

Life history traits of *Lepetodrilus nux* in the Okinawa Trough, based upon gametogenesis, shell size, and genetic variability

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ABSTRACT: Because marine benthic animals usually form metapopulations via larval dispersal, a firm grasp of life-history traits is essential to understand the larval dispersal processes and population dynamics of marine benthic communities. Deep-sea hydrothermal vents are ephemeral environments, but they support benthic communities of high biomass. *Lepetodrilus nux* is one of the most abundant and widely distributed limpets in deep-sea hydrothermal vent fields in the Okinawa Trough. In the present study, life-history traits of *L. nux* were investigated at 4 vent sites: Izena Hole, Minami-Ensei, Irabu, and Hatoma Knolls. New distributions of *L. nux* in the Minami-Ensei and Irabu Knolls are described, and genetic analyses suggest that *L. nux* exists as a metapopulation in the Okinawa Trough vent fields, with higher genetic diversity in the northern part and lower diversity in the southern part. Histological data reveal that *L. nux* is gonochoristic, and that it employs internal fertilization and possibly continuous reproduction. Individuals mature at relatively smaller size compared with those of other *Lepetodrilus* species in the East Pacific Rise (EPR) and Mid-Atlantic Ridge (MAR). Population size-frequency distributions differed among vent sites. Size-frequency differences among populations may result from complex environmental variability, such as hydrothermal vent activity and ocean current systems, which influence dispersal and succession of deep-sea vent fauna. These life history traits suggest that *L. nux* is an opportunistic colonizer, and explain its wide distribution in patchy and ephemeral deep-sea vent fields.

KEY WORDS: Metapopulation · Hydrothermal vents · Limpets · Reproduction · Recruitment

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INTRODUCTION

Deep-sea hydrothermal vents comprise patchy, ephemeral environments in marine ecosystems. Hundreds to thousands of kilometers may separate individual vents, the life spans of which may vary from years to centuries (Macdonald 1982, Grassle 1985, Hessler et al. 1988, Lalou 1991, Haymon et al. 1993). In addition, temporal fluctuations in venting

activity caused by volcanic eruptions or tectonic events sometimes cause abrupt changes in the geochemical milieu, resulting in rapid habitat turnover and changes in organismal distribution (Shank et al. 1998, Mullineaux et al. 2010).

In spite of their inherent instability, hydrothermal vents support extensive animal communities with biomasses that are comparable to those of rainforests and coral reefs (e.g. Gaill et al. 1997, Govenar et al.

2004, Podowski et al. 2009). Most animals inhabiting hydrothermal vents have biphasic life cycles that include sessile or demersal adult stages, and planktonic larval stages. Planktonic larval stages could be the only highly mobile period during the life cycle, with larval transport potentially strongly influenced by ocean currents (Adams et al. 2012). In addition, most vent species harness energy from the vent flow (Sarrazin & Juniper 1999, Van Dover 2000). Therefore, faunal colonization and subsequent succession are strongly affected by dynamics of hydrothermal vents, which can cause local extinctions and new colonization events. In addition to the association with vent activity and hydrodynamics, spatial and temporal variation in animal distributions undoubtedly have a profound relationship on life-history traits, especially reproductive modes, larval dispersal, and subsequent recruitment processes (Adams et al. 2012).

Animals inhabiting hydrothermal vents are expected to possess *r*-type life history traits, including rapid growth, early and continuous reproduction, and high dispersal ability due to the nature of their habitat, although environmental instability is not the only selective pressure in vent ecosystems (Desbruyeres & Laubier 1983). Moreover, such generalizations may not always be applicable (e.g. Young 2003, Adams et al. 2012). To date, using direct and indirect methods, life-history characteristics of vent animals from the East Pacific Rise (EPR) and the Mid-Atlantic Ridge (MAR) have been examined (reviewed by Tyler & Young 1999, 2003, Young 2003, Adams et al. 2012). In these areas, vent animals tend to be mostly continuous reproducers, but there is some evidence of periodic reproduction. Dispersal ability is estimated to be high, but it could be limited by local hydrodynamics. Moreover, larval recruitment patterns, as estimated from size-frequency distributions, appear to be species-specific; some show potentially episodic larval supply while others manifest potentially continuous recruitment. In contrast, life-history characteristics of vent species in the western Pacific are little understood (Miyake et al. 2010, Watanabe et al. 2010).

In the western Pacific, deep-sea hydrothermal vent systems are geologically very different from those of mid-ocean ridge systems, as in the Atlantic Ocean (Ishibashi & Urabe 1995). In the western Pacific, most hydrothermal vent fields are associated with submarine volcanoes and back-arc basins. As well as a variety of tectonic settings, most vent fields associated with back-arc systems are located in the spreading part of the seafloor, and are surrounded by island

arcs, which are potential barriers for dispersing larvae that could otherwise leave the back-arc basins (Watanabe et al. 2005). Such geological characteristics may affect larval transport, faunal colonization, and subsequent succession. To date, genetic connectivity analysis has revealed that some vent animals in the Okinawa Trough are genetically different from those of populations in other chemosynthetic environments in Japanese waters (Watanabe et al. 2010). It may be that larval dispersal of vent animals in the Okinawa Trough is largely restricted to the Trough, except for tubeworms, which apparently do disperse to other regions (Watanabe et al. 2010). Comprehensive studies of vent animal life-history traits are required to understand colonization and succession of vent fauna in back-arc basin systems.

Limpets of the Genus *Lepetodrilus* are considered important components of vent communities due to their abundance and because they are pioneers in terms of ecological succession (Shank et al. 1998, Bates et al. 2005, Mullineaux et al. 2010). They are one of the most abundant animals at hydrothermal vents worldwide (Bates et al. 2005), in association with larger invertebrates (e.g. Craddock et al. 1997, Tsurumi & Tunnicliffe 2001). Examples include *L. elevatus* with tubeworms *Riftia pachyptila* and *Tevnia jerichonana* at the EPR, *L. fucensis* with tubeworms *Ridgeia* spp. at the Juan de Fuca Ridge, *L. atlanticus* with mussels *Bathymodiolus puteoserpentis* in the MAR, and *L. nux* with squat lobsters *Shinkaia crosnieri* and mussels *B. japonicus* and *B. platifrons* in the Okinawa Trough. Their broad distributions may partly reflect their feeding mechanisms, since feeding habits vary among species. Morphological evidence suggests that *Lepetodrilus* species tend to be suspension feeders and grazers (Fretter 1988). Additionally, *L. fucensis* and *L. gordensis* harbor epibiotic bacteria on their gill lamellae (Bates 2007a,b), which help *Lepetodrilus* to survive in a variety of habitats. Moreover, *Lepetodrilus* species have been reported as pioneer taxa following volcanic eruptions (e.g. Shank et al. 1998, Mullineaux et al. 2010). Their reproductive traits favor opportunistic colonization, maturing at small sizes (2.3 and 2.4 mm for males and females, respectively) and reproducing quasi-continuously (Bayer et al. 2011). Recent genetic analysis has revealed cryptic species of *Lepetodrilus* in the EPR (Johnson et al. 2008). Therefore, it is important to investigate ecological features of *Lepetodrilus* with accurate species identification, including DNA barcoding.

In spite of their ecological importance, *Lepetodrilus* species in hydrothermal vent fields in the Okinawa

Trough have scarcely been studied in terms of life history characteristics and population dynamics. Currently, 2 *Lepetodrilus* species, *L. nux* and *L. japonicus*, have been recognized in the Okinawa Trough (Okutani et al. 1993, Fujikura et al. 2008). However, *L. japonicus* is known from only 2 individuals at Minami-Ensei Knoll (Okutani et al. 1993). In contrast, *L. nux* is relatively abundant; one recent investigation found more than 1000 ind. m⁻² (H. Watanabe et al. unpubl. data). *Lepetodrilus nux* is almost ubiquitous in this region, from the northeast North Knoll of Iheya Ridge to the southeast Dai-Yon Yonaguni Knoll, but it has not been observed at Minami-Ensei Knoll, the northernmost deep-sea vent in the Okinawa Trough, or at Irabu Knoll, one of the southernmost (Okutani et al. 1993, Sasaki et al. 2005, 2010, Fujikura et al. 2008). Therefore, the present study was undertaken to (1) observe the reproductive characteristics of *L. nux* through examination of gonads, and (2) describe population structure and estimate recruitment dynamics based upon size-frequency and molecular analyses, in comparison with *Lepetodrilus* spp. found in other regions.

MATERIALS AND METHODS

Lepetodrilus species were sampled at Minami-Ensei Knoll, Izena Hole, Irabu Knoll, and Hatoma Knoll in the Okinawa Trough, using a suction sampler mounted on the Remotely Operated Vehicle (ROV) 'Hyper-Dolphin', during the NT11-20 cruise of R/V 'Natsushima' in September-October 2011 (Fig. 1, Table 1). During this cruise, we also conducted a ROV dive at Yoron Hole (Fig. 1); however, no *Lepetodrilus* were found at this site. When *Lepetodrilus* were collected, they were dissected into different parts for the following analyses. The shell was removed in order to make shell length measurements. Gonads were separated for determination of sexual maturity, and radula and muscle

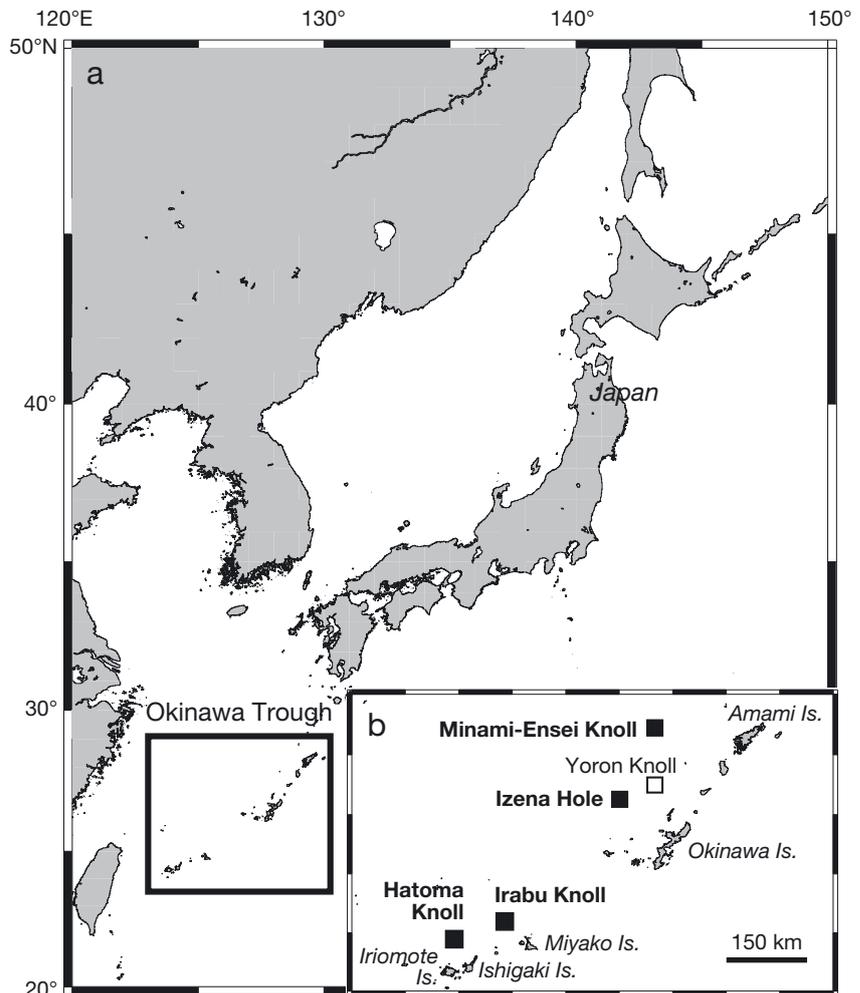


Fig. 1. Location of research sites showing (a) the Okinawa Trough and (b) target vent fields. Black boxes show vent fields where *Lepetodrilus* specimens were collected. White box shows vent field where *Lepetodrilus* was not collected

Table 1. Location of sampling sites in the Okinawa Trough. Sea water temperature (average \pm SD) was measured at 3 to 4 points around each sampling site using AERO-USB (JFE Alec). Measurement times were 16.6 ± 5.9 min (SD)

Vent site	Geographic coordinates	Depth (m)	Sea water temperature (°C)
Minami-Ensei Knoll	28° 23.47' N, 127° 38.39' E	~700	7.3 \pm 0.3
Izena Hole (Hakurei site)	27° 14.81' N, 127° 04.08' E	~1617	5.7 \pm 2.1
Irabu Knoll	25° 13.75' N, 124° 52.20' E	~1650	4.7 \pm 0.6
Hatoma Knoll	24° 51.47' N, 123° 50.50' E	~1477	4.7 \pm 1.0

were used for morphological and genetic species identification, respectively. Gonads were preserved in 10% seawater/formalin on board the ship and transferred to 70% ethanol in the laboratory. Other body parts were preserved in 99.5% ethanol.

Radulae were separated from soft parts under a stereomicroscope, cleaned in diluted (~5%) bleach, air-dried on stubs, coated with platinum-palladium, and imaged with scanning electron microscopy (Keyence VE-8800) at the University Museum of the University of Tokyo. Since the only *Lepetodrilus* species found at Minami-Ensei Knoll previously was *L. japonicus*, radulae of *Lepetodrilus* specimens found at Minami-Ensei Knoll were examined. Seven specimens were randomly chosen and examined (size range: 5.99 to 11.40 mm). *L. japonicus* and *L. nux* can be distinguished reliably by the acuteness of the cusps of rachidian and lateral teeth. In *L. japonicus*, cusps of all teeth are sharply pointed (Okutani et al. 1993, Fig. 33), while *L. nux* have blunt radular teeth (see Fig. 3).

Genomic DNA was extracted using a DNeasy Tissue Extraction Kit (QIAGEN) from ethanol-fixed limpet foot tissues. The target DNA sequence was mitochondrial cytochrome oxidase *c* subunit 1 (COI), which was amplified using primers LCO1490 and HCO2198 (Folmer et al. 1994), after GeneReleaser (BioVenture) treatment. Reactions were performed in 20 μ l reaction mixtures containing genomic DNA, (final concentration ~1 ng/ μ l), *ExTaq* buffer, 0.3 mM of dNTP mix, 1 mM of each primer, and 0.75 U of *ExTaq* DNA polymerase (TaKaRa). Cycling parameters were 94°C for 2 min, 30 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 1 min and a final elongation at 72°C for 2 min. PCR products were purified using Exo-SAP-IT (United States Biochemical) and were sequenced bidirectionally using the same primers as for PCR, and using a BigDye Terminator (BDT) Kit ver. 3.1 (Applied Biosystems). BDT products were purified by column chromatography (EdgeBio-systems) or X-Terminator (Applied Biosystems) before sequencing analysis on an ABI 3130 automated DNA sequencer (Applied Biosystems). Forward and reverse sequences were assembled into contigs with ATGC (Genetyx Version 6) and aligned by eye. All sequences were registered with DDBJ as well as EMBL and GenBank (accession numbers: AB820805–AB820839). Sequences were then used for population-level analyses, including estimation of parsimonious haplotype networks with TCS 1.21, and genetic diversity indices (\hat{H} , π , and mismatch distribution) for each population. The obtained mismatch distribution was compared to the simulated mismatch distribution by chi-squared test to examine confidence of estimated τ -value. Genetic differentiation among the 4 populations was examined by pairwise F_{ST} and Exact Test. Pairwise F_{ST} analysis was carried out for 2000 permutations, with the Kimura-2P distance

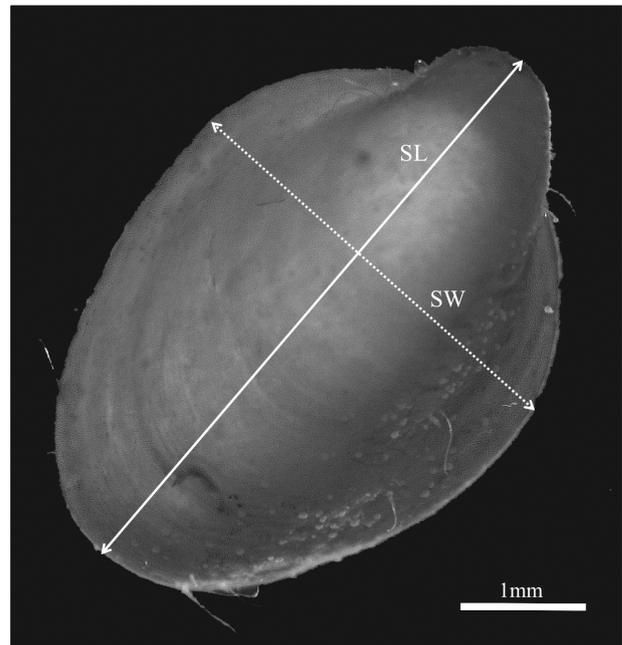


Fig. 2. Light micrograph of *Lepetodrilus nux*, indicating the shell measurements. SL = shell length (the longest length from the apex to the anterior edge); SW = shell width (the widest point along the dorsal side, perpendicular to SL)

matrix. The length of the Markov chain in the Exact Test was set as 100000 steps. All the above population-level analyses were carried out with Arlequin ver. 3.5.1.2 (Excoffier et al. 2010)

Shell lengths were measured as the longest distance from the shell apex to the anterior edge (SL), and shell width (SW) was measured at the widest point along the dorsal side (Fig. 2), to compare the size distribution and recruitment patterns among sites. Length-frequency distributions were analyzed using SL because SL and SW are strongly correlated ($R^2 = 0.97$). Length-frequency distributions were compared with a normal distribution using the Shapiro-Wilks *W* test. These were compared between vent sites using Kolmogorov-Smirnov 2-sample tests. Modal decompositions were assessed using the Mclust program developed for the R environment (R ver. 3.0.2).

Gonads were prepared for histological analysis to determine minimum mature size and reproductive continuity. An average of 8 gonads (4 to 20) were randomly chosen for every 1 mm size class in shell length, for all sites. Tissues were dehydrated in a graded ethanol series, cleared in xylene, and embedded in paraffin. Histological sections (6 μ m) were cut and stained with hematoxylin and eosin. All histological sections were photographed with an Olympus

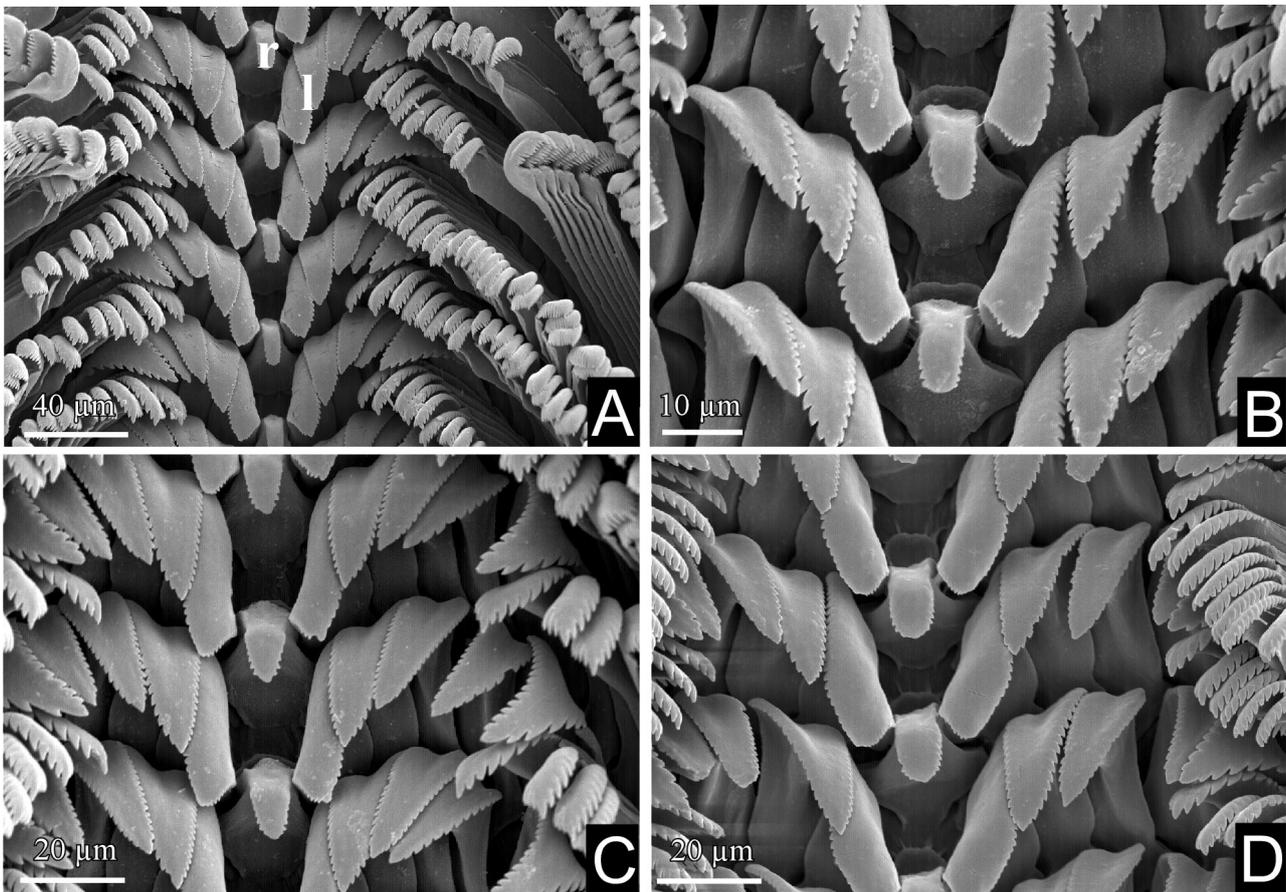


Fig. 3. Radulae of *Lepetodrilus nux*. A–D are different individuals, randomly chosen from the Minami-Ensei Knoll samples; r = rachidian tooth, l = lateral teeth

BX53 microscope equipped with an Olympus DP72 digital camera. Sexual maturity was defined as the stage at which a specimen had spermatozoa or vitellogenic oocytes in its gonads. For females, proportions of different developmental stages of oocytes were analyzed. Three sections were randomly chosen for each specimen, with sections separated by ~60 to 100 µm. Average proportions of vitellogenic oocytes were calculated from the 3 sections. They were compared among individuals among sites over size classes (S4 = 4 to <5 mm, S5 = 5 to <6 mm, S6 = 6 to <7 mm, S7 = 7 to <8 mm) using analysis of variance (ANOVA). Due to the small number of samples from a certain size class in a certain area, the following separate analyses were conducted: (1) comparison among individuals over sites (size classes were pooled) and (2) comparison among individuals over size classes (sites were pooled). Mean proportions of mature oocytes (VO) and immature oocytes (other than VO) were compared for sexually-mature females among size classes and among sites using fac-

torial ANOVA. All ANOVA tests were conducted with appropriate transformations to meet the assumptions of normality and of homogeneity of variance, where necessary. Data normality was checked using Shapiro-Wilks tests ($p > 0.05$), and homogeneity of variance was assessed using Levene's test ($p > 0.05$). Tukey's HSD test was used for multiple comparisons. Statistical analyses were performed with software R ver. 3.0.2.

RESULTS

Radular morphology confirmed that all specimens studied were *Lepetodrilus nux* (Okutani et al.1993), having blunt radular teeth (Okutani et al. 1993, their Figs. 28 & 29; our Fig. 3).

In total, 154 sequences (612 bp length) were obtained for the 4 sites. The haplotype network showed that *L. nux* populations in the Okinawa Trough share a single major haplotype, with some peripheral hap-

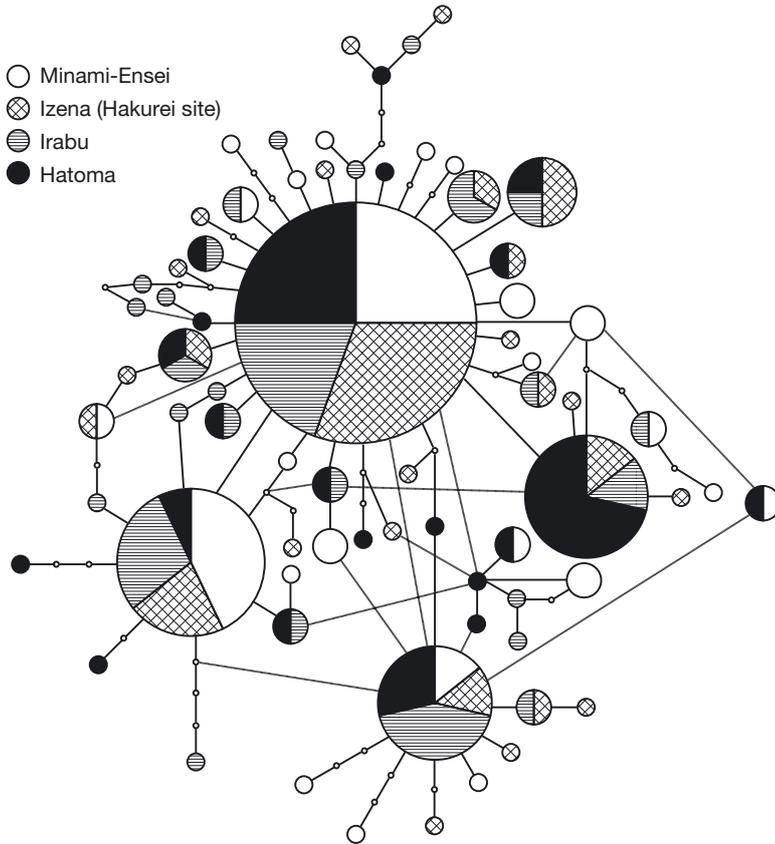


Fig. 4. Haplotype network of *Lepetodrilus nux* from the Okinawa Trough based on a partial sequence of the mitochondrial cytochrome oxidase I gene (COI). Each branch indicates a single base substitution, and the size of the circle indicates the number of individuals possessing the haplotype

lotypes (Fig. 4). Genetic diversity indices indicate that all sites show high diversity. Tajima's *D* and Fu's *FS* were estimated as negative values for all the analyzed populations (Table 2). Comparisons between observed and simulated mismatches using chi-squared tests showed no significant differences. Pairwise *F_{ST}* and Exact Test confirmed that there was no significant differentiation among populations in the 4 sites (Table 3).

Shell length of the examined *L. nux* ranged from 0.98 to 11.86 mm (Table 4). Large individuals with shell lengths >10 mm were only observed at Minami-Ensei Knoll. Accordingly, the mean shell length of individuals collected at Minami-Ensei Knoll was significantly greater than shell lengths at other sites (ANOVA, $F_{3,482} = 143.86$, $p < 0.0001$).

Length-frequency histograms showed normal distributions at Minami-Ensei Knoll ($W = 0.987$, $p = 0.164$) and Irabu Knoll ($W = 0.968$, $p = 0.085$). In contrast, length-frequency distributions at 2 other sites, Izena and Hatoma, deviated significantly from normality ($p < 0.0001$), and are assumed to be polymodal. Based on modal decomposition analysis, individuals from the latter 2 sites showed 2 modal components (Fig. 5, Table 5). Those 2 were positively skewed (Izena Hole: skewness = 1.104, $p < 0.0001$;

Table 2. Genetic diversity indices for the 4 sampling sites. N: number of analyzed individuals, \hat{H} : haplotype diversity (\pm SE), π : nucleotide diversity (\pm SE)

Vent site	N	\hat{H}	π	Tau	p of chi-squared	Tajima's <i>D</i>	Fu's <i>FS</i>
Minami-Ensei	42	0.9361 \pm 0.0255	0.004733 \pm 0.002817	2.992	0.65	-2.09628	-21.35435
Izena	39	0.9204 \pm 0.0374	0.004366 \pm 0.002640	1.920	0.70	-2.28994	-25.80727
Irabu	39	0.9582 \pm 0.0206	0.004520 \pm 0.002717	2.594	0.33	-2.24780	-26.32007
Hatoma	34	0.9162 \pm 0.0365	0.003658 \pm 0.002297	2.125	0.40	-2.23886	-19.71644

Table 3. The population differentiation test. Results of Pairwise *F_{ST}* (lower left) and of Exact Test (upper right). NS: not significant when $p = 0.01$

Vent site	Minami-Ensei	Izena	Irabu	Hatoma
Minami-Ensei		NS	NS	NS
Izena	0.00944 NS		NS	NS
Irabu	-0.00387 NS	-0.6007 NS		NS
Hatoma	0.00392 NS	-0.0096 NS	-0.00757 NS	

Table 4. *Lepetodrilus nux* shell length data at different vent sites in the Okinawa Trough

Vent site	Sample size	Shell length (mm)		
		Range	Mean	SD
Minami-Ensei Knoll	159	1.81–11.86	7.04	1.96
Izena Hole	155	0.98–8.94	3.35	1.90
Irabu Knoll	67	1.05–8.43	4.31	1.92
Hatoma Knoll	105	1.21–8.41	3.28	1.44

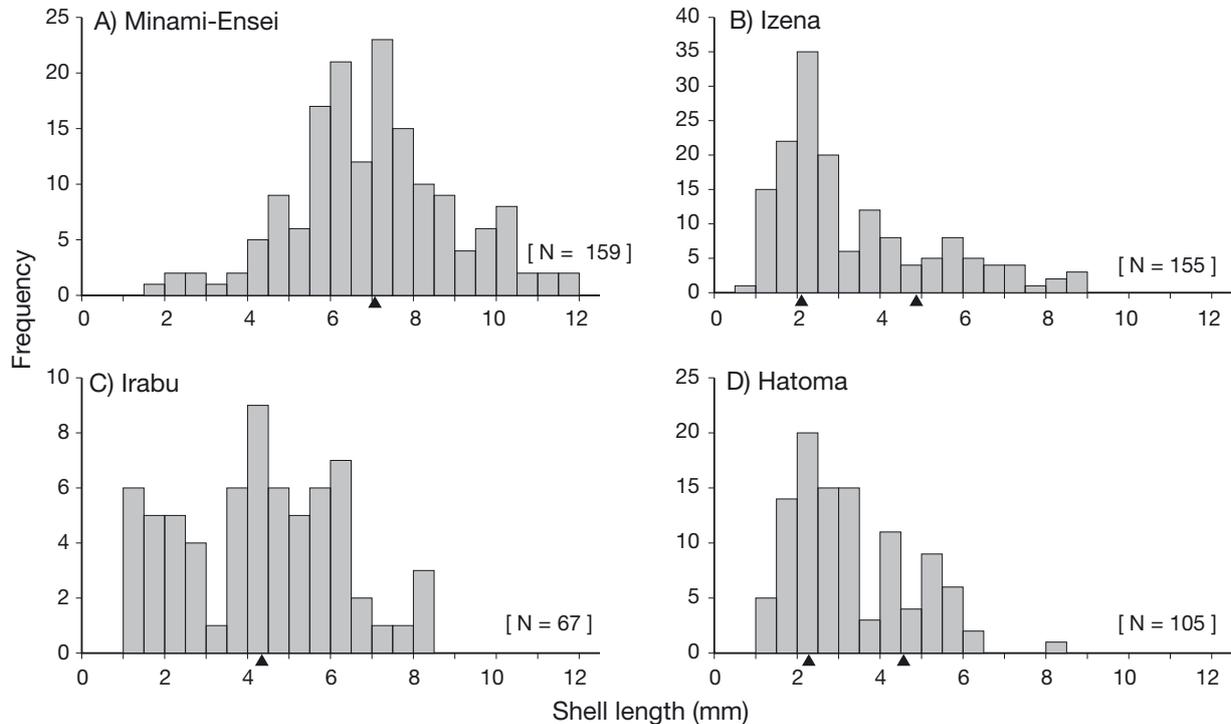


Fig. 5. Length-frequency distribution of *Lepetodrilus nux* at sampling sites (A) Minami-Ensei, (B) Izena, (C) Irabu, and (D) Hatoma. Arrowheads indicate the mean size for each cohort

Hatoma Knoll: skewness = 0.824, $p < 0.0003$). Approximately 70% of the individuals at Izena and Hatoma were < 4 mm, while a large number of individuals were > 4 mm at the other sites (Minami-Ensei = ~95%, Irabu = ~60%). Among sites, length-frequency distributions were significantly different (K-S 2-sample test, $p \leq 0.0001$), except between Izena and Hatoma Knolls (K-S 2-sample test, $p = 0.2898$).

Table 5. Modal components estimated from the length-frequency distributions of *Lepetodrilus nux* at different vent sites. μ : mean lengths (mm), σ : standard deviations, π : proportions. Populations in Minami-Ensei and Irabu Knolls showed only 1 modal component

Vent site	Modal component	Modal peaks	
		M1	M2
Minami-Ensei Knoll	μ	7.04	–
	σ	1.96	–
	π	1.00	–
Izena Hole	μ	2.08	4.88
	σ	0.53	1.82
	π	0.55	0.45
Irabu Knoll	μ	4.31	–
	σ	1.91	–
	π	1.00	–
Hatoma Knoll	μ	2.33	4.51
	σ	0.60	1.24
	π	0.56	0.44

All specimens dissected were gonochoric (male or female). The sex ratio approximated 1:1, except at Izena Hole, where there were twice as many females as males (Table 6). Mean size, size range, and minimum size at maturity were smaller for males than females (Table 6). Males were sexually mature in the low 2 mm range for all sites (Table 6). Females reached sexual maturity at slightly larger sizes, in the high 2 mm range for all sites (Table 6). Mature testes and ovaries contained different developmental stages of sperm and eggs at the same time (Fig. 6). In some females, the receptaculum seminis contained spermatozoa.

Proportions of vitellogenic oocytes differed significantly among individuals through size classes and sites ($p < 0.0001$). The proportion of immature oocytes was significantly higher than that of mature oocytes over size classes for all sites ($F_{16,256} = 2.963$, $p < 0.001$) (Fig. 7).

DISCUSSION

Our results indicate that *Lepetodrilus nux* is more widely distributed in the Okinawa Trough than previously thought. Previous studies have shown that *L. nux* was found in almost all deep-sea hydrothermal vent fields in the Okinawa Trough, except for

Table 6. Sexual properties in relation to shell length of *Lepetodrilus nux* at different vent sites in the Okinawa Trough

Vent site	Total no. of specimens	Sex	Sample size	Sex ratio (♀:♂)	χ^2 (p value)	Shell length (mm)			
						Size range	Average size	SD	Min. size at maturity
Minami-Ensei	125	♂	63	0.98	0.008 (0.929)	2.17–8.76	6.08	1.49	2.17
		♀	62			1.81–11.86	8.11	2.31	2.81
Izena	84	♂	28	2.00	9.333 (0.002)	1.91–6.86	3.30	1.32	2.32
		♀	56			1.85–8.94	4.91	1.98	2.49
Irabu	58	♂	26	1.23	0.621 (0.431)	1.64–5.86	3.85	1.14	2.33
		♀	32			1.66–8.43	5.32	1.80	2.86
Hatoma	78	♂	42	0.86	0.463 (0.497)	1.53–6.38	3.38	1.11	2.16
		♀	36			1.48–8.41	4.06	1.66	2.72

Minami-Ensei Knoll, where *L. japonicus* was collected instead (Fujikura et al. 2008), and Irabu Knoll, where reports on vent communities were scarce. Analyses of radular morphology and DNA sequences showed that all specimens examined from Minami-Ensei Knoll were *L. nux*. However, we only sampled at Depression C, one of 2 depressions at Minami-Ensei. *L. japonicus* may occur in the other depression (Depression B) or in some other microhabitat in Depression C. Moreover, DNA sequences demonstrated that *L. nux* also inhabits Irabu Knoll.

L. nux is not significantly differentiated genetically among 4 different sites in the Okinawa Trough, even though these local populations are separated by distances of 100 to 300 km. This indicates that these 4 sites represent a metapopulation, linked by dispersal of planktonic larvae, although *Lepetodrilus* larvae

are considered to be nonplanktotrophic (Craddock et al. 1997). In general, lecithotrophic larvae of shallow water invertebrates, which derive energy from yolk sac contents, have more limited planktonic larval periods (PLD) because they do not feed during the larval stage (Levin 2006). However, this general rule may not apply to deep-sea vent fauna because of the cold temperatures which could affect larval physiology, resulting in low metabolism, developmental arrest, delay of metamorphosis, and subsequently longer PLD (Pradillon et al. 2001, Young 2003, Adams et al. 2012). From metabolic rate, mean larval duration for non-feeding larvae of the vent tubeworm *Riftia pachyptila* was estimated as 38 d at 2°C and 250 atm, which would enable them to disperse as much as 100 km (Marsh et al. 2001). Lecithotrophic larvae of the vent barnacle *Neoverruca* sp. can

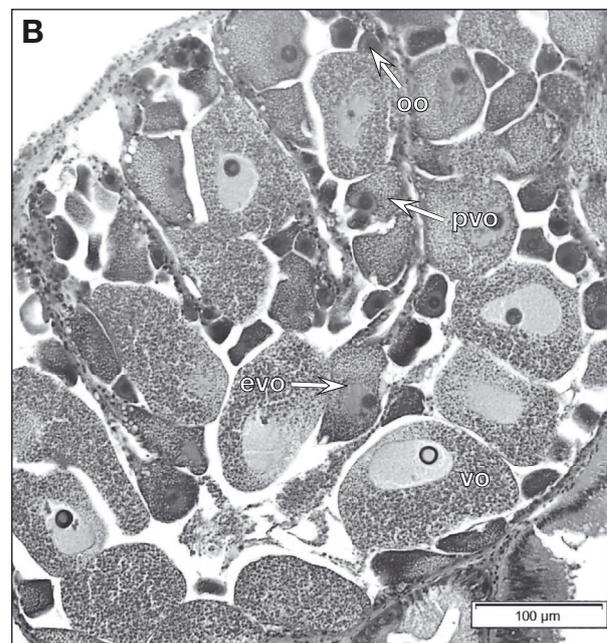
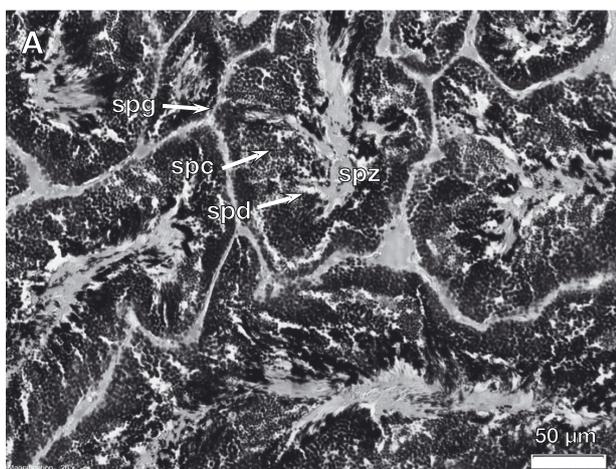


Fig. 6. Histological section of *Lepetodrilus nux* gonads. (A) Testis of an adult male showing spermatogonia (spg), spermatocytes (spc), spermatids (spd), and spermatozoa (spz). (B) Ovary of an adult female showing oogonia (oo), previtellogenic (pvo), early vitellogenic (evo), and vitellogenic oocytes (vo)

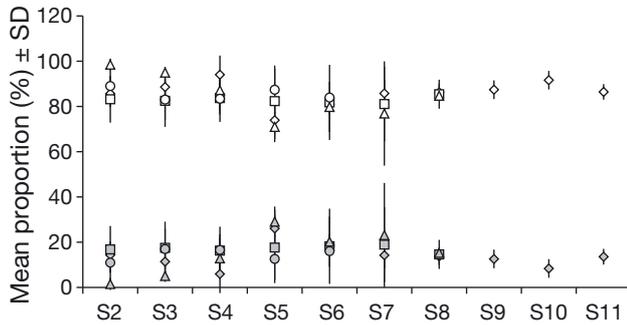


Fig. 7. Mean proportion of *Lepetodrilus nux* mature (gray) and immature (white) oocytes over size classes among vent sites. Diamond = Minami-Ensei Knoll; Square = Izena Hole; Triangle = Irabu Knoll; Circle = Hatoma Knoll. S2 = 2 to <3 mm, S3 = 3 to <4 mm, S4 = 4 to <5 mm, S5 = 5 to <6 mm, S6 = 6 to <7 mm, S7 = 7 to <8 mm, S8 = 8 to <9 mm, S9 = 9 to <10 mm, S10 = 10 to <11 mm, S11 \geq 11 mm. Sample sizes of each size class at each site were as follows: Minami-Ensei: S2 (1), S3 (2), S4 (2), S5 (8), S6 (3), S7 (8), S8 (11), S9 (10), S10 (9), S11 (4); Izena: S2 (5), S3 (10), S4 (8), S5 (8), S6 (8), S7 (5), S8 (4); Irabu: S2 (3), S3 (1), S4 (1), S5 (8), S6 (9), S7 (2), S8 (2); Hatoma: S2 (2), S3 (5), S4 (5), S5 (12), S6 (1)

extend the PLD at low temperatures (4°C) to more than 1 yr, suggesting the ability to disperse among different vent fields (Watanabe et al. 2006). Accordingly, the latter has been shown to form metapopulations in the Okinawa Trough (Watanabe et al. 2005). As seen in these previous studies, in deep-sea vent systems it may be possible to form and maintain large-scale metapopulations with non-planktonic larvae. Therefore, as genetic analysis demonstrated that *L. nux* form one population in the Okinawa Trough, lecithotrophic larvae of *L. nux* may have the physiological capacity in cold water to traverse distances of 100 to 300 km between different sites.

Size range and mean size of *L. nux* at Minami-Ensei Knoll were significantly larger than that at other 3 vent sites. Interpopulational variability in terms of body size may reflect differences in environmental conditions. Within the thermotolerance of the species, higher ambient temperature would facilitate growth (Hoegh-Guldberg & Pearse 1995). In deep-sea hydrothermal vent environments, metabolism of *L. elevatus* was higher in warmer areas of the EPR (Mullineaux et al. 1998). Larger individuals of *L. fucensis* occurred at higher temperatures; the largest individual was found at a vent area where water temperature exceeded 10°C (Bates 2008). In the present study, the ambient temperature was about $7.3 \pm 0.27^\circ\text{C}$ at Minami-Ensei Knoll, about 2 to 3°C higher than at other vent sites (Table 1). Therefore, one potential explanation for the larger size in Minami-

Ensei Knoll could be that higher temperature favors growth. An alternative explanation may be related to segregation of growth stages. Kelly & Metaxas (2008) found that *L. fucensis* in the NE Pacific demonstrated spatial segregation of life stages with ambient temperature; settlers were more likely to be abundant at lower temperature (3.2 to 8°C) and adults were more numerous at higher temperature (10.5 to 10.7°C). They hypothesized that this distribution might reflect (1) low thermotolerance of the settlers and (2) the distance from a venting source. In the present study, segregation of growth stages could not be observed because we did not consider this matter in the sampling design; thus, we may not have conducted sampling near enough to venting sources where larger individuals could be found, except at Minami-Ensei Knoll. Further studies will be required to examine the factors that account for size differences among *L. nux* populations in the different vent fields in the Okinawa Trough.

Length-frequency distributions demonstrated 2 distinct patterns. Minami-Ensei and Irabu Knolls displayed normal distributions. However, the sample number from Irabu Knoll was very small (approximately half those at other sites), yielding an unreliable result. At Izena Hole and Hatoma Knoll, frequency distributions were positively skewed, with 2 distinct modes. The length-frequency distribution at Minami-Ensei Knoll suggests the potential of continuous recruitment, but the 2 latter populations imply that *L. nux* in Izena Hole and Hatoma Knoll are composed of distinct cohorts. These differences between Minami-Ensei Knoll and the latter 2 sites could result from a complex dispersal process affected by local hydrodynamics (Adams et al. 2012). That is, larvae could be transported to Minami-Ensei Knoll constantly, but larval dispersal patterns could be changed by local hydrodynamics in Izena Hole and Hatoma Knoll, resulting in discontinuous recruitment. Another potential factor affecting the size-frequency distribution could be differences in post-settlement pressure. Mortality could vary among sites due to different environmentally induced physiological stresses, inter- and intraspecific competition, and predation (Adams et al. 2012). As mentioned above, because Minami-Ensei Knoll has a warmer environment, it could favor survival in addition to more rapid development. In contrast, larval supply may occur continuously in Izena Hole and Hatoma Knoll, but geothermal events may have killed many individuals in the community at once. Interestingly, distribution patterns found in these 2 sites showed similar size ranges and 2 distinct size

modes, with similar mean lengths in each mode. If compared with congeneric species based upon size-frequency distributions, *L. elevatus* from EPR have been reported to experience discontinuous and episodic larval supply (Sadosky et al. 2002), but recruitment patterns for *L. fucensis* from the northeastern Pacific were concluded to be continuous (Kelly & Metaxas 2007). These studies show that recruitment patterns deduced from size-frequency distributions vary among *Lepetodrilus* species. This difference may be a result of complex dispersal processes, determined by the interaction between larval life history parameters (e.g. spawning timing, planktonic larval duration, vertical larval distribution in the water column), and environmental properties (e.g. hydrodynamics, topography). In contrast, our results showed that the distributional patterns of *L. nux* differ among sites, suggesting that site-specific environmental factors during larval and post-recruitment processes are important to structure the population.

The minimum mature size of *L. nux* was smaller than previously reported for other *Lepetodrilus* species, especially for males (Table 7). *L. fucensis* has been observed to be reproductively mature at 3.8 and 3.9 mm SL, for males and females, respectively (Kelly & Metaxas 2007). From growth rates, both sexes are estimated to reach sexual maturity in ~13 mo (Kelly & Metaxas 2008). Temperatures for *L. nux* habitats ranged from 4 to 8°C, lower than those recorded for adult specimens of *L. elevatus* and *L. fucensis* (Bates 2008, Kelly & Metaxas 2008). Assuming that its growth rate is similar to those of congeneric species, *L. nux* appears to have the potential for early reproduction.

The potential for continuous reproduction for a certain period of the year was observed histologically for *L. nux* at both individual and population levels. Continuous reproduction is the most common strategy for deep-sea limpets in the genus *Lepetodrilus* (Kelly & Metaxas 2007, Bayer et al. 2011, Tyler et al. 2008). In the Northeast Pacific, *L. fucensis* has demonstrated the continuous release of vitellogenic oocytes throughout the sampling periods (Kelly & Metaxas 2007). Gametogenesis of *L. tevnianus* from the EPR was considered quasi-continuous based upon histological analysis (Bayer et al. 2011). Tyler et al. (2008) demonstrated that there was no evidence of temporal periodicity in reproductive patterns for the 3 *Lepetodrilus* species (*L. elevatus*, *L. putsulosus*, and *L. ovalis*) in the EPR and a single species (*L. atlanticus*) in the MAR. In the present study, a full range of gamete developmental stages were observed in gonads of females and males, suggesting continuous

Table 7. Comparison of the minimum size of mature adults of *Lepetodrilus* spp.

Species	Sex	Size (mm)	Reference
<i>L. fucensis</i>	♂	≥3.8	Kelly & Metaxas (2007)
	♀	≥3.9	
<i>L. tevnianus</i>	♂	≥2.3	Bayer et al. (2011)
	♀	≥2.4	
<i>L. elevatus</i>	♂	≥2.9	Tyler et al. (2008)
	♀	≥2.85	
<i>L. nux</i>	♂	≥2.16 (at Hatoma Knoll)	This study
	♀	≥2.49 (at Izana Hole)	

production of gametes within individuals, at least during the observation periods. In addition, proportions of vitellogenic oocytes were significantly different among individuals, and there were many fewer vitellogenic than immature oocytes. These observations imply that release of vitellogenic oocytes could occur asynchronously within populations, but quasi-continuously for individuals; hence, at the population level, vitellogenic oocytes could be released continuously. Additional time points would be desirable to confirm the complete absence of temporal periodicity in reproduction. On the other hand, many reproductive traits are considered to be phylogenetically constrained for archaeogastropods (Gustafson & Lutz 1994). Some similarity in gametogenesis with congeneric species (e.g. existence of all stages of gametes in the gonads, a large pool of immature gametes in a gonad), supports the potential of reproductive quasi-continuity or continuity in *L. nux*.

In summary, populations of *L. nux* in the Okinawa Trough appear to form a metapopulation, probably linked by dispersal of planktonic larvae. The reproductive traits of *L. nux* seem geared for opportunistic colonization, as seen in other *Lepetodrilus* spp.; maturity at small size, potential quasi-continuous or continuous reproduction. The difference is that *L. nux* matures at smaller sizes than other *Lepetodrilus* species for which data is available. In addition, *L. nux* probably reproduces continuously, but data from other seasons are required to confirm this. Two significant trends in size-frequency distribution were found among vent sites in the Okinawa Trough. This suggests the influence of environmental factors during larval and post-recruitment processes. In particular, due to the complexity of ocean currents, larval supply to a given area varies over time (Mullineaux et al. 2005, Adams & Mullineaux 2008, Adams et al.

2011). Transportation of *Lepetodrilus* larvae could be affected by near-bottom currents (Mullineaux et al. 2005), which are considered too slow to transport infauna, but are strong enough to affect infaunal distribution in some areas in the deep sea (Hollister et al. 1984, Kontar & Sokov 1994, Paterson & Lambshhead 1995, Thistle & Levin 1998). In addition, such near-bottom currents could be affected by diurnal and semidiurnal tides (Adams & Mullineaux 2008) and also by surface-generated mesoscale eddies (Adams et al. 2011). Therefore, knowledge of both surface and near-bottom currents and their variability will help to elucidate dispersal processes and subsequent succession in deep-sea vent faunal communities with the knowledge of their life-history traits.

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