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Vertical transmission of sponge microbiota is weak and inconsistent

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Abstract

Classic coevolutionary theory predicts that if beneficial microbial symbionts improve host fitness, they should be faithfully transmitted to offspring. More recently, the hologenome theory of evolution predicts resemblance between parent and offspring microbiomes, and high partner fidelity between host species and their vertically transmitted microbes. Here, we test these ideas for the first time in multiple coexisting host species with highly diverse microbiota, leveraging known parent-offspring pairs sampled from eight species of wild marine sponges (*Porifera*). Contrary to the prediction that vertically transmitted microbes represent a completely random subset of the microbes found in the adults, we find indirect evidence that the processes governing vertical transmission are both neutral and selective. Specifically, over 40% of symbiotic microbes which are likely selected or maintained by individual adults occur more frequently than expected in larval offspring. In spite of this, we find that the signature of vertical transmission is detectable but incomplete; that is, larval offspring share only a fraction of their microbes with their parents that are not shared with adults of the same species. Furthermore, while we find that siblings consistently receive a set of identical microbes from their parents, this set of microbes is very small, and most of them are also detected in the environment. Finally, we did not find any evidence for host species fidelity; that

is, conspecific adults and larvae did not share more microbes than hosts from different species. Our study demonstrates that common predictions of vertical transmission that stem from species-poor systems are not necessarily true when scaling up to diverse and complex microbiomes.

Introduction

All animals are colonized by microbes. These microbes live in communities, called microbiomes, that can exhibit astonishing diversity and complexity and have profound effects on host health and fitness [1, 2, 3]. However, despite their importance, we still do not understand how most organisms acquire their microbiomes: are they largely inherited from parents via vertical transmission, or acquired horizontally from the environment? In the last five years, the literature has provided widely divergent answers to this question [4, 5, 6, 7]. A recent meta-analysis of 528 host-microbe symbioses found that 42.8% of symbioses were strictly vertical, 21.2% were strictly horizontal, and 36% exhibited a combination of transmission modes [7]. Understanding how animals acquire their microbiomes, especially microbial symbionts, is necessary to learn how environments shape host phenotypes via host-microbe interactions and whether hosts and their microbiomes represent an important unit of natural selection (i.e., the hologenome theory of evolution) [8, 9, 10, 11, 12].

Classic coevolutionary theory predicts that: (i) if microbial symbionts are beneficial, they should be vertically transmitted (as the host is assured gaining a compatible partner), and (ii) the more a host depends on its microbial partners, the higher the expected incidence of vertical transmission [13, 14, 15, 16, 17, 18, 19, 20]. In support, many obligate insect-microbe interactions, such as those described between *Buchnera*-aphid [21], *Wolbachia*-nematode [22], and *Ishikawaella*-stinkbug [23] are transmitted from parents to offspring. Yet, despite this theory, evidence for symbioses involving horizontal transmission is common—at least in hosts with relatively simple microbiota [6, 19, 24, 25, 26, 27]. Two examples include the facultative symbiosis between the luminescent *Vibrio fischeri* and the bobtail squid *Euprymna scolopes* [28], and the obligate symbiosis between chemolithoautotrophic bacteria and the hydrothermal vent tubeworm *Riftia pachyptila* [29]. Furthermore, Mushegian and colleagues recently demonstrated that, in water fleas (*Daphnia magna*), microbes that are essential to host functioning are acquired from the environment and not maternally derived [30]. However, we currently do not understand if the patterns and processes observed in these relatively species-poor systems can be extrapolated to highly diverse microbiomes. With increasing community complexity, do parents transmit a representative sample of the whole microbial community or select only a subset of the most beneficial microbes? How does vertical transmission interact with other community assembly processes shown to be

important in complex communities, including ecological drift, priority effects, and environmental selection?

The present study is, to our knowledge, the first in-depth analysis of the strength and consistency of vertical transmission in multiple coexisting host species from an animal phylum with diverse and complex microbiomes. By characterizing signatures of vertical transmission in multiple, related host species, we also test, for the first time, partner fidelity between vertically transmitted microbes and their hosts. Partner fidelity is predicted by the hologenome theory of evolution because if vertically transmitted microbes occur in multiple host species, this weakens the coherence of the unit of selection [11]. Here we test these ideas in marine sponges, an evolutionary ancient phylum with a fossil record dating back over 600 million years [31]. Indeed, Porifera are the oldest metazoan group with known microbial symbioses [32]. Marine sponges are filter feeders with a simple body plan consisting of canals embedded in an extracellular matrix called the mesohyl. Within the mesohyl, sponges maintain diverse microbial communities that contribute to host functioning by e.g., cycling nitrogen, fixing carbon dioxide, producing secondary metabolites, and acquiring and converting dissolved organic matter—tasks that, in many cases, the sponge cannot perform without microbial symbionts [32, 33, 34]. Sponge larvae are lecithotrophic which means that they do not receive any external energy sources until they start filter feeding after metamorphosis and settlement [35]. However, some larvae travel long distances, much farther than what is expected from the energy content in their yolk. Evidence suggest that this is because some larvae have the capacity to phagocytose on vertically transmitted symbionts [36, 37].

While the prevailing transmission model in marine sponges is a mixture of horizontal and vertical transmission [38], at least three lines of evidence suggest that vertical transmission plays an important role in the assembly of sponge microbiome. First, sponges appear to have coevolved with a unique set of microbial symbionts that form so-called *sponge-enriched* 16S rRNA gene sequence *clusters* [39, 40]. These *sponge-enriched clusters* span 14 known bacterial and archaeal phyla, many of which are highly specific to the phylum Porifera (e.g., phyla such as Poribacteria, Chloroflexi and PAUC34f) [39, 40]. Unlike any other group of animal associated microbial symbionts described to date, each *sponge-enriched cluster* is monophyletic, indicating that microbes assigning to these clusters have diverged from their free-living relatives [39, 40]. Second, electron micrographs have revealed that sponge oocytes, embryos, and larvae contain free-swimming or vacuole-enclosed endosymbiotic bacteria that are morphologically identical to those found in the mesohyl of the parent [41, 42, 43, 44]. The mechanisms for microbial selection and transference to the oocytes can vary between sponge species [44], as does the density and diversity of microbes that are incorporated into the oocytes [36, 45, 46]. Third, multiple studies, largely based on non-high-throughput sequencing methods, have found similar microbial phylotypes in adults and larvae from the same species [47, 48, 38, 49, 50]. One study also found that three pre-selected bacterial taxa that were present in the embryos of the tropical sponge *Corticium*

sp. persisted throughout development and were consistently detected in adult samples over a period of three years [51]. These lines of evidence altogether strongly suggest that vertical transmission may be a frequent phenomenon that ensures the assembly of a functioning and beneficial microbiota in many species of marine sponges.

Here we test four hypotheses of complex microbiome acquisition by hosts. We use high-throughput sequencing to test for evidence of vertical and horizontal transmission by comparing microbial sharing in known parent-offspring pairs from wild sponges that are generalizable to any host-microbe system. Our approach helps to shed light on the prevalence and role of both vertical and horizontal transmission in an animal phylum with diverse microbiota that has important ramifications for understanding coevolution between hosts and their associated microbiota in general. Firstly, we test whether the processes underlying vertical and horizontal transmission are neutral or selective. If the processes underlying vertical transmission are neutral, microbes that are abundant inside the adults are expected to be widespread among their offspring. Alternatively, if the processes underlying vertical transmission are selective, then microbes found inside larval offspring should occur more frequently than expected, given their abundances in adults. Secondly, we test the hypothesis that sponges exhibit comprehensive vertical transmission, such that microbiota in larval offspring are either a perfect replica or a substantial subset of the microbes found in their adult parents. Alternatively, vertical transmission might be incomplete or undetectable; if incomplete, larval offspring will share only a fraction of their microbes with their parents, but this proportion will be higher than the proportion of microbes they share with other adults of the same species. If vertical transmission is undetectable, then larval offspring will be just as likely to share microbes with other conspecific adults as they are with their parents. Thirdly, we test the consistency of vertical transmission between parents and offspring. We hypothesize that if a specific set of symbionts has coevolved with their sponge host, and if it is adaptive for parents to transmit this set of symbionts, then all offspring from the same parent should receive an identical or highly consistent set of beneficial symbionts. Alternatively, if consistent vertical transmission is not important to parental fitness, or if parents benefit from transmitting different symbionts to each offspring (e.g., if larvae settle in variable environments where only a subset of symbionts is beneficial), then larvae might receive a variable or even random subset of microbes from their parents that is inconsistent between siblings. Finally, we test whether vertically transmitted taxa exhibit partner fidelity. If symbionts have coevolved with a particular sponge species, then conspecific sponge adults and larvae should share more vertically transmitted microbes with each other than with heterospecific hosts.

Results and Discussion

Taxonomic diversity is distributed along a *sponge-specific axis*

To establish parent-offspring relationships for wild sponges, we placed mesh traps around adult sponges living close to the Islas Medas marine reserve in the northwestern Mediterranean Sea. We sampled 24 adults from a total of eight sponge species spanning five orders (Figure S1; Table ??) and collected 63 larval offspring from 21 of these adults (1 to 5 larvae sampled per adult; Table ??). To control for environmental microbes as a potential source pool, we simultaneously collected seawater samples from seven locations within the area where the adult sponges were found (seawater samples were never taken in direct proximity to any sponge specimen).

After quality control, we obtained 11,375,431 16S rRNA gene amplicon reads from these 94 samples (mean=121,015 reads per sample; min=1116, max=668,100 reads), resulting in 12,894 microbial ASVs (Amplicon Sequence Variants). Of these, 9,030 ASVs were present in the 24 sponge adults, 5,786 were found in their 63 larval offspring, and 9,802 ASVs occurred in the seven seawater samples. The 12,894 ASVs were classified to over 30 bacterial phyla and candidate phyla, five of which were only detected in the surrounding seawater. One class of Proteobacteria was unique to sponge adults, and two phyla, *Deferribacteres* and *Fibrobacteres*, were especially enriched in larval offspring, but present in low abundances in the other two environments (Figure 1 A). While several phyla (classes for Proteobacteria) were shared between all three environments (circles close to the center in Figure 1 A), likely representing horizontally acquired ASVs, a large fraction of the observed taxonomic diversity was only shared between sponge adults and larvae, distributed along a *sponge-specific axis* (left-hand side of the ternary plot in Figure 1 A). These included many common sponge-associated phyla, such as *Poribacteria*, *Chloroflexi*, and *PAUC34f*, but also more arcane phyla like *Tectomicrobia* and *SBR1093* (Figure 1 A). Many of the sponge-associated phyla include microbes with known symbiotic features and functional capabilities. For example, members of *Poribacteria* and *Chloroflexi* harbor eukaryote-like protein domains which are suspected to be involved in preventing phagocytosis by the sponge host [52, 53]. Several genomic features in *Chloroflexi* are related to energy and carbon converting pathways, including amino and fatty acid metabolism and respiration, that directly benefit the sponge host [53]. Microbes from *PAUC34f* have the capacity to produce, transport and store polyphosphate granules, likely representing a phosphate reservoir for the sponge host in periods of deprivation [54]. This type of evidence strongly suggests that microbes from these phyla indeed represent beneficial symbionts for sponge hosts.

The ASVs we found also assigned to 105 different *sponge-enriched clusters* from 13 different bacterial phyla, of which Proteobacteria, *Chloroflexi* and *Poribacteria* represented the three most common (*PAUC34f* came in 5th

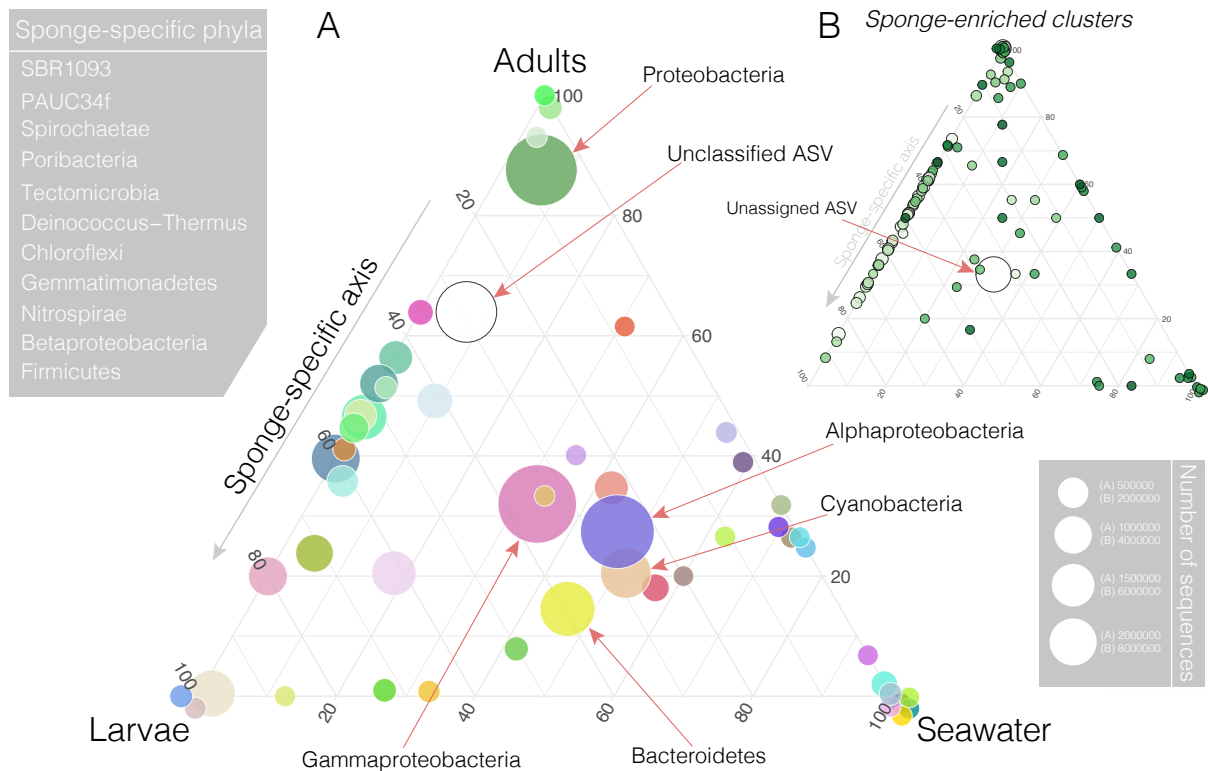


Figure 1: Ternary plots indicating the fraction of microbial ASVs classifying to (A) phyla, and assigning to (B) *sponge-enriched clusters*, present in three environments: seawater (bottom right corner); sponge adults (top corner); and larval offspring (bottom left corner). Figure A shows the distribution of all microbial ASVs at the phylum level (class level for Proteobacteria). Each circle represents a different phylum, and the size of the circle corresponds to the number of reads all ASVs classifying to that particular phylum amounts to. The color legend for (A) is shown in Figure 4. The phyla that lie along the sponge specific axis are listed in the grey table to the left of plot A. Figure B shows the diversity of all ASVs assigning to *sponge-enriched clusters*. Each circle (and shade of green) represents a different *sponge-enriched cluster*, and the size of the circle corresponds to the number of reads assigning to that particular cluster. ASVs that classify to phyla and *sponge-enriched clusters* that are unique to any of the three environments occur in their respective corners (100%); ASVs that classify to phyla and *sponge-enriched clusters* that are shared between any two environments occur along their focal axis. ASVs that classify to phyla and *sponge-enriched clusters* present in all three environments occur in the center of the ternary plots.

place) (Figure 1 B). These *sponge-enriched clusters* accounted for 9.6% of the total ASV richness and 25.5% of the total sequence count across samples. 94 *sponge-enriched clusters* were found in seawater, however, these only accounted for about 5% of ASV richness and 0.23% of sequences from seawater. Out of these 94 *sponge-enriched clusters*, only 4 were not detected in the sponge hosts, supporting the idea that a rare biosphere functions as a seed bank for colonization of sponge hosts [55, 56]. While very few *sponge-enriched clusters* were present in all three environments (circles close to the center in Figure 1 B), 62 were distributed along the sponge-specific axis (with a

relative abundance of <0.01% in the seawater). Sponge larvae do not filter feed prior to settlement and metamorphosis [35]. Concurrently, just one phyla and two *sponge-enriched clusters*, were shared between larvae and seawater only (bottom axis of Figure 1 B), showing that, at least at these higher taxonomic levels, there is a signature of microbial dispersal and subsequent enrichment between adults and larvae.

The processes underlying symbiont acquisition are both neutral and selective

To test whether the processes underlying horizontal and vertical transmission are neutral or selective, we fitted the neutral model developed by [57]. This model predicts the relationship between the occurrence frequency of microbes in individual hosts (here either adults or larvae), and their abundances in a larger metacommunity consisting of microbes found in either (i) adults, including those that are shared with larvae and seawater ($\text{adults} \cap \{\text{larvae}, \text{seawater}\}$; Figure 2 A), or (ii) larvae, including those shared with adults and seawater ($\text{larvae} \cap \{\text{adults}, \text{seawater}\}$; Figure 2 B). Given neutral assembly processes, the model predicts that microbes with high abundances in the metacommunity should be frequently found in individual hosts. Microbes that fall above the neutral prediction occur more frequently than expected, indicating that they are selectively acquired and/or maintained by the sponge host, whereas microbes that fall below the neutral prediction occur less frequently than expected, and may therefore either be selected against or dispersal limited [58].

In support of the hypothesis that neutral processes play an important role in vertical transmission in marine sponges, we found that the neutral model was a better fit to larval than the adult microbiota ($R^2 = 0.27$ in adults vs 0.66 in larvae; Figure 2 C and Figure 2 D). This pattern suggests that the importance of non-neutral processes increase as the sponge host matures, including selective acquisition of symbionts, active curation of the microbiota, and microbe-microbe interactions within the host. In sponges, microbes are actively transmitted and incorporated into the oocytes [44], but these results suggest that the mechanisms underlying this process can be either neutral and/or selective. Indeed, recent evidence from electron micrographs suggests that this is in fact the case; if microbes are collected by amoeboid nurse cells and subsequently engulfed by the oocytes, the process is selective. However, in the absence of nurse cells, microbes are incorporated into the oocytes solely based on their abundance (i.e., neutral processes; Riesgo, personal communication).

If the processes underlying vertical transmission are neutral, then microbes that are abundant across individual adults should be widespread amongst their larval offspring. To test this prediction, we examined whether microbes that fell above, within and below the neutral prediction across individual adults were also found within the same partition across individual larvae (transmission of microbes goes from parent to offspring, not vice versa). We found

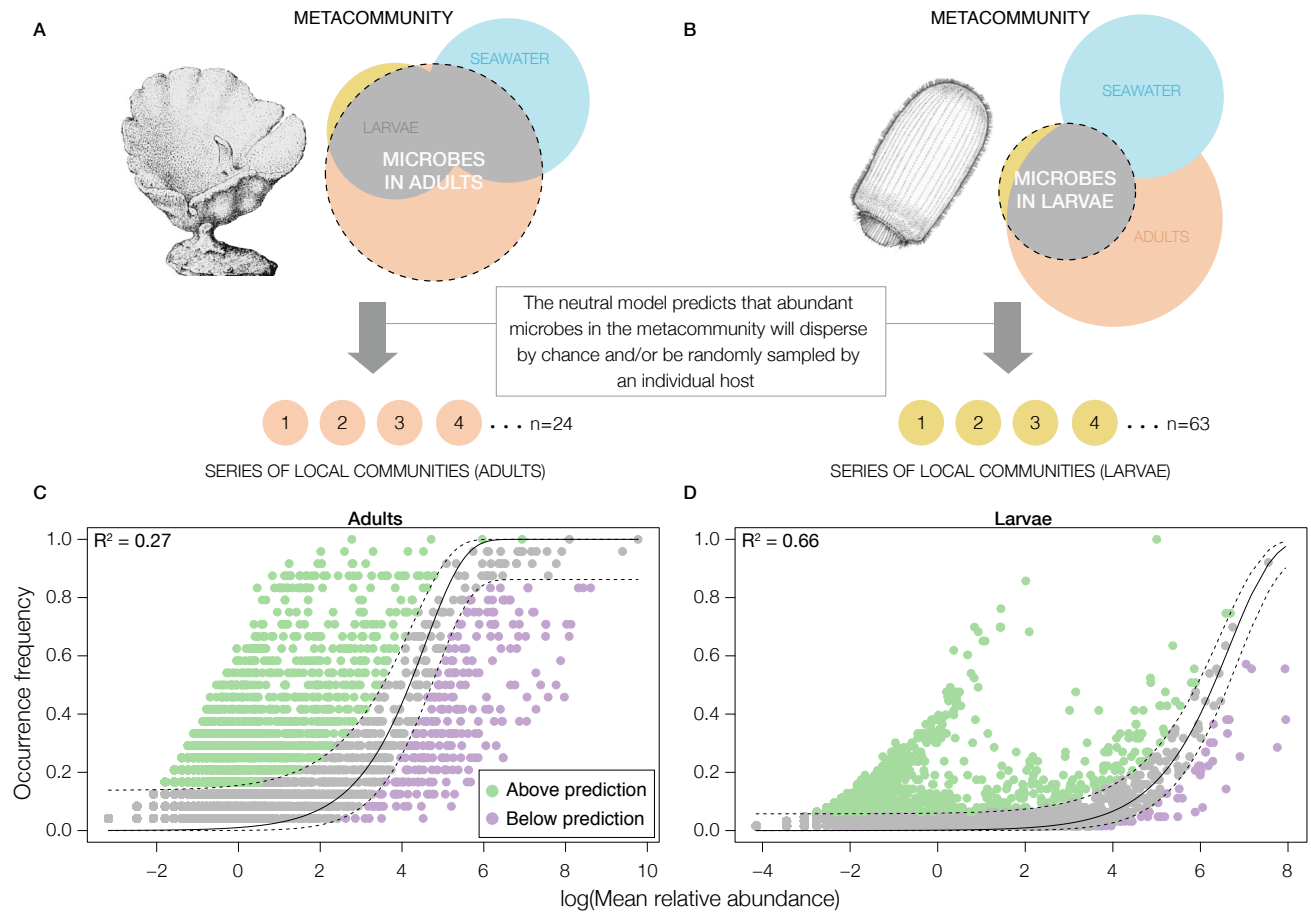


Figure 2: Panels A and B illustrate conceptual diagrams of the constructed metacommunities for (A) adults and (B) larvae. In the Venn diagrams, the microbial community associated to adults, larvae and seawater are depicted by circles colored in peach, yellow, and turquoise, respectively. The focal metacommunity is circled by a dashed black line, and the local host communities are represented as four circles below each Venn diagram, representing either individual adults (peach) or larvae (yellow). The bottom panel shows the fit of the neutral model for adults (C) and larvae (D). ASVs that fit the neutral model are colored gray; ASVs that occur more frequently than predicted by the model are colored green; and ASVs that occur less frequently than predicted are colored purple. Dashed black lines represent the 95% confidence interval around the model prediction (solid black line). The R^2 for the model fit is shown in the upper left-hand corner of each plot. The percentage of microbes that fall above, within, and below the neutral prediction for adults and larvae are 23.7%, 73.4%, 3%, and 20.4%, 78.8%, 0.8%, respectively. This indicates that both neutral and non-neutral processes governs microbial acquisition in marine sponges.

evidence that vertical transmission is govern by both neutral and non-neutral processes. Owing to their filter feeding activities, adults harbor a large number of transient visitors, including food microbes. Congruently, we found that 73.4% of the adult microbiota consisted of neutral ASVs (i.e., gray dots in Figure 2 C). However, larvae only shared 41.7% of these ASVs; 10.7% and 88.5% fell above and within the neutral prediction. While this indicates that larvae

receives less of these neutral ASVs, it also suggests that microbes which are neutral in adults also tend to be neutral across individual larvae. Furthermore, of the 23.7% ASVs that fell above the neutral prediction in adults (i.e., green dots in Figure 2 C), 79% were present in larvae; 42.6% and 56.5% fell above and within the neutral prediction. While this indicates that symbionts which are selectively acquired and/or maintained by individual adults also are frequent across individual larvae, it also suggests that almost 43% are transmitted and incorporated into the oocytes by selective processes. The 10.7% that fell above the neutral prediction in larvae may represent symbionts that were haphazardly filtered by a few individual adults, and subsequently incorporated into the oocytes. Finally, of the 3% ASVs that fell below the neutral prediction in adults (i.e., purple dots Figure 2 C), 86.5% were present in larvae; 54.1% and 43.3% fell above and within the neutral prediction, suggesting that most of microbes that fall below the neutral prediction in adults represent dispersal limited symbionts.

From Figure S2 A, an interesting pattern emerged: a “peak” consisting of microbes above the neutral prediction, which largely disappeared when microbes shared with the seawater were removed (Figure S2 B). Compared to adults, larvae do not filter feed prior to settlement and metamorphosis [35]. This, therefore, suggests that the “peak” consists of (i) a mixture of symbionts and other microbes that the adults acquire from the seawater (which are subsequently incorporated into the oocytes), and/or (ii) environmental microbes that populate the outer surface of the free-swimming larvae prior to settlement. While we can not exclude the latter, it is less likely for four reasons: (1) we rinsed sponge larvae with sterilized filtered seawater prior to DNA extraction; (2) evidence from electron micrographs suggest that microbes are not frequently present on the surface of sponge larvae [36, 59]; (3) most of the ASVs forming the “peak” are also present above the neutral prediction in adults, indicating that they are selectively acquired and/or maintained across individual adults (Figure S3); and (4) several of these ASVs assigned to *sponge-enriched clusters* (Figure S4).

An interesting consequence of the above results is 1st and 2nd generation of vertically transmitted symbionts; that is, microbes which are acquired each generation by the adult and subsequently incorporated into the oocytes (1st generation), and vertically transmitted symbionts which originate from the adult’s parent (i.e., present in the larva from which the adult developed from; 2nd generation). For example, postulate that both symbiont A and B are present in an adult host, and transmitted to its offspring. While symbiont A were acquired from the seawater, symbiont B were vertically transmitted from the adult’s parent. Then, symbiont A and B can be said to represent a 1st and 2nd generation of vertically transmitted symbionts, respectively. However, to fully verify the existence of 1st and 2nd generation vertical transmission, it will be necessary to trace microbial transmission at the microbial strain level through multiple generations of sponge hosts. A strain level analysis rely on identity by common descent and require more extensive genetic data, generated by either shotgun metagenomic sequencing or whole microbial genome

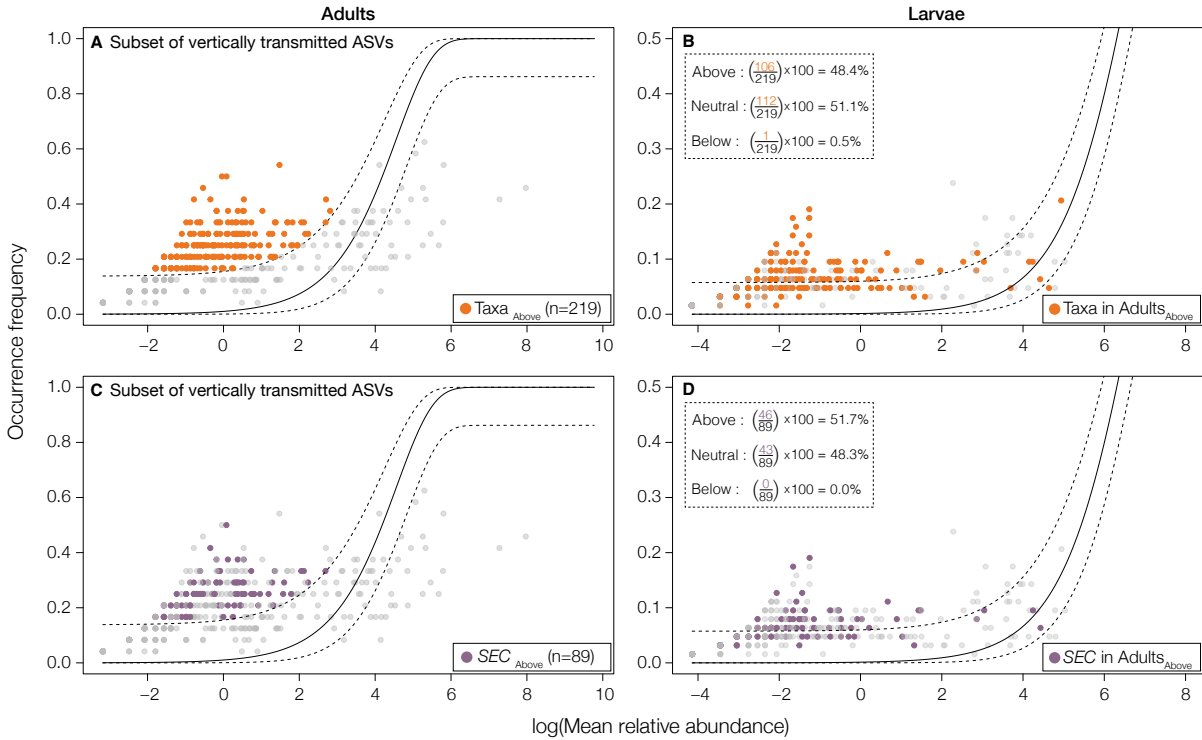


Figure 3: The processes underlying vertical transmission is both neutral and selective. Both the top and bottom panel corresponds to ASVs shared between parents and their offspring, but not detected in seawater. In the top panel (A-B), orange dots correspond to ASVs that are above the neutral prediction in individual adults; 48.4% of these vertically transmitted ASVs are also above the prediction in the larvae. In the bottom panel (C-D), purple dots correspond to *sponge-enriched clusters* that are above the neutral prediction in individual adults; 51.7% of these vertically transmitted *sponge-enriched clusters* are also above the prediction in the larvae. Microbes that fall within or below the neutral prediction are colored in gray.

We next focused on the subset of ASVs in Figure 2 C and Figure 2 D that most closely correspond to 2nd generation vertically transmitted symbionts, i.e., those shared between any given parent-offspring pair but not detected in seawater (note that we only fitted the neutral model once for adults and larvae). We found that 50.0%, 44.1% and 5.9% of the ASVs fell above, within and below the neutral prediction across individual adults, suggesting that at least half of the vertically transmitted ASVs are selected and/or maintained by individual adults. Of these 50%, 48.4% and 51.1% fell above and within the neutral prediction in larvae (Figure 3 A and Figure 3 B). Interestingly, of the ASVs that fell above the neutral prediction in adults, 40.6% assigned *sponge-enriched clusters*, and of these, 51.7% and 48.3% fell above and within the neutral prediction in larvae (Figure 3 C and Figure 3 D), further indicating that adults transmit beneficial symbionts to offspring that may likely be important during microbiome assembly. Of the 44.1% ASVs that

were neutral across individual adults, 22.3% and 77.2% fell above and within the neutral prediction in larvae (Figure 3 A and Figure 3 B), suggesting that offspring also receives microbes which likely serve as an additional energy reserve until larval settlement. Finally, of the 5.9% of ASVs that fell below the prediction across individual adults, 42.3% and 53.8% fell above and within the neutral prediction in larvae (Figure 3 A and Figure 3 B). These percents are altogether very similar to the ones found for the overall microbiota. This indicates that the relative importance of the neutral and non-neutral processes that governs vertical transmission is similar regardless of whether microbes detected in seawater are considered or not. This further indicates that microbes associated to larvae which are also detected in seawater, does not represent environmental microbes populating the surface of the larvae.

In the remaining series of analyses, we introduce one broad (*overall*) and one narrow (*sponge-specific*) definition of vertical transmission. In (1) *overall* vertical transmission, we consider all ASVs that are shared between parents and their offspring, whereas in the definition of (2) *sponge-specific* vertical transmission, we only include ASVs that are shared between parents and offspring, but not detected in seawater. The definition of *sponge-specific* vertical transmission is nested within the definition of *overall* vertical transmission. Specifically, the definition of *sponge-specific* vertical transmission is restricted to symbionts that are not detected (or under detection limit) in seawater, such as members of the rare biosphere, or symbionts which were present in the larval phase of the adult host. Apart from these symbionts, the definition of *overall* vertical transmission also encompasses transient microbes passing through the adult host, and symbionts which are selectively acquired from the seawater. This distinction allowed us to disentangle some of the processes underlying vertical transmission in marine sponges.

Vertical transmission in sponges is detectable but incomplete

We next tested whether patterns of vertical transmission were detectable in sponges, and if so, whether these patterns were comprehensive or incomplete. A visual inspection of taxonomic profiles of the microbiota between parents and offspring indicated that offspring often harbor similar microbial phyla to their parents, as well as to non-parental conspecific adults (Figure 4 A and Figure S5). Part of this similarity at the phylum level persisted when we refocused our analyses at the level of individual ASVs. For instance, across all sponge species, larval offspring shared, on average, 44.8% of their *overall* ASVs with their adult parents (Figure 5 A and Figure S6). Parents and offspring also shared, on average, 60.7% of their *overall sponge-enriched clusters* (Figure S7 Aa and Figure S8). These results suggest that vertical transmission is comprehensive, at least when considering all microbes found in larvae. However, these percents of sharing were not different than the percents of ASVs and *sponge-enriched clusters* larvae shared with conspecific adults living nearby (ASVs: 44.8% vs 44.5%, $\Delta=-0.34$, 95% CI [-4.49,3.87], Mann-Whitney U=3917.5, $P>0.1$, Fig-

A. aerophoba

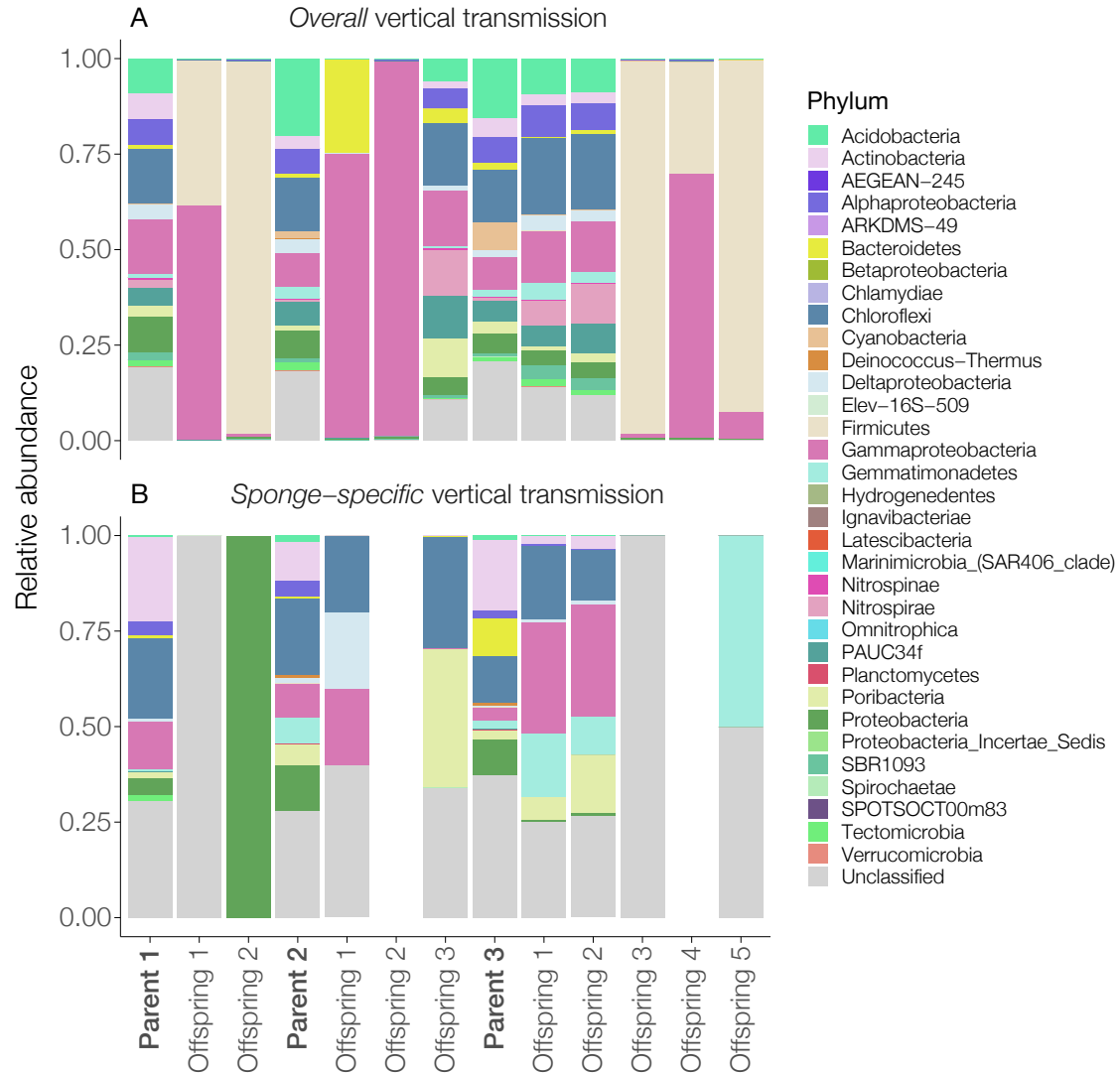


Figure 4: The relative contribution of vertically transmitted ASVs classifying to different microbial phyla (classes for Proteobacteria) in parents and the offspring of sponge species *A. aerophoba*. The top panel (A) shows the relative contribution of phyla for the *overall* definition of vertical transmission, and the bottom panel (B) shows the relative contribution of phyla for *sponge-specific* vertical transmission. Parents (in bold) and offspring are shown on the x-axis. Note that when microbes detected in seawater are removed, this sometimes leaves no vertical transmitted ASVs for the *sponge-specific* vertical transmission. Colors represent different microbial phyla (classes for Proteobacteria).

ure 5 A; *sponge-enriched clusters*: 60.7% vs 61.3% $\Delta=-0.36$, 95% CI [-6.94,5.73], Mann-Whitney U=3916.5, $P>0.1$,
 Figure S7 Aa), indicating that, at the level of all the microbes found in larvae, the signature of vertical transmission is
 essentially undetectable.

However, the analysis above included ASVs found in seawater, which may represent transient microbes passing through the adult hosts that are not consistent or important members of the sponge microbiota. Removing ASVs detected in seawater not only reduced the taxonomic diversity found in larvae, but also decreased the overlap of microbes between adults and their larval offspring. On average, offspring only shared 11.3% and 18.6% of their *sponge-specific* ASVs (Figure 5 Ba and Figure S6) and *sponge-enriched clusters* (Figure S7 Ba and Figure S8) with their parents, indicating that, at the level of microbes found in larvae that are not detected in seawater, vertical transmission is very incomplete. Nevertheless, the detectability of vertical transmission increased as microbes detected in seawater were removed; the percent of sharing between parents and offspring was higher than the percent of ASVs larvae shared with the conspecific adults living nearby (11.3% vs 8.8%, $\Delta=2.23$, 95% CI [0.00,5.00], Mann-Whitney U=4685, P=0.04; Figure 5 Ba). Conspecific larvae and adults shared, on average, 14.12% of their *sponge-specific* *sponge-enriched clusters*, but this percent of sharing was not different than from what offspring shared with their parents (18.6% vs 14.12, $\Delta=0.00$, 95% CI [-0.00,0.00]; Mann-Whitney U=4388, P>0.1; Figure S7 Ba). We observed the same patterns in data where we applied more stringent filtering criteria of sequencing depths. However, with more samples lost, the power to detect differences decreases (see Supplementary material ??).

To further characterize patterns of vertical transmission, we computed modularity (DIRT_LPA_wb_plus, [62]) on bipartite networks constructed for each sponge species. In the ecological network literature, modules are groups of species that “interact” more among themselves than with groups of other species (e.g., flowers and their pollinators, or fruits and their seed dispersers). If modules are perfectly separated; that is, no species interact with species from other modules, they are called compartments. Weighted modularity has been shown to be positively correlated with network specialization (H'_2), reinforcing the idea that modules exist because species only interact with a small number of other coevolved species [63]. Computing modularity on weighted bipartite networks allows for weighting species by their relative abundances, such that rare microbes are down-weighted and modules are formed around the most common host–microbe associations [63, 62]. We computed modularity on two sets of bipartite networks: (1) the *overall* networks which contain conspecific hosts and all ASVs detected in those hosts; (2) the *sponge-specific* networks that contain conspecific hosts and ASVs detected in those hosts, but not in seawater. The networks will be organized into compartments corresponding to parents and offspring if they harbor the same set of microbes at similar abundances, and if those microbes are unique to those parents and offspring. We tested whether the observed modules deviated from the prior expectation of perfectly separated parent-offspring compartments using the Normalized Mutual Information (NMI) criterion [64, 65, 66]. NMI ranges between 0 and 1, where 0 indicates complete dissimilarity between expected and observed modules, and 1 indicates that the observed modules only contain nodes corresponding to parents and

offspring. We found that, while both types of network were modular (*overall*: 0.48 ± 0.17 ; *sponge-specific*: 0.57 ± 0.14 ; Table ??), the observed modules were not comprised of nodes corresponding to parents and offspring (Figure S11). The *sponge-specific* networks had, on average, the highest NMI score but these networks were still quite far from the prior expectation of perfectly separated parent-offspring compartments (*overall*: 0.49 ± 0.08 and *sponge-specific*: 0.36 ± 0.13 ; Figure S12 a). We also computed modularity on unweighted bipartite networks. While these results were quantitatively different (e.g., different modules formed) from the weighted analysis (this has been demonstrated by others, see e.g., [63, 62]), it did not change the overall conclusion (Figure S12 b).

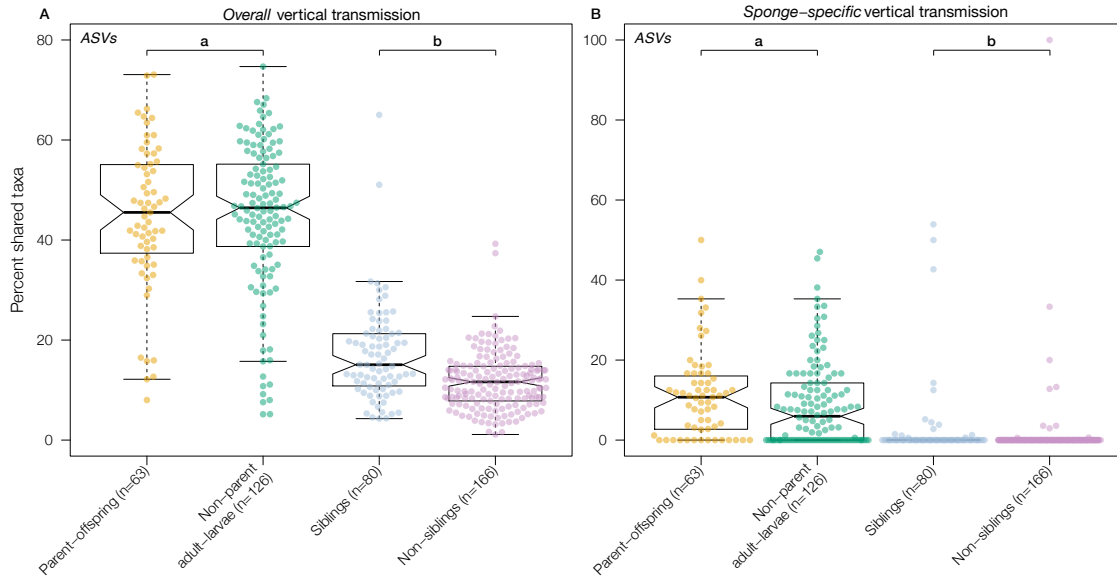


Figure 5: Percent shared ASVs in the (A) *overall* and (B) *sponge-specific* definition of vertical transmission. Boxplots (a) show the percent shared ASVs between sponge larvae and either (i) their known parents (yellow dots), or (ii) non-parental conspecific adults (green dots). In boxplots (a), each dot represents one parent-offspring pair, or one non-parent adult-larva pair across all sponge species (see Figure S6). For *overall* vertical transmission (A), parents and offspring shared, on average, 44.8% of the ASVs, whereas non-parental conspecific adults and larvae shared, on average, 44.5% of the ASVs ($P > 0.1$). For *sponge-specific* vertical transmission (B), parents and offspring shared, on average, 11.3% of the ASVs, whereas non-parental conspecific adults and larvae shared, on average, 8.8% of the ASVs ($P = 0.04$). Boxplots (b) show the percent shared vertically transmitted ASVs between (i) siblings (blue dots), and (ii) non-siblings (purple dots). In boxplots (b), each dot represents one sibling pair, or one pair of non-siblings (see Figure S9). For *overall* vertical transmission (A), siblings shared, on average, 17.0% of their vertically transmitted ASVs, while non-siblings only shared 11.7% ($P < 0.001$). For *sponge-specific* vertical transmission (B), siblings shared, on average, only 2.4% of their vertically transmitted ASVs, whereas non-siblings shared 1.0% ($P = 0.001$). While these are significantly different, the effect size (i.e., the difference in location, Δ), is effectively zero.

Vertical transmission is largely inconsistent; but each offspring receives a small set of identical microbes from their parent

If symbiotic microbes have coevolved with their sponge host, and if it is adaptive for parents to transmit these microbes, then we would expect all offspring from the same parent to receive an identical or highly consistent set of beneficial symbionts. Alternatively, if consistent vertical transmission is not important to parental fitness, or if parents benefit from transmitting different symbionts to each offspring, then we would expect larvae to receive a variable or even random subset of microbes from their parents that is inconsistent between siblings.

We tested this prediction by calculating the proportion of *overall* and *sponge-specific* vertically transmitted ASVs shared between siblings and non-siblings. Across all sponge species, siblings shared, on average, 17.0% and 18.8% of their *overall* vertically transmitted ASVs (Figure 5 Ab and Figure S9 A) and *sponge-enriched clusters* (Figure S7 Ab and Figure S10 A), respectively. These percents of sharing were higher than the percents of vertically transmitted ASVs and *sponge-enriched clusters* non-siblings shared (ASVs: 17.0% vs 11.7%, $\Delta=4.48$, 95% CI [2.68,6.26], Mann-Whitney U=9145, $P<0.001$, Figure 5 Ab; *sponge-enriched clusters*: 18.8% vs 12.4%, $\Delta=4.61$, 95% CI [2.08,7.21], Mann-Whitney U=8531.5, $P<0.001$, Figure S7 Ab), indicating that each offspring receives a small number of identical microbes from their parent. However, when we removed ASVs detected in seawater, siblings and non-siblings only shared 2.4% and 1.0%, and 1.85% and 0.6% of their vertically transmitted ASVs ($\Delta=0.00$, 95% CI [-0.00,0.00], Mann-Whitney U=6383, $P=0.001$, Figure 5 Ab and Figure S9 A) and *sponge-enriched clusters* ($\Delta=0.00$, 95% CI [-0.00,0.00], Mann-Whitney U=6076, $P=0.024$, Figure S7 Ab and Figure S10 A), respectively. Note that while these differences are significant, the effect sizes are practically zero. Overall, the above results indicate that siblings receive a small set of identical symbionts, but that the majority of these microbes originate from the seawater where they have been selectively acquired by the adult parent prior of being transmitted to offspring.

The absence of a large consistent set of microbes transmitted between a given parent and its offspring could have at least three explanations. First, perhaps only a few symbiotic microbes are required to establish a functioning and beneficial microbiota; hence, parents might only “selectively” transmit a few of the most important symbionts to offspring. Second, parents may benefit from varying the microbes transmitted to each offspring. Such variability might be important if offspring disperse long distances and settle in diverse and varying environments. In this case, the identity of the most favorable set of microbes may vary across environments. This explanation is analogous to the idea that a genetically diverse cohort of offspring is more likely to succeed than a genetically uniform cohort (in this case, the genetic diversity is microbial, and not from the host). Third, previous research have suggested that larvae

can phagocytose on vertically transmitted microbes [36, 37]. Thus, to maximize their offspring's chances of survival until settlement, parents may “neutrally” transmit a large number microbes as an additional energy source. All of these explanations are congruent with the finding that the mechanisms underlying vertical transmission are likely both neutral and selective.

Vertically transmitted microbes are not host species-specific

At the time many sponge species reach adulthood, they have converged on highly similar and species-specific microbiota [67, 68], including the eight sponge species analyzed here [69, 70, 67, 71]. Variation in the microbiota among conspecific hosts may reflect the nature and strength of host-microbe interactions. When the microbiota are highly similar among conspecific sponges, this may indicate strong selection for certain symbionts at the host species level. Furthermore, if this selection is a result of strong coevolution between microbes and hosts, then we would expect high levels of host species fidelity; that is, conspecific adults and larvae should share more vertically transmitted microbes than they do with individuals from different host species. While previous studies, largely based on non-high-throughput sequencing methods, have indeed found similar microbial phylotypes in adults and larvae from the same sponge species [47, 48, 38, 49, 50], little is known whether this is also the case when larvae from multiple species are compared.

We tested this prediction by calculating the percent of shared vertically transmitted ASVs among offspring from all possible combinations of adults. We found that larvae were not more likely to share vertically transmitted ASVs or *sponge-enriched clusters* with larvae from their own species as compared to larvae of other species (ASVs: 17.4% vs 15.5%, $\Delta=1.72$, 95% CI [-2.71,6.27], Mann-Whitney U=1928, $P>0.1$; *Sponge-enriched clusters*: 21.3% vs 18.6%, $\Delta=2.81$, 95% CI [-2.93,8.67], Mann-Whitney U=1966.5, $P>0.1$). This was also the case when we considered the relative abundances of the vertically transmitted ASVs in larvae (6.6% vs 6.2%, $\Delta=-0.05$, 95% CI [-1.07,1.04], Mann-Whitney U=1691, $P>0.1$) and *Sponge-enriched clusters* (20.4% vs 15.6%, $\Delta=3.75$, 95% CI [-1.04,15.20], Mann-Whitney U=1973, $P>0.1$). Removing microbes detected in seawater, conspecific larvae shared, on average, only 3.5% and 9.5% of their *sponge-specific* vertically transmitted ASVs and *sponge-enriched clusters*, respectively. These percents of sharing were not different than the percent of vertically transmitted ASVs larvae from different species shared (ASVs: 3.5% vs 2.7%, $\Delta=-0.000$, 95% CI [-0.41,0.00], Mann-Whitney U=1651, $P>0.1$; *sponge-enriched clusters*: 9.5% vs 8.6%, $\Delta=-0.000$, 95% CI [-2.65,3.61], Mann-Whitney U=1696, $P>0.1$). Similar results were also observed when we considered the relative abundance of vertically transmitted ASVs (5.0% vs 1.8%, $\Delta=-0.000$, 95% CI [-0.01,0.00], Mann-Whitney U=1614.5, $P>0.1$) and *Sponge-enriched clusters* (10.1% vs 8.5%, $\Delta=-0.000$, 95% CI

348 [-0.07,0.01], Mann-Whitney U=1591, P>0.1).

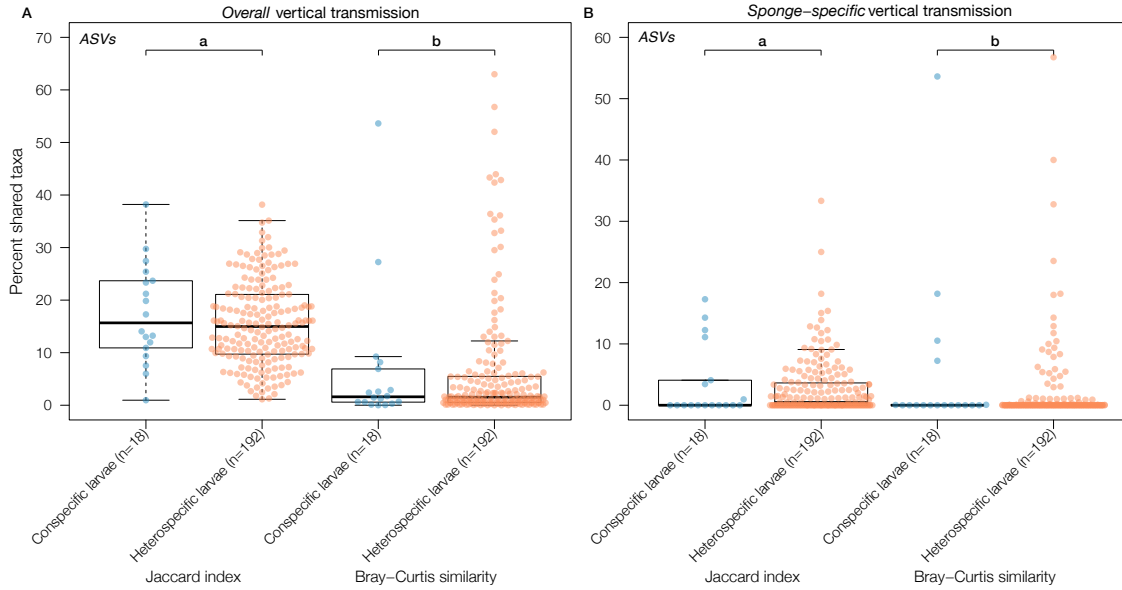


Figure 6: Percent shared (A) *overall* and (B) *sponge-specific* vertically transmitted ASVs among offspring from all possible combinations of adults calculated as either the (a) Jaccard index (see Figure S13 A and Figure S14 A), or (b) Bray-Curtis similarity (see Figure S13 B and Figure S14 B). Each dot represents all offspring from either (i) adults belonging to the same species (blue dots), or (ii) adults from different species (orange dots). While the Jaccard index calculates similarity between two samples based on the presence-absence of taxa, Bray-Curtis similarity also weights taxa by their relative abundance. For *overall* vertical transmission (A), conspecific larvae shared, on average, 17.4% (Jaccard) and 6.6% (Bray-Curtis) of the ASVs, whereas heterospecific larvae shared, on average, 15.5% (Jaccard) and 6.2% (Bray-Curtis) of the ASVs ($P>0.1$). For *sponge-specific* vertical transmission (B), parents and offspring shared, on average, 3.5% (Jaccard) and 5.0% (Bray-Curtis) of the ASVs, whereas non-parental conspecific adults and larvae shared, on average, 2.7% (Jaccard) and 1.8% (Bray-Curtis) of the ASVs ($P>0.1$). Note that the group *conspecific larvae* has much lesser number of observations (n) compared to the group *heterospecific larvae*.

349 To test this beyond pairwise comparisons, we computed weighted modularity on two bipartite networks: (i) the
 350 *overall* network which contains all hosts and ASVs detected in those hosts, and (ii) the *sponge-specific* network that
 351 contains all hosts and ASVs detected in those hosts, but not in seawater. If conspecific adults and larvae harbor the
 352 same microbes at similar abundances, and do not share those with other species, then the networks will be organized
 353 in compartments consisting of conspecific adults and larvae. While we found that both the *overall* and *sponge-specific*
 354 network were highly modular ($Q=0.71$ and $Q=0.79$), modules rarely consisted of adults and larvae from the same
 355 species ($NMI=0.51$ and $NMI=0.41$). For instance, in the *overall* network, apart from the two species *A. aerophoba*
 356 and *I. oros* that together formed one module, all other adults formed their own species-specific modules. While some
 357 modules containing adults also contained larvae, they rarely corresponded to offspring or even larvae of the same

species. Similarly, in the *sponge-specific* network, only adults from the species *I. fasciculata* and *I. oros* formed their own species-specific modules; instead modules consisted of either (1) adults and larvae from different species; (2) a mix of heterospecific larvae, (3) or single larva. We also computed unweighted modularity on the same bipartite networks. While these results were quantitatively different from the weighted analysis, it did not change the overall conclusion (table).

Conclusion

Vertical transmission is proposed to be a primary mechanism by which parents transmit assemblages of beneficial microbes to offspring in a way that maintains both these microbes' interactions with each other and the beneficial functions that emerge from their interactions [17]. However, contrary to these theoretical expectations, evidence is mounting that this view of vertical transmission is rare in animal microbiomes—especially when microbiomes are highly diverse (see [72] for a review). We find that marine sponges also do not fit the classic mold. While previous research based on electron micrographs has detected mechanisms by which parents transmit microbes to offspring [43, 44], our results support the findings that these processes can be neutral and/or selective, and that this may help to explain why several of our findings cast doubt on the consistency and faithfulness of these transmissions. Specifically, across eight sponge species, we show that: (1) vertical transmission is detectable, but weak and incomplete such that offspring do not receive a replica of their parent's microbiome; (2) parents do not transmit the same suite of microbes to each offspring; and (3) vertically transmitted microbes are not host species-specific and therefore unlikely to have coevolved with particular sponge species.

Our findings highlight the need for new theory to explain how hosts ensure the faithful transmission of beneficial microbiomes (see e.g., [73]). While the classic model may sometimes work well when the microbial symbionts consist of just one or a few species [6, 26], when microbiomes are very diverse and complex, transferring thousands of microbial species such that their interaction structures and emergent functions are preserved seems highly improbable. So, how do sponge parents ensure that offspring get the microbes they need? We know that such mechanisms exist because by the time sponge juveniles reach adulthood, they have converged on highly similar and species-specific microbiomes [67, 68]. The application of the neutral model did not only highlight the potential importance of neutral processes, but deviations from the model's prediction lead to a better understanding of the potential role of both horizontal and vertical transmission in shaping the sponge microbiome. Assuming that offspring do not acquire microbes independently from their parent [74], our results suggest that the “selective process” occurs when adults horizontally acquire

symbionts from the seawater. However, adults filter vast quantities of water, up to 24,000 liters (24 m^3) of water per kilogram and day [75]. Thus, at any time, adults harbor a large number of transient visitors. However, once these microbes are inside the host, the innate immune defenses of some sponge species can differentiate between pathogens, food bacteria and symbionts in a manner similar to the adaptive immune system of vertebrates [76, 77, 78, 79, 80, 81]. In addition, for some microbes the host niche also provides a more favorable environment than seawater; in turn, some symbionts have molecular structures that facilitate recognition by the sponge host [52, 82]. We found indirect evidence that the mechanisms by which microbes are incorporated into the oocytes are both neutral and selective. The direct consequences of this is first and second generation vertical transmission; that is, microbes that are acquired each generation by the adult and subsequently incorporated into the oocytes (first generation), and vertically transmitted symbionts which originate from the adult's parent (second generation). Such mixture of microbes may have at least three advantages. First, as dispersing larvae may not settle for several days [83], it may provide larvae with an additional nutritional boost until settlement. Second, evidence from other ecological communities, including the human gut microbiome, suggests that priority effects strongly influence community assembly [84, 85, 86, 87]. Therefore, a few symbionts may quickly reach carrying capacity while simultaneously modifying the initial host niche in their favor, thereby altering the ability of subsequent microbial immigrants to colonize. Hence, vertical transmission of a few beneficial symbionts may, via priority effects, help build the microbiome anew generation after generation. Third, it may provide a mechanisms by which adults transmit new “local” symbioses to offspring.

Finally, some of our results are relevant to the predictions put forward by of the hologenome theory of evolution [8, 9, 12]. This theory proposes that there may be value in treating hosts and their microbiota as a single evolutionary unit. This comes with an important expectation: high partner fidelity—if the collection of genomes varies within and between host generations, then it is not a coherent unit of selection [10, 11]. Such tight partner fidelity is typically only found among host-microbe symbioses with obligate vertical transmission. On the contrary, we found that many vertically transmitted microbes, including many *sponge-enriched clusters*, were not faithfully transmitted by parents to offspring nor were they host species-specific. As such, their evolution is likely shaped by multiple host species across the phylum Porifera, as well as by the marine environment where the sponge hosts live. Overall, our study demonstrates that common predictions of vertical transmission that stem from species-poor systems are not necessarily true when scaling up to diverse and complex microbiomes.

Methods

We collected sponge and seawater samples between July and August 2012, close to the Islas Medas marine reserve in the northwestern Mediterranean Sea $42^{\circ}3'0''N$, $3^{\circ}13'0''E$ by SCUBA at depths between 5-15 m. The analyzed species are common Mediterranean sponges and were identified based on their distinct morphological features. The sampling site consisted of a relatively small bay (roughly 18,000 m²). All sampled sponge species live in rocky overlapping habitats, and all species could be found within the same depth range. However, some specimens were found in more shaded areas than others.

Larval sponge collection

We constructed larvae traps by modifying the traps used in [88] (Figure S18). In order to collect offspring from known parents, traps were mounted over individual adult sponges by SCUBA. To minimize stress to individual adults, traps were removed after one week. During this time, sample bottles were collected and replaced each day. Bottles were placed on ice in insulated coolers and transported to the laboratory (<2 hours). Larvae were identified using a stereolupe. In order to remove loosely associated microbes, larvae were carefully rinsed with filter-sterilized seawater (0.20 μ m filter) before preservation in RNA later. All larval samples were stored at -80°C until DNA extraction.

Adult sponge collection

After larvae offspring were collected, three adults per sponge species were sampled. These individuals corresponded to the same adults that larvae had been collected for. However, for a few species, larvae could only be collected for two adults. In these cases, a third adult was still sampled. Specimens were sub-lethally sampled by removing a small sample of tissue. Excised tissue was placed in separate plastic tubes and brought to the surface where they were preserved in RNA later and placed on ice in insulated coolers and transported to the laboratory (<2 hours). Seawater samples were collected at 5 m depth and at seven locations within the sampling area. The water was always collected at deeper locations (>5m) within the sampling area, and never in direct proximity to the benthic community. All seven water samples were poured into separate, sterile 5 L jars. Aliquots of seawater (300-500 mL each, 1 aliquot per sample jar) were concentrated on 0.2 μ m polycarbonate filters, and submerged in lysis buffer. All samples were stored at -80°C until DNA extraction.

DNA extraction and sequencing

DNA was extracted from ≈ 0.25 g of adult sponge tissue using the PowerSoil DNA extraction kit (MoBio). DNA from larvae (one larva per adult) was extracted using the XS-RNA extraction kit (Macherey-Nagel) because of its capacity to extract DNA from small samples, i.e., one larva. All DNA extractions were performed according to standard protocols. The seven seawater samples were processed by passing 2 L (from the 5 L) of seawater through $0.2\mu\text{m}$ Sterivex filters, and DNA was extracted from these filters as described by [50]. All extractions included a negative control without sponge tissue, and the lack of amplified DNA was examined with the universal bacterial primers 27F and 1492R. The V4 region of the 16S rRNA gene was amplified using the primer set 515FB-806RB [89], and sequenced using the Illumina HiSeq2500 platform. Sequencing was performed by the Earth Microbiome Project [90].

Sequencing analysis

Illumina-sequenced, single-read fastq files were processed and cleaned in R [91] using the default settings in DADA2 [92] to produce an amplicon sequence variant (ASV) table (Appendix ??), and SILVA (v128) [93] was used to create the ASV taxonomy. The Phyloseq R package [94] was used to filter out sequences classifying to *Archaea* and *Eukaryota*. We also removed singleton ASVs, and phyla that occurred in less than two samples (Appendix ??). The analyzed dataset contained samples with at least 1,000 sequences.

Identification of *sponge-enriched clusters*

A representative sequence from each ASV was taxonomically assigned using a BLAST 62 search against a curated ARB-SILVA database containing 178 previously identified *sponge-specific clusters* [40]. For each BLAST search, the 10 best hits were aligned to determine sequence similarities. The most similar ASV sequence to the respective reference sequence within the database was then assigned to an *sponge-specific clusters* based on a 75% similarity threshold: (i) a sequence was only assigned to any given *sponge-specific clusters* if its similarity was higher to the members of the cluster than to sequences outside the cluster; and (ii) if its similarity to the most similar sequence within the cluster was above 75%. A majority rule was applied in cases where the assignment of the most similar sequences was inconsistent, and the ASV sequence was only assigned to the *sponge-specific clusters* if at least 60% of the reference sequences were affiliated with the cluster.

Data analyses

To partition data into the different bipartite networks and to find vertically transmitted microbes, we used set theory (e.g., `setdiff(x, y)` and `intersect(x, y)` functions in R). Modularity was analyzed using the `DIRT_LPA_wb_plus` algorithm in R [62]. We further used Normalized Mutual Information (NMI) criterion, calculated through the `NMI : :NMI(x, y)` function in R, to test whether observed modules deviated from prior expectations [64, 66]. In the few cases where statistical analyses were performed, we used estimation statistics to directly report and visualize effect sizes (point estimates) and their confidence intervals (precision estimates) [95]. More specifically, we used DABEST (“Data Analysis with Bootstrap-coupled ESTimation”) to compute the 95% confidence interval of the mean difference by bootstrap resampling from the observed data, and to visualize the result in a Gardner-Altman comparison plot [96]. This allows for an intuitive and transparent way to compute and visualize the difference between two or more groups by focusing on the magnitude of the effect and its precision. Groups are deemed significantly different if the 95% confidence interval of the mean difference excludes zero; that is, the probability of the mean difference being significantly different from zero exceeds 95%. This was done using the DABEST Python package in R via the `reticulate` package. Lastly, we used the logit transformation as a variance-stabilizing transformation of proportions. The logit transformation is the log of the odds ratio; that is, the log of the proportion divided by one minus the proportion. In practice, the transformation expands the ends of the scale, such that small differences in the proportions have a larger difference on the logit scale.

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Conflict of interest

The authors declare that they have no conflict of interest.

Authors' contributions

J.R.B. and J.M.M. conceived the study. J.R.B. performed the fieldwork and analyzed the data. J.R.B. and J.M.M. drafted the first versions of the manuscript, and J.R.B. and E.A. refined the ideas and wrote the final version of the paper. C.A.G. identified the *sponge-specific clusters*. All authors commented and approved of later versions of the paper.

Data and code availability

All data and code will be available on Open Science Framework with an R Markdown document such that all analyses and figures can be reproduced.

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1 Supplementary material

Vertical transmission of sponge microbiota is weak and inconsistent

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This file includes:

Supplementary figures 1-13

Supplementary tables 1-5

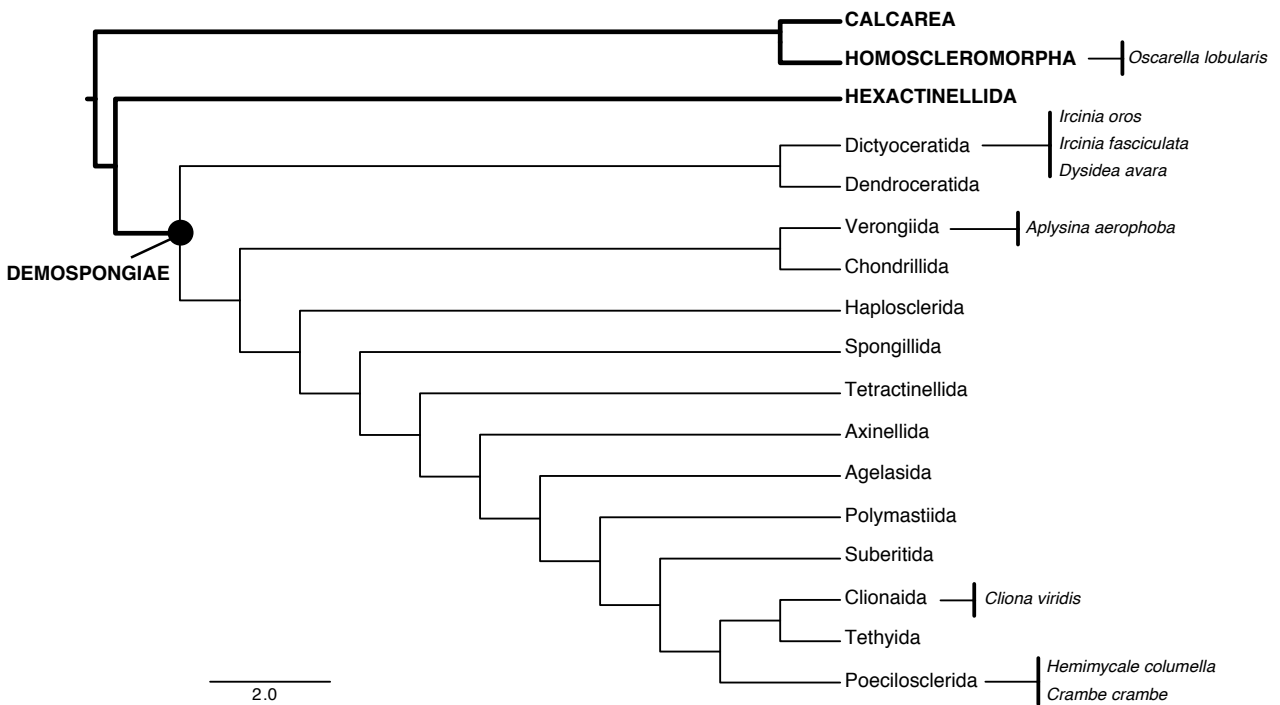


Figure S1: The phylogenetic relationship between the analyzed sponge species. The thin branches display orders within the class Demospongiae which contain over 75% of all sponges species worldwide. The eight analyzed sponge species span two classes (Homoscleromorpha and Demospongiae) and five orders (Homosclerophorida, Dictyoceratida, Verongiida, Clionaida and Poecilosclerida). All of the eight sponge species have the core of their known species range distribution within the Mediterranean Sea (which includes the Western Mediterranean Sea, the Adriatic Sea, the Ionian Sea, the Aegean Sea, and the Levantine Sea). More specifically, *Oscarella lobularis* has its known distribution in the Mediterranean Sea, part of the North Sea (the Swedish west coast), and part of the South Atlantic Ocean (the Azores, the Canary Islands, and Cape Verde); *Ircinia oros* has its known distribution in the Mediterranean Sea, and part of the south Atlantic Ocean (the Canary Islands); *Ircinia fasciculata* has its known distribution in the Mediterranean Sea; *Dysidea avara* has its known distribution in the Mediterranean Sea, the Black Sea, and part of the South Atlantic Ocean (the French coast); *Aplysina aerophoba* has its known distribution is in the Mediterranean Sea, and part of the South Atlantic Ocean (the Azores, the Canary Islands, and Cape Verde); *Cliona viridis* has its known distribution in the Mediterranean Sea, part of the South Atlantic Ocean (the Spanish and Portuguese coast, the Azores, the Canary Islands, and Cape Verde); *Hemimycale columella* has its known distribution in the Mediterranean Sea, part of the South Atlantic Ocean (the Spanish and Portuguese coast, the Azores, the Canary Islands, and Cape Verde), part of the North Sea (the Swedish west coast), and the Celtic Sea, including the English Channel; and finally, *Crambe crambe* has its known distribution in the Mediterranean Sea, and part of the South Atlantic Ocean (the Spanish and Portuguese coast). Source for the species range distribution: World Porifera Database.

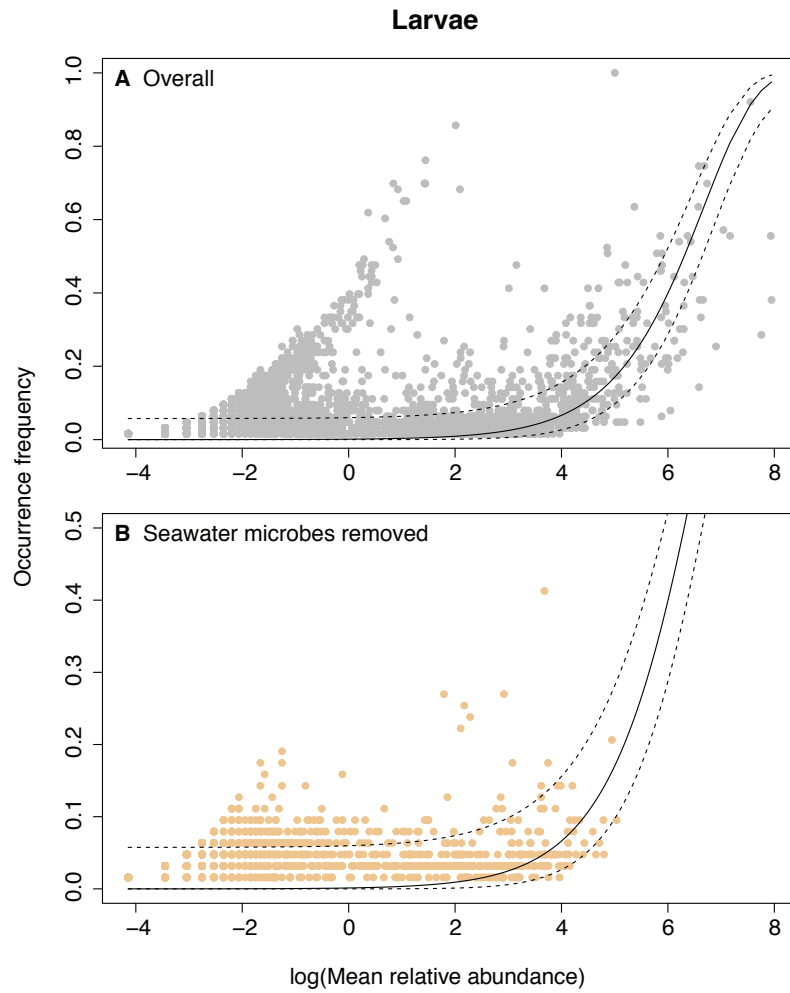


Figure S2: A “peak” consisting of taxa above the neutral prediction across individual larvae. In panel A, gray dots correspond to the overall larval microbiota. In the bottom panel B, yellow dots correspond to microbes not detected in seawater. When microbes detected in seawater are removed, much of the “peak” largely disappears.

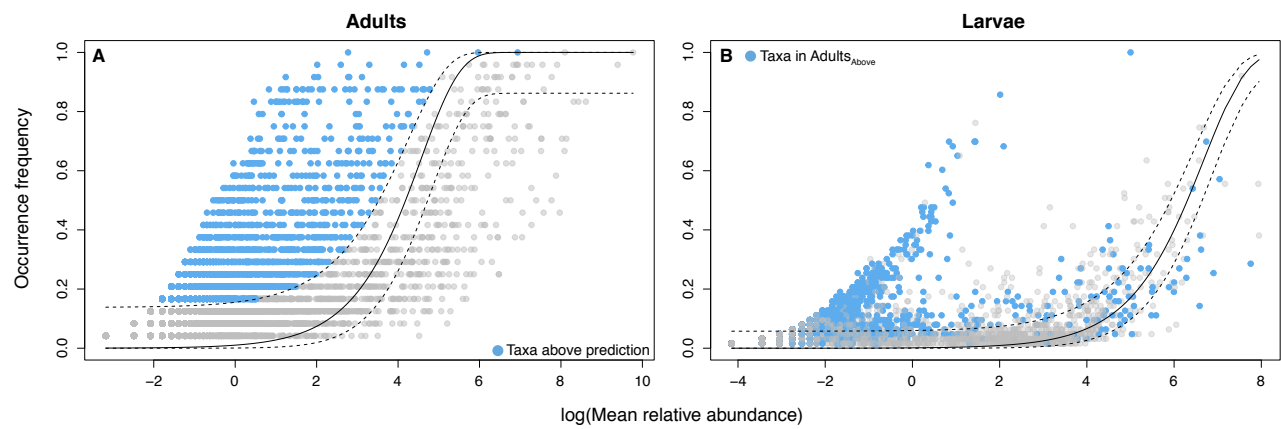


Figure S3: Most of the microbes forming the “peak” in the larval (B) are also present above the neutral prediction in adults (A). In panel A, microbes that fall above the neutral prediction in adults are colored blue. In panel B, the same microbes that fall above the neutral prediction in adults that are present in larvae are colored blue.

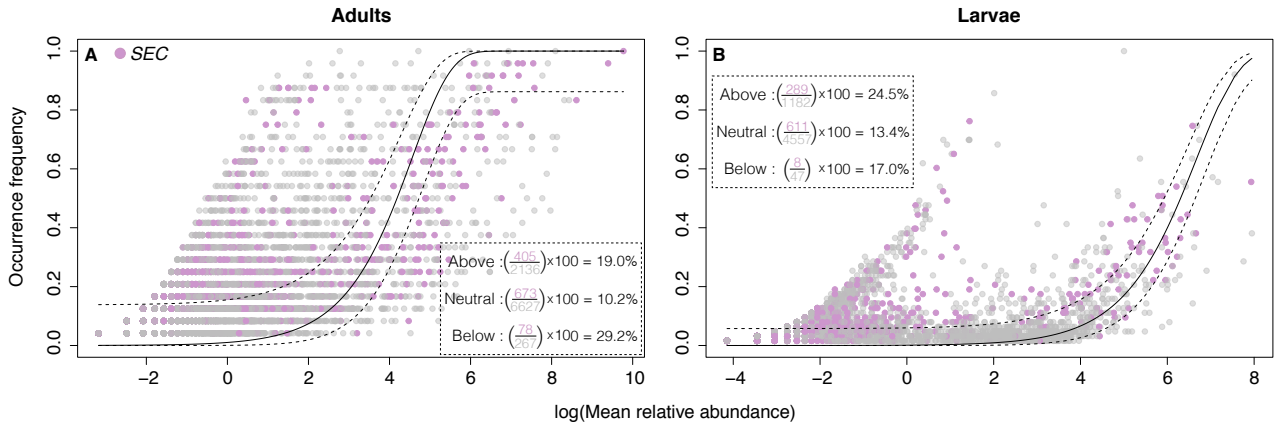
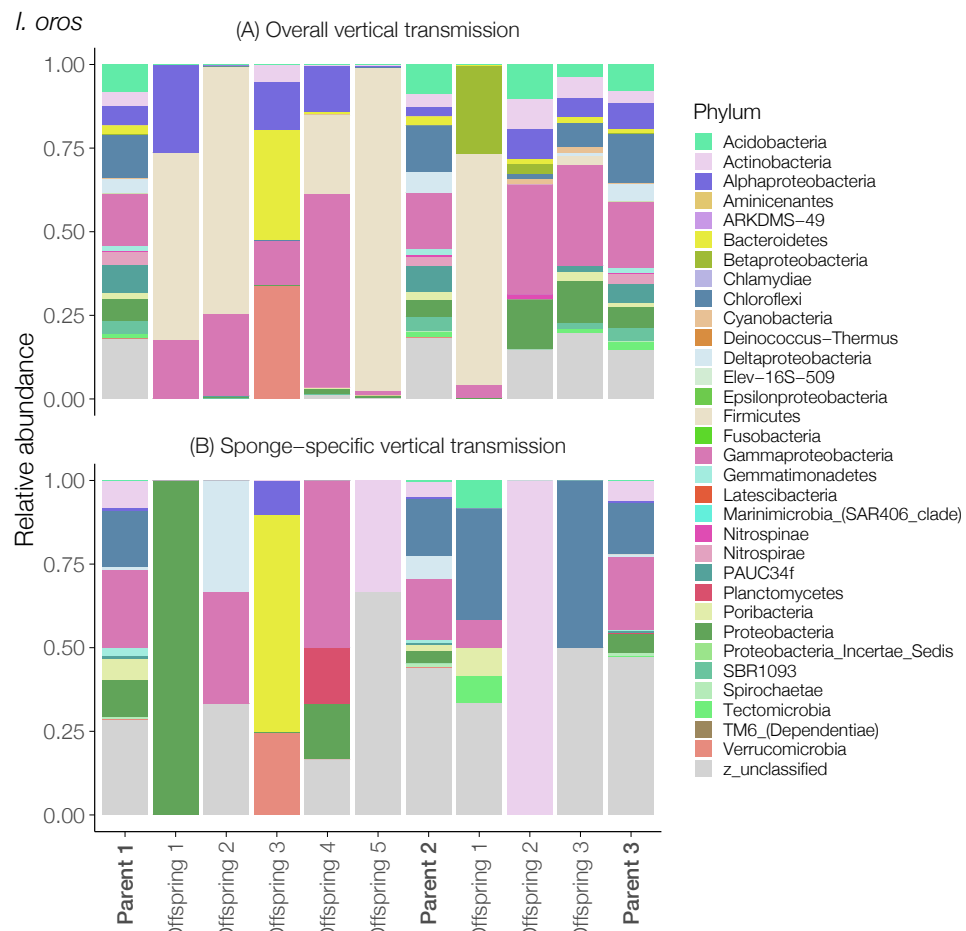


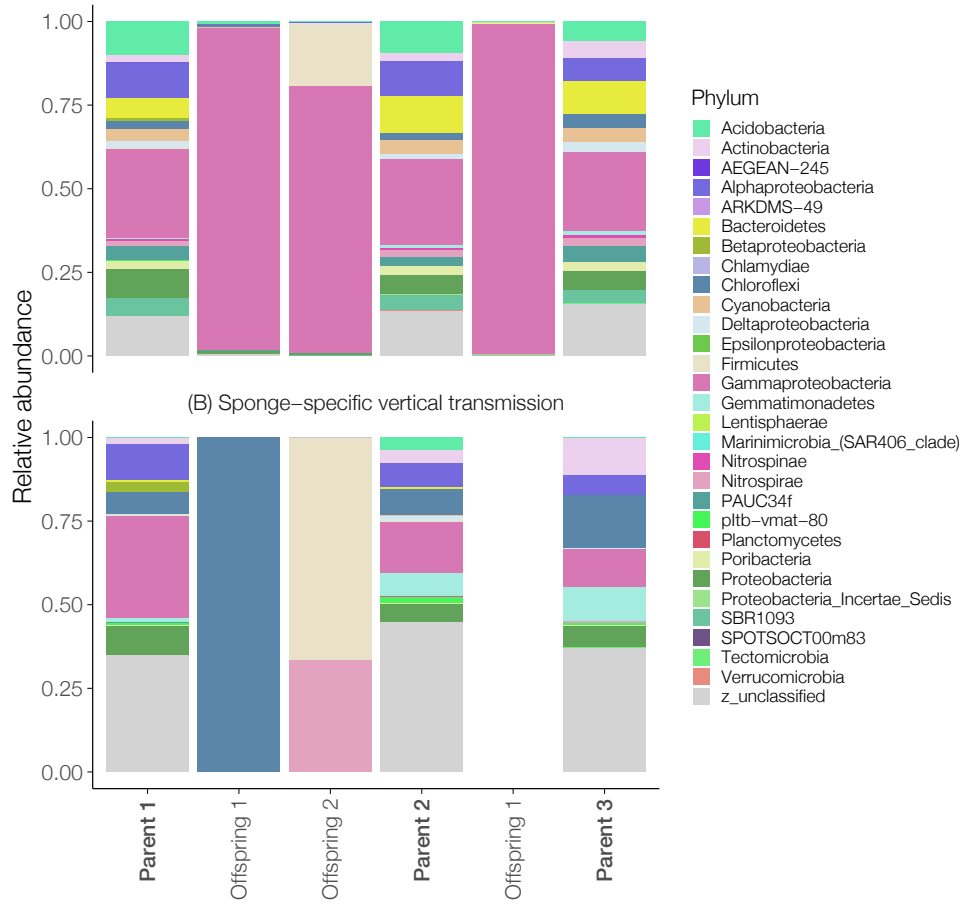
Figure S4: The distribution of *sponge-enriched clusters* (pink dots) above, within and below the neutral prediction for the (A) adults and (B) larvae. Gray dots correspond to ASVs that does not assign to *sponge-enriched clusters*. For each partition (i.e., above, within, and below), the percentage of *sponge-enriched clusters* has been calculated.



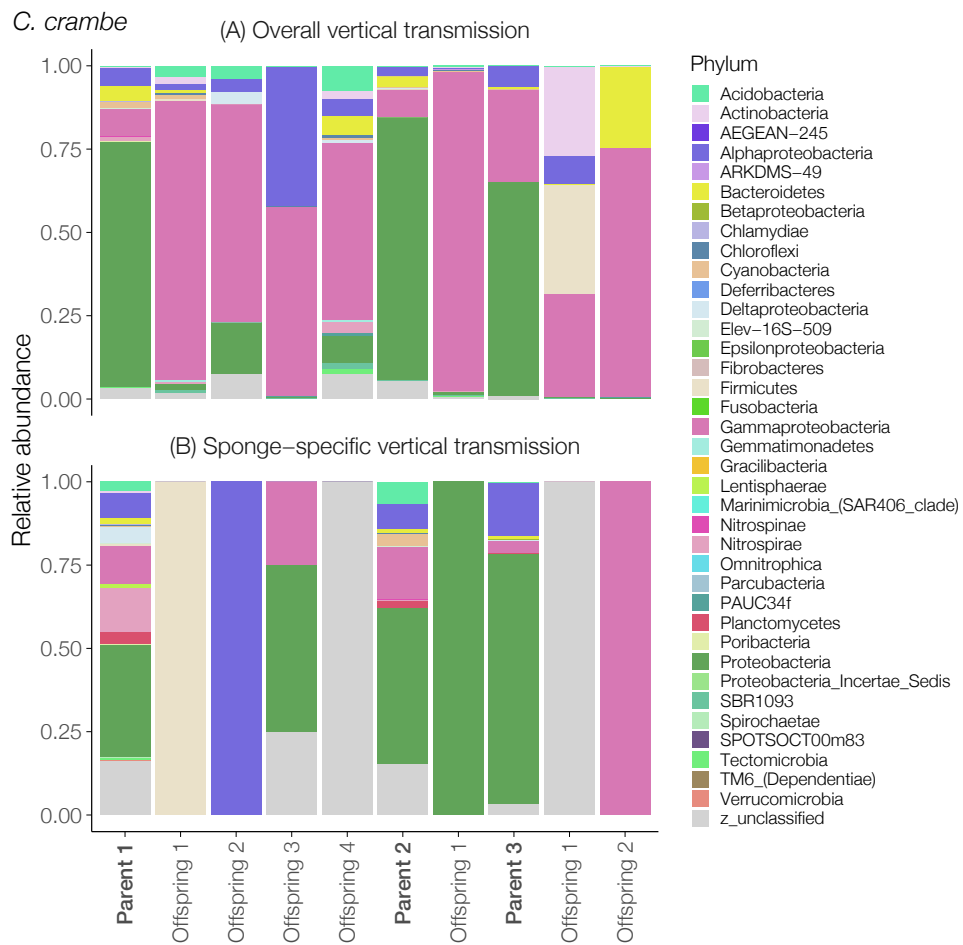
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I. fasciculata

(A) Overall vertical transmission



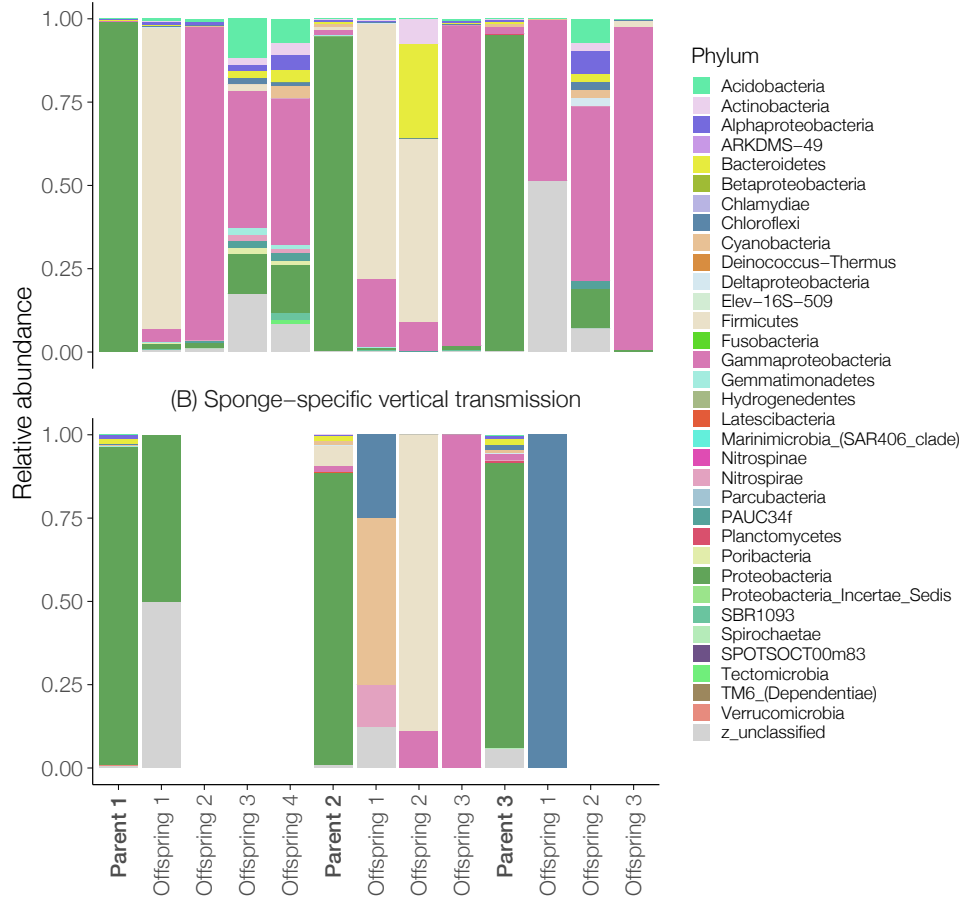
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C. viridis

(A) Overall vertical transmission

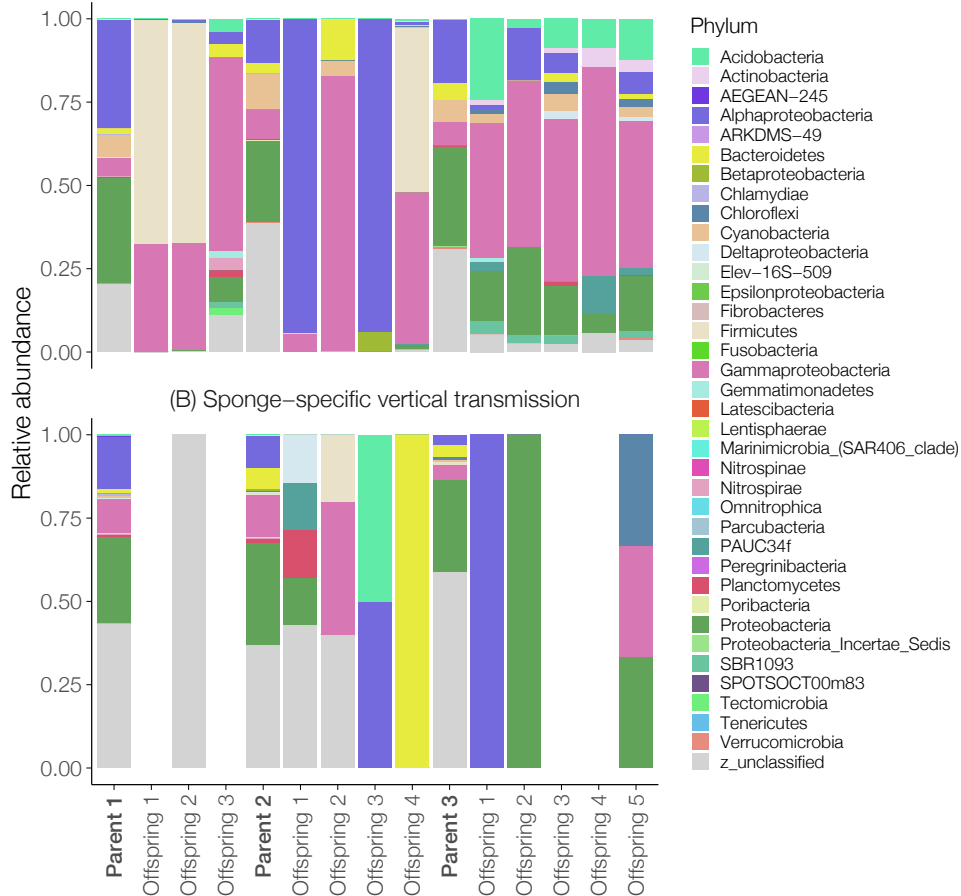


(B) Sponge-specific vertical transmission

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D. avara

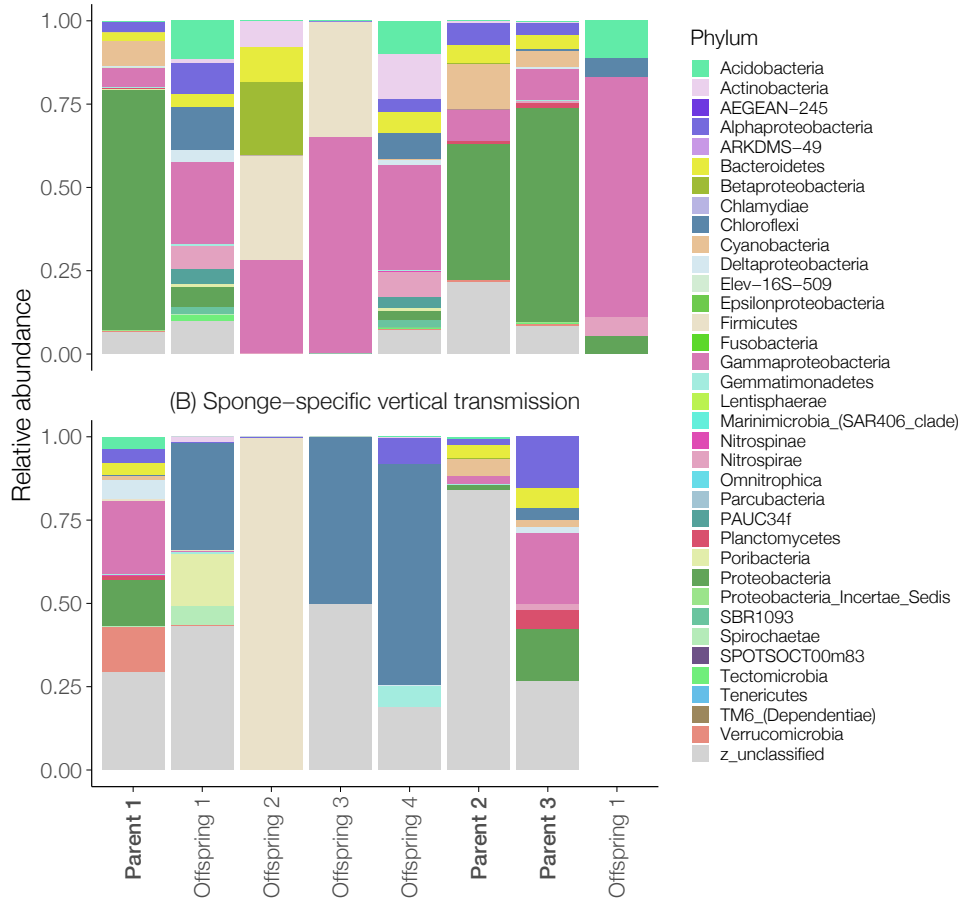
(A) Overall vertical transmission



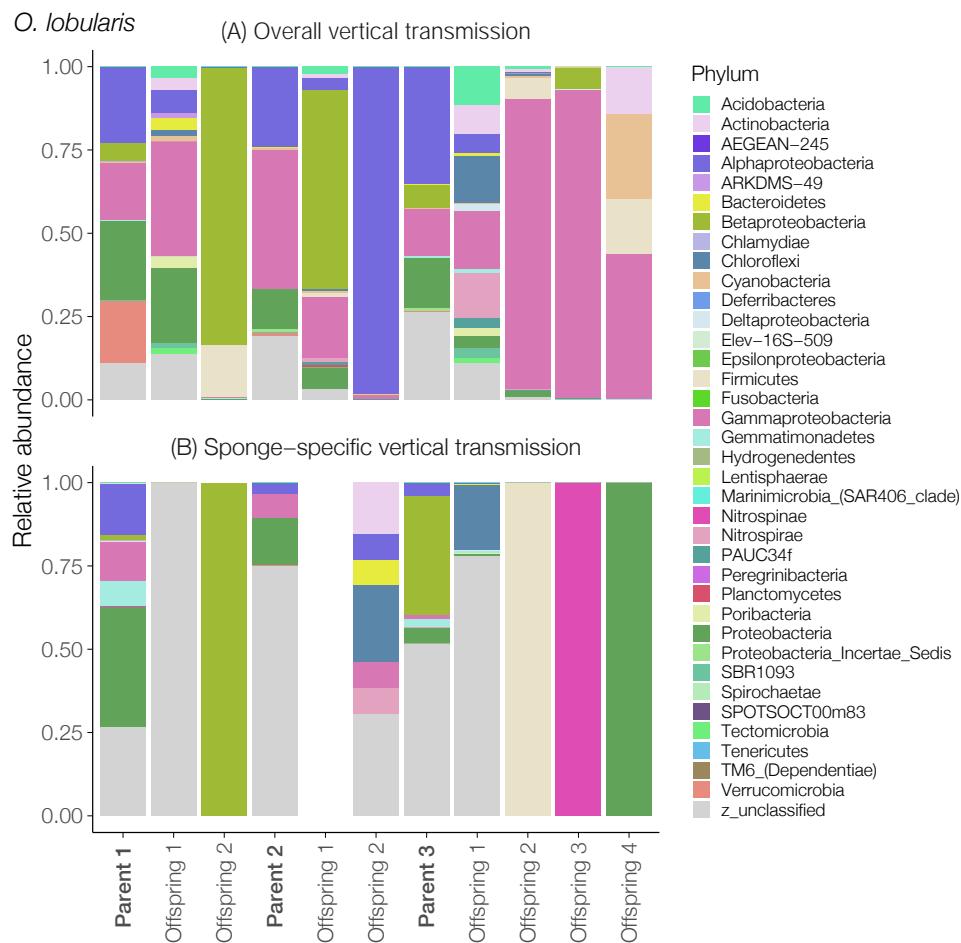
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H. columella

(A) Overall vertical transmission



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Figure S5: The relative contribution of vertically transmitted ASVs classifying to different microbial phyla (classes for Proteobacteria) in parents and the offspring of the remaining seven sponge species. The top panel (A) shows the relative contribution of phyla for the *overall* definition of vertical transmission, and the bottom panel (B) shows the relative contribution of phyla for *sponge-specific* vertical transmission. Parents (in bold) and offspring are shown on the x-axis. Note that when microbes detected in seawater are removed, this sometimes leaves no vertical transmitted ASVs for the *sponge-specific* vertical transmission. Colors represent different microbial phyla (classes for Proteobacteria). Panels A-G corresponds to host species: *I. oros*; *I. fasciculata*; *C. crambe*; *C. viridis*; *D. avara*; *H. columella*; and *O. lobularis*.

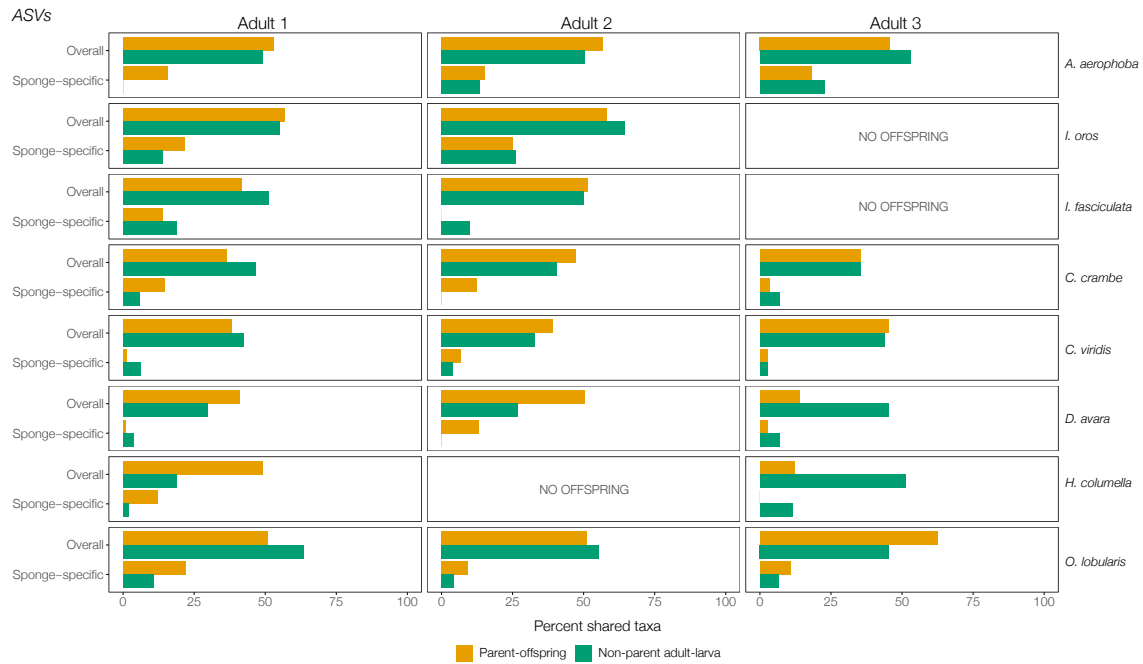


Figure S6: The average percent shared ASVs in the (A) *overall* and (B) *sponge-specific* definition of vertical transmission across adults and sponge species. Barplots show the average percent shared ASVs between sponge larvae and either (i) their known parents (yellow bars), or (ii) non-parental conspecific adults (green bars). Each row corresponds to a sponge species, and each column one of the three adults for that focal species.

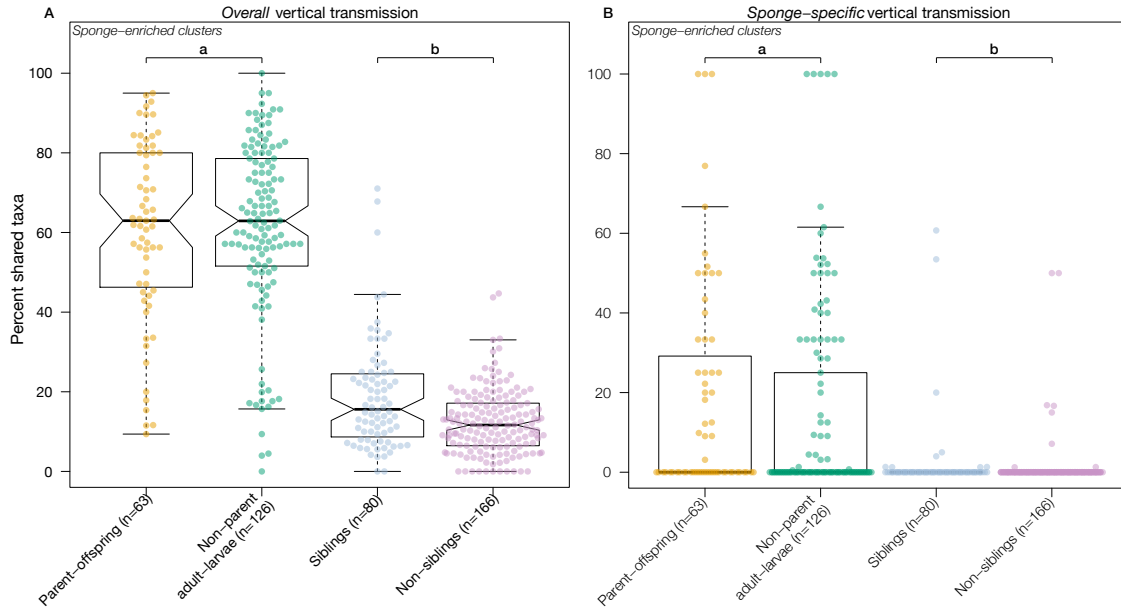


Figure S7: Percent shared *sponge-enriched clusters* in the (A) *overall* and (B) *sponge-specific* definition of vertical transmission. Boxplots (a) show the percent shared *sponge-enriched clusters* between sponge larvae and either (i) their known parents (yellow dots), or (ii) non-parental conspecific adults (green dots). In boxplots (a), each dot represents one parent-offspring pair, or one non-parent adult-larva pair across all sponge species (see Figure S8). For *overall* vertical transmission (A), parents and offspring shared, on average, 60.7% of the ASVs, whereas non-parental conspecific adults and larvae shared, on average, 61.3% of the *sponge-enriched clusters* ($P>0.1$). For *sponge-specific* vertical transmission (B), parents and offspring shared, on average, 18.6% of the *sponge-enriched clusters*, whereas non-parental conspecific adults and larvae shared, on average, 14.12% of the *sponge-enriched clusters* ($P>0.1$). Boxplots (b) show the percent shared vertically transmitted *sponge-enriched clusters* between (i) siblings (blue dots), and (ii) non-siblings (purple dots). In boxplots (b), each dot represents one sibling pair, or one pair of non-siblings (see Figure S9). For *overall* vertical transmission (A), siblings shared, on average, 18.8% of their vertically transmitted ASVs, while non-siblings only shared 12.4% ($P<0.001$). For *sponge-specific* vertical transmission (B), siblings shared, on average, only 1.85% of their vertically transmitted ASVs, whereas non-siblings shared 0.6% ($P=0.024$). While these are significantly different, the effect size (i.e., the difference in location, Δ), is effectively zero.

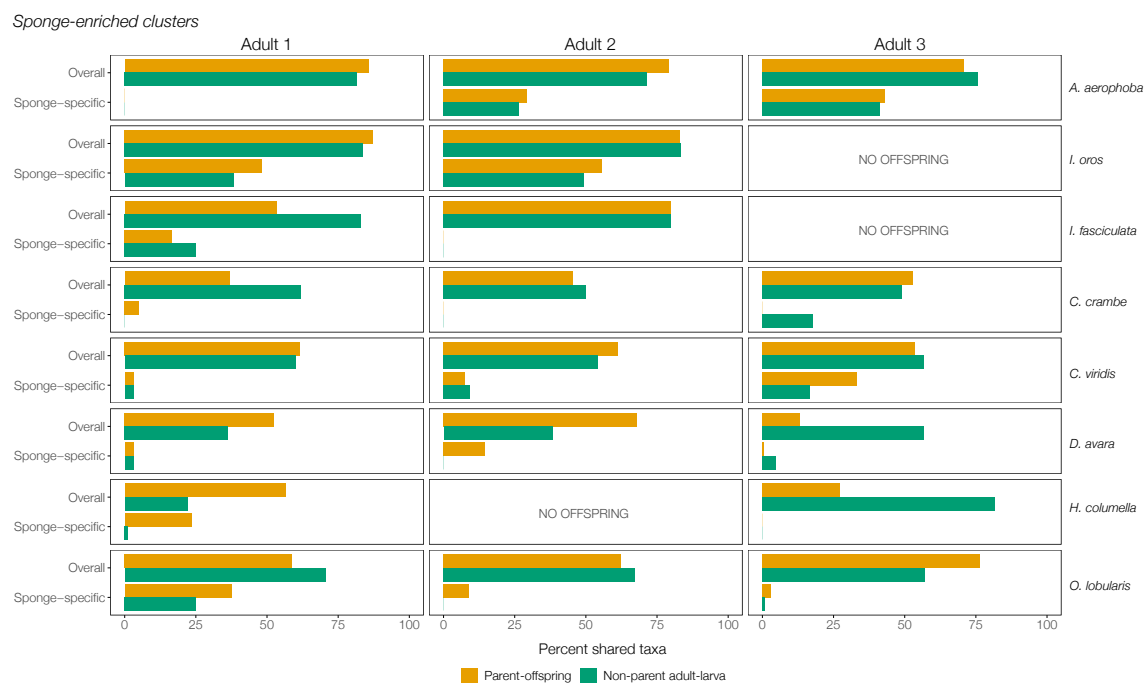


Figure S8: The average percent shared *sponge-enriched clusters* in the (A) *overall* and (B) *sponge-specific* definition of vertical transmission across adults and sponge species. Barplots show the average percent shared *sponge-enriched clusters* between sponge larvae and either (i) their known parents (yellow bars), or (ii) non-parental conspecific adults (green bars). Each row corresponds to a sponge species, and each column one of the three adults for that focal species.

ASVs

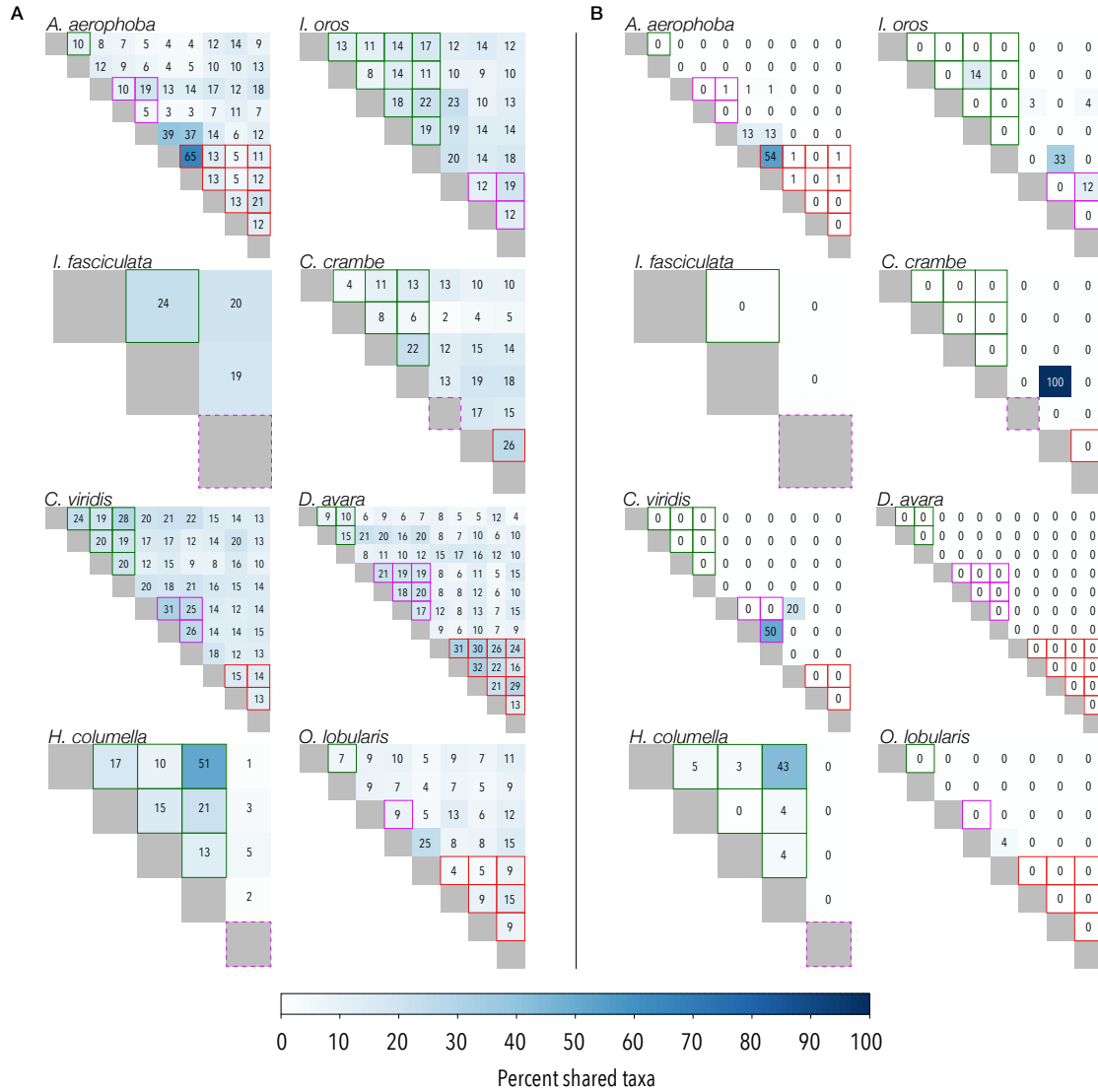


Figure S9: Percent shared ASVs of (A) overall and (B) sponge-specific vertical transmission between siblings and non-siblings. Each cell represents a larva, and sets of siblings from the same parent are indicated by cells bordered by the same color (green, purple, or red). In cases where parents only had one offspring, the diagonal is bordered by a dashed line. Cells with no borders correspond to the percent of vertically transmitted ASVs that are shared between non-siblings, i.e., conspecific larvae that did not share the same parent. Solid gray cells (i.e., the diagonal) represent the comparison with self. The white-blue continuous color legend corresponds to 0% (no ASVs shared) in white and 100% (all ASVs shared) in dark blue).

Sponge-enriched clusters

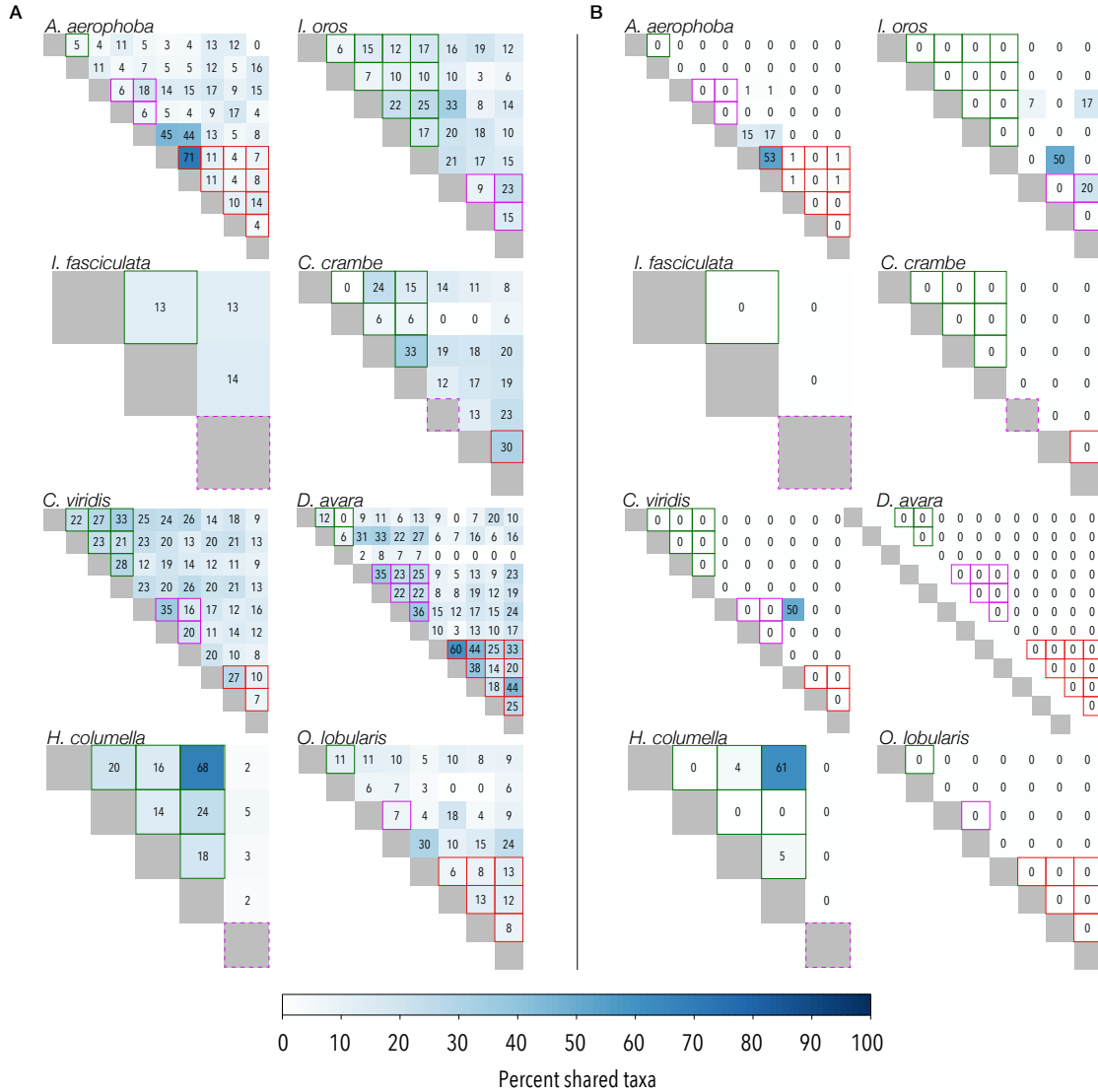
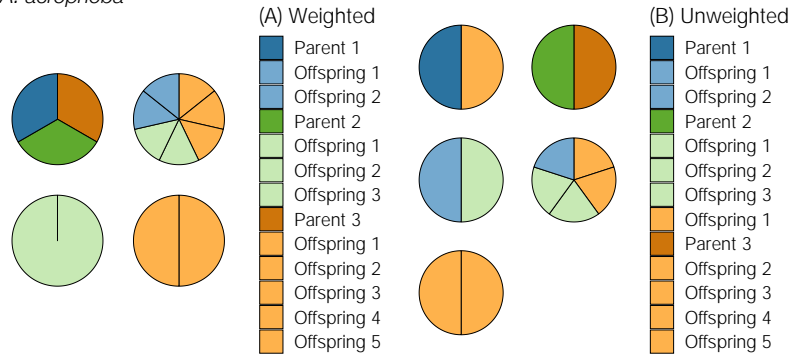


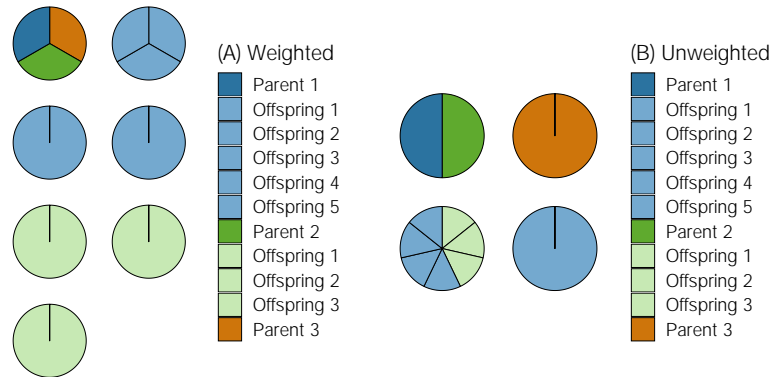
Figure S10: Percent shared *sponge-enriched clusters* of (A) overall and (B) *sponge-specific* vertical transmission between siblings and non-siblings. Each cell represents a larva, and sets of siblings from the same parent are indicated by cells bordered by the same color (green, purple, or red). In cases where parents only had one offspring, the diagonal is bordered by a dashed line. Cells with no borders correspond to the percent of vertically transmitted ASVs that are shared between non-siblings, i.e., conspecific larvae that did not share the same parent. Solid gray cells (i.e., the diagonal) represent the comparison with self. The white-blue continuous color legend corresponds to 0% (no *sponge-enriched clusters* shared) in white and 100% (all *sponge-enriched clusters* shared) in dark blue.

A. aerophoba



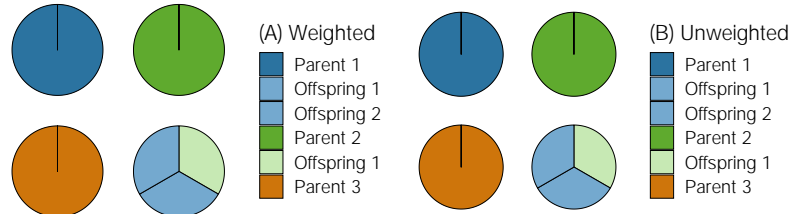
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I. oros



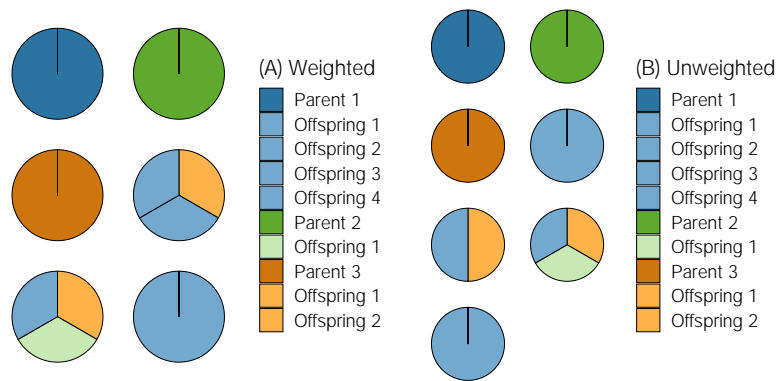
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I. fasciculata



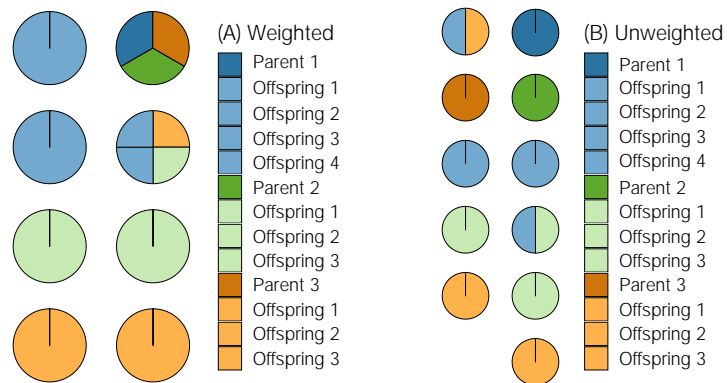
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C. crame



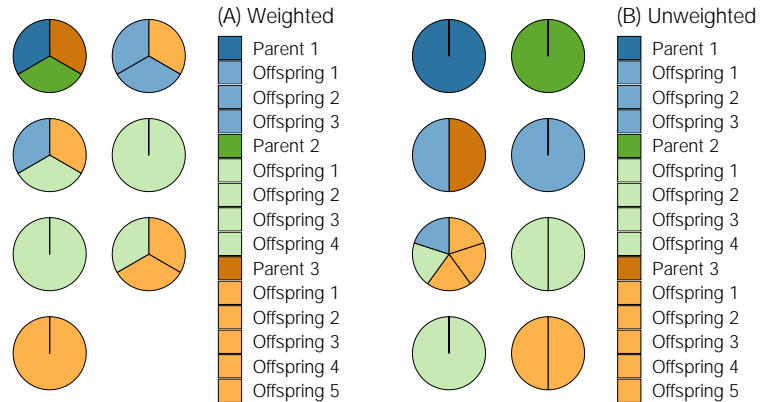
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C. viridis



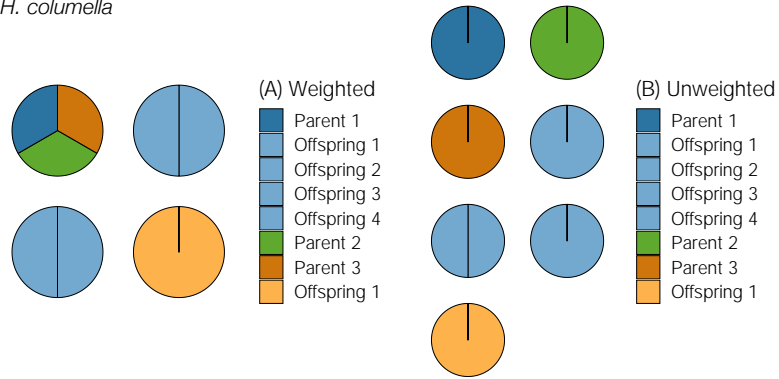
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D. avara



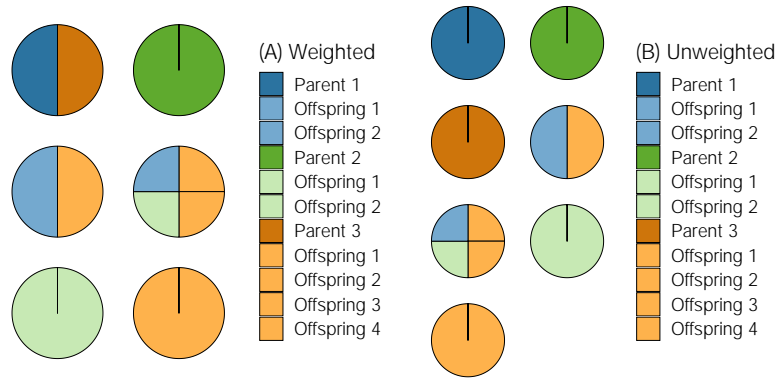
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H. columella



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O. lobularis



See figure legend below

Figure S11: Host composition of the identified modules. If parents and offspring harbor the same microbes and at similar abundances, then parents and offspring would be expected to form perfect compartments (i.e., modules only containing parents and their offspring). Panel A shows weighted modularity where ASVs have been weighted by their relative abundances, while panel B shows unweighted modularity where only presences and absences of ASVs were considered. Each color represent one parent and its offspring, and circles represent the identified modules (the number of circles represent the number of identified modules). In the case of perfect parent-offspring compartments, the number of modules would be three and they would contain multiple slices of the same color. Panels A-H corresponds to host species: *A. aerophoba*, *I. oros*; *I. fasciculata*; *C. crambe*; *C. viridis*; *D. avara*; *H. columella*; and *O. lobularis*.

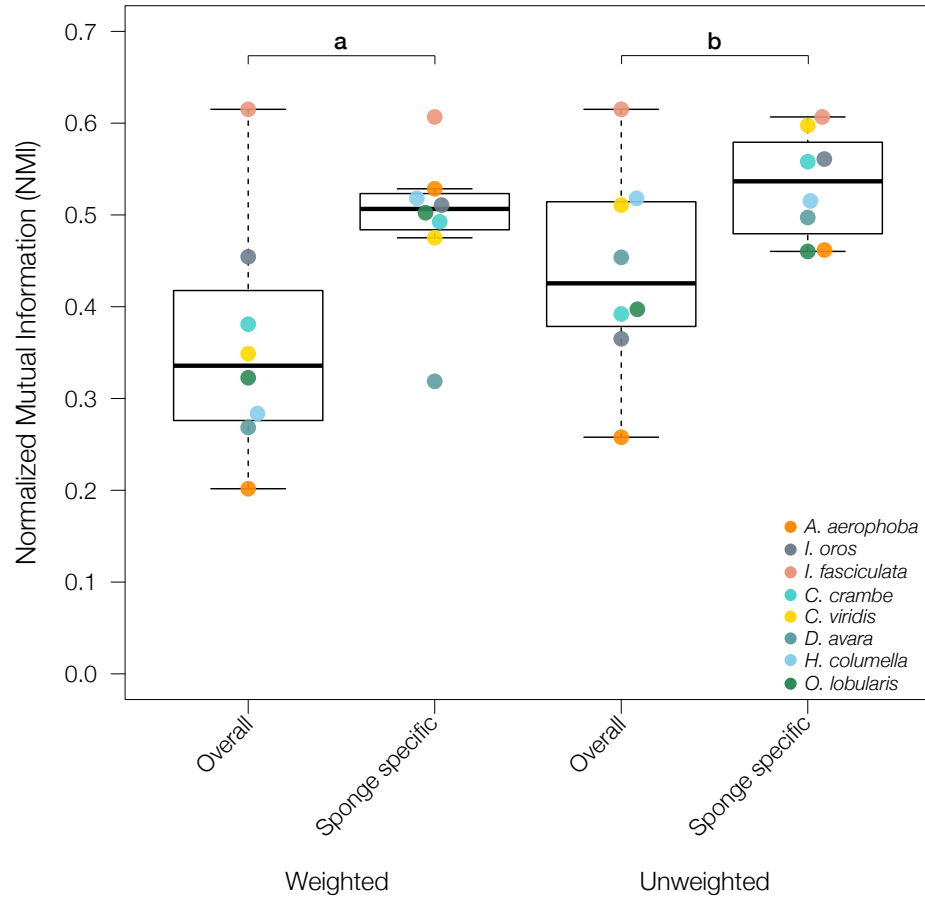


Figure S12: Boxplots for Normalized Mutual Information (NMI) criterion calculated for (a) weighted and (b) unweighted networks only containing conspecific hosts and their ASVs for *overall* and *sponge-specific* vertical transmission. Each colored dot corresponds to one sponge species. NMI ranges between 0 and 1, where 0 indicates complete dissimilarity between expected and observed modules, thus values closer to 1 corresponds to host species whose networks contain modules, and these modules contain nodes corresponding to parents and offspring.

Overall vertical transmission (ASVs)

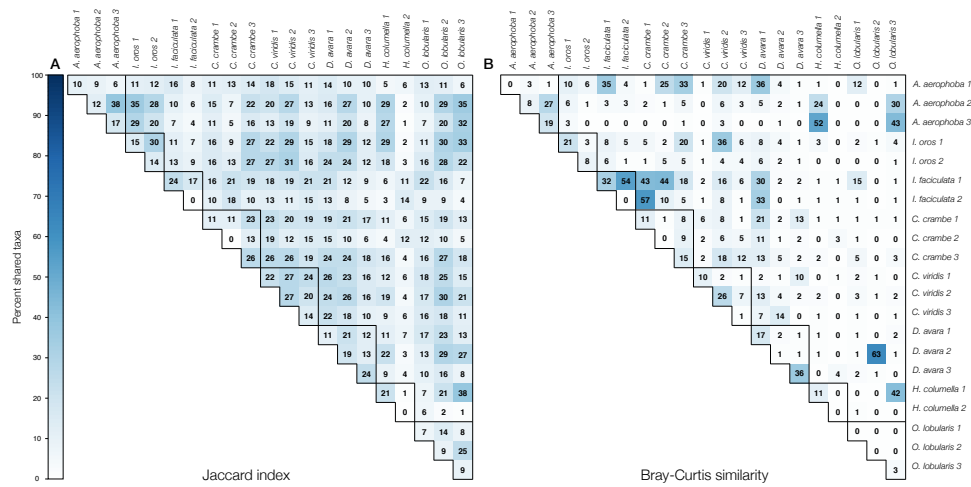


Figure S13: Similarity of *overall* vertically transmitted ASVs among all the offspring from to any given pair of adults. The figure shows similarity both within and between host species calculated as the (A) Jaccard index, or (B) Bray-Curtis similarity. In both panels, the diagonal corresponds to the average similarity among siblings (i.e., offspring from the same adult). While the Jaccard index calculates similarity between two samples based on the presence-absence of taxa, Bray-Curtis similarity also weights the coefficient by the number of individuals of each taxon. By converting the similarity coefficients to percents, their values range between 0% (no taxa shared; white), and 100% (all taxa shared; dark blue).

Sponge-specific vertical transmission (ASVs)

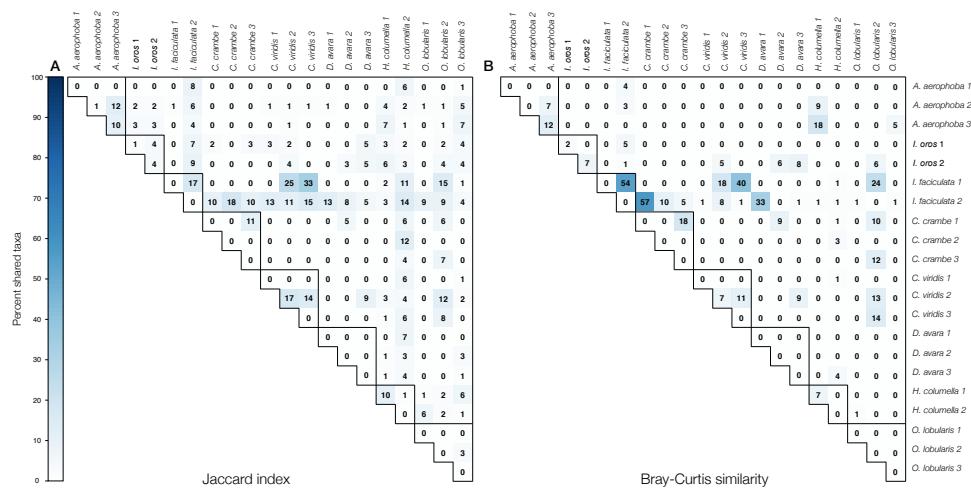


Figure S14: Similarity of *sponge-specific* vertically transmitted ASVs among all the offspring from to any given pair of adults. The figure shows similarity both within and between host species calculated as the (A) Jaccard index, or (B) Bray-Curtis similarity. In both panels, the diagonal corresponds to the average similarity among siblings (i.e., offspring from the same adult). While the Jaccard index calculates similarity between two samples based on the presence-absence of taxa, Bray-Curtis similarity also weights the coefficient by the number of individuals of each taxon. By converting the similarity coefficients to percents, their values range between 0% (no taxa shared; white), and 100% (all taxa shared; dark blue).

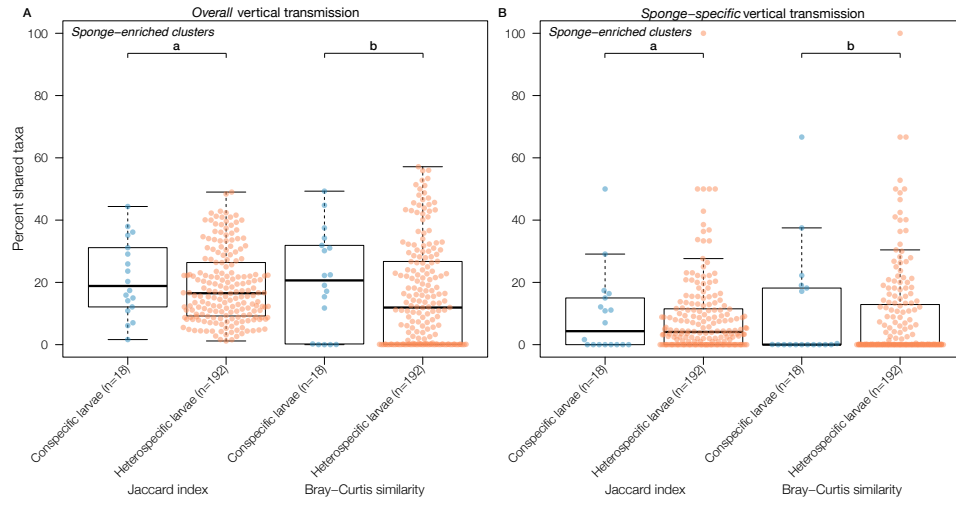


Figure S15: Percent shared (A) *overall* and (B) *sponge-specific* vertically transmitted *sponge-enriched clusters* among offspring from all possible combinations of adults calculated as either the (a) Jaccard index (see Figure S16 A and Figure S17 A), or (b) Bray-Curtis similarity (see Figure S16 B and Figure S17 B). Each dot represents all offspring from either (i) adults belonging to the same species (blue dots), or (ii) adults from different species (orange dots). While the Jaccard index calculates similarity between two samples based on the presence-absence of taxa, Bray-Curtis similarity also weights taxa by their relative abundance. For *overall* vertical transmission (A), conspecific larvae shared, on average, 21.3% (Jaccard) and 20.4% (Bray-Curtis) of the ASVs, whereas heterospecific larvae shared, on average, 18.6% (Jaccard) and 15.6% (Bray-Curtis) of the ASVs ($P>0.1$). For *sponge-specific* vertical transmission (B), parents and offspring shared, on average, 9.5% (Jaccard) and 10.1% (Bray-Curtis) of the ASVs, whereas non-parental conspecific adults and larvae shared, on average, 8.6% (Jaccard) and 8.5% (Bray-Curtis) of the ASVs ($P>0.1$). Note that the group *conspecific larvae* has much lesser number of observations (n) compared to the group *heterospecific larvae*.

Overall vertical transmission (*sponge-enriched clusters*)

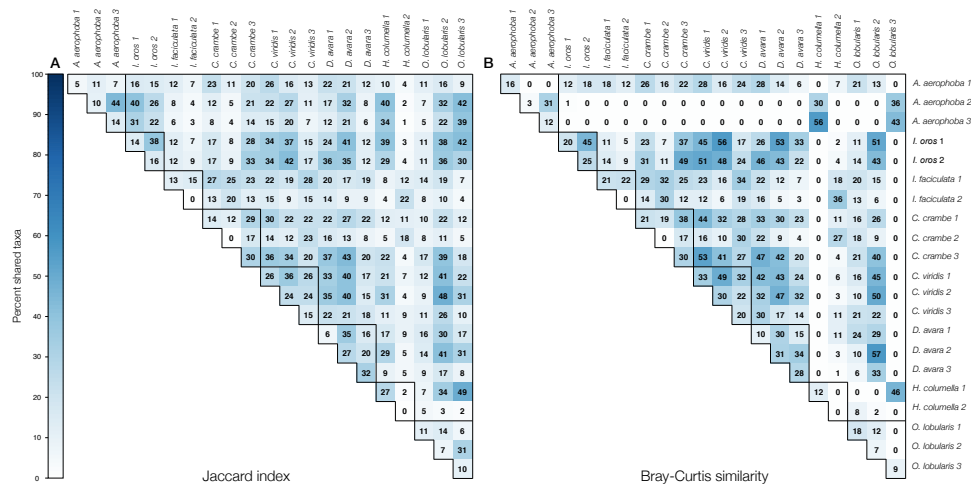


Figure S16: Similarity of *overall* vertically transmitted *sponge-enriched clusters* among all the offspring from to any given pair of adults. The figure shows similarity both within and between host species calculated as the (A) Jaccard index, or (B) Bray-Curtis similarity. In both panels, the diagonal corresponds to the average similarity among siblings (i.e., offspring from the same adult). While the Jaccard index calculates similarity between two samples based on the presence-absence of taxa, Bray-Curtis similarity also weights the coefficient by the number of individuals of each taxon. By converting the similarity coefficients to percents, their values range between 0% (no taxa shared; white), and 100% (all taxa shared; dark blue).

Sponge-specific vertical transmission (*sponge-enriched clusters*)

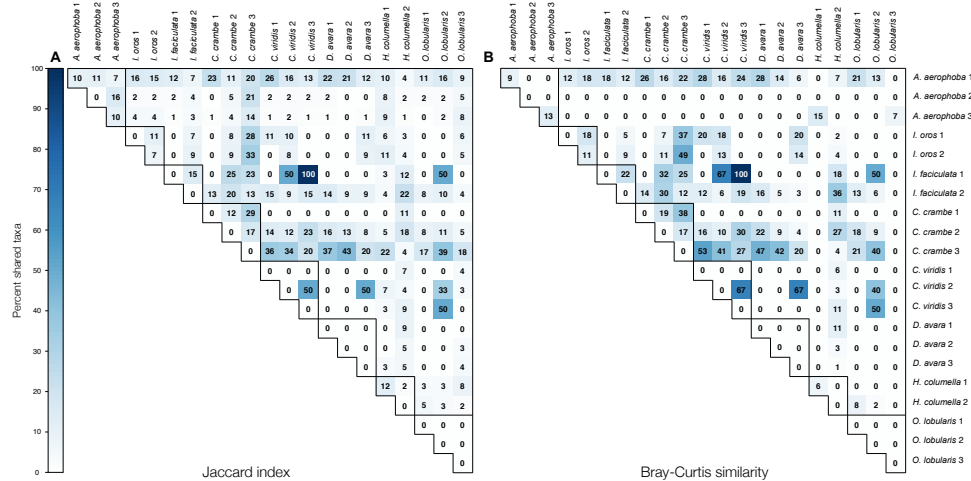


Figure S17: Similarity of *sponge-specific* vertically transmitted *sponge-enriched clusters* among all the offspring from to any given pair of adults. The figure shows similarity both within and between host species calculated as the (A) Jaccard index, or (B) Bray-Curtis similarity. In both panels, the diagonal corresponds to the average similarity among siblings (i.e., offspring from the same adult). While the Jaccard index calculates similarity between two samples based on the presence-absence of taxa, Bray-Curtis similarity also weights the coefficient by the number of individuals of each taxon. By converting the similarity coefficients to percents, their values range between 0% (no taxa shared; white), and 100% (all taxa shared; dark blue).

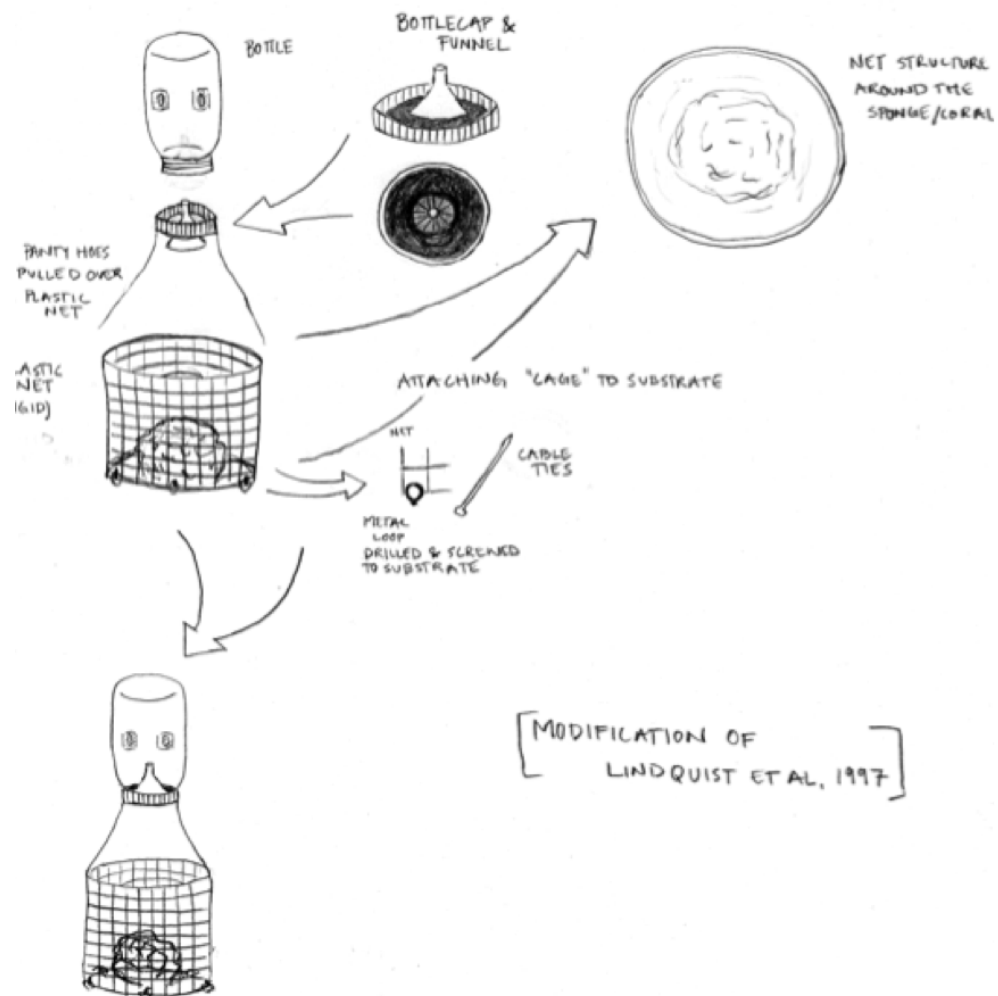


Figure S18: Sketch of the constructed traps that were used to capture dispersing larvae from adult sponges. Sketch by J.R.B.