

REVIEW

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# Symbiont transmission in marine sponges: reproduction, development, and metamorphosis

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## Abstract

Marine sponges (phylum Porifera) form symbioses with diverse microbial communities that can be transmitted between generations through their developmental stages. Here, we integrate embryology and microbiology to review how symbiotic microorganisms are transmitted in this early-diverging lineage. We describe that vertical transmission is widespread but not universal, that microbes are vertically transmitted during a select developmental window, and that properties of the developmental microbiome depends on whether a species is a high or low microbial abundance sponge. Reproduction, development, and symbiosis are thus deeply rooted, but why these partnerships form remains the central and elusive tenet of these developmental symbioses.

**Keywords:** Evolution, Holobiont, Host-microbe, Invertebrate, Microbiome, Porifera

“The ultimate goal is to characterize the mechanisms by which this fidelity is achieved and to define the impact of these associations on the evolution of developmental patterns in animals” –McFall-Ngai (2002)

## Background

Marine sponges (phylum Porifera) arose >600 million years ago and are among the oldest extant Metazoan lineages [1]. The >9300 species of marine sponges are grouped into four major classes: Calcarea (calcareous sponges; 8%), Demospongiae (demosponges; 83%), Hexactinellida (glass sponges; 7%), and Homoscleromorpha (1%) [2]. These sessile invertebrates filter-feed by pumping thousands of liters of seawater per kilogram of sponge per day, making them vital to benthic-pelagic coupling and the biogeochemical cycling of nutrients in the tropical, temperate, and polar ecosystems that they inhabit

[3–5]. One of the most notable characteristics of marine sponges is how they interact with the microbial world and, particularly, in the symbioses that they form with microorganisms [6–13].

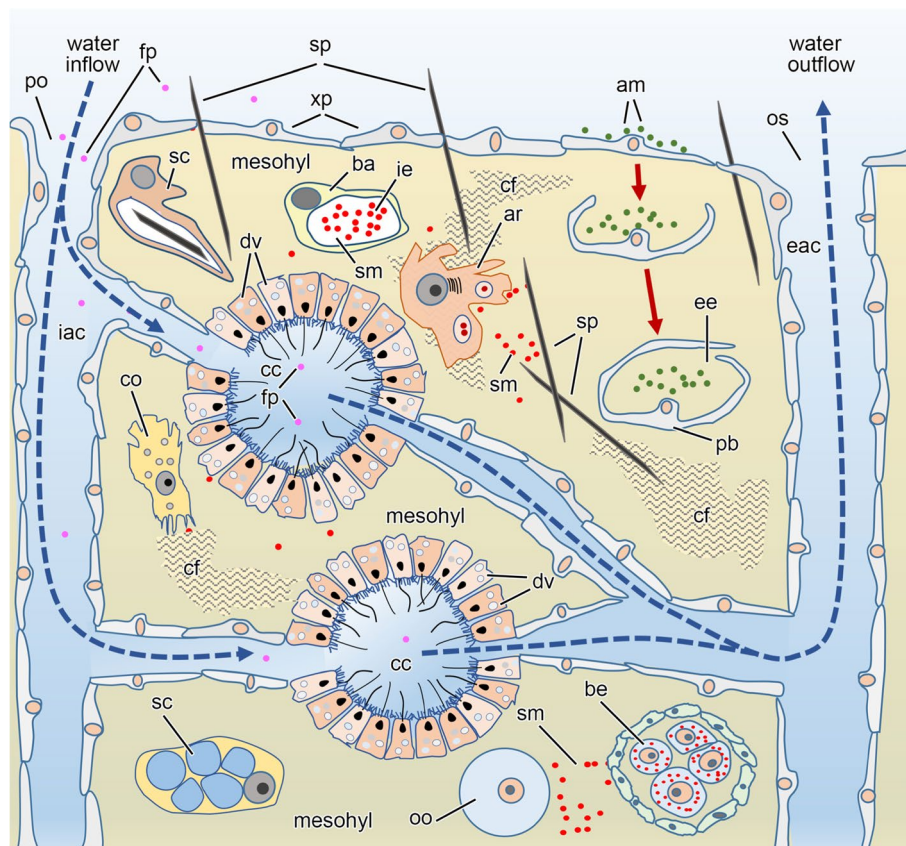
Sponges can interact with trillions of microbes each day by filter-feeding alone [5, 14], and they use an intricate set of morphological, cellular, and molecular dialogs to distinguish between friend, foe, and food [7, 15–17]. Microorganisms suspended in the seawater flow through the pores and inhalant aquiferous canals to the choanocyte chambers (Fig. 1). A fraction of these microbes are able to elude digestion and migrate through the epithelia of the aquiferous canal or the choanocyte chambers. These microbes will ultimately enter the mesohyl, where they can proliferate and provide a functional contribution to the sponge holobiont [6, 16, 24, 25]. In the sponge mesohyl (Fig. 1), there is typically an assemblage of microorganisms (~10<sup>6</sup> to ~10<sup>9</sup> cells per mL) that, in some species, can equate to ~40% of sponge tissue by volume [26]. Symbionts, defined as “dissimilar organisms living closely together in a sustained interaction” [27, 28],

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**Fig. 1** Schematic diagram summarizing the essential cytology known for demosponges, as reported previously (e.g., [18–23]). Sponges are bi-epithelial organisms. The external epithelium consists of pavement-like cells (exopinacocytes, xp). The internal epithelium delimits internal aquiferous canals and also consists of pavement-like cells (endopinacocytes, xp). However, at some points, the aquiferous canals expand into chambers, in which the pavement-like cells are replaced by cuboidal (cells provided with distal microvilli and a flagellum), the choanocytes. The beating of the choanocyte flagellum creates an inflow of ambient seawater (dashed arrows), which enters the inhalant aquiferous canals (iac) through the pores (po) at the sponge “skin,” carrying particles (fp) in suspension to the choanocyte chambers (cc). The choanocytes retain and engulf microorganisms or other particles (fp) that arrive at the lumen of the chambers, where digesting food particles into digesting vesicles (dv) begins. The seawater that is cleared of particles flows out through the exhalant aquiferous canals (eac) and the oscule (os). Between the internal and external epithelia, there is a thick region—a sort of internal “tissue”—known as the mesohyl. It consists of a gel-like intercellular medium with abundant collagen fibrils (cf) in which several types of amoeboid cells wander around. Among others, there are the collencytes (which produce the collagen fibrils, cf), sclerocytes (sc; which build the spicules, sp), and the archeocytes (which serve as cellular defense system). Archeocytes are totipotent and can transdifferentiate into almost any other cell type. In the mesohyl, there are also free-living symbiotic microbes (sm) as well as cells hosting microbes. The bacteriocytes (ba) are cells that host symbiotic microbes (sm) in the intracellular environment (ie) of a large intracytoplasmic vesicle. A peculiar type of bacteriocyte is the pocket bacteriocyte (pb). It is believed to derive from an epithelial cell that attracts free-living microbes (am) from the ambient water to the sponge surface. Then, the cell leaves the epithelium to enter the mesohyl (red arrows), folding over itself to form an extracellular sack-like cavity, in which microbes are host in the extracellular environment (ee). Oocytes (oo) and brooded embryos (be) also develop in the mesohyl

of sponges are taxonomically and functionally diverse. Sponges harbor 63 bacterial phyla (e.g., Proteobacteria, Actinobacteria, and Chloroflexi) that are often metabolically intertwined with the host through the utilization of dissolved organic and inorganic carbon and nitrogen as well as through amino acid and vitamin biosynthesis [6, 7, 9, 11, 29–31]. In addition to the bacterial symbionts, the microbial communities associated with sponges also includes archaea, viruses, and unicellular eukaryotes

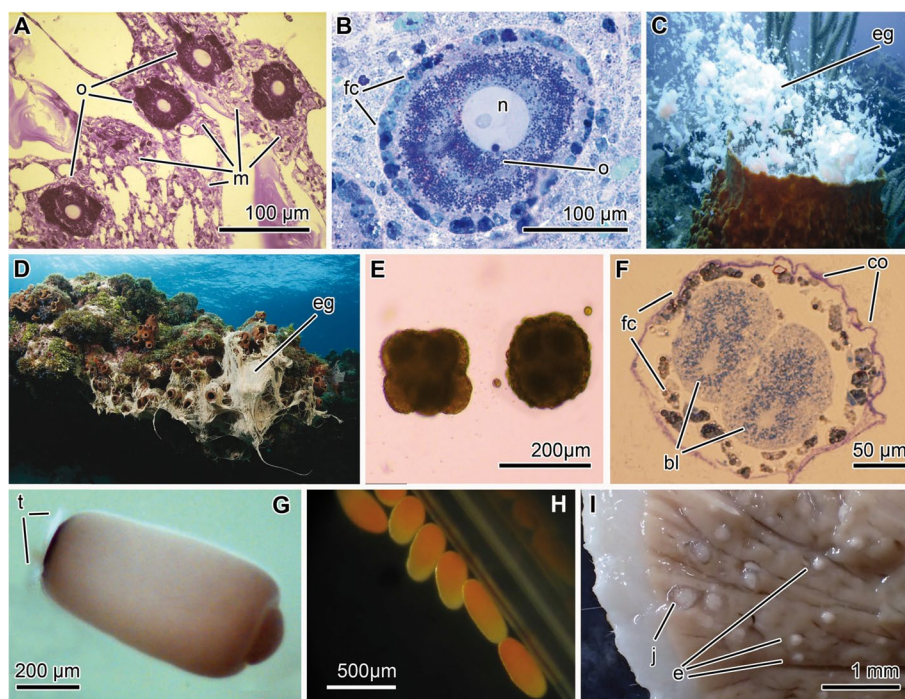
(e.g., *Symbiodinium*, other dinoflagellates, and diatoms) [9, 18].

Properties of these symbiont communities depend on whether a species is a “low microbial abundance” (LMA) or “high microbial abundance” (HMA) sponge. LMA sponges harbor microbial populations that are roughly equal to the microbial densities of the surrounding seawater while those of HMA sponges—formerly called bacteriosponges—are two to four orders of magnitude

higher than LMA sponges [7, 14, 32, 33]. These two patterns of microbial abundance have evolved independently on numerous occasions and have resulted in fundamental differences in the taxonomic composition and function of sponge symbiont communities [32, 33]. HMA sponges are comparably enriched in Acidobacteria, Chloroflexi, and Poribacteria, while LMA sponges are comparably enriched in Cyanobacteria and Proteobacteria [31, 33]. Moreover, symbionts of HMA sponges are primarily responsible for autotrophic and heterotrophic metabolism while these processes are primarily carried out by the host in LMA sponges [34, 35]. The provisioning of these nutritional resources from parent to offspring are then essential for reproduction [36–38].

Most marine invertebrates, including sponges, exhibit a biphasic (indirect) life cycle, where adults reside on the seafloor and the developmental stages are suspended in the water column [39–42]. Sponges predominately

fertilize internally, brood their embryos, and then release swimming larvae (viviparity). Fewer species broadcast spawn their gametes for external development (oviparity), while others develop directly by bypassing the larval stage and having juveniles crawl out from the parent (Fig. 2). Demosponges can either be viviparous or oviparous while Homosclerophorida and Calcarea are viviparous [46, 47]. The reproductive tendencies of Hexactinellida remain largely uninvestigated, but the few reported cases show that embryos are brooded [48, 49], which should lead to viviparism. Once the larva forms internally or externally, it undergoes a lecithotrophic (non-feeding) development that generally lasts a few hours to several days, during which the larva may disperse a few meters to hundreds of kilometers [41, 50–54]. The developmental stages of sponges also serve as the predominant vector of symbiont transmission between generations.



**Fig. 2** Summary of the main events in sponge reproduction and development. **A** Amoeboid oocytes (o) in the mesohyl (m) of the demersal sponge *Agelas oroides* collected in August 2018 and first described here. **B** Late-stage oocyte with nucleolated nucleus surrounded by a layer of follicular cells (fc) within the mesohyl of the demersal sponge *Aplysina aerophoba* [43]. **C** Release of egg (eg) masses by the demersal sponge *Xestospongia muta* (unpublished, UH). **D** Release of eggs (eg) in mucous strands by the demersal sponge *Agelas sceptrum* (photo by Stephen Frink). **E** Morules of *A. aerophoba* developing externally in the water column [43]. **F** Section of a morula of *A. aerophoba* similar to those in Fig. 1E, showing that they are surrounded by a collagen envelope (co) and a layer of follicle cells (fc). **G** A free-swimming parenchymella larva of the demersal sponge *Ircinia felix*, showing a posterior tuft of long cilia, which are part of a larval photoreceptor organ [44]. **H** Non-tufted parenchymella of the demersal sponge *Ectyoplasia ferox*, with a translucent posterior pole [45]. **I** Hand-made section through the body of an individual of demersal sponge *Craniella zetlandica* that was collected from a reef of the cold-water coral *Lophelia pertusa* in the Stjersundet area of a Norwegian fjord in August 2018 (unpublished, MM). This species undergoes a direct development and, therefore, lacks the larval stage. The picture shows smaller embryos (e) being brooded in deepest regions of the body, which migrate towards the surface of the sponge during development to be finally released as miniature sponge (juvenile = j) by squeezing themselves through the cells and spicules of the sponge cortex



Symbioses can be maintained with a high fidelity through both horizontal and vertical transmission [55–58]. Horizontal transmission occurs when a symbiosis is non-continuous throughout the host life cycle and resumes following an environmental acquisition [55, 57]. One example is the mutualism between the Hawaiian bobtail squid *Euprymna scolopes* and the luminous bacterium *Vibrio fischeri*. In this partnership, embryonic squid lack symbionts until post-hatching juveniles recruit *V. fischeri* from the seawater [59–61]. Vertical transmission, on the other hand, are symbioses that are maintained throughout the host's life cycle and across generations [55, 57]. This type of transmission is well-studied in aphids, where cells of the nutritional mutualist *Buchnera* sp. are endocytosed at the posterior pole of mature eggs [62]. Both transmission modes are ancient, evolutionarily advantageous, and not mutually exclusive [10, 13, 55, 57, 63, 64]. Horizontal and vertical transmission both occur in sponges and enable their symbiotic lifestyles to be maintained.

Researchers in the 1930s first realized that microorganisms were present in the tissues of marine sponges [65]. These microbes were initially regarded as pathogens, but were reinterpreted as probable mutualists once microbes were consistently observed in abundance across sponge species [66]. The transmission of microorganisms in marine sponges was not identified until the 1960s when Lévi and Porte [67] used transmission electron microscopy to describe the cellular structures of *Oscarella lobularis* larvae. Symbiont transmission has since become a fundamental subdiscipline of sponge microbiology, and this manuscript is an effort to synthesize ~60 years of research on the diversity and intricacies of symbiont transmission. We will qualitatively address three main questions. First, is symbiont transmission universal in this early-diverging lineage and what are the underlying mechanisms? Second, are patterns of microbial abundance in the adults reflected in the diversity and composition of the transmitted symbionts? Lastly, does symbiont function influence the larvae while in the pelagic environment? We conclude by providing a framework to assess the functional mechanisms of developmental symbioses in this emerging experimental system.

### Paths and patterns of symbiont transmission

Vertical transmission requires coordination between the developmental programs of the host and the microorganisms [57]. To understand how symbionts are maintained between generations, we must first briefly address how gametes arise (gametogenesis) and how they develop (embryogenesis). This baseline will set the stage to detail the morphogenetic events involved in symbiont transmission during reproduction and development. We then

describe the transmission of microbes from parent to offspring, including the types of microbes and the developmental stages transmittal occurs. We conclude this section with a meta-analysis of the bacterial communities associated with the developmental stages of marine sponges.

### Reproduction and development

Marine sponges can either be separate sexes (gonochoristic) or both sexes occur simultaneously in an individual (hermaphroditic). Gametoblasts (i.e., mother cells of gametes) are often motile and capable of actively migrating towards their nutrition source [68, 69]. Gametogenesis is initiated when the expression of genes relating to cell proliferation and pluripotency induce choanocytes and pluripotent archaeocytes to transdifferentiate into oogonia and spermatogonia [46, 70]. Established oogonia undergo meiosis, with the subsequent oocytes maturing during vitellogenesis as the oocyte becomes enriched with yolk [68, 69]. Spermatogonia reach maturation through two rounds of meiosis that greatly reduce cell volume and cytoplasm content. A flagellum is then built to become a mature spermatozoon [46, 70].

Mature spermatozoa are released through the excurrent flow of males (or hermaphroditic sponges serving as functional males), and mature oocytes may be fertilized in one of two ways. First, spermatozoa of broadcast spawning (oviparous) sponge species may encounter a conspecific oocyte that was released into the water column [71]. Second, gravid females (or hermaphroditic individuals serving as functional females) of internally developing species (viviparous) may capture conspecific spermatozoa in their inhalant flow [72, 73]. These spermatozoa are transferred to choanocyte chambers that phagocytose, but do not digest, the engulfed spermatozoa through unknown molecular signaling processes (e.g., [74]). The choanocytes with spermatozoa then leave the epithelium and become amoeboid cells that wander through the mesohyl seeking a mature oocyte. Each spermatozoon is carried individually within a cytoplasmic vesicle (spermiocyst) that transfers the spermatozoon nucleus directly to an oocyte for fertilization. This internal fertilization typically—but not always—leads to the subsequent embryo being brooded in the mesohyl until the larva is released from the parental sponge [50].

Embryogenesis in these early-diverging metazoans is complex and is best studied in species that develop internally [51, 72, 75, 76]. Depending on the sponge lineage, cleavage is holoblastic, equal or unequal, and may either be synchronous or asynchronous. Embryogenesis takes between a few hours to a couple days in externally developing species (Fig. 1E, F [43, 46, 77]) to a couple weeks to months in brooding species [75]. Embryos typically

develop into one of eight larval types recognized to date: the amphiblastula, calciblastula, cinctoblastula, clavablastula, dispherula, hoplitomella, parenchymella, or trichimella [51, 75]. Alternatively, some sponge species (e.g., sphirophorid and stylocordylid demosponges) lack this all together by developing directly into juveniles that crawl out of the parental body [78–80].

### Symbiont transmission during reproduction and development

Microbes within the sponge mesohyl are either free-living (Figs. 3A, B and 4A) or within specialized vesicles (bacteriocytes) (Figs. 1, 3C–E, and 4B) [18, 84, 85]. Symbionts living in the intercellular medium of the mesohyl are transferred from adult to offspring that are brooded (black arrows in Fig. 4) or that develop externally (blue arrows in Fig. 4). Pre-vitellogenic oocytes, which are amoeboid cells moving through the mesohyl, can actively engulf microbes with their pseudopodia (Fig. 3G), but these microbes are thought to serve as a nutritional resource for the young oocyte [18, 86]. Just prior to vitellogenesis, the zone directly surrounding the oocyte becomes densely populated with microbes, which can be incorporated into the oocyte at the onset of vitellogenesis [18, 44, 87–89]. It is unclear whether these microbial aggregations are the result of migration or enhanced rates of proliferation around the oocyte. While the mechanisms involved are unknown, it seems likely that this process is stimulated by chemical cues from the oocyte.

Symbionts that are transmitted during vitellogenesis reach the cytoplasm of the oocyte through two primary processes. First, microbial cells are recognized at the cell surface of the oocyte and the expression of genes corresponding with vesicle transport, endocytosis, and phagocytosis—and the subsequent production of those proteins—signal for these cells to be engulfed (Fig. 3G) [70]. Second, microbial cells in the mesohyl are taken up by nurse cells (Figs. 3I, J and 4D), the cell type that is primarily responsible for provisioning materials to the oocyte [68, 69]. Nurse cells then migrate to the vicinity of the oocyte's membrane, and microbes can be transferred through transient cytoplasmic bridges (Fig. 4E) [18, 87, 88, 90, 91]. Alternatively, entire nurse cells can be engulfed by the oocyte and its symbiont-containing vesicles are exocytosed into the cytoplasm of the oocyte (Figs. 3H and 4F, G). Near the end of vitellogenesis, nurse cells are organized into an epithelium (the follicle) that closely surrounds the oocyte (Fig. 2B and 4). This structure forms in species where the oocyte develops externally or is brooded (Fig. 4).

Once the oocyte is fertilized, the zygote begins to cleave and the multicellular embryo becomes a morula (Fig. 4G,

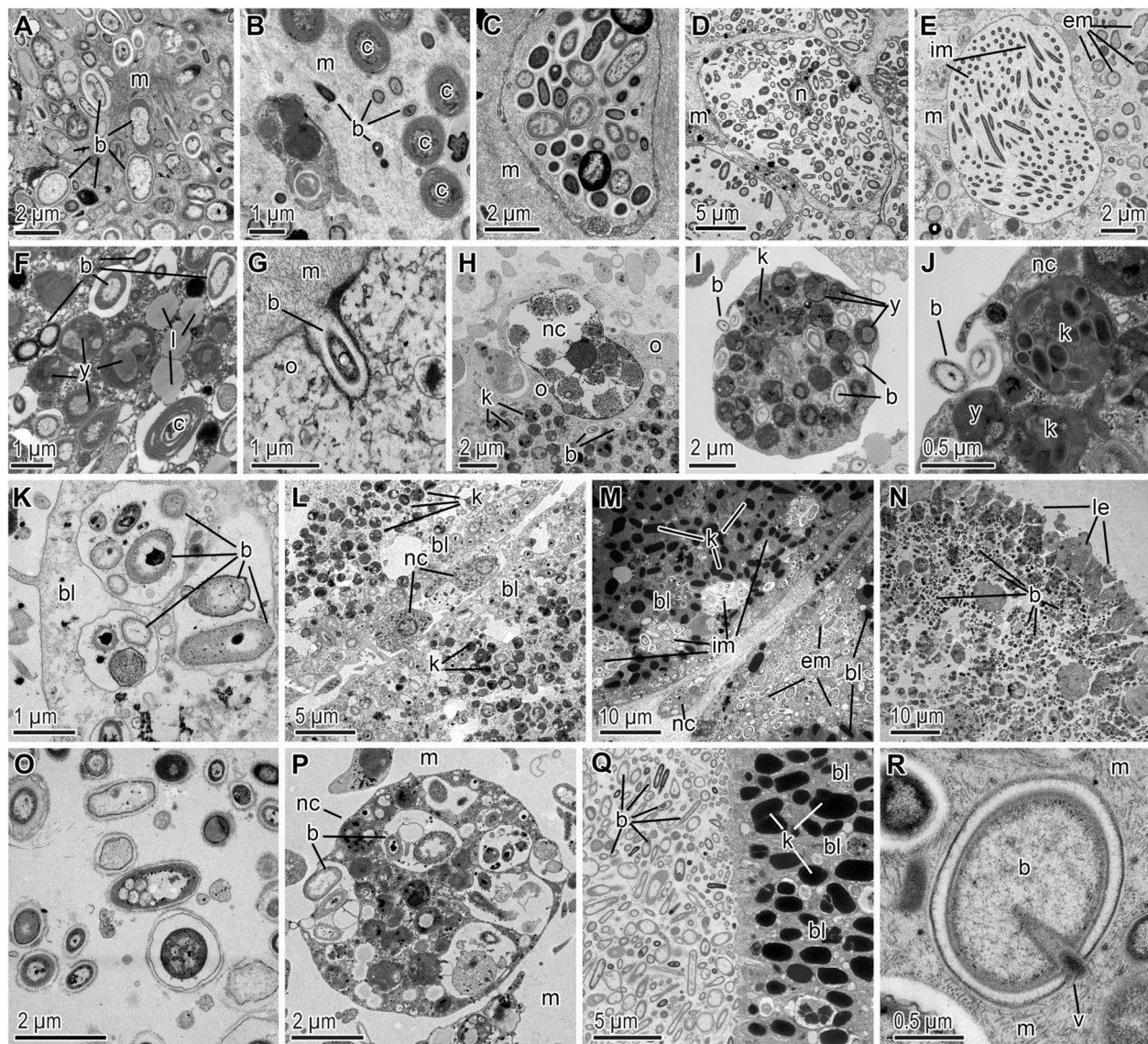
H, K–M for internal development and Fig. 4I, J for external development). If symbionts had previously been transmitted to the cytoplasm of the oocytes (Fig. 4C–G) then those symbionts are transferred to the blastomeres of the morula (Fig. 4H, J). If symbionts had not previously been transmitted (Fig. 3K for internal development and Fig. 4N for external development), then there are two primary pathways for a morula to obtain parental symbionts. First, symbionts may be incorporated by nurse cells and/or follicle cells (Fig. 3L for internal development and Fig. 4N, Q for external development), which subsequently migrate into the morula through the spaces between the blastomeres [43, 51, 92, 93]. Symbionts of nurse cells may also be transmitted to larvae that develop externally (Fig. 4N–Q), where nurse cells degrade while the embryo is in the water column and allow for microbes to be released into the blastocoel [93]. Second, a morula may acquire symbionts when microbial cells enter the space between the follicle and the oocyte (Fig. 4M for internal development and Fig. 4O–P for external development). Symbionts may then proliferate throughout early development and then enter the morula through spaces between the blastomeres [18, 94]. To our knowledge, it remains unknown: (i) whether embryos and larvae have bacteriocytes or (ii) if bacteriocytes are also transferred through cleavage furrows. These are herein hypothesized as a plausible pathway to also transmit symbionts across sponge generations.

As a result of the processes described above, early-stage larvae are transmitted a combination of extracellular (Fig. 4S–U) and intracellular (Fig. 4R) microbes. Solid larvae (e.g., parenchymella, trichimella, hoplitomella) (Fig. 4S, U) typically have extracellular symbionts in the intercellular medium underneath the larval epithelium (Fig. 3N–P). Hollow larvae (e.g., amphiblastula, calciblastula, cinctoblastula, clavablastula) predominately have symbionts in the larval cavity (Figs. 3Q and 4T), irrespective of whether this is a blastocoel or a secondary cavity [18, 90, 95–98]. During subsequent morphogenic events, intracellular symbionts remain mostly in the cell types derived from the larval epithelium while the extracellular microbial populations grow as the central cavity of the embryo expands throughout development (Table S1) [18, 75, 90, 95, 98–101].

### Global patterns in vertical transmission

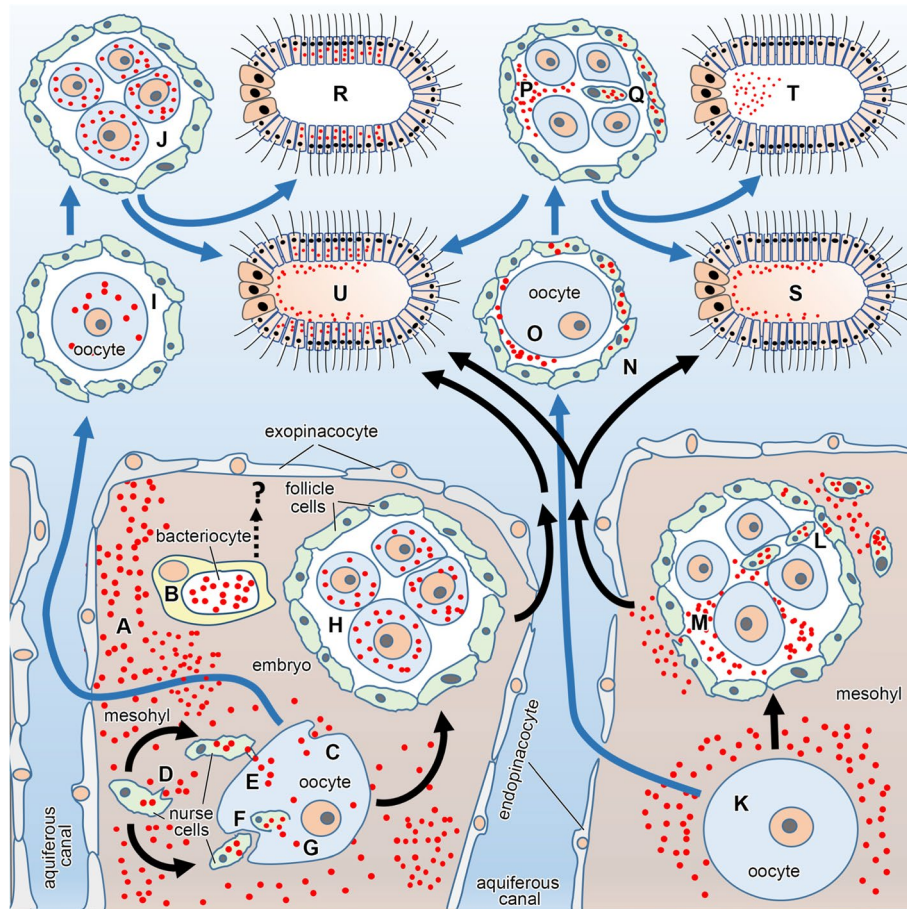
We utilized the literature on sponge reproduction and development to summarize the patterns of symbiont transmission from parent to offspring in this early-diverging lineage (see, Table S1). Vertical transmission of microbial symbionts has been documented in most, but not all, of the sponge species where it has been studied (Table S1). Three of these species (*Halisarca dujardini*,





**Fig. 3** Transmission electron micrographs documenting the variety of microbes in sponges and the main mechanisms of transference. **A–C** Diversity of microbes in an undescribed *Thymosia* sp. (an HMA demosponge), where bacteria+archaea (**A**) as well as and cyanobacteria (**B**) freely co-exist in the mesohyl and with microbial population in bacteriocytes (**C**) [81]. **D, E** Mesohyl of HMA demospoges *Petrosia ficiformis* (**D**) and *Aplysina cavernicola* (**E**). The former shows a pocket bacteriocyte that has a mix of microbes while the latter is a regular bacteriocyte with a single strain of a Spirochaete-like bacteria [73, 81]. **F** Cytoplasm of a *Chondrilla caribbea* (HMA demosponge) oocyte filled with bacteria+archaea, cyanobacteria, yeasts, and lipid bodies [82]. **G** Young oocyte of *Corticium candelebrum* (HMA homosclerophorid) phagocytosing a bacterium from the mesohyl [74]. **H** Oocyte of *Aplysina aerophoba* (HMA demosponge) phagocytosing a nurse cell. **I, J** Nurse cell of *C. caribbea* charged with bacteria+archaea, yeast, and yolk precursors. Note that the nurse cell is also in the process of phagocytosing two bacteria (**J**). **K, L** Morula of *A. aerophoba* that has incorporated bacteria+archaea into the blastomeres (**K**) and with nurse cells being incorporated by immigration through the cleavage furrows (**L**). **M** Morula of *C. candelebrum* in which both free-living microbes and nurse cells are migrating through the cleavage furrows between the blastomeres. Note that blastomeres have also phagocytosed groups of bacteria to make them intracellular. **N–P** Swimming larva of *A. aerophoba* that has accumulated bacteria+archaea below the larval epithelium (**N**) and making of a diverse microbial array (**O**), which is also phagocytosed and used as a food source by amoeboid cells in the larval interior (**Q**). Swimming larva of *C. candelebrum*, showing a dense diverse assemblage of bacteria+archaea within its internal, central cavity. **R** Coccoid bacterium in the mesohyl of *Thymosia* sp. nov being infected by a bacteriophage virus. Labels: “b”= bacteria+archaea, “c”= cyanobacterium, “y”= yeast, “v”= virus, “m”= intracellular microbes, “em”= extracellular microbes, “m”= mesohyl, “ns”= nurse cell, “o”= oocyte, “n”= cell nucleus, “l”= lipid body, “k”= yolk bodies, “bl”= blastomere, “le”= larval epithelium. All samples studied were collected and pictured by MM





**Fig. 4** Comprehensive schematic summarizing vertical transmission of microorganisms in sponges, as reported previously [18, 19, 46, 75, 83]. Microbes (red dots) occur freely in the sponge mesohyl (A) or within bacteriocytes (B). While it is not known how microbes in bacteriocytes are transferred (?), microbes from the mesohyl are transmitted from mother to offspring through a variety of pathways (C–Q), irrespective of whether oocytes and embryos are brooded (black arrows) or develop externally (blue arrows). Oocytes can acquire microbes by direct engulfing them (C) or through nurse cells. Nurse cells engulf microbes from the mesohyl (D), which are subsequently transferred to the oocyte through cytoplasmic bridges (E) or by nurse cells being phagocytosed by the oocyte (F). Oocytes charged with microbes give rise to embryos that have intracellular microbes in the blastomeres, irrespective of whether the embryo was brooded (H) or was released into the environment (I) for external fertilization and development (J). Alternatively, oocytes may not acquire microbes (K) but an embryo (morula) can acquire them through two pathways. First, follicle cells and/or nurse cells charged with microbe that enter the embryo by migrating through the cleavage furrows (L–Q). Second, microbes infiltrated in the space between the follicle and the embryo migrating themselves through the spaces between blastomeres (M–P). These two extracellular transfers of microbes to embryos occur both in brooded embryos (L–M) and externally fertilizing oocytes (N) leading to externally developing embryos (P–Q). Therefore, the resulting larvae may have microbes in their epithelial cells (R), their internal extracellular medium (S), or their internal cavity (T), if any. By combining mechanisms for intracellular and extracellular transference, some species have larvae containing intracellular and extracellular vertically transmitted microbes (U)

*Mycale laxissima*, and *Petrosia ficiformis*) have also been reported not to vertically transmit microbial symbionts [73, 102–104]. These sponge species have been sampled globally but come predominately from the Mediterranean Sea, Northwestern Atlantic Ocean, and Caribbean Sea. A few species have also been studied in the Coral Sea/Great Barrier Reef, Scotia Sea, and Celtic Sea. Moreover, all but two species are from coastal waters, with *Craniella zetlandica* and *C. infrequens* being from deep waters in the North Atlantic Ocean. Thus, there is a strong taxonomic,

geographical, and habitat bias that may confound our understanding of the major patterns of symbiont transmission in sponges.

Marine sponges vertically transmit multiple types of microorganisms, which includes archaea, bacteria, and eukaryotes. Bacteria were vertically transmitted in most, but not all, of sponge species known to inherited microbes while few species inherited archaea and eukaryotes (i.e., *Symbiodinium* spp.) (Table S1). Different types of microorganisms can be vertically transmitted

simultaneously. Several sponge species inherit both archaea and bacteria, while *Chondrilla nucula* vertically transmits bacteria and yeast (Figs. 2F and 4B; Table S1) [18, 82, 105]. Interestingly, no studies have noted whether sponges vertically transmit viruses (Fig. 3R), despite their extraordinary diversity, functional relevance, and ecological importance to the adults [106–110]. The methodology for profiling, annotating, and imaging sponge viruses has developed significantly in recent years [111, 112], and it may now be methodologically feasible to determine whether sponges also vertically transmit viruses. Moreover, it remains unknown whether bacteria are the primary type of microbe that is vertically transmitted or whether this is due to a methodological bias.

The majority of sponges brood their offspring until they are early-stage larvae and microbial symbionts can, in principle, be incorporated at any time during this developmental window. Microorganisms have been detected in each of the three major developmental stages (oocytes, embryos, and larvae), with detection in larvae seemingly twice as common as in oocytes or embryos (Table S1). This distribution, however, is suspected to be confounded by a methodological bias. Studies supporting that vertical transmission occurs in oocytes and embryos predominately use electron microscopy (e.g., [18, 94]) while those suggesting the same for larvae use next-generation sequencing (e.g., [113, 114]). Therefore, we suspect that this distribution is more skewed towards oocytes and embryos than is currently represented.

Symbionts may also enter sponge sperm (Table S1). About 1.6% of *Chondrilla australiensis* spermatozoa harbor cyanobacteria, and these microbes actively divide in the sperm head [91]. It remains unknown whether a fitness benefit—possibly through a metabolic contribution—provided by these cyanobacteria would compensate for the inconvenience of the increased mass during the race to fertilize an oocyte [115]. Moreover, spermatozoa in the few congeneric species that have been examined by transmission electron microscopy lack cyanobacteria [43]. Vertical transmission through spermatozoa appears to be the exception and not the rule in marine sponges.

### Bacterial communities of the developmental stages

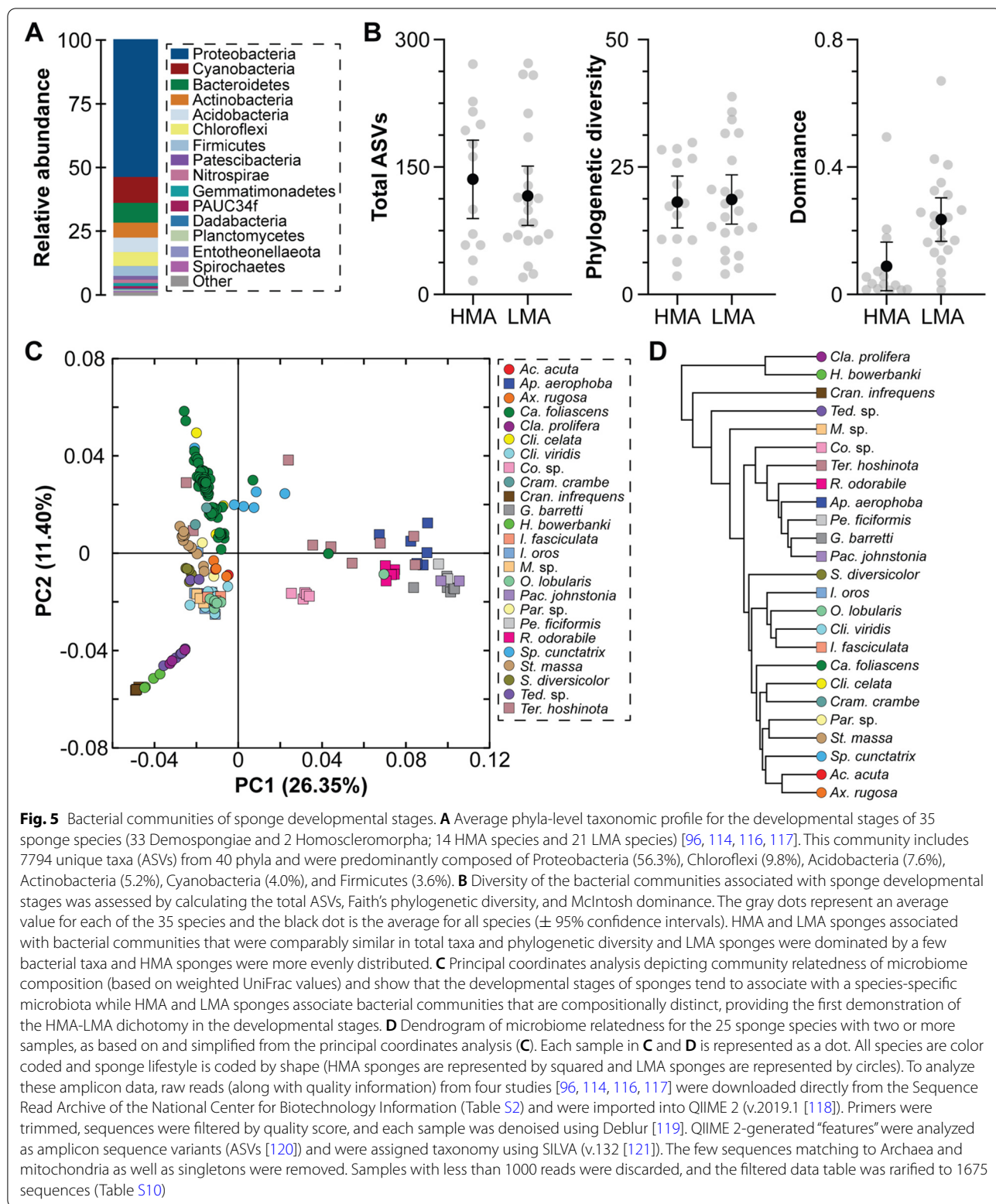
Amplicon (*16S* rRNA gene) data are available for the developmental stages of 35 sponge species (33 Demospongiae and 2 Homoscleromorpha; Additional file 1: Table S2) [96, 114, 116, 117]. Like the adults, sponge developmental stages associate with a remarkable diversity of bacteria that collectively includes 7794 unique taxa from 40 phyla (Fig. 5A; Additional file 1: Table S2). Any given sponge species associates with between 16 and 272 unique taxa from 4 to 35 phyla. This includes the

Proteobacteria (56.3%), Chloroflexi (9.8%), Acidobacteria (7.6%), Actinobacteria (5.2%), Cyanobacteria (4.0%), and Firmicutes (3.6%) (Fig. 5A; Table S3-4). The developmental stages of HMA (14 species) and LMA (21 species) sponges associated with bacterial communities that were comparably similar in total taxa and phylogenetic diversity (*t*-test, observed ASVs:  $p = 0.474$ ; Faith's:  $p = 0.886$ ; Fig. 5B; Table S5-6). However, the bacterial communities of LMA sponge developmental stages were represented by only a few bacterial taxa while the bacterial communities of HMA sponge developmental stages were more evenly distributed (*t*-test, Mcintosh dominance:  $p = 0.005$ ; Fig. 5B; Table S5).

Two widespread phenomena of sponge-microbe symbioses are that hosts associate with a species-specific and lifestyle-specific microbiome [31, 33]. The developmental stages of HMA and LMA sponges are associated with bacterial communities that are compositionally distinct (PERMANOVA,  $p < 0.001$ ), providing the first demonstration of the HMA-LMA dichotomy in sponge developmental stages. Moreover, the developmental stages of sponges generally, but not always, have a species-specific bacterial community (PERMANOVA,  $p < 0.001$ ; Fig. 5C; Table S7). Pairwise comparison of the 25 sponge species with at least two samples suggest that species specificity occurred in 73.7% (221/300) of the unique combinations (Fig. 5C; Table S7). An inconsistent species-specific signature occurred for 80% (20/25) of the studied sponge species, suggesting that the majority of these species associate with bacterial communities that are compositionally similar to the developmental stages of at least one other sponge. Despite an inconsistent species-specific signature, we compared host phylogeny (using the *18S* rRNA gene; Table S8) and bacterial dendrogram to determine if the symbiont communities covaried with the evolutionary history of the host. Consistent with adult sponges [122], there is topological congruency and, thus, a phylogenetic signal in the symbiont communities of sponge developmental stages (Robinson-Foulds,  $p = 0.025$ ; normalized score 0.904; Fig. 5D; Table S9 [123]).

The diverse bacterial community that the developmental stage of sponges inherit is not composed solely of faithfully transmitted symbionts [44, 113]. Comparisons between adult and larval sponges estimate that larvae share ~45% of their bacterial taxa with the parents, implying that ~55% of the bacterial taxa associated with sponge larvae are acquired horizontally. Moreover, a portion of the vertically transmitted symbionts are microbes that adults acquired from the seawater and subsequently incorporated into the development stages. Patterns of transmission is also inconsistent between siblings, as larvae within a clutch share a small set of identical bacterial taxa (~17%) [89, 113]. Therefore, while adult sponges





seed the next generation, there is a degree of stochasticity that has led others to hypothesize that marine sponges exhibit a “leaky” vertical transmission [63, 113]. High taxonomic variability yet functional stability is increasingly common for holobionts that associate with diverse microbial communities [124, 125]. We hypothesize that microbiome function is stable across the sponge life cycle and between generations. This hypothesis remains untested because the meta-omic techniques used on adult sponges have yet to be applied to their developmental stages [30, 126–129].

The vertical transmission of microbial symbionts is a widespread phenomenon among marine sponges. Our understanding of this biological feature stems from taxonomic, geographical, and ecological biases, but, nevertheless, there is a clear spatial coordination during development (Figs. 3 and 4). For a sub-set of these sponge species, we know which symbionts serve as the inoculum for the next generation but not why they are transmitted. The assumption is that there are functional links to the biology and ecology of the dispersing larvae, but data supporting these hypotheses are currently scarce. Microbial symbionts may provide complementary resources to the developing larva once it is no longer sheltered by the adult (Fig. 3P). This may include coping with the dynamic oceanographic environment and seeking out a suitable location to settle and undergo metamorphosis. These are the focal point of the next section: to describe how the microbial communities associated with sponge larvae influence life in the plankton, how these symbiont communities are influenced by the environment, and how they may be key to transition back to the seafloor.

## Life in the plankton

### Pelagic larvae

Marine invertebrates use environmental cues to time reproduction and the release of their eggs, sperm, embryos, and/or larvae to increase fertilization success, dispersal, and food availability [71, 130, 131]. Sponges exhibit reproductive synchrony with annual or bi-annual spawning events, where offspring are released from the excurrent flow of a female sponge in the hours following sunrise or proceeding sunset during particular days or weeks [50, 51, 132–134]. Alternatively, some tropical and subtropical demosponges release small quantities of larvae throughout the year [50, 135]. Upon being released, sponge larvae are between 100  $\mu\text{m}$  and 6 mm in length (i.e., microplankton or mesoplankton), may swim between 0.1 and 1 cm per second, and most have neutral or positive buoyancy [50, 136, 137].

Living in the water column comes with an added mortality risk. The five main sources of mortality for marine invertebrate larvae are temperature, nutritional

conditions, dispersal, predation, and metamorphosis [41, 138, 139], but dysbiosis should also be considered [140]. Sponge larvae are non-feeding (lecithotrophic) and spend only a few hours to a few days in the plankton (anchiplanic). Therefore, they are unaffected by external food availability (but not internal nutritional condition) and are less susceptible to being transported off the continental shelf. Mortality due to predation is also likely to be minimal because sponge larvae are briefly in the water column and because they appear to have chemical defenses that make them less palatable [141, 142]. Chemical defenses are symbiont-derived for larvae of the bryozoan *Bugula neritina* [143, 144], and this may also occur in sponges because they are a group rich in secondary metabolites with defensive roles (e.g., [145]). These defenses combined with a short pelagic duration may make sponges favorable for vertical transmission because the microbial cells are less likely to be wasted as a result of larval mortality.

Sponges are released as nearly competent or competent larvae that typically spend minutes to a couple weeks in the water column. Marine invertebrate larvae use a hierarchical suite of ecological signals to locate a suitable site to settle and undergo metamorphosis [50, 134, 146–149]. In the brief hiatus from the benthos, sponge larvae can face substantial local variation in temperature, salinity, and light that influence their dispersal through disruptions in development, locomotion, and behavior [149, 150]. If these ecological variables cause a prolonged larval duration and a delay in metamorphosis, then juvenile fitness will likely be compromised [136, 151]. These same ecological factors may influence animal microbiomes and, in particular, those of marine invertebrate larvae [152, 153]. Larvae from other groups of marine invertebrates horizontally acquire microbes from the seawater in response to variation in abiotic variables [154]. It is estimated that ~55% of the microbial taxa associated with sponge larvae are acquired horizontally [44, 113], but very few experiments have determined whether these horizontal acquisitions also aid in coping with environmental variation.

As human behaviors disproportionately influence global climate, sponge larvae now face an acidified sea with more frequent and prolonged heatwaves that may ultimately compromise their survival, dispersal, and settlement success [155–158]. However, little is known about how these rapid environmental changes affect sponge larval holobionts and their capacity to cope through horizontal acquisitions. Most studies to date have either used *Rhopaloeides odorabile* or *Carteriospongia foliascens*, two sponge species from the Great Barrier Reef. Larvae of these sponges are highly resistant to both acidification and warming, such that an elevated

mortality is barely detectable under predicted end-of-the-century conditions [159–162]. These sponge species exhibit taxonomic shifts in their bacterial community by horizontally acquiring microbes in response to temperature as well as crude oil [161, 163]. If and how microbial symbionts influence the larval life stage is unknown, but these pieces of information may be fundamental to predicting the success of sponge larvae in future climatic scenarios.

### Settlement and metamorphosis

As marine invertebrate larvae near competency for metamorphosis, they are guided towards the seafloor by environmental cues and behavioral shifts that are not yet well-understood. Larvae of some species become denser and negatively phototactic, allowing them to sink through the water column, enter the benthic boundary layer, and explore settlement cues [50, 164]. However, this transition cannot occur without the input of specific hormones and/or other signaling compounds that may be acquired exogenously [165, 166] or from symbionts that are both vertically transmitted and metabolically active [45]. One example of the latter is the demosponge *Amphimedon queenslandica*. This sponge requires nitric oxide signaling to stimulate larval settlement, but the host is unable to biosynthesize arginine [167, 168]. Instead, arginine is produced by vertically transmitted bacteria, taken up by the larval cells, and incorporated into the arginine-citrulline loop to produce nitric oxide [169–171]. Metabolic complementation between host and symbiont(s) is widespread [62, 172], but *A. queenslandica* is unique in that this principle extends to a major developmental and life cycle transition [171]. We hypothesize that the symbiont incorporation of signaling that enables larval holobionts to undergo metamorphosis is more widespread among marine invertebrates. Such symbioses may stimulate settlement, but they can only be truly beneficial if the correct benthic substrate is found [173].

The most widespread settlement cue to induce metamorphosis for marine invertebrate larvae are biofilms, complex assemblages of microorganisms that coat most surfaces in the sea [173–177]. Larvae of some marine invertebrates (e.g., the polychaete worm *Hydroides elegans*) must come in contact with a specific bacterium (i.e., *Pseudoalteromonas luteoviolacea*) in the biofilm. In this case, *P. luteoviolacea* uses a contractile injection system to induce the settlement of *H. elegans* [174, 178–180]. Our present understanding is that sponge larvae locate a biofilm or conspecific cue to undergo metamorphosis [147, 173, 176]. These responses may be because sponge larvae often have a short planktonic life, and thus, being selective is less advantageous. Alternatively, specific bacterial strains on the seafloor may induce metamorphosis

and involve select mechanical triggers [174, 178, 179]. These details remain unresolved in marine sponges.

Metamorphosis involves an extensive reorganization and transdifferentiation of larval cells into adult structures [181–185]. This transition takes hours to days and can be accompanied by a major reconstruction in the associated bacterial community [117, 169]. For example, *Carteriospongia foliascens* has a stable bacterial community throughout this transition, while that of *A. queenslandica* changes drastically due to an influx of environmentally derived bacteria [160, 169]. These associations are short-lived for *A. queenslandica*. Juveniles upregulate genes involved in innate immunity (e.g., scavenger receptors) and host-microbe crosstalk (e.g., ankyrins) that allow for the vertically inherited symbionts to replace these environmental bacteria as the sponge reaches adulthood [169]. The shift away from resident members of the symbiont community would appear to be maladaptive and this interpretation may simply be due to our limited understanding of metamorphosis in sponges.

### Post-metamorphosis

For species that did not inherit a full collection of necessary symbionts for life on the benthos, the return to the adult microbiota requires bacterial taxa to be acquired post-metamorphosis via horizontal transmission. The best example of this comes from *Petrosia ficiformis*, an HMA demosponge that does not vertically transmit symbionts to the developmental stages, but associates with archaea and bacteria as adults [73, 103]. The adults are postulated to attract and accumulate microbes from the seawater on its external surface by unknown mechanisms (Fig. 1). Epithelial cells then leave the epithelium to enter the mesohyl (Fig. 1), while folding over itself to form a large extracellular pocket that encloses the symbionts. The resulting mesohyl cell is known as a pocket bacteriocyte (Figs. 1 and 3D) [18]. These are atypical bacteriocytes, in which microbes remain in the extracellular space but still “under control” within the cell pocket. This is in contrast to conventional bacteriocytes in other sponges that host symbionts in true endocyttoplasmic vesicles (Figs. 1 and 3C, D).

The mode of horizontal transmission described above utilizes “pocketing”—but not endocytosis—of microbes by epithelial cells. Alternatively, symbionts may be acquired from the environment by squeezing between epithelial cells. Sponges have epithelia that are maintained through simple non-tight junctions, allowing a variety of cellular materials (e.g., sponge cells, compounds, and microbes) to enter or exit the mesohyl. Laboratory experiments indicate that microbes may immigrate from the seawater to the mesohyl by squeezing between the epithelial cells [16]. This squeezing is a type



of horizontal acquisition that may explain how sponges larvae and juveniles that do not vertically transmit symbiont end up having microbes as the adults. Moreover, “pocketing” may serve as one of the mechanisms used by sponges to exhibit a “leaky” vertical transmission [63, 113].

Our understanding of sponges, their microorganisms, and the transmission of symbionts is deeply rooted in comparative biology (e.g., [35]), and this has allowed for an extensive catalog of microbiome compositions [31], gene inventories [30], and expression profiles [128] to be established in the adults. However, establishing experimental (model) systems often lags when a broad understanding of a given process is first described across multiple species. This is particularly true in marine sponges [8] and their developmental stages, where a single or small set of experimental systems have yet to emerge; the tropical demosponge *A. queenslandica* is currently the frontrunner. In the following section, we outline three groups of techniques that we feel should be established in sponges and their developmental stages to transition research efforts towards characterizing the functional mechanisms of symbiont transmission during reproduction and development.

### Towards the function of developmental symbionts

Experimentally tractable systems in animal-microbe research generally require several desirable properties [8, 186–188]. Establishing a wide array of techniques and accessible genomic data has only been achieved for several animal species, which collectively represent a slim portion of the biological world. Notable advancements have been made on “non-model” animals as holobiont and metaorganism research has popularized, and this is particularly evident in marine sponges [8, 189, 190]. However, there are three primary reproduction and development-focused obstacles that should be overcome for this system to become more experimentally tractable. Specifically, reproduction must be reliably induced in the laboratory, individuals must be amenable to gnotobiotic conditions, and molecular and multi-omic techniques must be developed for host and symbiont.

### Reproduction in the laboratory

Marine invertebrates from most phyla can be reliably induced to reproduce and their offspring can be cultured under laboratory conditions [191, 192]. For example, echinoderms that are collected during their reproductive period can be spawned by an intracoelomic injection of potassium chloride [191–193], and spawning frequency can be manipulated by controlling both temperature and photoperiod [194]. This is also true for the sea anemone *Nematostella vectensis*, which may spawn weekly when

provided the proper dietary conditions and photoperiod-temperature combination [195, 196]. Similarly, sponges from a number of genera exhibit predictable and synchronous reproductive events in response to an increase in temperature and a lengthening of the photoperiod (e.g., [76, 197–199]). This reliability has yet to translate to the laboratory. In order to bridge this gap between field and laboratory conditions, we suggest that the thermal regime and photoperiod of spawning events to be characterized for species of interest, and for these conditions to be replicated and manipulated in the laboratory.

### Germ-free sponge larvae

An overarching goal in animal-microbe symbiosis research is to determine the suite of host life-history functions that are impacted by the associated microbial community. An effective way of doing this is by establishing germ-free (axenic) animals, but this is notoriously difficult. For example, it took more than a decade to reliably produce germ-free *Hydra* [200]. Gnotobiotic individuals, where the reduction of the bacterial community is quantified, are more obtainable with the “non-model” animals that disproportionately populate holobiont and metaorganism research. Techniques to generate gnotobiotic marine invertebrate embryos and larvae are limited to the sea anemone *Nematostella vectensis* [201], the sea urchin *Strongylocentrotus purpuratus* [202], and the tunicate *Ciona intestinalis* [203].

The commonalities between these systems are that the developmental stages or early juveniles were incubated in an antibiotic cocktail and subsequently cultured in sterile artificial saltwater, but natural seawater that was fine-filtering and incubated overnight at high temperatures that maintain seawater chemistry can also be used [204]. These protocols have been adopted from the freshwater polyp *Hydra vulgaris* [205] and the zebrafish *Danio rerio* [206], which may collectively serve as a baseline for generating gnotobiotic or germ-free sponge larvae. Preliminary attempts to make the Caribbean sponge *Ectyoplasia ferox* germ-free were promising, as the bacterial community of antibiotically treated larvae—that are contained in a gelatinous sheet rather than disappearing in the open ocean—was significantly reduced but not eliminated [45].

### A molecular toolkit

Much of this synthesis has addressed sponge-symbiont dynamics throughout reproduction and development, but the functional benefits of these partnerships remain largely unknown. Answers to these questions require function-oriented techniques. For example, metagenomic (e.g., [126, 129, 207, 208]) and metatranscriptomic (e.g., [127, 128, 209]) protocols, which are regularly applied to the benthic adults, have yet to be adopted to

sponge larvae. The primary bottleneck inhibiting these techniques from being applied to larvae is that they produce insufficient template quantity for sequencing. However, sequencing and nucleic acid extraction sensitivity have advanced substantially in recent years, and technologies maximizing sequencing depth should overcome this barrier, allowing for both symbiont functional potential and activity in the developmental stages to be determined. Establishing these protocols would also afford the opportunity to determine if viruses are vertically transmitted, characterize virome diversity, composition, and structure, and how these relate to the bacterial population [106–109].

The mechanisms underpinning sponge-symbiont interactions during transmission and embryonic development cannot be determined solely by meta-omics, as these techniques only provide snapshots into the biology of sponge holobionts. Meta-omics techniques should be complemented with tools to genetically manipulate both host and symbiont (e.g., CRISPR/Cas9 [210–212]), track host- and symbiont-derived products over time [35, 171], and novel visualization techniques to localize processes of both live and fixed individuals [111, 213]. This information may serve as the basis to use mathematical and computational modeling (e.g., integrated network analysis) to interpret interactions between sponge developmental stages and their symbionts as well as between symbionts [214, 215]. Lastly, this array of techniques can be applied across environmental gradients and over the course of generations to assess hologenomic acclimation and adaptation in vivo [126, 160, 216].

### Developmental symbioses beyond sponges

Coordination between the developing animal host and symbiont community is not unique to marine sponges, as these associations are common among marine and terrestrial animals. The eggs, embryos, and larvae of diverse marine invertebrates (e.g., annelids [217], bivalves [218, 219], bryozoans [143, 220], cnidarians [221, 222], crustaceans [223, 224], echinoderms [152, 225], and gastropods [226]), all associate with microbial symbionts that are interconnected with the holobiont during development [154, 217, 227]. For example, bacteria reside in the external hyaline layer of sea urchin embryos and flood the maturing larval gut lumen once it becomes active [202, 228], where they are able to influence various aspects of aspects of host biology and ecology [154, 225, 227].

Nearly 60 years of research on marine sponges demonstrates that reproduction, development, and symbiont transmission have been deeply rooted for >600 million years [6, 55]. As such, it is increasingly apparent that the internalist view of animal development—where an organism is a mere product of self—will continue to

fade as the symbiotic influence comes into focus [229–231]. The ultimate goal in developmental symbiosis of marine sponges, or elsewhere, is not who but why. This—the why—must take center stage in the decades to come because understanding how these interactions and their transmission influence development and the phenotype remain the future of developmental biology.

### Conclusions

In this Review, we have described the general patterns of symbiont transmission in marine sponges during reproduction, development, and metamorphosis. Here, we highlight 5 key points from this Review:

- (1) Vertical transmission of microbial symbionts through the developmental stages is widespread but not universal, with the majority of species being demosponges from coastal waters of the Mediterranean Sea, Northwestern Atlantic Ocean, and Caribbean Sea.
- (2) The developmental stages of marine sponges form symbioses with archaea, bacteria, and various eukaryotes (e.g., *Symbiodinium* spp.), with bacteria being the most common and abundant. It remains unknown if viruses are vertically transmitted, but we suspect they are.
- (3) Symbionts are transmitted to oocytes, embryos, and larvae, with transference beginning at the onset of vitellogenesis and continuing during embryonic cleavage as well as while larvae develop externally.
- (4) The developmental stages of sponges associate with 10s to 100s of bacterial taxa from upwards of 40 different bacterial phyla and do not always comprise a species-specific microbiome, and species with the HMA and LMA lifestyles have microbiomes distinct in composition. This provides the first demonstration of the HMA-LMA dichotomy in the developmental stages.
- (5) Little is known about the functional roles of the symbionts associated with the developmental stages and we propose that tools for meta-omic and genetic manipulation of host and symbionts, gnotobiotic culturing of the developmental stages, and crosstalk techniques need to be established.

### Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12915-022-01291-6>.

**Additional file 1: Table S1.** Sponge species with known relations to microorganisms during development and corresponding characteristics.

**Table S2.** Sponge developmental stages microbiome sampled used in meta-analysis. **Table S3.** Alpha diversity measures for each sponge developmental stages. **Table S4.** Relative abundance of bacterial phyla for all species of sponge developmental stages. **Table S5.** Summary of statistical tests for alpha diversity between HMA and LMA sponges. **Table S6.** Sponge species with profiled bacterial communities and known as an LMA or HMA. **Table S7.** Summary of PERMANOVA results for compositional comparisons between sponge species and symbiont life-style. **Table S8.** GenBank accession numbers for 18S rRNS sequences used in gene tree as part of phylosymbiosis. **Table S9.** Statistical tables for testing phylosymbiosis across geminate species pairs. **Table S10.** Average alpha rarefaction values for each species based on observed ASVs and phylogenetic diversity [232–267].

**Additional file 2.** The bioinformatic commands used to process and analyze the 16S rRNA sequences that are presented in Figure 6.

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### Authors' contributions

T.J.C. conceived this study, performed meta-analyses, and drafted and edited the manuscript; M.M. performed a meta-analysis, electron microscopy, and drafted and edited the manuscript; L.S. drafted and edited the manuscript; L.P. drafted and edited the manuscript; T.C.G.B. drafted and edited the manuscript; and U.H. conceived this study and edited the manuscript. The authors have read and approved the final manuscript.

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### Availability of data and materials

All data used in our 16S rRNA meta-analysis can be found in Additional file 1: Tables S2 and S8.

### Declarations

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

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