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Camille Poitrimol, Marjolaine Matabos, Alicia Veuillot, Annah Ramière, Thierry Comtet, et al.. Reproductive biology and population structure of three hydrothermal gastropods (*Lepetodrilus schrolli*, *L. fijiensis* and *Shinkailepas tollmanni*) from the South West Pacific back-arc basins. *Marine Biology*, 2024, 171 (1), pp.31. 10.1007/s00227-023-04348-4 . hal-04452291

HAL Id: hal-04452291

<https://hal.science/hal-04452291v1>

Submitted on 6 Nov 2024

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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

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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

47 **Introduction**

48 Understanding the processes involved in the colonisation of new sites and the connectivity is critical to
49 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 Szafranski 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 nautiliei*, 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

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

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

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

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

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

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

153

154 **Materials and methods**

155 **Sampling**

156 All specimens of *Lepetodrilus schrolli*, *L. fijiensis* and *Shinkailepas tollmanni* were sampled using the
157 hydraulic arm of the Remotely Operated Vehicle (ROV) *Victor6000* during the CHUBACARC cruise
158 (Hourdez and Jollivet 2019) held onboard the French research vessel *L'Atalante* between March and
159 June 2019. Samples were collected in *Ifremeria nautilei* and *Bathymodiolus* habitats from low to
160 moderate diffused flow areas, in several hydrothermal vent fields from three back-arc basins of the
161 South West Pacific, i.e. the Manus, North Fiji and Lau basins, and one volcanic arc, i.e. Futuna
162 (Figure 1A, Table 1). To roughly characterise environmental conditions in the sampling habitat and to
163 investigate potential variation in reproductive features and population structure in relation to
164 environmental conditions, physico-chemical measurements were conducted in each sampling area
165 prior to sampling. *In situ* temperature was measured with the high-temperature probe of the ROV, and
166 free inorganic sulphides [$\Sigma\text{S(-II)} = \text{H}_2\text{S} + \text{HS}^- + \text{S}^{2-}$] were measured with the *in situ* chemical
167 miniaturised analyser CHEMINI (Vuillemin et al. 2009). As the sample was pumped without any
168 filtration, the chemical species analysed with CHEMINI correspond to an operationally defined
169 fraction of sulphides called free inorganic sulphides which includes dissolved and particulate sulphides
170 that are enough labile to be measured by the colorimetric method (Cotte et al. 2020). Diluted fluid
171 samples were collected above the substrate with an *in situ* water sampler mounted on the ROV. The
172 collected fluids were then analysed on board for pH, and methane concentrations were measured by
173 gas chromatography after gas extraction (Donval et al. 2008). While temperature and free inorganic
174 sulphides were measured on three replicate points, diluted fluid was sampled on one point because of
175 logistic limitations.

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

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

181 **Population structure**

182 To infer population structure, size-frequency distributions were analysed by measuring the curvilinear
183 shell length (i.e. the longest distance from the apex to the anterior edge of the shell along the dorsal
184 side, Sadosky et al. 2002; Matabos et al. 2008) for *Lepetodrilus* species and the maximal antero-
185 posterior shell length for *S. tollmanni* (Figure 1B). The measured length was chosen according to the
186 shape of the shell and previous studies (Sadosky et al. 2002; Matabos et al. 2008). Only samples with
187 at least 100 individuals were selected (considered as the minimum required for size-frequency
188 distributions), and a random sub-sample of 500 individuals was used for larger samples.

189 Measurements were conducted with the Leica Application Suite software linked to a Leica MC 170
190 HD camera mounted on a Leica M125 stereoscopic microscope for *Lepetodrilus* species, and through
191 the ZEN pro 3.2 software connected to a ZEISS AxioCam 208 Color camera mounted on a ZEISS
192 SteREO Discovery.V20 stereoscopic microscope for *S. tollmanni*. Post-larval and juvenile shells of *S.*
193 *tollmanni* were photographed under an Olympus SX16 microscope linked by an Infinity 1 Camera to
194 the Infinity capture software and measured using ImageJ (Schneider et al. 2012). Measurement error
195 was determined using the maximum difference among ten repeated measures of the same individual
196 on 10 specimens covering the whole size range of both species. It was fixed at 0.213 mm for
197 *Lepetodrilus* species and 0.125 mm for *S. tollmanni*. Length-class interval was then set at 0.5 and 0.4
198 mm for *Lepetodrilus* species and *S. tollmanni*, respectively, according to the three criteria proposed by
199 Jollivet et al. (2000): (1) most size-classes must have at least five individuals; (2) the number of
200 adjacent empty classes must be minimised; and (3) the interval has to be much greater than the error of
201 measurement.

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

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

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

226

227 **Sex ratio and reproductive biology**

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

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

238 Reproductive characteristics of each species were assessed through gonad histology. Individuals were
239 removed from their shell with forceps after having been measured as described above. The whole soft-
240 body part was then dehydrated with a series of increasing concentration of ethanol (starting with 80%,
241 then 95% and finally 100%), cleared in xylene, infiltrated of liquid paraffin and embedded into
242 paraffin blocks. Serial 9- μ m and 7- μ m thick sections of gonads were produced with a microtome for
243 *Lepetodrilus* spp. and *Shinkailepas tollmanni* individuals, respectively. Sections were mounted onto
244 microscope slides and stained with haematoxylin and eosin following the protocol proposed by Gabe
245 (1968). Photographs of histological sections were taken using the Leica Application Suite AF software
246 connected to a Leica DFC 450C camera on a Leica DMI6000 B inverted videomicroscope. Oocytes
247 were then measured and counted using the ImageJ software.

248 For gametogenesis studies, two oocyte development stages were targeted: the vitellogenic stage which
249 represents mature oocytes, and the previtellogenic stage considered as non-mature. Oogonia were not
250 considered as their first stages are very difficult to identify. Maximum and minimum Feret’s diameter
251 of at least 100 previtellogenic and vitellogenic oocytes per individual were measured from two to five
252 sections selected in the beginning, middle and end part of the gonad. Only oocytes that have been
253 sectioned through the nucleus were considered. As oocyte shape is variable, maximum and minimum
254 Feret’s diameter were used to calculate the area of an ellipse to infer the area-equivalent diameter

255 which is $\sqrt{\frac{4 \times area}{\pi}}$. This is the estimated diameter of a circle with the same area as the object.

256 Measurement error, fixed at 5 μ m, was determined using the maximum difference between ten

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

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

271

272 **Results**

273 **Environmental conditions**

274 Environmental conditions are presented in Table 1. Maximal temperature recorded within the
275 *Ifremeria* habitat ranged from 4.26 to 21.33°C. The mean $\Sigma\text{S(-II)}$ concentrations ranged from $0.50 \pm$
276 0.00 to $170.17 \pm 167.55 \mu\text{M}$, while CH_4 concentrations ranged from 0.07 to 1.03 μM . The pH varied
277 from 5.88 to 7.49. Within the *Bathymodiolus* habitat, maximal temperature ranged from 3.25°C to
278 19.28°C. The mean $\Sigma\text{S(-II)}$ concentrations ranged from 1.99 ± 1.91 to $21.13 \pm 3.84 \mu\text{M}$; CH_4
279 concentrations varied from 0.07 to 0.61 μM ; pH ranged from 6.43 to 7.57. A significant difference
280 between habitats was detected for the mean $\Sigma\text{S(-II)}$ concentration only (Wilcoxon-Mann-Whitney test:
281 $w = 33$, p value = 0.041).

282 *Lepetodrilus schrolli* and *L. fijiensis*

283 **Population structure**

284 In total, length-frequency distributions were established from 6 079 individuals of *Lepetodrilus*
285 *schrolli* from the Manus Basin and 3 405 individuals of *L. fijiensis* from the Lau Basin, sampled in the
286 *Ifremeria* and *Bathymodiolus* habitats (Table 2). *Lepetodrilus schrolli* shell length ranged from 0.51 to
287 9.51 mm, with population mean lengths varying from 2.89 ± 0.81 to 5.93 ± 1.14 mm. *Lepetodrilus*
288 *fijiensis* shell length ranged from 0.74 to 9.86 mm, with population mean lengths varying from $3.48 \pm$
289 1.14 to 5.43 ± 1.29 mm (Table 2). Except for *L. schrolli* in the PM2, PM4 and PM6 samples collected
290 at Pacmanus in either *Ifremeria* or *Bathymodiolus* habitats, all length-frequency distributions of
291 *Lepetodrilus* populations significantly differed from a normal distribution (Lilliefors test, p values $<$
292 0.05 , see Table 2 for specific p values). However, most were unimodal and characterised by a large
293 number of medium-size individuals (~ 60-90% of the sample for both species, considering the four
294 size classes around the mean) coupled with a few small and/or large individuals, which led to a strong
295 asymmetry for some samples (Figure 4A-B, see supplementary material for all size-frequency
296 histograms). For both species, between one and three Gaussian components with varying proportions
297 were identified through modal decomposition with Mixdist. However, analyses did not allow to infer
298 biologically meaningful cohorts (i.e. a group of individuals from the same population and born at the
299 same time, see discussion) and were hence not shown. The Kruskal-Wallis tests highlighted significant
300 differences in shell length among samples for both species (*L. schrolli*: $H = 1884.5$, $df = 11$, p value $<$
301 $2.2e-16$; *L. fijiensis*: $H = 948.2$, $df = 8$, p value $<$ $2.2e-16$). The Nemenyi and Dunn multiple pairwise
302 comparisons test among samples of *L. schrolli* showed variability at the vent field scale. Within
303 Pacmanus, all but three pairs of samples out of fifteen significantly differed (i.e. PM3 vs. PM4 both
304 from *Ifremeria* habitats, PM2 from *Bathymodiolus* vs. PM3 from *Ifremeria* and PM5 from
305 *Bathymodiolus* vs. PM3 from *Ifremeria*). Among Susu samples, all but four pairs out of fifteen
306 significantly differed (i.e. SU1 vs. SU4 and SU5 from *Ifremeria* and *Bathymodiolus* habitat, SU4 vs.
307 SU2 and SU5 from *Ifremeria* habitat). Within-field variability also occurred among the *L. fijiensis*
308 samples. Samples AB1 and AB2 from ABE were significantly different (p value = $4.3e-09$); only TC1

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

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

322 **Sex ratio and reproductive biology**

323 For both *Lepetodrilus* species, sexing was possible for the individuals longer than 2 mm (curvilinear
324 length). In total, 509 individuals of *L. schrolli* and 493 individuals of *L. fijiensis* were sexed (Table 3).
325 None of the sexed individuals had a penis. In *L. schrolli*, female shell length ranged from 2.29 to 9.63
326 mm with a mean length of 5.31 ± 1.38 (SD) mm while male shell length ranged from 2.22 to 6.92 mm
327 with a mean length of 4.53 ± 0.96 mm. In *L. fijiensis*, female shell length ranged from 2.51 to 9.02 mm
328 with a mean length of 5.40 ± 1.22 mm while male shell length ranged from 2.74 to 6.42 mm with a
329 mean length of 4.40 ± 0.70 mm. For each sample, and for both species, females were significantly
330 larger than males (unilateral Wilcoxon-Mann-Whitney tests: p values < 0.01, see Table 3 for specific p
331 values). The sex ratio was not significantly different from 1:1 (chi-square goodness-of-fit: p values >
332 0.05, see Table 3 for specific p values), except for one sample of *L. schrolli* from the Pacmanus vent
333 field that displayed a female-biased sex ratio (i.e. PM5; M:F = 0.66, p value = 0.039).

334 For both *Lepetodrilus* species, ovary and testis were posteriorly located, underlying the digestive gland
335 and rising on its left as reported on Figure 2A-D for *L. fijiensis*. Gametogenesis was described through
336 the analysis of 93 females of *L. schrolli* and 89 females of *L. fijiensis*, collected in *Ifremeria* and
337 *Bathymodiolus* habitats (Table 4), with a curvilinear shell length ranging from 3.72 to 8.14 mm and
338 3.48 to 9.13 mm, respectively. For both species, three stages of oocyte development were observed
339 and present in all gonads: oogonia, and previtellogenic and vitellogenic oocytes. Oogonia seemed to
340 develop from the germinal epithelium along the entire gonad. Although they had been observed in
341 each female, oogonia were not considered for oocyte size-frequency distribution analyses, as their
342 proportion would have been underestimated due to photographs quality. Previtellogenic oocytes,
343 considered as non-mature, presented a smooth and dark cytoplasm while the vitellogenic mature
344 oocytes were distinguishable by their pink coloured granular yolk in their voluminous cytoplasm
345 (Figure 2E). In *L. schrolli*, previtellogenic oocytes diameter ranged from 7.96 to 94.22 μm with a
346 mean diameter of $26.84 \pm 10.91 \mu\text{m}$. Vitellogenic oocytes diameter ranged from 19.57 to 124.13 μm
347 with a mean diameter of $74.58 \pm 14.97 \mu\text{m}$. In *L. fijiensis*, previtellogenic oocytes diameter ranged
348 from 9.22 to 95.6 μm with a mean diameter of $26.1 \pm 10.86 \mu\text{m}$. Vitellogenic oocytes diameter ranged
349 from 42.20 to 126.23 μm with a mean diameter of $75.61 \pm 13.60 \mu\text{m}$. Whatever the species, all
350 females presented the same pattern of oocytes size-frequency distribution with a large proportion of
351 previtellogenic oocytes and a smaller proportion of vitellogenic oocytes (Figure 5A-B, see
352 supplementary material for all *L. schrolli* and *L. fijiensis* oocytes size-frequency histograms). The
353 proportion of vitellogenic oocytes in *L. schrolli* ranged from 2 to 34% (Table 4) with a mean
354 proportion of $13 \pm 6\%$ and was independent on the female size (Spearman correlation test $\rho = -0.13$,
355 $n = 93$, p value = 0.22) (Figure 6A). The proportion of vitellogenic oocytes in *L. fijiensis* ranged from
356 1 to 29% (Table 4) with a mean proportion of $14 \pm 5\%$ and correlation between female size and the
357 proportion of vitellogenic oocyte only appeared significant when considering the largest female
358 (Spearman correlation test $\rho = -0.23$, $n = 89$, p value = 0.03). (Figure 6B). Otherwise, it was also
359 independent on the female size (Spearman correlation test $\rho = -0.20$, $n = 88$, p value = 0.06). The
360 proportion of vitellogenic oocytes differed significantly between samples for both *Lepetodrilus* species
361 (Kruskal-Wallis test, *L. schrolli*: $H = 39.032$, $df = 10$, p value = $2.5e-05$; *L. fijiensis*: $H = 16.833$, $df =$

362 8, p value = 0.032). For *L. schrolli*, pairwise comparison tests showed that only two samples from
363 Pacmanus, for which the highest proportions of oocytes were observed (i.e. PM3 and PM4 from
364 *Ifremeria* habitat), differed significantly from some Susu samples (i.e. SU1, SU3, SU5 for both
365 samples and SU6 for PM4 only from *Ifremeria* and *Bathymodiolus* habitats) in the Manus Basin. No
366 difference was detected for *L. fijiensis* between samples.

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

384 Actual fecundity could be estimated from 4 females of *L. schrolli* and 7 females of *L. fijiensis*
385 randomly selected among different samples (Table 4), with a shell length ranging from 5.07 to 7.83
386 mm and from 3.75 to 6.77 mm, respectively. The number of mature oocytes varied between 52 and
387 205 vitellogenic oocytes per female in *L. schrolli* with a mean of 119 ± 74 vitellogenic oocytes, and
388 between 80 and 605 for *L. fijiensis*, with a mean of 366 ± 183 vitellogenic oocytes. For both, fecundity

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

392 *Shinkailepas tollmanni*

393 **Population structure**

394 Length-frequency distributions were established from 3 599 individuals of *S. tollmanni* from the
395 Futuna Volcanic Arc, and the Manus, North Fiji and Lau basins, sampled in *Ifremeria* and
396 *Bathymodiolus* habitats (Table 2). Shell length ranged from 0.55 to 12.20 mm. All length-frequency
397 distributions, except the one from the FK1 sample (Fatu Kapa vent field at the Futuna Volcanic Arc),
398 differed from a normal distribution (Lilliefors test, *p* values < 0.05, see Table 2 for specific *p* values).
399 Length-frequency distributions were characterised by a large number of medium-sized individuals (~
400 60-90% of the sample considering the four size classes around the mean) and a few small and/or large
401 individuals (Figure 7, see supplementary material for all size-frequency histograms). Between two to
402 four Gaussian components of varying proportions were identified by modal decomposition with
403 Mixdist, yet, analyses did not allow to infer biologically meaningful cohorts (i.e. a group of
404 individuals from the same population and born at the same time, see discussion) and were not shown.
405 Kruskal-Wallis tests highlighted significant differences in shell length among samples at the scale of
406 the southwestern Pacific ($H = 2099.6$, $df = 9$, *p* value < 2.2e-16). The Nemenyi and Dunn multiple
407 pairwise comparisons test among samples showed significant variations among samples within a
408 basin. The three pairs of samples from the Manus Basin and all six pairs but one from the Lau Basin
409 samples differed significantly; only the samples TM2 and TM3 were not significantly different from
410 each other. As only two samples were collected from *Bathymodiolus* habitat and both were from the
411 Lau Basin, we focussed on this basin to observe differences according to the habitat. All four pairs of
412 samples from the two distinct habitats but one differed significantly (*p* values < 0.001), as did the two
413 pairs from a same habitat (i.e. *Ifremeria* vs. *Ifremeria* and *Bathymodiolus* vs. *Bathymodiolus*) (*p* values
414 < 0.001). The forward selection conducted prior to RDA analysis selected two explanatory variables

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

417 **Sex ratio and reproductive biology**

418 In total, sexing was possible for 3 297 individuals longer than 4.4 mm (Table 3). Female shell length
419 ranged from 4.93 to 12.20 mm with a mean length of 7.90 ± 1.40 mm. Male shell length ranged from
420 4.42 to 11.80 mm with a mean length of 7.84 ± 1.32 mm. Female mean length was significantly larger
421 than male mean length in four samples from the Pacmanus, Susu, Fatu Kapa and Tow Cam vent field
422 (i.e. PM7, SU4, FK1 and TC3, unilateral Wilcoxon-Mann-Whitney tests: p values < 0.01 , see Table 3
423 for specific p values). Three samples out of ten from the Pacmanus, Susu and Tow Cam vent field
424 were significantly different from a balanced sex ratio and were all in favour of males (i.e. PM7, SU8
425 and TC3, Table 3).

426 Ovary and testis were dorsally located and extend downward on the left and backward of the digestive
427 gland (Figure 3C-D). Gametogenesis could be described from 16 females ranging from 9.21 to 13.83
428 mm, collected in two samples from the two very distant Manus and Lau basins in the *Ifremeria* habitat
429 (Table 4). Three stages of development were observed (Figure 3E). Oogonia developed from the
430 germinal epithelium along the entire gonad. Oogonia have been observed in each female, but as their
431 proportion would have been underestimated due to photographs quality, they were not considered for
432 oocyte size-frequency distribution analyses. Previtellogenic oocyte diameter ranged from 13.28 to
433 $109.37 \mu\text{m}$ with a mean of $38.47 \pm 15.38 \mu\text{m}$ and vitellogenic oocyte diameter ranged from 55.92 to
434 $152.92 \mu\text{m}$ with a mean of $92.95 \pm 15.58 \mu\text{m}$. All females presented the same pattern of oocytes size-
435 frequency distribution with a large proportion of previtellogenic oocytes and a smaller proportion of
436 vitellogenic oocytes (Figure 5C). Vitellogenic oocytes proportion ranged from 16 to 44% (Table 4)
437 with a mean proportion of $27 \pm 8\%$ and was independent of the female size (Spearman correlation test
438 $\rho = 0.02$, $n = 16$, p value = 0.95) (Figure 6C). The Kruskal-Wallis test highlighted significant
439 differences in oocyte size-frequency distribution among females within the two samples observed
440 (Table 4). According to the Nemenyi and Dunn multiple pairwise comparisons test two females were
441 responsible of these differences in both cases. Among the females in sample PM7, the two individuals

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

445

446 **Discussion**

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

454 Individuals of *Lepetodrilus* spp. and *Shinkailepas tollmanni* colonised the shells of *Bathymodiolus* and
455 *Ifremeria* which inhabit areas of low and intermediate diffuse areas, respectively (Podowski et al.
456 2010). In the Lau Basin, Podowski et al. (2010) argued that (1) temperatures of about 20°C correspond
457 to one end of *Bathymodiolus* spectra of ecological niche, while another one is defined by its minimum
458 sulphide requirements (temperature on that end being lower and close to open deep seawater); (2) the
459 ecological niche of *Ifremeria nautilei* is more constrained by biotic interactions with *Bathymodiolus*
460 and *Alviniconcha* although this last genus was present at greater temperatures and sulphide
461 concentrations. The ranges of physico-chemical parameters observed in our study are in agreement
462 with previous observations by Podowski et al. (2010) and confirm that *Ifremeria* is present in more
463 intense diffusion (and/or more focussed and hence less diluted) zones with higher sulphide
464 concentrations. No significant differences between the two habitats were observed for the other
465 physico-chemical variables, underlining the difficulty in discriminating them on the basis of these
466 variables only. Indeed, methane concentrations in diffuse flow areas are low, close to the detection
467 limit, and the range of pH within these areas is narrow (between 6 and 7.8), with micro-scale

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

470 **Recruitment patterns**

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

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

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

522 conditions structure organisms spatial distribution, and hydrothermal communities organise
523 themselves according to temperature, pH, O₂ concentration, and chemical composition (Kelly and
524 Metaxas 2008; Matabos et al. 2008; Podowski et al. 2010; Sen et al. 2013; Mullineaux et al. 2018).
525 However, none of the environmental conditions measured in this study nor the habitat type (*Ifremeria*
526 or *Bathymodiolus*) explained the variability observed among size-frequency distributions of the two
527 *Lepetodrilus* species and *S. tollmanni*. In addition, unlike previous findings in *L. fucensis* (Bates 2008)
528 and *L. nux* (Nakamura et al. 2014) the largest individuals of *L. schrolli*, *L. fijiensis* and *S. tollmanni*
529 were not found at the warmest vents. On the other hand, the supply of larvae in a given area is
530 influenced by variations in local hydrodynamics, such as currents and turbulence levels (Mullineaux et
531 al. 2005; Adams and Mullineaux 2008; Adams et al. 2011). This could result in episodic larval
532 supplies differing among fields or among vents within a field, which could influence size-frequency
533 distributions. Mesoscale eddies created on the ocean's surface can also impact the transport of both
534 hydrothermal vent efflux and larvae, creating episodic opportunities for vent species to disperse their
535 larvae across large distances (Adams et al. 2011). Lastly, the lack of information on settlement cues
536 prevents from assessing how physico-chemical conditions influence larval settlement and recruitment.

537

538 **Reproductive biology**

539 Our results brought new insights into the reproductive biology and anatomy of three abundant
540 gastropod vent species. All three species were gonochoric and both sexes were equally represented in
541 most populations, except for some cases where sex ratio was biased towards females for *L. schrolli* in
542 one sample, and towards males for *S. tollmanni* in three samples. Such a balanced sex ratio combined
543 with a high population density, as observed here, could increase the chances of mating and allow
544 multi-male fertilisation, potentially favouring genetic diversity (Xue et al. 2016). Unlike other
545 *Lepetodrilus* species, *L. schrolli* and *L. fijiensis* lack a penis (Beck 1993; Chen and Sigwart 2023). The
546 presence of a penis was reported for few individuals in the North Fiji Basin (Warén and Bouchet
547 2001) for the species now named as *L. fijiensis*, but none of the males analysed in the present study

548 had one, yet their gonads were full of spermatozoa (Figure 2D, F). This is not consistent with Beck
549 (1993)'s hypothesis of a seasonal reproduction with the penis appearing at the time of breeding. The
550 absence of a penis could suggest either an external fertilisation or a pseudo-copulation, with physical
551 contact between males and females that would be facilitated by high densities of individuals with 1:1
552 sex ratio populations. Pseudo-copulation could allow semi-internal (or entaquatic) fertilisation in the
553 mantle cavity like in other *Lepetodrilus* species (Fretter 1988). It has already been hypothesised for
554 Lepetodrilidae of the genus *Pseudorimula* which males also lack a secondary reproductive organ
555 (Haszprunar 1989; Marticorena et al. 2020). Internal fertilisation might be favoured by hydrothermal
556 gastropods to protect gametes from possible harmful conditions of their environment (Fretter 1988;
557 Matabos and Thiébaud 2010). Some species such as *Shinkailepas tollmanni* produce egg capsules to
558 protect their developing embryos (Beck 1992).

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

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

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

579 For the three species, female gonad analysis showed a similar pattern with all oocyte development
580 stages present (i.e. oogonia, previtellogenic oocytes, and vitellogenic oocytes), suggesting potential
581 continuous or quasi-continuous reproduction (Berg 1985), with a much higher proportion of
582 previtellogenic than vitellogenic oocytes (on average 87% for *L. schrolli*, 86% for *L. fijiensis* and 73%
583 for *S. tollmanni*). The large overlap in size between previtellogenic and vitellogenic oocytes could be
584 due to their irregular shape. The measurement of the equivalent diameter could either overestimate or
585 underestimate the oocyte size, especially for the larger ones, and thus increase the size range of the
586 different development stages (Copley and Young 2006; Kelly and Metaxas 2007). The high variability
587 of oocyte size distributions among females within a site, and among sites and fields, supported the
588 hypothesis of a continuous or quasi-continuous and an asynchronous gametogenesis between females.
589 Among the Lepetodrilidae as well as other gastropod families, such as Peltospiridae, Sutilizonidae,
590 and Skeneidae, continuous reproduction is widespread (Tyler et al. 2008; Matabos and Thiébaud 2010;
591 Bayer et al. 2011; Nakamura et al. 2014; Marticorena et al. 2020). Continuous or quasi-continuous
592 gametogenesis is also common in other vent taxa including polychaetes (Zal et al. 1995; Faure et al.
593 2007) and shrimps (Ramirez-Llodra 2002). This type of gametogenesis can be explained by the
594 regular energy flows provided by chemosynthesis (Tyler et al. 1994; Marticorena et al. 2020).
595 Individuals can therefore allocate part of the consumed food resources to produce eggs continuously.
596 This reproductive trait would allow hydrothermal species to maintain viable populations and quickly
597 adapt to changes in environmental conditions and venting activity (Tyler et al. 2008; Matabos and
598 Thiébaud 2010).

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

602 Atlantic ridge (Matabos and Thiébaud 2010; Bayer et al. 2011; Marticorena et al. 2020). However, the
603 maximum vitellogenic oocyte size in the two *Lepetodrilus* species studied here was slightly larger as
604 compared to other species of *Lepetodrilus*, generally below 100 μm (Tyler et al. 2008). Although egg
605 size often correlates with the development mode of marine invertebrates, large oocytes being
606 associated with lecithotrophic development and smaller ones with planktotrophic development, this is
607 not true for some gastropods, such as lepetodrilids, which have small eggs ($<200 \mu\text{m}$) but larval shell
608 morphology suggesting non-planktotrophic development (Lutz et al. 1986; Craddock et al. 1997).
609 Conversely, *S. tollmanni* carried vitellogenic oocytes also below 200 μm but a recent study reported a
610 planktotrophic development for this species (Yahagi et al. 2020). Recent studies suggested long-
611 distance larval dispersal capacities, up to a year, in *S. tollmanni* and another Phenacolepadidae (i.e. *S.*
612 *myojinensis*) (Yahagi et al. 2017, 2020). Although *Lepetodrilus* larvae are thought to be non-
613 planktotrophic (Lutz et al. 1986; Craddock et al. 1997; Plouviez et al. 2019), the cold temperature at
614 the seafloor may imply metabolism reduction and thus allow also long-distance dispersal (Young et al.
615 1997; Mullineaux et al. 1998).

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

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

629 oocyte development rate fluctuate according to the habitat, with low oocyte development and low
630 fecundity in individuals living in senescent habitats (i.e. hydrothermally inactive areas with no
631 temperature anomaly, see Kelly and Metaxas (2007) for more details). In addition, Marticorena et al.
632 (2020) suggested that warmer habitats could provide a greater food resource and allow individuals to
633 allocate more energy to vitellogenesis. Despite this, we did not identify any relationships between the
634 gametogenesis variability among samples or the actual fecundity of the two *Lepetodrilus* species and
635 environmental conditions measured or the habitat. This may simply be due to the fact that we did not
636 sample senescent habitats as defined by Kelly and Metaxas (2007) and that both species are rather well
637 adapted to the range of physico-chemical conditions prevailing in the *Bathymodiolus* and *Ifremeria*
638 habitats. Indeed, Kelly and Metaxas (2007) only observed differences between actively venting and
639 senescent sources in *L. fucensis* gametogenesis and fecundity but not between active sources of
640 different intensity. The limited role of the physico-chemical environment may also be a consequence
641 of our limited ability to accurately measure environmental conditions at the individual's scale. In fact,
642 the measurement tools used do not allow for the characterisation of the environmental conditions in
643 direct contact with the organisms. While this is possible for temperature sensor, the risk of damaging
644 the water sampling probe prevents the characterisation of fluid chemistry at this scale. Therefore, it is
645 necessary to place the probe at about 1 cm from the organisms, although the environment present high
646 fluctuations at the centimetre scale. Furthermore, conditions vary greatly in space and time, ranging
647 from seconds (related to turbulence) to several years (linked to flow modifications). The low number
648 of replicates and the one-time measurements may not necessarily represent the dominant
649 environmental conditions encountered by individuals that could influence reproductive traits or
650 population dynamics. Multiple spatial and temporal measurements would be required to effectively
651 characterise the vent environment (Le Bris et al. 2005; Lee et al. 2015; Van Audenhaege et al. 2022).

652 **Conclusion**

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

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

682

683 **Statements and Declarations**

684

685 **Funding**

686 This work was supported by the ANR CERBERUS (contract number ANR-17-CE02-0003). Author
687 Camille Poitrimol PhD was funded by the ANR CERBERUS and IFREMER.

688 **Competing interest**

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

690 **Author Contribution**

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

696 **Data Availability**

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

700 **Ethics approval**

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

706 **Consent to publish**

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

711 **Acknowledgments**

712 We are deeply grateful to the chief scientists of the CHUBACARC cruise, Didier Jollivet and
713 Stéphane Hourdez. We thank the captain and crew members of the RV *L'Atalante*, as well as the ROV
714 *Victor 6000* team for their valuable work at sea. We also thank Mathilde Le Pans for her work on the
715 acquisition of biological data. We are grateful to Thomas Broquet, Stéphane L'Haridon and Loïc
716 Michel for their help in sorting the samples on board and to Vincent Mouchi for his help regarding the
717 measurement of some juveniles. We also thank O. Rouxel, E. Rinnert, A. Boissier and N. Gayet for
718 their help at sea for deploying *in situ* PIF and CHEMINI instrument and recovering and processing
719 fluid samples on board. We are grateful to A. Laes for lending us the CHEMINI instrument. We would
720 also like to thank O. Rouxel for his comments on an early version of the manuscript and the three
721 reviewers for their comments, which have helped to improve the manuscript. This work was supported
722 by the ANR CERBERUS (contract number ANR-17-CE02-0003).

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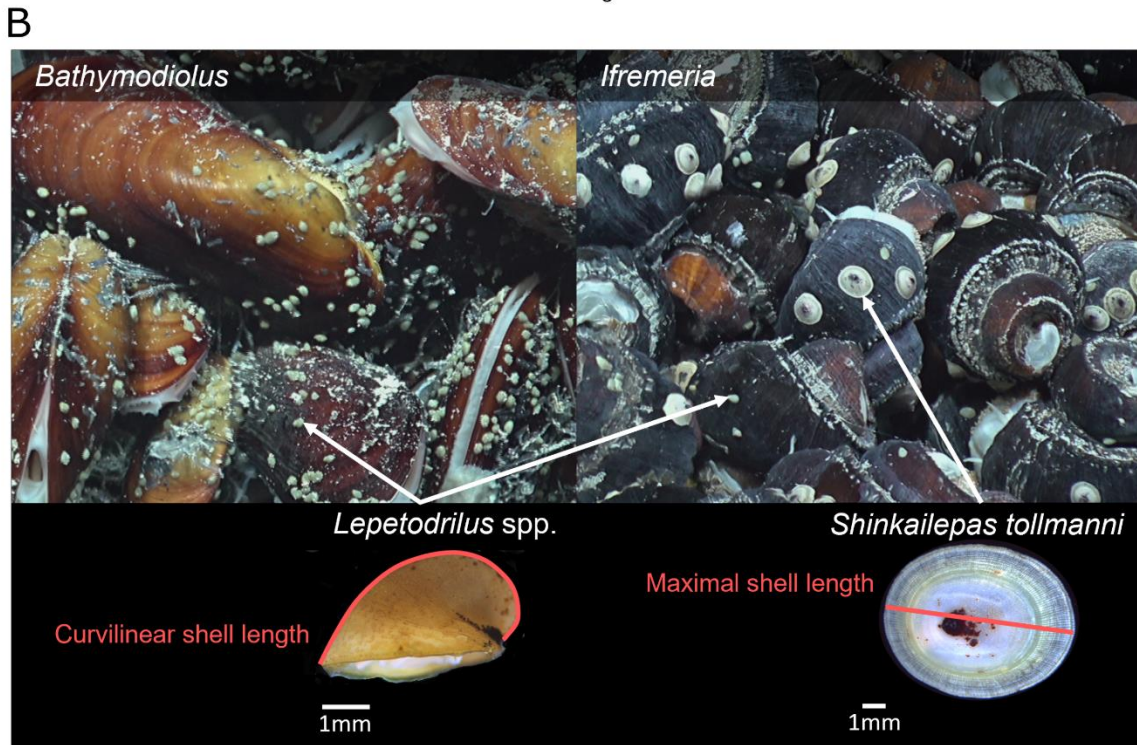
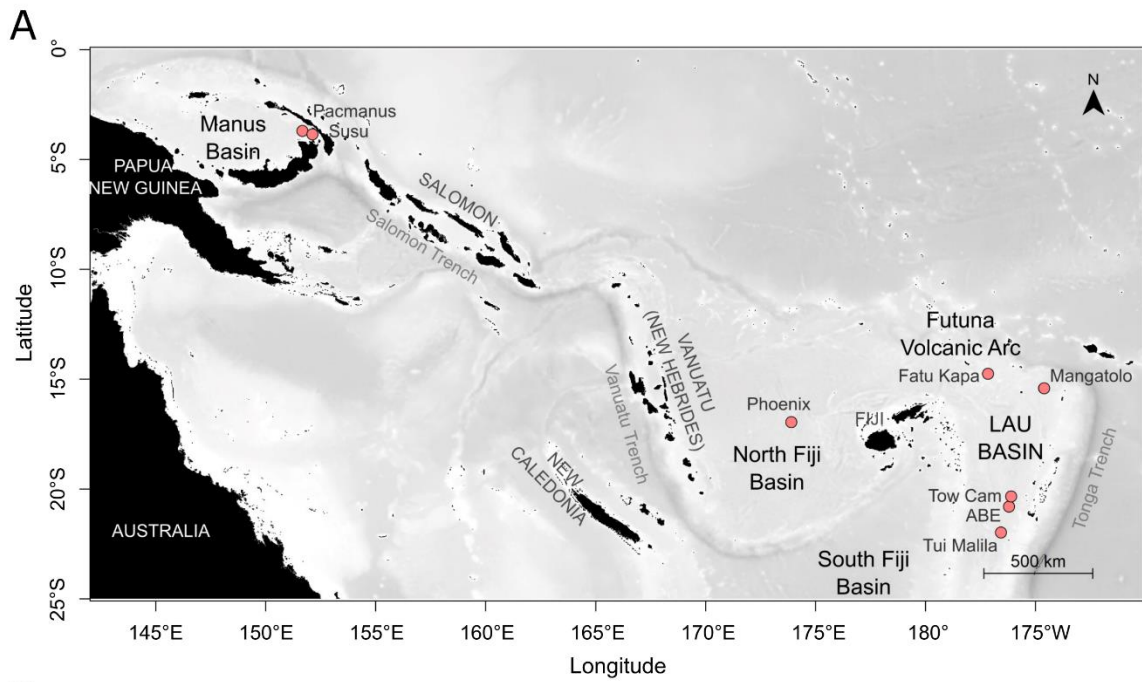


Fig. 1 **A** Back-arc basins and volcanic arc sampling area from the CHUBACARC cruise in the South West Pacific. Red dots represent sampled vent field. **B** Illustration of *Lepetodrilus* spp. and *Shinkailepas tollmanni* inhabiting the complex three-dimensional habitat formed by *Bathymodiolus* and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

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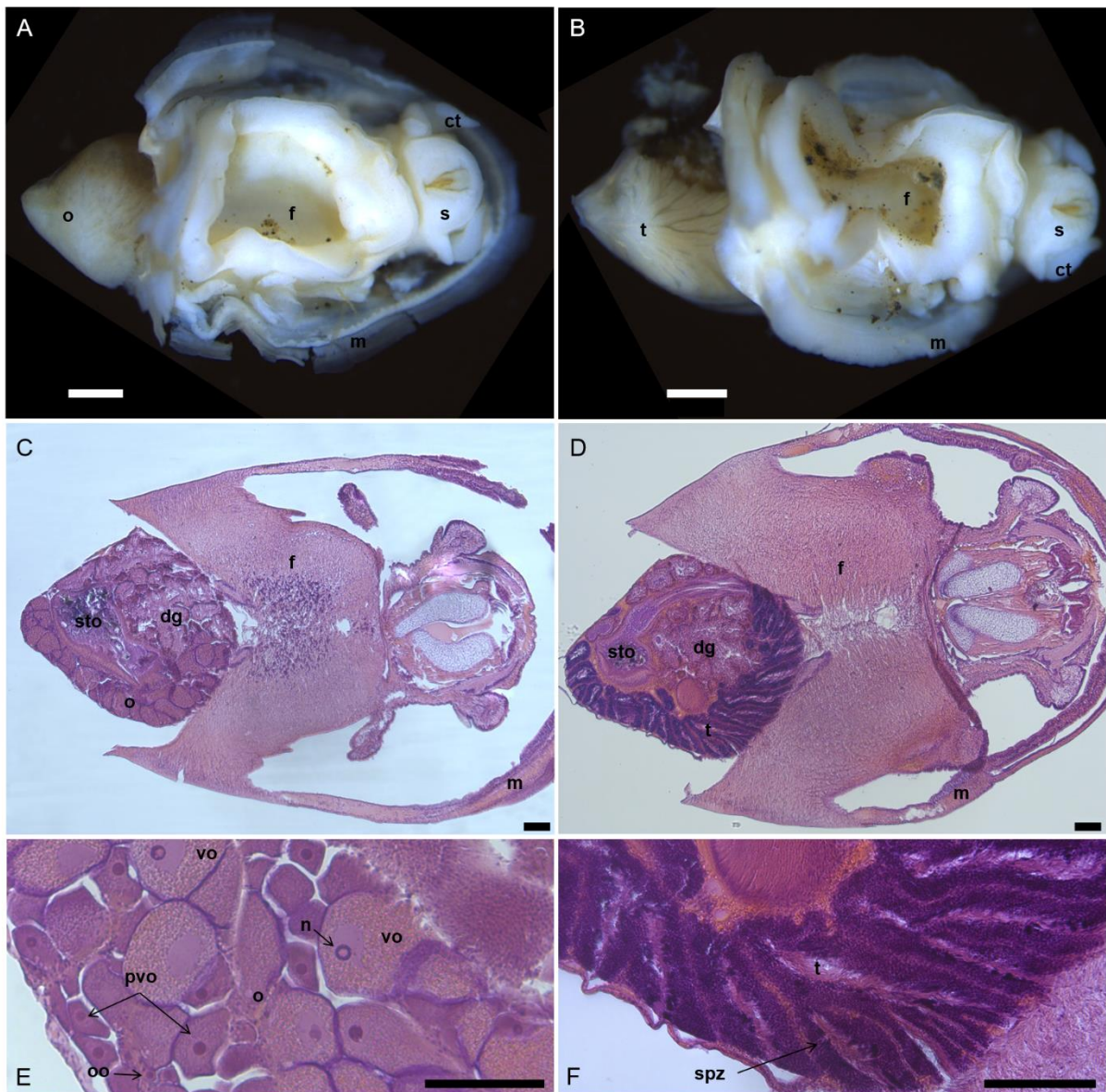


Fig. 2 *Lepetodrilus fijiensis* female and male reproductive structures. **A** Female soft-body, ventral view. **B** Male soft-body, ventral view. **C** General view of female transversal section, ventral view. **D** General view of male transversal section, ventral view. **E** Detailed view of ovary. **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 μ m. Black scale bar: 100 μ m

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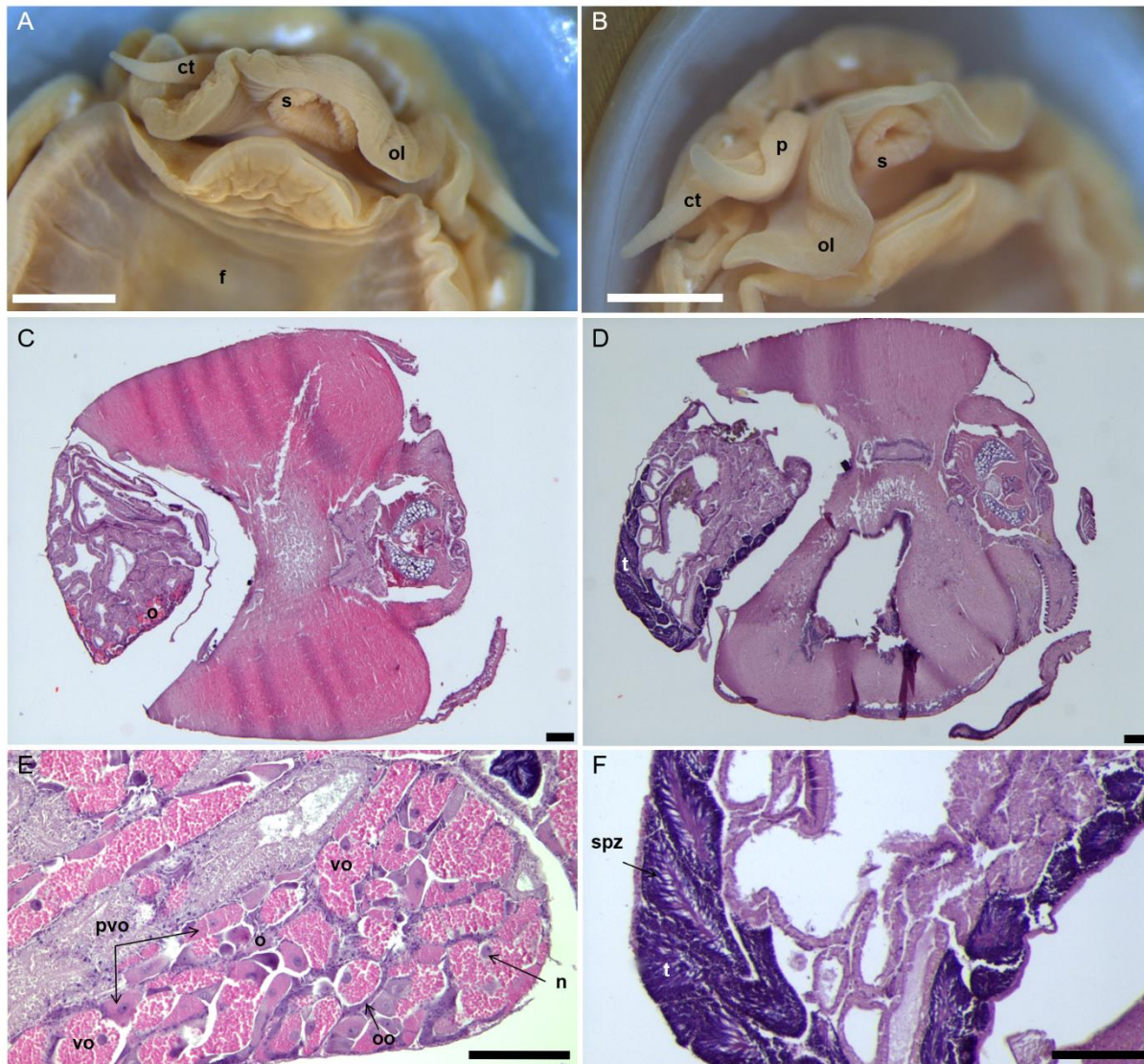


Fig. 3 *Shinkailepas tollmanni* female and male reproductive structures. **A** Detailed view of female anterior part, ventral view. **B** Detailed view of male anterior part, ventral view. **C** General view of female transversal section, ventral view. **D** General view of male transversal section, 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 μ m

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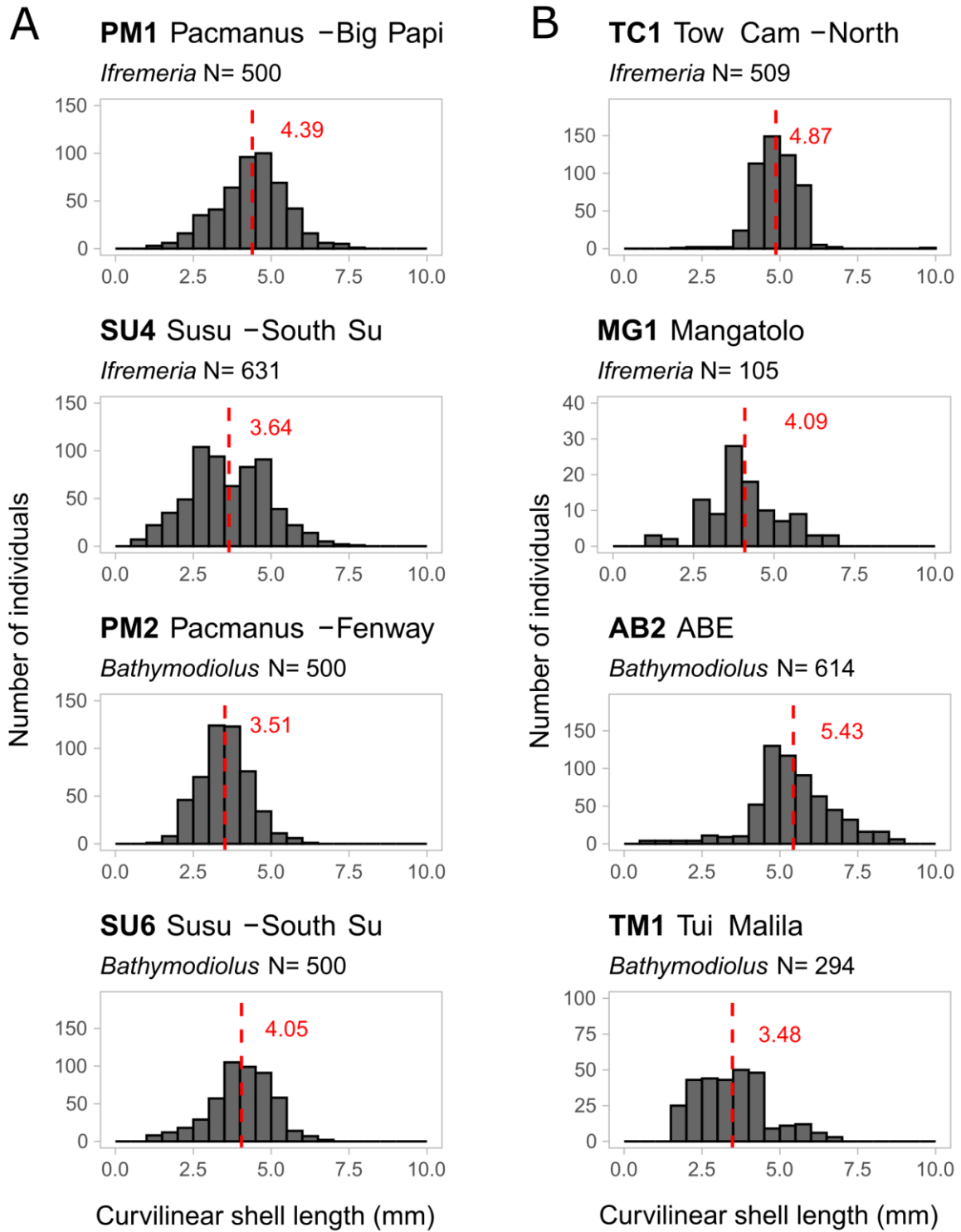


Fig. 4 Example of size-frequency histograms of *Lepetodrilus schrolli* (A) and *L.fijiensis* (B) collected in *Ifremeria* and *Bathymodiolus* habitats in the South West Pacific. N = number of measured individuals. Mean size is indicated in red

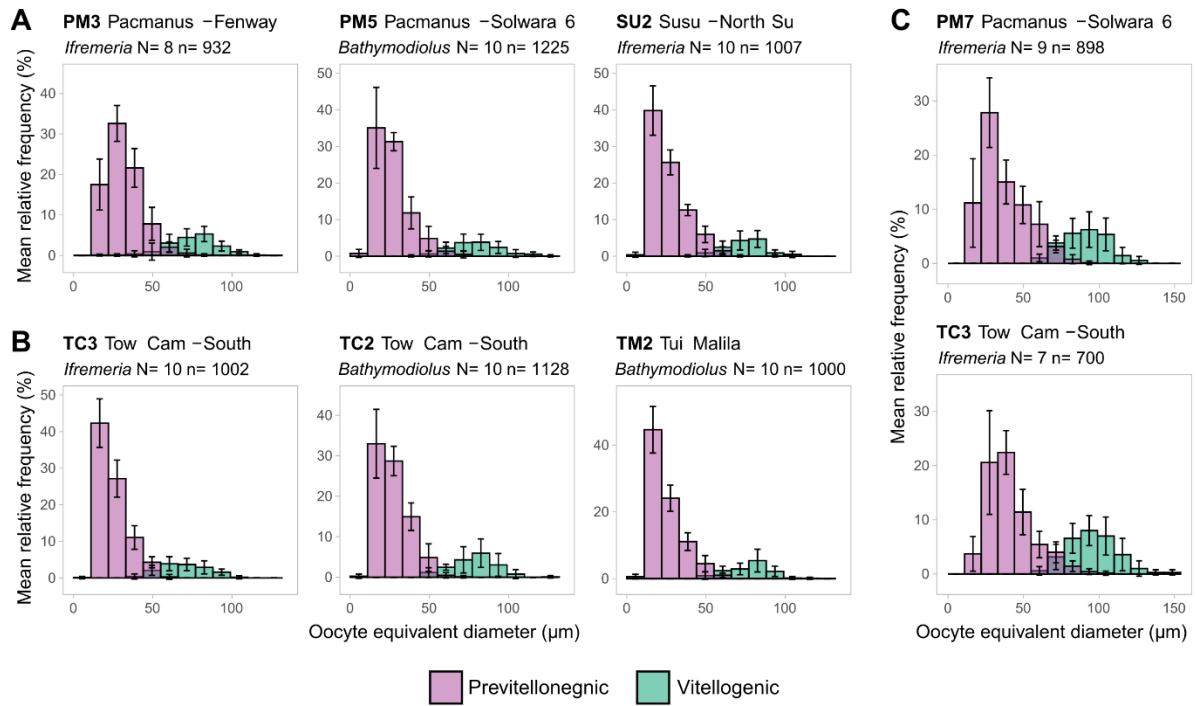


Fig. 5 Examples of mean oocyte size-frequency histograms for females of *Lepetodrilus schrolli* (A), *L. fijiensis* (B) and *Shinkailepas tollmanni* (C) collected in the South West Pacific. N = number of measured individuals; n= number of measured oocytes

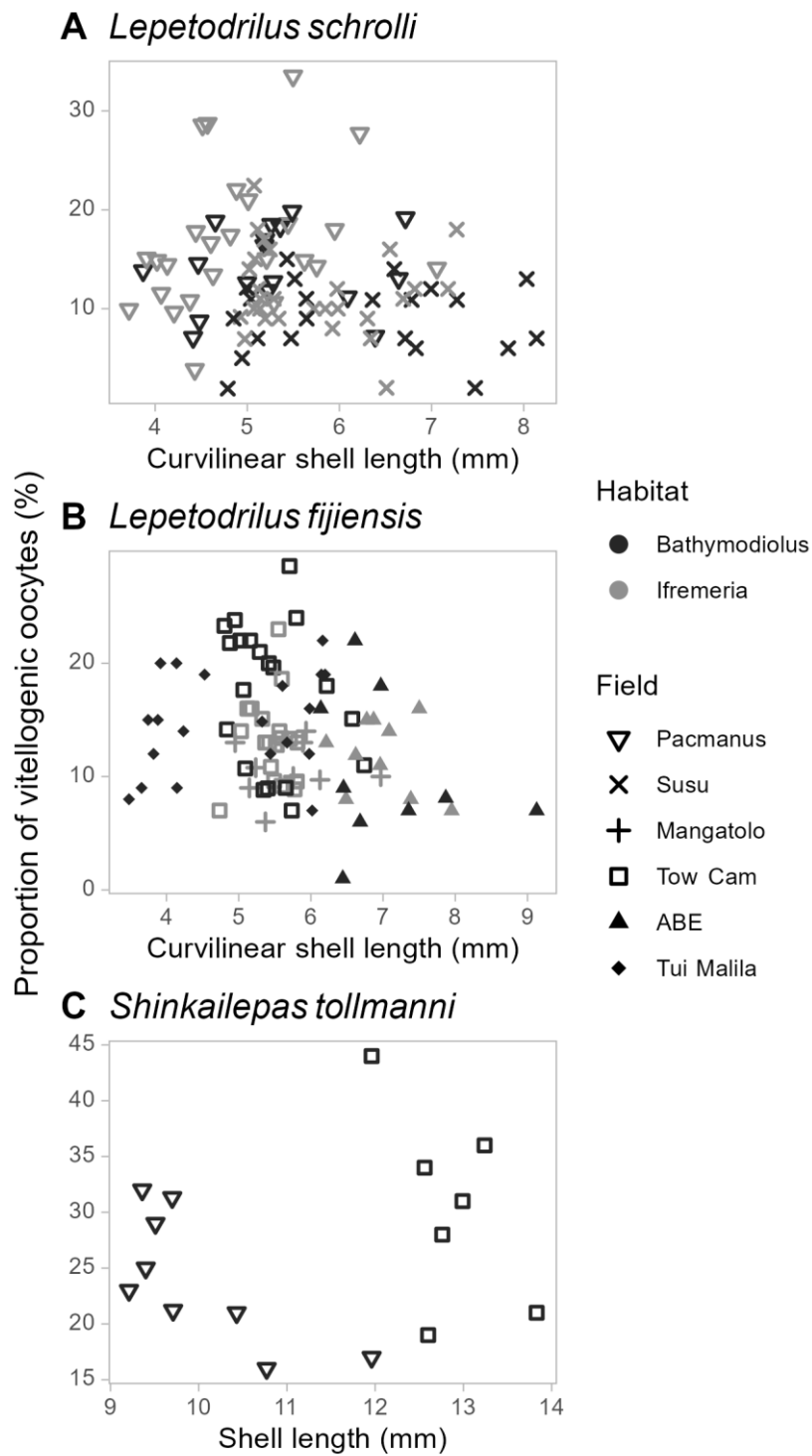


Fig. 6 Proportion of vitellogenic oocytes according to females' size for *Lepetodrilus schrolli* (A), *L. fijiensis* (B) and *Shinkailepas tollmanni* (C) collected in the South West Pacific

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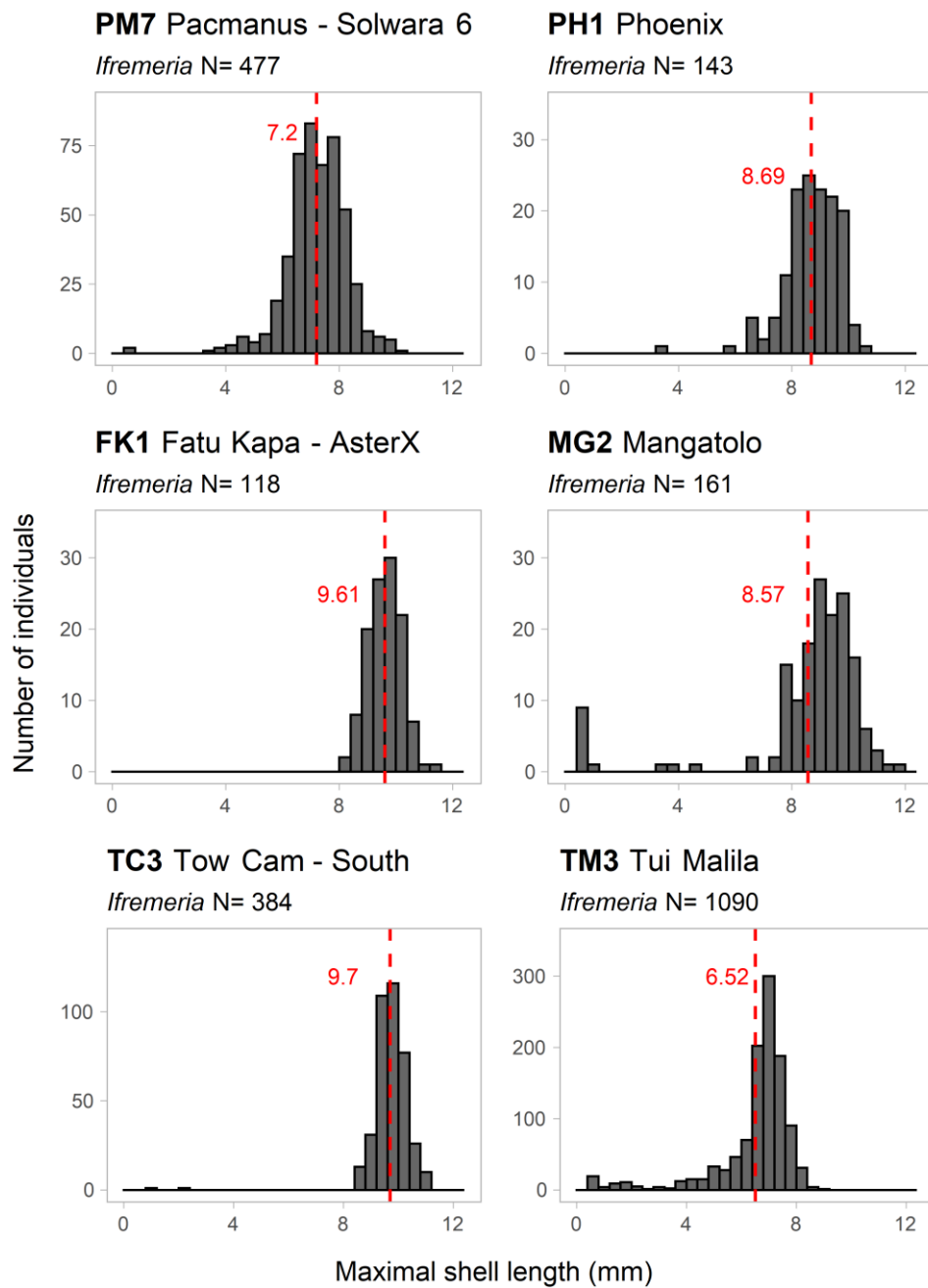


Fig. 7 Example of size-frequency histograms of *Shinkailepas tollmanni* collected in *Ifremeria* and *Bathymodiolus* habitats in the South West Pacific. N = number of measured individuals. Each mean is indicated in red

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 \pm SD (Σ S(-II)), methane (CH₄) concentrations and pH

Basin	Field	Site	Sample	Hab.	Sampling date (2019)	Longitude	Latitude	Depth (m)	T°C (max)	Σ S(-II) (μ M)	CH ₄ (μ M)	pH	
<i>Lepetodrilus schrolli</i>													
Manus	Pacmanus	Big Papi	PM1	I	16/05	151° 40.342'E	03° 43.707'S	1703	12.07	15.93 \pm 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 \pm 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 \pm 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 \pm 4.63	0.18	7.47	
			SU2	I	23/05	152° 06.046'E	03° 47.935'S	1216	7.73	40.16 \pm 12.71	0.44	6.93	
			SU3	B	23/05	152° 06.089'E	03° 47.957'S	1195	10.40	2.52 \pm 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 \pm 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 \pm 76.32	0.58	6.62	
		SU6	B	24/05	152° 06.310'E	03° 48.583'S	1352	7.35	10.09 \pm 8.72	0.56	6.43		
<i>Lepetodrilus fijiensis</i>													
Lau	Mangatolo		MG1	I	16/04	174° 39.208'W	15° 24.874'S	2031	17.45	61.77 \pm 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 \pm 0.60	0.07	7.35
				TC4	B	01/04	176° 08.211'W	20° 19.051'S	2696	4.24	4.09 \pm 0.79	0.07	7.43
		South	TC2	B	31/03	176° 08.250'W	20° 19.074'S	2711	11.98	21.13 \pm 3.84	-	6.92	
				TC3	I	01/04	176° 08.263'W	20° 19.084'S	2711	7.09	13.27 \pm 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 \pm 2.74	-	6.02	
			AB2	B	27/04	176° 11.480'W	20° 45.784'S	2154	3.25	3.00 \pm 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 \pm 8.72	0.61	7.31	
			TM2	B	03/04	176° 34.088'W	21° 59.351'S	1874	8.84	5.17 \pm 3.48	0.12	7.20	
	<i>Shinkailepas tollmanni</i>												
Manus	Pacmanus	Solwara 6	PM7	I	20/05	151° 40.852'E	03° 43.653'S	1729	12.70	25.98 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 8.72	0.61	7.31	
			TM2	B	03/04	176° 34.088'W	21° 59.351'S	1874	8.84	5.17 \pm 3.48	0.12	7.20	
			TM3	I	04/04	176° 34.098'W	21° 59.355'S	1886	18.83	8.93 \pm 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 (\pm SD)	Median	Normality test <i>p</i> value
<i>L. schrolli</i>	Pacmanus	I	PM1	500	1.13 - 7.80	4.39 \pm 1.10	4.46	0.019
		B	PM2	500	1.42 - 6.20	3.51 \pm 0.80	3.51	0.770
		I	PM3	500	1.79 - 6.96	3.70 \pm 0.87	3.63	0.004
		I	PM4	440	1.54 - 5.77	3.88 \pm 0.75	3.90	0.120
		B	PM5	500	1.33 - 8.27	4.71 \pm 1.14	4.62	0.004
		I	PM6	500	0.75 - 5.25	2.89 \pm 0.81	2.95	0.056
	Susu	B	SU1	500	1.52 - 8.16	3.87 \pm 0.96	3.82	0.1e-03
		I	SU2	500	1.72 - 5.45	3.58 \pm 0.57	3.54	0.004
		B	SU3	500	1.99 - 9.51	5.93 \pm 1.14	5.65	< 2.2e-16
		I	SU4	631	0.51 - 7.84	3.64 \pm 1.27	3.55	0.002
		I	SU5	500	1.00 - 7.13	3.78 \pm 1.09	3.84	4.8e-05
		B	SU6	500	1.13 - 6.81	4.05 \pm 0.99	4.10	0.6e-03
<i>L. fijiensis</i>	Mangatolo	I	MG1	105	1.31 - 6.98	4.09 \pm 1.17	3.96	0.004
	Tow Cam	I	TC1	509	1.85 - 9.86	4.87 \pm 0.66	4.83	0.013
		B	TC2	501	2.52 - 6.52	4.70 \pm 0.60	4.61	3.6e-06
		I	TC3	500	2.72 - 6.17	4.76 \pm 0.54	4.67	4.2e-09
		B	TC4	501	2.40 - 6.94	4.79 \pm 0.67	4.78	0.041
	ABE	I	AB1	182	1.57 - 7.95	4.80 \pm 1.10	4.84	0.004
		B	AB2	614	0.74 - 8.94	5.43 \pm 1.29	5.29	1.6e-13
	Tui Malila	B	TM1	294	1.71 - 6.90	3.48 \pm 1.14	3.41	0.006
		B	TM2	199	2.16 - 5.50	3.61 \pm 0.53	3.55	0.010
<i>S. tollmanni</i>	Pacmanus	I	PM7	477	0.55 - 10.24	7.20 \pm 1.08	7.21	7.7e-06
	Susu	I	SU8	355	0.68 - 12.20	9.09 \pm 1.41	9.31	9.3e-13
		I	SU4	319	3.22 - 11.66	8.40 \pm 1.33	8.40	0.004
	Phoenix	I	PH1	143	3.21 - 10.46	8.69 \pm 0.98	8.76	0.016
	Futuna	I	FK1	118	8.15 - 11.56	9.61 \pm 0.60	9.66	0.843
	Mangatolo	I	MG2	161	0.74 - 11.74	8.57 \pm 2.31	9.13	< 2.2e-16
	Tow Cam	I	TC3	384	1.17 - 11.16	9.70 \pm 0.76	9.72	< 2.2e-16
	Tui Malila	B	TM1	202	0.76 - 9.22	6.98 \pm 1.58	7.28	< 2.2e-16
		B	TM2	350	3.43 - 8.43	6.88 \pm 0.47	6.87	0.010
	I	TM3	1090	0.70 - 8.90	6.52 \pm 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

Field	Hab.	S.	Sex ratio								
			N	Female		Male		WMW <i>p</i> value	M/F	X ²	<i>p</i> value
				Mean length (mm) ± SD	min - max	Mean length (mm) ± SD	min - max				
<i>Lepetodrilus schrolli</i>											
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
<i>Lepetodrilus fijiensis</i>											
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
<i>Shinkailepas tollmanni</i>											
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 <i>p</i> value	Prop. vo min - max (%)	N	Nb of vo
<i>Lepetodrilus schrolli</i>								
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		
<i>Lepetodrilus fijiensis</i>								
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
<i>Shinkailepas tollmanni</i>								
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