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**Original Article**

# Distinct development trajectories and symbiosis modes in vent shrimps

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## Abstract

Most animal species have a singular developmental pathway and adult ecology, but developmental plasticity is well-known in some such as honeybees where castes display profoundly different morphology and ecology. An intriguing case is the Atlantic deep-sea hydrothermal vent shrimp pair *Rimicaris hybisae* and *R. chacei* that share dominant COI haplotypes and could represent very recently diverging lineages or even morphs of the same species. *Rimicaris hybisae* is symbiont-reliant with a hypertrophied head chamber (in the Mid-Cayman Spreading Centre), while *R. chacei* is mixotrophic with a narrow head chamber (on the Mid-Atlantic Ridge). Here, we use X-ray micro-computed tomography and fluorescence in situ hybridization to show that key anatomical shifts in both occur during the juvenile–subadult transition, when *R. hybisae* has fully established symbiosis but not *R. chacei*. On the Mid-Atlantic Ridge, the diet of *R. chacei* has been hypothetically linked to competition with the obligatorily symbiotic congener *R. exoculata*, and we find anatomical evidence that *R. exoculata* is indeed better adapted for symbiosis. We speculate the possibility that the distinct development trajectories in *R. hybisae* and *R. chacei* may be determined by symbiont colonization at a "critical period" before subadulthood, though further genetic studies are warranted to test this hypothesis along with the true relationship between *R. hybisae* and *R. chacei*.

**Keywords:** development, phenotypic plasticity, symbiosis

## Introduction

Each animal species is generally constrained to a single mode of ontogenetic development, leading to individuals (of the same sex) exhibiting similar ecology at each life stage. Nevertheless, there are some well-documented cases where individuals of the same species can develop into profoundly different forms with distinct "ways of life." Castes of eusocial insects are a well-known example, where nutritional input typically has a key role in determining the developmental outcome. Whether a female larva of the honeybee *Apis mellifera* develops into worker or queen, for example, is almost entirely dependent on if it is fed a large quantity of royal jelly (Schwander et al., 2010). Another example is the cave fish *Astyanax mexicanus*, whose morphology differs greatly among individuals living in rivers and surrounding caves; cave morphotypes have lost their eyes and show reduced pigmentation, but have larger body sizes and other sensory organs (McGaugh et al., 2014). At hydrothermal vents, a significant case is the *Ridgeia piscesae* tubeworm, which develops into a "short-fat" or a "long-skinny" morphotype depending on the vent-flow regime (Tunnicliffe et al., 2014; Urcuyo et al., 2007).

The formation of symbioses, intimate and long-term biological association between two or more organisms (McFall-Ngai et al., 2013), is also a powerful evolutionary force that can have fundamental impacts on the developmental processes and ecology of the host animal (Bosch & McFall-Ngai, 2021; Carrier & Bosch, 2022; Hammer & Moran, 2019). For example, the bobtail squid *Euprymna scolopes* houses the bioluminescent symbiont *Vibrio fisheri* for counter-illumination in its light organ, which requires signaling molecules from the symbiont in its morphogenesis (Nyholm & McFall-Ngai, 2021). Deep-sea hydrothermal vent ecosystems are unusual in that intimate symbiotic relationships between chemoautotrophic bacteria and endemic invertebrate animals form the basis for the entire system (Dubilier et al., 2008). Vent holobionts usually acquire their symbionts from the environment after they settle in the vent field as juveniles, with the exception of vesicomyid clams that have maternal inheritance of chemosynthetic symbionts (Ikuta et al., 2016). As significant transitions in ecological niche within the life cycle of a species often involve metamorphosis, where conspicuous anatomical and physiological shifts occur, symbiont acquisition is often linked to such metamorphosis involving the loss or the reduction of the digestive organs and an enlargement of the symbiont-hosting structures (Bright & Bulgheresi, 2010; Nussbaumer et al., 2006; Wentrup et al., 2014). These reconfigurations of internal organs post-settlement are not necessarily apparent from the external morphology, as seen with the "cryptometamorphosis" of *Gigantopelta* vent snails where the symbiont-hosting "trophosome" suddenly expands

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internally (Chen et al., 2018), or the straightening of the digestive tube in bathymodiolin mussels (Franke et al., 2021).

Among vent animals, alvinocaridid shrimps exhibit a wide range of feeding strategies and reliance on symbiosis. A strict dependence on symbiosis is found in two closely related species, *Rimicaris exoculata* and *R. kairei*, hosting dense communities of filamentous bacterial symbionts inside their expanded "head" (the cephalothorax) that provide most of their diet (Guri et al., 2012; Methou, Hikosaka, et al., 2022; Ponsard et al., 2013; Zbinden et al., 2004). These symbiotic species exhibit striking morphological differences from other alvinocaridid shrimps, with the enlargement of their symbiont-hosting cephalothoracic cavities and mouthparts starting during the post-settlement metamorphosis between juveniles and adults (Guri et al., 2012; Methou et al., 2020). Conversely, several other species of vent shrimps such as *Nautilocaris saintlaurentae* and *Rimicaris variabilis* harbor few to no symbiotic bacteria and instead feed on other food sources such as bacterial mats or detritus (Gebruk et al., 2000; Methou et al., 2023).

One species complex of alvinocaridid shrimps from Atlantic vents—the *Rimicaris hybisae* and *R. chacei* complex—is exceptional as they share dominant haplotypes in genetic barcodes (*COI*: Figure 1; H3, 28S, 16S, 18S: Supplementary Figure S1) but exhibit drastically distinct adult morphologies and occupy disparate ecological niches (Plouviez et al., 2015; Versteegh et al., 2022). *Rimicaris hybisae* occurs in the Mid-Cayman Spreading Centre (MCSC), has an external morphology resembling *R. exoculata* with an inflated cephalothorax (Nye et al., 2012; Streit et al., 2015). It appears to still retain an ability to feed on alternative sources outside of its symbiosis, with evidences of facultative carnivory/scavenging in individuals distributed at the periphery of vent fluid emissions (Versteegh et al., 2022). In contrast, *R. chacei* from the Mid-Atlantic Ridge (MAR) co-occur with *R. exoculata* (Methou, Hernández-Ávila, et al., 2022) and lacks an enlarged cephalothorax; it has a mixed diet where it also feeds on bacterial mat and scavenges in addition to some contribution from symbiosis (Apremont et al., 2018; Methou et al., 2020). Though indistinguishable based on barcodes available at present (Figure 1; Supplementary Figure S1), these are still limited and it remains inconclusive whether *R. chacei* and *R. hybisae* represent recently diverged lineages or morphs of a single species (Teixeira et al., 2013)

Given their close genetic relationship, the morphology of *R. chacei* and *R. hybisae* adults could result from: (1) a recent allopatric speciation in distinct geographical areas if gene flow has ceased between the two, or (2) developmental plasticity, potentially triggered by different environmental conditions. It has been hypothesized that juveniles of *R. chacei* are competitively excluded from the vigorously venting environment by *R. exoculata* of similar life-history stages (Methou, Hernández-Ávila, et al., 2022). This contrasts with the situation at MCSC where *R. hybisae* is the only shrimp species dominating similar venting areas (Nye et al., 2012). Thus, we hypothesize that this environment may be necessary during the juvenile phase for the development into the symbiont-reliant *R. hybisae* adult.

Anatomy and food source are closely linked in animals, and organ volumetric is a useful estimator of feeding ecology for

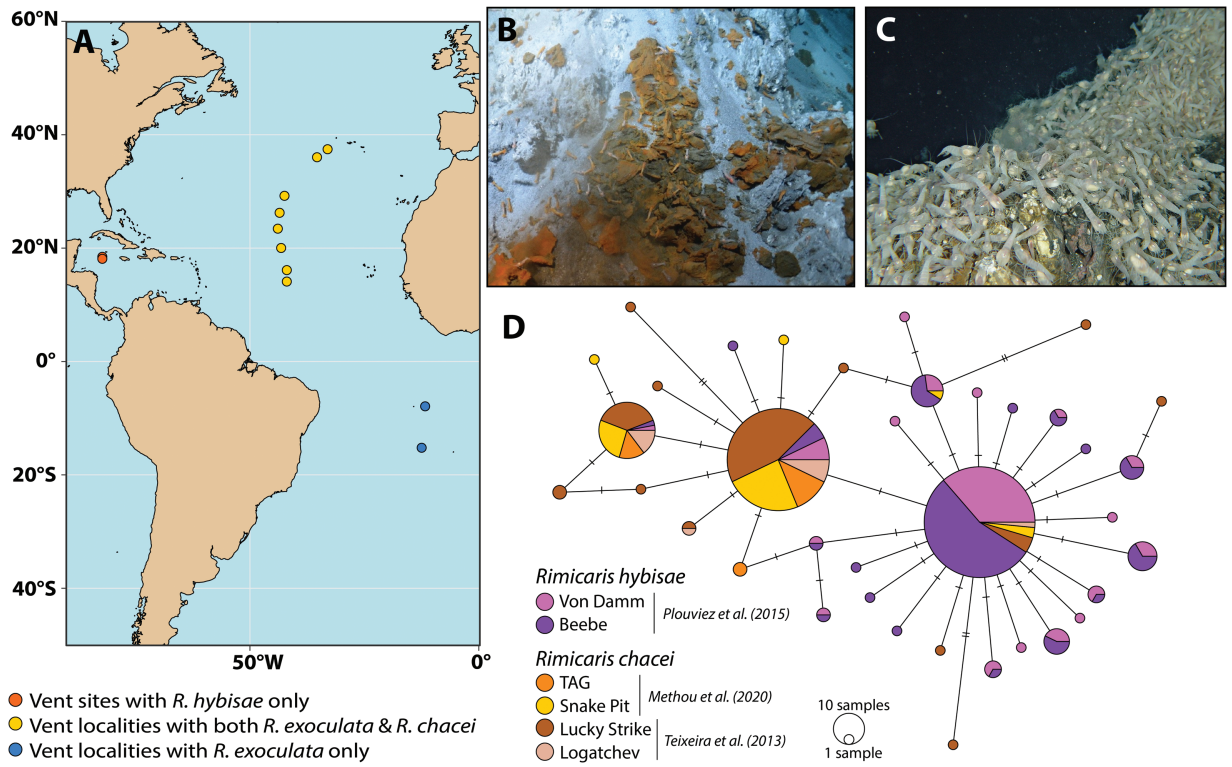


**Figure 1.** Geographic context and genetic relationship of *Rimicaris hybisae* and *Rimicaris chacei*. (A) Distribution of *Rimicaris* shrimps in hydrothermal vent fields within the Atlantic Ocean. (B) Peripheral habitat with scattered adults of *R. chacei* shrimps (TAG vent field, BICOSE 2). (C) Active venting habitat with dense aggregates of *R. hybisae* shrimps. (D) *COI* haplotype network obtained with a median-joining method in PopART (Leigh & Bryant, 2015) from published sequences of *R. chacei* (Methou et al., 2020; Teixeira et al., 2013) and *R. hybisae* (Plouviez et al., 2015). Sizes of colored circles indicate relative haplotype frequencies.

hydrothermal vent species where detailed in situ studies are difficult (Chen et al., 2022). Until now, no direct comparisons in the anatomy, ontogenetic changes, and symbiont acquisition between *R. hybisae* versus *R. chacei* have been made, or how they compare to the obligatorily symbiotic *R. exoculata*. In order to shed light on our hypothesis, we combine quantitative 3D anatomy by X-ray micro-computed tomography (µ-CT) and fluorescence in situ hybridization (FISH) to answer three questions: (1) at which life stage does the split in development trajectories occur between *R. hybisae* and *R. chacei*, (2) whether developmental trajectory is correlated with symbiont acquisition, and (3) if there is anatomical evidence that *R. chacei* would indeed be outcompeted by *R. exoculata*.

## Methods

Specimens of the three main target shrimp species—*R. exoculata*, *R. hybisae*, and *R. chacei*—were collected at hydrothermal vent fields during several sea-going expeditions: *R. exoculata* and *R. chacei* were collected at the Trans-Atlantic Geotraverse (TAG) and Snake Pit vent fields during the BICOSE 2 (R/V *Pourquoi pas?*, 2018, doi: https://doi.org/10.17600/18000004) expedition using suction sampler of the human-occupied vehicle (HOV) *Nautile*; *R. hybisae* were collected at the Von Damm and Beebe vent fields during the expeditions JC82 (RRS *James Cook*, 2013) and YK13-05 (R/V *Yokosuka*, 2013), respectively, with suction samplers on the remotely operated vehicle (ROV) *Isis* or HOV *Shinkai 6500*. In addition, we also studied the anatomy of *R. kairei* to confirm that it does not differ significantly from that of its closest relative, *R. exoculata* using specimens collected from the Kairei and Edmond vent fields during expeditions YK09-13 (R/V *Yokosuka*, 2009) and YK16-E02 (R/V *Yokosuka*, 2016) using suction samplers mounted on the HOV *Shinkai 6500*. Shrimps were preserved in 10% formalin or 99% ethanol and measured with vernier calliper using carapace length (CL) for standardized measurement, upon recovery on-board.

Individuals were identified to their life stages, according to Methou et al. (2020). Subadults of all species were characterized as small individuals morphologically similar to adults with sizes below the onset of sexual maturity, whereas stage A and stage B juveniles of *R. exoculata* were distinguished by the morphology of their eyes (Methou et al., 2020). Given that *R. chacei* juveniles settle and begin their anatomical transitions at smaller sizes than *R. exoculata*, their adult stages were separated into two categories: "small adults" of similar sizes to that of *R. exoculata* juvenile stages (CL = 8–10 mm) and "adults" (CL = 14–16 mm). Life stages of *R. kairei* were defined following the established nomenclature for *R. exoculata* and the same was done for *R. hybisae* based on *R. chacei*. Additional subadult specimens of *R. exoculata*, *R. chacei*, and *R. hybisae* were fixed in 3% paraformaldehyde for 3 h on-board, rinsed with phosphate-buffered saline (PBS)/sterile seawater buffer, and stored in 50:50 2× PBS/ethanol at –20 °C for FISH analyses (Supplementary Table S1).

A total of 47 individuals, including a triplicate of each life stage for the four shrimp species (except where only two individuals of stage A juveniles were available for *R. hybisae*), were used for X-ray µ-CT scanning. Shrimps preserved in 99% ethanol were progressively rehydrated in Milli-Q water in four steps (25:75, 50:50, and 75:50 Mili-Q:ethanol), and then stained for 24 h in 0.05 mol/L iodine solution. Scanning was performed using a ScanXmate-D160TSS105 (Comscantecno, Japan) commercial µ-CT at 60–90 kV/25–92 µA, with a resolution of 10.904–49.587 µm per pixel, at 992 × 992 pixels resolution per slice (for details see Supplementary Table S2). Images obtained were imported into the specialist software Amira version 2022 (Thermo Fisher) for visualization and manual segmentation of the different organs of interest. This included the symbiont-hosting branchiostegite (i.e., wall of the cephalothoracic gill chamber) and mouthparts (scaphognathites and exopodites), as well as the digestive system (stomach, digestive tube, and hepatopancreas). Only the anterior part of the cephalothoracic cavities was reconstructed, since posterior components facing the gills are known to be not colonized by bacterial symbionts (Apremont et al., 2018; Methou, Hikosaka, et al., 2022; Zbinden et al., 2004). Prior to smoothing, organ volumes and surface areas were calculated also in Amira based on the segmentation. As pereopods and antennae were often fully or partially broken, these parts were removed from all our 3D reconstructions and thus the measurement of total body volumes and surface areas (see Supplementary Table S3 for detailed body volumes and surface areas measurements and Supplementary Material for 3D interactive models). The final surface renderings used for the figures were generated post-smoothing and complexity reduction. Cephalothoracic cavities of five specimens at each life stage of *R. chacei* and *R. hybisae* (except where only two individuals available for stage A juveniles of *R. hybisae*) were also dissected to observe the distribution of bacteriophore setae on their mouthparts. Mouthparts of one *R. exoculata* adult were also dissected and observed. As the anatomical features and patterns seen in *R. kairei* generally matched *R. exoculata*, for simplicity and clarity we only displayed the three main study species in the main figures and the *R. kairei* data are figured in Supplementary Figures S2–S4.

For FISH analyses of subadult symbiont colonization, the cephalothorax of *R. exoculata* and *R. chacei* subadults were progressively dehydrated in a PBS–ethanol series (ambient temperature) before embedding in polyethylene glycol distearate-1-hexadecanol (9:1) resin (Sigma, St. Louis, MO). Resin blocks were stored in –20 °C until trimming into 10-µm sections with an RM 2255 microtome (Leica Biosystems, Nussloch, Germany). Sections were placed on adhesive glass slides (Menzel-Gläser Superfrost® Plus), residues of resin were removed prior to hybridization with three baths of 96% ethanol for 5 min each and tissues were partially rehydrated with a bath of 70 ethanol. The cephalothorax of *R. hybisae* subadults were cut from the abdomens and rehydrated in 50:50 PBS–ethanol bath followed by 75:25 PBS–ethanol (30 min each) and then rinsed three times in 1× PBS buffer for 5 min each. Specimens were then progressively transferred to an optimum temperature compound (OCT) (Tissue-Tek; Sakura Finetek, Osaka, Japan): first in 15% sucrose–PBS, followed by 30% sucrose–PBS, and finally in 30% sucrose–PBS/OCT, for 1 h 30 min each, before embedding in OCT in a plastic holder at 4 °C. Sectioning of OCT-embedded specimens was done with a cryostat (CM1520; Leica Biosystems) in a chamber at –20 °C. Sections of 8-µm thickness were thaw-mounted on adhesive glass slides. Before hybridization, mounted sections were washed in 1× PBS two times for 5 min each, then post-fixed 1× PBS–10% formalin for 10 min, and finally washed again in 1× PBS for three times at 5 min each. These two different embedding and sectioning protocols have already been used for FISH experiments with *Rimicaris* shrimps and are

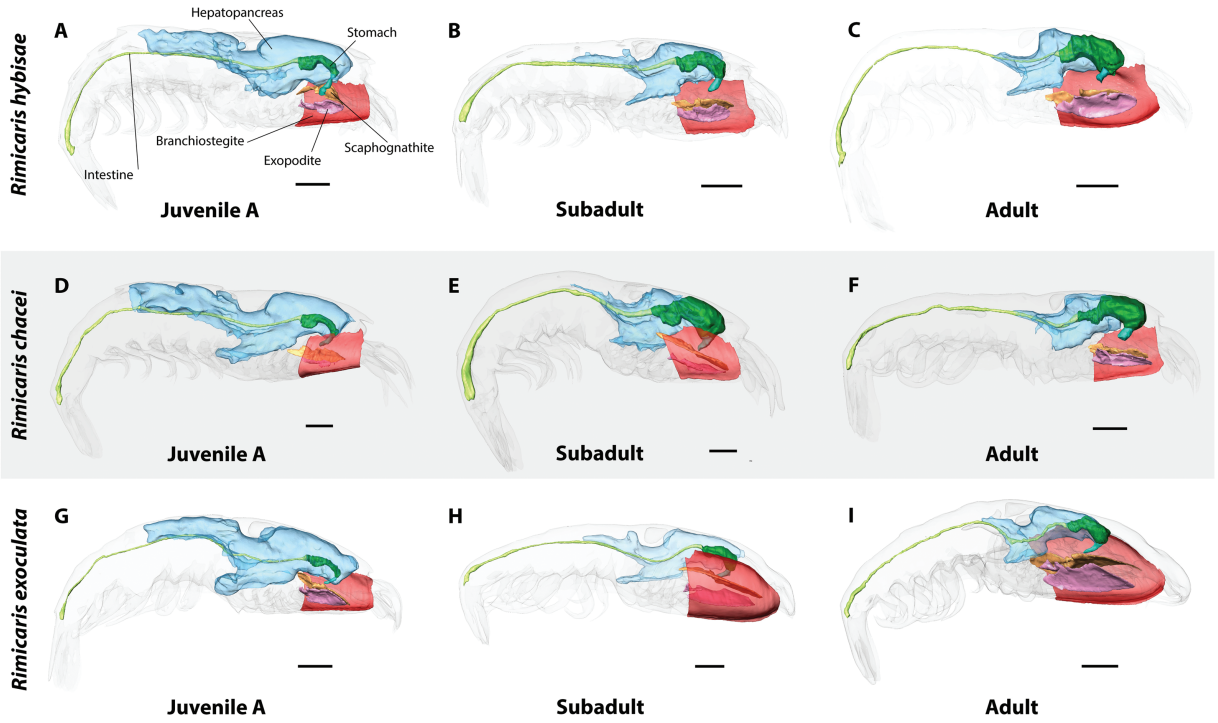


**Figure 2.** 3D anatomical reconstructions of symbiont-hosting organs (branchiostegite in red, scaphognathites in orange, and exopodites in pink) and digestive organs (stomach in dark green, hepatopancreas in blue, digestive tube in light green) in three *Rimicaris* shrimps across post-settlement ontogeny of (A–C) *R. hybisae*, (D–F) *R. chacei*, (G–I) *R. exoculata*.

not expected to impact the detection of symbionts (Guri et al., 2012; Methou, Hikosaka, et al., 2022).

For all species, sections were hybridized in a mix containing 0.5 mM (0.5 µM final concentration) of the Eub338 (eubacteria) probe in a 30% (*R. chacei* and *R. exoculata*) or 35% (*R. hybisae*) formamide hybridization buffer for 3 h at 46 °C following a published protocol (Guri et al., 2012). Sections were washed at 48 °C for 30 min in a washing buffer (Guri et al., 2012), rinsed briefly with Mili-Q water, air-dried, and covered with SlowFade Gold antifade reagent mounting medium containing DAPI (Invitrogen) and a cover slip. Observations of *R. exoculata* and *R. chacei* subadults were made using a Zeiss Imager.Z2 microscope equipped with the Apotome.2® sliding module and Colibri.7 light technology (Zeiss, Oberkochen, Germany) and processed with the Zen software (Zeiss). Observations of *R. hybisae* subadults were made using a Leica Stellaris STED confocal scanning microscope (Leica, Germany) and analyzed with the LAS X software.

Haplotypes networks were constructed with the median-joining method in POPART (Leigh & Bryant, 2015) using mostly sequences previously published and available in GenBank. For some individual, additional sequences of 16S, 28S, H3, and 18S genes were added (Supplementary Table S4). For these sequences, genomic DNA was extracted from muscle pieces with the DNeasy Blood & Tissue kit (Qiagen) following manufacturer's instructions. Polymerase chain reactions were conducted following (Aznar-Cormano et al., 2015) with the corresponding primers: 16-CariF and 16S-CariR for 16S; 28S-C1 and 28S-D2 for 28S; H3F1 and H3R1 for H3, and 18S-1F and 18S-5R for 18S.

## Results

Anatomy of *Rimicaris* species was reconstructed across four stages of development from recently settled juvenile to adult stages (Figure 2; Supplementary Figures S2 and S3). Early juvenile stages of all species had very small stomach volumes, ranging from 0.12 ± 0.01% of the total body volume in *R. exoculata* to 0.32 ± 0.04% in *R. chacei* (Figures 2 and 3). In adults, *R. chacei* and *R. hybisae* both possessed large stomach volumes representing 1.73 ± 0.84% and 2.18 ± 1.3% of their body volumes, respectively (Figures 2–4), whereas adult stages of *R. exoculata* had much smaller stomachs at 0.69 ± 0.32% of the body volume (Figures 2–4). However, their stomachs expanded differently during their transition from juvenile to subadult with a dramatic increase, although variable among individuals, up to 2.03 ± 0.99% right from the subadult stages of *R. chacei* while it was more gradual in *R. hybisae* with an increase up to 0.86 ± 0.09% in subadults and 0.77 ± 0.13% in small adults (Figures 2 and 3). On the other hand, the hepatopancreas followed a similar trajectory in the three species, occupying a very large volume in juvenile stages (*R. exoculata*: 18.6 ± 1.3%; *R. hybisae*: 17.2 ± 0.96%; *R. chacei*: 17.3 ± 4.0%) representing lipid reserves, and then decreasing rapidly when metamorphosizing into subadults (*R. exoculata*: 8.55 ± 1.93%; *R. hybisae*: 6.61 ± 1.07%; *R. chacei*: 8.02 ± 2.85%). No clear variation could be seen in the volumes of digestive tubes along the metamorphosis of the three species (Figure 2 and Supplementary Figure S4). Overall, the anatomical development of the *R. kairei* digestive system exhibited similar patterns as seen in *R. exoculata*, although with an earlier stomach enlargement in stage B juveniles (*R. kairei*: 0.60 ± 0.26%; *R. exoculata*: 0.15 ± 0.04%) as well as an earlier decrease of hepatopancreas volume (juveniles B; *R. kairei*: 8.08 ± 3.23%; *R. exoculata*: 18.6 ± 1.3%) (Supplementary Figures S3 and S4).

The symbiont-hosting branchiostegite (wall of the gill chamber) surface area was found to exhibit a sudden expansion in *R. exoculata* between juvenile B (1.96 ± 0.52% of total body surface) and subadult stages (3.72 ± 0.56% of the

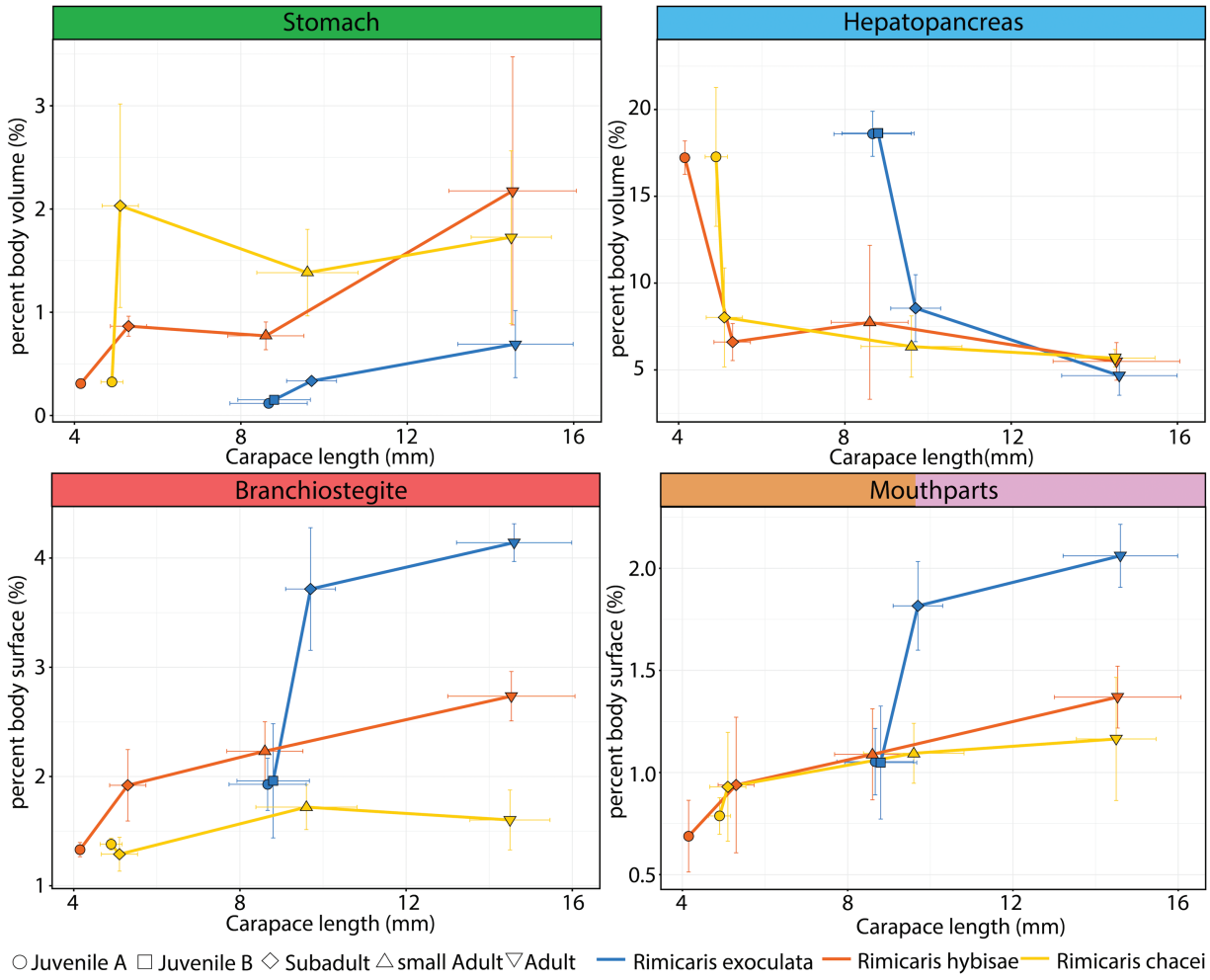


**Figure 3.** Relationship between body size (carapace length) and the relative percent body volumes or body surfaces of symbiont-hosting organs (branchiostegite, mouthparts) and digestive organs (stomach, hepatopancreas).

total body surface), and then up to 4.14 ± 0.17% in adult stages (Figures 2–4). Surfaces of other symbiont-hosting mouthparts—scaphognathites and exopodites—similarly expanded between juvenile B and subadult stages of *R. exoculata* from 1.05 ± 0.28% to 1.82 ± 0.22% of the total body surfaces (Figure 3). This pattern of branchiostegites and mouthparts expansion was also seen in *R. kairei* with rapid expansion before subadult, main differences being in *R. kairei* the expansion was also evident between juveniles A and B and the expansion being less and more variable in adults (see Supplementary Figures S3 and S4). The increase of branchiostegite surfaces was more rapid in *R. hybisae* between juvenile and subadult stages and more or less isometric thereafter, from 1.33 ± 0.06% of the total body surface in stage A juveniles to 1.92 ± 0.33% in subadults and 2.74 ± 0.23% in adults. Conversely, surfaces of *R. chacei* branchiostegites expanded only slightly along their ontogeny from 1.38 ± 0.05% to 1.60 ± 0.05%. For mouthparts, the increment was less marked than *R. exoculata* for both *R. hybisae* and *R. chacei*, with a limited growth of both mouthparts from 0.69 ± 0.18% to 1.37 ± 0.15% between stage A juveniles and adults in *R. hybisae* and 0.79 ± 0.09% to 1.16 ± 0.30% in *R. chacei* (Supplementary Figure S4). Dissection of the adult mouthparts between *R. hybisae* and *R. chacei*, however, showed a striking difference in the coverage of bacteria-hosting setae, being much less dense on *R. chacei* mouthparts than on *R. hybisae* and *R. exoculata* mouthparts (Figure 4). The dense setae would mean a much greater colonizable surface area in *R. hybisae* mouthparts than *R. chacei* (Figure 4). Our FISH observations of subadult specimens indicate that the three taxa differed in their complete bacterial colonization patterns (Figure 5). Although mouthparts of subadults were all well-colonized by filamentous bacteria in the three species, the branchiostegites structures of *R. exoculata* and *R. hybisae* subadults were densely colonized across the entire surface. On the other hand, the same structures in *R. chacei* was only partly colonized with large parts of the branchiostegites being devoid of filamentous bacteria.

## Discussion

Our results confirm that *R. hybisae* and *R. chacei* indeed display striking differences in anatomical development, despite the lack of genetic differentiation based on mitochondrial *COI* gene (Plouviez et al., 2015; Teixeira et al., 2013) and other available markers (Supplementary Figure S1) indicating they either diverged very recently or even represent morphs of the same species. The symbiont-hosting organs of *R. hybisae* increased in percentage surface area (as a fraction of the overall body surface) throughout growth with the fastest

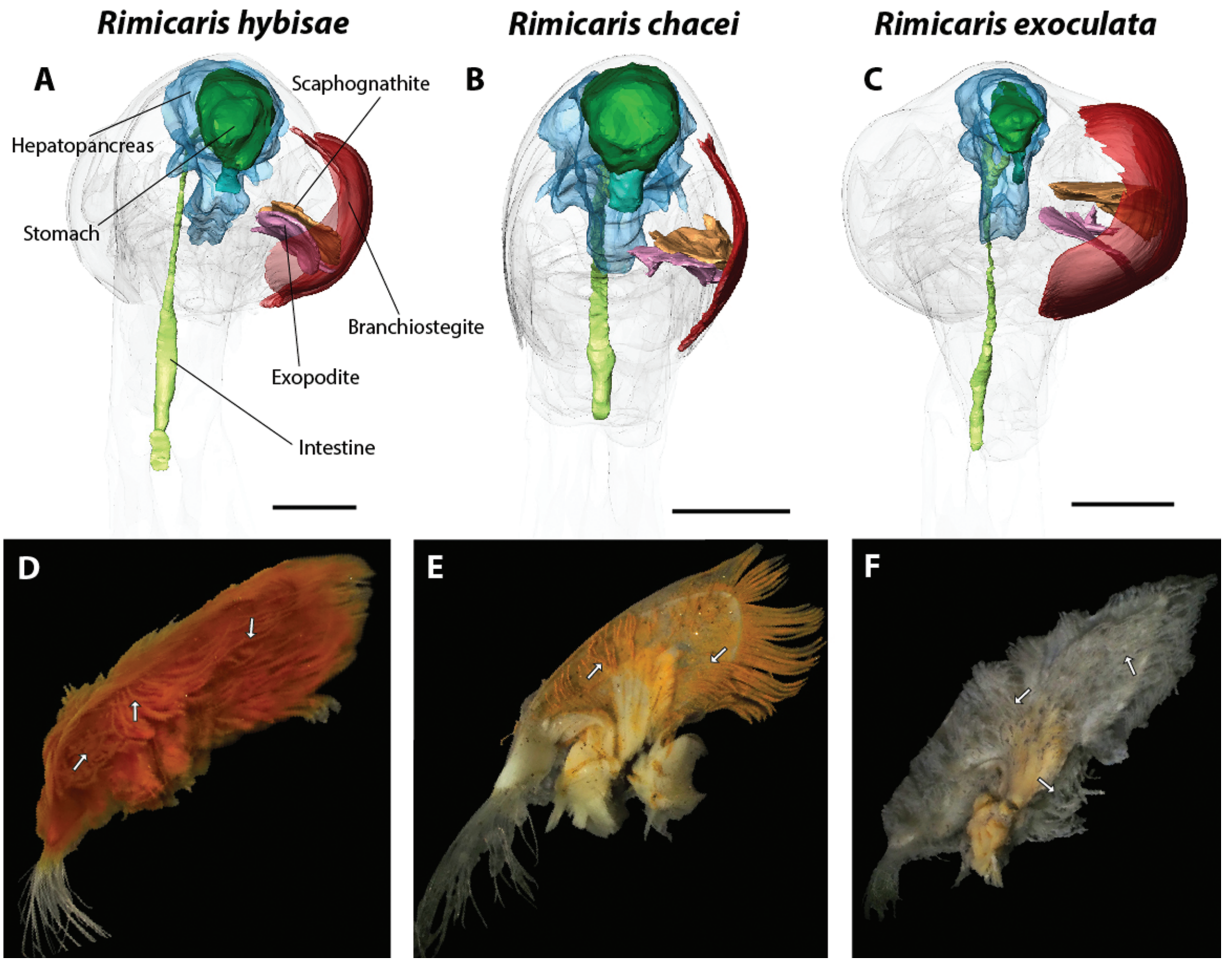


**Figure 4.** Symbiont-hosting organs in adult stages of the three *Rimicaris* shrimps. (A–C) 3D reconstructions of hosting organs in frontal view. (D–F) Dissected mouthparts covered with bacteriophore setae (indicated by white arrows).

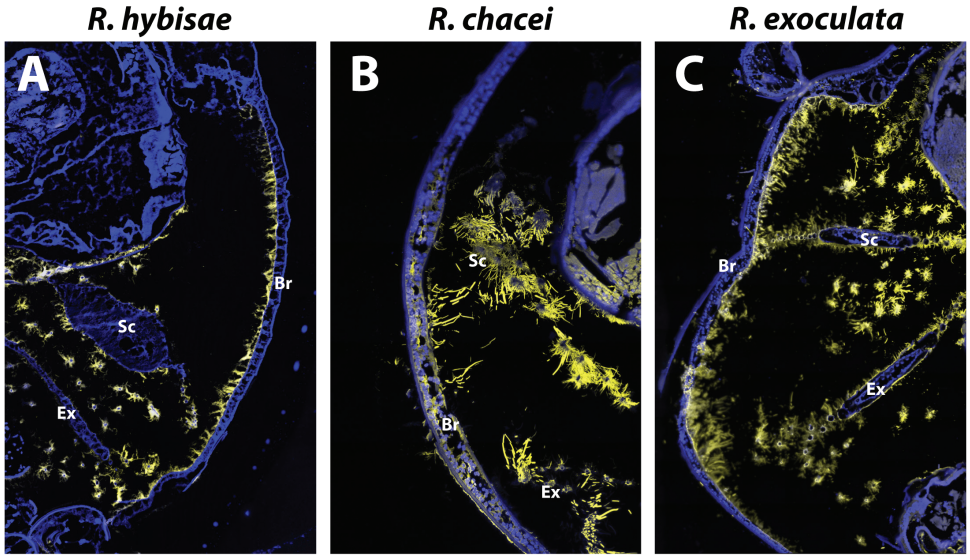


**Figure 5.** FISH observations of cephalothoracic cavities of *Rimicaris* subadults with universal bacterial probes (yellow) on semi-thin sections (7 µm) stained with DAPI (blue). Br: Branchiostegites; Sc: Scaphognathite; Ex: Exopodite. (A) *R. hybisae* subadult; (B) *R. chacei* subadult; (C) *R. exoculata* subadult. FISH = fluorescence in situ hybridization.

increment between juvenile and subadult stages, whereas there was almost no increase in *R. chacei*. The symbiont-hosting mouthparts were not different in the overall surface area between the two, but they were densely covered by setae only in *R. hybisae* (like *R. exoculata*) meaning a greater realized surface area for colonization in *R. hybisae*. The stomach

size drastically increased before the subadult stage in *R. chacei*, but not in *R. hybisae*. Nevertheless, there are two key anatomical characters shared between *R. hybisae* and *R. chacei* that distinguish them from *R. exoculata* (and *R. kairei*). At adult, (1) the final stomach volumes of *R. hybisae* and *R. chacei* are equivalent and much larger than *R. exoculata* and (2) the surface areas of mouthparts are smaller in *R. hybisae* and *R. chacei* than *R. exoculata*. Thus, the internal anatomy of *R. hybisae* is actually much more similar to *R. chacei*, in line with their genetic closeness (Plouviez et al., 2015; Teixeira et al., 2013). As such, the most significant shifts in the volume or surface area of key organs in these two closely related species were clearly between juvenile and subadult stages (Figure 3), indicating that their development trajectory could be determined in a "critical period" between these two stages. Overall, this total anatomical shift between juvenile and subadult stages may be sufficient to be considered a post-settlement metamorphosis (Chen et al., 2018).

In species with differential adult phenotypes and therefore developmental pathways, an individual's fate could be determined purely by environmental factors or genetic factors, or anything in the spectrum in-between (Schwander et al., 2010). For example, *A. mexicanus* fishes have repeatedly evolved during a few million years toward a blind phenotype by a recurring geographic isolation in several cave systems with all individuals remaining interfertile (Gore et al., 2018; Krishnan & Rohner, 2017). In these fishes, phenotype variations appear to be mostly determined by genetic and epigenetic mechanisms (Gore et al., 2018; Krishnan & Rohner, 2017; McGaugh et al., 2014). On the other hand, caste determination in different species of eusocial insects stems from a combination of various environmental stimuli and epigenetics (Schwander et al., 2010). The two distinct morphotypes of *R. piscesae* tubeworms is a similar case at hydrothermal vents, influenced by vent-flow dynamics (Tunnicliffe et al., 2014). This rapid divergence among populations is generally facilitated by phenotypic plasticity or by standing genetic variation with preexisting polymorphism of relevant alleles in ancestral populations (Barrett & Schluter, 2008; Pfennig et al., 2010; Rohner et al., 2013). Freshwater populations of the stickleback fish *Gasterosteus aculeatus* repeatedly diverged from marine ancestors in several rivers and lakes around the world after the last glaciation (Colosimo et al., 2005). They exhibit a high level of phenotypic variability, with the freshwater ones exhibiting reduction in bony plates linked to an allele that was already present at low frequency in the ancestral population (Barrett & Schluter, 2008; Colosimo et al., 2005). Conversely, divergences among stream and lake ecotypes of this species in terms of body depth, head shape, or morphology of the digestive system were found to be related to an interplay of phenotypic plasticity and genetic predisposition (Lucek et al., 2014). The absence of genetic differentiation with the existing markers suggests a similarly recent divergence—or even an absence of divergence—between *R. hybisae* and *R. chacei* as the abovementioned cases. Although additional genetic investigations are warranted to clarify the relationship between *R. hybisae* and *R. chacei*, similar mechanisms such as standing genetic variation could be at play in determining the development outcome in these shrimps.

Another possibility, especially in the possible scenario where *R. hybisae* and *R. chacei* are not genetically different, is that the development outcome could also be driven environmentally. Rapid reduction in hepatopancreas volume before reaching the subadult stage indicates the depletion of lipid reserves accumulated before settlement (Methou et al., 2020). This means the shrimps must become energetically self-sustaining before becoming subadults. Juveniles of *R. chacei* and *R. exoculata* on the MAR have been suggested to directly compete for access to the vent fluid source, with *R. exoculata* outcompeting *R. chacei* (Methou, Hernández-Ávila, et al., 2022). Being distant from vent orifices, *R. chacei* juveniles may be limited in their ability to acquire an energetically sufficient chemosymbiosis, promoting a shift toward a mixotrophy (Methou, Hernández-Ávila, et al., 2022). Conversely, *R. hybisae* has no known competitor at the MCSC vents (Nye et al., 2013; Plouviez et al., 2015). We speculate that the ecological niche occupied by juveniles of *R. hybisae* and *R. chacei* before reaching subadult size could play a role in deciding their development trajectory. The bacterial colonization patterns on branchiostegites—densely colonized in subadult *R. hybisae* but not *R. chacei*—coincide well with key anatomical characters before reaching subadult and this may be a "critical period" for determining the development trajectory.

If this scenario is true, then symbionts may drive the morphogenesis of enlarged symbiont-hosting organ in these vent shrimps. Though this is still far from proven in our case, similar cases are known such as the morphogenesis of the light organ in juveniles of the bobtail squid *E. scolopes* mediated by its *Vibrio fischeri* symbionts upon acquisition (Nyholm & McFall-Ngai, 2021). Moreover, *E. scolopes* juveniles treated with antibiotics enter in a refractory state after 5 days and cannot be recolonized by *V. fischeri* symbionts, suggesting a "critical period" (Koch et al., 2014). Similarly, in the same animals, the accessory nidamental gland does not develop in individuals raised without the symbiont consortium hosted within this organ (McAnulty et al., 2023). Likewise, the jellyfish *Cassiopea xamachana* rely on the colonization of its *Symbiodinium* symbionts as a developmental trigger to perform its lifecycle transitions (Ohdera et al., 2018). In juvenile *Rimicaris* shrimps, the transition from juvenile to subadult is certainly aided by red lipid reserves stored in the hepatopancreas and the depletion of this may mark the end of the "critical period." Although *R. hybisae* preferentially occupy areas of vigorous venting emissions and *R. chacei* more peripheral areas, adults are sometimes found in the opposite habitat (Methou, Hernández-Ávila, et al., 2022; Nye et al., 2013; Versteegh et al., 2022). Large variations in stomach volumes could be seen among adults of both species, potentially related to plasticity in feeding ecology such as facultative carnivory/scavenging reported for some peripheral *R. hybisae* (Versteegh et al., 2022) or mixed diets among *R. chacei* depending on the habitat (Apremont et al., 2018; Methou et al., 2020). However, this is not accompanied by changes in the cephalothorax morphology—which may be proportionally fixed after the "critical period" like the organs of *E. scolopes* (Koch et al., 2014). Future work, including population genomics, are still required to clarify the evolutionary relationship between *R. chacei* and *R. hybisae* and disentangle the respective influence of environment versus genetics on their phenotype and ecology.

Understanding how chemosymbiotic relationships are established is a major challenge because symbiosis is such a successful and effective strategy that most hosts energetically reliant on symbionts have become obligate holobionts that cannot survive or develop into adulthood to reproduce without their partners (Koga et al., 2021; Nussbaumer et al.,

2006). Although whether *R. hybisae* and *R. chacei* represent two recently speciated lineages or morphs of the same species remain inconclusive at this time, they are undoubtedly very closely related. As is true for molecular and epigenetic factors, environmental conditions may be important drivers behind the distinct development trajectories seen between these two shrimps. We speculate that individuals might have a limited "critical period" before reaching the subadult stage for deciding their developmental trajectory, likely by whether an energetically viable symbiosis has been established by then. Depending on the outcomes of future studies on the relationship between *R. hybisae* and *R. chacei*, these shrimps can be a valuable model for understanding how chemosymbioses are established and function, providing comparable data for genetically equivalent individuals in different symbiotic conditions and also during transitions, similar to how the blind cavefish model has proved useful for studying eye loss (Krishnan & Rohner, 2017). Our works lays the morphological foundation for understanding the evolutionary adaptation of vent shrimps that allow them to "feed on Earth" through symbiosis.

## Supplementary material

Supplementary material is available online at *Evolution*.

## Data availability

3D interactive models from each stage and shrimp species are available as Supplementary Material. Metadata associated to each shrimp individuals including collection date, sampling location, storage conditions, and body size are displayed in Supplementary Table S1. Surfaces and volumes measurement of shrimp organs obtained from 3D anatomical reconstructions are given in Supplementary Table S3. Genes sequences used in this work for Supplementary Figure S1 are given in Supplementary Table S4 with their GenBank ID. Among them, new sequences are highlighted in bold and have been submitted to the NCBI GenBank repository.

## Author contributions

P.M. collected shrimps during the BICOSE 2 expedition, carried out the identification and measurement of specimens, realized fluorescent in situ hybridization experiments on *R. hybisae* specimens, conducted data analysis of 3D anatomical reconstruction, lead the conception and design of the study, and drafted the original manuscript; M.G. conducted FISH analyses on *R. exoculata* and *R. chacei*, and critically revised the manuscript; J.T.C. collected specimens from the JC82 expedition and critically revised the manuscript. H.K.W. collected specimens from YK13-05 and critically revised the manuscript. F.P. participated in the design and conception of the study and critically revised the manuscript. M.-A.C.-B. participated in the design and conception of the study and critically revised the manuscript. C.C. collected specimens from YK16-06 expeditions, assisted in drafting the original manuscript, participated in the design and conception of the study, and coordinated the study. All authors gave final approval for submission and publication of the manuscript in its current form.

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