environmental
microbiology reports

Environmental Microbiology Reports (2021) **13**(2), 104–111 doi:10.1111/1758-2229.12909

Brief Report

Contrasting influences on bacterial symbiont specificity by co-occurring deep-sea mussels and tubeworms

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Summary

Relationships fueled by sulfide between deep-sea invertebrates and bacterial symbionts are well known, yet the diverse overlapping factors influencing symbiont specificity are complex. For animals that obtain their symbionts from the environment, both host identity and geographic location can impact the ultimate symbiont partner. Bacterial symbionts were analysed for three co-occurring species each of *Bathymodiolus* mussels and vestimentiferan tubeworms, from three deep methane seeps off the west coast of Costa Rica. The bacterial internal transcribed spacer gene was analysed via direct and barcoded amplicon sequencing to reveal fine-scale symbiont diversity. Each of the three mussel species (*B. earlougheri*, *B. billschneideri* and *B. nancyschneideri*) hosted genetically distinct thiotrophic endosymbionts, despite living nearly side-by-side in their habitat, suggesting that host identity is crucial in driving symbiont specificity. The dominant thiotrophic symbiont of co-occurring tubeworms *Escarpia spicata* and *Lamellibrachia* (*L. barhami* and *L. donwalshi*), on the other hand, was identical regardless of host species or sample location, suggesting lack of influence by either factor on symbiont selectivity in this group of animals. These findings highlight the specific, yet distinct, influences on the environmental acquisition of symbionts in two foundational invertebrates with similar lifestyles, and provide a rapid, precise method of examining symbiont identities.

Introduction

In chemosynthetic habitats, both biological and thermal production of high concentrations of hydrogen sulfide fuel unique animal communities reliant upon partnerships with bacteria. Since the discovery of hydrothermal vent ecosystems in the late 1970s, vent physiologists and ecologists have studied the enormously successful symbiotic relationships between deep-sea marine invertebrates and chemoautotrophic microbes (Corliss *et al*., 1979; Felbeck, 1981; Cavanaugh, 1983; Fiala-Medioni and Le Pennec, 1987; Childress *et al*., 1991; to name a few). Deep-sea bathymodiolin mussels and vestimentiferan tubeworms, for example, are among the dominant deep-sea animals that form symbiotic associations and make up much of the biomass in many chemosynthetic habitats (reviewed in the study by Dubilier *et al*., 2008 and Sogin *et al*., 2020). *Bathymodiolus* mussels have a greatly reduced digestive tract (Le Pennec *et al*., 1990; Barry *et al*., 2002) and rely instead on endosymbiotic bacteria, residing in gill tissues, which produce organic carbon via energy from hydrogen sulfide oxidation (Rau and Hedges, 1979; Dattagupta *et al*., 2004; Wentrup *et al*., 2014). Similarly, although larval vestimentiferan tubeworms possess a transient digestive system that is lost during development, adult tubeworms rely entirely on gammaproteobacteria endosymbionts for their nutrition and are housed in a specialized organ known as the trophosome (Jones, 1981; Nussbaumer *et al*., 2006).

Both vestimentiferan tubeworms and *Bathymodiolus* mussels, some of the very first described chemosynthetic symbioses (Felbeck, 1981; Cavanaugh, 1983; Childress *et al*., 1986), acquire their symbionts horizontally (Won *et al*. 2003; Nussbaumer *et al*., 2006). The free-living population of bacteria in the deep sea serve as the inoculum for their hosts (Harmer *et al*., 2008) and must overcome many physical and biochemical hurdles to ensure the formation of a successful association. For instance, symbiont infection in both vestimentiferan tubeworms and bathymodiolin mussels is restricted to particular life stages including post-settlement larvae in tubeworms and juveniles in mussels (Nussbaumer *et al*., 2006; Laming *et al*., 2014). Additionally, during the initial stages of symbiont acquisition, both dominant chemosynthetic marine invertebrates restrict symbiont infection to a specific area. For the Bathymodiolinae, hypertrophic gills in early developmental stages recruit environmental symbionts and

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later elongate to accommodate increased symbiont infection of the tissue (Laming *et al*., 2014; Laming *et al*., 2018). On the other hand, symbionts of vestimentiferan tubeworms enter the larval host worm through the epidermis; a process that continues as the juvenile trophosome develops (Nussbaumer *et al*., 2006). Even in the case of horizontal transmission and the associated risks involved in finding the correct partner, these successful symbioses are persistent across generations and are often highly selective (Duperron, 2010; Vrijenhoek, 2010; Laming *et al*., 2018; Sogin *et al*., 2020). The factors that influence selectivity and specificity in these foundational, often co-occurring, invertebrates and their environmentally acquired symbionts, however, remain ambiguous due to the limitations of sampling co-occurring mussel species and tubeworm species across multiple vent or seep sites.

Previous studies have demonstrated, albeit some indirectly, that symbiont specificity and diversity in *Bathymodiolus* mussels, and other bathymodiolins, can depend on geographic location, habitat type, and host identity (Won *et al*., 2003; DeChaine *et al*., 2006; Laming *et al*., 2015; Uecker *et al*., 2020). For instance, *Bathymodiolus azoricus* and *B. puteoserpentis*, from hydrothermal vents along the northern and southern Mid-Atlantic Ridge (MAR), respectively, shared nearly identical thiotrophic endosymbiont 16S rRNA phylotypes (Won *et al*., 2003; DeChaine *et al*., 2006; Duperron *et al*., 2006). By examining the internal transcribed spacer (ITS) gene; however, DeChaine *et al*. (2006) identified two distinct clades of mussel gammaproteobacteria symbionts along the MAR: one from the southern *B. puteoserpentis* population and one from two northern *B. azoricus* and *B*. aff *azoricus* populations (DeChaine *et al*., 2006). While the authors concluded that the acquired symbiont is most likely dependent on the local environment and not host species identity, given that no ITS genotype was shared among the three sites, it must be noted that none of the mussel species were co-occurring and, thus, inconclusive with regard to lack of host fidelity versus geographic isolation of differing free-living bacterial populations. A related study by Uecker *et al*. (2020) revealed that geographic distance alone had the strongest effect on the variation of three thiotrophic phylotypes among the northern and southern MAR mussel populations, as well as *B. puteoserpentis* in a hybrid zone between them, supporting the previous observations made by DeChaine *et al*. (2006). They suggested that each site hosts its own unique free-living bacterial populations available for symbiont infection, restricted to that particular site by geographic faults along the MAR (Uecker *et al*., 2020). However, Laming *et al*. (2015) revealed that co-occurring bathymodioline mussels, *Idas simpsoni* and *I. modiolaeformis*, from the Mediterranean Sea and adjoining East Atlantic Ocean, hosted distinct sulfur-oxidizing symbionts. Interestingly, the dominant symbiont of *I. modiolaeformis* varied depending on location, further illustrating the overlapping factors that influence mussel endosymbiont specificity. While these studies have furthered our current understanding of inter- and intra-specific symbiont diversity and composition, their conclusions are restricted by the limited number of co-occurring mussels across multiple sites.

Genetic analyses of vestimentiferan tubeworm symbionts suggests that the gammaproteobacterial thiotrophic symbiont 16S rRNA phylotype(s) are primarily influenced by the specific chemosynthetic habitat in which the host resides (e.g. seep or vent site), regardless of host species (Feldman *et al*., 1997; Nelson and Fisher, 2000; Di Meo *et al*., 2000; McMullin *et al*., 2003; Reveillaud *et al*., 2018). In addition to habitat type, water depth (and the associated physical parameters such as pressure, temperature, density) has been proposed to structure the surrounding free-living bacterial populations available for symbiont infection (McMullin *et al*., 2003; Thiel *et al*., 2012; Zimmermann *et al*., 2014). More recent studies have suggested that individual vestimentiferans can host multiple 16S rRNA symbiont phylotypes (Duperron *et al*., 2009; Zimmermann *et al*., 2014; Patra *et al*., 2016) and by using variable genetic markers, such as the symbiont ITS gene, metagenomic studies, and DNA fingerprinting of different vestimentiferan host species, we now know of much greater intra-host diversity beyond the 16S rRNA gene marker level (Di Meo *et al*., 2000; Vrijenhoek *et al*., 2007; Harmer *et al*., 2008; Reveillaud *et al*., 2018; Polzin *et al*., 2019; Breusing *et al*., 2020b). With the discovery of greater intra-host symbiont variation in vestimentiferan tubeworms, the influence of the environment on symbiont composition and diversity at local levels offers an interesting point of comparison to co-occurring *Bathymodiolus* mussels in the current study.

Here, we examined three species of co-occurring *Bathymodiolus* mussels (*B. earlougheri*, *B. nancyschneideri*, and *B. billschneideri*; McCowin et al. 2020) and three species of co-occurring vestimentiferan tubeworms (*Lamellibrachia barhami*, *L. donwalshi*; McCowin and Rouse, 2018, and *Escarpia spicata*) from three distinct deep-sea methane seeps located off the west coast of Costa Rica (1000–1742 m; Fig. 1, Supporting Information Table S1). By examining the symbiont ITS phylotypes of multiple co-occurring host species, the current study is specifically intended to clarify whether host species or the local environment influences the identity of thiotrophic endosymbionts acquired by the mussels and tubeworms. Using a novel high-throughput ITS barcode sequencing approach allowed us to uncover strain-level symbiont diversity and thus insights into factors that influence the varying layers of symbiont specificity in deep-sea chemosynthetic marine invertebrates.

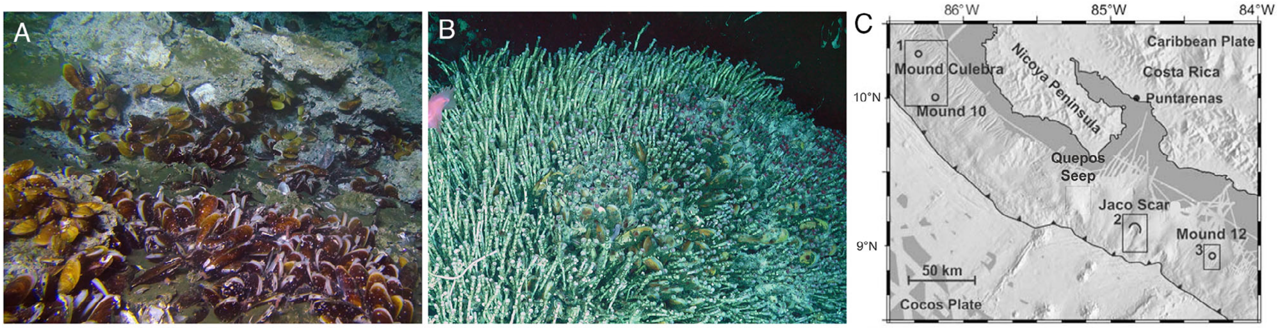

Fig. 1. Specimens, collected from the west coast off of Costa Rica. A. *Bathymodiolus* mussels and B. *Lamellibrachia* and *Escarpia* tubeworms. Photo credits HOV *Alvin*, Woods Hole Oceanographic Institute. C. Bathymetry of Costa Rican margin of sampled cold seep sites, Mound 12 (8° 55.794 N/84° 18.780 W), Jaco Scar (9° 7.071 N/84° 50.445 W), and Quepos Seep (9° 1.904 N/84° 37.294 W). Map reprinted and modified from Mau *et al*. (2007). Three co-occurring mussel species (*B. earlougheri* from Mound 12 and Jaco Scar, *B. billschneideri* from Jaco Scar and Quepos Seep, and *B. nancyschneideri* from Mound 12) and co-occurring vestimentiferans, *Escarpia spicata* and two species of *Lamellibrachia* (*L. barhami* from Jaco Scar and Quepos Seep and *L. donwalshi* from Mound 12 (McCowin and Rouse, 2018) were sampled from three seep sites in 2017 and 2018 ranging from 1000 m-1742 m depth, off the west coast of Costa Rica (Quepos Seep, Mound 12, Jaco Scar).

ITS barcoding

Total genomic DNA was extracted from symbiont-bearing tissues using the Qiagen DNeasy kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. In addition to 16S rRNA sequencing, the ITS region was deemed appropriate for assessing substrain variability in these hosts based on previous studies in which symbionts of *Bathymodiolus* mussels, for example, shared identical 16S rRNA phylotypes, but much more variable ITS regions (Won *et al*., 2003; DeChaine *et al*., 2006). Based on the primer sets of Won *et al*. (2003), a ~400 bp region of the ITS gene was amplified using primers Sym-ITS-1322F (5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTTGGAATCGCTAGTAATCG–3′) and Sym-ITS-356R (5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-ACTGAGCTACAGACCCGAAT-3′), both with Illumina adapters on the 5′ end, specifically following the method described in the study by Goffredi *et al*. (2020), including thermal cycling specifics, barcode attachment, sample purification/quantification, and sequencing, via Laragen (Culver City, CA, USA) on the Illumina MiSeq platform. Data was processed in Quantitative Insights Into Microbial Ecology (v1.8.0), also following Goffredi *et al*. (2020) and is available on the Dryad Digital Repository (reviewer link: https://doi.org/10.5061/dryad.7pvmcvdqt).

Results and discussion

The phylogenetic relationships of co-occurring *Bathymodiolus* mussel and *Escarpia*/*Lamellibrachia* tubeworm symbionts was initially characterized by direct sequencing of the 16S rRNA gene from symbiont-bearing tissues. As expected, the endosymbiont 16S rRNA sequences grouped into two host-specific clades (with 12%–14% divergence), regardless of location, and supported previous observations of distinct 16S rRNA symbiont phylotypes from mussels versus tubeworms (e.g. Distel *et al*., 1988). The symbiont 16S rRNA sequences from *Bathymodiolus* mussels from Quepos Seep, Jaco Scar, and Mound 12 were nearly identical, with only one base pair difference in *B. earlougheri* (from Mound 12 and Jaco Scar; out of 634 bp). With few exceptions, previous studies have similarly observed that neighbouring *Bathymodiolus* mussels share highly similar thiotrophic endosymbiont 16S rRNA ribotypes (e.g. >99.8% similar to those from the Lau Basin hydrothermal vents and Gulf of Mexico; Won *et al*., 2003; Duperron *et al*., 2006; DeChaine *et al*., 2006; Duperron *et al*., 2007; Raggi *et al*., 2013; Fontanez and Cavanaugh, 2014). Likewise, the tubeworms *E. spicata*, *L. barhami*, and *L. donwalshi* from all three sites hosted symbionts with >99.8% 16S rRNA sequence similarity, confirming past results for these specific tubeworm genera (Reveillaud *et al*., 2018), as well as nearly identical thiotrophic endosymbionts from many other vestimentiferan taxa (e.g. those from the Gulf of California, Gulf of Mexico, and Gulf of Guinea; Vrijenhoek *et al*., 2007; Raggi *et al*., 2013; Duperron *et al*., 2014). Variability in natural bacterial populations is often invisible at the 16S rRNA gene level (Jaspers and Overmann, 2004), thus genomic regions with lower selective constraint are useful for assessing microheterogeneity. The bacterial internal transcribed spacer (ITS) region separating the 16S and 23S rRNA genes, for example, is one such region that has been applied to studies on *Bathymodiolus* mussels and vestimentiferan tubeworms (Won *et al*., 2003; DeChaine *et al*., 2006; Stewart and Cavanaugh, 2007; Harmer *et al*., 2008; Reveillaud *et al*., 2018).

Rapid, high-throughput ITS amplicon sequencing revealed that all tubeworms, regardless of sampling location or host species, shared a primary dominant ITS symbiont phylotype (shown in red in Fig. 2B). Previous observations of tubeworms at other hydrothermal vent and seep sites suggest the lack of influence of local sampling sites or host species on symbiont composition in co-occurring vestimentiferan tubeworms (McMullin *et al*., 2003; Zimmermann *et al*., 2014; Reveillaud *et al*., 2018). With regard to their selection of a particular symbiont from the environment, Li *et al*. (2019) have identified a number of toll-like receptors, and other genes (NLRP gene family) in the genome of *Lamellibrachia luymesi* speculated to be involved in the innate immune recognition of infectious pathogens or symbionts. Additionally, comparative genomic studies of the chemoautotrophic symbionts of *Escarpia* and *Lamellibrachia* from the Gulf of Mexico, and *Riftia pachyptila* from the East Pacific Rise, revealed several adhesion-related proteins in the symbiont genomes, including a fibronectin type III domain, so far only found to date in vent-dwelling vestimentiferan symbionts and thought to be involved in initial attachment to the host surface (Gardebrecht *et al*., 2012; Li *et al*., 2018). Vestimentiferan tubeworms are clearly able to distinguish symbiont from infectious pathogen during symbiont acquisition at the larval stage and may also scrutinize their environment for symbionts that encode for adhesion-related proteins, likely beneficial for initial attachment to tubeworm larvae.

For the Costa Rica tubeworms, however, direct sequencing of the ITS region revealed two symbiont ITS phylotypes that shared ∼97% sequence identity; one recovered from *E. spicata* from Jaco Scar and *L. barhami* from both Quepos Seep and Jaco Scar, the two deeper sites (= the ITS phylotype noted above), and a secondary ITS phylotype recovered from *L. donwalshi* and *E. spicata* from Mound 12 (Fig. 2C). This secondary ITS phylotype, based on ITS amplicon sequencing, comprised 7%–14% of the symbiont composition in *E. spicata* and 13%–41% of the endosymbiont composition in *L. donwalshi* (McCowin and Rouse, 2018; shown in green in Fig. 2B). The presence of additional closely related (>99% similarity) ITS phylotypes in *E. spicata* and *L. donwalshi* from Mound 12 were also uncovered, including one most closely related to those recovered from the *Bathymodiolus* mussels (which comprised ∼1% of the recovered ITS amplicons; indicated in purple in Fig. 2B). Other studies, using both 16S rRNA surveys, conserved, yet variable, gene markers (e.g. ITS, RuBisCo, and gyrB), and high-coverage metagenomics, have similarly concluded that multiple phylotypes of very closely-related symbionts can exist within the

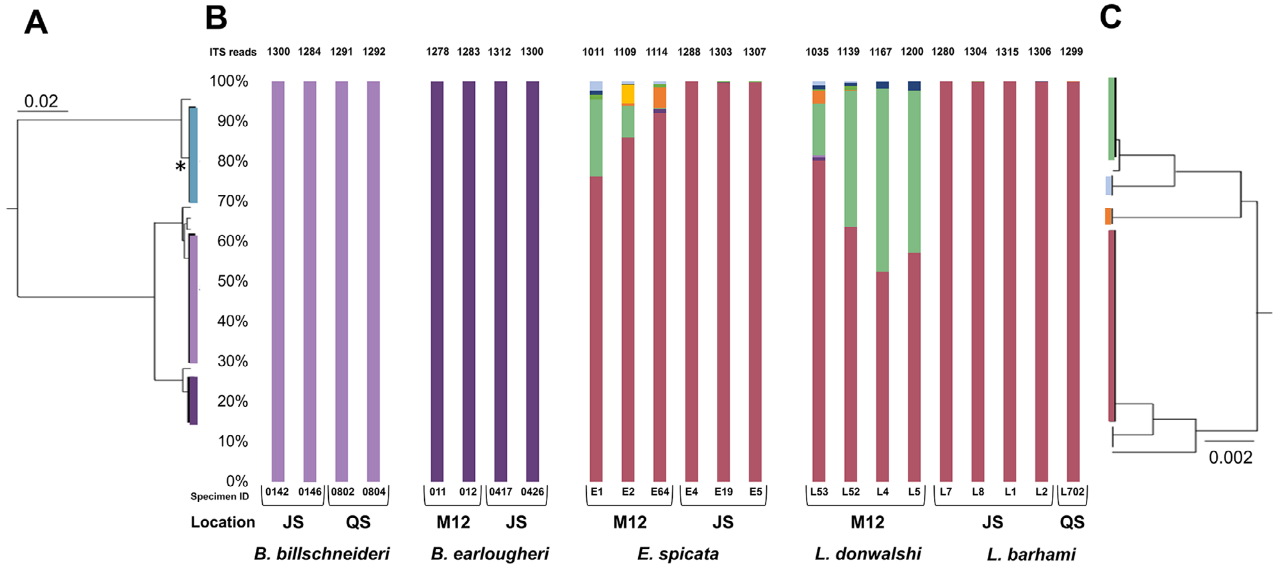

Fig. 2. Endosymbiont composition of mussel and worms, based on internal transcribed spacer region (ITS) direct and amplicon sequencing. A. Relationships among *Bathymodiolus* mussel thiotrophic symbionts, based on direct sequencing of the ITS region (∼700 bp; scale = 0.02). Both 16S rRNA and ITS sequences for every host-symbiont pair are available via GenBank (accession #'s MW158830-MW158839 and MW148226-MW148235, resp. B. Stacked bar graph representing the microbial community of two species of *Bathymodiolus* mussels and two species of vestimentiferan tubeworms using novel ITS barcode-amplicon sequences. Each colour represents a unique bacterial ribotype indicated by the ITS gene tree, based on the ITS barcode. Specimen identification numbers written directly below each individual column. Total ITS reads (normalized) are written above each individual column. *An insert of 60 bp in the *B. nancyschneideri* symbiont ITS gene (represented in blue) makes the product too long to barcode. *B. nancyschneideri* individuals were collected from Mound 12. C. Relationships among *Lamellibrachia* and *Escarpia* tubeworm thiotrophic symbionts, based on direct sequencing of the ITS region (∼700 bp; scale = 0.002). JS = Jaco Scar, QS = Quepos Seep, M12 = Mound 12.

trophosome of a single vent tubeworm (Vrijenhoek *et al*., 2007; Duperron *et al*., 2009; Zimmermann *et al*., 2014; Reveillaud *et al*., 2018; Polzin *et al*., 2019; Breusing *et al*., 2020a). This pattern underscores the importance of using rapid barcode sequencing of more variable genes to expose underlying endosymbiont diversity and fine-scale differences, previously hidden by traditional direct 16S rRNA sequencing approaches.

Although neither host species nor sampling location influenced the dominant symbiont ITS phylotype observed in all tubeworms at all locations, the occurrence of multiple thiotrophic endosymbiont ITS phylotypes in Mound 12 tubeworms raises a number of questions regarding the factors that affect symbiont diversity in vestimentiferan tubeworms. The presence of only a single dominant symbiont in tubeworms from the deeper Jaco Scar (1600–1742 m) and Quepos seeps (1400 m) might be attributed to the constraint of water depth on the availability of thiotrophic symbionts for uptake by larval vestimentiferan tubeworms. Accordingly, the second most dominant ITS phylotype, seen only in Mound 12 tubeworms, may be restricted primarily to shallower depths (e.g. Mound 12 at ~1000 m). Previous studies have shown that at ~1000 m depth, there is a compositional change in free-living microbial groups (Galand *et al*., 2010; Agogué *et al*., 2011). Certain tubeworm symbionts are known to group according to depth (e.g. as little as 350 m vertical distance differences), suggesting this factor as limiting the range of available free-living symbionts (McMullin *et al*., 2003; Thiel *et al*., 2012; Zimmermann *et al*., 2014). Additionally, Fagervold *et al*. (2013) noted that microbial diversity in the western Mediterranean Sea was significantly different at 900 m compared to 1200–1500 m depth. While it remains unclear whether vestimentiferans acquire fully free-living symbionts or those recently released into the environment by nearby decaying tubeworms (Klose *et al*., 2015), Szafranski *et al*. (2015) noted that water depth and the associated physical parameters including pressure, density, and temperature account for roughly 38% of the variance in diversity and composition of free-living, deep-sea bacterial populations. It would be interesting to consider differences in potential geochemistry on the free-living populations of symbionts at the three different sites, however these data, at the scale of individual tubeworm clumps, do not yet exist.

For the co-occurring *Bathymodiolus* mussels from Mound 12, Jaco Scar, and Quepos Seep, ITS sequencing revealed a unique symbiont hosted by each species, regardless of location. DeChaine *et al*. (2006) and Uecker *et al*. (2020) suggested that free-living mussel symbionts are structured by geographic isolation. If the population structure of the thiotrophic symbionts in the Costa Rica mussels was primarily due to geographic location or environmental structuring of the free-living symbiont population; however, all *Bathymodiolus* mussel species inhabiting the same location would be expected to share the same symbiont ITS phylotype. Our findings, in contrast, align with patterns observed for *Idas* mussels by Laming *et al*. (2015), where symbiont composition was primarily dependent on host identity. *Bathymodiolus earlougheri* and *B. billschneideri* symbionts share only 97% ITS sequence identity, while the *B. nancyschneideri* symbiont was even more divergent at 85% similarity (Fig. 2A), based on direct ITS sequencing. Amplicon barcode sequencing of the symbiont ITS gene confirmed the presence of a single distinct ITS phylotype in both *B. billschneideri* and *B. earlougheri* (Fig. 2B); however, this was unavailable for *B. nancyschneideri* due to a 60-bp insert within the ITS gene, rendering the gene product too long for current barcode sequencing technologies. Unlike the tubeworms, the occurrence of a unique symbiont ITS phylotype in each *Bathymodiolus* mussel species across its geographic range suggests that neither water depth nor the numerous bathymetric faults and mounds separating these mussels, influence symbiont composition. Interestingly, recent studies on the life cycle of bathymodiolins have proposed that symbiont acquisition in mussels may occur through the release of symbionts into the environment by proximal mussels, which could contribute to the persistence of faithful symbiotic transfer across generations (Génio *et al*., 2015; Laming *et al*., 2018).

Whether symbiont specificity in deep-sea mussels is initiated and/or enforced by the host or symbiont is not yet known. In a comparative proteome study of thiotrophic symbionts of *B. thermophilus* and *B. azoricus*, which are closely related phylogenetically yet oceans apart, Ponnudurai *et al*. (2020) detected an abundance of symbiont attachment-related proteins involved in surface-binding and cell–cell adhesion in the proteome of the *B. thermophilus* symbiont, yet very few in the *B. azoricus* symbiont. These proteins (e.g. adhesins, invasins, cadherins, and integrins) are potentially related to symbiont colonization and establishment of the host gill tissue (Ponnudurai *et al*., 2020). Other ‘omics studies have demonstrated abundant toxin-related genes (TRGs) in the genomes of *Bathymodiolus* mussel symbionts, which could play a role in selection of a specific symbiont (McFall-Ngai *et al*., 2013; Sayavedra *et al*., 2015; Sayavedra *et al*., 2019). The number and types of these TRGs varied greatly between *Bathymodiolus* host species (Sayavedra *et al*., 2019), suggesting that the symbionts likely enact unique and host-specific selective interactions at the molecular level. Further studies are still needed to understand whether there are underlying differences in recognition of symbionts between *Bathymodiolus* species, which may contribute to the highly specific relationships observed between species-specific thiotrophic endosymbionts and their host *Bathymodiolus* mussels.

Conclusions

Co-occurring *Bathymodiolus* mussels and vestimentiferan tubeworms at methane seeps acquire their nutritional symbionts horizontally (whether through free-living forms in the local environment or from symbionts released by nearby hosts), yet the present molecular analysis using amplicon barcode sequencing of the bacterial ITS gene revealed differing layers of symbiont specificity for each of these foundational deep-sea animals. *Bathymodiolus* mussels exhibited highly specific relationships with their symbionts with no regard for geographic location, while vestimentiferan tubeworm-symbiont relationships lacked this host species fidelity, but rather a single dominant symbiont colonized all three species at all three sites. For the latter, a secondary closely related symbiont appeared in both Mound 12 tubeworm species, suggesting a separate influence of host locale (i.e. water depth). This study raises a number of questions for future investigation. First, do differing expression levels of host protein recognition receptors or symbiont attachment-related proteins or toxins determine specific symbiont recognition in *Bathymodiolus* mussels? Second, does the dominant symbiont of all three tubeworm species in this study encode for vestimentiferan-specific adhesion proteins, which are unaffected by host species or geographic location? As more high-throughput amplicon sequencing studies and metagenomic studies uncover increased symbiont diversity in both *Bathymodiolus* mussels and vestimentiferan tubeworms that were previously undetected at the gene marker level (e.g. Reveillaud *et al*., 2018; Ansorge *et al*., 2019), insight will be gained regarding the impacts of the free-living bacterial population on the eventual endosymbiont population, particularly in vestimentiferan tubeworms. While the current study sought mainly to focus on the influence of host identity and host locale on symbiont specificity, many missing factors that assemble symbiotic relationships at seeps and vents remain. These findings imply that influences on symbiont specificity in deep-sea mussel and tubeworm symbioses can be selective, yet distinct, despite living nearly side-by-side in the methane seep environment. Future molecular studies will surely untangle the complex host–symbiont interactions that drive symbiont-selectivity and specificity in environmentally acquired symbiont partnerships.

Acknowledgements

The authors thank the captains and crew of the R/V *Atlantis*, HOV *Alvin* pilots and technicians, as well as scientific participants of AT37-13 and AT42-03, chief scientist E. Cordes (Temple University), co-principal investigators V. Orphan (California Institute of Technology), G. Rouse and L. Levin (Scripps Institution of Oceanography), and especially J. Cortes Nunez (University of Costa Rica, international collaborator), who played an essential role in acquiring sample collection and export permits through the Costa Rican Ministry of the Environment and Energy (SINAC-CUSBSE- PI-R-032-2018 and Academic Licence SINAC-SE-064-2018). The research expeditions were supported in conjunction with U.S. NSF grants OCE 1635219, OCE 1634172, and OCE 1634002. In addition, Occidental College undergraduate C. Brzechffa was sponsored by the Southern California Academy of Sciences and the Occidental College Undergraduate Research Center.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Supplementary Table 1. Brzechffa and Goffredi.