

Feeding strategy, morphological specialisation and presence of bacterial episymbionts in lepetodrilid gastropods from hydrothermal vents

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ABSTRACT: Hydrothermal vent gastropods use diverse feeding mechanisms and can gain nutrition from symbionts. The Juan de Fuca Ridge limpet *Lepetodrilus fucensis* forms conspicuous stacks in hydrothermal flows and hosts filamentous bacterial episymbionts on its gill. The present study investigates whether its feeding strategy differs from those of several *Lepetodrilus* species without epibionts. A comparative approach was used to detect morphological features of the gill and digestive tissues that would indicate feeding specialisation. The gill lamellae of *L. fucensis* possess distinct features that are shared by several suspension-feeding gastropod genera: dense spacing of enlarged, unattached lamellae that do not narrow towards the tip and are stabilised by ciliary junctions. These modifications increase surface area and fluid velocities across the gill. Furthermore, the radular ribbon length, tooth cusp area and stomach volume of adult *L. fucensis* are significantly reduced, indicating that grazing may not be as efficient a feeding mechanism in comparison to non-symbiotic *Lepetodrilus*. Next, the feeding abilities of *L. fucensis* were evaluated using carmine red as a tracer for particle uptake in shipboard pressure vessels. Occasional grazing and active suspension feeding were documented. Dissections of animals and microscopy revealed that bacterium-like filaments accumulate at the lamellar tips, are formed into a cylindrical mass that is moved by cilia to the neck, and are sorted into accepted (passes to the mouth) and rejected material. The morphological specialisations of *L. fucensis* gills allow effective processing of suspended particles and provide a pathway whereby the episymbionts can be cultivated and ingested. The reduction in the radula and stomach are consistent with the hypothesis that adult *L. fucensis* primarily suspension feed and/or farm their gill symbionts. However, in peripheral locations, where suspended particle concentrations and chemical fluxes are low, grazing may be the only feasible option. Thus, *L. fucensis* can survive in a variety of habitats by using multiple feeding mechanisms.

KEY WORDS: Suspension feeding · Symbiont farming · Grazing · Comparative morphology · Gill specialisations · Radula reduction · *Lepetodrilus* · Gastropod · Hydrothermal vent

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INTRODUCTION

Gastropods exhibit great variety in feeding strategy and morphology, and as a result are found in a wide array of niches where they exploit many food sources. For instance, the radula functions in tasks as diverse as harvesting biofilms, tearing algae, drilling shells and manipulating particle-filled mucous nets (see review by Kohn 1983). Several gastropod families also suspen-

sion feed by using their gills to concentrate and sort particles suspended in seawater, and these families exhibit convergent gill specialisations, such as more numerous and longer lamellae (reviewed in Declerck 1995). Some species also use a combination of grazing and suspension feeding to access different food sources, thus maximising their food intake and overall nutrition (as suggested by Chaparro et al. 2002), e.g. *Crepidula fecunda* is an intertidal limpet that can rasp

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biofilms from surfaces with its radula and ingest food particles entrained and processed by the gill (Chaparro et al. 2002).

Hydrothermal vents are a unique ecosystem because chemosynthetic production by bacteria is the major food resource available to primary consumers. Bacterial densities on surfaces and suspended in vent effluent are relatively high (Giere et al. 2003), and the majority of vent gastropods feed on free-living bacteria. In addition, several species harbour endosymbiotic bacteria in their gill tissues that appear to translocate fixed organic carbon compounds directly to their hosts and/or are digested by lysosomal bodies (e.g. *Hirtopelta tufari*: Beck 2002; *Ifremeria nautiliei*: Okutani & Ohta 1988, Windoffer & Giere 1997; *Alviniconcha hessleri*: Bouchet & Warén 1991). In addition, an Indian Ocean species harbours bacteria in its oesophageal gland (Goffredi et al. 2004). In other taxa, such as rimicarid shrimp (Casanova et al. 1993), episympiotic bacteria are cultivated on animal surfaces and ingested by their host, a feeding mechanism unknown in gastropods.

Comparative morphological studies can highlight differences among species in feeding strategy (Wainwright 1991, Honkoop et al. 2003). A characteristic of species that gain nutritional benefit from their symbionts is that the bacterium-harbouring tissues or structures are enlarged, presumably to favour the symbiosis. For example, the vent shrimp *Rimicaris exoculata* cultivates epibacteria on the inner sheet of its carapace in an enlarged gill chamber, a specialisation thought to increase the area available for symbiont growth (Casanova et al. 1993). Furthermore, while many related species possess identical feeding capabilities, they often use different feeding mechanisms and possess distinguishing morphological traits related to their feeding strategy. For instance, comparisons between 2 mixotrophic gastropods (family Provanidae) that host endosymbionts in their gills reveal that one species possesses a relatively enlarged gill and reduced digestive organs; this species appears to depend more heavily on its symbionts for nutrition (Windoffer & Giere 1997).

The *Lepetodrilus* genus (order Vetigastropoda) is represented globally, and species are common in the hydrothermal vent and cold-seep assemblages where they occur (Fretter 1988, McLean 1993, Warén & Bouchet 2001). Feeding mechanisms may vary among *Lepetodrilus* species, thus providing an excellent model for studying the relationship between feeding strategy and morphological specialisation. While *Lepetodrilus* species tend to exhibit morphological evidence of suspension feeding (e.g. modified frontal pads on lamellar tips) and grazing (e.g. radular wear) (Fretter 1988), 2 sister species from the northeast

Pacific (*L. fucensis* McLean, 1988 from vents on the Juan de Fuca and Explorer Ridges and *L. gordensis* Johnson et al., 2006 from the Gorda Ridge) also host filamentous bacteria found partially embedded in the gill epithelium (de Burgh & Singla 1984, Bates 2007). Fox et al. (2002) suggest that the bacteria are chemoautotrophic based on results from gill tissue assays that returned high levels of RubisC/O, nitrate reductase and ATP-sulphurylase. The function of the symbiosis is probably nutritional in nature, and there are 3 ways in which the gill bacteria may contribute organic carbon to their limpet hosts: (1) fixed carbon compounds could pass from the bacteria to the limpet across the gill epithelium, (2) the bacteria may be farmed and ingested and (3) significant numbers of bacteria may be digested by the lysosomal bodies present in symbiont-harbouring tissues (as suggested by de Burgh & Singla 1984).

While the feeding repertoire of *Lepetodrilus fucensis* has not been determined using live specimens, limpets do form prominent stacks in warm vent flows, where their densities ($>100\,000\text{ m}^{-2}$) are an order of magnitude greater than congeners and co-occurring gastropods (Bates et al. 2005). This stacking behaviour may be driven by a requirement to access vent fluids laden with organic particles for suspension feeding, and reduced chemicals for the provisioning of its episympionts (Bates 2007). *L. fucensis* also occurs in waning and peripheral habitats at low densities (Bates et al. 2005), where suspended particle and reduced chemical fluxes are relatively low and, consequently, where suspension feeding or reliance on bacterial symbionts for organic carbon presumably yields minimal nutritional gain. Therefore, in peripheral habitats, grazing may be a primary feeding mechanism.

The prominent stacks formed by *Lepetodrilus fucensis* and the presence of gill symbionts suggest that this limpet may rely on a diet consisting largely of suspended bacterial particles and/or symbiont-derived organic carbon. If so, its feeding structures will presumably exhibit functional modifications reflecting a specialised feeding strategy relative to *Lepetodrilus* species that do not stack or host gill symbionts. To test this, I designed a comparative study to identify morphological features and/or specialised traits indicative of innovative feeding mechanisms: in addition to *L. fucensis*, 6 *Lepetodrilus* species with ($n = 1$) and without ($n = 5$) gill symbionts were selected from different vent sites in the Pacific Ocean to quantify gill, radula and digestive tract features. Next, to characterise the feeding capabilities of *L. fucensis*, I observed live animals in shipboard pressure vessels in the presence of attached and suspended food particles. To visualise particle transport across the gill, I exposed live animals to suspended carmine red for different time intervals

and then dissected these specimens to examine the material processed by the gill and transported to the mouth and stomach. This work was complemented with microscope observations of particles on the gill and neck. Last, I determined if symbiont-like particles are present in the ingested material to see whether *L. fucensis* farms and ingests its symbionts.

MATERIALS AND METHODS

Morphological comparisons. Collections: Specimens of 7 *Lepetodrilus* species from the east and west Pacific were collected by remotely operated vehicle ('Ropos') and manned submersible ('Alvin') and preserved in either 7% buffered formalin or 2.5% glutaraldehyde. Collection information includes ridge, vent field, latitude, longitude and depth for each species: *L. fucensis* McLean, 1988 (Juan de Fuca Ridge, Axial Volcano, 45° 56' N, 130° 00' W, 1570 m; Endeav-

our Segment, 47° 57' N, 129° 06' W, 2220 m), *L. gorden-sis* Johnson et al., 2006 (Gorda Ridge, SeaCliff Site, 42° 45' N, 126° 42' W, 2750 m), *L. guaymasensis* McLean, 1988 (Guaymas Basin, Southern Guaymas, 27° 1' N, 111° 40' W, 2000 m), *L. elevatus* McLean, 1988, *L. ovalis* McLean, 1988, *L. pustulosus* McLean, 1988 (East Pacific Rise, 9° 50' N, 104° 17' W, 2500 m) and *L. affinis schrolli* (Mariana Arc, NW Eifuku Seamount, 21° 29' N, 144° 2' E, 1576 m). *L. affinis schrolli* has not been described, but resembles *L. schrolli* Beck, 1993 (A. Warén pers. comm.).

Dissections: Thirty specimens of each *Lepetodrilus* species (5 to 7 mm shell length) were transferred to 70% EtOH for dissection under a light microscope. I used an optical micrometer to measure the dimensions of several morphological features of the gill, radula and digestive tract. The lengths of the gill axis and the longest lamellae were measured (Fig. 1). The radula ribbon was removed from the buccal cavity by dissection and its length determined. The length, width and

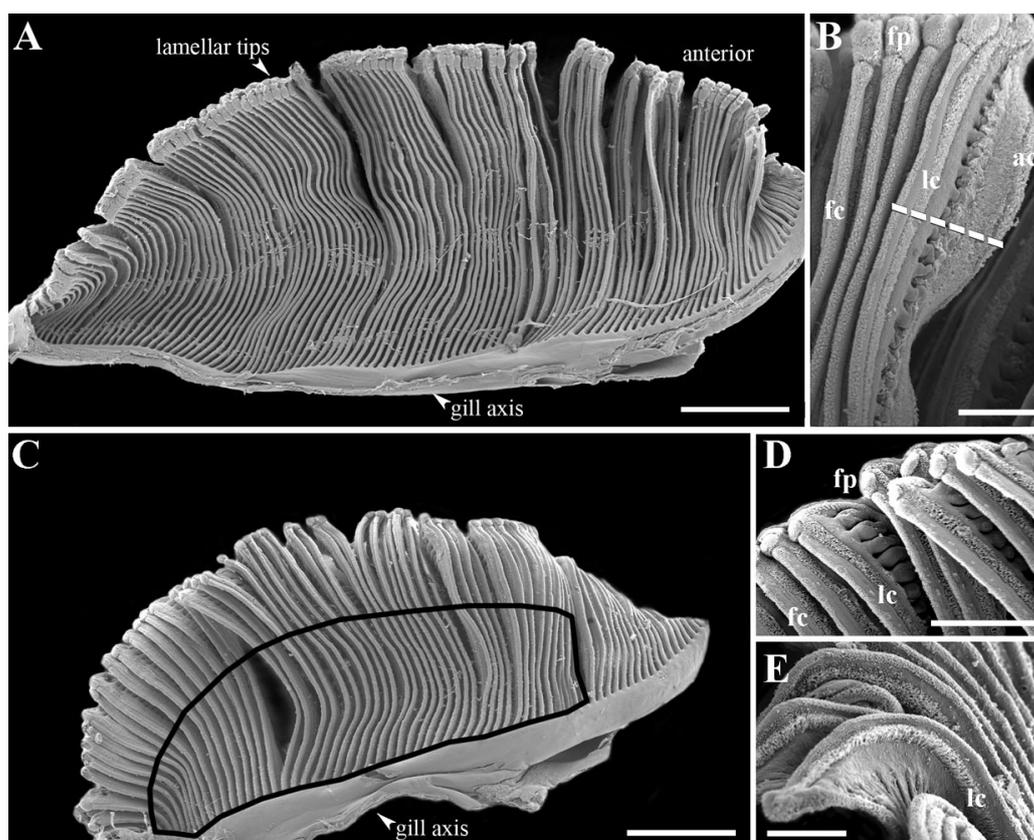


Fig. 1. (A,B) *Lepetodrilus fucensis*, (C,D) *L. guaymasensis*, (E) *L. elevatus*. Scanning electron micrographs of gills removed from specimens 8 mm in shell length. Dorsal surface of the (A) gill and (B) lamellae of *L. fucensis*, showing the ciliated frontal pads (fp) and frontal (fc), lateral (lc) and abfrontal cilia (ac). 'Lamella depth' is the length of the dashed line (transverse section through the gill lamella). (C) Gill and (D) lamellae of *L. guaymasensis* and (E) lamellae from *L. elevatus* are shown for visual comparisons. Gill axis length and lamella length are greater in *L. fucensis*. Lamellae of *L. fucensis* are free of the mantle along their entire length (B). In *L. guaymasensis* the dorsal surface of lamellae (opposite to the surface shown in C) are attached to the mantle for at least one-half their length (except the anterior lamellae), as indicated by the black outline (C). The free lamellar tips are shown in (D) & (E). Scale bars = 1 mm

height of the digestive gland were measured to estimate volume (approximately rectangular shape) (I flattened the *L. affinis schrolli* digestive gland using tweezers during measurements because it was L-shaped). The digestive tract was excised by dissection, and its length was measured (from the point of entry to the exit from the digestive gland). Last, the length and diameter (at a middle position) of the stomach were measured to estimate volume (assuming a cylindrical shape).

Microscopy: Radulae were dissected from the buccal cavity and cleaned in a 1% bleach solution at room temperature for 15 min. The dorsal surface of excised gills and radulae from 10 specimens (5 mm in shell length) of each *Lepetodrilus* species were prepared for scanning electron microscopy (SEM). Tissues were critical-point dried and sputter-coated in gold to generate micrographs on a Hitachi S-3500N scanning electron microscope. Gill lamellae were counted along a 1 mm length of the gill axis. The cusp areas of the central and lateral teeth were measured using Image-Pro Plus 4.5. In addition, scanning electron micrographs of the dorsal surface of the intact gill and food mass of *L. fucensis* (n = 3) were generated from specimens with their shell and mantle removed.

Gill lamellae (n = 10 for each species) from anterior, middle and posterior regions (Fig. 1) were post-fixed with osmium tetroxide, dehydrated in a graded ethanol series, transferred to 100% propylene dioxide and embedded in Epon 812 resin (TAAB). Semi-thin transverse sections (500 nm) of individual filaments were treated with 1% methylene blue (Richardson's stain) and viewed using a Zeiss Universal compound light microscope. Thin sections (70 nm) were stained with lead citrate and uranyl acetate and imaged using a Hitachi H-7000 transmission electron microscope. Light and transmission electron micrographs were examined to verify the presence or absence of gill bacteria and ciliary junctions, and to determine the morphology of gill lamellae for the 7 *Lepetodrilus* species.

Feeding capabilities. Pressure vessels: Feeding studies were conducted in pressure vessels described in Bates et al. (2005). Pressure was maintained (at 13 789 kPa; depth equivalent = 1406 m); at less than the vessel capacity to ensure long-term functioning by a Whitey backpressure regulator on the chamber outflow tubing (full pressure was achieved after ~15 min). Filtered seawater was pumped into the chamber inlet with a Beckman 110B high pressure liquid chromatography pump at a rate of 2 ml min⁻¹.

Grazing: Bacterium-like material from the shells of live animals was removed and stained red with carmine, and then smeared onto the inside of the viewing window in a pressure vessel. *Lepetodrilus fucensis* specimens (n = 30, collected at a depth of ~1500 m from

Axial Volcano in 2001) were placed in the pressure vessel in the presence of 2 grazing gastropods (to confirm that the experimental set-up did not deter grazing): *Depressigyra globulus* Warén and Bouchet, 1989 (order Neomphalida) and *Provanna variabilis* Warén and Bouchet, 1989 (order Caenogastropoda). Representative specimens from each species (n = 5) were observed on the viewing window individually for 15 min for evidence of grazing, and, after 6 h, all animals were fixed in 7% seawater formalin. The stomach of each specimen was removed by dissection for examination of its contents under a light microscope.

Suspension feeding: Feeding observations with suspended carmine red (<0.5 mm) were carried out on-ship (1) to document suspension feeding by live animals and (2) to track the movement of particles on the gill. *Lepetodrilus fucensis* were collected at Axial Volcano (~1500 m depth). Carmine red was suspended in seawater (5% solution) and injected into the pressure vessel for 1 h at a rate of 1 ml min⁻¹ (using a Beckman 110B high pressure liquid chromatography pump), followed by a 'chase' with filtered seawater (2 ml min⁻¹) that varied in duration: 1, 3, 7 and 11 h (n = 20 for each duration). Animals were then preserved in 7% seawater formalin for dissection under a light microscope. The gill, food mass, radula, stomach content and faecal pellets were examined for the presence of carmine particles.

Next, to describe the particle-processing regions of the gill and neck, whole *Lepetodrilus fucensis* (collected from Endeavour) were processed (with the shells and mantle removed) for SEM. Specimens were dehydrated in a graded ethanol series, critical-point dried and sputter-coated in gold for viewing with a Hitachi S-3500N scanning electron microscope.

Symbiont farming: The regions between the lamellar tips and the mantle on *Lepetodrilus fucensis* (Axial and Endeavour specimens were imaged) and *L. gordensis* gills were examined in light and transmission electron micrographs (see 'Morphological comparisons; Microscopy' above). The accepted food material in *L. fucensis* specimens was also imaged with SEM (see preceding paragraph).

RESULTS

Morphological comparisons

The gills of *Lepetodrilus fucensis* and *L. gordensis* are ~30% larger than non-symbiont-hosting species and possess several specialised traits (summarised in Table 1, Figs. 1, 2 & 3). Individual lamellae are not markedly tapered, while the lamellae of congeners are wider at the base and narrow toward the tip (compare

Table 1. *Lepetodrilus* spp. Comparative observations for gill features among 7 *Lepetodrilus* species. Specimens were whole (SEM: scanning electron microscopy; LM: light microscopy) or tissue sections (TEM: transmission electron microscopy; LM). Gill lamellae were examined for symbiotic bacteria and ciliary junctions (Fretter 1988) (Pr: present; Ab: absent). In all species where symbiotic bacteria were absent, the mantle was attached to at least 30% of the dorsal surface of gill lamellae (except for the free anterior tip) and the base of each lamella was deeper than at the tip (Tap: tapered). In the 2 species that host bacteria, the gill lamellae were entirely free of the mantle and were a similar depth at the tip and base (Not tap: not tapered). Non-symbiotic species had less filaments for the same length of gill axis (n = 5; ~8 mm shell length)

Species	Bacteria (TEM/SEM)	Ciliary junction (SEM)	Mantle (LM: whole)	Lamella shape (LM: section)	No. of lamellae mm ⁻¹ (SEM)
<i>L. fucensis</i>	Pr	Pr	Free	Not tap	1.3
<i>L. gordensis</i>	Pr	Pr	Free	Not tap	1.3
<i>L. guaymasensis</i>	Ab	Ab	Attached	Tap	1.1
<i>L. affinis schrolli</i>	Ab	Ab	Attached	Tap	1.1
<i>L. elevatus</i>	Ab	Ab	Attached	Tap	1.1
<i>L. ovalis</i>	Ab	Ab	Attached	Tap	1.1
<i>L. pustulosus</i>	Ab	Ab	Attached	Tap	1.1

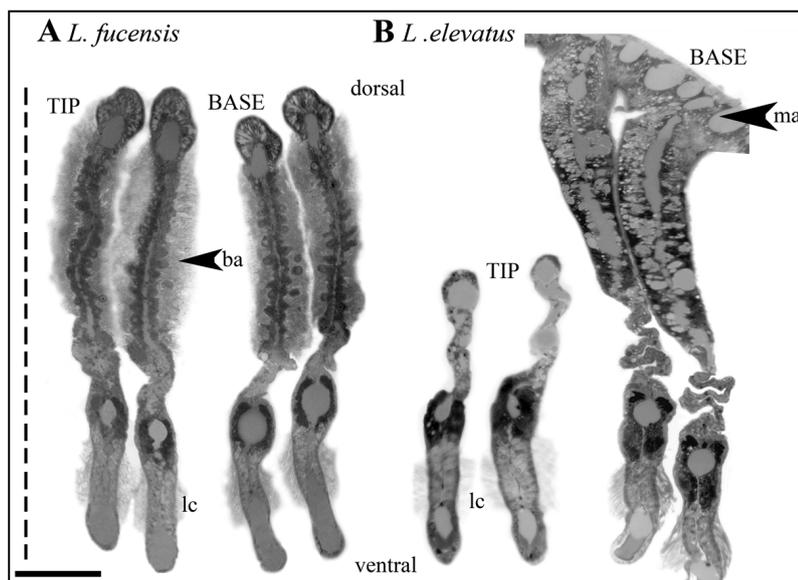


Fig. 2. (A) *Lepetodrilus fucensis* and (B) *L. elevatus*. Light micrographs showing gill lamellae in transverse section. Lamella depth (indicated by the dashed line) of *L. fucensis* is similar at the tip and base (see Fig. 1B), lamellae are free of the mantle, and bacterial filaments (ba) are present (*L. gordensis* is similar). In *L. elevatus* (and all other aposymbiotic species) lamellae taper towards their tips and the dorsal base of lamellae are attached to the mantle (ma) for at least one-third of the lamella length (except for the anterior lamellae: see Fig. 1C). Specimens are from animals 8 mm in shell length. lc: lateral cilia. Scale bar = 250 μ m

the depth of the lamellar tips between *L. fucensis* and *L. elevatus* in Fig. 2). Bacteria are visible on the afferent region (sensu Fretter 1988) of lamellae for the 2 symbiont-hosting species, but are absent from the corresponding region in congeners (Fig. 2). The lamellae of *L. fucensis* and *L. gordensis* are free of the mantle along

the entire length of their dorsal surface (between the mantle and the abfrontal cilia) and are stabilised by lateral ciliary junctions (see Fretter 1988). In comparison, the basal portion of the dorsal edge of lamellae (except anterior lamellae) of all non-symbiont-hosting species is attached to the mantle roof (for at least one-third of the lamellar length starting at the base), and ciliary junctions are not observed (Table 1, Figs. 1 & 2). Lamellae are more densely spaced in *L. fucensis* and *L. gordensis* by 2 more lamellae for every ~10 mm of gill axis (compare *L. fucensis* to *L. guaymasensis*: Fig. 1A,C), and the lengths of the gill axis and lamellae are >25% longer than congener lamellae (planned comparison ANOVA, $t > 26$, $p < 0.001$).

The radulae of the 2 symbiont-hosting species are smaller than aposymbiotic congeners (compare *Lepetodrilus fucensis* to *L. elevatus* in Fig. 4). The length of the radula is reduced by ~25% (Fig. 5A); likewise, the areas of the cusps on the central and lateral teeth are reduced by ~50% (Fig. 5B,C). These differences are significant between symbiont- and non-symbiont-hosting species (planned comparison ANOVA, $t < -10$, $p < 0.001$).

While the length of the gut and digestive gland volume of *Lepetodrilus fucensis* and *L. gordensis* fell within values obtained for congeners, the stomach volume was significantly smaller (reduced by ~50%) than that of the congener group (Fig. 6A) (planned comparison ANOVA, $t < -3$, $p < 0.05$). In addition, species-specific stomach volumes (standardised by shell volume) and radula lengths (standardised by shell length) are significantly correlated (Fig. 6B) (Pearson product moment correlation: $p < 0.001$, $r = 0.96$).

Grazing

Lepetodrilus fucensis protract their radulae to remove red-stained particulates from the surfaces of a pressure vessel window (ca. once every 15 min). For comparison, *Depressigyra globulus* and *Provanna variabilis* (2 active grazers) frequently rasp particulates from the glass with their radulae (ca. once per minute). After 6 h of exposure

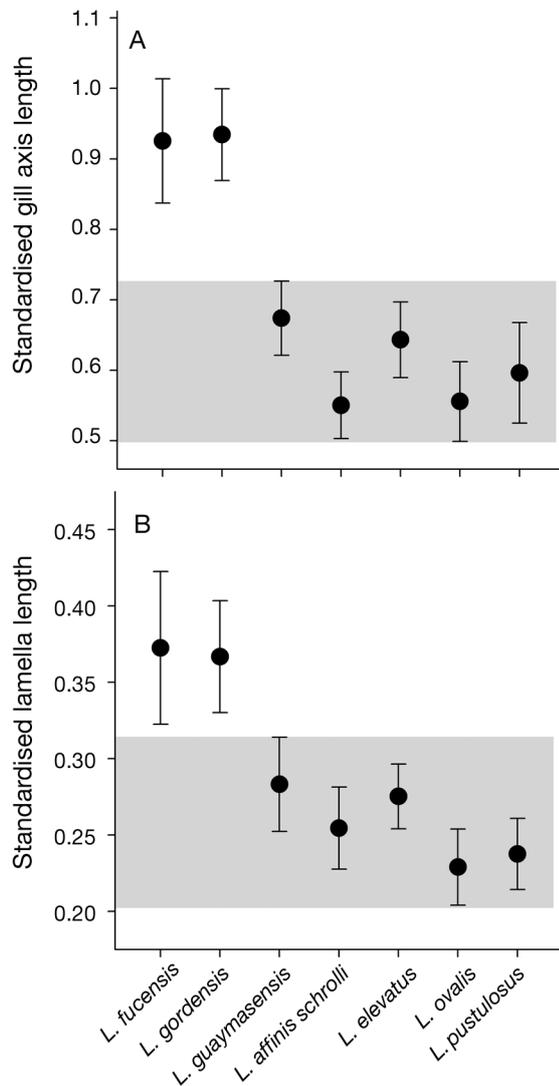


Fig. 3. *Lepetodrilus* spp. Measures of mean (± 1 SD) (A) gill axis and (B) lamella lengths (gill and lamella lengths are divided by shell length; values are dimensionless). Data range for the non-symbiont-hosting species is shaded in grey. Both the gill axis and lamella lengths of *L. fucensis* and *L. gordensis* are significantly enlarged (ANOVA, $p < 0.001$)

to the stained, attached material, the stomach contents of 2 *L. fucensis* specimens (out of 30) contained red material visible under a light microscope, while the stomach contents of all (30 out of 30) *D. globulus* and *P. variabilis* were red.

Suspension feeding

Based on whole-animal examinations of *Lepetodrilus fucensis*, fluids enter the mantle cavity anteriorly and deposit larger particles on the ventral surface of the gill (Fig. 7). The morphology of the frontal and lat-

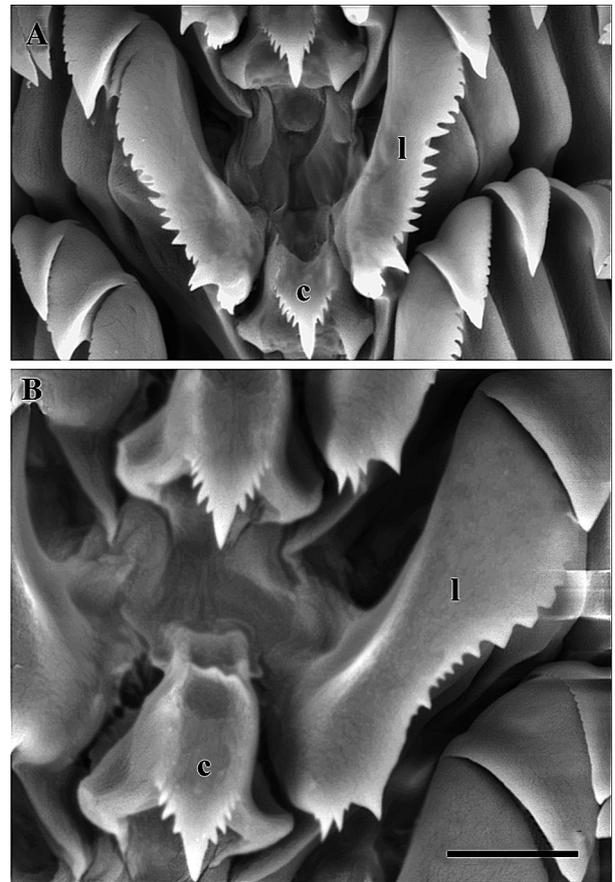


Fig. 4. (A) *Lepetodrilus fucensis* and (B) *L. elevatus*. Scanning electron micrographs of radulae. Animals are 8 mm in shell length. Central (c) and lateral (l) tooth cusps are visible. Radular teeth of *L. fucensis* are smaller than those of similar-sized congeners. Scale bar = 25 μ m (applies to both images)

eral cilia suggest that they push water between the gill lamellae (from ventral to dorsal: see Fig. 7); in addition, the frontal cilia also move particles ventrally to the right side of the gill (A. Warén pers. comm.). Smaller particles are moved in between the lamellae to the dorsal surface of the gill, are transported by the abfrontal cilia (Fig. 7) along the lamellar length, and are incorporated into the food material at the lamellar tips (Fig. 8).

Lepetodrilus fucensis ($n = 80$) concentrate carmine with other particles into a cylindrical mass that is moved from the gill to the mouth. After 2 and 4 h, the particle mass at the lamellar tips contains carmine (Fig. 8A,B). This material is transported on the right side of the neck (Fig. 8C) in a ciliated tract that splits into an accepted and rejected tract (see Figs. 8D & 9). Scanning electron micrographs of the accepted and rejected material are different in their composition (Fig. 9). The accepted tract contains high abundances of filaments and some spheroid shapes (Fig. 9A: image is representative of multiple specimens). The rejected material contains debris-like

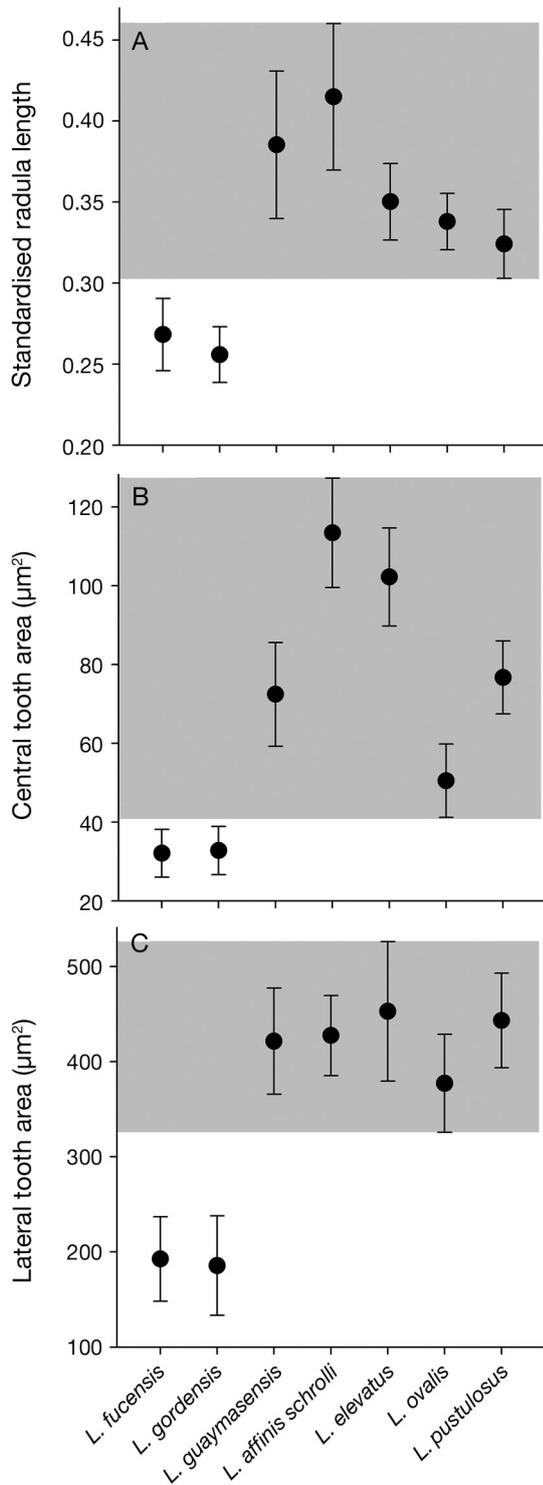


Fig. 5. *Lepetodrilus* spp. Measures of mean (± 1 SD) (A) radula lengths (divided by shell length; values are dimensionless), (B) central tooth area and (C) lateral tooth area. Data range for the non-symbiont-hosting species is shaded in grey. Length of the radula ribbon and the areas of the central and lateral teeth are significantly reduced in *L. fucensis* and *L. gordensis* specimens (ANOVA, $p < 0.001$)

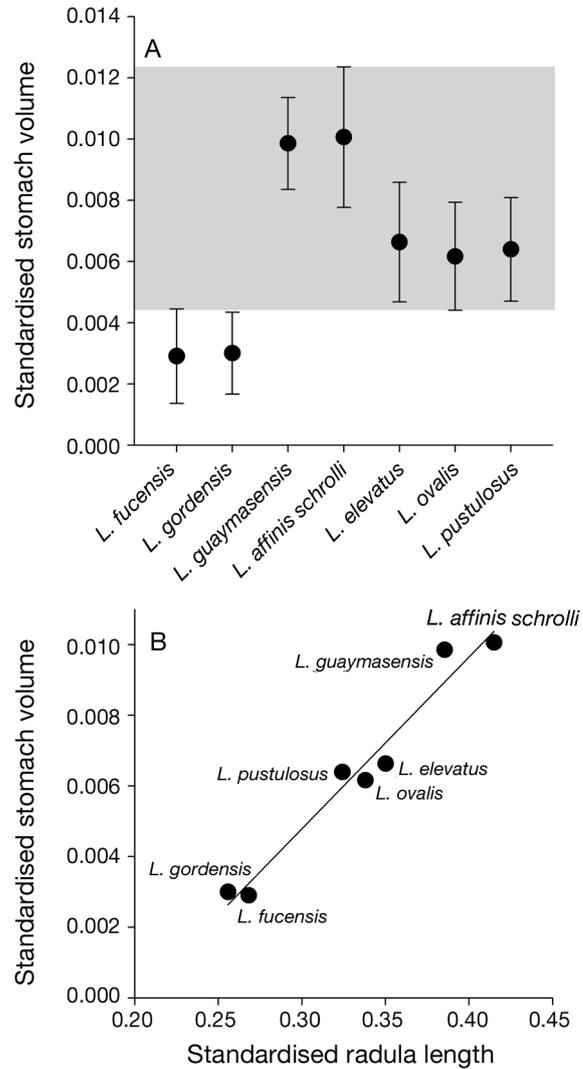


Fig. 6. *Lepetodrilus* spp. (A) Mean (± 1 SD) stomach volume (divided by shell volume; values are dimensionless). Data range for the non-symbiont-hosting species is shaded in grey. Stomach volumes of *L. fucensis* and *L. gordensis* are significantly reduced (ANOVA, $p < 0.001$). (B) Standardised stomach volume of each species is significantly correlated ($p < 0.001$, Pearson's $r = 0.96$) with standardised radula ribbon length (see Fig. 5)

particles, and relatively fewer filaments and spheroids (Fig. 9B). The particles in the accepted tract are transported above the cephalic tentacle to the mouth (Fig. 8D), and, within 4 h, carmine particles are present on the radular teeth and in the stomach. After 8 h, faecal pellets contain carmine (Fig. 8E).

Symbiont farming

Lepetodrilus fucensis hosts epibiotic bacteria attached to the gill epithelium that are bathed in fluids

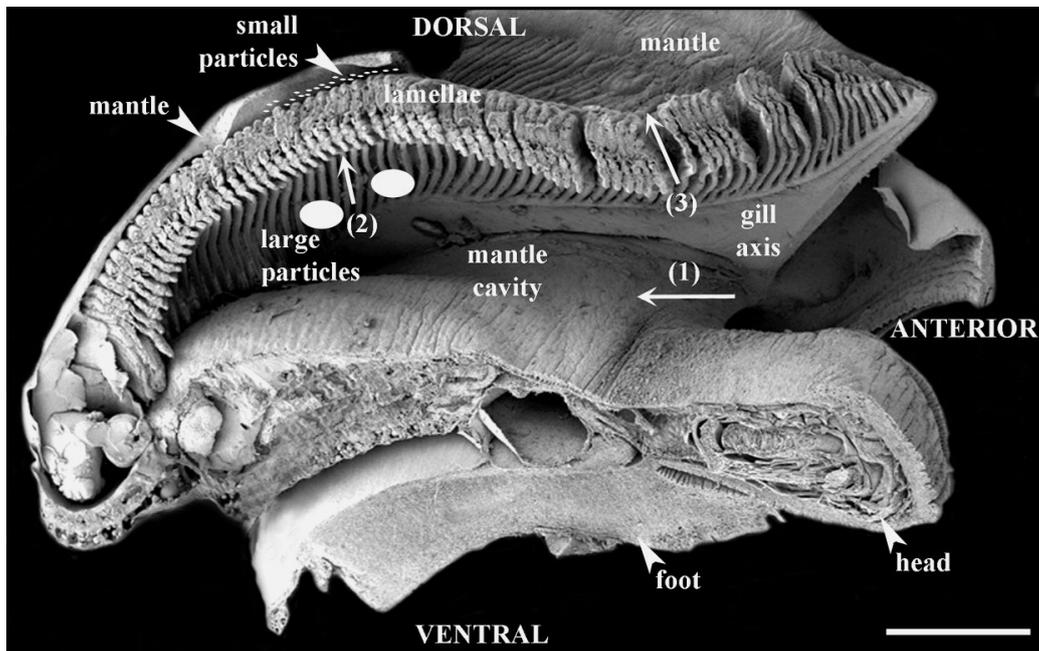


Fig. 7. *Lepetodrilus fucensis*. Whole animal longitudinal section after critical-point drying (courtesy of A. Warén). Based on dissections of animals at varying time intervals following exposure to carmine, fluids enter the mantle cavity anteriorly (Arrow 1). The frontal cilia on the ventral surface of the gill accumulate large-sized particles into mucous chords that are transported at right angles to the lamellae (Arrow 2); this material is then transported to the lamellar tips and incorporated into the food mass (see Fig. 8A,B). Water appears to be pushed from the ventral surface in between the lamellae (Arrow 3) by the lateral cilia. Small particles also accumulate on the dorsal surface of the gill, between the mantle and the lamellar abfrontal cilia, and are moved at right angles to the lamellae and coalesced into the food mass shown in Fig. 8A,B. The food mass is then transported to the neck region and sorted into accepted and rejected ciliated tracts (see Fig. 9). Scale bar = 1 mm

pushed from the ventral to the dorsal surface by the lateral cilia (Figs. 7 & 10). Detached bacterium-like filaments are present dorsally in the region between the mantle and the abfrontal cilia on *L. fucensis* gills and appear to be sloughed from the symbiont colonies (Fig. 10A,B); these filaments share the following traits with the symbiotic bacteria: diameter $\approx 1 \mu\text{m}$, length $> 200 \mu\text{m}$ and a double-membrane. The filaments are transported to a sorting region on the neck (Fig. 8) and are present in the accepted tract that leads to the mouth (Fig. 9A). Likewise, *L. gordensis* accumulates bacterium-like filaments between the abfrontal cilia and mantle, where a furrow is also present on the dorsal surface of lamellae (Fig. 10) (see also Johnson et al. 2006). In other *Lepetodrilus* species without gill bacteria, the dorsal surface of the lamella is partially fused to the mantle (Figs. 1 & 2), and, where the lamellae are free from the mantle, high abundances of particles are not observed.

DISCUSSION

Species in the *Lepetodrilus* genus possess morphological and behavioural traits characteristic of suspen-

sion feeding and grazing. For example, the frontal and abfrontal ciliary pads at the free tip of lamellae are enlarged, a specialisation to transport high particle loads; radular tooth wear (which occurs during grazing) is also common (Fretter 1988). However, suspension feeding may be a more efficient feeding mode for *L. fucensis* and *L. gordensis*, 2 sister species from northeast Pacific hydrothermal vents that are unique in hosting episymbiotic bacteria on their gill lamellae, because they possess several highly specialised gill traits that have evolved independently in several other suspension-feeding gastropod families (Declerck 1995). Relative to their congeners, *L. fucensis* and *L. gordensis* have longer gill axes and longer, more densely spaced lamellae. Furthermore, their gill lamellae do not taper, are connected by lateral ciliary junctions and are free of the mantle along their entire dorsal surface. In other gastropods, greater numbers of longer lamellae that are unattached to the mantle increase the gill's surface area and fluid current velocities (Vogel & Gutmann 1980), thus enhancing particle processing and delivery (reviewed in Declerck 1995).

The morphology of *Lepetodrilus fucensis* gills and progression of carmine particles from the gill to the mouth indicate directions of fluid flow and a sequence

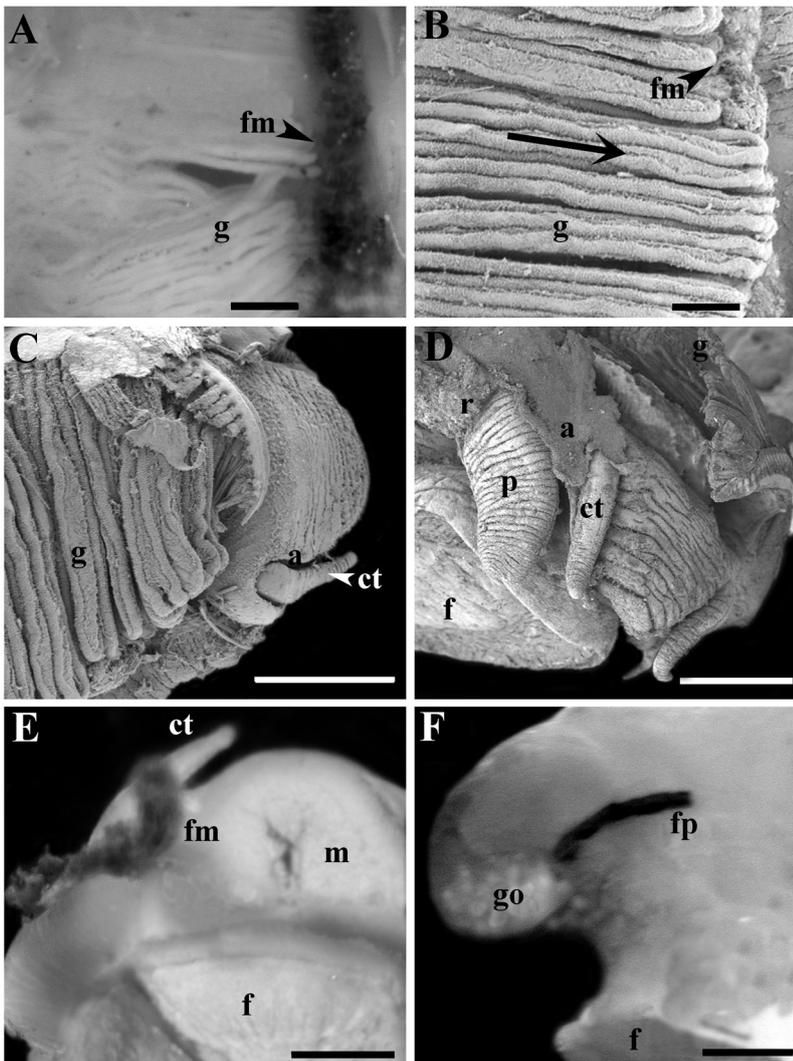


Fig. 8. *Lepetodrilus fucensis*. (A,E,F) Light and (B,C,D) scanning electron micrographs documenting the accumulation of particles at the lamellar tips into a food mass (fm) (sensu Fretter 1988). (A) Carmine particles are removed from suspension and accumulate in the food mass. (B) Small particles are observed on the ventral surface of the gill (g) and are transferred to (arrow), and incorporated into, the food mass. (C,D) The food mass is then transported to the neck and sorted into 2 ciliated tracts: the accepted material (a) passes to the right of the cephalic tentacle (ct), while rejected material (r) passes to the right of the penis (p) (in the case of males). (E) The accepted material continues to the mouth (m) (see also Fretter 1988). Animals protract their radula to ingest the food mass. (F) Carmine is observed in stomach contents and faecal pellets (fp). f: foot; go: gonad. Scale bars = 1 mm

of particle transport that supports suspension feeding. Based on the orientation and length of the frontal and lateral cilia, fluids are pushed at right angles to the lamellae on the ventral surface of the gill (A. Warén pers. comm.), where large particles (>0.5 mm) accumulate and are then transported by the frontal cilia to the lamellar tips. Similarly, in the suspension-feeding limpet *Crepidula fecunda*, material on the gill's ventral surface comprises larger particulates that settle from

fluids as current passes in between the lamellae; these particles are subsequently rejected (Chaparro et al. 2002). On *L. fucensis* gills, fluids are also pushed in between the lamellae, presumably providing the blood-rich regions of the gill with oxygen and the chemosynthetic bacterial symbionts with access to chemical substrates, and then along the dorsal surface of the gill. This current includes primarily small filamentous particles (<0.5 mm) that are moved along the gill's dorsal surface between the abfrontal cilia and the mantle to the lamellar tips, where they are bound into a cylindrical-shaped mass that is transported by cilia to the neck and subsequently sorted into an accepted or rejected tract. The accepted tract contains the food material that is ingested.

Particle transport and processing by the gill and neck of *Lepetodrilus fucensis* confirm that this species is an active suspension feeder, but also suggests a pathway whereby the gill episybionts can be farmed and ingested. Symbiont-like particles are found in the food debris in light and electron micrographs (*L. fucensis* and *L. gordensis*), and preliminary fluorescent *in situ* hybridisation studies show that the symbiont phylotype can dominate the material incorporated into the accepted food material (*L. fucensis*: Bates 2006). Although free-living filamentous bacteria are common in vent habitats, the high abundance of symbiont-like bacteria (based on diameter, length and the presence of a double membrane) in the food material on the gill (present study) and gut (de Burgh & Singla 1984) is consistent with the hypothesis that the symbionts are cultivated, transported to the mouth and ingested. However, it is important for further studies to investi-

gate whether symbiont farming is a significant feeding mechanism used by *L. fucensis*; once firmly documented, this would represent the first report of a gastropod that receives nutrition by farming gill episybionts.

In fact, the modified gill traits shared by *Lepetodrilus fucensis* and *L. gordensis* not only increase their ability to process suspended particles (based on comparisons with other suspension-feeding genera in the class Gas-

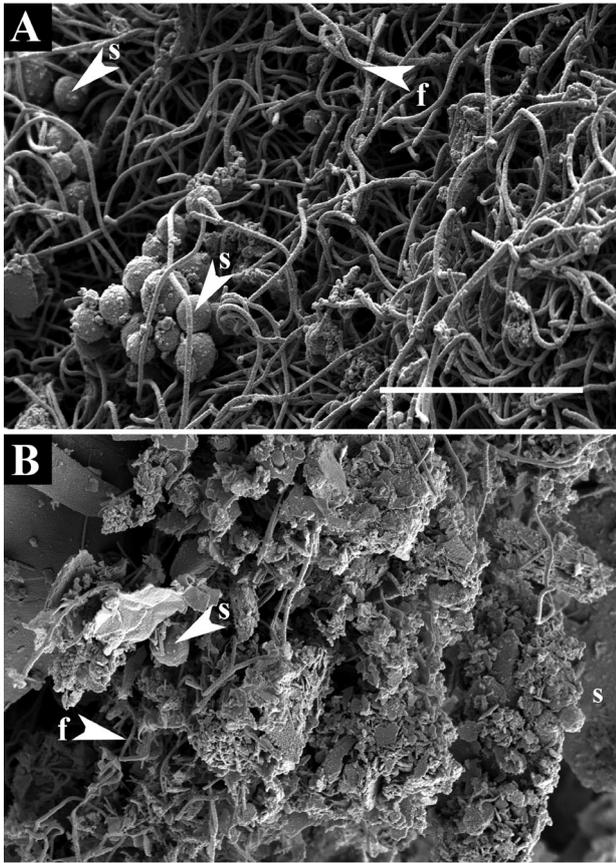


Fig. 9. *Lepetodrilus fucensis*. Scanning electron micrographs of particles in accepted and rejected ciliary tracts on the neck. (A) Accepted food mass showing spheroid (s) and filamentous (f) shapes that resemble bacteria. The filaments are similar in length and diameter to the *L. fucensis* symbiont. (B) Rejected material that passes to the right of the cephalic tentacle. Amorphous and mineralised particles are more common. Scale bar (for both A & B) = 200 μ m. Images provided by A. Warén

tropoda: Declerck 1995), but also to cultivate their gill symbionts. Larger, longer, more densely spaced lamellae that are unattached to the mantle favour symbiont farming by: (1) increasing fluid velocities and, thus, facilitating greater chemical exchange to promote bacterial growth, (2) allowing the symbionts to be concentrated into a food mass and (3) increasing the gill's surface area for greater cultivation potential. Therefore, suspension feeding and symbiont farming may be additive selective forces driving the gill traits of the 2 symbiont-hosting species. It is also possible, since aposymbiotic species in the *Lepetodrilus* genus feed on suspended particles (see Fretter 1988), that episympiosis is a key innovation that has driven the suite of highly specialised gill traits possessed by *L. fucensis* and *L. gordensis* (Liem 1974, Wainwright 1991, Vermeij 2001).

However, while both *Lepetodrilus fucensis* and *L. gordensis* host gill episympionts, they do exhibit differences in their lamellae, which may influence their relative feeding capabilities. *L. gordensis* gill lamellae have a furrow on the dorsal surface that, in other suspension-feeding species, increases food capture and processing efficiency (Declerck 1995). Furthermore, *L. gordensis* hosts remarkably dense colonies of bacteria and other debris in comparison to the same area of *L. fucensis* gills (I have examined the gill lamellae of 300 *L. fucensis*: Bates 2007). The significance and generality of this furrow and the high particle density on the *L. gordensis* gills at the population level are unknown, and a more detailed study aiming to compare the feeding strategy of these sister species and their reliance on their symbionts may be worthwhile.

In the present study, I also found that adult *Lepetodrilus fucensis* and *L. gordensis* have significantly smaller radular ribbon lengths and stomach volumes in comparison to their aposymbiotic congeners. Molluscs with endosymbionts typically have reduced structures and tissues that are associated with food ingestion and digestion, because the host receives organic carbon via more direct processes: translocation of fixed carbon from symbiont to host and/or digestion of the endosymbionts directly in lysosomes (e.g. *Ifremeria nautiliei*: Windoffer & Giere 1997). Hence, one explanation for the reduction in the radula and stomach of the 2 symbiont-hosting species is that their nutrition is supplemented by translocation or digestion of the gill bacteria in epithelial lysosomes. Yet the gut length and digestive gland volume of *L. fucensis* and *L. gordensis* were not reduced, indicating that digestion of ingested food material is significant and is therefore probably not supplemented by high rates of carbon translocation. Likewise, direct ingestion of bacteria by the gill epithelium is not an important feeding mechanism because the occurrence of lysosomes in the gill epithelium is not consistently related to the abundance of bacteria on the gill (for *L. fucensis*: Bates 2007).

In fact, the relative sizes of the radular ribbon and stomach displayed a tight linear relationship for the 7 *Lepetodrilus* species examined. Therefore, a parsimonious hypothesis explaining the reduction in the radula and stomach of the 2 symbiont-hosting species must also explain why these 2 structures are enlarged in several aposymbiotic species. It is possible that differences in radula and stomach dimensions reflect the importance of grazing in the feeding strategy of each limpet species and, ultimately, in the inter-specific differences in the quality of ingested food material. Radulae are relatively shorter when grazing activity is minimal because mature teeth do not need to be replaced as quickly when mechanical abrasion is less frequent (e.g. Ito et al. 2002). The size of the radular teeth is also

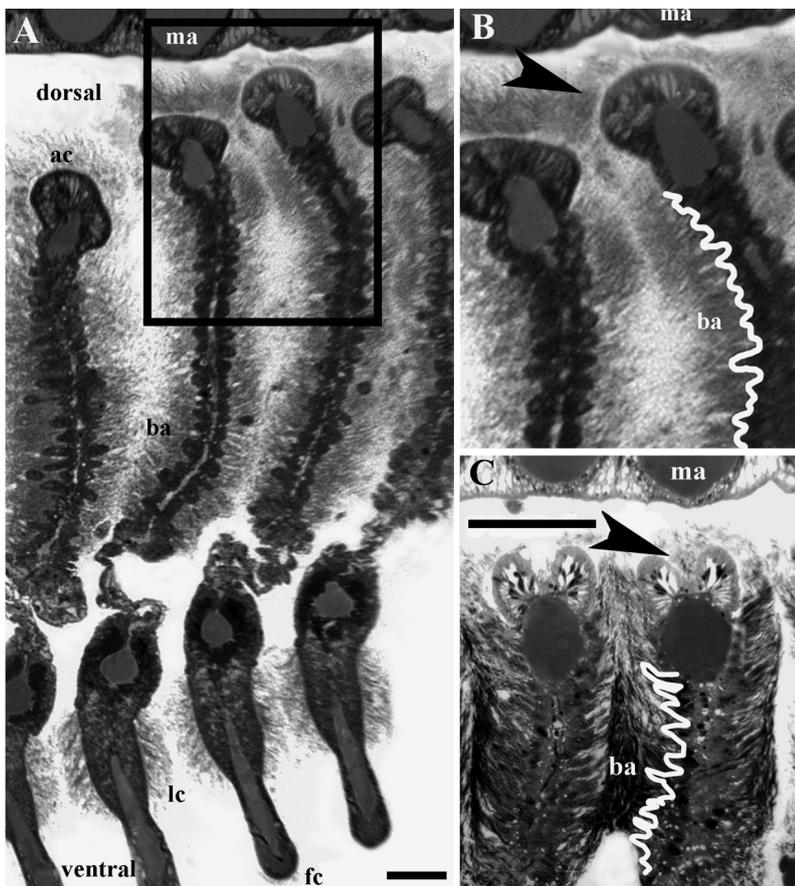


Fig. 10. *Lepetodrilus* spp. (A) Light micrograph of *L. fucensis* gill lamellae in transverse section with the mantle (ma) present. lc: lateral cilia; fc: frontal cilia; ac: abfrontal cilia. (B) Epibiotic bacteria (ba) are present in between the lamellae and are attached to the gill epithelium along the white line. Detached bacterium-like filaments (arrowhead) are found between the abfrontal cilia on the dorsal edge of lamellae and the mantle. (C) Light micrograph of *L. gordenis* gill lamellae in similar orientation to (A) & (B). Epibiotic bacteria (ba) are attached to the gill epithelium (white line). Detached bacterium-like filaments are also present on the dorsal edge of lamellae and within a furrow (arrowhead) that is absent from *L. fucensis* lamellae. Scale bars = 10 μm (B is the same scale as C)

positively related to how resistant teeth are to damage (Hickman 1980) (the teeth of *L. fucensis* and *L. gordenis* are smaller and, consequently, less robust than similar-sized specimens of species without symbionts). Grazed material will most likely contain more inorganic debris than a diet of suspended particles (because this material is sorted on the gill) or symbionts; thus, the stomach can be reduced when grazing is minimal because it does not need to process the same volumes of material to gain the same nutritional benefit. Therefore, co-reduction in the radula and stomach support the hypothesis that adults of the symbiont-hosting species specialise on suspended material and/or their symbionts for nutrition. Conversely, *L. guaymasensis* and *L. affinis schrolli* have long radula ribbons and large stomach volumes, and may

rely more heavily on grazing than the other 5 species. However, this hypothesis remains to be tested.

In all likelihood, the primary feeding mechanism used by *Lepetodrilus fucensis* probably varies in accordance with food availability (as predicted by optimal foraging theory: MacArthur & Pianka 1966) and size (ontogenetic shifts in the diets of invertebrates are common: Werner & Gilliam 1984). In active vent flows, suspended particles (Giere et al. 2003) and symbiont biomass are high (Bates 2007). In these habitats, *L. fucensis* populations are dominated by adult sizes (at least 90% of the population is >1 mm in shell length) and appear to rely primarily on suspension feeding and/or symbiont farming by forming sessile stacks in venting fluids (Bates et al. 2005). In contrast, habitats on the periphery of vents and waning flows have relatively low concentrations of suspended particles, and the abundance of gill symbionts on limpets in these habitats is minimal (Bates 2007), making grazing the only feasible feeding mechanism. However, adult-sized animals found in low flux habitats have poor tissue condition (Bates 2007), indicating that nutrition from grazing alone in such habitats cannot maintain healthy tissues for long durations (this may be because the radula and stomach are reduced: see preceding paragraph). Furthermore, although adult *L. fucensis* do graze from the surfaces of aquaria, their rasping rates are low (~1 per 15 min). While

a low grazing rate can be explained in a number of ways (e.g. *L. fucensis* may be highly selective in its food material), this result is consistent with the hypothesis that adults are suspension-feeding and/or symbiont-farming specialists. In comparison, juvenile limpets are found almost exclusively in peripheral locations (Marcus & Tunnicliffe 2002, Bates 2006). It is probable that grazing is the primary feeding mechanism used by smaller *L. fucensis* because suspension feeding and symbiont farming are limited by size (small gills do not process and/or cultivate bacteria with the same efficiency as large gills). Whether *L. fucensis* shows size- or habitat-specific variation in feeding is an open question.

In summary, *Lepetodrilus fucensis* presumably uses a combination of different feeding mechanisms, which

may explain its ability to persist in habitats with very different levels of hydrothermal fluid flux where the availabilities of suspended and attached particles vary (Tsurumi & Tunnicliffe 2003). *L. fucensis* shows morphological and behavioural evidence of both suspension feeding and grazing, and, in addition, may farm its epibionts for the purposes of ingestion. The symbionts associated with the gill appear to be sloughed with suspended particles into a food mass that is transported from the gill to the mouth in a ciliated tract, although the importance of this feeding mechanism requires further investigation. While the specialised gill traits of *L. fucensis*, e.g. unattached and enlarged lamellae, have evolved independently in other suspension-feeding gastropod genera, these specialisations also promote high symbiont abundance by increasing the gill's surface area and the velocity of the gill currents. The symbiosis may be a key innovation that has driven the gill modifications displayed by the 2 species hosting bacteria.

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LITERATURE CITED

- Bates AE (2006) Population and feeding characteristics of hydrothermal vent gastropods along environmental gradients with a focus on a bacterial symbiosis hosted by *Lepetodrilus fucensis* (Vetigastropoda). PhD dissertation, Department of Biology, University of Victoria, BC
- Bates AE (2007) Persistence, morphology and nutritional state of a gastropod hosted bacterial symbiosis in different levels of hydrothermal vent flux. *Mar Biol* 152(3):557–568
- Bates AE, Tunnicliffe V, Lee RW (2005) Role of thermal conditions in habitat selection by hydrothermal vent gastropods. *Mar Ecol Prog Ser* 305:1–15
- Beck LA (2002) *Hirtopelta tufari* sp. n., a new archaeogastropod hot vent limpet (Rhipidoglossa, Peltospiridae) harbouring endocytosymbiotic bacteria in its gill. *Arch Moll* 132:23–34
- Bouchet P, Warén A (1991) *Ifremeria nautiliei*, a new gastropod from hydrothermal vents, probably associated with symbiotic bacteria. *CR Acad Sci Paris Ser III* 312:495–501
- Casanova B, Brunet M, Ségonzac M (1993) Impact of bacterial epibiosis on functional-morphology of shrimp associated with the mid-Atlantic hydrothermal conditions. *Cah Biol Mar* 34:573–588
- Chaparro O, Thompson R, Pereda S (2002) Feeding mechanisms in the gastropod *Crepidula fecunda*. *Mar Ecol Prog Ser* 234:171–181
- Chelazzi G, Parpagnoli D, Santini G (1998) A satiation model for the temporal organization of grazing in limpets. *Funct Ecol* 12:203–210
- de Burgh ME, Singla CL (1984) Bacterial colonization and endocytosis on the gill of a new limpet species from a hydrothermal vent. *Mar Biol* 84:1–6
- Declerck C (1995) The evolution of suspension feeding in gastropods. *Biol Rev* 70:549–569
- Fox M, Juniper SK, Vali H (2002) Chemoautotrophy as a possible nutritional source in the hydrothermal vent limpet *Lepetodrilus fucensis*. *Cah Biol Mar* 43:371–376
- Fretter V (1988) New archaeogastropod limpets from hydrothermal vents; superfamily Lepetodrilacea II. In: Cann JR, Elderfield H, Laughton A (eds) Mid-ocean ridges: dynamics of processes associated with creation of new ocean crust, Vol 318. *Phil Trans R Soc Lond B* 319:33–82
- Giere O, Borowski C, Prieur D (2003) Biological productivity in hydrothermal systems. In: Halbach PE, Tunnicliffe V, Hein JR (eds) Energy and mass transfer in marine hydrothermal systems. Dahlem University Press, Berlin, p 211–234
- Goffredi SK, Warén A, Orphan VJ, Dover CLV, Vrijenhoek RC (2004) Novel forms of structural integration between microbes and a hydrothermal vent gastropod from the Indian Ocean. *Appl Environ Microbiol* 70:3082–3090
- Hickman CS (1980) Gastropod radulae and the assessment of form in evolutionary paleontology. *Paleobiology* 6: 276–294
- Honkoop P, Bayne B, Drent J (2003) Flexibility of size of gills and palps in the Sydney rock oyster *Saccostrea glomerata* (Gould, 1850) and the Pacific oyster *Crassostrea gigas* (Thunberg, 1793). *J Exp Mar Biol Ecol* 282:113–133
- Ito A, Ilano AS, Goshima S, Najao S (2002) Seasonal and tidal-height variations in body weight and radular length in *Nodolittoria radiata* (Eydoux & Souleyet, 1852). *J Molluscan Stud* 68:197–203
- Johnson SB, Young CR, Jones WJ, Warén A, Vrijenhoek RC (2006) Migration, isolation and speciation of hydrothermal vent limpets (Gastropoda; Lepetodrilidae) across the Blanco Transform Fault. *Biol Bull (Woods Hole)* 210: 140–157
- Kohn AJ (1983) Feeding biology of gastropods. In: Saleuddin ASM, Wilbur KM (eds) *The Mollusca, physiology*, Vol 5, Part 2. Academic Press, New York, p 1–63
- Liem K (1974) Evolutionary strategies and morphological innovations: cichlid pharyngeal jaws. *Syst Zool* 22:425–441
- MacArthur RH, Pianka ER (1966) On optimal use of a patchy environment. *Am Nat* 100:603
- Marcus J, Tunnicliffe V (2002) Living on the edges of diffuse vents on the Juan de Fuca Ridge. *Cah Biol Mar* 43:263–266
- McLean JH (1993) New species and records of *Lepetodrilus* (Vetigastropoda: Lepetodrilidae) in the hydrothermal vent habitat. *Veliger* 36:27–35
- Okutani T, Ohta S (1988) A new gastropod mollusc associated with hydrothermal vents in the Mariana backarc basin, western Pacific. *Venus Jpn J Malacol* 47:1–9
- Tsurumi M, Tunnicliffe V (2003) Tubeworm-associated communities at hydrothermal vents on the Juan de Fuca Ridge, northeast Pacific. *Deep-Sea Res I* 50:611–629
- Vermeij GJ (2001) Innovation and evolution at the edge: origins and fates of gastropods with a labral tooth. *Biol J Linn Soc* 72:461–508
- Vogel K, Gutmann W (1980) The derivation of pelecypods: role of biomechanics, physiology and environment.

- Lethaia 13:269–275
- Wainwright PC (1991) Ecomorphology—experimental functional anatomy for ecological problems. *Am Zool* 31: 680–693
- Warén A, Bouchet PH (2001) Gastropoda and Monoplacophora from hydrothermal vents and seeps: new taxa and records. *Veliger* 44:116–231
- Werner EE, Gilliam JF (1984) The ontogenetic niche and species interactions in size-structured populations. *Annu Rev Ecol Syst* 15:393–425
- Windoffer R, Giere O (1997) Symbiosis of the hydrothermal vent gastropod *Ifremeria nautilei* (Provannidae) with endobacteria—structural analysis and ecological considerations. *Biol Bull (Woods Hole)* 193:368–380

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