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Reproductive biology and population structure of three hydrothermal gastropods (*Lepetodrilus schrolli*, *L. fijiensis* and *Shinkailepas tollmanni*) from the South West Pacific back-arc basins

Poitrimol Camille [1, 2, *], Matabos Marjolaine [2], Veuillot Alicia [2], Ramière Annah [1], Comtet Thierry [1], Boulart Cedric [1], Cathalot Cecile [3], Thiébaut Éric [1]

[1] Sorbonne Université, CNRS, Station Biologique de Roscoff, UMR 7144 Adaptation Et Diversité en Milieu Marin, 29680, Roscoff, France
[2] University Brest, CNRS, Ifremer, UMR 6197 Biologie Et Ecologie Des Ecosystèmes Marins Profonds, 29280, Plouzané, France
[3] University Brest, University Bretagne Sud, CNRS, Ifremer, UMR 6538 Geo-Ocean, 29280, Plouzané, France

* Corresponding author : Camille Poitrimol, email address : camille.poitrimol@gmail.com

Abstract :

Hydrothermal vents host fragmented habitats and are increasingly becoming the target of deep-sea mining projects for their mineral resources. Managing a future sustainable exploitation requires a good understanding of the resilience of biological populations to natural and anthropogenic disturbances, hence a better knowledge of species life history traits and their capacity to replenish local populations or colonise distant sites. In this context, we studied the reproductive biology and recruitment patterns of three main representative hydrothermal vent limpets of the South West Pacific back-arc basins, Lepetodrilus schrolli, Lepetodrilus fijiensis and Shinkailepas tollmanni, in relation to habitats and environmental conditions. Limpets were collected in Bathymodiolus and Ifremeria nautilei habitats at several vent sites in the Manus, North Fiji and Lau back-arc basins, and the Futuna Volcanic Arc during the CHUBACARC cruise in 2019. Population structure, gonad morphology, and gametogenesis were analysed for each species, and fecundity was analysed for the two Lepetodrilus species. Both Lepetodrilus spp. were gonochoric and displayed a sexual size dimorphism with females larger than males. Gametogenesis was continuous or quasi-continuous with all stages of oocyte development present in the gonad and a maximum oocyte size of 124 µm for L. schrolli and 126 µm for L. fijiensis. Fecundity varied between 52 and 205 with a mean of 119 ± 74 (SD) matured oocytes per female in L. schrolli and between 80 and 605 with a mean of 366 ± 183 (SD) matured oocytes per female in L. fijiensis, and was independent of the limpet size for both species. Shinkailepas tollmanni is also a gonochoric gastropod with continuous gametogenesis and a maximum oocyte size of 153 µm. For each species, size-frequency distributions were consistent with a continuous recruitment although episodic larval supply could blur the signal. There was no evidence of an influence of the habitat type nor environmental conditions on population structures.

Keywords : Lepetodrilidae, Phenacolepadidae, Gametogenesis, Fecundity, Bathymodiolus, Ifremeria

Introduction

Understanding the processes involved in the colonisation of new sites and the connectivity is critical to assess the resilience of benthic communities to natural and anthropogenic disturbances, and their long-

50 term persistence. In the deep sea, hydrothermal vents, reported along mid-ocean ridges, back-arc
51 basins and volcanic arcs (Beaulieu et al. 2015; Beaulieu and Szafrański 2020), form fragmented and
52 ephemeral systems whose spatio-temporal variability (e.g. vent distribution, disturbance rate) greatly
53 influences the dynamics of the communities they harbour (Mullineaux et al. 2018). Natural
54 disturbances, which result from volcanic eruptions and tectonic events, may remove local benthic
55 communities partially or totally, or create new suitable habitats. Depending on vent systems,
56 disturbance rate due to volcanic eruptions ranges from very high (e.g. several eruptions per year on
57 submarine arc volcanoes) to very low (e.g. one every 10,000 years at slow spreading mid-oceanic
58 ridges, Perfit and Chadwick 1998). Finally, physico-chemical conditions can be highly variable in
59 space and time. Vent fluid chemical composition can vary at 10-km scale in some back-arc basins (e.g.
60 Eastern Lau Spreading Center and Manus Spreading Center, Mottl et al. 2011; Reeves et al. 2011) or
61 slow spreading ridges (e.g. Lucky Strike vent field, Chavagnac et al. 2018) with significant impact on
62 the composition of vent communities (Mullineaux et al. 2018). At the vent scale, the mixing of vent
63 fluids with seawater generates strong horizontal and vertical gradients in environmental conditions
64 (e.g. temperature, reduced compounds concentrations, Le Bris et al. 2006; Sarradin et al. 2009)
65 resulting in a zonation in the distribution of the benthic fauna (e.g. Shank et al. 1998; Podowski et al.
66 2010). In the South West Pacific, hydrothermal vent communities are distributed along an
67 environmental gradient, from the large gastropods *Alviniconcha* clumps in high diffuse flow areas to
68 clumps of a second large gastropod, *Ifremeria nautilei*, in moderate flow areas, and beds of the
69 mussels *Bathymodiolus* in low flow areas (Podowski et al. 2010).

70 Over the last decades, connectivity between vent fauna populations has been mainly assessed through
71 population-genetics or modelling studies that rely on ocean circulation and selected species life history
72 traits, such as planktonic larval duration and larval vertical distribution (Mitarai et al. 2016; Breusing
73 et al. 2021). However, these methods provide very different measures of connectivity. While most
74 population genetics studies inform on connectivity over several generations, biophysical models
75 measure connectivity only from larval release to larval settlement following one or several spawning
76 events. Fully understanding persistence requires the complementary knowledge of life-history traits

(Burgess et al. 2014). Studying life history traits related to the reproductive effort, such as age at first reproduction, fecundity, reproductive pattern, and sex ratio, is essential to improve our understanding of the distribution and the colonisation potential of species. In vent systems, fast growth, early reproduction and long-distance larval dispersal have long been considered life-history strategies adapted to the properties of the hydrothermal environment (Ramirez-Llodra 2002). However, life-history traits can be much more diverse than expected, even between closely related species (e.g. larval behavior, Metaxas 2011; Yahagi et al. 2017), and are still unknown for numerous deep-sea vent organisms.

Recruitment patterns have been established for several hydrothermal vent taxa. Discontinuous recruitment has been described within polychaetes (Zal et al. 1995; Thiébaut et al. 2002), bivalves (Comtet and Desbruyères 1998) and gastropods (Sadosky et al. 2002), whereas continuous recruitment has been reported for some gastropods and bivalves (Berg 1985; Hessler et al. 1988; Kelly and Metaxas 2008; Marticorena et al. 2020). Except for a few exceptions (e.g. *Bathymodiolus azoricus*, Comtet et al. 1999; Dixon et al. 2006), a majority of hydrothermal species exhibit a continuous or quasi-continuous reproduction whatever the recruitment pattern (Tyler and Young 1999; Matabos and Thiébaut 2010). This is the case for several gastropod species from the Lepetodrilidae, Sutilizonidae, Skeneidae, and Peltospiridae (Gustafson and Lutz 1994; Kelly and Metaxas 2007; Tyler et al. 2008; Matabos and Thiébaut 2010; Bayer et al. 2011; Marticorena et al. 2020). In addition, hydrothermal vent fluid properties could play an important role on reproduction and population structure of vent species by influencing temperature, food availability (e.g. sulphide resources for autotrophic bacteria) and quality, and by generating a potential toxic environment. As an example, gamete maturation and fecundity of *Lepetodrilus fucencis* differed between actively venting and senescent habitats (Kelly and Metaxas 2007). The venting conditions, i.e. high flow or waning vents, also influenced the sex ratio of this species with a dominance of females in very active vents and a dominance of males in peripheric and senescent vents (Bates 2008). Finally, biotic interactions can influence recruitment and population structure through predation, grazing or competition for space and/or resources (Micheli et al. 2002; Sancho et al. 2005; Lenihan et al. 2008).

To date, most studies on reproductive biology and recruitment of vent species have focussed on specimens that colonise mid-oceanic ridges, mainly the East Pacific Rise, the North East Pacific Ridges and the Mid-Atlantic Ridge. Apart from Nakamura et al. (2014)'s study on *Lepetodrilus nux* from the North West Pacific, reproductive and recruitment studies on benthic invertebrates in the West Pacific are still very scarce and generally limited to anatomical studies (Beck 1992, 1993). Yet there are at least two reasons to consider the reproductive biology and recruitment of the species in this region. First, in comparison with almost continuous mid-oceanic ridges where connectivity is only interrupted by transform faults and/or microplates (Plouviez et al. 2009), the back-arc basins of the West Pacific form a system of discontinuous newly-formed ridges. In the South West Pacific, the few studies that have analysed the effective dispersal of the associated vent fauna highlighted a relatively complex and contrasting evolutionary history linked to the complex tectonic history of the region and contrasting species life-history traits (Thaler et al. 2011; Plouviez et al. 2019; Poitrimol et al. 2022). Second, emerging mining activities targeting hydrothermal vent sulphide mounds focus mainly on this region suggesting that these areas will be facing new anthropogenic disturbances in a near future (Boschen et al. 2013; Petersen et al. 2016; Thaler and Amon 2019).

The Lepetodrilidae *Lepetodrilus schrolli* L. Beck, 1993 and *L. fijiensis* L. Beck, 2023, and the Phenacolepadidae *Shinkailepas tollmanni* (L. Beck, 1992) are dominant species in the West Pacific, widely distributed and representative of the small vent fauna inhabiting the complex three-dimensional habitats (shells and/or crevices) formed by *Ifremeria nautilei* clumps and *Bathymodiolus* beds. *Lepetodrilus schrolli* was previously considered as a species complex comprising three genetically-distinct lineages: *L. schrolli* from the Manus Basin, *L.* aff. *schrolli* from the North Fiji, Lau and Manus basins and *L.* aff. *schrolli* from the Mariana Trough (Johnson et al. 2008; Plouviez et al. 2019). Poitrimol et al. (2022) suggested the presence of a single species that extends from the Manus Basin to the Kermadec Volcanic Arc through the Woodlark, North Fiji, Lau basins and Futuna Volcanic Arc, but with a strong geographical structure: two lineages opposing the Manus populations from those further east, both lineages being present in the Woodlark Basin (Poitrimol et al. 2022). However, very recently, following the discovery of Lothar Beck's unpublished work before his death, Chen and

Sigwart (2023) have described as a new distinct species named *L. fijiensis* the individuals previously described as *L.* aff. *schrolli* in the North Fiji and Lau basins. The two species are morphologically and anatomically very similar (Chen and Sigwart 2023) with *L. fijiensis* present in the North Fiji and the Lau basins, and the Futuna and Kermadec Volcanic Arcs, and *L. schrolli* found in the Manus Basin. As previously mentioned, both species coexist in the Woodlark Basin. *Lepetodrilus schrolli* and *L. fijiensis* are gonochoric species but, unlike the other *Lepetodrilus* species described, most individuals lack a penis. Just a few specimens with a well-developed penis were reported from the North Fiji Basin for *L. fijiensis* (Warén and Bouchet 2001). Ova fertilisation is thought to take place in the mantle cavity but this has not been proven (Beck 1993). *Shinkailepas tollmanni*, formerly known as *Olgasolaris tollmanni* L. Beck, 1992, is also gonochoric. The right cephalic lappet is transformed into a penis with dorsal seminal groove in males, and fertilisation is internal (Sasaki et al. 2010). *S. tollmanni* lays egg capsules that can be found attached to shells of living *Ifremeria nautilei*. The present study aims at providing new insights into the reproductive biology and recruitment patterns of these three vent gastropod species. This will lead to a better understanding of the distribution and the colonisation potential of the species and thus be used to set up larval dispersal models between populations in the South West Pacific.

From a spatially nested sampling design, the objectives of this study are to: (1) explore spatial variability in population structure within and between back-arc basins and one volcanic arc according to the environmental variability and habitat (*Bathymodiolus* beds *vs. Ifremeria nautilei* clumps), and infer their recruitment strategy (continuous *vs.* discontinuous); (2) determine the sex ratio of the populations; (3) study the gametogenesis through histology to describe the variability of females' reproduction status; and (4) estimate the fecundity of the two *Lepetodrilus* species.

Materials and methods

Sampling

All specimens of *Lepetodrilus schrolli, L. fijiensis* and *Shinkailepas tollmanni* were sampled using the hydraulic arm of the Remotely Operated Vehicle (ROV) *Victor6000* during the CHUBACARC cruise (Hourdez and Jollivet 2019) held onboard the French research vessel *L'Atalante* between March and June 2019. Samples were collected in *Ifremeria nautilei* and *Bathymodiolus* habitats from low to moderate diffused flow areas, in several hydrothermal vent fields from three back-arc basins of the South West Pacific, i.e. the Manus, North Fiji and Lau basins, and one volcanic arc, i.e. Futuna (Figure 1A, Table 1). To roughly characterise environmental conditions in the sampling habitat and to investigate potential variation in reproductive features and population structure in relation to environmental conditions, physico-chemical measurements were conducted in each sampling area prior to sampling. *In situ* temperature was measured with the high-temperature probe of the ROV, and free inorganic sulphides [$\Sigma S(-II) = H_2S+HS^-+S^{2-}$] were measured with the *in situ* chemical miniaturised analyser CHEMINI (Vuillemin et al. 2009). As the sample was pumped without any filtration, the chemical species analysed with CHEMINI correspond to an operationally defined fraction of sulphides called free inorganic sulphides which includes dissolved and particulate sulphides that are enough labile to be measured by the colorimetric method (Cotte et al. 2020). Diluted fluid samples were collected above the substrate with an *in situ* water sampler mounted on the ROV. The collected fluids were then analysed on board for pH, and methane concentrations were measured by gas chromatography after gas extraction (Donval et al. 2008). While temperature and free inorganic sulphides were measured on three replicate points, diluted fluid was sampled on one point because of logistic limitations.

On board, the collected specimens were washed through a 250-µm sieve and individuals of *Lepetodrilus* spp. and *S. tollmanni* were sorted. Fifty individuals per sample were stored in 4% buffered seawater formalin and transferred to 80% ethanol after 4-5 months for histological

observations, while all other individuals were preserved in 96% ethanol for population structure studies.

Population structure

To infer population structure, size-frequency distributions were analysed by measuring the curvilinear shell length (i.e. the longest distance from the apex to the anterior edge of the shell along the dorsal side, Sadosky et al. 2002; Matabos et al. 2008) for *Lepetodrilus* species and the maximal antero-posterior shell length for *S. tollmanni* (Figure 1B). The measured length was chosen according to the shape of the shell and previous studies (Sadosky et al. 2002; Matabos et al. 2008). Only samples with at least 100 individuals were selected (considered as the minimum required for size-frequency distributions), and a random sub-sample of 500 individuals was used for larger samples. Measurements were conducted with the Leica Application Suite software linked to a Leica MC 170 HD camera mounted on a Leica M125 stereoscopic microscope for *Lepetodrilus* species, and through the ZEN pro 3.2 software connected to a ZEISS AxioCam 208 Color camera mounted on a ZEISS SteREO Discovery.V20 stereoscopic microscope for *S. tollmanni*. Post-larval and juvenile shells of *S. tollmanni* were photographed under an Olympus SX16 microscope linked by an Infinity 1 Camera to the Infinity capture software and measured using ImageJ (Schneider et al. 2012). Measurement error was determined using the maximum difference among ten repeated measures of the same individual on 10 specimens covering the whole size range of both species. It was fixed at 0.213 mm for *Lepetodrilus* species and 0.125 mm for *S. tollmanni*. Length-class interval was then set at 0.5 and 0.4 mm for *Lepetodrilus* species and *S. tollmanni*, respectively, according to the three criteria proposed by Jollivet et al. (2000): (1) most size-classes must have at least five individuals; (2) the number of adjacent empty classes must be minimised; and (3) the interval has to be much greater than the error of measurement.

Size-frequency distributions were compared to a normal distribution using a Kolmogorov-Smirnov one-sample test adapted by Lilliefors (1967) which is less sensitive to *ex æquo*. When distribution differed significantly from a normal distribution, modal decomposition, assuming that gastropod sizes

follow a Gaussian distribution within cohorts, was performed using the Mixdist Package in R. Gaussian component number and, associated mean and standard deviation were first estimated through Bhattacharya (1967)'s method adapted by Pauly and Caddy (1985). Non-parametric Kruskal-Wallis tests were used to test for differences in shell lengths among samples within a basin, followed by a Nemenyi and Dunn multiple comparison test to identify pairwise differences. As the number of samples did not make it possible to perform numerous Kruskal-Wallis tests to assess significant effects of habitat or vent field, only one Kruskal-Wallis test was performed per species. Variations among habitats within a vent field and among fields within a basin were highlighted from the analysis of the results of the Nemenyi and Dunn multiple comparison test.

To determine the relationships between the size-frequency distribution data (i.e. response variables) and the environmental variables (i.e. explanatory variables), a Redundancy Analysis (RDA) was performed on Hellinger-transformed size-class abundance data (Legendre and Gallagher 2001). This transformation corresponds to the square root of relative abundances of size classes and has the advantage of fulfilling the Euclidean metric properties. Environmental variables considered included depth, maximal temperature (°C), mean concentration of ΣS(-II) (µM) and CH_4 (µM), mean pH, basin and vent field; these two latter were coded as dummy variables. Samples with missing data were not considered for this analysis. Prior to the RDA, a forward selection was applied to select significant environmental variables using the *forward.sel* function of the R package adespatial (Dray et al. 2022). Wilcoxon-Mann-Whitney test was computed to test for differences in the physico-chemical variables between habitats. All statistical analyses were performed with R statistical software 4.0.3 (R Core Team 2020).

Sex ratio and reproductive biology

Due to the lack of penis in *Lepetodrilus schrolli* and *L. fijiensis*, about 100 individuals randomly selected among five samples from various fields and habitats for each species were measured and sexed by examination of gonad aspect after shell removal (see Table 3). For both *Lepetodrilus* species,

male gonad could be identified by series of white strips while “grain-like” structures could be distinguished in female ones (Figure 2A-B). All specimens of *S. tollmanni* were sexed when possible (i.e. 3 297 individuals sexed, Table 3). Males and females could easily be distinguished by the presence of a penis (Figure 3A-B) beside the right cephalic tentacle (Beck 1992). To assess if mean length of females was greater than that of males, a unilateral Wilcoxon-Mann-Whitney test was computed. Finally, to test for the deviation from a balanced 1:1 sex ratio, a chi-square goodness-of-fit test was applied.

Reproductive characteristics of each species were assessed through gonad histology. Individuals were removed from their shell with forceps after having been measured as described above. The whole soft-body part was then dehydrated with a series of increasing concentration of ethanol (starting with 80%, then 95% and finally 100%), cleared in xylene, infiltrated of liquid paraffin and embedded into paraffin blocks. Serial 9-µm and 7-µm thick sections of gonads were produced with a microtome for *Lepetodrilus* spp. and *Shinkailepas tollmanni* individuals, respectively. Sections were mounted onto microscope slides and stained with haematoxylin and eosin following the protocol proposed by Gabe (1968). Photographs of histological sections were taken using the Leica Application Suite AF software connected to a Leica DFC 450C camera on a Leica DMI6000 B inverted videomicroscope. Oocytes were then measured and counted using the ImageJ software.

For gametogenesis studies, two oocyte development stages were targeted: the vitellogenic stage which represents mature oocytes, and the previtellogenic stage considered as non-mature. Oogonia were not considered as their first stages are very difficult to identify. Maximum and minimum Feret’s diameter of at least 100 previtellogenic and vitellogenic oocytes per individual were measured from two to five sections selected in the beginning, middle and end part of the gonad. Only oocytes that have been sectioned through the nucleus were considered. As oocyte shape is variable, maximum and minimum Feret’s diameter were used to calculate the area of an ellipse to infer the area-equivalent diameter which is $\sqrt{\frac{4 \times area}{\pi}}$. This is the estimated diameter of a circle with the same area as the object. Measurement error, fixed at 5 µm, was determined using the maximum difference between ten

repeated measurements of the same ten oocytes of various size and shape. According to the three criteria mentioned earlier (Jollivet et al. 2000), oocytes sizes were grouped into 11-µm size classes and the relative frequencies of oocyte size class were computed for each female. To test for synchrony in reproductive development, size-frequency distributions of oocyte size among females within a vent site and among vent sites within a basin or volcanic arc were compared using a Kruskal-Wallis multisample test. When significant differences occurred, a Nemenyi and Dunn *post hoc* test was performed. Gametogenic maturity defined as the percentage of vitellogenic oocytes per female was inferred and difference among samples was analysed through a Kruskal-Wallis multisample test followed by a Nemenyi and Dunn *post hoc* test. The relationship between the proportion of vitellogenic oocytes and the female size was analysed using the Spearman rank correlation coefficient.

Actual fecundity was estimated by counting and measuring the total number of vitellogenic oocytes within the gonad of four females of *L. schrolli* from the Manus Basin and seven females of *L. fijiensis* from the Lau Basin, using the measurement methods detailed above. Spearman correlation test was used to test whether fecundity depended on size.

Results

Environmental conditions

Environmental conditions are presented in Table 1. Maximal temperature recorded within the *Ifremeria* habitat ranged from 4.26 to 21.33°C. The mean ΣS(-II) concentrations ranged from 0.50 ± 0.00 to 170.17 ± 167.55 µM, while CH_4 concentrations ranged from 0.07 to 1.03 µM. The pH varied from 5.88 to 7.49. Within the *Bathymodiolus* habitat, maximal temperature ranged from 3.25°C to 19.28°C. The mean ΣS(-II) concentrations ranged from 1.99 ± 1.91 to 21.13 ± 3.84 µM; CH_4 concentrations varied from 0.07 to 0.61 µM; pH ranged from 6.43 to 7.57. A significant difference between habitats was detected for the mean ΣS(-II) concentration only (Wilcoxon-Mann-Whitney test: w = 33, *p* value = 0.041).

Lepetodrilus schrolli and *L. fijiensis*

Population structure

In total, length-frequency distributions were established from 6 079 individuals of *Lepetodrilus schrolli* from the Manus Basin and 3 405 individuals of *L. fijiensis* from the Lau Basin, sampled in the *Ifremeria* and *Bathymodiolus* habitats (Table 2). *Lepetodrilus schrolli* shell length ranged from 0.51 to 9.51 mm, with population mean lengths varying from 2.89 ± 0.81 to 5.93 ± 1.14 mm. *Lepetodrilus fijiensis* shell length ranged from 0.74 to 9.86 mm, with population mean lengths varying from 3.48 ± 1.14 to 5.43 ± 1.29 mm (Table 2). Except for *L. schrolli* in the PM2, PM4 and PM6 samples collected at Pacmanus in either *Ifremeria* or *Bathymodiolus* habitats, all length-frequency distributions of *Lepetodrilus* populations significantly differed from a normal distribution (Lilliefors test, p values < 0.05, see Table 2 for specific p values). However, most were unimodal and characterised by a large number of medium-size individuals (~ 60-90% of the sample for both species, considering the four size classes around the mean) coupled with a few small and/or large individuals, which led to a strong asymmetry for some samples (Figure 4A-B, see supplementary material for all size-frequency histograms). For both species, between one and three Gaussian components with varying proportions were identified through modal decomposition with Mixdist. However, analyses did not allow to infer biologically meaningful cohorts (i.e. a group of individuals from the same population and born at the same time, see discussion) and were hence not shown. The Kruskal-Wallis tests highlighted significant differences in shell length among samples for both species (*L. schrolli*: H = 1884.5, df = 11, p value < 2.2e-16; *L. fijiensis*: H = 948.2, df = 8, p value < 2.2e-16). The Nemenyi and Dunn multiple pairwise comparisons test among samples of *L. schrolli* showed variability at the vent field scale. Within Pacmanus, all but three pairs of samples out of fifteen significantly differed (i.e. PM3 *vs.* PM4 both from *Ifremeria* habitats, PM2 from *Bathymodiolus vs.* PM3 from *Ifremeria* and PM5 from *Bathymodiolus vs.* PM3 from *Ifremeria*). Among Susu samples, all but four pairs out of fifteen significantly differed (i.e. SU1 *vs.* SU4 and SU5 from *Ifremeria* and *Bathymodiolus* habitat, SU4 *vs.* SU2 and SU5 from *Ifremeria* habitat). Within-field variability also occurred among the *L. fijiensis* samples. Samples AB1 and AB2 from ABE were significantly different (p value = 4.3e-09); only TC1

and TC2 differed significantly (*p* value = 0.004) within Tow Cam, while the Tui Malila samples TM1 and TM2 were not significantly different.

The habitat type did not seem to impact shell length. Twenty-six out of thirty-five (i.e. 74%) and fifteen out of twenty pairs of samples (i.e. 75%) from different habitats (*Ifremeria vs. Bathymodiolus*) differed significantly in *L. schrolli* and *L. fijiensis*, respectively. By comparison, twenty-four out of thirty-one pairs of *L. schrolli* samples (i.e. 77%) and eleven out of sixteen pairs of *L. fijiensis* samples (i.e. 69%) from a same habitat (i.e. *Ifremeria vs. Ifremeria* or *Bathymodiolus vs. Bathymodiolus*) differed significantly. The forward selection prior to RDA analysis did not select any of the explanatory variables for *L. schrolli*. However, it selected depth as the only explanatory variable for *L. fijiensis* with an adjusted R^2 of 0.66. Depth which was negatively correlated with the first axis of the RDA, separated the three deeper Tow Cam samples (i.e. TC1, TC3 and TC4) from the others (i.e. MG1, TM1 and TM2). Whatever the habitat, Tow Cam samples were characterised by a narrow range of curvilinear shell length around 4.7-4.8 mm (Figure 4B).

Sex ratio and reproductive biology

For both *Lepetodrilus* species, sexing was possible for the individuals longer than 2 mm (curvilinear length). In total, 509 individuals of *L. schrolli* and 493 individuals of *L. fijiensis* were sexed (Table 3). None of the sexed individuals had a penis. In *L. schrolli*, female shell length ranged from 2.29 to 9.63 mm with a mean length of 5.31 ± 1.38 (SD) mm while male shell length ranged from 2.22 to 6.92 mm with a mean length of 4.53 ± 0.96 mm. In *L. fijiensis*, female shell length ranged from 2.51 to 9.02 mm with a mean length of 5.40 ± 1.22 mm while male shell length ranged from 2.74 to 6.42 mm with a mean length of 4.40 ± 0.70 mm. For each sample, and for both species, females were significantly larger than males (unilateral Wilcoxon-Mann-Whitney tests: *p* values < 0.01, see Table 3 for specific *p* values). The sex ratio was not significantly different from 1:1 (chi-square goodness-of-fit: *p* values > 0.05, see Table 3 for specific *p* values), except for one sample of *L. schrolli* from the Pacmanus vent field that displayed a female-biased sex ratio (i.e. PM5; M:F = 0.66, *p* value = 0.039).

For both *Lepetodrilus* species, ovary and testis were posteriorly located, underlying the digestive gland and rising on its left as reported on Figure 2A-D for *L. fijiensis*. Gametogenesis was described through the analysis of 93 females of *L. schrolli* and 89 females of *L. fijiensis*, collected in *Ifremeria* and *Bathymodiolus* habitats (Table 4), with a curvilinear shell length ranging from 3.72 to 8.14 mm and 3.48 to 9.13 mm, respectively. For both species, three stages of oocyte development were observed and present in all gonads: oogonia, and previtellogenic and vitellogenic oocytes. Oogonia seemed to develop from the germinal epithelium along the entire gonad. Although they had been observed in each female, oogonia were not considered for oocyte size-frequency distribution analyses, as their proportion would have been underestimated due to photographs quality. Previtellogenic oocytes, considered as non-mature, presented a smooth and dark cytoplasm while the vitellogenic mature oocytes were distinguishable by their pink coloured granular yolk in their voluminous cytoplasm (Figure 2E). In *L. schrolli*, previtellogenic oocytes diameter ranged from 7.96 to 94.22 µm with a mean diameter of 26.84 ± 10.91 µm. Vitellogenic oocytes diameter ranged from 19.57 to 124.13 µm with a mean diameter of 74.58 ± 14.97 µm. In *L. fijiensis*, previtellogenic oocytes diameter ranged from 9.22 to 95.6 µm with a mean diameter of 26.1 ± 10.86 µm. Vitellogenic oocytes diameter ranged from 42.20 to 126.23 µm with a mean diameter of 75.61 ± 13.60 µm. Whatever the species, all females presented the same pattern of oocytes size-frequency distribution with a large proportion of previtellogenic oocytes and a smaller proportion of vitellogenic oocytes (Figure 5A-B, see supplementary material for all *L. schrolli* and *L. fijiensis* oocytes size-frequency histograms). The proportion of vitellogenic oocytes in *L. schrolli* ranged from 2 to 34% (Table 4) with a mean proportion of 13 ± 6% and was independent on the female size (Spearman correlation test rho = -0.13, n = 93, *p* value = 0.22) (Figure 6A). The proportion of vitellogenic oocytes in *L. fijiensis* ranged from 1 to 29% (Table 4) with a mean proportion of 14 ± 5% and correlation between female size and the proportion of vitellogenic oocyte only appeared significant when considering the largest female (Spearman correlation test rho = -0.23, n = 89, *p* value = 0.03). (Figure 6B). Otherwise, it was also independent on the female size (Spearman correlation test rho = -0.20, n = 88, *p* value = 0.06). The proportion of vitellogenic oocytes differed significantly between samples for both *Lepetodrilus* species (Kruskal-Wallis test, *L. schrolli*: H = 39.032, df = 10, *p* value = 2.5e-05; *L. fijiensis*: H = 16.833, df =

8, *p* value = 0.032). For *L. schrolli*, pairwise comparison tests showed that only two samples from Pacmanus, for which the highest proportions of oocytes were observed (i.e. PM3 and PM4 from *Ifremeria* habitat), differed significantly from some Susu samples (i.e. SU1, SU3, SU5 for both samples and SU6 for PM4 only from *Ifremeria* and *Bathymodiolus* habitats) in the Manus Basin. No difference was detected for *L. fijiensis* between samples.

Significant differences in oocyte size distribution occurred among *L. schrolli* females within a sample, except for females in one sample from the Susu vent field (SU1, Table 4). Significant differences in oocyte size distribution also occurred among *L. fijiensis* females within a sample, except for females in two samples from the Mangatolo and ABE vent field (MG1 and AB1, Table 4). For both species, the Nemenyi and Dunn multiple pairwise comparisons test among females of a sample showed that the observed differences were attributed to a variable number of females, ranging from one to four. For *L. fijiensis*, no significant differences among females from Tow Cam TC3 and Tui Malila TM2 samples were identified from the multiple comparisons test, although the Kruskal-Wallis test detected a significant difference among females. Kruskal-Wallis tests also showed significant variations in oocyte size distribution among samples (*L. schrolli*: $H = 815.11$, df = 10, p value < 2.2e-16; *L. fijiensis*: $H = 109.43$, df = 8, p value < 2.2e-16), and the differences were attributed to specific samples. For *L. fijiensis*, three samples were found to differ from all other samples (p values < 0.001) but not from each other (the ABE AB1 and AB2, and Tow Cam TC2). For *L. schrolli*, *Ifremeria* habitat samples SU2 and SU4 differed from SU1, SU3 and SU6 collected in *Bathymodiolus* habitat, however, SU5 also from an *Ifremeria* habitat differed only from SU3. All Pacmanus samples differed from Susu samples, except PM5 and SU2 from various habitats. The habitat type did not seem to have an impact on gametogenesis neither for *L. schrolli* nor *L. fijiensis*.

Actual fecundity could be estimated from 4 females of *L. schrolli* and 7 females of *L. fijiensis* randomly selected among different samples (Table 4), with a shell length ranging from 5.07 to 7.83 mm and from 3.75 to 6.77 mm, respectively. The number of mature oocytes varied between 52 and 205 vitellogenic oocytes per female in *L. schrolli* with a mean of 119 ± 74 vitellogenic oocytes, and between 80 and 605 for *L. fijiensis*, with a mean of 366 ± 183 vitellogenic oocytes. For both, fecundity

was independent of size (Spearman correlation test: *L. schrolli* rho = 0.2, n = 4, *p* value = 0.92; *L. fijiensis* rho = 0.64, n = 7, *p* value = 0.14) although the very low sample size reduces the statistical power of the test.

Shinkailepas tollmanni

Population structure

Length-frequency distributions were established from 3 599 individuals of *S. tollmanni* from the Futuna Volcanic Arc, and the Manus, North Fiji and Lau basins, sampled in *Ifremeria* and *Bathymodiolus* habitats (Table 2). Shell length ranged from 0.55 to 12.20 mm. All length-frequency distributions, except the one from the FK1 sample (Fatu Kapa vent field at the Futuna Volcanic Arc), differed from a normal distribution (Lilliefors test, *p* values < 0.05, see Table 2 for specific *p* values). Length-frequency distributions were characterised by a large number of medium-sized individuals (~ 60-90% of the sample considering the four size classes around the mean) and a few small and/or large individuals (Figure 7, see supplementary material for all size-frequency histograms). Between two to four Gaussian components of varying proportions were identified by modal decomposition with Mixdist, yet, analyses did not allow to infer biologically meaningful cohorts (i.e. a group of individuals from the same population and born at the same time, see discussion) and were not shown. Kruskal-Wallis tests highlighted significant differences in shell length among samples at the scale of the southwestern Pacific (H = 2099.6, df = 9, *p* value < 2.2e-16). The Nemenyi and Dunn multiple pairwise comparisons test among samples showed significant variations among samples within a basin. The three pairs of samples from the Manus Basin and all six pairs but one from the Lau Basin samples differed significantly; only the samples TM2 and TM3 were not significantly different from each other. As only two samples were collected from *Bathymodiolus* habitat and both were from the Lau Basin, we focussed on this basin to observe differences according to the habitat. All four pairs of samples from the two distinct habitats but one differed significantly (*p* values < 0.001), as did the two pairs from a same habitat (i.e. *Ifremeria vs. Ifremeria* and *Bathymodiolus vs. Bathymodiolus*) (*p* values < 0.001). The forward selection conducted prior to RDA analysis selected two explanatory variables

(i.e. the Tui Malila and Pacmanus vent fields). Only the first axis of the RDA was significant and correlated to Tui Malila (adjusted $R^2 = 0.64$).

Sex ratio and reproductive biology

In total, sexing was possible for 3 297 individuals longer than 4.4 mm (Table 3). Female shell length ranged from 4.93 to 12.20 mm with a mean length of 7.90 ± 1.40 mm. Male shell length ranged from 4.42 to 11.80 mm with a mean length of 7.84 ± 1.32 mm. Female mean length was significantly larger than male mean length in four samples from the Pacmanus, Susu, Fatu Kapa and Tow Cam vent field (i.e. PM7, SU4, FK1 and TC3, unilateral Wilcoxon-Mann-Whitney tests: *p* values < 0.01, see Table 3 for specific *p* values). Three samples out of ten from the Pacmanus, Susu and Tow Cam vent field were significantly different from a balanced sex ratio and were all in favour of males (i.e. PM7, SU8 and TC3, Table 3).

Ovary and testis were dorsally located and extend downward on the left and backward of the digestive gland (Figure 3C-D). Gametogenesis could be described from 16 females ranging from 9.21 to 13.83 mm, collected in two samples from the two very distant Manus and Lau basins in the *Ifremeria* habitat (Table 4). Three stages of development were observed (Figure 3E). Oogonia developed from the germinal epithelium along the entire gonad. Oogonia have been observed in each female, but as their proportion would have been underestimated due to photographs quality, they were not considered for oocyte size-frequency distribution analyses. Previtellogenic oocyte diameter ranged from 13.28 to 109.37 µm with a mean of 38.47 ± 15.38 µm and vitellogenic oocyte diameter ranged from 55.92 to 152.92 µm with a mean of 92.95 ± 15.58 µm. All females presented the same pattern of oocytes size-frequency distribution with a large proportion of previtellogenic oocytes and a smaller proportion of vitellogenic oocytes (Figure 5C). Vitellogenic oocytes proportion ranged from 16 to 44% (Table 4) with a mean proportion of 27 ± 8% and was independent of the female size (Spearman correlation test rho = 0.02, n = 16, *p* value = 0.95) (Figure 6C). The Kruskal-Wallis test highlighted significant differences in oocyte size-frequency distribution among females within the two samples observed (Table 4). According to the Nemenyi and Dunn multiple pairwise comparisons test two females were responsible of these differences in both cases. Among the females in sample PM7, the two individuals

that differed had slightly smaller previtellogenic oocytes as compared to the others. This was also the case for one of the females in sample TC3, while the other had the highest proportion of vitellogenic oocytes.

Discussion

The CHUBACARC cruise, held in 2019, visited a large number of vent fields over three back-arc basins and one volcanic arc in the South West Pacific. The associated large-scale sampling allowed the study of population structure and reproductive traits of three dominant gastropod species, *Lepetodrilus schrolli, L. fijiensis* and *Shinkailepas tollmanni*, providing the first combined population and reproductive study of vent species in the southwestern Pacific. To date, in the western Pacific, only the gastropod *L. nux* at the Okinawa Trough (northwestern Pacific) was investigated for population and reproductive biology (Nakamura et al. 2014).

Individuals of *Lepetodrilus* spp. and *Shinkailepas tollmanni* colonised the shells of *Bathymodiolus* and *Ifremeria* which inhabit areas of low and intermediate diffuse areas, respectively (Podowski et al. 2010). In the Lau Basin, Podowski et al. (2010) argued that (1) temperatures of about 20°C correspond to one end of *Bathymodiolus* spectra of ecological niche, while another one is defined by its minimum sulphide requirements (temperature on that end being lower and close to open deep seawater); (2) the ecological niche of *Ifremeria nautilei* is more constrained by biotic interactions with *Bathymodiolus* and *Alviniconcha* although this last genus was present at greater temperatures and sulphide concentrations. The ranges of physico-chemical parameters observed in our study are in agreement with previous observations by Podowski et al. (2010) and confirm that *Ifremeria* is present in more intense diffusion (and/or more focussed and hence less diluted) zones with higher sulphide concentrations. No significant differences between the two habitats were observed for the other physico-chemical variables, underlining the difficulty in discriminating them on the basis of these variables only. Indeed, methane concentrations in diffuse flow areas are low, close to the detection limit, and the range of pH within these areas is narrow (between 6 and 7.8), with micro-scale

variations very difficult to assess with discrete sampling and a limited number of measurement points (Le Bris et al. 2001; Sarradin et al. 2009).

Recruitment patterns

Lepetodrilus schrolli, *L. fijiensis* and *Shinkailepas tollmanni* demographic structures varied along the South West Pacific, but we were not able to detect any particular pattern. While some populations presented a polymodal distribution, it is unlikely that the identified Gaussian components corresponded to a cohort as defined in ecology, which is a group of individuals from the same population and born at the same time. The size distributions were quite variable in terms of number of Gaussian components and mean size in all samples whatever the spatial scale of observation (e.g. between samples from the same site, between vent fields or between basins), and it is difficult to infer from this number the frequency of recruitment events or the existence of recruitment failures. However, they had some common characteristics. All samples were dominated by a large number of sexually mature medium-size individuals (representing over 60-90% of the local population) with a few large and/or small individuals. Only in a few cases slightly more juveniles were observed, particularly in the Tui Malila and Mangatolo samples of *S. tollmanni* where individuals from 0.4 to 1.2 mm represented between 2 to 6% of the population. Therefore, the absence of a visible massive cohort of small individuals suggested the absence of a major recruitment event at the time of sampling. Three main mechanisms can be proposed to explain these results. First, it could be due to a discontinuous recruitment with a massive arrival of larvae that was responsible of the group of medium-size individuals and other very minor recruitment events, or a chronic failure of the following recruitment events. According to the observed continuous gametogenesis, this pattern could result from a decoupling between reproduction and larval supply with episodic massive recruitment events. For instance, following a massive volcanic eruption in the East Pacific Rise, Mullineaux et al. (2010) highlighted massive recruitment of the limpet *Ctenopelta porifera* from distant areas. However, this hypothesis seems unlikely unless one assumes the same events on the scale of a site as on the scale of the study area. A more likely alternative hypothesis would be that this pattern could result from a continuous recruitment with the regular arrival of a small number of young individuals that grow fast

and accumulate in one or two Gaussian components of medium-sized individuals. Finally, another hypothesis of a settlement outside the *Ifremeria* and *Bathymodiolus* habitats followed by a migration towards these habitats is also unlikely as we sampled other habitats never observing any settlers of the three species. The absence of any recruitment signals, that would have resulted from the settlement of a significant number of larvae in a short period of time, suggests that there is no larval aggregation process and could instead reflect the occasional arrival of larvae from distant locations as hypothesised by Van Dover et al. (2001) for vent invertebrates.

Among Lepetodrilidae, discontinuous recruitment was suggested for *Lepetodrilus elevatus* (Sadosky et al. 2002), while *L. fucensis* appeared to show continuous recruitment (Kelly and Metaxas 2008), which seems here a more likely interpretation for *L. schrolli, L. fijiensis* and *S. tollmanni* limpets. The assumption of continuous recruitment could be supported by the histological observations of the female gonads of the three species, which showed all gamete development stages simultaneously, indicative of continuous gametogenesis (see below). In the particular case of *S. tollmanni*, we observed on most sampled sites that different egg capsules contained embryos at different development stages (same stage within a single capsule), so that all development stages were present simultaneously (authors' personal observations). This further supported the hypothesis of continuous release of larvae and continuous recruitment in this species. On the other hand, the hypothesis of a massive recruitment event at the scale of the different basins of the South West Pacific was unlikely. The observed heterogeneity of size structures would be the result of specific local processes. Indeed, the probability of successful settlement and the mortality rate can be influenced by biotic or abiotic factors, such as competition, predation, or physico-chemical variations, which in turn may also affect demographic structures and induce heterogeneous patterns (Kelly and Metaxas 2008). For example, zoarcid fish along the East Pacific Rise showed a selective predation on *L. elevatus*, especially large individuals (Sancho et al. 2005), while mobile grazers such as snails or filter-feeders such as mussels may also increase juveniles mortality in areas of high faunal density (Micheli et al. 2002; Mullineaux et al. 2003; Lenihan et al. 2008). In areas of strong diffuse venting, competition for space and/or resources can affect community composition (Mullineaux et al. 2003). Physical and chemical

conditions structure organisms spatial distribution, and hydrothermal communities organise themselves according to temperature, pH, O_2 concentration, and chemical composition (Kelly and Metaxas 2008; Matabos et al. 2008; Podowski et al. 2010; Sen et al. 2013; Mullineaux et al. 2018). However, none of the environmental conditions measured in this study nor the habitat type (*Ifremeria* or *Bathymodiolus*) explained the variability observed among size-frequency distributions of the two *Lepetodrilus* species and *S. tollmanni*. In addition, unlike previous findings in *L. fucensis* (Bates 2008) and *L. nux* (Nakamura et al. 2014) the largest individuals of *L. schrolli*, *L. fijiensis* and *S. tollmanni* were not found at the warmest vents. On the other hand, the supply of larvae in a given area is influenced by variations in local hydrodynamics, such as currents and turbulence levels (Mullineaux et al. 2005; Adams and Mullineaux 2008; Adams et al. 2011). This could result in episodic larval supplies differing among fields or among vents within a field, which could influence size-frequency distributions. Mesoscale eddies created on the ocean's surface can also impact the transport of both hydrothermal vent efflux and larvae, creating episodic opportunities for vent species to disperse their larvae across large distances (Adams et al. 2011). Lastly, the lack of information on settlement cues prevents from assessing how physico-chemical conditions influence larval settlement and recruitment.

Reproductive biology

Our results brought new insights into the reproductive biology and anatomy of three abundant gastropod vent species. All three species were gonochoric and both sexes were equally represented in most populations, except for some cases where sex ratio was biased towards females for *L. schrolli* in one sample, and towards males for *S. tollmanni* in three samples. Such a balanced sex ratio combined with a high population density, as observed here, could increase the chances of mating and allow multi-male fertilisation, potentially favouring genetic diversity (Xue et al. 2016). Unlike other *Lepetodrilus* species, *L. schrolli* and *L. fijiensis* lack a penis (Beck 1993; Chen and Sigwart 2023). The presence of a penis was reported for few individuals in the North Fiji Basin (Warén and Bouchet 2001) for the species now named as *L. fijiensis*, but none of the males analysed in the present study

had one, yet their gonads were full of spermatozoa (Figure 2D, F). This is not consistent with Beck (1993)'s hypothesis of a seasonal reproduction with the penis appearing at the time of breeding. The absence of a penis could suggest either an external fertilisation or a pseudo-copulation, with physical contact between males and females that would be facilitated by high densities of individuals with 1:1 sex ratio populations. Pseudo-copulation could allow semi-internal (or entaquatic) fertilisation in the mantle cavity like in other *Lepetodrilus* species (Fretter 1988). It has already been hypothesised for Lepetodrilidae of the genus *Pseudorimula* which males also lack a secondary reproductive organ (Haszprunar 1989; Marticorena et al. 2020). Internal fertilisation might be favoured by hydrothermal gastropods to protect gametes from possible harmful conditions of their environment (Fretter 1988; Matabos and Thiébaut 2010). Some species such as *Shinkailepas tollmanni* produce egg capsules to protect their developing embryos (Beck 1992).

A sexual dimorphism was observed for the two *Lepetodrilus* species with females larger than males but with a large overlap. According to histological observations, a sequential hermaphroditism seems unlikely. Such a dimorphism in size with large overlap has already been observed in *L. nux* from the North West Pacific vent sites (Nakamura et al. 2014). It was also reported in different costal gastropods such as littorinids in which males are usually smaller and grow more slowly than females (Chow 1987; Riascos and Guzman 2010). Such a dimorphism is commonly explained by fecundity selection (Riascos and Guzman 2010), females might grow to a larger size to physically accommodate the development of a large gonad for egg provisioning, although in females of *L. schrolli* and *L. fijiensis* there was no significant relationship between fecundity and size.

Early maturity is expected at vents where resources are not limited and environmental conditions are highly dynamic, and contribute to maximise the number of offspring produced (Ramirez-Llodra 2002). Size at first maturity of females varies among *Lepetodrilus* species: the presence of mature oocytes starts at around 2 mm for *L. nux* (Nakamura et al. 2014), 2.4 mm for *L. tevnianus* (Bayer et al. 2011; Nakamura et al. 2014) and 3.9 mm for *L. fucensis* (Kelly and Metaxas 2007). The smallest female in *L. schrolli* and *L. fijiensis* we observed in our histological analysis was 3.72 mm and 3.48 mm long respectively, and had mature oocytes, while the smallest female sexed based on gonad morphology

was 2.29 mm and 2.51 mm. Unfortunately, females for histological analyses were sorted on board and the absence of females smaller than 3.48 mm in these samples prevented us from defining the size at first sexual maturity for these species. The same was true for *S. tollmanni*, where the smallest female observed on histology was 9.21 mm, while the smallest female sexed was 4.93 mm.

For the three species, female gonad analysis showed a similar pattern with all oocyte development stages present (i.e. oogonia, previtellogenic oocytes, and vitellogenic oocytes), suggesting potential continuous or quasi-continuous reproduction (Berg 1985), with a much higher proportion of previtellogenic than vitellogenic oocytes (on average 87% for *L. schrolli*, 86% for *L. fijiensis* and 73% for *S. tollmanni*). The large overlap in size between previtellogenic and vitellogenic oocytes could be due to their irregular shape. The measurement of the equivalent diameter could either overestimate or underestimate the oocyte size, especially for the larger ones, and thus increase the size range of the different development stages (Copley and Young 2006; Kelly and Metaxas 2007). The high variability of oocyte size distributions among females within a site, and among sites and fields, supported the hypothesis of a continuous or quasi-continuous and an asynchronous gametogenesis between females. Among the Lepetodrilidae as well as other gastropod families, such as Peltospiridae, Sutilizonidae, and Skeneidae, continuous reproduction is widespread (Tyler et al. 2008; Matabos and Thiébaut 2010; Bayer et al. 2011; Nakamura et al. 2014; Marticorena et al. 2020). Continuous or quasi-continuous gametogenesis is also common in other vent taxa including polychaetes (Zal et al. 1995; Faure et al. 2007) and shrimps (Ramirez-Llodra 2002). This type of gametogenesis can be explained by the regular energy flows provided by chemosynthesis (Tyler et al. 1994; Marticorena et al. 2020). Individuals can therefore allocate part of the consumed food resources to produce eggs continuously. This reproductive trait would allow hydrothermal species to maintain viable populations and quickly adapt to changes in environmental conditions and venting activity (Tyler et al. 2008; Matabos and Thiébaut 2010).

Vitellogenic oocytes of *L. schrolli* and *L. fijiensis* had a maximum size of 124 µm and 126 µm, respectively, while those of *S. tollmanni* reached 153 µm, which are in the same order of magnitude as those observed in other hydrothermal limpets from the Juan de Fuca Ridge, East Pacific Rise and Mid-

Atlantic ridge (Matabos and Thiébaut 2010; Bayer et al. 2011; Marticorena et al. 2020). However, the maximum vitellogenic oocyte size in the two *Lepetodrilus* species studied here was slightly larger as compared to other species of *Lepetodrilus*, generally below 100 µm (Tyler et al. 2008). Although egg size often correlates with the development mode of marine invertebrates, large oocytes being associated with lecithotrophic development and smaller ones with planktotrophic development, this is not true for some gastropods, such as lepetodrilids, which have small eggs (<200 µm) but larval shell morphology suggesting non-planktotrophic development (Lutz et al. 1986; Craddock et al. 1997). Conversely, *S. tollmanni* carried vitellogenic oocytes also below 200 µm but a recent study reported a planktotrophic development for this species (Yahagi et al. 2020). Recent studies suggested long-distance larval dispersal capacities, up to a year, in *S. tollmanni* and another Phenacolepadidae (i.e. *S. myojinensis*) (Yahagi et al. 2017, 2020). Although *Lepetodrilus* larvae are thought to be non-planktotrophic (Lutz et al. 1986; Craddock et al. 1997; Plouviez et al. 2019), the cold temperature at the seafloor may imply metabolism reduction and thus allow also long-distance dispersal (Young et al. 1997; Mullineaux et al. 1998).

In *L. schrolli and L. fijiensis*, the number of vitellogenic oocytes ranged between 52 and 205 per female with a mean value of 119 ± 74 and between 80 and 605 with a mean value of 366 ± 183, respectively. Fecundity was relatively high in comparison with mean values already reported for different Lepetodrilidae: 27.9 for *L. ovalis*, 37.2 for *L. atlanticus*, 53.9 for *L. pustulosus*, 125.7 for *L. fucensis* and 187 for *Pseudorimula atlantica* (Kelly and Metaxas 2007; Tyler et al. 2008; Marticorena et al. 2020). However, it is lower than the maximum observed values of 850 for *L. pustulosus* (Tyler et al. 2008) and 5 149 for *L. fucensis* (Kelly and Metaxas 2007). Unlike other *Lepetodrilus* species (Kelly and Metaxas 2007; Tyler et al. 2008; Bayer et al. 2011), the fecundity is not linked to the size of the female, at least in the observed size range. Furthermore, the proportion of vitellogenic oocytes decreased with female size suggesting that the reproductive effort could decrease for the largest individuals.

Finally, our study showed that there was no influence of the habitat type on neither the reproductive biology or population structure. Kelly and Metaxas (2007) showed that in *L. fucensis* fecundity and

oocyte development rate fluctuate according to the habitat, with low oocyte development and low fecundity in individuals living in senescent habitats (i.e. hydrothermally inactive areas with no temperature anomaly, see Kelly and Metaxas (2007) for more details). In addition, Marticorena et al. (2020) suggested that warmer habitats could provide a greater food resource and allow individuals to allocate more energy to vitellogenesis. Despite this, we did not identify any relationships between the gametogenesis variability among samples or the actual fecundity of the two *Lepetodrilus* species and environmental conditions measured or the habitat. This may simply be due to the fact that we did not sample senescent habitats as defined by Kelly and Metaxas (2007) and that both species are rather well adapted to the range of physico-chemical conditions prevailing in the *Bathymodiolus* and *Ifremeria* habitats. Indeed, Kelly and Metaxas (2007) only observed differences between actively venting and senescent sources in *L. fucensis* gametogenesis and fecundity but not between active sources of different intensity. The limited role of the physico-chemical environment may also be a consequence of our limited ability to accurately measure environmental conditions at the individual's scale. In fact, the measurement tools used do not allow for the characterisation of the environmental conditions in direct contact with the organisms. While this is possible for temperature sensor, the risk of damaging the water sampling probe prevents the characterisation of fluid chemistry at this scale. Therefore, it is necessary to place the probe at about 1 cm from the organisms, although the environment present high fluctuations at the centimetre scale. Furthermore, conditions vary greatly in space and time, ranging from seconds (related to turbulence) to several years (linked to flow modifications). The low number of replicates and the one-time measurements may not necessarily represent the dominant environmental conditions encountered by individuals that could influence reproductive traits or population dynamics. Multiple spatial and temporal measurements would be required to effectively characterise the vent environment (Le Bris et al. 2005; Lee et al. 2015; Van Audenhaege et al. 2022).

Conclusion

The three species analysed in this study display a continuous and asynchronous gametogenesis as reported for the majority of vent molluscs (Tyler et al. 2008), and a likely continuous recruitment with a rapid growth and an early age at first maturity but exhibit contrasting phylogeographic patterns

(Poitrimol et al. 2022) and distribution range. While *Shinkailepas tollmanni* has the broadest distribution in the South West Pacific, *Lepetodrilus fijiensis* has a large distribution, from the Woodlark Basin to the Kermadec Volcanic Arc, and *L. schrolli* is distributed only in the Manus and Woodlark basins. *Lepetodrilus schrolli* may then face more challenges in maintaining its populations if mining activities were initiated in the Manus Basin where exploration contracts have been already awarded. Although the impact of disturbances will depend on their attributes (e.g. intensity, timing and extent of a single event, frequency of events, spatial pattern), the resilience of local populations in response to disturbances relies on a balance between increased mortality/emigration, the opportunities to use newly released resources (e.g. food, space) and the ability of species to recolonize sites, which is influenced by factors, such as distance between sites, habitat availability, larval dispersal, and reproductive traits (e.g. age at first maturity, energy allocation to reproduction). In this context, long-living species characterised by episodic recruitment and/or low dispersal are generally reported to be more sensitive to disturbances than fast-growing species with high dispersal capabilities as expected for *Lepetodrilus* species and *S. tollmanni.* Although larval characteristics of the studied species are unknown, population genetics suggested high dispersal capabilities (Yahagi et al. 2020; Poitrimol et al. 2022). On the other hand, continuous and asynchronous gametogenesis will promote extended reproductive period that increases the likelihood that some larvae contribute to the recruitment in a highly variable environment and the resilience of local populations. However, continuous recruitment and reproduction could be effective against natural small-scale disturbances but may not withstand chronic and large-scale commercial mineral mining (Gollner et al. 2017). Finally, for species with high dispersal abilities, larval supply which determines colonisation success will depend on the larval production at the regional scale which is related to individual reproductive effort but also to habitat availability and occupancy frequency of suitable habitat. As suggested by Gollner et al. (2017), large-scale mining may reduce species population size and habitat availability including habitat formed by foundation species, such as *Bathymodiolus* and *Ifremeria,* with negative impact on connectivity and recolonization processes.

Statements and Declarations

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Competing interest

The authors have no relevant financial or non-financial interests to disclose.

Author Contribution

CP, ET and MM conceived and designed the study. CP, ET, MM, TC, AV and AR acquired the biological data. CB and CC were in charge of the acquisition of chemical data. CP, AV, AR, MM and ET analysed the data. CP, AV and AR wrote the first draft of the manuscript and ET and MM commented and revised the first versions. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request. The biological data used in this study are available online (https://doi.org/10.17882/96476).

Ethics approval

All applicable international, national, and/or institutional guidelines for sampling for the study have been followed and all necessary approvals have been obtained. Permission for sampling in Exclusive Economic Zones (EEZ) was issued by the Papua New Guinea, The Republic of Fiji and Kingdom of Tonga. We obtained the agreement to sample in Wallis et Futuna waters by the Haut Commissariat à la République in New Caledonia and the Préfecture in Wallis and Futuna.

Consent to publish

The authors consent to the publication of their work in all Springer publications. They guarantee that the work has not been published elsewhere in any form other than as a preprint, that it has not been submitted in parallel to any other publisher, and that it is not currently under review by any other publisher.

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References

Adams DK, Mullineaux LS (2008) Supply of gastropod larvae to hydrothermal vents reflects transport from local larval sources. Limnol Oceanogr 53:1945–1955. https://doi.org/10.4319/lo.2008.53.5.1945

Adams DK, McGillicuddy DJ, Zamudio L, Thurnherr AM, Liang X, Rouxel O, German CR, Mullineaux LS (2011) Surface-generated mesoscale eddies transport deep-sea products from hydrothermal vents. Science 332:580–583. https://doi.org/10.1126/science.1201066

Bates AE (2008) Size- and sex-based habitat partitioning by *Lepetodrilus fucensis* near hydrothermal vents on the Juan de Fuca Ridge, Northeast Pacific. Can J Fish Aquat Sci 65:2332–2341. https://doi.org/10.1139/F08-139

733 Bayer SR, Mullineaux LS, Waller RG, Solow AR (2011) Reproductive traits of pioneer gastropod
734 species colonizing deep-sea hydrothermal vents after an eruption. Mar Biol 158:181–192.
735 https://doi.org/10.1007/s00227-010-1550-1

736 Beaulieu SE, Szafrański KM (2020) InterRidge Global Database of Active Submarine Hydrothermal
737 Vent Fields Version 3.4.

738 Beaulieu SE, Baker ET, German CR (2015) Where are the undiscovered hydrothermal vents on oceanic
739 spreading ridges? Deep Sea Res Part II Top Stud Oceanogr 121:202–212.
740 https://doi.org/10.1016/j.dsr2.2015.05.001

741 Beck LA (1992) Two new neritacean limpets (Gastropoda: Prosobranchia: Neritacea:
742 Phenacolepadidae) from active hydrothermal vents at Hydrothermal Field 1 "Wienerwald" in
743 the Manus Back-Arc Basin (Bismarck Sea, Papua-New Guinea). Ann Naturhistorischen Mus
744 Wien Ser B Für Bot Zool 93:259–275.

745 Beck LA (1993) Morphological and anatomical studies on a new lepetodrilacean limpet (Gastropoda,
746 Prosobranchia) from hydrothermal vents at the Manus Back-Arc Basin (Bismarck Sea, Papua
747 New Guinea). Ann Naturhistorischen Mus Wien Ser B Für Bot Zool 94/95:167–179.

748 Berg C (1985) Reproductive strategies of mollusks from abyssal hydrothermal vent communities. Bull
749 Biol Soc Wash 6:185–197.

750 Bhattacharya CG (1967) A simple method of resolution of a distribution into Gaussian components.
751 Biometrics 23:115–135. https://doi.org/10.2307/2528285

752 Boschen RE, Rowden AA, Clark MR, Gardner JPA (2013) Mining of deep-sea seafloor massive
753 sulfides: A review of the deposits, their benthic communities, impacts from mining, regulatory
754 frameworks and management strategies. Ocean Coast Manag 84:54–67.
755 https://doi.org/10.1016/j.ocecoaman.2013.07.005

756 Breusing C, Johnson SB, Mitarai S, Beinart RA, Tunnicliffe V (2021) Differential patterns of
757 connectivity in Western Pacific hydrothermal vent metapopulations: A comparison of
758 biophysical and genetic models. Evol Appl 16:22–35. https://doi.org/10.1111/eva.13326

759 Burgess SC, Nickols KJ, Griesemer CD, Barnett LAK, Dedrick AG, Satterthwaite EV, Yamane L,
760 Morgan SG, White JW, Botsford LW (2014) Beyond connectivity: how empirical methods can
761 quantify population persistence to improve marine protected-area design. Ecol Appl 24:257–
762 270. https://doi.org/10.1890/13-0710.1

763 Chavagnac V, Leleu T, Fontaine F, Cannat M, Ceuleneer G, Castillo A (2018) Spatial variations in vent
764 chemistry at the Lucky Strike hydrothermal field, Mid-Atlantic Ridge (37°N): Updates for
765 subseafloor flow geometry from the newly discovered capelinhos vent. Geochem Geophys
766 Geosystems 19:4444–4458. https://doi.org/10.1029/2018GC007765

767 Chen C, Sigwart JD (2023) The lost vent gastropod species of Lothar A. Beck. Zootaxa 5270:401–436.
768 https://doi.org/10.11646/zootaxa.5270.3.2

769 Chow V (1987) Patterns of growth and energy allocation in northern California populations of *Littorina*
770 (Gastropoda: Prosobranchia). J Exp Mar Biol Ecol 110:69–89. https://doi.org/10.1016/0022-
771 0981(87)90067-0

772 Comtet T, Desbruyères D (1998) Population structure and recruitment in mytilid bivalves from the
773 Lucky Strike and Menez Gwen hydrothermal vent fields (37°17'N and 37°50'N on the Mid-
774 Atlantic Ridge). Mar Ecol Prog Ser 163:165–177. https://doi.org/10.3354/meps163165

775 Comtet T, Pennec ML, Desbruyères D (1999) Evidence of a sexual pause in *Bathymodiolus azoricus*
776 (Bivalvia: Mytilidae) from hydrothermal vents of the Mid-Atlantic Ridge. J Mar Biol Assoc U
777 K 79:1149–1150. https://doi.org/10.1017/S0025315499001514

778 Copley JTP, Young CM (2006) Seasonality and zonation in the reproductive biology and population
779 structure of the shrimp *Alvinocaris stactophila* (Caridea: Alvinocarididae) at a Louisiana Slope
780 cold seep. Mar Ecol Prog Ser 315:199–209. https://doi.org/10.3354/meps315199

781 Cotte L, Chavagnac V, Pelleter E, Laës-Huon A, Cathalot C, Dulaquais G, Riso RD, Sarradin P-M,
782 Waeles M (2020) Metal partitioning after in situ filtration at deep-sea vents of the Lucky Strike
783 hydrothermal field (EMSO-Azores, Mid-Atlantic Ridge, 37°N). Deep Sea Res Part Oceanogr
784 Res Pap 157:103204. https://doi.org/10.1016/j.dsr.2019.103204

785 Craddock C, Lutz RA, Vrijenhoek RC (1997) Patterns of dispersal and larval development of
786 archaeogastropod limpets at hydrothermal vents in the eastern Pacific. J Exp Mar Biol Ecol
787 210:37–51. https://doi.org/10.1016/S0022-0981(96)02701-3

788 Dixon DR, Lowe DM, Miller PI, Villemin GR, Colaço A, Serrão-Santos R, Dixon LRJ (2006) Evidence
789 of seasonal reproduction in the Atlantic vent mussel *Bathymodiolus azoricus*, and an apparent
790 link with the timing of photosynthetic primary production. J Mar Biol Assoc U K 86:1363–
791 1371. https://doi.org/10.1017/S0025315406014391

792 Donval J-P, Charlou J-L, Lucas L (2008) Analysis of light hydrocarbons in marine sediments by
793 headspace technique: Optimization using design of experiments. Chemom Intell Lab Syst
794 94:89–94. https://doi.org/10.1016/j.chemolab.2008.06.010

795 Dray S, Bauman D, Blanchet G, Borcard D, Clappe S, Guenard G, Jombart T, Larocque G, Legendre P,
796 Madi N, Wagner HH (2022) adespatial: Multivariate Multiscale Spatial Analysis.

797 Faure B, Chevaldonné P, Pradillon F, Thiébaut E, Jollivet D (2007) Spatial and temporal dynamics of
798 reproduction and settlement in the Pompeii worm *Alvinella pompejana* (Polychaeta:
799 Alvinellidae). Mar Ecol Prog Ser 348:197–211. https://doi.org/10.3354/meps07021

800 Fretter V (1988) New Archaeogastropod limpets from hydrothermal vents; superfamily Lepetodrilacea
801 II. Anatomy. Philos Trans R Soc Lond B Biol Sci 319:33–82.

802 Gabe M (1968) Techniques histologiques. Masson et Cie, Paris, FR

803 Gollner S, Kaiser S, Menzel L, Jones DOB, Brown A, Mestre NC, Van Oevelen D, Menot L, Colaço A,
804 Canals M, Cuvelier D, Durden JM, Gebruk A, Egho GA, Haeckel M, Marcon Y, Mevenkamp
805 L, Morato T, Pham CK, Purser A, Sanchez-Vidal A, Vanreusel A, Vink A, Martinez Arbizu P
806 (2017) Resilience of benthic deep-sea fauna to mining activities. Mar Environ Res 129:76–101.
807 https://doi.org/10.1016/j.marenvres.2017.04.010

808 Gustafson RG, Lutz RA (1994) Molluscan life history traits at deep-sea hydrothermal vents and cold
809 methane/sulfide seeps. In: Reproduction, Larval Biology, and Recruitment of the Deep-sea
810 benthos. Columbia University Press, New York, pp 76–97

811 Haszprunar G (1989) New slit-limpets (Scissurellacea and Fissurellacea) from hydrothermal vents. Part
812 2. Anatomy and relationships. Ser Publ Nat Hist Mus Los Angel Cty 408:1–17.
813 https://doi.org/10.5962/p.226814

814 Hessler RR, Smithey WM, Boudrias MA, Keller CH, Lutz RA, Childress JJ (1988) Temporal change in
815 megafauna at the Rose Garden hydrothermal vent (Galapagos Rift; eastern tropical Pacific).
816 Deep Sea Res Part Oceanogr Res Pap 35:1681–1709. https://doi.org/10.1016/0198-
817 0149(88)90044-1

Hourdez S, Jollivet D (2019) CHUBACARC cruise, L'Atalante R/V. https://doi.org/10.17600/18001111

Johnson SB, Warén A, Vrijenhoek RC (2008) DNA barcoding of *Lepetodrilus* limpets reveals cryptic species. J Shellfish Res 27:43–51. https://doi.org/10.2983/0730-8000(2008)27[43:DBOLLR]2.0.CO;2

Jollivet D, Empis A, Baker MC, Hourdez S, Comtet T, Jouin-Toulmond C, Desbruyères D, Tyler PA (2000) Reproductive biology, sexual dimorphism, and population structure of the deep sea hydrothermal vent scale-worm, *Branchipolynoe seepensis* (Polychaeta: Polynoidae). J Mar Biol Assoc UK 80:55–68. https://doi.org/10.1017/S0025315499001563

Kelly NE, Metaxas A (2007) Influence of habitat on the reproductive biology of the deep-sea hydrothermal vent limpet *Lepetodrilus fucensis* (Vetigastropoda: Mollusca) from the Northeast Pacific. Mar Biol 151:649–662. https://doi.org/10.1007/s00227-006-0505-z

Kelly NE, Metaxas A (2008) Population structure of two deep-sea hydrothermal vent gastropods from the Juan de Fuca Ridge, NE Pacific. Mar Biol 153:457–471. https://doi.org/10.1007/s00227-007-0828-4

Le Bris N, Sarradin P-M, Pennec S (2001) A new deep-sea probe for in situ pH measurement in the environment of hydrothermal vent biological communities. Deep Sea Res Part Oceanogr Res Pap 48:1941–1951. https://doi.org/10.1016/S0967-0637(00)00112-6

Le Bris N, Zbinden M, Gaill F (2005) Processes controlling the physico-chemical micro-environments associated with Pompeii worms. Deep Sea Res Part Oceanogr Res Pap 52:1071–1083. https://doi.org/10.1016/j.dsr.2005.01.003

Le Bris N, Govenar B, Le Gall C, Fisher CR (2006) Variability of physico-chemical conditions in 9°50′N EPR diffuse flow vent habitats. Mar Chem 98:167–182. https://doi.org/10.1016/j.marchem.2005.08.008

Lee RW, Robert K, Matabos M, Bates AE, Juniper SK (2015) Temporal and spatial variation in temperature experienced by macrofauna at Main Endeavour hydrothermal vent field. Deep Sea Res Part Oceanogr Res Pap 106:154–166. https://doi.org/10.1016/j.dsr.2015.10.004

Legendre P, Gallagher ED (2001) Ecologically meaningful transformations for ordination of species data. Oecologia 129:271–280. https://doi.org/10.1007/s004420100716

Lenihan HS, Mills SW, Mullineaux LS, Peterson CH, Fisher CR, Micheli F (2008) Biotic interactions at hydrothermal vents: Recruitment inhibition by the mussel *Bathymodiolus thermophilus*. Deep Sea Res Part Oceanogr Res Pap 55:1707–1717. https://doi.org/10.1016/j.dsr.2008.07.007

Lilliefors HW (1967) On the Kolmogorov-Smirnov test for normality with mean and variance unknown. J Am Stat Assoc 62:399–402. https://doi.org/10.1080/01621459.1967.10482916

Lutz RA, Bouchet P, Jablonski D, Turner RD, Warén A (1986) Larval ecology of mollusks at deep-sea hydrothermal vents. Am Malacol Bull 4:49–54.

Marticorena J, Matabos M, Sarrazin J, Ramirez-Llodra E (2020) Contrasting reproductive biology of two hydrothermal gastropods from the Mid-Atlantic Ridge: implications for resilience of vent communities. Mar Biol 167:109. https://doi.org/10.1007/s00227-020-03721-x

Matabos M, Thiébaut E (2010) Reproductive biology of three hydrothermal vent peltospirid gastropods (*Nodopelta heminoda*, *N. subnoda* and *Peltospira operculata*) associated with Pompeii worms on the East Pacific Rise. J Molluscan Stud 76:257–266. https://doi.org/10.1093/mollus/eyq008

Matabos M, Le Bris N, Pendlebury S, Thiébaut E (2008) Role of physico-chemical environment on gastropod assemblages at hydrothermal vents on the East Pacific Rise (13°N/EPR). J Mar Biol Assoc UK 88:995–1008. https://doi.org/10.1017/S002531540800163X

Metaxas A (2011) Spatial patterns of larval abundance at hydrothermal vents on seamounts: evidence for recruitment limitation. Mar Ecol Prog Ser 437:103–117. https://doi.org/10.3354/meps09283

Micheli F, Peterson CH, Mullineaux LS, Fisher CR, Mills SW, Sancho G, Johnson GA, Lenihan HS (2002) Predation structures communities at deep-sea hydrothermal vents. Ecol Monogr 72:365–382. https://doi.org/10.1890/0012-9615(2002)072[0365:PSCADS]2.0.CO;2

Mitarai S, Watanabe H, Nakajima Y, Shchepetkin AF, McWilliams JC (2016) Quantifying dispersal from hydrothermal vent fields in the western Pacific Ocean. Proc Natl Acad Sci 113:2976–2981. https://doi.org/10.1073/pnas.1518395113

Mottl MJ, Seewald JS, Wheat CG, Tivey MK, Michael PJ, Proskurowski G, McCollom TM, Reeves E, Sharkey J, You C-F, Chan L-H, Pichler T (2011) Chemistry of hot springs along the Eastern Lau Spreading Center. Geochim Cosmochim Acta 75:1013–1038. https://doi.org/10.1016/j.gca.2010.12.008

Mullineaux LS, Mills SW, Goldman E (1998) Recruitment variation during a pilot colonization study of hydrothermal vents (9°50′N, East Pacific Rise). Deep Sea Res Part II Top Stud Oceanogr 45:441–464. https://doi.org/10.1016/S0967-0645(97)00045-3

Mullineaux LS, Peterson CH, Micheli F, Mills SW (2003) Successional mechanism varies along a gradient in hydrothermal fluid flux at deep-sea vents. Ecol Monogr 73:523–542. https://doi.org/10.1890/02-0674

Mullineaux LS, Mills SW, Sweetman AK, Beaudreau AH, Metaxas A, Hunt HL (2005) Vertical, lateral and temporal structure in larval distributions at hydrothermal vents. Mar Ecol Prog Ser 293:1–16. https://doi.org/10.3354/meps293001

Mullineaux LS, Adams DK, Mills SW, Beaulieu SE (2010) Larvae from afar colonize deep-sea hydrothermal vents after a catastrophic eruption. Proc Natl Acad Sci 107:7829–7834. https://doi.org/10.1073/pnas.0913187107

Mullineaux LS, Metaxas A, Beaulieu SE, Bright M, Gollner S, Grupe BM, Herrera S, Kellner JB, Levin LA, Mitarai S, Neubert MG, Thurnherr AM, Tunnicliffe V, Watanabe HK, Won Y-J (2018) Exploring the ecology of deep-sea hydrothermal vents in a metacommunity framework. Front Mar Sci 5:49. https://doi.org/10.3389/fmars.2018.00049

Nakamura M, Watanabe H, Sasaki T, Ishibashi J, Fujikura K, Mitarai S (2014) Life history traits of *Lepetodrilus nux* in the Okinawa Trough, based upon gametogenesis, shell size, and genetic variability. Mar Ecol Prog Ser 505:119–130. https://doi.org/10.3354/meps10779

Pauly D, Caddy JF (1985) A modification of Bhattacharya's method for the analysis of mixtures of normal distributions. FAO Fisheries Circular

Perfit MR, Chadwick WW (1998) Magmatism at mid-ocean ridges: Constraints from volcanological and geochemical investigations. Geophys Monogr-Am Geophys Union 106:59–116.

Petersen S, Krätschell A, Augustin N, Jamieson J, Hein JR, Hannington MD (2016) News from the seabed – Geological characteristics and resource potential of deep-sea mineral resources. Mar Policy 70:175–187. https://doi.org/10.1016/j.marpol.2016.03.012

Plouviez S, Shank TM, Faure B, Daguin-Thiébaut C, Viard F, Lallier FH, Jollivet D (2009) Comparative phylogeography among hydrothermal vent species along the East Pacific Rise reveals vicariant processes and population expansion in the South. Mol Ecol 18:3903–3917. https://doi.org/10.1111/j.1365-294X.2009.04325.x

Plouviez S, LaBella AL, Weisrock DW, Von Meijenfeldt FAB, Ball B, Neigel JE, Van Dover CL (2019) Amplicon sequencing of 42 nuclear loci supports directional gene flow between South Pacific populations of a hydrothermal vent limpet. Ecol Evol 9:6568–6580. https://doi.org/10.1002/ece3.5235

Podowski EL, Ma S, Luther GW, Wardrop D, Fisher CR (2010) Biotic and abiotic factors affecting distributions of megafauna in diffuse flow on andesite and basalt along the Eastern Lau Spreading Center, Tonga. Mar Ecol Prog Ser 418:25–45. https://doi.org/10.3354/meps08797

Poitrimol C, Thiébaut É, Daguin-Thiébaut C, Port A-S, Ballenghien M, Tran Lu Y A, Jollivet D, Hourdez S, Matabos M (2022) Contrasted phylogeographic patterns of hydrothermal vent gastropods along South West Pacific: Woodlark Basin, a possible contact zone and/or stepping-stone. PLOS ONE 17:e0275638. https://doi.org/10.1371/journal.pone.0275638

R Core Team (2020) R: A language and environment for statistical computing.

Ramirez-Llodra E (2002) Fecundity and life-history strategies in marine invertebrates. In: Southward AJ, Tyler PA, Young CM, Fuiman LA (eds) Advances in Marine Biology. Academic Press, London, UK, pp 87–170

Reeves EP, Seewald JS, Saccocia P, Bach W, Craddock PR, Shanks WC, Sylva SP, Walsh E, Pichler T, Rosner M (2011) Geochemistry of hydrothermal fluids from the PACMANUS, Northeast Pual and Vienna Woods hydrothermal fields, Manus Basin, Papua New Guinea. Geochim Cosmochim Acta 75:1088–1123. https://doi.org/10.1016/j.gca.2010.11.008

Riascos JM, Guzman PA (2010) The ecological significance of growth rate, sexual dimorphism and size at maturity of *Littoraria zebra* and *L. variegata* (Gastropoda: Littorinidae). J Molluscan Stud 76:289–295. https://doi.org/10.1093/mollus/eyq011

Sadosky F, Thiébaut E, Jollivet D, Shillito B (2002) Recruitment and population structure of the vetigastropod *Lepetodrilus elevatus* at 13°N hydrothermal vent sites on East Pacific Rise. Cah Biol Mar 43:399–402. https://doi.org/10.21411/CBM.A.3C09EBF1

Sancho G, Fisher CR, Mills S, Micheli F, Johnson GA, Lenihan HS, Peterson CH, Mullineaux LS (2005) Selective predation by the zoarcid fish *Thermarces cerberus* at hydrothermal vents. Deep Sea Res Part Oceanogr Res Pap 52:837–844. https://doi.org/10.1016/j.dsr.2004.12.002

Sarradin P-M, Waeles M, Bernagout S, Le Gall C, Sarrazin J, Riso R (2009) Speciation of dissolved copper within an active hydrothermal edifice on the Lucky Strike vent field (MAR, 37°N). Sci Total Environ 407:869–878. https://doi.org/10.1016/j.scitotenv.2008.09.056

Sasaki T, Warén A, Kano Y, Okutani T, Fujikura K (2010) Gastropods from recent hot vents and cold seeps: systematics, diversity and life strategies. In: Kiel S (ed) The Vent and Seep Biota. Springer Netherlands, Dordrecht, pp 169–254

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9:671–675. https://doi.org/10.1038/nmeth.2089

Sen A, Becker EL, Podowski EL, Wickes LN, Ma S, Mullaugh KM, Hourdez S, Luther GW, Fisher CR (2013) Distribution of mega fauna on sulfide edifices on the Eastern Lau Spreading Center and

943 Valu Fa Ridge. Deep Sea Res Part Oceanogr Res Pap 72:48–60.
944 https://doi.org/10.1016/j.dsr.2012.11.003

945 Shank TM, Fornari DJ, Von Damm KL, Lilley MD, Haymon RM, Lutz RA (1998) Temporal and spatial
946 patterns of biological community development at nascent deep-sea hydrothermal vents (9°50′N,
947 East Pacific Rise). Deep Sea Res Part II Top Stud Oceanogr 45:465–515.
948 https://doi.org/10.1016/S0967-0645(97)00089-1

949 Thaler AD, Amon D (2019) 262 Voyages beneath the sea: a global assessment of macro- and megafaunal
950 biodiversity and research effort at deep-sea hydrothermal vents. PeerJ 7:e7397.
951 https://doi.org/10.7717/peerj.7397

952 Thaler AD, Zelnio K, Saleu W, Schultz TF, Carlsson J, Cunningham C, Vrijenhoek RC, Van Dover CL
953 (2011) The spatial scale of genetic subdivision in populations of *Ifremeria nautilei*, a
954 hydrothermal-vent gastropod from the southwest Pacific. BMC Evol Biol 11:372.
955 https://doi.org/10.1186/1471-2148-11-372

956 Thiébaut E, Huther X, Shillito B, Jollivet D, Gaill F (2002) Spatial and temporal variations of
957 recruitment in the tube worm *Riftia pachyptila* on the East Pacific Rise (9°50[1]N and 13°N). Mar
958 Ecol Prog Ser 234:147–157. https://doi.org/10.3354/meps234147

959 Tyler PA, Young CM (1999) Reproduction and dispersal at vents and cold seeps. J Mar Biol Assoc U
960 K 79:193–208. https://doi.org/10.1017/S0025315499000235

961 Tyler PA, Campos-Creasey, LS, Giles LA (1994) Environmental control of quasi-continuous and
962 seasonal reproduction in deep-sea benthic invertebrates. In: Young CM, Eckelbarger KJ (eds)
963 Reproduction, larval biology, and recruitment of the deep-sea benthos. Columbia University
964 Press, pp 158–178

965 Tyler PA, Pendlebury S, Mills SW, Mullineaux L, Eckelbarger KJ, Baker M, Young CM (2008)
966 Reproduction of gastropods from vents on the East Pacific Rise and the Mid-Atlantic Ridge. J
967 Shellfish Res 27:107–118. https://doi.org/10.2983/0730-8000

968 Van Audenhaege L, Matabos M, Brind'Amour A, Drugmand J, Laës-Huon A, Sarradin P-M, Sarrazin
969 J (2022) Long-term monitoring reveals unprecedented stability of a vent mussel assemblage on
970 the Mid-Atlantic Ridge. Prog Oceanogr 204:102791.
971 https://doi.org/10.1016/j.pocean.2022.102791

972 Van Dover CL, Jenkins CD, Turnipseed M (2001) Corralling of larvae in the deep sea. J Mar Biol Assoc
973 U K 81:823–826. https://doi.org/10.1017/S0025315401004659

974 Vuillemin R, Le Roux D, Dorval P, Bucas K, Sudreau JP, Hamon M, Le Gall C, Sarradin PM (2009)
975 CHEMINI: A new in situ CHEmical MINIaturized analyzer. Deep Sea Res Part Oceanogr Res
976 Pap 56:1391–1399. https://doi.org/10.1016/j.dsr.2009.02.002

977 Warén A, Bouchet P (2001) Gastropoda and Monoplacophora from hydrothermal vents and seeps; new
978 taxa and records. The Veliger 44:116–231.

979 Xue D-X, Zhang T, Liu J-X (2016) Influences of population density on polyandry and patterns of sperm
980 usage in the marine gastropod *Rapana venosa*. Sci Rep 6:23461.
981 https://doi.org/10.1038/srep23461

982 Yahagi T, Watanabe HK, Kojima S, Kano Y (2017) Do larvae from deep-sea hydrothermal vents
983 disperse in surface waters? Ecology 98:1524–1534. https://doi.org/10.1002/ecy.1800

984 Yahagi T, Thaler AD, Van Dover CL, Kano Y (2020) Population connectivity of the hydrothermal-vent
985 limpet Shinkailepas tollmanni in the Southwest Pacific (Gastropoda: Neritimorpha:
986 Phenacolepadidae). PLoS ONE 15:e0239784. https://doi.org/10.1371/journal.pone.0239784

987 Young CM, Sewell MA, Tyler PA, Metaxas A (1997) Biogeographic and bathymetric ranges of Atlantic
988 deep-sea echinoderms and ascidians: the role of larval dispersal. Biodivers Conserv 6:1507–
989 1522. https://doi.org/10.1023/A:1018314403123

990 Zal F, Jollivet D, Chevaldonné P, Desbruyères D (1995) Reproductive biology and population structure
991 of the deep-sea hydrothermal vent worm *Paralvinella grasslei* (Polychaeta: Alvinellidae) at
992 13°N on the East Pacific Rise. Mar Biol 122:637–648. https://doi.org/10.1007/BF00350685

993

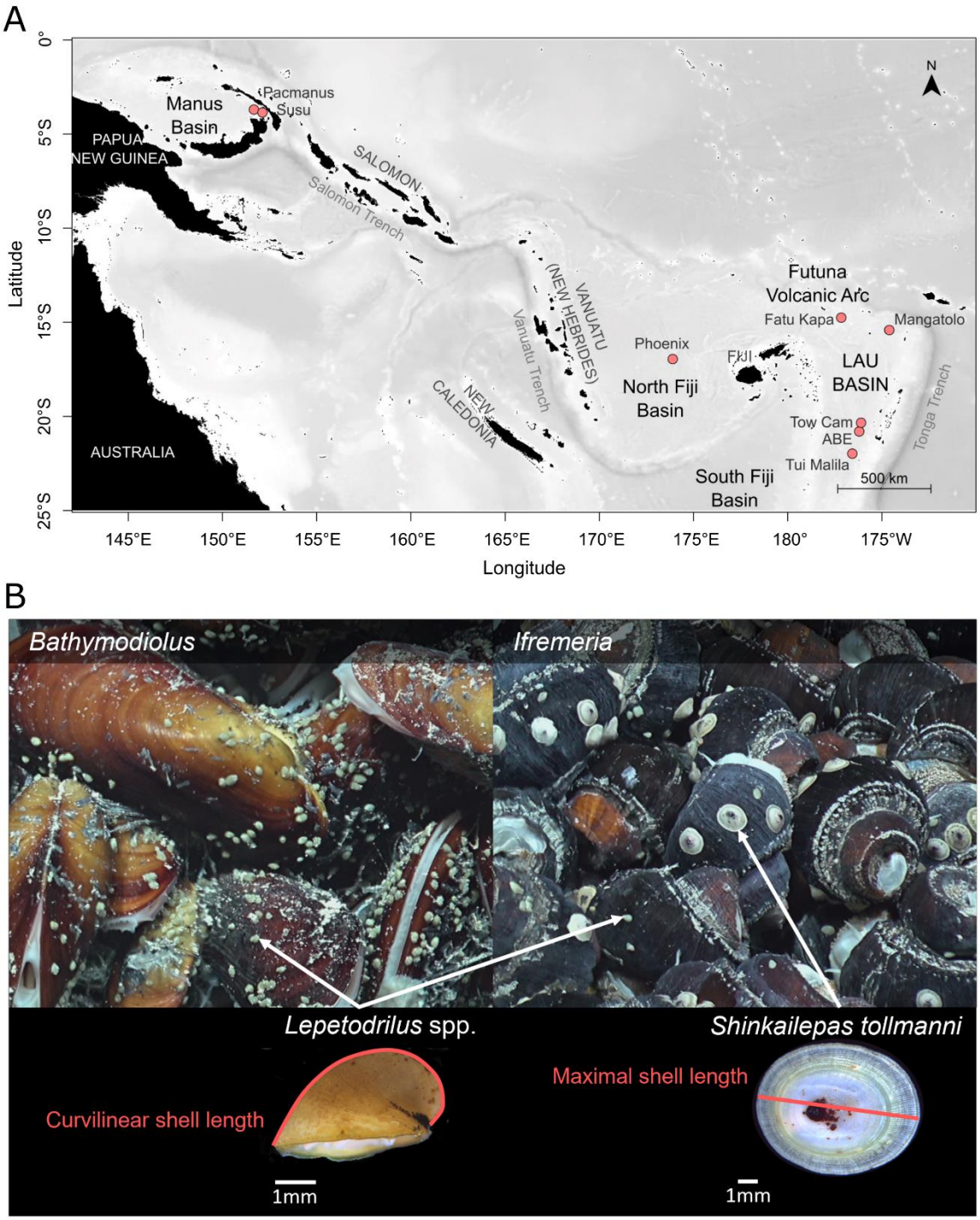

Fig. 1 A Back-arc basins and volcanic arc sampling area from the CHUBACARC cruise in the South West Pacific. Red dots represent sampled vent field. **B** Illustration of *Lepetodrilus* spp. and *Shinkailepas tollmanni* inhabiting the complex three-dimensional habitat formed by *Bathymodiolus* and *Ifremeria nautilei*. The different shell measurements for both taxa were also shown in red.

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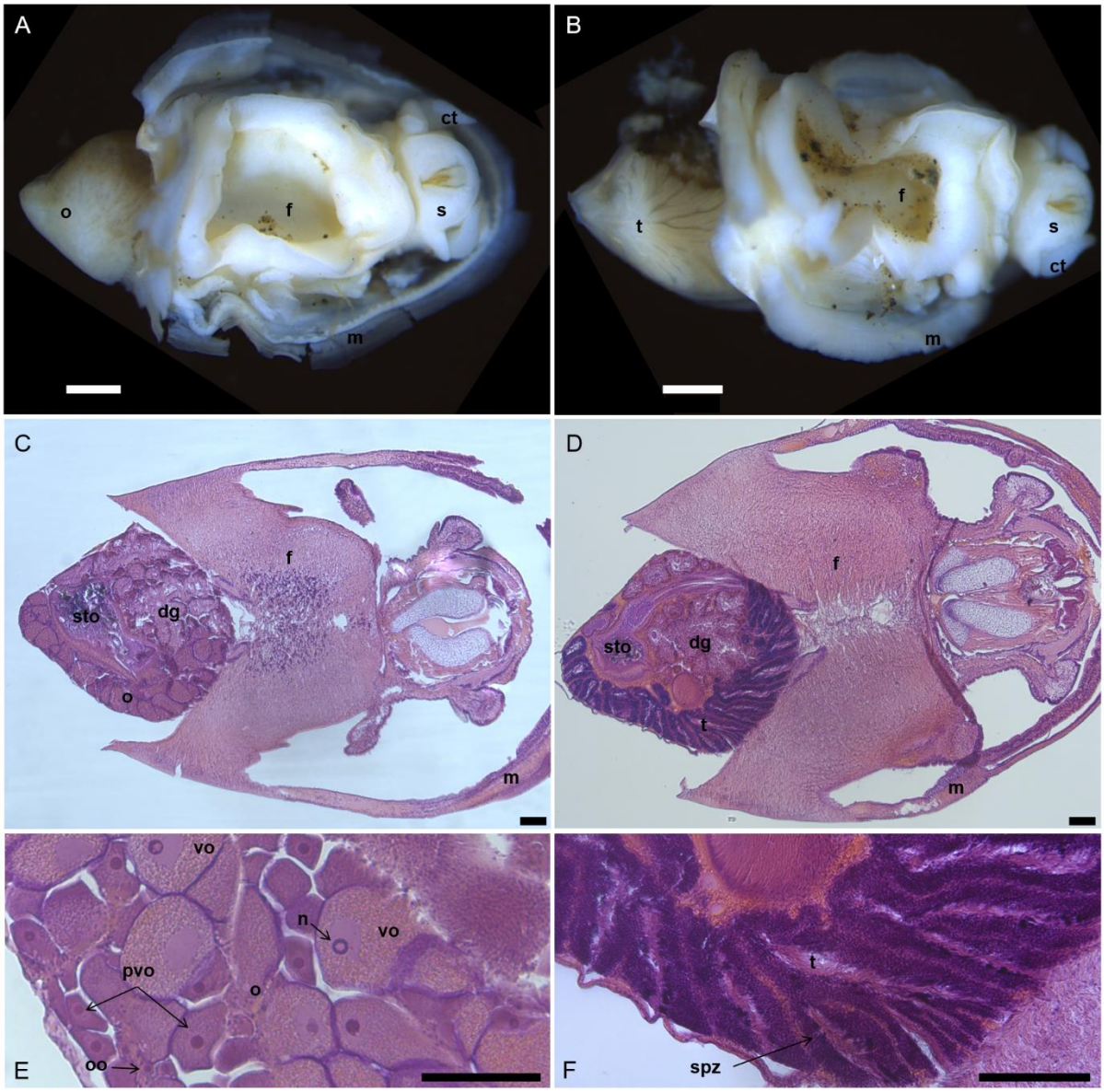

Fig. 2 *Lepetodrilus fijiensis* female and male reproductive structures. **A** Female soft-body, ventral view. **B** Male soft-body, ventral view. **C** General view of female transversal section, ventral view. **D** General view of male transversal section, ventral view. **E** Detailed view of ovary. **F** Detailed view of testis. Abbreviations: *ct* cephalic tentacle; *dg* digestive gland; *f* foot; *m* mantle; *n* nucleus; *o* ovary; *oo* oogonia; *pvo* previtellogenic oocyte; *s* snout; *spz* spermatozoa; *sto* stomach; *t* testis; *vo* vitellogenic oocyte. White scale bar: 500 µm. Black scale bar: 100 µm

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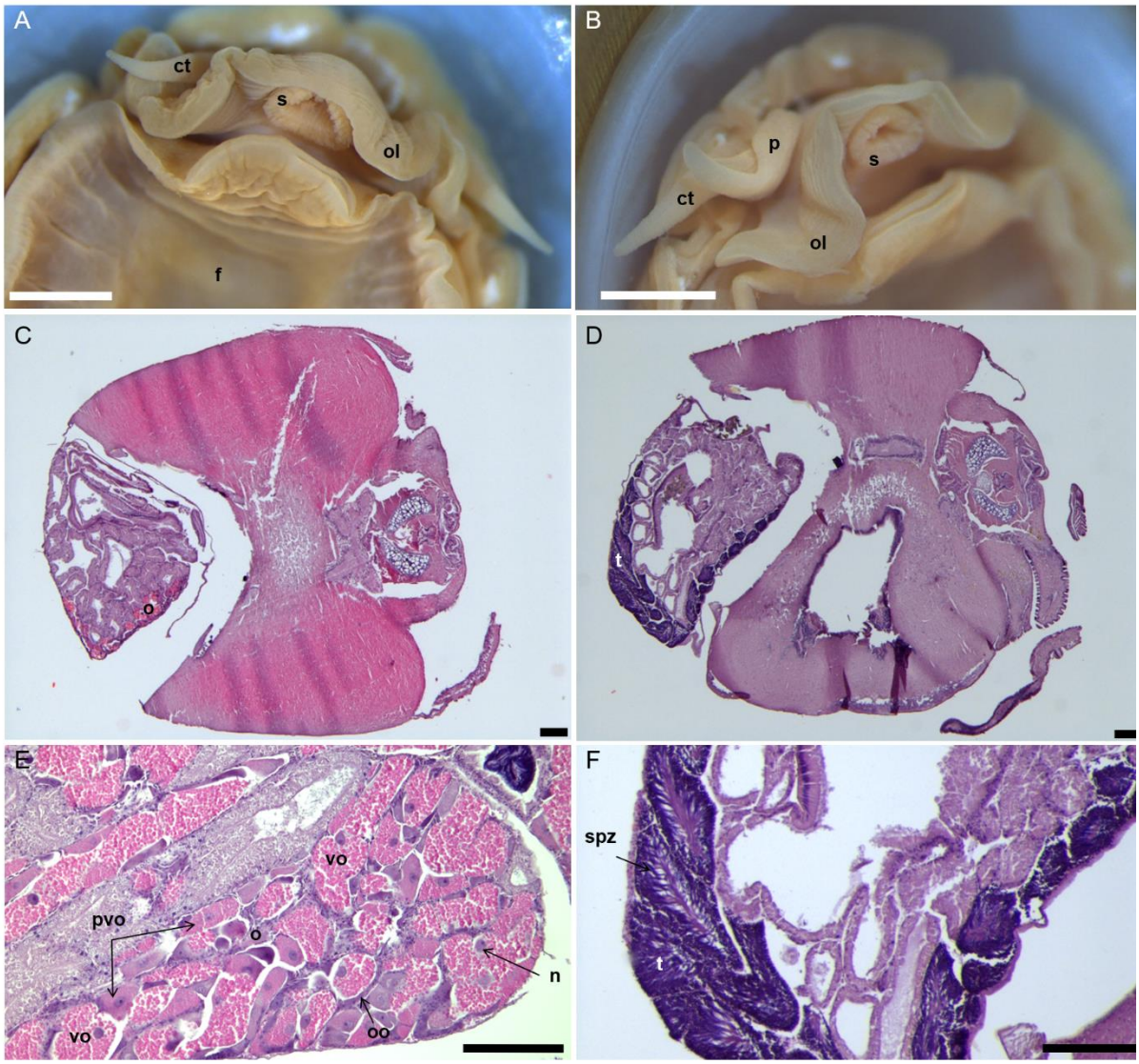

Fig. 3 *Shinkailepas tollmanni* female and male reproductive structures. **A** Detailed view of female anterior part, ventral view. **B** Detailed view of male anterior part, ventral view. **C** General view of female transversal section, ventral view. **D** General view of male transversal section, ventral view. **E** Detailed view of ovary. **F** Detailed view of testis. Abbreviations: *ct* cephalic tentacle; *f* foot; *n* nucleus; *o* ovary; *oo* oogonia; *ol* oral lobe; *p* penis; *pvo* previtellogenic oocyte; *s* snout; *t* testis; *spz* spermatozoa; *vo* vitellogenic oocyte. White scale bar: 1 mm. Black scale bar: 200 µm

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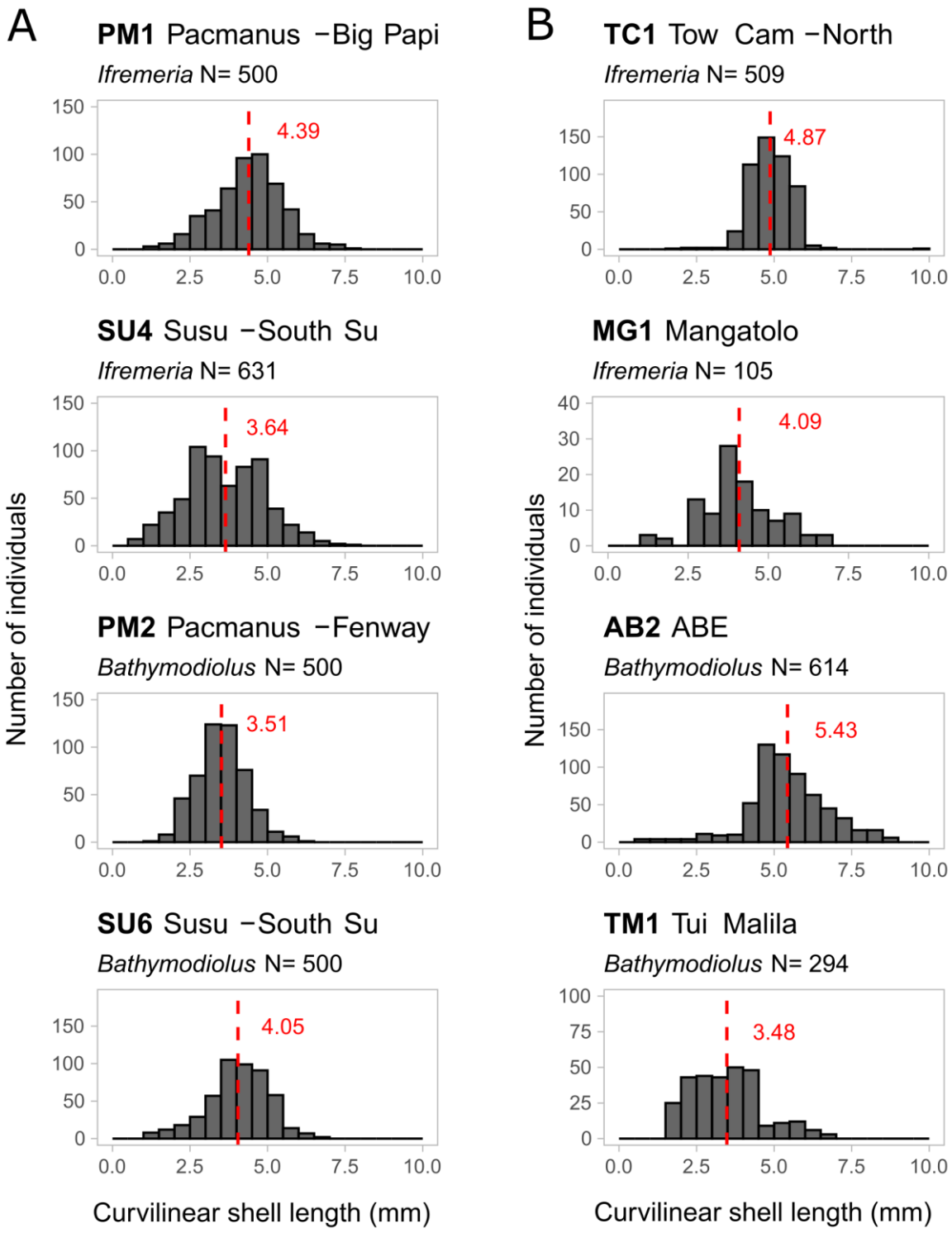

Fig. 4 Example of size-frequency histograms of *Lepetodrilus schrolli* (**A**) and *L.fijiensis* (**B**) collected in *Ifremeria* and *Bathymodiolus* habitats in the South West Pacific. N = number of measured individuals. Mean size is indicated in red

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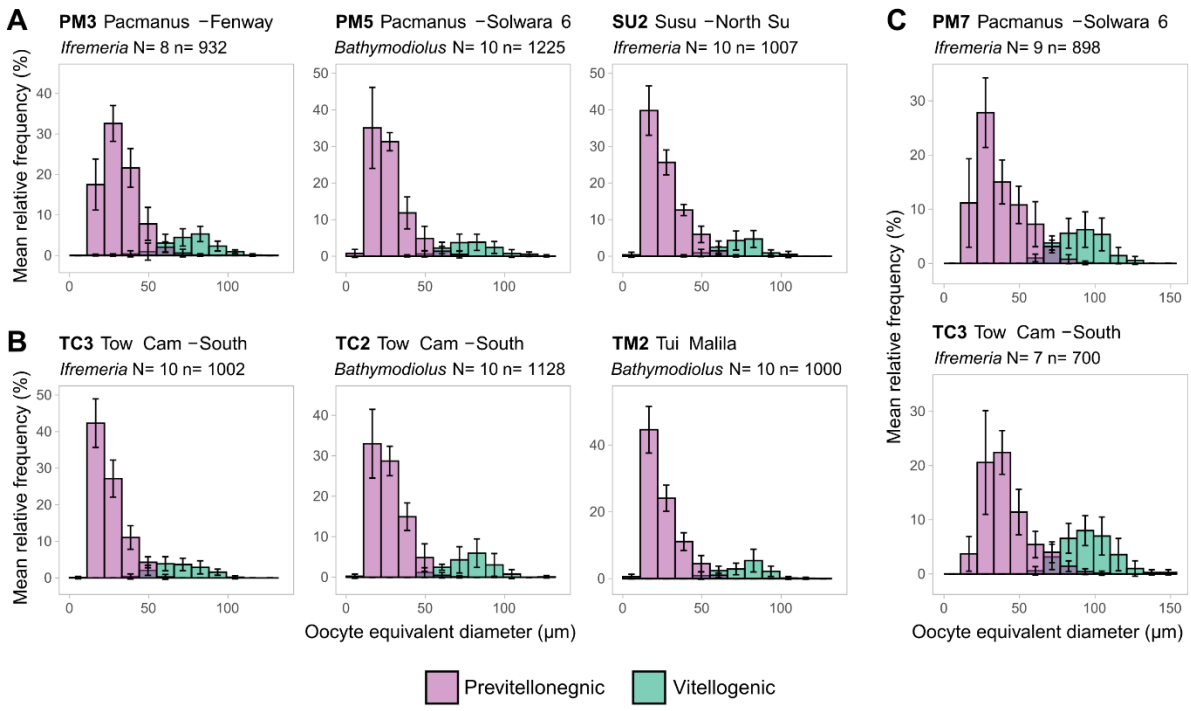

Fig. 5 Examples of mean oocyte size-frequency histograms for females of *Lepetodrilus schrolli* (**A**), *L. fijiensis* (**B**) and *Shinkailepas tollmanni* (**C**) collected in the South West Pacific. N = number of measured individuals; n= number of measured oocytes

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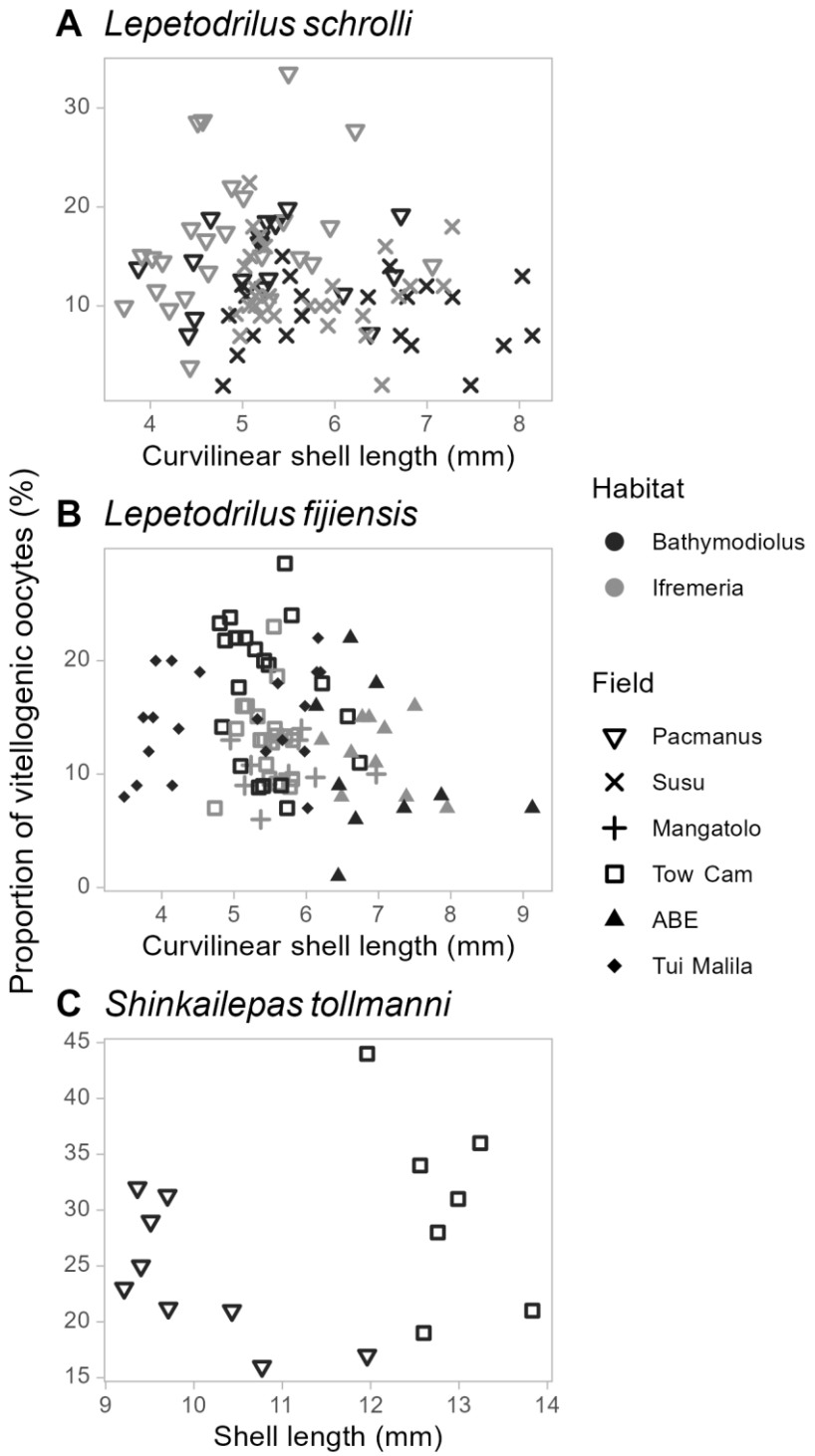

Fig. 6 Proportion of vitellogenic oocytes according to females' size for *Lepetodrilus schrolli* (**A**), *L. fijiensis* (**B**) and *Shinkailepas tollmanni* (**C**) collected in the South West Pacific

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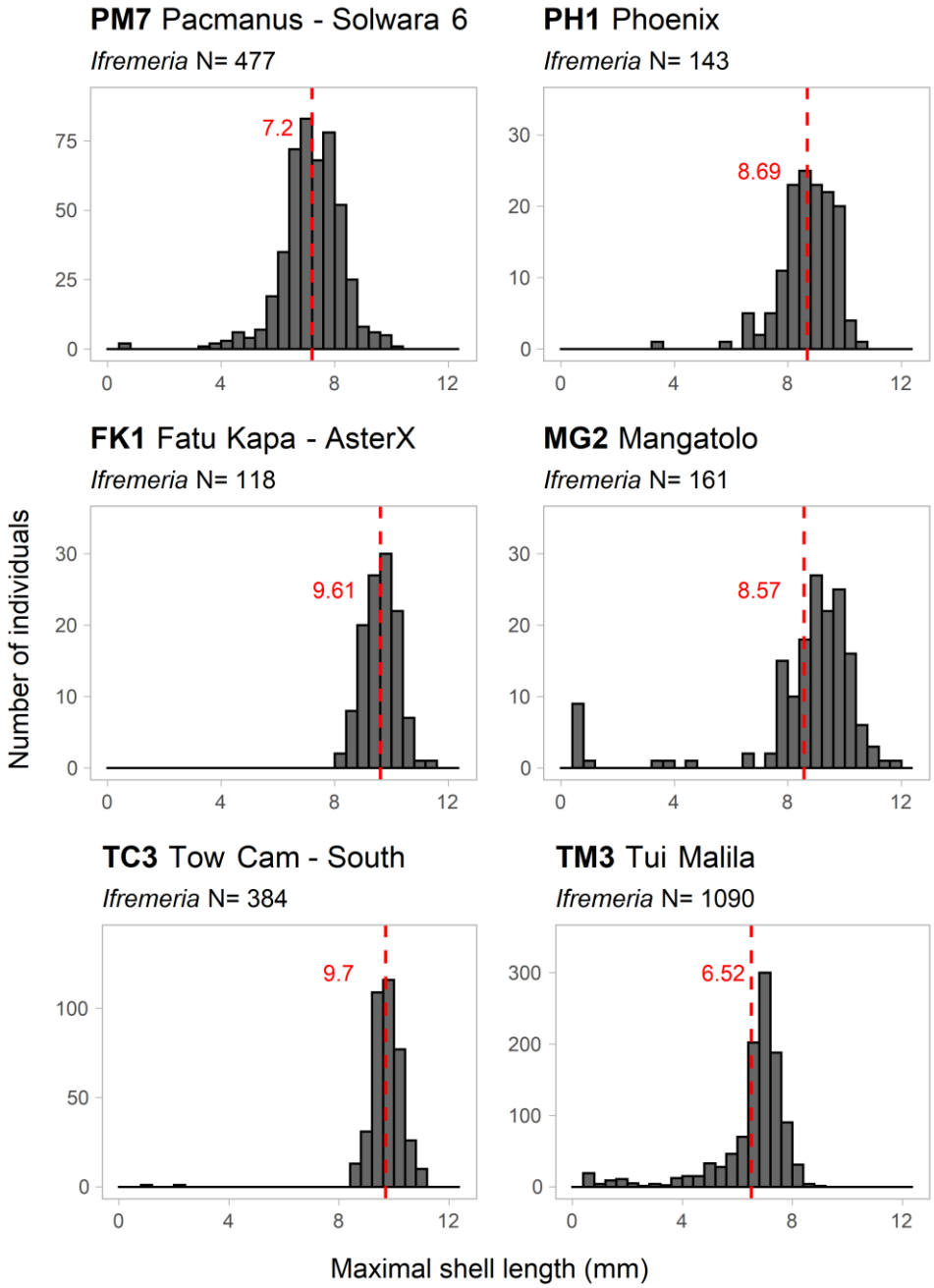

Fig. 7 Example of size-frequency histograms of *Shinkailepas tollmanni* collected in *Ifremeria* and *Bathymodiolus* habitats in the South West Pacific. N = number of measured individuals. Each mean is indicated in red

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Table 1 *Lepetodrilus schrolli, L. fijiensis* and *Shinkailepas tollmanni* sampling locations in the South West Pacific. For each sample, environmental data are provided: the habitat type (Hab: I *Ifremeria*, B *Bathymodiolus*), depth, *in situ* maximal temperature (T°C), mean free inorganic sulphides ± SD (ΣS(-II)), methane (CH_4) concentrations and pH

Basin	Field	Site	Sample	Hab.	Sampling date (2019)	Longitude	Latitude	Depth (m)	T°C (max)	ΣS(-II) (µM)	CH4 (µM)	pH
Lepetodrilus schrolli												
Manus	Pacmanus	Big Papi	PM1	I	16/05	151° 40.342'E	03° 43.707'S	1703	12.07	15.93 ± 8.57	0.29	-
		Fenway	PM2	B	17/05	151° 40.370'E	03° 43.681'S	1698	19.28	-	-	-
			PM3	I	17/05	151° 40.367'E	03° 43.665'S	1699	19.16	-	-	-
		Solwara 8	PM4	I	18/05	151° 40.441'E	03° 43.825'S	1739	10.36	-	-	-
		Solwara 6	PM5	B	19/05	151° 40.861'E	03° 43.649'S	1725	5.44	1.99 ± 1.91	0.12	7.40
		Solwara 7	PM6	I	19/05	151° 40.374'E	03° 43.040'S	1769	6.43	9.88 ± 10.62	0.20	7.28
	Susu	North Su	SU1	B	22/05	152° 06.060'E	03° 47.942'S	1210	9.96	4.63 ± 4.63	0.18	7.47
			SU2	I	23/05	152° 06.046'E	03° 47.935'S	1216	7.73	40.16 ± 12.71	0.44	6.93
			SU3	B	23/05	152° 06.089'E	03° 47.957'S	1195	10.40	2.52 ± 3.51	0.11	7.31
		South Su North	SU4	I	25/05	152° 06.291'E	03° 48.499'S	1341	5.80	170.17 ± 167.55	0.18	7.14
		South Su South	SU5	I	24/05	152° 06.310'E	03° 48.583'S	1352	9.35	81.51 ± 76.32	0.58	6.62
			SU6	B	24/05	152° 06.310'E	03° 48.583'S	1352	7.35	10.09 ± 8.72	0.56	6.43
Lepetodrilus fijiensis												
Lau	Mangatolo		MG1	I	16/04	174° 39.208'W	15° 24.874'S	2031	17.45	61.77 ± 58.09	1.03	6.36
	Tow Cam	North	TC1	I	31/03	176° 08.203'W	20° 19.047'S	2698	4.26	3.36 ± 0.60	0.07	7.35
			TC4	B	01/04	176° 08.211'W	20° 19.051'S	2696	4.24	4.09 ± 0.79	0.07	7.43
		South	TC2	B	31/03	176° 08.250'W	20° 19.074'S	2711	11.98	21.13 ± 3.84	-	6.92
			TC3	I	01/04	176° 08.263'W	20° 19.084'S	2711	7.09	13.27 ± 2.40	0.13	7.30
	ABE	ABE	AB1	I	26/04	176° 11.479'W	20° 45.784'S	2153	7.64	16.02 ± 2.74	-	6.02
			AB2	B	27/04	176° 11.480'W	20° 45.784'S	2154	3.25	3.00 ± 0.97	-	7.57
	Tui Malila	Tui Malila	TM1	B	04/04	176° 34.096'W	21° 59.352'S	1886	5.57	5.92 ± 8.72	0.61	7.31
			TM2	B	03/04	176° 34.088'W	21° 59.351'S	1874	8.84	5.17 ± 3.48	0.12	7.20
Shinkailepas tollmanni												
Manus	Pacmanus	Solwara 6	PM7	I	20/05	151° 40.852'E	03° 43.653'S	1729	12.70	25.98 ± 22.55	0.20	7.38
	Susu	Suzette	SU8	I	22/05	152° 05.783'E	03° 47.368'S	1506	9.19	2.14 ± 0.57	0.10	7.49
		South Su North	SU4	I	25/05	152° 06.291'E	03° 48.499'S	1341	5.80	170.17 ± 167.55	0.18	7.14
North Fiji	Phoenix	Phoenix North	PH1	I	10/04	173° 55.111'E	16° 56.936'S	1974	9.15	12.44 ± 1.10	0.15	7.35
Futuna	Fatu Kapa	AsterX	FK1	I	18/04	177° 09.134'W	14° 45.110'S	1562	12.89	0.5 ± 0.00	0.23	5.88
Lau	Mangaloto	Mangatolo South	MG2	I	17/04	174° 39.330'W	15° 24.958'S	2040	21.33	7.11 ± 5.73	0.51	6.72
	Tow Cam	South	TC3	I	01/04	176° 08.263'W	20° 19.084'S	2711	7.09	13.27 ± 2.40	0.13	7.30
	Tui Malila	Tui Malila	TM1	B	04/04	176° 34.096'W	21° 59.352'S	1886	5.57	5.92 ± 8.72	0.61	7.31
			TM2	B	03/04	176° 34.088'W	21° 59.351'S	1874	8.84	5.17 ± 3.48	0.12	7.20
			TM3	I	04/04	176° 34.098'W	21° 59.355'S	1886	18.83	8.93 ± 5.34	0.34	6.53

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Table 2 Number, shell length range, mean and median (mm) of individuals of *L. schrolli*, *L. fijiensis* and *S. tollmanni* used for demographic analysis within each sample collected in the South West Pacific

Species	Field	Hab.	Sample	Number of individuals	Shell length min - max	Mean (± SD)	Median	Normality test *p* value
L. schrolli	Pacmanus	I	PM1	500	1.13 - 7.80	4.39 ± 1.10	4.46	**0.019**
		B	PM2	500	1.42 - 6.20	3.51 ± 0.80	3.51	0.770
		I	PM3	500	1.79 - 6.96	3.70 ± 0.87	3.63	**0.004**
		I	PM4	440	1.54 - 5.77	3.88 ± 0.75	3.90	0.120
		B	PM5	500	1.33 - 8.27	4.71 ± 1.14	4.62	**0.004**
		I	PM6	500	0.75 - 5.25	2.89 ± 0.81	2.95	0.056
	Susu	B	SU1	500	1.52 - 8.16	3.87 ± 0.96	3.82	**0.1e-03**
		I	SU2	500	1.72 - 5.45	3.58 ± 0.57	3.54	**0.004**
		B	SU3	500	1.99 - 9.51	5.93 ± 1.14	5.65	**< 2.2e-16**
		I	SU4	631	0.51 - 7.84	3.64 ± 1.27	3.55	**0.002**
		I	SU5	500	1.00 - 7.13	3.78 ± 1.09	3.84	**4.8e-05**
		B	SU6	500	1.13 - 6.81	4.05 ± 0.99	4.10	**0.6e-03**
L. fijiensis	Mangatolo	I	MG1	105	1.31 - 6.98	4.09 ± 1.17	3.96	**0.004**
	Tow Cam	I	TC1	509	1.85 - 9.86	4.87 ± 0.66	4.83	**0.013**
		B	TC2	501	2.52 - 6.52	4.70 ± 0.60	4.61	**3.6e-06**
		I	TC3	500	2.72 - 6.17	4.76 ± 0.54	4.67	**4.2e-09**
		B	TC4	501	2.40 - 6.94	4.79 ± 0.67	4.78	**0.041**
	ABE	I	AB1	182	1.57 - 7.95	4.80 ± 1.10	4.84	**0.004**
		B	AB2	614	0.74 - 8.94	5.43 ± 1.29	5.29	**1.6e-13**
	Tui Malila	B	TM1	294	1.71 - 6.90	3.48 ± 1.14	3.41	**0.006**
		B	TM2	199	2.16 - 5.50	3.61 ± 0.53	3.55	**0.010**
S. tollmanni	Pacmanus	I	PM7	477	0.55 - 10.24	7.20 ± 1.08	7.21	**7.7e-06**
	Susu	I	SU8	355	0.68 - 12.20	9.09 ± 1.41	9.31	**9.3e-13**
		I	SU4	319	3.22 - 11.66	8.40 ± 1.33	8.40	**0.004**
	Phoenix	I	PH1	143	3.21 - 10.46	8.69 ± 0.98	8.76	**0.016**
	Futuna	I	FK1	118	8.15 - 11.56	9.61 ± 0.60	9.66	0.843
	Mangatolo	I	MG2	161	0.74 - 11.74	8.57 ± 2.31	9.13	**< 2.2e-16**
	Tow Cam	I	TC3	384	1.17 - 11.16	9.70 ± 0.76	9.72	**< 2.2e-16**
	Tui Malila	B	TM1	202	0.76 - 9.22	6.98 ± 1.58	7.28	**< 2.2e-16**
		B	TM2	350	3.43 - 8.43	6.88 ± 0.47	6.87	**0.010**
		I	TM3	1090	0.70 - 8.90	6.52 ± 1.43	6.90	**< 2.2e-16**

Hab Habitat, *I Ifremeria*, *B Bathymodiolus*. Normality test: Kolmogorov-Smirnov one-sample test adapted by Lilliefors. Significant values are shown in bold

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Table 3 Number and characteristics of individuals of *Lepetodrilus schrolli, L. fijiensis* and *Shinkailepas tollmanni* used for the sex ratio analysis within each sample from the South West Pacific

			Sex ratio								
				Female		Male					
Field	Hab.	S.	N	Mean length (mm) ± SD	min - max	Mean length (mm) ± SD	min - max	WMW *p* value	M/F	X^2	*p* value
Lepetodrilus schrolli											
Pacmanus	I	PM1	103	5.77 ± 1.33	2.91 - 9.63	5.24 ± 0.79	3.78 - 6.92	**0.002**	1.06	0.09	0.768
	I	PM3	100	4.32 ± 0.92	2.70 - 6.20	3.66 ± 0.59	2.46 - 4.71	**5.1e-05**	1.27	1.44	0.230
	B	PM5	103	5.32 ± 0.98	3.30 - 7.79	4.66 ± 0.62	3.41 - 6.04	**0.3e-03**	0.66	4.28	**0.039**
Susu	B	SU3	102	6.68 ± 1.07	3.09 - 8.70	5.33 ± 0.45	4.41 - 6.30	**2.4e-13**	0.96	0.04	0.843
	I	SU5	101	4.30 ± 0.95	2.29 - 6.56	3.84 ± 0.74	2.22 - 5.31	**0.006**	0.94	0.09	0.765
Lepetodrilus fijiensis											
Mangatolo	I	MG1	91	4.56 ± 1.26	2.51 - 6.98	3.76 ± 0.62	2.74 - 5.21	**0.002**	0.90	0.27	0.600
Tow Cam	I	TC1	101	5.26 ± 0.38	4.56 - 6.01	4.52 ± 0.35	3.80 - 5.18	**1.4e-13**	1.24	1.20	0.274
	B	TC2	100	5.01 ± 0.49	4.04 - 6.51	4.18 ± 0.28	3.69 - 5.10	**4.6e-15**	0.96	0.04	0.842
ABE	B	AB2	100	6.99 ± 1.00	4.56 - 9.02	5.38 ± 0.55	4.17 - 6.43	**1.4e-13**	0.89	0.36	0.549
Tui Malila	B	TM1	101	4.96 ± 0.78	2.81 - 6.52	4.20 ± 0.53	3.18 - 5.78	**4.8e-08**	1.46	3.57	0.059
Shinkailepas tollmanni											
Pacmanus	I	PM7	440	7.40 ± 0.81	4.93 – 9.64	7.14 ± 0.94	4.42 – 10.24	**0.4e-03**	1.22	4.40	**0.036**
Susu	I	SU8	326	9.16 ± 1.33	4.93 – 12.20	9.13 ± 1.17	4.79 – 11.80	0.174	1.26	4.43	**0.035**
	I	SU4	287	8.59 ± 1.25	5.53 – 11.42	8.20 ± 1.04	5.19 – 11.66	**0.002**	1.04	0.09	0.768
Phoenix	I	PH1	118	8.61 ± 0.80	5.88 – 10.03	8.51 ± 0.87	6.47 – 10.37	0.192	1.11	0.31	0.581
Futuna	I	FK1	94	9.80 ± 0.67	8.15 – 11.56	9.35 ± 0.51	8.41 – 10.46	**0.2e-03**	1.35	2.09	0.149
Mangatolo	I	MG2	148	9.31 ± 0.98	6.41 – 11.74	9.10 ± 0.84	6.58 – 10.92	0.080	1.21	1.32	0.250
Tow Cam	I	TC3	358	9.85 ± 0.51	8.43 – 11.15	9.65 ± 0.46	8.50 – 10.92	**0.1e-03**	1.34	7.55	**0.006**
Tui Malila	B	TM1	348	7.38 ± 0.72	5.02 – 9.22	7.30 ± 0.68	4.96 – 9.06	0.188	1.07	0.19	0.663
	B	TM2	988	6.90 ± 0.46	5.79 – 8.43	6.86 ± 0.40	5.70 – 8.31	0.288	1.10	0.76	0.391
	I	TM3	190	6.88 ± 0.72	4.93 – 8.90	6.92 ± 0.63	4.95 – 8.59	0.689	1.12	2.95	0.085

Hab Habitat, *I Ifremeria*, *B Bathymodiolus*, *N* Number of individuals, *S* Sample, *F* Female, *M* Male, *WMW* Wilcoxon-Mann-Whitney test (testing if *F* lengths > *M* lengths), X^2 Chi-squared goodness-of–fit test (testing the deviation from a balanced sex ratio). Significant values are shown in bold

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Table 4 Number and characteristics of females of *Lepetodrilus schrolli*, *L. fijiensis* and *Shinkailepas tollmanni* used for gametogenesis and fecundity analysis within each sample from the South West Pacific

Field	Hab.	S.	Gametogenesis				Fecundity	
			N	oocyte size min - max (µm)	KW *p* value	Prop. vo min - max (%)	N	Nb of vo
Lepetodrilus schrolli								
Pacmanus	B	PM2	7	11.17 - 110.43	**1.4e-09**	7 - 19		
	I	PM3	8	11.71 - 112.75	**0.031**	14 - 28		
	I	PM4	9	8.99 - 117.19	**1.9e-09**	10 - 34		
	B	PM5	10	7.96 - 121.70	**<2.2e-16**	7 - 20	1	205
	I	PM6	9	12.65 - 124.13	**3.7e-09**	4 - 15		
Susu	B	SU1	5	11.29 - 118.23	0.547	2 - 11		
	I	SU2	10	8.59 - 110.00	**0.004**	9 - 22		
	B	SU3	10	9.35 - 106.84	**8.1e-11**	2 - 15	2	156; 63
	I	SU4	10	9.34 - 104.45	**0.002**	7 - 18		
	I	SU5	8	11.23 - 98.63	**1.5e-09**	2 - 14	1	52
	B	SU6	7	9.91 - 105.18	**0.003**	7 - 13		
Lepetodrilus fijiensis								
Mangatolo	I	MG1	10	10.31 - 106.86	0.154	6 - 14	2	538; 338
Tow Cam	I	TC1	10	10.53 - 113.35	**7.3e-09**	9 - 16		
	B	TC2	10	10.27 - 126.23	**2.2e-10**	9 - 29		
	I	TC3	10	10.16 - 109.15	**0.009**	7 - 23		
	B	TC4	10	9.22 - 116.65	**2.8e-09**	7 - 24	1	197
ABE	I	AB1	10	10.46 - 99.25	0.144	1 - 16	1	605
	B	AB2	9	11.57 - 106.23	**1.2e-08**	1 - 22	1	411
Tui Malila	B	TM1	10	10.44 - 98.04	**0.003**	7 - 22	1	393
	B	TM2	10	9.84 - 111.00	**0.004**	8 - 20	1	80
Shinkailepas tollmanni								
Pacmanus	I	PM7	9	13.28 - 130.61	**6.0e-06**	16 - 32		
Tow Cam	I	TC3	7	14.47 - 152.92	**2.4e-08**	19 - 44		

Hab Habitat, *I Ifremeria*, *B Bathymodiolus*, *N* Number of individuals, *S* Sample, *vo* Vitellogenic oocyte, *KW* Kruskal-Wallis test (testing differences among female oocyte sizes). Significant values are shown in bold