veirs, S.R., Stahr, F.R., 2003.
70 Constrained circulation at Endeavour ridge facilitates colonization by vent larvae. *Nature* 424, 545-549.

71 Tunnicliffe, V., Embley, R.W., Holden, J.F., Butterfield, D.A., Massoth, G.J., Juniper, S.K., 1997.
72 Biological colonization of new hydrothermal vents following an eruption on Juan de Fuca Ridge. *Deep-*
73 *Sea Research Part I* 44, 1627-1644.

74 Tyler, P.A., Young, C.M., 1999. Reproduction and dispersal at vents and cold seeps. *J. Mar. Biol. Ass.*
75 *U.K.* 79, 193-208.

76 Tyler, P.A., Young, C.M., Dolan, E., Arellano, S.M., Brooke, S.D., Baker, M., 2007. Gametogenic
77 periodicity in the chemosynthetic cold-seep mussel "*Bathymodiolus*" *childressi*. *Mar. Biol.* 150, 829-840.

78 Tyler, P.A., Pendlebury, S., Mills, S.W., Mullineaux, L., Eckleberger, K.J., Baker, M., Young, C.M.,
79 2008. Reproduction of gastropods from vents on the East Pacific Rise and the Mid-Atlantic Ridge.
80 *Journal of Shellfish Research* 27, 107-118.

81 Van Dover, C.L., Berg, C.J., Turner, R.D., 1988. Recruitment of marine invertebrates to hard substrates at
82 deep-sea hydrothermal vents on the East Pacific Rise and Galapagos spreading. *Deep-Sea Research Part A*
83 35, 1833-1849.

84 Van Dover, C.L., 2014. Impacts of anthropogenic disturbances at deep-sea hydrothermal vent
85 ecosystems: A review. *Marine Environmental Research* 102, 59-72.

86 **Figure Legends**

87 Fig 1. Locations of research sites showing (a) the Iheya North hydrothermal field, (b) IODP 331 Drilling
88 sites (C0013 to C0017, stars) in the Iheya-North hydrothermal field, and (c) drilling holes at C0014.

89 Fig 2. Colonization plates at: a) Site 1, b) Site 2, c) Site 3. These photographs were taken during the dive
90 of ROV Kaiko 7000 II.

91 Fig 3. Differences in community composition of benthic animals at three sites 22 months after
92 deployment of colonization plates. a) Site 1, b) Site 2, c) Site 3.

93 Fig 4. Similarity of benthic community composition between colonization plates (P1 and P2) at Sites 1, 2
94 and 3 after 22 months of deployment. Numbers and contour lines show degrees of similarity.

95 Fig 5. Size-frequency distribution of *Lepetodrilus nux* on colonization plates (P1 and P2) deployed at
96 Sites 1 and 3 for 22 months. a) Site 1, b) Site 3.

97 Fig S1. Results of principal coordinates analysis (PCA) from the covariance matrix with data
98 standardization calculated by GenAlEx for genetic structure of *Lepetodrilus nux* among size cohorts
99 (showing M) of each colonization plate (P1 and P2) at Sites 1 and 3. The first two axes explain 45.88 %
00 of variation (the first explains 28.95% and the second does 16.93%).

01

02 **Table Legends**

03 Table 1. Latitudes and longitudes of study sites with presence (+) and absence (-) of visible vent fauna.

04 Table 2. Microsatellite markers of *Lepetodrilus nux* used in this study.

05 Table 3. Benthos observed on colonization plates at Sites 1, 2, and 3 in the Iheya North hydrothermal
06 field.

07 Table 4. Modal components showing different cohorts (M1 to 9), estimated from the length-frequency
08 distributions of *Lepetodrilus nux* on colonization plates (P1 and P2) at different vent sites in the North
09 Iheya Knoll. μ : mean length (mm), σ : standard deviation, π : proportions, $()$: skewness.

10 Table 5. Population genetics indices for *Lepetodrilus nux* among cohorts (showing Modal Components
11 from Table 4) of each colonization plate (P1 and P2) between Sites 1 and 3 at the North Iheya Knoll:
12 numbers of analyzed *Lepetodrilus nux* (N), numbers of alleles (N_A), observed (H_O), expected (H_E)
13 heterozygosities, deviation index from Hardy-Weinberg equilibrium (F_{IS}), and the number of private
14 alleles (PVA) for each locus and size cohort of each colonization plate. F_{IS} values underlined in italics
15 indicate significant deviations from Hardy-Weinberg equilibrium at $p < 0.05$.

16 Table 6. *Lepetodrilus nux* pairwise Fst values estimated among cohorts (showing Modal Components
17 from Table 4) of each colonization plate (P1 and P2) for Sites 1 and 3 in the North Iheya Knoll. Statistical
18 significance was calculated, and probability values based on 999 permutations are shown. Statistical
19 significance levels for all pairwise test were $p < 0.05$. Values underlined in italics are significant.
20 Table S1. Univariate ANOVA of benthos settled on colonization plates.

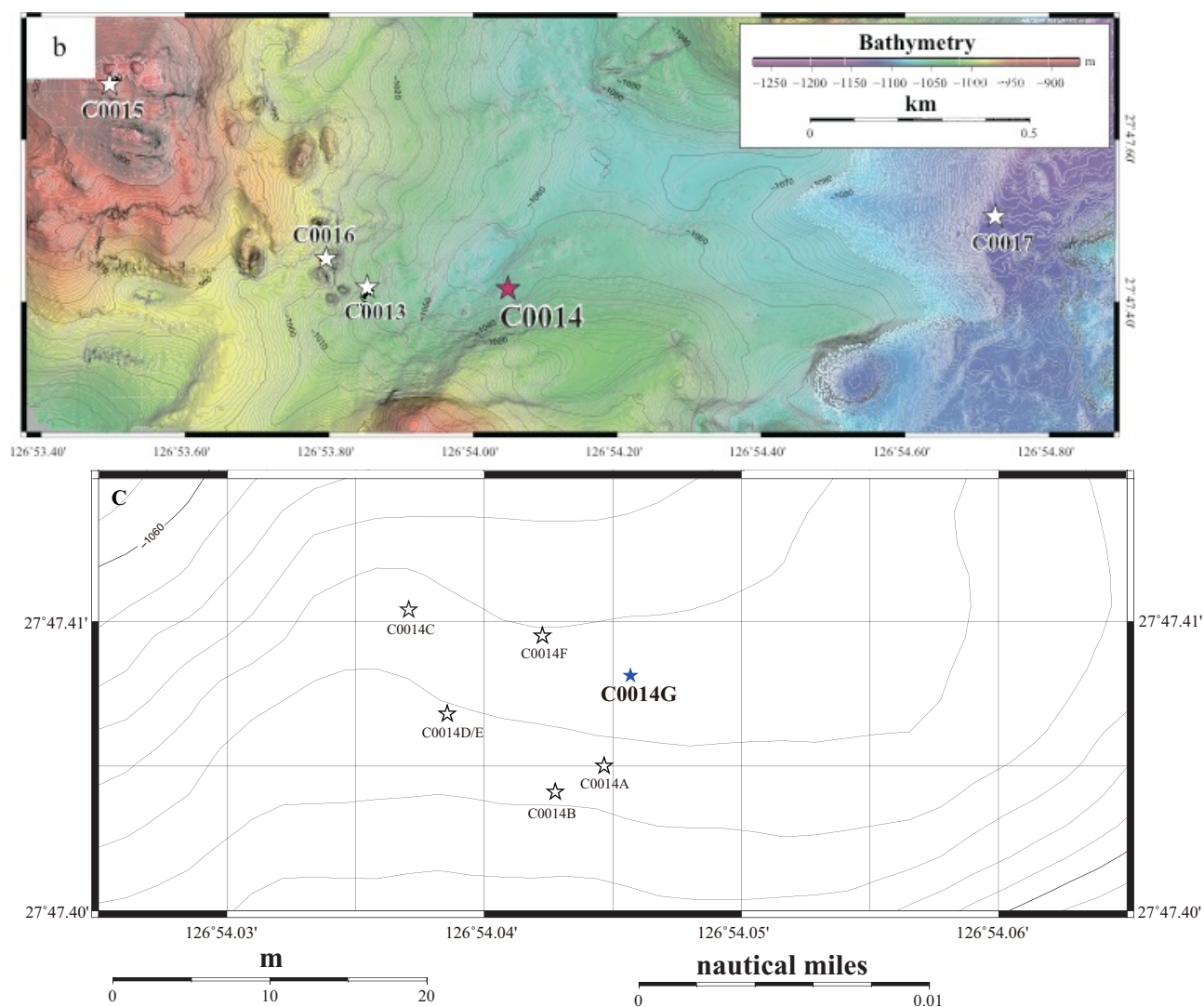
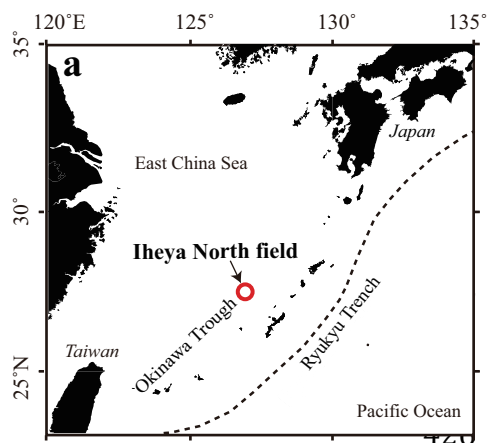
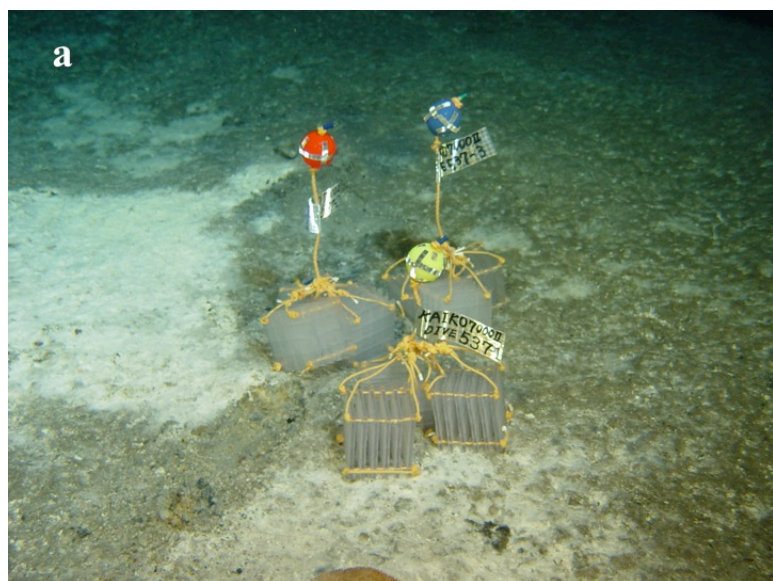
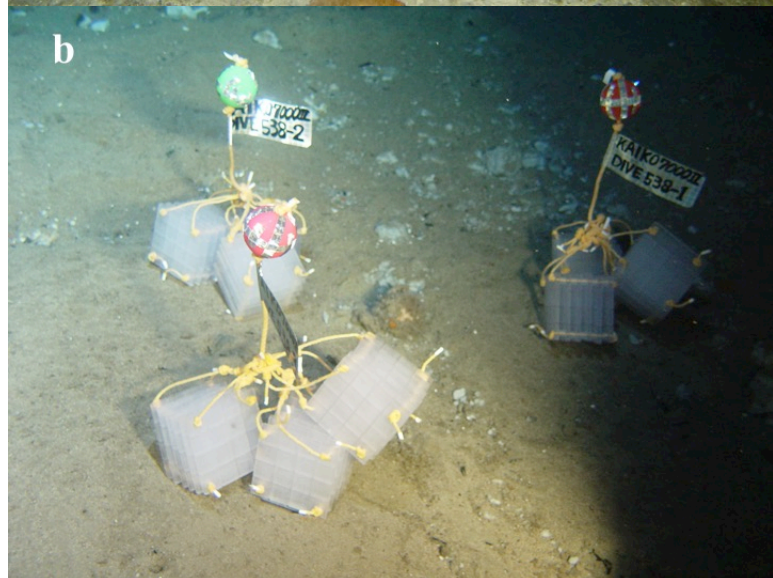


Fig 1. Locations of research areas showing (a) the Iheya-North hydrothermal field, (b) IODP 331 Drilling sites (C0013 to C0017, stars) in the Iheya North hydrothermal field, and (c) drilling holes at C0014.

42

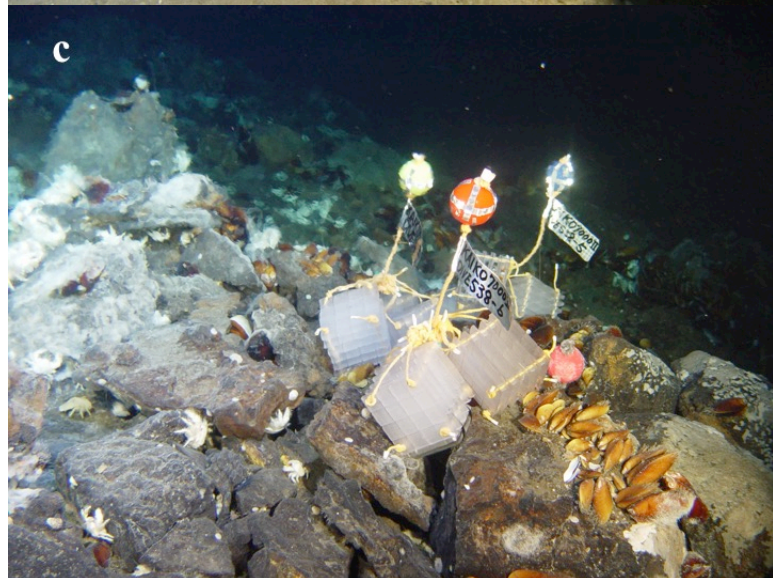


43



44

45

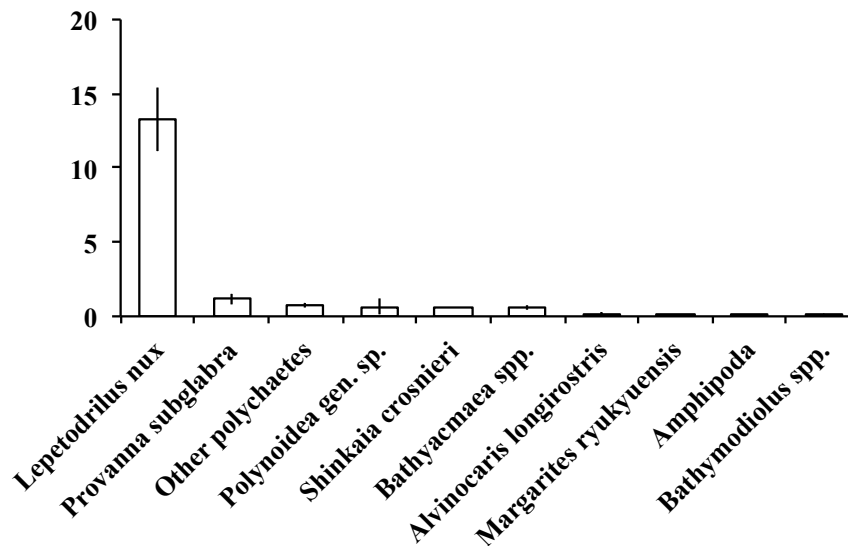


46

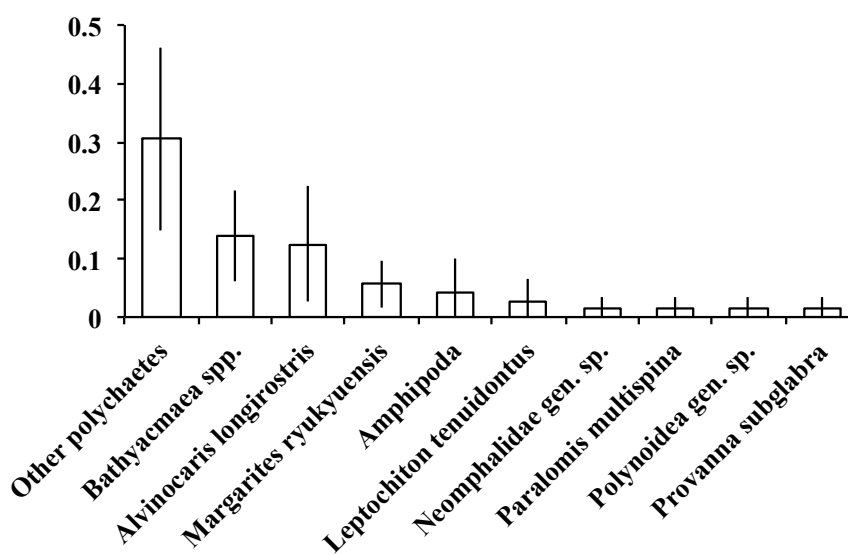
47

Fig 2. Colonization plates at: a) Site 1, b) Site 2, c) Site 3. These photographs were taken during the dive of ROV Kaiko 7000 II.

a) Site 1



b) Site 2



c) Site 3

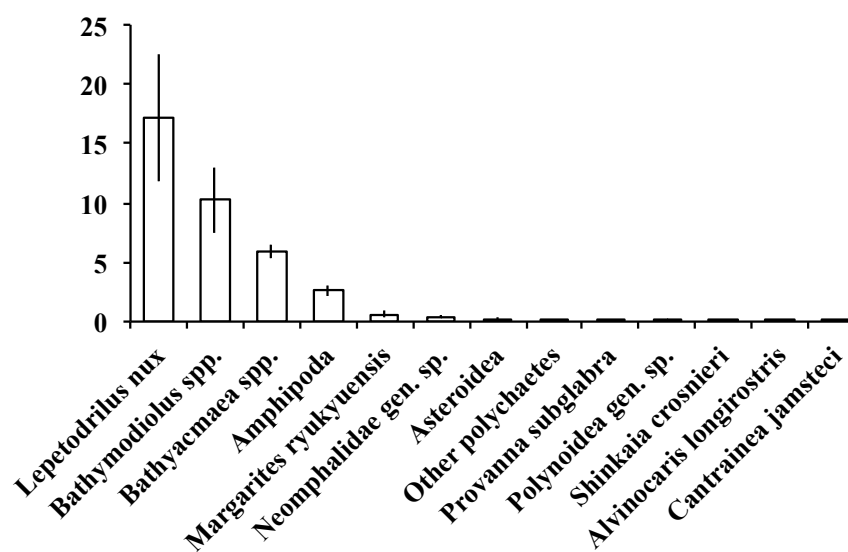
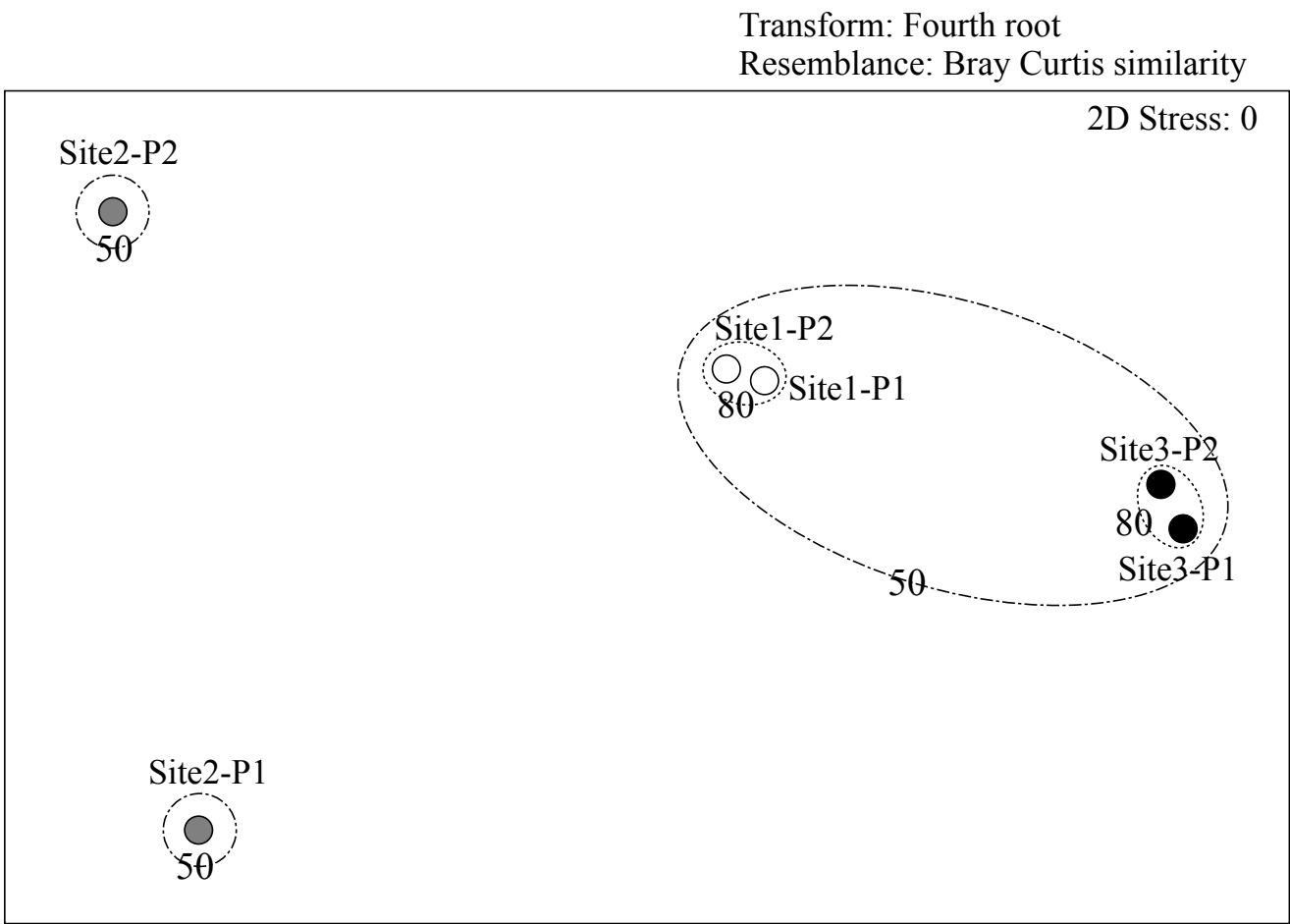


Fig 3. Differences in community composition of benthic animals at three sites 22 months after deployment of colonization plates. a) Site 1, b) Site 2, c) Site 3.

51



52

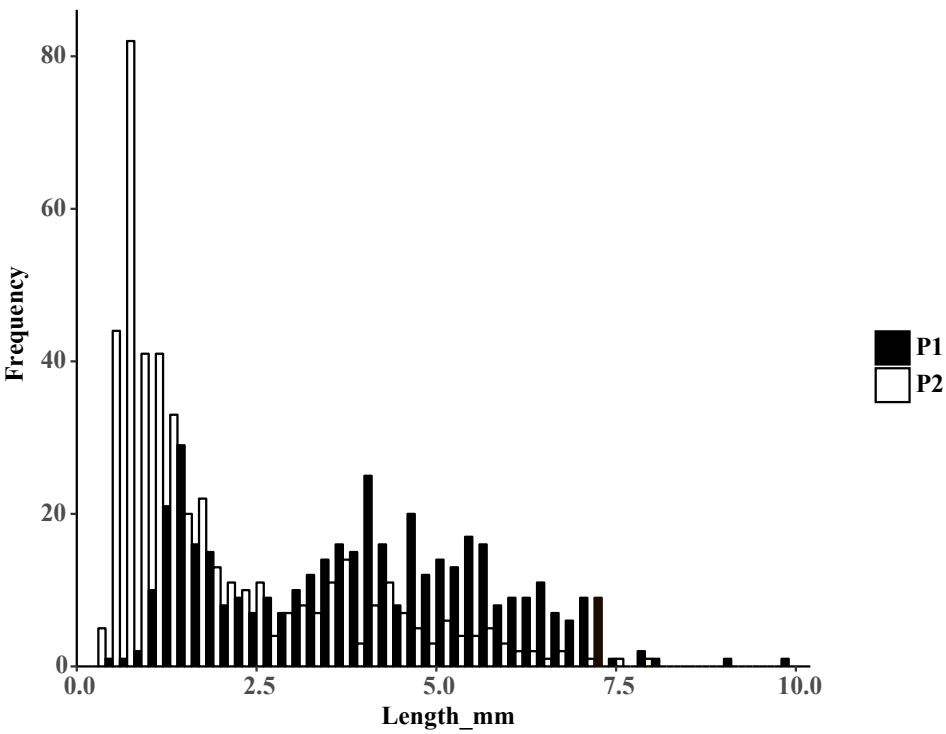
53

54

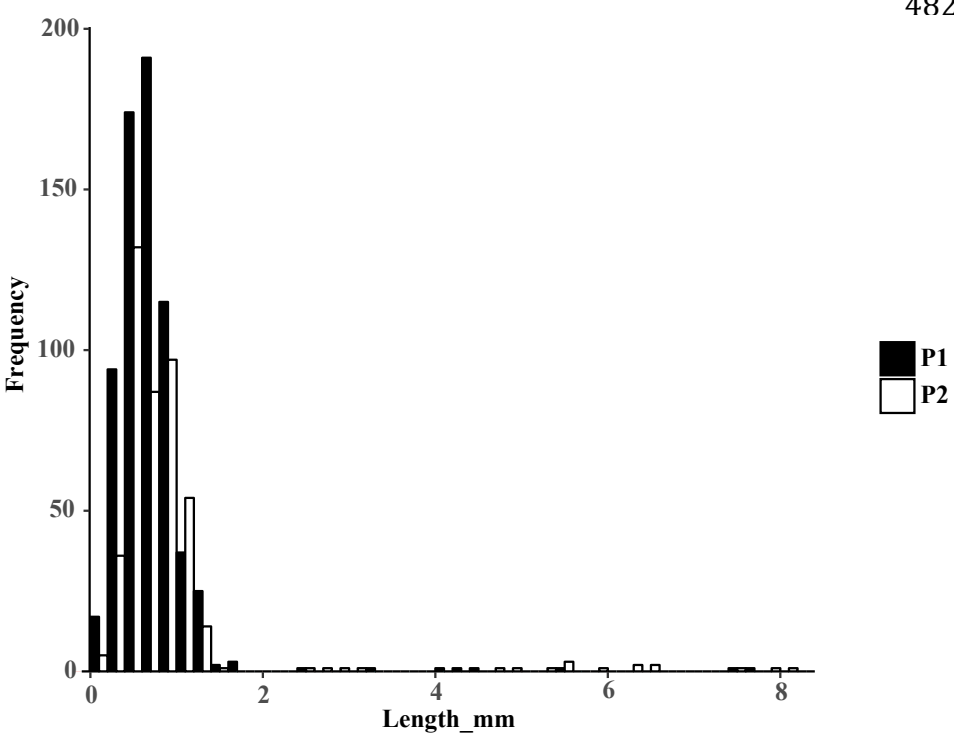
55 Fig 4. Similarity of benthic community composition between colonization plates (P1 and P2) among Sites
56 1, 2 and 3 after 22 months of deployment. Numbers and contour lines show degrees of similarity.

57

58 a)



81 b)



95 Fig 5. Size-frequency distribution of *Lepetodrilus nux* on colonization plates (P1 and P2) deployed at
96 Sites 1 and 3 for 22 months. a) Site 1, b) Site 3.

Principal Coordinates (PCoA)

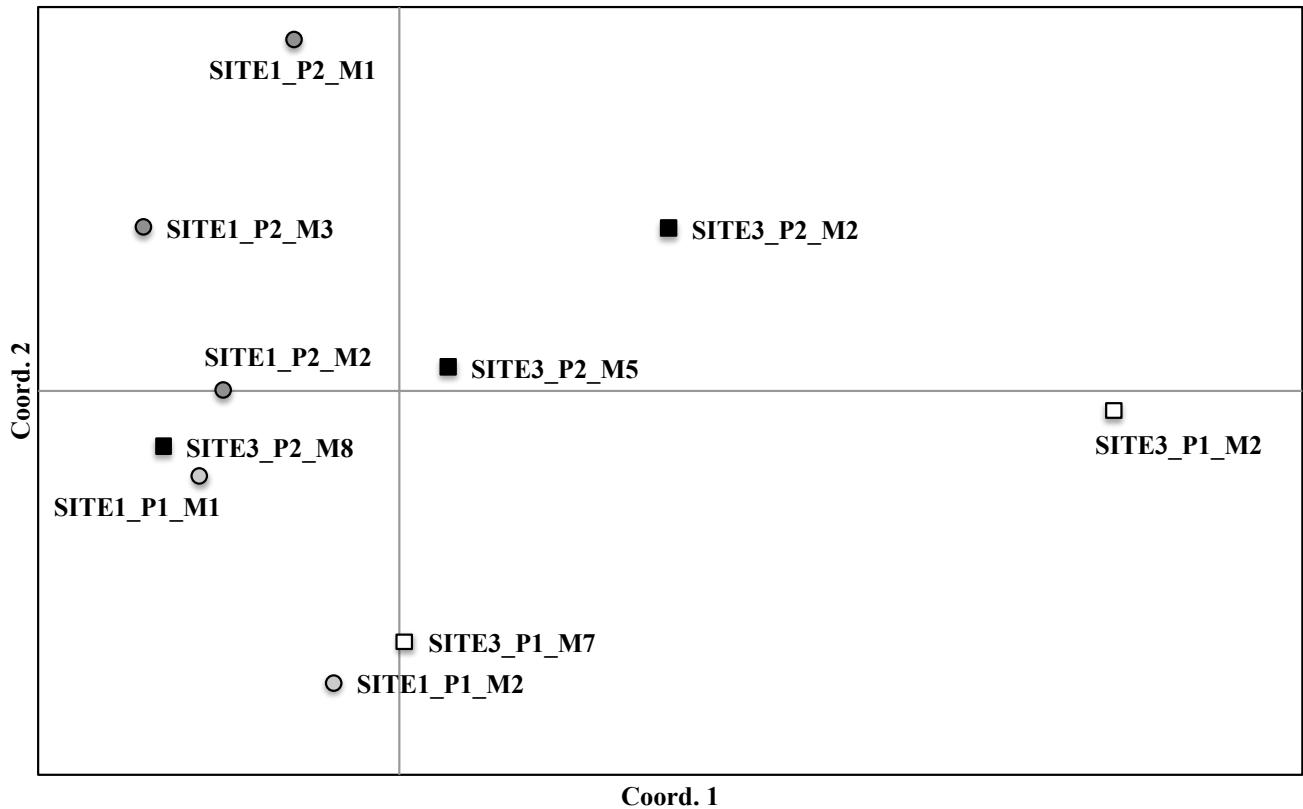


Fig S1. Results of PCA from the covariance matrix with data standardization calculated by GenAlEx for genetic structure of *Lepetodrilus nux* among size cohorts (showing M) of each colonization plate (P1 and P2) at Sites 1 and 3. The first two axes explain 45.88 % of variation (the first explains 28.95% and the second does 16.93%).

03 Table 1. Latitudes and longitudes of study sites with presence (+) and absence (-) of visible vent fauna.

Site	Latitudes	Longitudes	Depth	Visible Vent Fauna	
				before drilling	after drilling
SITE 1	27 ° 47' 24.78" N	126 ° 54' 2.22" N	1,056 m	—	+
SITE 2	27 ° 47' 25.14" N	126 ° 54' 3.42" N	1,056 m	—	—
SITE 3	27 ° 47' 25.02" N	126 ° 53' 59.28" N	1,060 m	+	+

04

05

06

07

08

09

10 Table 2. Microsatellite markers of *Lepetodrilus nux* used in this study.

Locus	Primer sequence (5'-3')	Accession No.
LN12	F: U19-TCGGATCGGGGTTGG	AB971596
	R: GACCTTGAGCTGGGTTTCG	
LN16	F: ATCATTATCGGTGAAATTCG	AB971597
	R: U19-TGCATAGAACGTTTGG	
LN30	F: U19-ACCGTTACACAGGGATGC	AB971604
	R: CCACGATTTTCTTAAAGGC	
LN42	F: CCCTTGTGAAGGTACTTGCG	AB971608
	R: U19-ACAAGCATGGGAGCATGG	
LN83	F: U19-CTGAATGCAGCCCTGG	AB971625
	R: TCATGAAAAGGGTGTATTGG	
LN84	F: U19-ACCACTGAGCACCTTCGG	AB971626
	R: CCTGGAGGAGGGACAAGG	
LN94	F: U19-TTGGTGAAATGTCATGAGG	AB971630
	R: AGTGGGTGGATATGGCG	
LN95	F: U19-GGTGAAATATTCCCACTGC	AB971631
	R: CACGGTTAAAACTGAATGGG	

11

12 Table 3. Benthos observed on colonization plates at Sites 1, 2, and 3 in the Iheya North hydrothermal
 13 field.

14

Phylim	Class	Order	Family	Genus / Species	Density per dm ² ± SD (Total on two sets of plates)					
					SITE1		SITE2		SITE3	
Annelida	Polychaeta	Phyllodocida	Polynoidae	<i>Polynoidae</i> gen. sp.	0.64 ± 0.51	(46)	0.01 ± 0.02	(1)	0.08 ± 0.08	(6)
	Other polychaetas				0.77 ± 0.20	(56)	0.31 ± 0.16	(22)	0.17 ± 0.08	(12)
Arthropoda	Malacostraca	Amphipoda			0.08 ± 0.00	(6)	0.04 ± 0.06	(3)	2.57 ± 0.41	(185)
	Malacostraca	Decapoda	Alvinocarididae	<i>Alvinocaris longirostris</i>	0.19 ± 0.04	(14)	0.13 ± 0.10	(9)	0.07 ± 0.10	(5)
	Malacostraca	Decapoda	Lithodidae	<i>Paralomis multispina</i>	–	(0)	0.01 ± 0.02	(1)	–	(0)
	Malacostraca	Decapoda	Munidopsidae	<i>Shinkaia crosnieri</i>	0.63 ± 0.02	(45)	–	(0)	0.08 ± 0.00	(6)
	Asteroidea				–	(0)	–	(0)	0.22 ± 0.24	(16)
Mollusca	Bivalvia	Mytiloidea	Mytilidae	<i>Bathymodiolus</i> spp.	0.07 ± 0.10	(5)	–	(0)	10.22 ± 2.75	(736)
	Gastropoda		Colloniidae	<i>Cantrainea jamsteci</i>	–	(0)	–	(0)	0.01 ± 0.02	(1)
	Gastropoda		Lepetodrilidae	<i>Lepetodrilus nux</i>	13.25 ± 2.12	(954)	–	(0)	17.11 ± 5.34	(1232)
	Gastropoda		Neomphalidae	<i>Neomphalidae</i> gen. sp.	–	(0)	0.01 ± 0.02	(1)	0.38 ± 0.10	(27)
	Gastropoda		Pectinodotidae	<i>Bathyaemaea</i> spp.	0.57 ± 0.14	(41)	0.14 ± 0.08	(10)	5.88 ± 0.53	(423)
	Gastropoda		Provannidae	<i>Provanna subglabra</i>	1.18 ± 0.37	(85)	0.01 ± 0.02	(1)	0.15 ± 0.02	(11)
	Gastropoda		Turbinidae	<i>Margarites ryukyuensis</i>	0.13 ± 0.06	(9)	0.06 ± 0.04	(4)	0.60 ± 0.26	(43)
	Polyplocophora	Neoloricata	Leptochitonidae	<i>Leptochiton tenuidentus</i>	–	(0)	0.03 ± 0.4	(2)	–	(0)

15

16

Table 4. Modal components showing different cohorts (M1 to 9), estimated from the length-frequency distributions of *Lepetodrilus nux* on colonization plates (P1 and P2) at different vent sites in the North Iheya Knoll. μ : mean length (mm), σ : standard deviation, π : proportions, (): skewness.

Vent Site	Plate Number	Modal components									
		Modal peaks									
			M1	M2	M3	M4	M5	M6	M7	M8	M9
SITE 1	P1 (0.142)	μ	1.518	4.633	–	–	–	–	–	–	–
		σ	0.277	1.546	–	–	–	–	–	–	–
		π	0.208	0.792	–	–	–	–	–	–	–
	P2 (1.271)	μ	0.647	1.203	3.621	–	–	–	–	–	–
		σ	0.105	0.386	1.468	–	–	–	–	–	–
		π	0.256	0.371	0.373	–	–	–	–	–	–
	P1 (7.738)	μ	0.169	0.441	0.536	0.608	0.700	0.750	0.941	3.658	–
		σ	0.000	0.085	0.000	0.002	0.015	0.006	0.183	2.183	–
		π	0.026	0.308	0.087	0.078	0.093	0.076	0.313	0.019	–
SITE 3	P2 (4.879)	μ	0.326	0.494	0.527	0.587	0.710	0.858	0.995	1.125	5.095
		σ	0.063	0.019	0.000	0.012	0.031	0.029	0.034	0.100	1.922
		π	0.094	0.133	0.047	0.131	0.180	0.148	0.093	0.130	0.044

Table 5. Population genetic indices for *Lepetodrilus nux* among cohorts (showing Modal Components from Table 4) of each colonization plate (P1 and P2) between Sites 1 and 3 at the North Iheya Knoll: numbers of analyzed *Lepetodrilus nux* (N), numbers of alleles (N_A), observed (H_O), expected (H_E) heterozygosities, deviation index from Hardy-Weinberg equilibrium (F_{IS}), and the number of private alleles (PVA) for each locus and size cohort of each colonization plate. F_{IS} values underlined in italics indicate significant deviations from Hardy-Weinberg equilibrium at $p < 0.05$.

Locus		SITE1_P1_M1	SITE1_P1_M2	SITE1_P2_M1	SITE1_P2_M2	SITE1_P2_M3	SITE3_P1_M2	SITE3_P1_M7	SITE3_P2_M2	SITE3_P2_M5	SITE3_P2_M8	Total
LN12	N	25	18	26	28	16	25	24	18	20	23	223
	Na	11	14	11	14	12	9	14	11	10	8	
	Ho	0.739	0.765	0.500	0.739	0.938	0.500	0.708	0.529	0.526	0.579	
	He	0.778	0.865	0.737	0.791	0.818	0.770	0.855	0.827	0.792	0.677	
	F_{IS}	0.050	0.116	<u>0.321</u>	0.066	-0.146	0.351	0.172	<u>0.360</u>	<u>0.336</u>	<u>0.145</u>	
	PVA		3		2			3	1			
LN16	Na	12	12	10	10	9	14	14	9	11	10	
	Ho	0.880	0.722	0.760	0.846	0.875	0.680	0.667	0.611	0.737	0.909	
	He	0.850	0.887	0.807	0.852	0.795	0.857	0.888	0.824	0.856	0.844	
	F_{IS}	-0.036	0.186	0.058	0.007	<u>-0.101</u>	0.206	<u>0.249</u>	<u>0.258</u>	<u>0.139</u>	<u>-0.077</u>	
	PVA		1			1	1		1		1	
	Na	11	13	9	14	8	6	11	8	12	11	
LN30	Ho	0.696	0.944	0.571	0.720	0.933	0.308	0.773	0.600	1.000	0.900	
	He	0.850	0.872	0.748	0.839	0.833	0.775	0.850	0.795	0.871	0.813	
	F_{IS}	0.181	-0.083	<u>0.236</u>	<u>0.142</u>	-0.120	<u>0.603</u>	0.091	<u>0.245</u>	-0.148	-0.108	
	PVA	2								3	1	
	Na	11	12	12	13	14	11	11	7	10	12	
	Ho	0.680	0.500	0.500	0.520	0.750	0.385	0.238	0.500	0.533	0.773	
LN42	He	0.855	0.866	0.809	0.769	0.869	0.891	0.880	0.836	0.858	0.833	
	F_{IS}	<u>0.205</u>	<u>0.422</u>	<u>0.382</u>	<u>0.324</u>	0.137	<u>0.568</u>	<u>0.729</u>	0.402	<u>0.378</u>	0.072	
	PVA	3	1	1	1	4		1			1	
	Na	5	4	8	6	2	5	3	5	4	3	
	Ho	0.348	0.188	0.409	0.250	0.067	0.318	0.091	0.286	0.158	0.182	
	He	0.309	0.277	0.392	0.230	0.064	0.353	0.241	0.260	0.323	0.168	
LN83	F_{IS}	-0.125	0.324	<u>-0.045</u>	-0.086	-0.034	0.099	<u>0.622</u>	-0.098	<u>0.511</u>	-0.080	
	PVA	1	1	4			1		2	1		
	Na	10	9	8	10	7	11	8	10	12	5	
	Ho	0.565	0.375	0.714	0.679	0.875	0.609	0.609	0.563	0.722	0.550	
	He	0.558	0.682	0.737	0.661	0.680	0.696	0.751	0.793	0.765	0.555	
	F_{IS}	<u>-0.014</u>	<u>0.450</u>	0.031	-0.027	-0.287	0.125	<u>0.190</u>	0.291	0.056	0.009	
LN84	PVA	2	1	1		1	1					
	Na	6	5	6	8	6	4	6	4	4	7	
	Ho	0.522	0.625	0.435	0.571	0.375	0.591	0.783	0.563	0.556	0.591	
	He	0.502	0.594	0.372	0.646	0.539	0.532	0.684	0.512	0.566	0.574	
	F_{IS}	-0.040	-0.053	-0.168	0.115	0.304	-0.111	<u>-0.144</u>	-0.099	0.019	-0.029	
	PVA	1			1	1					1	
LN94	Na	11	7	12	9	11	11	11	12	7	14	
	Ho	0.870	0.750	0.818	0.786	1.000	0.727	0.957	0.625	0.833	0.905	
	He	0.836	0.773	0.819	0.797	0.850	0.885	0.859	0.791	0.813	0.878	
	F_{IS}	-0.041	0.030	0.001	<u>0.014</u>	-0.177	<u>0.179</u>	-0.113	<u>0.210</u>	-0.025	-0.031	
	PVA	2		2			2		3		4	
	Na	9.625	9.500	9.500	10.500	8.625	8.875	9.750	8.250	8.750	8.750	9.213 (± 0.213)
Mean	Ho	0.662	0.609	0.588	0.639	0.727	0.515	0.603	0.535	0.633	0.674	0.618 (± 0.020)
	He	0.692	0.727	0.678	0.698	0.681	0.720	0.751	0.705	0.731	0.668	0.705 (± 0.008)
	F_{IS}	0.023	0.174	0.102	0.069	-0.053	0.253	0.225	0.196	0.158	-0.012	
	PVA	11	7	8	4	7	5	4	7	4	8	

Table 6. *Lepetodrilus nux* pairwise Fst values estimated among cohorts (showing Modal Components from Table 4) of each colonization plate (P1 and P2) for Sites 1 and 3 in the North Iheya Knoll. Statistical significance was calculated, and probability values based on 999 permutations are shown. Statistical significance levels for all pairwise test were $p < 0.05$. Values underlined in italics are significant.

	SITE1_P1_M1	SITE1_P1_M2	SITE1_P2_M1	SITE1_P2_M2	SITE1_P2_M3	SITE3_P1_M2	SITE3_P1_M7	SITE3_P2_M2	SITE3_P2_M5	SITE3_P2_M8
SITE1_P1_M1										
SITE1_P1_M2	0.000									
SITE1_P2_M1	0.014	<i>0.021</i>								
SITE1_P2_M2	0.007	0.009	<i>0.017</i>							
SITE1_P2_M3	<i>0.019</i>	<i>0.021</i>	<i>0.028</i>	<i>0.019</i>						
SITE3_P1_M2	<i>0.086</i>	<i>0.075</i>	<i>0.060</i>	<i>0.080</i>	<i>0.102</i>					
SITE3_P1_M7	<i>0.011</i>	0.003	<i>0.026</i>	<i>0.016</i>	<i>0.018</i>	<i>0.069</i>				
SITE3_P2_M2	<i>0.056</i>	<i>0.048</i>	<i>0.028</i>	<i>0.050</i>	<i>0.062</i>	<i>0.017</i>	<i>0.038</i>			
SITE3_P2_M5	0.009	0.009	0.007	<i>0.010</i>	<i>0.018</i>	<i>0.043</i>	0.007	<i>0.018</i>		
SITE3_P2_M8	0.004	0.002	<i>0.015</i>	0.004	<i>0.010</i>	<i>0.077</i>	<i>0.008</i>	<i>0.048</i>	<i>0.012</i>	

51

Table S1. Univariate ANOVA of benthos settled on colonization plates.

Taxon	F	<i>p</i>
<i>Alvinocaris longirostris</i>	1.13	0.431
<i>Bathymacra</i> spp.	66.76	0.003
<i>Margarites ryukyuensis</i>	7.42	0.069
Polynoidae gen. sp.	6.95	0.075
<i>Provanna subglabra</i>	48.79	0.005

53