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REVIEW

Rimicaris exoculata: biology and ecology of a shrimp from deep-sea hydrothermal vents associated with ectosymbiotic bacteria

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ABSTRACT: *Rimicaris exoculata*, the 'blind shrimp,' is the most abundant species living on active hydrothermal edifices at deep-sea vents of the Mid-Atlantic Ridge. Its unusually enlarged branchial chamber houses a dense ectosymbiotic community of chemoautotrophic bacteria. Long debated, shrimp nutrition has been proven to be a kind of osmotrophy, whereby small organic molecules produced by the symbionts pass through the integument of the shrimp directly into the circulatory system, rather than through the digestive system. The broad phylogenetic and metabolic diversity of this epibiotic community suggests a highly flexible and adjustable microbial consortium, adapted to the chemically contrasting environments inhabited by the shrimp. To cope with the highly fluctuating oxygen and temperature conditions of its habitat, *R. exoculata* possesses hemocyanin with a strong oxygen affinity, and has developed both molecular and behavioral responses to heat stresses. If *R. exoculata* is able to detect very dim light or chemical compounds emitted by vents, the relatively small visual and olfactory areas in the brain, along with the disproportionately enlarged higher centers, argue for a significant involvement of navigational skills using learning and place memory to orient itself within its aphotic environment. This shrimp undergoes unconventional larval development, with a primary lecithotrophic stage followed by an extended planktotrophic period, allowing a huge potential for dispersion. In light of mining licenses posing a threat for deep-sea environments, this species is a model still to be studied to better understand life in extreme deep-sea ecosystems at the global scale of an ocean.

KEY WORDS: Alvinocaridid shrimp · Chemoautotrophic symbiont · Deep-sea · Epibiosis · Holobiont · Hydrothermal vents · Mid-Atlantic Ridge · *Rimicaris exoculata*

1. INTRODUCTION

1.1. Distribution of *Rimicaris exoculata*

The discovery of hydrothermal vents in 1977 on ocean ridge crests off the Galapagos Islands was quickly followed by vent discoveries along the East Pacific Rise (EPR) (Lonsdale 1977, Corliss et al. 1979). Exploration of the Mid-Atlantic Ridge (MAR) began in 1985. Observed faunal assemblages on the MAR were markedly different from those described a few years earlier on the EPR, with the notable exception of bathymodiolin mussels. The assemblages were characterized by the absence of alvinellids and vestimentiferan tubeworms, and the presence of huge motile aggregations of shrimp (Fig. 1a) of the family Alvinocarididae, later named *Rimicaris exoculata*, which dominated by far the communities of the visited deep MAR vent fields (Van Dover et al. 1988, Segonzac 1992, Gebruk et al. 1997a, Desbruyères et al. 2001).

R. exoculata (Williams & Rona 1986) has been reported on most of the known MAR sites, from 45° 28′ N (Moytirra) to 15° 10′ S (Deyin-1) (Fig. 1b, Table 1),

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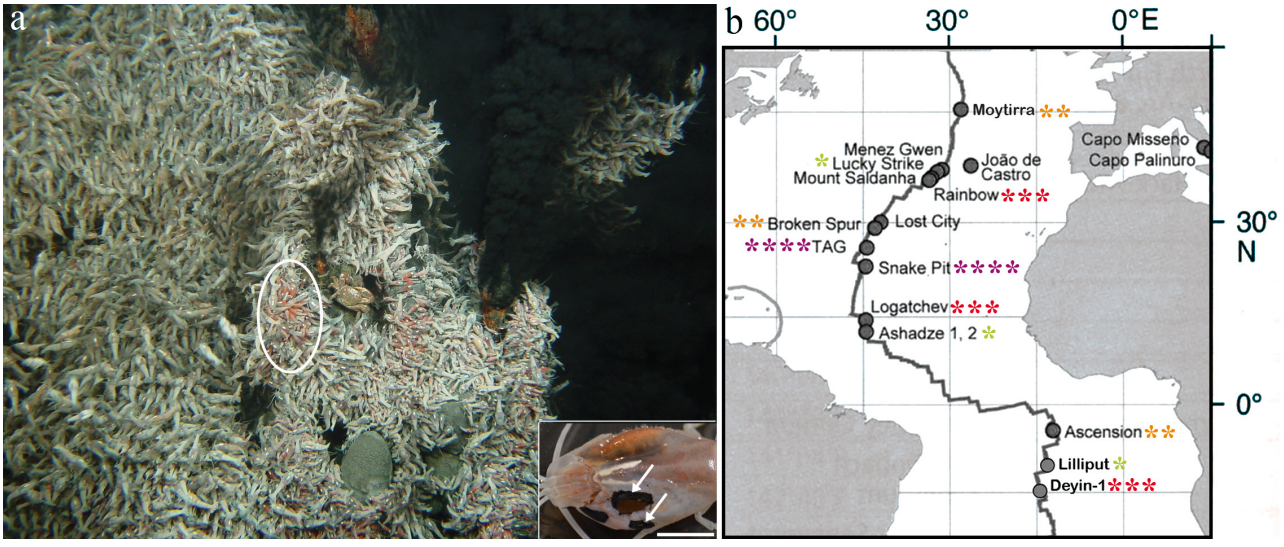

Fig. 1. (a) *Rimicaris exoculata* aggregation at the Trans-Atlantic Geotraverse (TAG) site (© Exomar / Ifremer). White circle shows a patch of orange juveniles close to a fluid exit and inset shows a shrimp with traces of burns (arrows) on the branchiostegite (© F. Pradillon). Scale bar = 1 cm. (b) Distribution of *R. exoculata* populations along the Mid-Atlantic Ridge, with density information from the least (1 star) to the most (4 stars) colonized sites (modified from Desbruyères et al. 2006)

Table 1. Locations of Mid-Atlantic Ridge vent fields hosting *Rimicaris exoculata* (data from InterRidge database: http://vents-data.interridge.org/). Shrimp densities based on personal observations (M.Z. and M.A.C.B.), Desbruyères et al. (2000), Haase et al. (2009), Wheeler et al. (2013) and Dong et al. (2019). 1: very rare or not observed during the most recent cruises, 2: rare, 3: abundant, 4: very abundant. TAG: Trans-Atlantic Geotraverse

Vent field	Latitude	Longitude	Depth (m)	Shrimp density	Discovery
Moytirra	45° 28′ N	27° 51′ W	2095	2	2011
Lucky Strike	37° 17′ N	32° 16′ W	1740	1	1993
Rainbow	36° 16′ N	33° 54′ W	2320	3	1997
Broken Spur	29° 10′ N	43° 10′ W	3100	2	1993
TAG	26° 08′ N	44° 49′ W	3670	4	1986
Snake Pit	23° 22′ N	44° 57′ W	3500	4	1985
Logatchev	14° 45′ N	44° 58′ W	3050	3	1995
Achadze	12° 58′ N	44° 51′ W	4200	1	2007
Ascension	4° 47′ S	12° 22′ W	3050	2	2005
Lilliput	9° 33′ S	13° 10′ W	1500	1	2005
Deyin-1	15° 10′ S	13° 21′ W	2850	3	2011

with the exception of the shallowest sites: Menez Gwen (850 m depth), Lilliput (1500 m depth) and Lucky Strike (1700 m depth), where no or only very few specimens have been observed (Gebruk et al. 2000, Pond et al. 2000a,b, Dong et al. 2019).

1.2. Habitat

R. exoculata, the most extensively studied hydrothermal shrimp species to date, occurs on active chimney walls, in astonishingly dense aggregations of up to 3000 ind. m^{-2} (Fig. 1a), packed side by side and piled on 2 or more layers (Van Dover et al. 1988, Segonzac 1992, Copley et al. 1997, Gebruk et al. 1997a, 2000). Adults of *R. exoculata* are generally found crawling on the substratum (Rona et al. 1986, Segonzac et al. 1993) and maintain high levels of activity on walls of active black smokers (Van Dover et al. 1988). When displaced from the substratum, the shrimp immediately seek to re-establish themselves near the source of hydrothermal fluid (Van Dover et al. 1988, Renninger et al. 1995), resulting in a teeming swarm oriented against the current of vent fluids (Copley et al. 1997). These dense aggregations were proposed to form in response to physical constraints such as the restricted availability of substratum exposed to hydrothermal fluids (Polz et al. 1998, Gebruk et al. 2000) or the selection of an appropriate thermal niche (Ravaux et al. 2009). However, Ravaux et al. (2019) observed shrimp aggregations in pressurized aquaria, in a homogeneous environment and in the absence of any environmental cues. They thus suggested for the first time that *R. exoculata* aggregations may form not only as a result of the independent attraction of individuals to a resource, but

could also be the result of some social behavior that provides benefits.

R. exoculata lives within steep thermal and chemical gradients, where hot, reduced hydrothermal fluid mixes turbulently with cold, oxygenated seawater (Schmidt et al. 2008b). The thermal environment within the aggregations has been well documented, with reported temperatures ranging from 2.8 to 32°C (Van Dover et al. 1988, Segonzac et al. 1993, Desbruyères et al. 2001, Geret et al. 2002, Zbinden et al. 2004, Schmidt et al. 2008a), although measurements taken within a few centimeters of the shrimp aggregations (Zbinden et al. 2004, Schmidt et al. 2008a) do not exceed 18°C. Many shrimp were reported with burns on the cuticle, pereopods, pleopods or antennae (Van Dover et al. 1988, Gebruk et al. 1993, Segonzac et al. 1993, Gebruk et al. 2000; see inset in Fig. 1), indicating that they likely sometimes encounter much higher temperature peaks. On the other hand, occasionally few shrimp occur, rather motionless, scattered at the periphery of a community, exposed to temperatures just above that of ambient seawater (2.4°C) (Gebruk et al. 1993, Segonzac et al. 1993).

R. exoculata colonizes sites along the MAR with chemically contrasting environments. Most active hydrothermal sites known to date are basaltic, i.e. based on underlying rocks of a basaltic nature. Water–rock interactions at basalt-hosted vents (such as the Trans-Atlantic Geotraverse [TAG], Broken Spur or Snake Pit) release end-member fluids enriched in hydrogen sulfide (H_2S), but are relatively depleted in hydrogen (H_2) and methane (CH_4) (Table 2). On the other hand, the hydrothermal circulation at ultramafic sites (Rainbow, Logatchev, Ashadze) is the result of interaction between seawater and mantle rocks outcropping at the seafloor. The end-member fluid composition of those vents departs from that of the typical basaltic end-member, and is relatively depleted in H_2S and enriched in H_2, ferrous iron (FeII) (notably at the Rainbow site) and CH_4, due to serpentinization processes (Charlou et al. 2002, 2010, Douville et al. 2002). Thus, *R. exoculata* encounters contrasting chemical habitats at the different sites. The chemical environment within the shrimp aggregations is less documented than the thermal environment. *In situ* measurements of temperature, pH, ferrous iron and sulfide concentrations within the aggregations reflect the moderate contribution of hydrothermal fluid in the mixing with seawater (Zbinden et al. 2004). Analyses at the Rainbow site revealed near-neutral pH (6.3–8), high levels of ferrous iron (0.13–265 µM), moderate methane levels (21 µM) and low levels of sulfide (0.4–22 µM) (Geret et al. 2002, Zbinden et al. 2004, Schmidt et al. 2009). Little or no data are available within the aggregations for other hydrothermal sites (see Schmidt et al. 2008a,b for data from the TAG site, showing lower methane and ferrous iron concentrations).

R. exoculata abundance varies among sites (see Fig. 1b), from isolated individuals at Achadzé (Fouquet et al. 2008) to aggregations of hundreds to thousands of individuals almost entirely covering active chimney walls at TAG (Williams & Rona 1986; Fig. 1a). However, the parameters influencing the distribution of this shrimp remain unknown. The importance of flow rate, chemical composition and/or temperature of the hydrothermal fluid, or even duration of activity of the site, remains to be determined (Schmidt et al. 2008b).

1.3. Brief morphological description

Following is a brief description of specific morphological characteristics of the species, according to Williams & Rona (1986), Segonzac et al. (1993) and Komai & Segonzac (2008) (Fig. 2). The size (total length measured from the anterior tip of the cephalothorax to the end of the telson) ranges from 31.3–50.2 mm for males, 42.3–57.4 mm for females and 23–47.4 mm for juveniles. The rostrum is strongly reduced. The antennular and antennal peduncles are hyper-

Table 2. Concentration of the main electron donors in the seawater (SW) and in the end-member fluid of different hydrothermal vent sites colonized by *Rimicaris exoculata* populations (data from Desbruyères et al. 2000, Douville et al. 2002, Charlou et al. 2002, 2010, Demina et al. 2013). TAG: Trans-Atlantic Geotraverse, –: no data available

	SW	Rainbow	Broken Spur	TAG	Snake Pit	Logatchev	Achadze
Type of host rock		Ultramafic	Basaltic	Basaltic	Basaltic	Ultramafic	Ultramafic
pH	7.8	2.8–3.1	2.9–3.2	2.5–3.4	3.7–3.9	3.3–3.9	3.1
H_2S (mM)	0	1.2–1.4	8.5–11	2.5–6.7	6.7	1.1–1.4	1
CH_4 (mM)	0.0003	1.6–2.5	0.065–0.13	0.124–0.147	0.046–0.062	2–2.6	0.5–1.2
H_2 (mM)	0.0004	13–16	0.43–1.03	0.15–0.37	–	9–12.5	8–19
Fe (µM)	<0.001	17700–24050	1680–2160	1640–5450	2400	1825–2500	9300

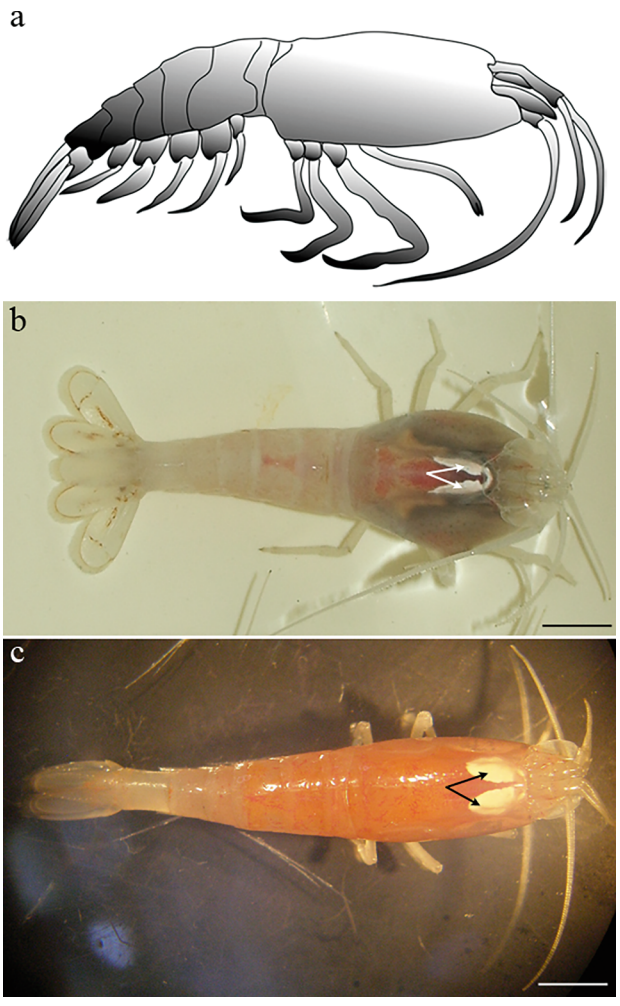

Fig. 2. (a) Lateral view of *Rimicaris exoculata*. (b) Dorsal view of an adult showing the strongly inflated cephalothorax. (c) Dorsal view of a juvenile showing the bright orange coloration and the narrow cephalothorax. Arrows in panels b and c show the dorsal eye. Scale bars = 1 cm (b), 0.2 cm (c)

trophied. The cephalothorax is dorsoventrally compressed, distinctly broader than the pleon, with strongly inflated branchial regions. The carapace extends forward both ventrally and encloses cephalic, but also anterior thoracic appendages up to the second pair of pereiopods, in an almost closed 'prebranchial' chamber. The structure of the mouthparts is particularly modified: exopodites of the second maxilla (hereafter called scaphognathites) and of the first maxillipeds (hereafter called exopodites) are enormously expanded anteriorly, occupying two-thirds of the prebranchial chamber. They are entirely covered on both sides with long and numerous bacteriophore setae (Fig. 3). The mandibles have regressed. The first 2 pereiopods are relatively small and probably cannot extend out of the branchial chamber. The strongly modified ocular region is characterized by transverse ocular plates, with no eye-stalks (see Section 3.2 for further description).

1.4. Classification

The generic name *Rimicaris* derives from the Latin 'rima,' meaning rift or fissure, with reference to the MAR, and from the Greek 'karis,' meaning shrimp. The specific epithet '*exoculata*' derives from the Latin term 'exoculo,' meaning deprived of eyes, with reference to the highly modified, non-image-forming, eyes (Williams & Rona 1986).

The genus *Rimicaris* was originally established (in the family Bresiliidae) for 2 species discovered from the hydrothermal vent field TAG on the MAR: *R. exoculata* (Williams & Rona 1986) and *R. chacei* (Williams & Rona 1986). The former was designated as the type species of the genus. The genus *Rimicaris* was later assigned to the family Alvinocarididae (Christoffersen 1986, Segonzac et al. 1993, Komai & Segonzac 2003). A new subfamily (Rimicaridinae) comprising the genus *Rimicaris* was created in 2015 (Vereshchaka et al. 2015).

Two 'small orange' species, namely *R. aurantiaca* (Martin et al. 1997), described from the Snake Pit site, and *Iorania concordia* (Vereshchaka 1996), described from the TAG site, were subsequently recognized to be *R. exoculata* juveniles. This initial taxonomic confusion was due to important morphological differences between juvenile and adult *R. exoculata* (Martin & Haney 2005). Coloration between adults of *R. exoculata* and the small bright orange shrimp were very different. The slightly inflated branchiostegites and oval ocular plates of the small orange shrimp contrasted with the highly inflated branchiostegites and wing-shaped ocular plates of *R. exoculata* adults (Vereshchaka 1996, Nuckley et al. 1996, Shank et al. 1998). However, the absence of brooding females and mature males with developed appendix interna in samples of the small orange shrimp, as well as the small size of these orange shrimp vs. adult *R. exoculata* raised some doubts. Allozymes and mitochondrial DNA sequences of samples from the Broken Spur, TAG and Snake Pit sites confirmed that these 2 small orange shrimp 'species' were indeed *R. exoculata* juveniles (Segonzac et al. 1993, Creasey et al. 1996, Shank et al. 1998).

The genus *Rimicaris* currently contains 10 species. *R. exoculata*, *R. kairei* (described from the hydrothermal vent field 'Kairei' on the Central Indian Ridge, Watabe & Hashimoto 2002) and *R. hybisae*

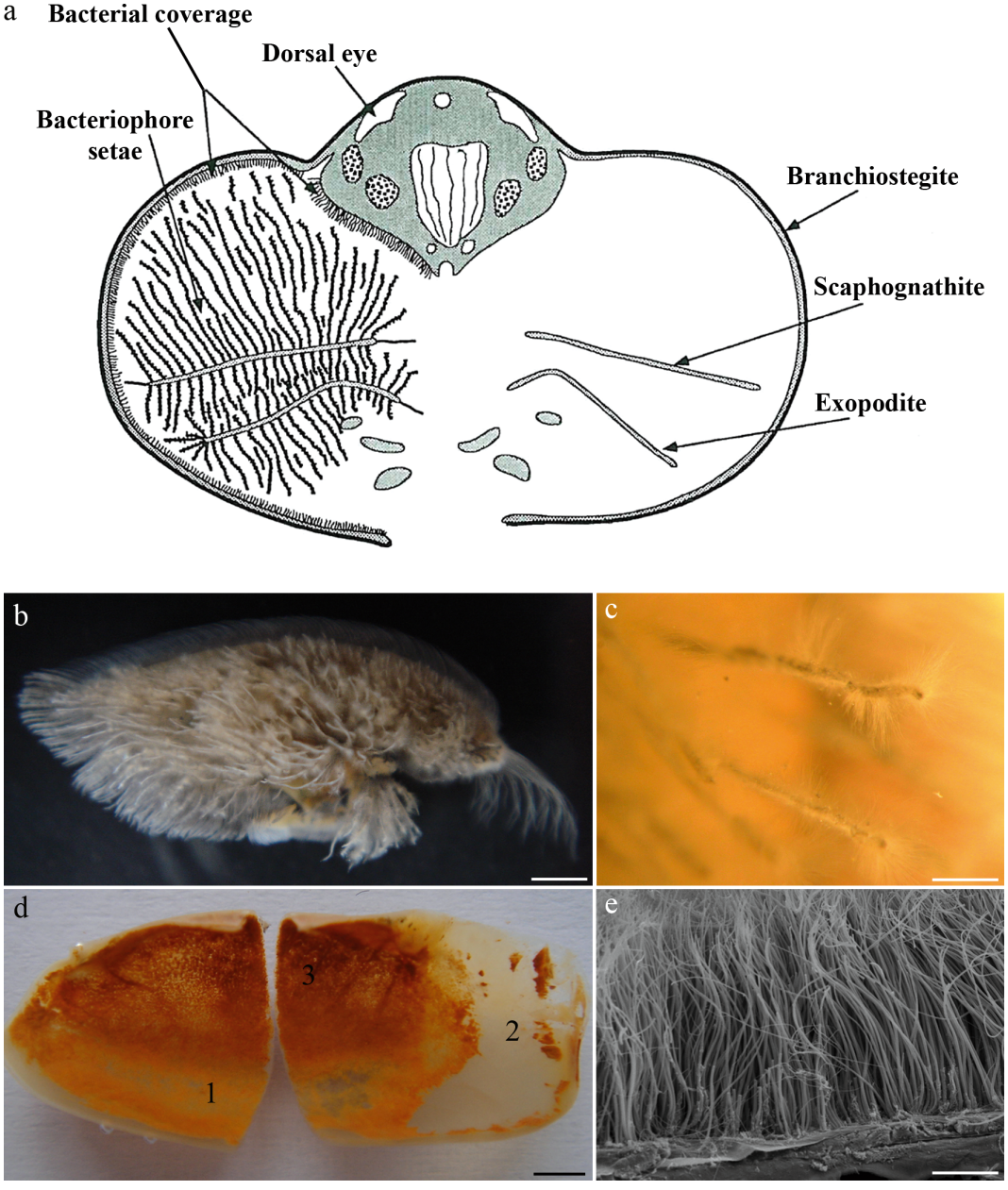

Fig. 3. *Rimicaris exoculata* epibiotic bacteria of the branchial chamber. (a) Schematic cross-section of the cephalothorax showing the localization of the bacteria (inner side of the branchiostegite, bacteriophore setae of the scaphognathites and exopodites) (modified from Segonzac et al. 1993). (b) Scaphognathite with bacteriophore setae. (c) Close-up of a seta covered by filamentous bacteria. (d) Inner side of the banchiostegite covered by orange iron oxides (1: lower pre-branchial chamber, 2: true branchial chamber, 3: upper pre-branchial chamber, housing the main ectosymbiotic bacterial community) (photo by P. Compère). (e) Scanning electron microscopy close-up of the mat of filamentous bacteria in the pre-branchial chamber (3 in panel d). Scale bars = 2 mm (b,d), 100 µm (c,e)

(described from hydrothermal vent fields on the Mid-Cayman Spreading Centre, Nye et al. 2012) were the 3 initially recognized *Rimicaris* species. Six species previously assigned to the genus *Chorocaris* were later synonymized with *Rimicaris* in 2015 (Vereshchaka et al. 2015), following previous recommendations (Creasey et al. 1996, Shank et al. 1999, Teixeira et al. 2013, Komai & Tsuchida 2015): *R. chacei* (Williams & Rona 1986), *R. vandoverae* (Martin & Hessler 1990), *R. paulexa* (Martin & Shank 2005), *R. susannae* (Komai et al. 2007), *R. parva* (Komai & Tsuchida 2015) and *R. variabilis* (Komai & Tsuchida 2015). Finally, Komai & Giguère (2019) described a new *Rimicaris* species (*R. falkorae*) from the Mariana

Back Arc Spreading Center. Within the current *Rimicaris* genus, *R. exoculata*, *R. kairei* and *R. hybisae* present an enlarged branchial chamber, which houses a dense epibiotic bacterial community. In the 7 other species, the scaphognathites are moderately broad and devoid of bacteriophore setae (except for *R. vandoverae* and *R. chacei*). Until now, no symbiotic association has been described for these species, except for *R. chacei*, which harbors a symbiotic community both in its gut and in its branchial chamber, although less developed than in *R. exoculata* (Casanova et al. 1993, Gebruk et al. 2000, Apremont et al. 2018).

2. DOUBLE SYMBIOSIS

Two microbial epibiotic communities are associated with *Rimicaris exoculata*: the most prominent (and most studied) one is located in the branchial chamber, the other in the gut.

2.1. Branchial chamber symbionts

2.1.1. Bacterial localization

Bacteria colonize almost all of the available surfaces of the branchial chamber (Fig. 3a), with the notable exceptions of the gills and inner side of the branchiostegites facing them (Zbinden et al. 2004). Bacteria are mostly present on the bacteriophore setae of the dorsal and ventral sides of the hypertrophied exopodites and scaphognathites (Fig. 3b,c), as well as on the internal tegument of the carapace (hereafter called branchiostegites) (Fig. 3d,e) (Casanova et al. 1993, Segonzac et al. 1993). Using oligonucleotide slot-blot hybridization, Polz & Cavanaugh (1995) estimated that an average shrimp would carry 8.5×10^6 bacteria in its branchial chamber, which would correspond to $(2–4) \times 10^{11}$ bacteria m^{-2} in the aggregations.

Investigation of bacteria (Fig. 3d) by photonic and scanning electron microscopy (SEM) (Fig. 3e) on the inner side of the branchiostegite revealed 3 distinct compartments in the branchial chamber: (1) the lower pre-branchial chamber, with only a thin bacterial coverage; (2) the 'true' branchial chamber, containing the gills and completely devoid of bacteria; and (3) the upper pre-branchial chamber, housing the main ectosymbiotic bacterial community. The limit between the upper and lower pre-branchial chamber corresponds to the position of the exopodites, the scaphognathites being entirely enclosed in the upper pre-branchial chamber (Zbinden et al. 2004). The water current, generated by the beating of the scaphognathites, enters ventrally, passes from the lower pre-branchial chamber (first compartment), to the gills (second compartment), and then through the upper pre-branchial chamber containing the main epibiont community (third compartment) (Casanova et al. 1993, Segonzac et al. 1993, Zbinden et al. 2004).

2.1.2. Trophic symbiosis

In deep-sea hydrothermal ecosystems, deprived of photosynthetic activity, chemoautotrophic bacteria fueled by hydrothermal fluid reduce compounds (mainly sulfur and methane, Nelson & Fisher 1995) to sustain a complex and dense food web. Here, the primary consumers gain their energy directly from these primary producers through direct consumption or through the establishment of symbioses. The huge densities of *R. exoculata* on the MAR, and their dominance at many sites, rapidly raised questions on how these populations were sustained (Van Dover et al. 1988). Furthermore, these shrimp cluster close to the mixing zone between the hot fluids and seawater, displaying intense activity, which is unusual for deep-sea organisms (Segonzac 1992).

The first investigations on the feeding biology of *R. exoculata* led Van Dover et al. (1988) to propose that these shrimp were normal heterotrophs, grazing on free-living microorganisms colonizing the black smoker chimney walls. This conclusion came from: (1) their results of lipopolysaccharide assays revealing large amounts of bacterial cell-wall material in the stomach content, along with the fact that the gut was filled with sulfide particles; (2) their data of $\delta^{15}N$ (+7.5‰) and $\delta^{34}S$ (+9.7‰) isotopic ratios; and (3) the fact that they were unable to detect any autotrophy activity (i.e. ribulose-1,5-bisphosphate carboxylase activity) in the studied tissues (gut, hepatopancreas, gonads, abdominal muscles). These authors, looking only for chemoautotrophic endosymbiotic bacteria, nevertheless acknowledged that they may have missed the correct host tissues. They also reported the occurrence of a dense coating of filamentous microorganisms on the setae of the scaphognathites and exopodites, which they proposed to constitute a second nutritional source.

It rapidly became clear that, if shrimp occupy a substratum directly exposed to the hydrothermal fluid flow, it would enable chemosynthetic primary production by the epibiotic chemosynthetic bacterial community hosted in their branchial chamber (Gebruk et al. 1993, Polz & Cavanaugh 1995). Indeed, some au-

thors argued that the amount of bacteria present on chimney walls was not enough to support the shrimp population. They rather proposed an alternative hypothesis to that of Van Dover et al. (1988), suggesting that *R. exoculata* gained most of its carbon from the epibiotic bacteria living within its carapace, by scraping and grazing on them (Casanova et al. 1993, Gebruk et al. 1993, Wirsen et al. 1993, Polz et al. 1998, Rieley et al. 1999). The morphology of the 2 pairs of chelipeds, which are relatively small, suggests that they probably cannot extend out of the branchial chamber to graze the chimney walls. By contrast, they are able to reach and clean the surfaces of all appendages within the branchial chamber, as well as the internal walls of the carapace, and then carry the scraped particles to the mouth (Van Dover et al. 1988).

The highly specialized morphological features of *R. exoculata* (hypertrophied branchial chamber and mouthparts, bacteriophore setae, Fig. 3) suggest an efficient bacterial culture in the shrimp pre-branchial chamber and argue for a trophic symbiosis (Gebruk et al. 1993). A number of lines of evidence converge towards the present consensus, i.e. that the epibionts are the major source of carbon (80%, according to a calculation by Polz et al. 1998): (1) close $\delta^{13}C$ values of the saturated and monounsaturated fatty acids isolated from the muscle tissues of *R. exoculata* (−13‰) and the epibionts (−12‰), (2) very high level of polyunsaturated (n-7) and (n-4) fatty acids, both in the epibionts and in *R. exoculata* tissues (Pond et al. 1997a, 2000a,b, Allen-Copley et al. 1998, Rieley et al. 1999). Many studies reached similar conclusions considering the close $\delta^{13}C$ signature between the epibiotic bacteria (−9.3 to −12.4‰; Polz et al. 1998, Gebruk et al. 2000) and *R. exoculata* tissues (−10 to −12.5‰, Van Dover et al. 1988, Gebruk et al. 1997b, 2000, Pond et al. 1997a, Polz et al. 1998, Vereshchaka et al. 2000).

Several years later, a third hypothesis argued for a trans-epidermal transfer of dissolved organic matter from the epibiotic bacteria through the branchiostegite cuticle (Casanova et al. 1993, Zbinden et al. 2004, Corbari et al. 2008a). Some authors (Gebruk et al. 2000) first rejected this hypothesis, on the basis of previous studies arguing that transport of dissolved organic matter across the chitinous exoskeleton in crustaceans is unlikely (Stephens 1988). This hypothesis is nevertheless supported by several points: (1) the thinness of the cuticle (as low as 0.5 µm) and the important hemolymphatic lacunas underlying it; (2) the numerous membrane infoldings and mitochondria in branchiostegite epithelium beneath the bacteria (Martinez et al. 2005), suggesting an important epidermal transport activity; and (3) the absence of any scraped areas on the inner side of branchiostegites or on scaphognathites despite a considerable number of observations (Zbinden et al. 2004, Corbari et al. 2008a). Although supporting the hypothesis that the shrimp grazed off their epibionts, Polz et al. (1998) noticed that only slightly more than 20% of the rRNA detected in the gut corresponds to that of the epibionts. In order to test the trans-tegumental hypothesis, Ponsard et al. (2013) performed *in vivo* shrimp incubations in a pressurized aquarium with isotope-labeled inorganic carbon ($NaH^{13}CO_3$ and $NaH^{14}CO_3$), to trace carbon assimilation by the epibionts and shrimp tissues. The high ^{13}C and ^{14}C incorporation in the bacterial biofilms of scaphognathites and branchiostegites (Fig. 4a) proves that they are indeed chemoautotrophs. The higher incorporation rates in the presence of iron or thiosulfate indicates that both sulfide and iron oxidation metabolism occurs among the epibionts. Among the shrimp tissues analyzed (outer branchiostegite [i.e. the branchiostegite epithelium and its outer cuticle], gills, hepatopancreas, digestive tract, abdominal muscles), the highest incorporation rate was measured in the outer branchiostegite (2.7 µmol C_{inc} gC^{-1} h^{-1}) and to a lesser extent the gills (0.2 µmol C_{inc} gC^{-1} h^{-1}). The rates recorded for the internal tissues of all shrimp (muscles: 0.01; digestive tract: 0.04; hepatopancreas: 0.01 µmol C_{inc} gC^{-1} h^{-1}) were low to very low. These results showed that chemosynthetic bacterial organic carbon passes to the host through a direct absorption across the branchial chamber integument (Fig. 4b–d), rather than via the digestive tract. They thus provide the first direct evidence of a true symbiotic trophic interaction between the host and its epibiotic bacteria.

2.1.3. Bacterial morphological diversity

Using SEM, the first observations of the epibionts revealed 3 bacterial morphotypes: 2 filamentous types (one thin: 0.2–1 µm; one thick: 1–3 µm in diameter, both up to 250 µm in length, Fig. 5a) and 1 rod-shaped type (0.5 × 1.5 µm, Fig. 5e) (Casanova et al. 1993, Gebruk et al. 1993, Segonzac et al. 1993, Zbinden et al. 2004).

Additional observations with transmission electron microscopy (TEM) on Rainbow samples refined these data, leading to the identification of 6 different morphotypes (Zbinden et al. 2008). Two sub-types of rods were distinguished based on size and intracellular aspect: (1) short and thick rods (0.6 × 1.25 µm), with a dense dark intracellular content and (2) longer and

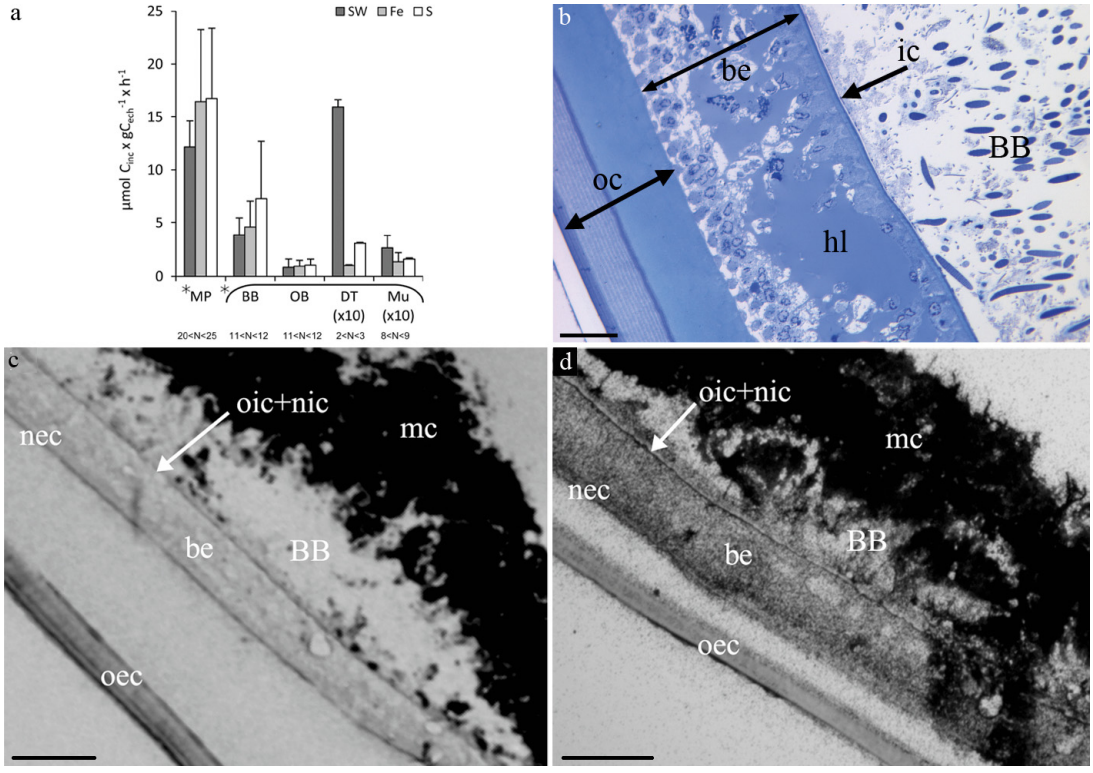

Fig. 4. Incorporation of inorganic carbon into bacterial mats and shrimp tissues. (a) ^{13}C fixation for specimens incubated for 10 h in $NaH^{13}CO_3$ in pure seawater (SW; dark grey), supplemented with reduced iron (Fe, light grey) or thiosulfate (S, white) (from Ponsard et al. 2013). Stars indicate significant differences between groups of tissues; '×10' indicates tissues for which the values were multiplied by 10 for graphical presentation; 'N' indicates the number of samples for each data bar. MP: mouth parts; BB: bacterial biofilm; OB: outer branchiostegite (= shrimp branchiostegite tissue); DT: digestive tract; Mu: muscles. (b) Semi-thin cross-section (toluidine blue stained) of the branchiostegite, showing the inner (ic) and outer cuticle (oc) and the bacterial biofilm (BB) growing on the inner side, close to the hemolymphatic lacunas (hl) of the branchiostegite epithelium (be). (c,d) Autoradiography of the branchiostegite of a specimen incubated with ^{14}C-acetate: (c) after only 3 d and (d) after 11 d of exposure (from Ponsard et al. 2013). oec: old external cuticle; oic: old internal cuticle; nic: new internal cuticle; nec: new external cuticle; mc: mineral crust. Scale bars = 50 µm (b), 100 µm (c,d)

thinner rods (0.3 × 3 µm), with a light intracellular content. Two sub-types of thin filaments were also described based on their cell morphology and intracellular aspect: (1) thin filaments with rectangular cells, no marked narrowing between 2 adjacent cells and a homogeneous and dense content and (2) thin filaments with ovoid cells, with a marked narrowing between 2 adjacent cells, a more heterogeneous intracellular content and occurrence of intracellular electron-dense granules (Fig. 5c). Finally, in addition to the previously described thick filaments, these authors reported the occurrence of bacteria with stacks of intracytoplasmic membranes typical of type I methanotrophs, bringing the diversity to 6 morphotypes (Fig. 5b).

Intracellular granules were observed in the thin filaments (but not in the other morphotypes). Energy dispersive x-ray microanalysis revealed 2 types of granules: one containing phosphorus and iron, probably as iron polyphosphate, and the other containing only sulfur (Zbinden et al. 2008). Gebruk et al. (1993) also reported the occurrence of granules of elemental sulfur in the filamentous bacteria of *R. exoculata*. Shrimp maintenance in a pressurized aquarium led to the emptying of most of the granules, thus suggesting a storage role (Zbinden et al. 2008).

2.1.4. Bacterial phylogenetic diversity

Hydrothermal endosymbioses were initially thought to rely on a limited number of symbionts (for example, only 1 symbiont, a sulfur-oxidizing *Gammaproteobacteria* species, was identified in *Riftia pachyptila*, *Calyptogena magnifica* and *Bathymodiolus thermophilus*, Distel et al. 1988). However, further studies showed a higher diversity of closely related bacterial lineages co-occurring in a single host (see Duperron

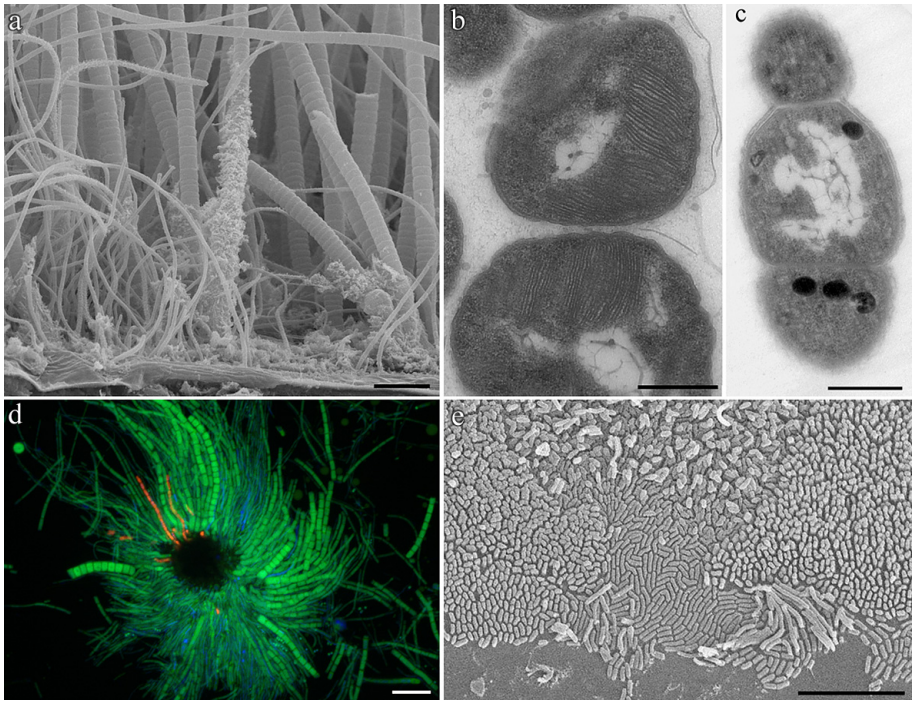

Fig. 5. Diversity of branchial chamber bacterial epibionts. (a) Scanning electron microscopy (SEM) view of the thick and thin filamentous bacteria on the inner face of the branchiostegite. (b) Transmission electron microscopy (TEM) view of methanotrophic bacteria characterized by stacks of intracytoplasmic membranes. (c) TEM view of intracellular granules in the thin filaments. (d) Fluorescence *in situ* hybridization view of a scaphognathite seta showing *Campylobacteria* thick and thin filaments (green) and *Gammaproteobacteria* thin filaments (red). (e) SEM view of a monolayer of prostrate and erect rod-shaped bacteria. Scale bars = 10 µm (a,d,e), 0.5 µm (b,c)

et al. 2008, Ansorge et al. 2019). Likewise, the first phylogenetic study (Polz & Cavanaugh 1995) showed that all *R. exoculata* epibionts belonged to a single bacterial lineage of *Campylobacteria* (previously assigned to *Epsilonproteobacteria*; Waite et al. 2017, 2018), which is no longer supported. The high morphological diversity observed among the *R. exoculata* symbiotic community (see references above) rather argues for a higher phylogenetic diversity, a hypothesis proposed by Polz & Cavanaugh (1995). Advances in sequencing technologies helped to refine the initial data and showed a much greater diversity. Two dominant groups were identified: *Campylobacteria* (up to 90% based on investigated specimens) and *Gammaproteobacteria* (10–30%). *Alpha-*, *Beta-*, *Delta-* and *Zetaproteobacteria*, as well as *Bacteroidetes*, *Flavobacteria*, *Bacilli*, *Clostridia* and even some *Aquificae* were also reported at lower abundance (Zbinden et al. 2008, Petersen et al. 2010, Hügler et al. 2011, Guri et al. 2012, Jan et al. 2014, Jiang et al. 2020).

Fluorescent *in situ* hybridization (FISH) observations allowed intersecting data of morphology and molecular biology, confirming the predominance of *Campylobacteria* with thick and thin filamentous morphologies and *Gammaproteobacteria* related to some thin filamentous morphologies and rods (Fig. 5d, Hügler et al. 2011, Guri et al. 2012, Liu et al. 2019). Type I methanotrophic *Gammaproteobacteria* resemble circular 'donuts' in FISH (Guri et al. 2012). *Zetaproteobacteria* appear as rod-shaped curved bacteria, fixed on the host tegument (Jan et al. 2014). Some *Deltatproteobacteria* also appear as rods, close to the shrimp tegument (Hügler et al. 2011, Liu et al. 2019, Jiang et al. 2020).

2.1.5. Bacterial metabolic diversity

The single phylotype initially described was inferred to be a sulfur-oxidizer, because of its morphological resemblance to *Thiothrix* (Wirsen et al. 1993) and the occurrence of sulfur granules within the cells (Gebruk et al. 1993). Several authors later suggested that, at hydrothermal vents, a number of other reduced inorganic compounds could be used as electron donors by chemoautotrophic bacteria, such as hydrogen, ammonia, and nitrite, and heavy metals such as iron or manganese (Jannash & Wirsen 1979, Segonzac et al. 1993). Attempts to cultivate these symbionts (and beyond, all other currently known symbionts) have remained unsuccessful to date. Potential metabolism can thus only be inferred from *in vivo* experiments on animals or fresh bacteria (Fisher et al. 1989, Zbinden et al. 2008, Scott et al. 2012, Ponsard et al. 2013), occurrence of specific genes, transcripts or other indirect assumptions, as described below.

2.1.5.1. Carbon fixation. Chemoautotrophic metabolism relies on bacterial oxidation of reduced compounds available in fluids, mostly H_2S, CH_4, Fe and H_2. Along the MAR, the fluids emitted at the different

sites colonized by *R. exoculata* show distinct chemical compositions, as summarized in Table 2 (see Section 1.2 for explanations).

Chemoautotrophy of the branchial chamber bacterial community was established early by reports of both ^{14}C-labeled bicarbonate incorporation and high ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity in the bacteria covering the shrimp mouthparts and carapace (Wirsen et al. 1993, Polz et al. 1998). Moreover, *in vivo* experiments demonstrated autotrophic capabilities of *Campylobacteria* and *Gammaproteobacteria* lineage morphotypes (Ponsard et al. 2013). According to molecular approaches (single-gene PCR amplification to metagenomic studies), 3 carbon fixation pathways co-occur in the *R. exoculata* epibiotic community. All chemoautotrophic *Campylobacteria* investigated to date (Campbell & Cary 2004, Hügler et al. 2005, 2011, Jan et al. 2014) use the reductive tricarboxylic acid (rTCA) cycle, highlighted by gene detection of its key enzyme: the ATP-dependent citrate lyase (Hügler et al. 2011). In the metagenome of *R. exoculata* mouthparts and branchiostegites, the rTCA complete set of genes was recovered (Jan et al. 2014). The second most important pathway is the Calvin-Benson-Bassham (CBB) cycle, highlighted by gene detection of its key enzyme: Rubisco. *Gammaproteobacteria* (Hügler et al. 2011, Beinart et al. 2012) typically use the CBB cycle. All of the CBB cycle genes were identified in the metagenome from *R. exoculata* mouthparts (Jan et al. 2014). Two notable exceptions to this usual pattern of CBB/*Gammaproteobacteria* and rTCA/*Campylobacteria* are: (1) the *Riftia pachyptila* gammaproteobacterial endosymbiont *Endoriftia persephone* (as well as other siboglinid endosymbionts) in which both carbon fixation pathways co-occur (Markert et al. 2007, Yang et al. 2020), and (2) campylobacterial epibionts of deep-sea mussels which use the CBB cyle for carbon fixation (Assié et al. 2020). Genes for the CBB cycle were also retrieved in *R. exoculata*-associated *Zetaproteobacteria* (Jan et al. 2014). Recently, a third carbon fixation pathway was highlighted among epibionts of the *R. exoculata* branchial chamber: the Wood-Ljungdahl pathway (Jiang et al. 2020), for which nearly all genes required were highlighted in the deltaproteobacterial epibiont '*Candidatus* Desulfobulbus rimicarensis' metagenome and transcriptome (Jiang et al. 2020).

Incorporation of radio-labeled small organic molecules (^{14}C-acetate and ^{3}H-lysine) in thick and thin filaments indicates that at least a fraction of the symbionts can switch to mixotrophic metabolism, depending on available energy and/or carbon sources (Ponsard et al. 2013). Genes for acetate assimilation were identified in the symbiont metagenomes (Jan et al. 2014, Jiang et al. 2020).

2.1.5.2. Sulfur metabolism. Sulfide oxidation was initially proposed as the sole metabolism of the symbiotic population (Wirsen et al. 1993), based on (1) the phylogeny of the symbionts (thought to belong to a single campylobacterial phylotype, Polz & Cavanaugh 1995), (2) the high level of H_2S at the Snake Pit site from where the shrimp and their bacteria were first described and (3) the observation of internal sulfur granules in shrimp ectosymbionts (Gebruk et al. 1993).

Successful amplification of several genes coding for subunits of key enzymes involved in the oxidation of sulfur compounds confirmed the occurrence of this type of metabolism within the epibiotic community: (1) genes coding for the adenosine-5′-phosphosulfate reductase (*aprA*), related to *Gammaproteobacteria* sulfur oxidizers and to *Deltaproteobacteria* sulfate reducers (Zbinden et al. 2008, Hügler et al. 2011) and (2) genes coding for sulfate thiohydrolase (*SoxB*), related to *Campylobacteria* (Hügler et al. 2011). Through metagenomic approaches, numerous *Sox* genes (*SoxAZYX*, *SoxB*, *SoxW*) and *aprA* genes affiliated to *Campylobacteria* and *Gammaproteobacteria* were also found (Jan et al. 2014). *Dsr* genes (coding for the dissimilatory sulfate reductase) were found in *Gammaproteobacteria* (Jan et al. 2014). This enzyme is known to promote the oxidation of stored elemental sulfur, and could in this case be used to take advantage of the reserves of intracellular elemental sulfur (Zbinden et al. 2008):

$$\underset{}{H_2S} \xrightarrow{Dsr} \underset{}{Sulfite\ (H_2SO_3)} \xrightarrow{Apr} \underset{}{APS} \rightarrow \underbrace{\underset{}{Sulfate\ (H_2SO_4)}}_{Sox}$$

where APS is adenosine-5′-phosphosulfate reductase.

Hügler et al. (2011) highlighted the co-occurrence of sulfur-oxidizing and sulfur-reducing epibionts among the symbiont community, suggesting that they may be involved in a syntrophic exchange of sulfur compounds, which could increase the overall efficiency of the *Rimicaris exoculata* cephalothoracic epibiotic community. More recently, genes implicated in the disproportionation of inorganic sulfur compounds were identified in the deltaproteobacterial epibiont '*Candidatus* Desulfobulbus rimicarensis' metagenome (Jiang et al. 2020). In this latter case, sulfur compounds (thiosulfate, sulfide, elemental sulfur) can be used as electron donors or acceptors depending on their degree of oxidation, which expands metabolic epibiont capabilities and may avoid substrate competition.

2.1.5.3. Methane oxidation. Aerobic methanotrophs can be recognized by their characteristic intracytoplasmic membrane stacking system in which methane oxidation occurs. Such morphotypes have been observed in *R. exoculata* epibionts from the Rainbow site, using TEM (Zbinden et al. 2008) and FISH (Guri et al. 2012). Bacterial 16S rDNA gene sequences obtained from these samples clearly belong to known methanotrophic *Gammaproteobacteria* symbionts (like *Bathymodiolus* symbionts) (Zbinden et al. 2008). Furthermore, particulate methane monoxygenase (*pmoA*) genes affiliated to the methylotrophic *Gammaproteobacteria* class (*Methylomonas* sp., *Methylobacter* sp. and *Bathymodiolus pmoA* gene sequences) were obtained (Zbinden et al. 2008).

Using ^{14}C-labeled methane incubation, Polz et al. (1998) failed to detect methane utilization in shrimp branchiostegites or scaphognathites from Snake Pit samples. The levels of CH_4 in the fluid, and consequently the proportion of this type of metabolism among the bacterial community, are probably too low at Snake Pit to detect any activity. Such activity could possibly be measured at ultramafic sites like Rainbow or Logatchev. Indeed, if the ratio of CH_4:H_2S in the fluids is less than 1 (as at Snake Pit), the thiotrophic symbionts in the mussel *Bathymodiolus azoricus* dominate, whereas if this ratio is higher than 2 (as at Rainbow or Logatchev), the methanotrophs dominate (Salerno et al. 2005). This may be true for *R. exoculata* symbionts as well, but remains to be tested.

2.1.5.4. Iron oxidation. Oxidation of sulfide and methane has long remained the only bacterial autotrophic chemosynthetic metabolism described in hydrothermal vent symbionts. However, the exceptional enrichment in iron in Rainbow fluids (see Table 2) and the fairly abundant and rusty mineral deposits surrounding the bacteria of the shrimp branchial chamber at that site (see Fig. 3d) suggest that iron oxidation could be another potential metabolism (Zbinden et al. 2004). Long based on indirect suggestions, a large body of evidence now indicates that iron oxidation must be present within the epibiosis. In oxic environments, spontaneous ferrous iron oxidation is extremely rapid at ambient temperature (25°C) and neutral pH (Emerson & Moyer 1997, Neubauer et al. 2002). Under these conditions, Fe-oxidizing bacteria may be unable to compete with the abiotic processes. However, in the shrimp environment, the temperature is usually lower than 25°C, and oxygen is reduced to about two-thirds of its level in air-saturated seawater (Zbinden et al. 2004). Under such conditions, abiotic oxidation is much slower. Based on the kinetic study of Millero et al. (1987), Zbinden et al. (2004) calculated that the half-life of ferrous iron would be about 35 h in a shrimp environment. Considering that the upper pre-branchial chamber (housing the main ectosymbiotic bacterial community) is located downstream of the gills in the water flow, leading to a pH reduction, a CO_2 increase and an O_2 decrease due to shrimp respiration, the abiotic oxidation rate should thus be even slower (Zbinden et al. 2004). Moreover, since the rusty minerals only appear following a recolonization of the branchial chamber by bacteria after each molt (Corbari et al. 2008a) and because of their tight association with microbial cells (Gloter et al. 2004, Anderson et al. 2008, Corbari et al. 2008b), a neutrophilic iron-oxidizing bacterial origin is strongly suspected.

Other data are in line with a bacterial origin of iron oxides. The very homogeneous composition and the uncommon mixed-valence of the nanocristalline 2-line ferrihydrite (Gloter et al. 2004) of these iron oxides are characteristic of bacteriogenic iron oxyhydroxides (Kennedy et al. 2004). Iron polyphosphate granules were detected inside some thin filamentous epibionts (Zbinden et al. 2008). Such granules are widely distributed in prokaryotes, and could serve as ATP substitutes, energy storage units or chelators of metal ions (Kornberg 1995). An iron storage role has been suggested here to fuel the metabolism in case of iron depletion in the environment. Shrimp maintenance in a pressurized aquarium without iron supply indeed led to the emptying of most of the granules (Zbinden et al. 2008). Finally, this hypothesis is supported by the fact that a 6 h shrimp incubation in the presence of ferrous iron increases the ^{14}C-carbon fixation rate, showing that ferrous iron contributes to fueling *R. exoculata* epibionts (Ponsard et al. 2013).

Molecular data were recently obtained regarding the occurrence of iron-oxidizing bacteria among the epibionts, revealing members of the *Zetaproteobacteria* through a metagenomic approach and FISH observations (Jan et al. 2014). Thus far, all characterized *Zetaproteobacteria* are iron oxidizers, able to grow at circumneutral pH and under microaerophilic conditions, resulting in the precipitation of large amounts of Fe-oxyhydroxides (Emerson et al. 2007, Jan et al. 2014, Scott et al. 2015, Henri et al. 2016). Furthermore, homologs of genes expressed during iron oxidation by *Acidithiobacillus ferrooxidans* (a molybdopterin oxidoreductase Fe_4S_4 region and ferredoxin encoding gene) were identified in the partial metagenome of *Zetaproteobacteria* epibionts of shrimp (Jan et al. 2014).

Based on geochemical calculations, Schmidt et al. (2008a) showed that at the Rainbow site, iron oxida-

tion appears to be the major energy source for chemosynthesis, due to its exceptional enrichment in fluids. However, kinetic modeling (Schmidt et al. 2009) suggested successive stages of iron oxide deposition, being first microbiologically driven and followed by an abiotic iron oxidation. The initial bacterial ferrihydrite deposition, which is a strong catalyst for abiotic iron oxidation, would favor the subsequent abiotic deposition. In the earliest stage of the molting cycle, the formation of iron oxyhydroxides would then be dominated by iron-oxidizing bacteria, and the resulting accumulation of biogenic iron oxide would subsequently favor abiotic iron oxidation and inhibit microbial growth.

2.1.5.5. Hydrogen. Hydrogen powers primary production in bacterial symbionts of the hydrothermal vent mussel *Bathymodiolus puteoserpentis*, from the Logatchev site on the MAR (Petersen et al. 2011). High concentrations of hydrogen are present in the fluids of ultramafic systems. It is a particularly favorable electron donor, as the energy yield from hydrogen oxidation is much higher than that from methane oxidation, sulfur oxidation and all other potential electron donors for chemolithoautotrophic growth under standard conditions (Petersen et al. 2011).

The key enzymes involved in hydrogen metabolism are hydrogenases, which catalyze the reaction: $H_2 \rightarrow 2H^+ + 2e^-$. The *HupL* gene, encoding the large subunit of a [NiFe] hydrogenase, was successfully amplified in *R. exoculata* and sequences were affiliated to *Campylobacteria* and *Deltaproteobacteria* relatives (Hügler et al. 2011). Jan et al. (2014) also found several hydrogenase genes (H_2 uptake and H_2 sensing) and relatives in the *Campylobacteria* and *Gammaproteobacteria* partial metagenome, suggesting that both dominant epibionts have the potential to use hydrogen as an energy source. Recently, the deltaproteobacterial epibiont '*Candidatus* Desulfobulbus rimicarensis' was also proposed to be capable of hydrogen oxidation (Jiang et al. 2020). Measures of H_2 consumption in the shrimp incubation medium (as demonstrated for *B. puteoserpentis* gill tissues by Petersen et al. 2011) or an increased carbon incorporation rate by *R. exoculata* bacteria in the presence of hydrogen (as shown in the presence of sulfide and iron by Ponsard et al. 2013) would prove that at least part of the symbiotic bacteria actually carry out this metabolism.

2.1.5.6. Metabolism plasticity. Since the concentration of major electron donors varies greatly among the end-member fluids at the different sites colonized by *R. exoculata* (see Table 2), it is likely that the activity level of metabolic pathways related to those compounds varies accordingly within the epibiosis. The energy budget provided by the oxidation of the various potential electron donors greatly depends on local environmental conditions, notably their concentrations, temperature and pH (Zbinden et al. 2004), which will define the energy that can be derived from the different oxidative pathways (Schmidt et al. 2008a). Using geochemical calculations, Schmidt et al. (2008a) determined the most favorable energy metabolism for the Rainbow and TAG sites. At Rainbow, iron oxidation appears to be the major energy source, due to its exceptional enrichment in fluids. The energy yield of hydrogen oxidation depends on how fast it reacts with oxygen, but could be in the same range as for iron oxidation. Sulfide and methane only represent secondary energy sources for microbial primary production at that site. At TAG, sulfide oxidation is the major energy source for microbial primary production; iron oxidation could still be substantial, while methane represents only a minor part of the primary production (Schmidt et al. 2008a). At the Logatchev vent field, aerobic hydrogen oxidation could provide up to 7 times more energy per kilogram of vent fluid than methane oxidation, and up to 18 times more energy per kilogram of vent fluid than sulfide oxidation, based on a thermodynamic model (Petersen et al. 2011). These data indicate that the chemical energy pathways used by *R. exoculata* branchial chamber symbionts differ depending on the fluid chemistry at a site, highlighting metabolic plasticity in the shrimp–microbe symbiosis.

In the light of all of these results, we can now consider the branchial chamber epibiont community of *R. exoculata* as a functional consortium, composed of different morphotypes and phylotypes, and possessing different complementary metabolisms. In summary, compiling all of these data, the following scheme can be drawn (Fig. 6):

(1) The bacterial community is numerically dominated by filamentous *Campylobacteria* and *Gammaproteobacteria* (respectively 39 and 30% of the bacterial sequences of the metagenome; Jan et al. 2014).

(2) *Campylobacteria* appear as thick filaments and some thin filaments. Both filament types are chemoautotrophic sulfur-oxidizers, using the Sox pathway for energy generation and the rTCA cycle for carbon fixation (*SoxB* and *aclA* gene sequences, respectively). Some of the *Campylobacteria* also possess hydrogenase genes and may use hydrogen oxidation for energy generation. Thick and thin filaments incorporate small organic molecules (acetate and lysine), indicating that at least a fraction of these symbionts can switch to mixotrophic metabolism, depending on available energy and/or carbon sources.

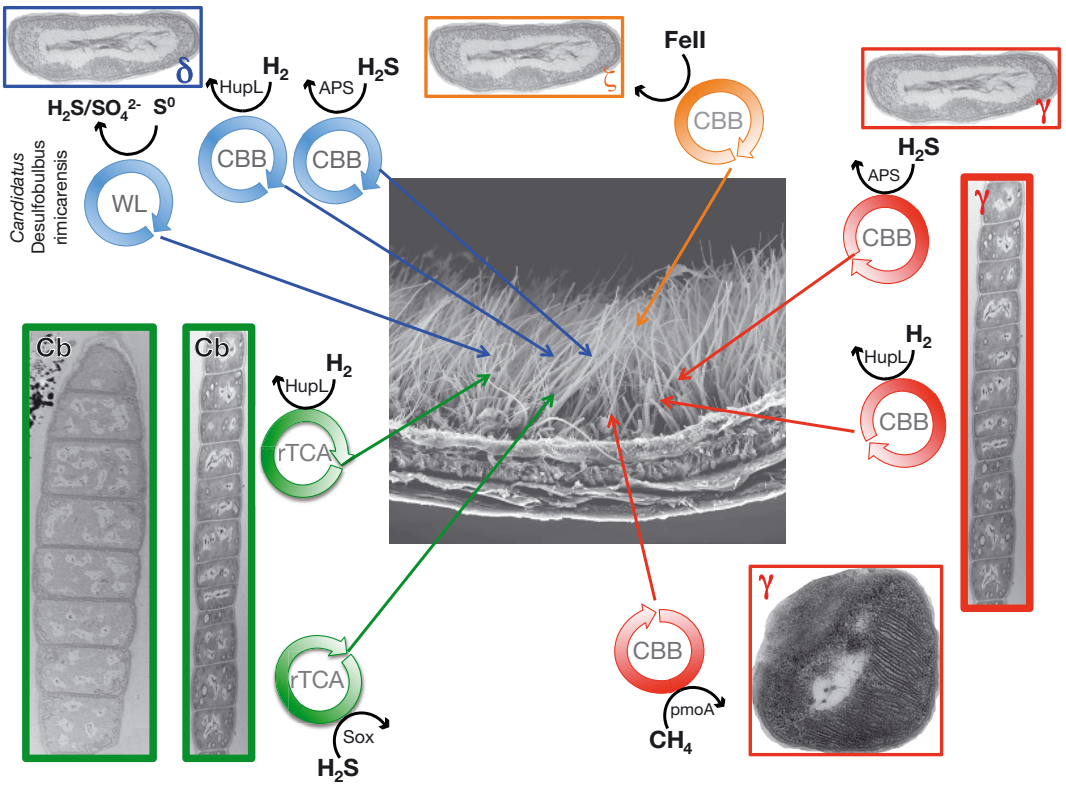

Fig. 6. Summary scheme of the diversity and functioning of the *Rimicaris exoculata* branchial chamber symbiosis. Dominant phylotypes are indicated by thicker boxes. Sox: sulfate thiohydrolase; HupL: [NiFe] hydrogenase; APS: adenosine-5′-phosphosulfate reductase; pmoA: particulate methane monoxygenase, A-subunit; rTCA: reductive tricarboxylic acid cycle; CBB: Calvin-Benson-Bassham cycle; WL: Wood-Ljungdahl pathway; Cb: *Campylobacteria*; γ, ζ, δ: respectively *Gamma-*, *Zeta-* and *Deltaproteobacteria*. Colors differentiate the phylotypes

(3) *Gammaproteobacteria* appear as thin filaments and rod-shaped bacteria. They are chemoautotrophic sulfur-oxidizers, using the APS pathway for energy generation and the CBB cycle for carbon fixation (*aprA*/*Sox* and *cbbM* gene sequences, respectively). *Gammaproteobacteria* also occur as coccoid-shaped cells with intracytoplasmic membranes, characteristic of type I methanotrophs, using methane oxidation for energy generation and probably also using the CBB cycle for carbon fixation.

(4) *Zetaproteobacteria* appear as rod curved bacteria and are chemoautotrophic iron-oxidizers (identified by a molybdopterin oxidoreductase Fe_4S_4 region and ferredoxin encoding genes), using the CBB cycle for carbon fixation (*cbbM* genes).

(5) *Deltaproteobacteria* appear as small cocci and possess *aprA* and hydrogenase genes, suggesting they can grow lithotrophically, using the Wood-Ljungdahl pathway. They can use both sulfide and hydrogen as electron donors for sulfate and/or sulfur reduction and are capable of disproportionation of inorganic sulfur compounds.

Observed diversity may depart from this general scheme as the relative abundance of the different phylotypes and/or the importance of different metabolic pathways may differ among sites (especially between basaltic and ultramafic sites). Plasticity could also depend on the molt stage, as shrimp seem to be predominantly colonized by *Campylobacteria* after the exuviation, while later molt stages appear to be dominated by *Gammaproteobacteria* (Petersen et al. 2010). More work is required to elucidate the functioning of this plasticity, which appears to enable the co-occurrence of similar metabolism, with no evidence of bacterial competition and maybe even cooperation. Complementary studies are underway through metagenomic but also metatranscriptomic approaches, together with the development of deep-sea sampling tools in order to better access *in vivo* functioning of this complex symbiosis.

2.2. Digestive tract symbionts

2.2.1. Digestive tract anatomy and content

Morphological descriptions of the digestive tract of *R. exoculata* reveal a small stomach with few grind-

ing structures, suggesting low mechanical digestive activity (Segonzac et al. 1993, Durand et al. 2010). The midgut, i.e. the central digestive absorption zone deprived of a cuticle, represents two-thirds of the total length of the digestive tract, and is significantly longer than in most crustaceans (Durand et al. 2010).

The first studies examining the digestive tract of *R. exoculata* reported a high sulfur mineral content, typical of chimney walls (chalcopyrite [$CuFeS_2$], pyrite [FeS_2] and sphalerite [ZnS]), along with iron oxides (Van Dover et al. 1988, Segonzac et al. 1993) (Fig. 7a,b). Elevated bacterial densities were also reported in the stomach (10^9 cells per ml of stomach content, Van Dover et al. 1988) and still increasing in the mid- and hindgut (Polz et al. 1998). A high CO_2 fixation rate in the mid- and hindgut (Polz et al. 1998) suggested the occurrence of a highly active chemoautotrophic bacterial community that could use the gut polymetallic sulfide content as an energy source (Pond et al. 1997a). These bacteria could be a nutritional source, through either a classical digestion process or by essential dietary component supply or synthesis of organic molecules (Pond et al. 1997a).

Shrimp cuticle fragments were also observed in the gut content (Zbinden et al. 2004). The consumption of freshly molted exuviae to ingest associated bacteria was suggested. But as the bacterial coverage decreases beneath the iron oxide crust before the molt (Zbinden et al. 2004, Corbari et al. 2008a), it seems more likely that *R. exoculata* eats the exuvium to recover the minerals (e.g. calcium) contained in the cuticle, as observed in other crustaceans (Steel 1993, Corbari et al. 2008a).

2.2.2. Bacterial localization and morphological diversity

Stomach bacteria seem to be subject to a digestion process, as bacterial DNA and lipopolysaccharides were detected (Van Dover et al. 1988, Durand et al. 2010), but SEM and TEM observations failed to detect intact microorganisms (Van Dover et al. 1988, Zbinden & Cambon-Bonavita 2003). Intact rods and cocci were observed within the gut content (Zbinden & Cambon-Bonavita 2003). Some of them are probably transient, but some may be part of a specific resident microflora (Zbinden & Cambon-Bonavita 2003, Durand et al. 2010, Cowart et al. 2017).

A dense bacterial community of thin and long (0.2 µm × 15 µm) single-celled bacteria is also present between the microvilli of the midgut epithelium

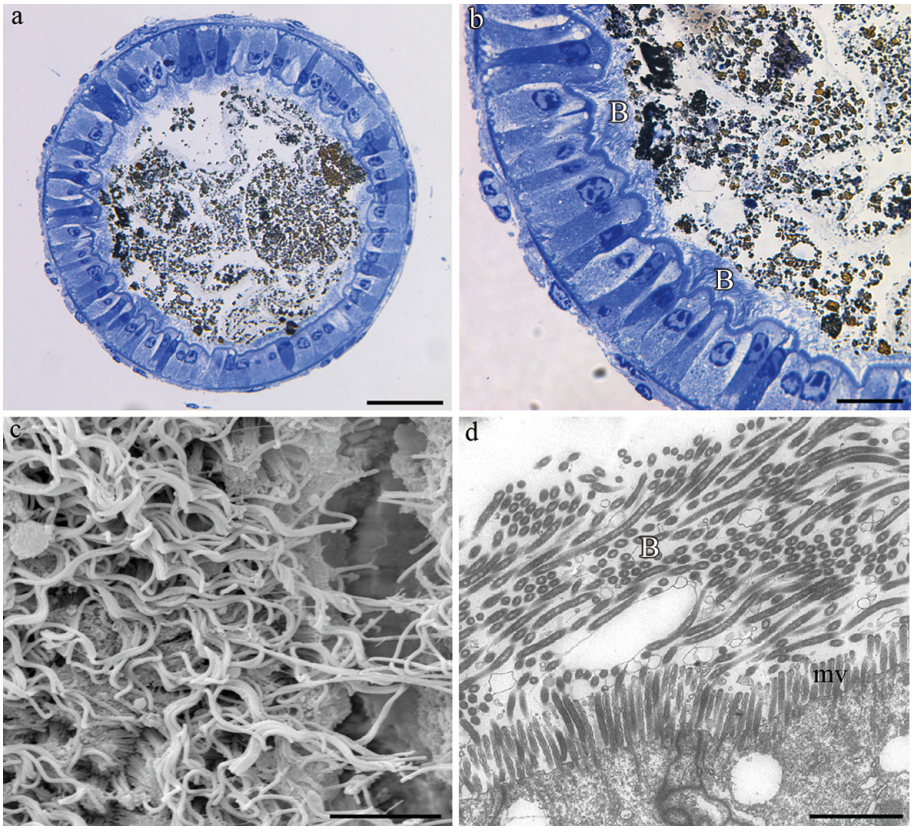

Fig. 7. *Rimicaris exoculata* gut epibionts. (a) Semi-thin section in the midgut showing the black and brown mineral particles (respectively iron sulfides and oxides). (b) Closer view of the epithelium, showing the abundance of filamentous epibiotic bacteria (B) all around the section. (c) Scanning electron microscopy view of the epibionts (picture by L. Durand). (d) Transmission electron microscopy observations of the bacterial gut epibionts (B) inserted between the microvilli (mv). Scale bars = 50 µm (a), 20 µm (b), 5 µm (c), 2 µm (d)

(Durand et al. 2010) (Fig. 7c,d). This community is separated from the bolus by the peritrophic membrane and is thus not part of the shrimp diet, at least not through a digestion process. As the midgut is deprived of cuticle, and thus not subject to exuviation, this probably favors long-term microbial colonization and interactions of this resident microbial community with its host (Durand et al. 2010). Possibly under host control of bacterial invasion, filamentous epibiont cells would grow, along with their host, without division, leading to this long, single-celled, filamentous morphology. Further investigations are required to confirm this hypothesis (Durand et al. 2015).

2.2.3. Bacterial phylogenetic diversity

The bacterial diversity in the midgut is largely represented by 3 major groups: *Deferribacteres*, *Mollicutes* and *Campylobacteria*. *Gammaproteobacteria* are also present to a lesser extent (Zbinden & Cambon-Bonavita 2003, Durand et al. 2010, Cowart et al. 2017). This diversity is relatively restricted compared to the environmental one (Durand et al. 2015). The 3 main lineages were still present after a 72 h starvation experiment leading to the complete elimination of the bolus, and were consequently designated as resident epibionts (Durand et al. 2010) rather than transient microflora. The 2 former phylotypes, absent from the environment (except for 1 clone retrieved from an environmental sample collected close to shrimp aggregations; Hügler et al. 2011), could be a specific and selected bacterial resident community (Zbinden & Cambon-Bonavita 2003, Durand et al. 2010, 2015). Conversely, gut and branchial chamber *Campylobacteria* epibionts are similar, suggesting that a part of gut *Campylobacteria* could be directly acquired from the branchial chamber and/or the environment, and may thus be a transient community remaining due to a permanent ingestion process (Durand et al. 2015). *In vivo* experiments using radiolabeled ^{14}C-bicarbonate have revealed poor digestive incorporation, suggesting that *Campylobacteria*, and to a lesser extent *Gammaproteobacteria*, would not be active at least under autotrophic conditions (Ponsard et al. 2013).

As no specific hybridization response has been obtained yet on the thin filamentous bacteria inserted between the microvilli, their phylotype assignation remains uncertain (Durand et al. 2010). A 72 h starvation experiment showed a low representation of *Deferribacteres* in the resulting clone library, suggesting that the filaments (which were unaffected by the starvation) would not belong to this group. Being mostly anaerobic and iron dependent, *Deferribacteres* may have lysed during the experiment, which does not fit natural conditions, preventing any definitive conclusion (Durand et al. 2010). Furthermore, it should also be noted that the *Deferribacteres* haplotype is mostly dominant at the Rainbow site, but less represented at TAG and even less at Logatchev (Cowart et al. 2017), whereas microscopic observations revealed the same occurrence of filamentous bacteria at these 3 sites (Durand et al. 2015). The thin filaments were also suspected to belong to *Mollicutes*, based on their morphology, but this has not been confirmed, as FISH attempts have been unsuccessful thus far (Durand et al. 2010, 2015). More work has still to be done using metagenomic and metatranscriptomic approaches in order to properly identify these filamentous bacteria and to reveal their potential role in holobiont fitness.

2.3. Symbiont transmission

2.3.1. Branchial chamber symbionts

The cyclic occurrence of aposymbiotic (before the symbiont acquisition) and symbiotic phases in the host life cycle is intrinsic to horizontally transmitted symbioses (Bright & Bulgheresi 2010). When present, larvae are also aposymbiotic during their pelagic dispersal (Bright & Bulgheresi 2010). To date, no bacteria have been observed inside eggs of *R. exoculata*, but *Gammaproteobacteria* and *Campylobacteria* have been reported on their surface (Guri et al. 2012, Cowart et al. 2017, Methou et al. 2019). In the same way, bacterial symbionts appear to be absent in the branchial chamber of post-hatching larvae of *R. exoculata* (Guri et al. 2012, Hernandez-Avila et al. 2015) or in their first juvenile stages after recruitment (Komai & Segonzac 2008). Some *Campylobacteria* and *Gammaproteobacteria* sequences retrieved from Rainbow seawater samples were closely related (99% similarity) to epibiont sequences from the branchial chamber of shrimp from the same site (Guri et al. 2012). A free-living form of the *Campylobacteria* symbiont was also detected in substantial proportions at the Snake Pit site (Polz & Cavanaugh 1995). Finally, using a 16S rDNA gene, segregation by site was observed among *Campylobacteria* and *Gammaproteobacteria* symbionts (Petersen et al. 2010, Durand et al. 2015), which was also supported using *Lux* genes (Le Bloa et al. 2017). These results suggest

the existence of horizontal (environmental) transmission for the shrimp branchial chamber epibionts, acquired in each generation and after each molt (Petersen et al. 2010). Potential symbionts may not always be abundant in the environment. For this reason, many aquatic hosts actively pump water against their tissues, enhancing the chance of entrapping potential symbionts (Bright & Bulgheresi 2010). The water currents generated by scaphognathite beating in the branchial chamber of *R. exoculata* may similarly enhance environmental colonization by symbionts.

2.3.2. Gut symbionts

Free-living *Campylobacteria* and *Gammaproteobacteria* are commonly identified in hydrothermal ecosystems, but the gut community of these groups in *R. exoculata* displays a lower diversity than in the environment (Flores et al. 2011). The gut symbionts could thus be acquired from the environment, but with a selective process (Durand et al. 2015). On the other hand, no *Deferribacteres* (Durand et al. 2015, Cowart et al. 2017) and only 1 sequence affiliated to *Mollicutes* (Hügler et al. 2010) were retrieved from the environment. The strong similarity of *Deferribacteres* associated with shrimp (>99%), regardless of site, suggests a specific long-term association between these bacteria and their host (Durand et al. 2015). Both their absence in the environment and the long-term association suggest vertical transmission of these symbionts. As previously mentioned, no bacteria have ever been observed inside the eggs (Guri et al. 2012, Cowart et al. 2017). Thus another, horizontal, transmission pathway is suggested. Epibionts could be acquired at early juvenile stages through anus-to-mouth fluid exchange with adults (proctodeal trophallaxis) (Durand et al. 2015), as observed in some social insects with intestinal symbionts, such as termites or cockroaches (Salem et al. 2015).

2.4. Ectosymbiosis and molt cycle

In the shrimp, as in other arthropods, growth occurs through cyclic molts, during which the cuticle is regularly renewed. At ecdysis, when the entire old cuticle is shed, the bacterial community of the branchial chamber is shed with it, and a new bare cuticle covers the freshly molted shrimp. As direct continuous observations during the molt cycle are not possible at hydrothermal sites, estimation of the absolute duration of the molt cycle (in days) can only be determined indirectly, averaged over an entire sample. The percentage of individuals at each molting stage was determined using the Drach method (Drach & Tchernigovtzeff 1967) and corresponds to the relative duration (in percentage of the absolute total duration of the complete molt cycle) of the stage (Corbari et al. 2008a). By compiling data from the literature, Corbari et al. (2008a) showed that the anecdysial period (stage C_4) varied considerably in different shrimp species of comparable size, whereas the pre-ecdysial period (stages D_0–D_4) was similar. Comparison with known cycles of similar-sized coastal shrimp allowed these authors to estimate that the interval between 2 successive exuviations in *R. exoculata* (Fig. 8a) could be as short as 10 d (compared to 21 d for *Penaeus japonicus* or 48–91 d for *Macrobrachium rosenbergii*) (Corbari et al. 2008a).

The abundance and distribution of the bacterial coverage are related to the molting cycle and depend on the time elapsed since the last molt (Zbinden et al. 2004, Corbari et al. 2008a). Bacterial re-colonization begins just after the exuviation, with only scarce and randomly distributed patches of short thin filaments and rods (Fig. 8b,c). Rapid bacterial growth quickly (within 2 d) leads to a dense bacterial mat composed of long thin and thick filaments (Corbari et al. 2008a). Evolution of bacterial coverage until the next molt consists mainly of the gradual accumulation of mineral deposits, mainly composed of iron oxides (see Section 2.1.5.4) at Rainbow and associated with iron sulfides at TAG (Corbari et al. 2008a). These mineral deposits ultimately turn into a thick crust isolating the bacterial community from the external environment (Fig. 8d,e) (Corbari et al. 2008a). A rapid molting rate is usually regarded as an antifouling mechanism contributing to the elimination of epibiotic bacteria (Bauer 1989). The rapid molting frequency in *R. exoculata* is thought to be critical in eliminating the mineral crust that isolates the bacteria from the reduced compounds of the fluid on which they rely, thus allowing the recolonization of a new and efficient fueling symbiotic community (Corbari et al. 2008a).

The color of the adult shrimp exoskeleton evolves throughout the molt cycle due to progressive mineral deposits in the branchial chamber. Color evolves from white and translucent specimens in post-ecdysis (Fig. 8b) to light red and finally dark red specimens in pre-ecdysis (Fig. 8d) at the Rainbow site, where only iron oxides are deposited. At TAG, where the fluid contains more sulfide and less iron than at Rainbow, grey shrimp appear between white and light red individuals in the molt cycle. The grey color corresponds to iron sulfide deposits on the bacterial

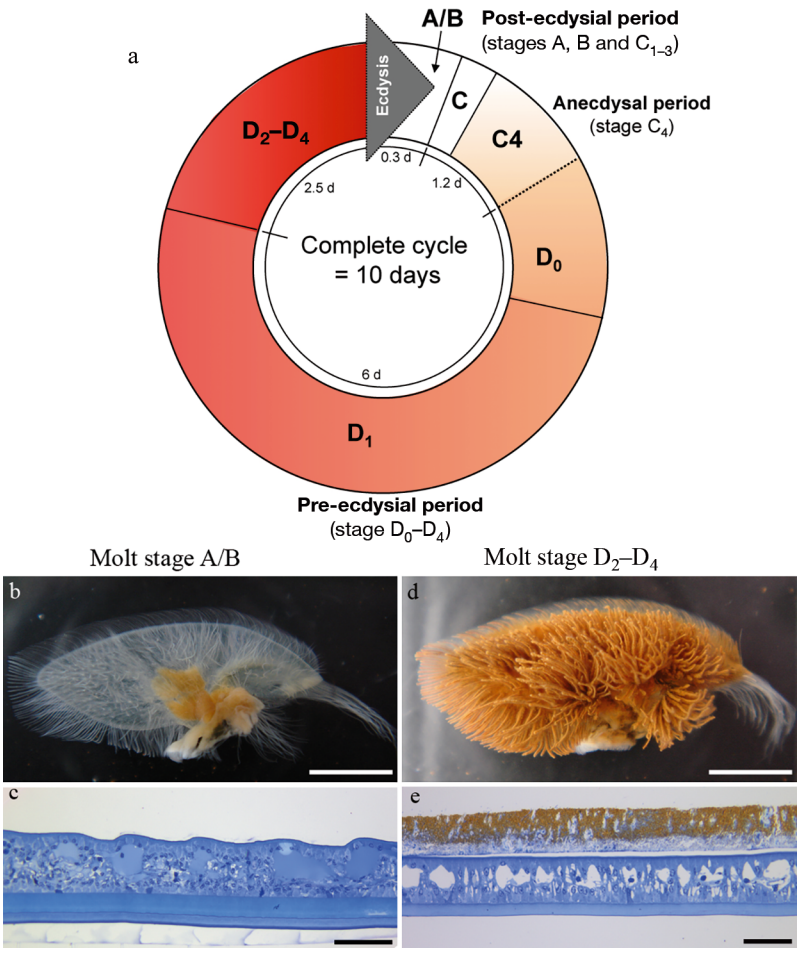

Fig. 8. (a) *Rimicaris exoculata* molt cycle with duration of the different stages (graphic courtesy of L. Corbari). (b,d) Coloration pattern of scaphognathites (specimens from the Trans-Atlantic Geotraverse [TAG] site) and (c,e) semi-thin sections of the branchiostegite (specimens from the Rainbow site) illustrating the bacterial and mineral coverage of a specimen in the A/B stages (b,c) and the D_2–D_4 stages (d,e). Scale bars = 5 mm (b,d), 100 µm (c,e)

biofilm. Late pre-ecdysial individuals are also dark red at TAG (Corbari et al. 2008a). Color can thus be used as a convenient proxy to determine specimen molting stage (Corbari et al. 2008a).

The bacterial community of the digestive tract is located in the midgut, devoid of cuticle, and thus is not submitted to the molt cycle. This symbiotic community does not need to be renewed from scratch at each molt.

2.5. Host–symbiont interactions

2.5.1. Host–symbiont recognition

The first essential step in the establishment of a symbiosis with horizontal transmission is the bilateral recognition of host and symbiont. The diversity of the epibiotic community of the *R. exoculata* branchial chamber is very stable in time and space (Zbinden et al. 2008, Petersen et al. Guri et al. 2012), and is reduced compared to the environmental bacterial diversity (Flores et al. 2011). It can therefore be assumed that a subset from the pool of diverse environmental free-living bacteria is selected during the stages of colonization. Host–bacteria recognition is usually facilitated by host lectin-like (or other types of) surface molecules that produce adhesion receptors that recognize and bind microbe-associated molecular patterns present on the microbial surface (Liu et al. 2019). In invertebrates, all symbiotic lectins identified to date belong to the subgroup of C-type lectins (CTLs), like in the nematode *Laxus oneistus* or the clam *Codakia orbicularis*, which both harbor sulfur-oxidizing symbionts (Bulgheresi et al. 2006, Gourdine et al. 2007). A CTL was identified in *R. exoculata*, termed RCTL (Liu et al. 2019), predominantly expressed in symbiotic (compared to non-symbiotic) tissue, reflecting its main function of agglutinating symbionts therein (Liu et al. 2019). A recombinant protein (rRCTL) was used for bacterial binding experiments on species of *Campylobacteria* (*Arcobacter nitrofigilis*) and *Gammaproteobacteria* (*Escherichia coli*). The results showed that the rRCTL recognizes and agglutinates only the *Gammmaproteobacteria*, but not the *Campylobacteria* species (Liu et al. 2019). Unlike conventional crustacean CTLs (i.e. non-symbiosis-related, pathogen-oriented lectins), rRCTL did not affect the normal growth of the bacteria that had been recruited and could thus play a role in the recognition and enrichment of the *Gammaproteobacteria* symbionts (Liu et al. 2019).

2.5.2. Quorum sensing perception and biofilm formation

The selection of a subset of microbial types from the environment could also result from interactions/regulation between the epibionts themselves. In a

biofilm such as that in the branchial chamber of *R. exoculata*, bacteria communicate with each other through the secretion of signaling molecules, such as the quorum sensing pathway, to coordinate the biofilm formation, exopolysaccharide production, virulence or cell aggregation (Montgomery et al. 2013). Genes coding for such signaling molecules (*luxS* in *Campylobacteria* and *luxR* in *Gammaproteobacteria*) were revealed in epibionts of the *R. exoculata* branchial chamber through a metagenomic study (Jan et al. 2014), but the expression of *luxS* and *luxR* genes was only detected for the pre-molting stages, at the peak of bacterial colonization (Le Bloa et al. 2017). Quorum sensing in the *R. exoculata* branchial chamber epibiosis could regulate bacterial growth to avoid an excessive development of the biofilm. It could also be involved in the dispersal of the epibiont biofilm, liberating some bacteria from the biofilm in the proximal environment of the shrimp just before the molt so that these bacteria can recolonize the freshly molted shrimp (Le Bloa et al. 2017).

Genes encoding proteins involved in attachment to surfaces (type IV pili, O antigen) and genes encoding for flagella, implied in bacterial motility and chemotaxis, were evidenced in the *R. exoculata* epibiont metagenome (Jan et al. 2014). They may be involved in *Campylobacteria* and *Gammaproteobacteria* attraction to the host and biofilm formation within the branchial chamber of the shrimp (Le Bloa et al. 2017).

2.5.3. Pathogen recognition and non-proliferation of symbionts

To maintain its epibiosis, the shrimp must at the same time select its epibionts among microbial communities, regulate their proliferation and prevent colonization by non-beneficial bacteria or pathogens. Permanently exposed to a large density and diversity of microorganisms, marine animals in general, and *R. exoculata* in the present case, must have an effective immune system to control microbial growth. Devoid of any specific immune system, shrimp rely only on innate immunity, a non-specific, rapid defense oriented towards all external aggressions (Rosa & Barracco 2010). Part of this defense occurs through antimicrobial peptides (AMPs), small-sized molecules naturally produced by all living organisms, which act as a first line of defense against bacterial infection (Rosa & Barracco 2010, Tasiemski et al. 2014). The first AMP isolated from a deep-sea hydrothermal species was described in the polychaete *Alvinella pompejana* (Tasiemski et al. 2014), which harbors a dense epibiotic community on the dorsal expansions of its tegument (Gaill et al. 1984) and therefore shares similarities with *R. exoculata* (Segonzac et al. 1997). Tasiemski et al. (2014) showed that AMP targets specifically one type of bacteria (the most abundant) but has no effect on the others, suggesting that this AMP (alvinellacin) selects and shapes the *A. pompejana* epibiotic microflora and prevents microbiota from proliferating. Gu et al. (2018) characterized another AMP (which they named RspALF1, for *Rimicaris* sp. anti-lipopolysaccharide factor) in a *Rimicaris* species from the Manus Basin. RspALF1 is mostly expressed in hepatopancreas and gills (but bacteriophore tissues, i.e. branchiostegite and scaphognathite, were not tested). RspALF1 exhibits anti-microbial activity, *in vitro* and *in vivo*, against a wide range of Gram-negative and Gram-positive bacteria, by binding to the cell wall components and damaging bacterial cellular structures. RspALF1 is more effective (i.e. inhibiting bacterial growth at lower concentrations) on bacteria isolated from the Manus Basin deep-sea hydrothermal vent field than on neritic bacteria. Anti-fungal activity was also demonstrated for RspALF1. Le Bloa et al. (2020) identified an AMP in *R. exoculata*, which they named *Re*-crustin. *Re*-crustin is mainly synthesized in the tissues on which the ectosymbiotic community of the branchial chamber develops, i.e. the branchiostegite and scaphognathite. As it is mostly expressed at the juvenile stage, *Re*-crustin has been proposed to be involved in symbiont attraction and adhesion (Le Bloa et al. 2020). AMPs could thus also play a role in host symbiont recognition and not just in the regulation of their proliferation.

All of these data lead to the hypothesis that there could be a stringent recognition system between the shrimp and its branchial chamber symbionts and strict control by the shrimp of the selected bacteria allowing a phylogenetically almost identical recolonization in the branchial chamber after each molt (Le Bloa et al. 2017).

3. PHYSIOLOGY

3.1. Respiration

Rimicaris exoculata features 10 pairs of phyllobranchiate gills, with single-layered branchial lamellae bordered by a thin cuticle (0.3–0.5 µm) (Martinez et al. 2005). The gills do not appear to be involved in osmoregulation (which seems only mediated by the epipodites in the branchial chamber), considering the

lack of Na^+-K^+ ATPase (Martinez et al. 2005). Gills are mainly involved in respiration, but a detoxification activity also occurs in the gills through sulfide-oxidizing bodies (Compère et al. 2002; see Section 3.4).

Living within steep chemical and thermal gradients, where hot, reduced hydrothermal fluid mixes turbulently with oxygenated seawater (Schmidt et al. 2008a), shrimp are exposed to highly fluctuating oxygen conditions, sometimes to hypoxic conditions. For example, O_2 concentrations of 63–236 µM have been measured in discrete samples of fluid in the vicinity of shrimp aggregations (Zbinden et al. 2004). For comparison, concentrations of 242 and 12.4 µM were measured from ambient seawater and a black smoker outlet at the Rainbow site, respectively (Zbinden et al. 2004). Few studies have been devoted to respiratory adaptations of *R. exoculata* (Lallier & Truchot 1997, Lallier et al. 1998), and to a broader extent to crustaceans at hydrothermal vents (Arp & Childress 1981, Sanders et al. 1988, Lallier et al. 1998, Chausson et al. 2001, 2004). Ravaux et al. (2003) measured oxygen consumption rates of 0.837–1.094 mg O_2 g^{-1} dry mass h^{-1} for *R. exoculata* at *in situ* pressure conditions (23 MPa) and at oxygen levels of the deep seawater surrounding the North Atlantic vent sites. These values are in good accordance with the relationship between oxygen consumption and dry mass obtained for crustaceans (see references in Ravaux et al. 2003).

The respiratory pigment of decapod crustaceans is hemocyanin (Hc), which reversibly binds oxygen to a pair of copper atoms. The Hc of *R. exoculata* is composed mostly of hexameric molecules (89%) of 469 ± 9 kDa (Lallier & Truchot 1997). For a majority of crustaceans, Hc–O_2 affinity is relatively low, with oxygen partial pressure at half-saturation (P_{50}) values >10 Torr under physiological conditions (Truchot 1992). For example, P_{50} = 27 Torr at pH 7.8, 10°C for the coastal shrimp *Palaemon elegans* (Bridges et al. 1984). However, Hc–O_2 affinity is rather well correlated with ambient oxygen content of the environment (Sanders & Childress 1990, Truchot 1992), and Hc affinity for oxygen of species living in hypoxic environments is higher than for those living in normoxic conditions. Lallier & Truchot (1997) showed that *R. exoculata* Hc has a high oxygen affinity (P_{50} = 3 Torr at pH 7.5, 15°C), which is also the case for *Segonzacia mesatlantica*, the endemic crab of the MAR vent sites (P_{50} = 2.2 Torr at pH 7.5, 15°C, Chausson et al. 2004), and for other vent endemic crustacean species (Hourdez & Lallier 2007). *R. exoculata* Hc also showed almost no temperature effect on P_{50} in the range 15–35°C, a large Bohr effect (oxygen affinity increases with pH) and a moderate lactacte (main end-product of anaerobiosis in Crustacea) effect (oxygen affinity increases with lactate) (Lallier & Truchot 1997). This is favorable in an environment where temperature and oxygen contents considerably fluctuate (Lallier et al. 1998). Lallier & Truchot (1997) also highlighted that native hemolymph shows a lower affinity for O_2 (P_{50} = 6) than when dialysed, suggesting the occurrence of a dialyzable factor in *R. exoculata* hemolymph that decreases Hc–oxygen affinity. The high affinity would thus allow better oxygen extraction in a frequently hypoxic environment, while the decrease in pH and the possible presence of other factors would increase the release of the oxygen to the tissues by decreasing the affinity of Hc for oxygen (Lallier et al. 1998).

3.2. Sensory perception

3.2.1. Localization of hydrothermal emissions

Since the very first publications describing hydrothermal vents, the question of the localization of vent emission by endemic species was put forward. Corliss et al. (1979, p. 1079) noted that 'there are many questions to be answered about these animal communities. One concerns how they locate and colonize new vents.' *R. exoculata* faces recognition of active hydrothermal sites at different times of its life. After dispersal in the water column, larvae need to find a vent site to settle and begin their development toward adult life (Pond et al. 1997b, Herring & Dixon 1998). As adults, shrimp have to navigate in the environment, find fluids fueling their symbiotic bacteria, or just remain in an appropriate range of physicochemical conditions (Desbruyères et al. 2000, 2001). Temperature, dim light emitted by vents, chemical compounds like sulfide or acoustic vibrations induced by hydrothermal fluid emissions have been proposed as navigational cues for the detection of hydrothermal vents (Van Dover et al. 1989, Segonzac et al. 1993, Renninger et al. 1995, Gaten et al. 1998a,b, Crone et al. 2006), but few studies have reported clear results on these assumptions.

3.2.1.1. Vision. As hydrothermal environment is devoid of light and as shrimp eyes present obvious modifications, the vision of the so-called 'eyeless shrimp' has been relatively well studied. Adults of *R. exoculata* (as well as *Mirocaris fortunata*, *R. chacei* and *Alvinocaris markensis*) lack the usual externally differentiated eye (eye-stalk) of shrimp, having instead a pair of large, highly reflective, dorsal

organs (Van Dover et al. 1989). These unusual eyes (see arrows in Fig. 2b) have no image-forming optics, but a solid wall of light-sensitive rhabdom consisting of 3500 photoreceptors (containing rhodopsin). Beneath the rhabdomeral segments, a thick white diffusing layer (tapetum) increases, by reflection, the quantity of light captured by the retina (O'Neill et al. 1995, Nuckley et al. 1996, Kuenzler et al. 1997, Lakin et al. 1997, Wharton et al. 1997, Jinks et al. 1998, Chamberlain 2000). These features are mostly understood as an adaptation for detection of extremely faint sources of light emitted by the vents (thermal radiations or sonoluminescence, i.e. glow produced by imploding bubbles of gas at high pressure; Pelli & Chamberlain 1989, Van Dover et al. 1989, 1996). The eyes of alvinocaridid shrimp present a clear evolution between the larvae and adults (also observed in the hydrothermal vent crab *Bythograea thermidron*, Jinks et al. 2002) from an imaging retina to the non-imaging retina of the adults: the zoeal eye is similar (eye-stalk) to those of other surface-dwelling decapod larvae (Gaten et al. 1998a,b), suggesting that the early stages are planktonic (Gaten et al. 1998b). Intense lights (and notably those of submersibles used for the collection) were shown to induce irreversible retinal damage in hydrothermal shrimp (*Rimicaris exoculata* and *Mirocaris fortunata*), with complete loss of the rhabdom layer (Herring et al. 1999).

3.2.1.2. Chemoreception. Chemoreception in decapods encompasses 2 modalities: the 'olfaction,' mediated by olfactory receptor neurons of specialized unimodal olfactory sensilla (the esthetascs), restricted to the lateral flagella of the antennules (Laverack 1964, Grünert & Ache 1988, Cate & Derby 2001), and the 'distributed chemoreception,' mediated by numerous bimodal sensilla (innervated by both mechano- and chemoreceptor neurons) occurring on all appendages (Schmidt & Mellon 2011, Mellon 2014, Derby et al. 2016). Renninger et al. (1995) suggested increased sensory capabilities in hydrothermal species due to the fact that the non-esthetasc bimodal chemosensilla of *R. exoculata* antenna were more numerous and innervated by 4 to 5 times more dendrites than in the coastal shrimp *Penaeus aztecus*. Zbinden et al. (2017) described a higher diversity of non-esthetasc setal types in the coastal shrimp *Palaemon elegans* than in several hydrothermal shrimp species. However, the role of these sensilla is still poorly known, and whether their diversity corresponds to a multiplicity of perceived stimuli remains an open question (Cate & Derby 2001). Focusing on esthetasc sensilla of *R. exoculata*, Zbinden et al. (2017) revealed no specific adaptation regarding the size or number of esthetascs between hydrothermal and coastal species. The anatomical features of esthetascs do not reveal striking differences between hydrothermal and coastal species, whether in terms of (1) cuticle thickness, which relates to permeability and thus to the ability to detect odor molecules, or (2) the number of olfactory sensory neurons and dendrites, which relates to the odorant discrimination ability (Machon et al. 2018). A specificity of hydrothermal species (*M. fortunata* and *R. exoculata*) is the occurrence of numerous pore-like structures in the esthetasc cuticle, absent from the coastal species, which could facilitate the passage of odor molecules through the cuticle to the olfactory receptors of the neurons (Machon et al. 2018). IR25a, an olfactory co-receptor, is expressed in the lateral antennular flagella, bearing the olfactory sensilla of the esthetascs, but also to a lesser extent in the medial antennular flagella and the antennae in *R. exoculata* (Zbinden et al. 2017). Molecular studies are underway to further investigate adaptations of hydrothermal species by identifying, quantifying and localizing the different chemoreceptors expressed by olfactory sensory neurons (Zbinden et al. 2017).

Electroantennography detection of food-related odor and sulfide (but only at the high concentrations encountered in the pure fluid), via both antennae and antennules, were evidenced in hydrothermal shrimps (*R. exoculata* and *Mirocaris fortunata*), as well as in coastal species (*Penaeus aztecus* and *Palaemon elegans*) (Renninger et al. 1995, Machon et al. 2018). Nevertheless, detection of the same chemical can trigger different behavioral reactions according to species. Renninger et al. (1995) observed *in situ* strong orientation behavior to a piece of sulfide removed from the chimney but reported unpublished data of Sofranko and Van Dover showing avoidance behavior to sulfide solution in the coastal shrimp *Palaemonetes vulgaris*.

A recent study investigated the brain architecture of adult *R. exoculata* (Machon et al. 2019). This study shows, as one would expect, a small visual area, as already observed in blind cave crustaceans (Stegner et al. 2015). Surprisingly, however, as opposed to most blind animals that rely on olfaction as a major sensory modality, *R. exoculata* also exhibits a small olfactory area. The striking features of the *R. exoculata* brain are the especially well developed higher brain centers (the hemiellipsoid bodies), which integrate all of the sensory information and are considered high-order learning and memory centers (Maza

et al. 2016). The unusual development of this brain part has also been observed in crustaceans with sophisticated navigation skills or spatial memory (like *Panulirus argus*, Sandeman et al. 2014, or mantis shrimp, Wolff et al. 2017). This suggests that adult *R. exoculata* may strongly rely on peculiar navigation skills with learning of place memory to locate in its environment and memorize emission sites of hydrothermal fluids, rather than on vision or chemodetection (Machon et al. 2019). A study of the brain architecture of larvae and juveniles is underway to determine whether the sensory modalities of early life stages differ from those of adults.

3.2.1.3. Thermoreception. Even though the thermal biology of *R. exoculata* has been investigated (see Section 3.3), very few studies have been conducted on thermosensibility. Peripheral specific or putative multimodal thermoreceptors are not known in crustaceans (Ache 1982). Nevertheless, *R. exoculata* seem able to detect thermal differences, as they avoid extreme thermal temperatures, showing quick escape behavior when coming in contact with hydrothermal fluid (Segonzac et al. 1993, M. A. Cambon-Bonavita & M. Zbinden unpubl. data). In addition, behavioral studies have shown that *R. exoculata* can discriminate temperatures in a gradient and is attracted by the hot points (10°C) in a cold environment (2°C, Ravaux et al. 2009).

3.2.2. Biotic interactions

For most crustaceans, chemodetection is the dominant sensory modality used in all important biological processes. Various chemical cues are used for the recognition of conspecifics, individual identity, social status or aggressiveness, for the localization of sexual partners and for the identification of injured or diseased conspecifics or in order to avoid predators (Derby & Weissburg 2014). To date, no data are available about such social interactions in *R. exoculata* aggregations. Known predators of *R. exoculata* are crabs (*Segonzacia mesatlantica*), gastropods (*Phymorhynchus* sp.), Actiniaria, bathyal fishes and possibly *R. chacei* (Gebruk et al. 1997a,b, 2000, Van Dover 2000, Colaço et al. 2002).

3.3. Thermal biology

The extreme trophic specialization of *R. exoculata* appears to force the shrimp to live close to superheated fluid discharges (Gebruk et al. 1997a). Damage (black burnt cuticles) caused by contact with superheated fluid has been observed on many specimens (Van Dover et al. 1988, Gebruk et al. 1993, 2000, Segonzac et al. 1993; Fig. 1). The proportion of damaged specimens with burnt pereopods, pleopods or antennae at the TAG site ranged from 33 to 50 % of the population (Gebruk et al. 1997a, Vereshchaka et al. 2000). Shrimp were often observed deviating very suddenly when entering in contact with hot fluid. Vereshchaka et al. (2015) related the efficiency of the shrimp escape behavior to a morphological characteristic of *R. exoculata*, which possess 2 strong movable spines instead of 1 on the tail fan.

These organisms thrive not only near very hot fluid outlets, but also in an environment where the thermal variability is the highest among aquatic habitats (Bates et al. 2010). What is the upper thermal limit tolerated by this shrimp, and what are the molecular mechanisms and/or behavioral strategies involved to cope with such deleterious temperatures and their fluctuations (Ravaux et al. 2019)? Answers to these questions came with the development of pressure aquaria allowing *in vivo* experiments (Ravaux et al. 2003, 2009, Cottin et al. 2010a,b, Shillito et al. 2014, Ravaux et al. 2019). The *R. exoculata* upper thermal limit (critical thermal maximum, CTmax) was first proposed to be in the 33–37°C range (Ravaux et al. 2003), but was later recalculated by Shillito et al. (2006) as 38.5 ± 2°C. This species can therefore not sustain prolonged exposure at this temperature, and has also been proven to trigger a heat stress response through the over-expression of the stress gene *hsp70* when exposed for 1 h to 30°C. No response was triggered when exposed to 20°C, or for shorter time exposure, whereas a 10 min shock peaking at 39°C also yielded a heat stress response (Cottin et al. 2010a, Ravaux et al. 2019). In addition to the molecular response to heat shock, *R. exoculata* presents 2 kinds of behavioral response to heat stress: (1) an escape response at temperatures above 24°C (Ravaux et al. 2003) and (2) thermoregulation behavior via increased activity among the swarm, leading to a mix between hot hydrothermal vent and cold seawater in their surroundings maintaining an optimal temperature in the swarm (Ravaux et al. 2019). This has previously been described for social insects like bees or wasps that cool the colony by wing fanning (Jones & Oldroy 2006). *R. exoculata* may therefore choose a microhabitat in a relatively narrow thermal window that would be defined by its nutritional demands, thermal tolerance and thermal preference, as evidenced by the low level of *hsp70* expression in natural populations (Ravaux et al. 2019).

Similar to their host, *R. exoculata* epibionts are punctually subjected to bursts of very hot temperatures. To date, however, only few data are available on their thermal sensitivity. Genes encoding heat shock proteins were recently identified in the metagenome for *Campylobacteria*, *Gammaproteobacteria* and *Zetaproteobacteria* epibionts, indicating their possible ability to cope with the fluctuating conditions of the hydrothermal habitat (Jan et al. 2014).

3.4. Detoxification

Hydrothermal vent invertebrates are surrounded by fluids containing numerous potential chemical threats, such as sulfide (a cellular respiration poison), methane and dissolved heavy metals like iron, copper, zinc, cadmium and lead (Charlou et al. 2002). An excessive accumulation of metals in the cells can lead to the formation of reactive oxygen species and cause severe cell damage. Hydrothermal fauna have developed adaptations to deal with these toxic compounds, excreting them from their bodies or storing them in less toxic forms (Childress & Fisher 1992, Rousse et al. 1998). The question of detoxification is all the more topical in the present circumstances, where the various metals (e.g. Cu, Ni, Zn, Mn, Co, Ag, Au) contained in the massive hydrothermal sulfide deposits are of growing economic interest to mining industries (Gramling 2014, Mengerink et al. 2014). It is likely that exploitation activities will expose the local fauna to intense plumes of sediments, metals and various chemical compounds (Van Dover 2014; see Section 6 on potential threats).

Various detoxification mechanisms have been suggested or described for *R. exoculata*. Metals can be stored as insoluble, non-circulating forms, thus avoiding disturbance of essential metabolic functions (Geret et al. 2002). For example, copper and sulfur are accumulated in the epithelial cells of the *R. exoculata* hepatopancreas as insoluble granules, thus decreasing the potential toxic effect of Cu present in the cytoplasm of cells (Auguste et al. 2016). Hepatopancreas and gills are the 2 tissues that accumulate the most trace metals, in concentrations that reflect their concentrations in the vent fluid (Geret et al. 2002, Kádár et al. 2006, Auguste et al. 2016). The most accumulated metals in *R. exoculata* soft tissues are Fe, Zn, Mn and Cu, whilst Ag and Cd are accumulated less (Gonzalez-Rey et al. 2008). However, compared with the concentration in their environment, only Cu and Cd are bioaccumulated (Geret et al. 2002). *R. exoculata* experimental exposure to 0.4 and 4 µM of Cu in pressurized aquaria did not show significant differences in Cu concentrations in the tissue analyzed between the 2 treatments, which could indicate a potential regulation mechanism of metal metabolism by the shrimp (Auguste et al. 2016). Compared with coastal shrimps, low levels of arsenic (Larsen et al. 1997, Taylor et al. 2012) and mercury (Martins et al. 2001, Kádár et al. 2006) have been detected in *R. exoculata*, probably related to their short food chain, which limits the accumulation of toxic compounds in tissues. Moreover, compared to *Mirocaris fortunata* that lives farther away from vent fluids, *R. exoculata* had similar or often less metal accumulated in selected organs, possibly the result of a higher degree of adaptation to hydrothermal conditions (Kádár et al. 2006), or a shorter food chain.

Sulfide detoxification was proposed to occur in the gill epithelium of the shrimp via sulfide-oxidizing bodies, electron-dense organelles containing membrane stacks (Compère et al. 2002). Such structures were also observed in different tissues of organisms from sulfide-rich habitats, such as the gills of the clam *Solemya reidi* or the hindgut and tegumental tissues of the echiuran worm *Urechis caupo*, in which enzymatic oxidation of sulfide to less toxic thiosulfate was demonstrated (Compère et al. 2002 and references therein). Iron detoxification was also proposed to occur in the gills of *R. exoculata*, thanks to a ferritin (H-ferritin RexFrtH) which rapidly converts and stores the ferrous iron contained in the fluid passing over the gills in a less toxic ferric form (Liu et al. 2019).

Another detoxification process, the metallothioneins (MTs), cystein-rich, low molecular weight proteins with a high affinity for physiological (Zn, Cu, Se) or xenobiotic (Cd, Hg, As, Ag) metal cations, have been proposed to be involved in binding metals and could also act as oxyradical scavengers, protecting cells against reactive oxygen species (Roesijadi 1992). Induction of metallothioneins was observed in the shrimp gill after an experimental exposure to 4 µM of Cu, probably linked to the role of MTs in the detoxification and/or storage of Cu (Auguste et al. 2016), but the presence of MTs, as well as activities of several antioxydant enzymes in control shrimp, showed the existence of constitutive mechanisms of protection against metal toxicity (Auguste et al. 2016). Compared to *Mirocaris fortunata*, *R. exoculata* showed much higher MT levels (approximately 6-fold higher), suggesting a higher exposure to metals, but lower antioxidant activities (Gonzalez-Rey et al. 2007). A possible explanation is that metal-induced reactive oxygen species are less likely to be formed when MT synthesis increases, leading to a natural decrease in

the antioxidant enzymatic protections (Gonzalez-Rey et al. 2007).

Following hypotheses proposed for *Alvinella pompejana* filamentous epibionts on the EPR (Cosson-Mannevy et al. 1988), detoxicating properties have also been proposed for the shrimp symbiotic bacteria at MAR (Segonzac et al. 1993, Jan et al. 2014). Due to their metabolic activities, symbionts convert harmful compounds such as sulfide, nitrite or FeII to other less toxic compounds, which in return may have a beneficial effect for their host. Some granules and polysaccharides synthesized by symbionts may also play a role in metal chelation (Jan et al. 2014). However, further investigations are needed to elucidate the role of symbionts in detoxification, including deeper metagenomics studies, transcriptomic and *in vivo* experiments.

4. REPRODUCTION AND DISPERSAL

4.1. Oogenesis and reproductive cycle timing

Based on specimens recovered at different periods of the year (June–July, September and November), Copley et al. (2007) and Ramirez-Llodra et al. (2000) respectively observed a continuous range in oocyte size–frequency distributions, from *R. exoculata* individuals with only small oocytes (76–108 µm) to individuals with much larger ones (466–601 µm). These findings led the researchers to conclude asynchronous gametogenesis. Copley et al. (2007) suggested a lack of seasonal reproduction in *Rimicaris exoculata* due to similar median sizes of oocytes in their 2 samplings (September and November), whereas Ramirez-Llodra et al. (2000) observed that females collected in September had larger oocytes than those collected in June, arguing for a more advanced stage of development. Sampling in August to October revealed that many of the females had well-formed eggs in their gonads and were ready to spawn (at the TAG and Broken Spur sites, Vereshchaka et al. 1998), whereas sampling in January and March showed brooding females, eggs and hatched larvae (at the Logatchev site, Guri et al. 2012; at the TAG site, Hernandez-Avila et al. 2015). Combining all these data argues for seasonal reproduction in *R. exoculata* (Hernandez-Avila 2016), with spawning occurring in late fall (October–November), females brooding embryos during winter and larvae hatching in the late winter to early spring (January–March). Seasonal reproduction has also been described for 2 other alvinocaridid species: *R. hybisae*, from the Mid-Cayman spreading center (Nye et al. 2012) and *Alvinocaris stactophila*, from the Brine Pool cold seep in the Gulf of Mexico (Copley & Young 2006).

4.2. Brooding and fecundity

In most caridean shrimps, females spawn soon after copulation, and mature oocytes are transferred to pleopods where they are fertilized by the stored sperm (Correa & Thiel 2003). In decapods, copulation (which, to our knowledge, has never been observed in *R. exoculata*) takes place after ecdysis, and the ovigerous females incubate the eggs, until hatching, on the pleopods, between the lateral sclerites that are usually enlarged at the pre-spawning molt to create a brood chamber (Ramirez-Llodra et al. 2000). Brooding females of *R. exoculata* have only recently (2007) been collected (M.-A. Cambon-Bonavita pers. data), despite numerous sampling cruises to all colonized sites since the discovery of the species in 1986. For example, Vereshchaka et al. (1998) noted that *R. exoculata* is represented in Russian collections by thousands of individuals, among which brooding females are absent. This absence was linked to seasonal breeding and sampling efforts almost entirely restricted to the summer months (Gebruk et al. 1997a, Herring 1998, Vereshchaka et al. 1998, Ramirez-Llodra et al. 2000, Guri et al. 2012, Hernandez-Avila et al. 2015, Hernandez-Avila 2016). It was also suggested that ovigerous *R. exoculata* leave the aggregations around high temperature vents, where samples are generally collected, to protect the embryos from hydrothermal fluids and from the risk of mechanical damage caused by the highly active shrimp aggregations (Ramirez-Llodra et al. 2000). Brooding females were finally collected among the shrimp aggregations, first in March 2007 at the Logatchev site (Guri et al. 2012) and more recently from January to March 2014 and 2018, at the TAG and Snake Pit sites (Hernandez-Avila et al. 2015, Methou et al. 2019). This supports the first hypothesis of seasonal reproduction.

Within *R. exoculata* broods, the embryos develop synchronously and all eggs are at the same stage, which can differ from one female to another (Ramirez-Llodra et al. 2000, Guri et al. 2012). Egg sizes of 0.2–0.4 mm (Guri et al. 2012), 0.6 mm (Ramirez-Llodra et al. 2000) and 0.8–1 mm (Vereshchaka et al. 1998) have been reported, probably reflecting different stages of embryo development. A few fecundity values have also been published to date: 988 eggs for 1 female (Ramirez-Llodra et al. 2000, who also reported a

value of 836 eggs for 1 female from the PhD thesis of J. Copley).

4.3. Larval morphology and development strategy

The first larvae of *R. exoculata* were sampled in March 2007 at the Logatchev site (Guri et al. 2012) and were described from a January 2014 collection at the TAG site (Hernandez-Avila 2016). They were obtained either from onboard hatching of brooding females or, using a larval pump, from the water column near adults (Hernandez-Avila et al. 2015). The detailed description of the first larval stage (zoea I) of *R. exoculata* brought important cues about the early life history of the species. The notable absence of both masticatory processes in the mandible and setation in mouthparts (which usually participate in the process of capture and manipulation of food) suggests that the *R. exoculata* first larval stage is a non-feeding larva (Hernandez-Avila et al. 2015). Both the lack of developed feeding structures and the accumulation of triacylgycerols, wax esters and monounsaturated fatty acids in eggs of alvinocaridid species (Pond et al. 1997c) support the occurrence of primary lecithotrophy in the early stage of alvinocaridids (Hernandez-Avila et al. 2015). Nevertheless, the size of the postlarvae and their photosynthetically derived fatty acid contents suggest that larvae must have fed and survive for extended periods as planktotrophic organisms (Pond et al. 1997b, 2000b, Allen-Copley et al. 1998, Dixon et al. 1998, Herring 1998). Combining these data suggests that the early lecithotrophic period would be followed by a feeding period during larval development (Hernandez-Avila et al. 2015).

It should be noted that Alvinocarididae is the only taxon among caridean decapods combining lecithotrophy in the first larval stage (suggested by undeveloped mouthparts) and extended larval development (suggested by the lack of pereiopods and pleopods). Indeed, in all other currently known cases of carideans, lecithotrophy is always associated with abbreviated development, as a mechanism of larval retention (Hernandez-Avila et al. 2015).

4.4. Larval dispersal

Given the apparent discontinuous nature of vent sites along the MAR (even if the number of active vent sites may be higher than actually reported, and may thus correspond to lower inter-site spacing, Baker et al. 2016), vent shrimp populations are faced with the threat of local extinction due to the seismic/volcanic nature of hydrothermal emissions (Dixon et al. 1998). The strong convection current of hot water rising from the vents forms a buoyant plume that is likely to transport larvae away from vents in the water column (Tyler & Young 2003). Along the MAR, the flow remains mostly in the axis of the ridge, constrained by the steep walls of the graben (Tyler & Young 2003, Fouquet et al. 2010). Nevertheless, several geological structures could act as barriers against larval dispersal (such as the Atlantis and Kane Fracture Zones, the Azores islands, etc.). Active swimming and changes of buoyancy would seem necessary to rise above the basin and then sink down to a new site (Herring & Dixon 1998).

Several facts suggest that *R. exoculata* larvae have high dispersal abilities that would facilitate long-distance colonization: (1) the lack of pereiopods and pleopods in the first zoeal stage suggests an extended larval development, (2) the large number of small-sized eggs (Ramirez-Llodra et al. 2000), together with the photosynthetically derived fatty acid contents of the postlarvae (Pond et al. 1997b) suggest that larvae feed for extended periods as planktotrophic organisms, and (3) the genetic analysis of adult *R. exoculata* from several sites that shows no significant genetic differentiation along the MAR (see Section 5). However, to date, no data on larval dispersal of *R. exoculata* are currently available, as the rare larvae collected off-site so far could not be definitively affiliated to a specific species. The data therefore concern Alvinocarididae as a whole. Using a midwater trawl, Herring & Dixon (1998) collected unidentified alvinocarid shrimp post-larvae between 1990 and 3060 m around Broken Spur (without knowing if they were dispersing from the vent site or converging on it; Herring 1998). Although larval stages of some alvinocarid shrimp appear to feed on photosynthetically derived organic matter (Allen-Copley et al. 1998), it is still unclear which habitat the larval stages use (Hernandez-Avila et al. 2015). Although some Alvinocarididae early stages seem to tolerate large pressure variation and survive for a while at atmospheric pressure (Tyler & Dixon 2000, Koyama et al. 2005), temperature seems to determine the upper limit of the bathypelagic habitat. Based on laboratory experiments, Tyler & Dixon (2000) reported that 1 atm pressure (surface) and 20°C conditions are fatal to larvae of the shrimp *Mirocaris fortunata*. The alvinocarid larvae probably do not rise up to the euphotic zone, limited by the thermocline in their upward dispersal (~200 m depth), and feed on particles descending to the aphotic zone (Tyler & Dixon 2000, Hernandez-Avila et al. 2015).

4.5. Settlement

The settlement stage is a key step in reproduction, where larvae have to find a location which confers high probability of survival and successful reproduction (Rittschof et al. 1998). Environmental (chemical and physical) cues, such as inorganic and organic compounds like conspecific odors, surface energy, vibration or light, determine where the larvae settle (Rittschof et al. 1998). At hydrothermal vents, sulfide, as an obvious signature for hydrothermal habitats, was proposed to be a settlement cue (Rittschof et al. 1998). *In situ* experiments at the Juan de Fuca Ridge confirmed attraction of alginate gels containing sulfide on larvae of vent polychaete worms (Cuomo 1985). However, to date, nothing is known on *R. exoculata* larvae settlement cues or sensory abilities. Vereshchaka (1997) indicated a settling stage between 3 and 5 mm in carapace length. The smallest stage A juvenile collected, close to adults, by Methou et al. (2020) was 5.7 mm in carapace length.

4.6. Juveniles

Upon examination of the abundant specimens of postlarval stages, covering a wide range of sizes (rostral carapace length between 4.8 and 17.4 mm), Komai & Segonzac (2008) described 4 ontogenetic stages (A, B, C and D-subadult), with a dramatic morphological change at size of 7–9 mm in carapace length. In a recent study, Methou et al. (2020) revised the ontogenetic description of *R. exoculata* larvae combining morphological observations and DNA identification. These authors proposed 3 ontogenetic stages, A, B and C-subadult. During these stages, no significant increase in size was noticed, with the shrimp probably spending most of its energy for great morphological changes rather than increases in body size (Komai & Segonzac 2008). The most notable changes are the progressive reduction of the rostrum, the fusion of the eye-stalk, the inflation of the branchiostegite, the appearance of numerous plumose setae on the swelling scaphognathites and exopodites (Komai & Segonzac 2008, Methou et al. 2020) and a gradual increase in bacterial abundance between 8 and 10 mm in carapace length (Vereshchaka 1996, Gebruk et al. 2000). Among the 519 juveniles examined from the July 1997 expedition, Shank et al. (1998) found no males and suggested that: (1) male sexual characteristics were not displayed by juveniles, or (2) *R. exoculata* could be a protogynous hermaphrodite. Komai & Segonzac (2008) reported that the smallest male they determined by the pleopod morphology was 10.1 mm in carapace length, corresponding to their stage D, or subadults (stage C-subadults for Methou et al. 2020), corroborating the first hypothesis of Shank et al. (1998).

When arriving at vents, juveniles carry abundant stores of lipids of photosynthetic origin. The carbon stable isotope ratio ($\delta^{13}C$) showed a graded increase from juveniles, through smaller adults to the older specimens (Gebruk et al. 1997b, 2000, Polz et al. 1998, Methou et al. 2020), corresponding to the gradual introduction in the diet of carbon of bacterial chemosynthetic origin. There is also an increase in the proportion of the heavy isotope of nitrogen between the juvenile and the adult stages, which is probably due to epibiont metabolism processes using nitrate (Methou et al. 2020). The lipid data (Pond et al. 2000a,b, Allen et al. 2001), showing an evolution from photosynthetically derived n-3 fatty acids to bacterially derived n-4 fatty acids, also evidence a change in diet during metamorphosis and maturation (Gebruk et al. 2000). The transition between the 2 diets occurs at sizes >10 cm carapace length, when juveniles are mostly sexually mature and have an inflated branchial chamber (Gebruk et al. 2000, Methou et al. 2020), probably mixing at the beginning the remaining reserves of accumulated photosynthetic carbon and carbon of bacterial origin.

Aggregations of hundreds to thousands of juveniles were reported in the vicinity (1–5 m) of the aggregations of *R. exoculata*, at lower habitat temperatures than the adults (10 vs. 28°C), in areas with lower hydrothermal flow, with the juveniles also being less active than the adults (Nuckley et al. 1996, Shank et al. 1998, Gebruk et al. 2000). Patches of juveniles (clearly visible due to their bright orange color) were also observed within adult aggregations, sometimes in direct proximity to fluid emissions (see white circle in Fig. 1). Their habitat could evolve with the progressive acquisition of the symbionts, their transition to a symbiotic diet and thus their requirements for fluid compounds.

Although knowledge on the reproduction and life cycle of *R. exoculata* has progressed in recent years (Hernandez-Avila et al. 2015, Hernandez-Avila 2016, Methou et al. 2019, 2020), there are still many unresolved questions. The main ones focus on the larval stages (larval life span, number of molts, distance, duration and area of dispersal), and on their recruitment (recognition factors for hydrothermal activity, recruitment stage) (Fig. 9).

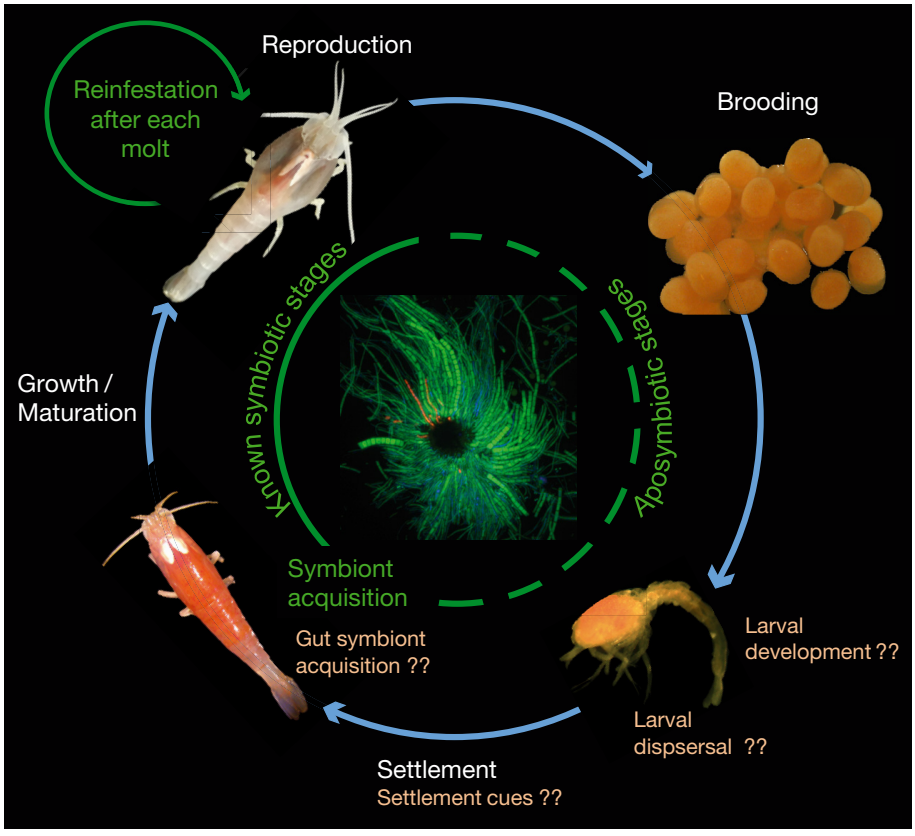

Fig. 9. Life cycle of the vent shrimp *Rimicaris exoculata* and its symbionts. Known symbiotic and aposymbiotic stages, for both gut and branchial chamber symbionts, are indicated. Branchial chamber symbionts are acquired by juveniles at a size range of 7–9 mm in carapace length. Gut symbionts are probably acquired at the juvenile stage, but no precise data are currently available. Current major gaps in the knowledge of the shrimp life cycle are indicated by question marks

5. BIOGEOGRAPHY AND CONNECTIVITY

5.1. Host biogeography and connectivity

The discovery and faunal descriptions of hydrothermal vent sites all around the world gave rise to the description of biogeographic provinces based on faunal distribution (Van Dover et al. 2002, Moalic et al. 2012). Due to the discontinuous and ephemeral nature of these habitats, questions arose about colonization pathways and dispersal capabilities of hydrothermal vent communities. Indeed, the high rate of large-scale dispersal, although potentially generating a significant loss of larvae in the water column, ensures long-term persistence in these fragmented ephemeral habitats (Teixeira et al. 2011). Discontinuities of the ridges (transform faults and fracture zones), as well as hydrological barriers, are thought to impede the along-axis dispersal of a number of vent species and to favor allopatric speciation (Bachraty et al. 2009, Vrijenhoek 2010). The first study of *Rimicaris exoculata* biogeography (Creasey et al. 1996) was conducted using allozyme polymorphisms, between TAG and Broken Spur, 2 sites separated by 370 km. The results showed no significant genetic differentiation, indicating a high gene flow between the 2 populations. The authors calculated a high migration rate between the 2 vent fields exceeding 100 individuals per generation. Using the mitochondrial COI gene from juveniles and adults from Broken Spur, TAG and Snake Pit, Shank et al. (1998) came to the same conclusion that the different individuals analyzed were conspecific. Several studies using mtDNA haplotypes (Teixeira et al. 2011), different mitochondrial marker genes (COI, Dong et al. 2019; COI and CytB, Petersen et al. 2010) and microsatellites (Teixeira et al. 2012) of shrimp from 6 sites along the MAR (between 36° 16′ N and 9° 33′ S) showed an overall high genetic haplotype diversity within the populations, but a lack of strict genetic structure among the different populations. Results also revealed no genetic differentiation among samples separated by up to 11 yr for 2 vent sites (Logatchev and Rainbow). Teixeira et al. (2011, 2012)

suggested that such a pattern could be explained by a recent (about 250 Kyr ago) common bottleneck or (re)colonization event of *R. exoculata* populations along the MAR, followed by an ongoing population extension, with a large panmixia and a probably high larval dispersal capacity (see Section 4.4).

All of these results indicate that neither the distance between vent fields (up to 1000s of km, Vrijenhoek 2010), nor the broad range of depths (2300–4040 m) and the occurrence of the different transform faults or fracture zones along the MAR are obstacles to dispersal and gene flow between the studied *R. exoculata* populations. Teixeira et al. (2012) even reported that of all the species studied to date on hydrothermal vent habitats, *R. exoculata* was the first to exhibit no barriers to dispersal across such a large geographic extent.

5.2. Symbiont biogeography

5.2.1. Branchial chamber symbionts

In contrast with their hosts, a phylogenetic analyses of the 16S rRNA gene of *R. exoculata* branchial chamber epibionts from 3 vent sites (Rainbow, Logatchev and South MAR) showed spatial clustering of bacterial sequences. This genetic differentiation is correlated with geographic distances between the sites rather than with vent geochemistry (i.e. ultramafic- vs. basalt-hosted vent fields) (Petersen et al. 2010). The authors proposed 2 hypotheses to explain the spatial structuring of symbiont communities, without being able to favor one or the other, due to limited data on free-living relatives of the shrimp symbionts: (1) free-living symbiont communities are genetically isolated due to the existence of barriers to gene flow between vent fields, and the spatial structuring of symbiont communities reflects the diversity of free-living forms of the symbionts; (2) the free-living symbiont communities are not spatially structured along the MAR, and the structuring of symbiont communities at each vent site reflects a specific colonization of the hosts by their symbionts from the pool of diverse free-living forms. Using *lux* genes, Le Bloa et al. (2017) also showed a geographical clustering of the epibionts according to their vent site origins (Rainbow, TAG, Snake Pit and Logatchev).

Geographical segregation of the symbionts (absent in hosts) may be attributable either to the influence of the environment on symbionts or to the very recent common history of *R. exoculata* populations, with recent divergence genetically detectable only in short-generation symbionts and still undetectable in the genetics of their hosts (Teixeira et al. 2011).

5.2.2. Gut symbionts

As for the branchial chamber symbionts, *Campylobacteria* and *Gammaproteobacteria* gut symbionts showed differentiation according to the sampled site (Durand et al. 2015, Cowart et al. 2017). This was interpreted as a local acquisition and or selection to confer better fitness to the holobiont best adapted to the local geochemical environment. In contrast, *Deferribacteres* and *Mollicutes* phyla, that have not yet been retrieved in the environment (seawater and chimney samples, Flores et al. 2011, Durand et al. 2015), showed a pattern of phylogenetic differentiation very similar to that of the host, revealing no clear geographical differentiation among the TAG, Logatchev and Ashadze sites. These results are interpreted, for hosts and these symbionts, as significant recurrent gene flow and/or a recent and common bottleneck–expansion history across MAR sites (Teixeira et al. 2011, 2012). This would also be in favor of a vertical transmission for these symbiont lineages (Durand et al. 2015, Cowart et al. 2017). The epibiont divergence pattern at the ribotype level contrasted with that of the hosts only at the Rainbow site (Durand et al. 2015). Both physical (the shallower depth compared to the other sites and its peculiar position at the intersection between the nontransform fault system and the ridge fault; Charlou et al. 2010), as well as the unique geochemical conditions prevailing at Rainbow, could be a factor affecting selective pressures acting on epibionts (Durand et al. 2015). Durand et al. (2015) proposed that the observed epibiont biogeography would reflect an ongoing MAR colonization from the north to the south.

6. KEY FEATURES OF SHRIMP ECOLOGY, POTENTIAL THREATS AND RESILIENCE

The shrimp *Rimicaris exoculata* represents the dominant faunal biomass at many deep-sea hydrothermal vent ecosystems along the MAR, and can be regarded as a keystone species on the MAR hydrothermal vents. Key ecological features of the species are summarized in Fig. 10. The knowledge of the requirements and range of tolerance of *R. exoculata* would help to predict how this species will respond to specific environmental changes. Steep modifi-

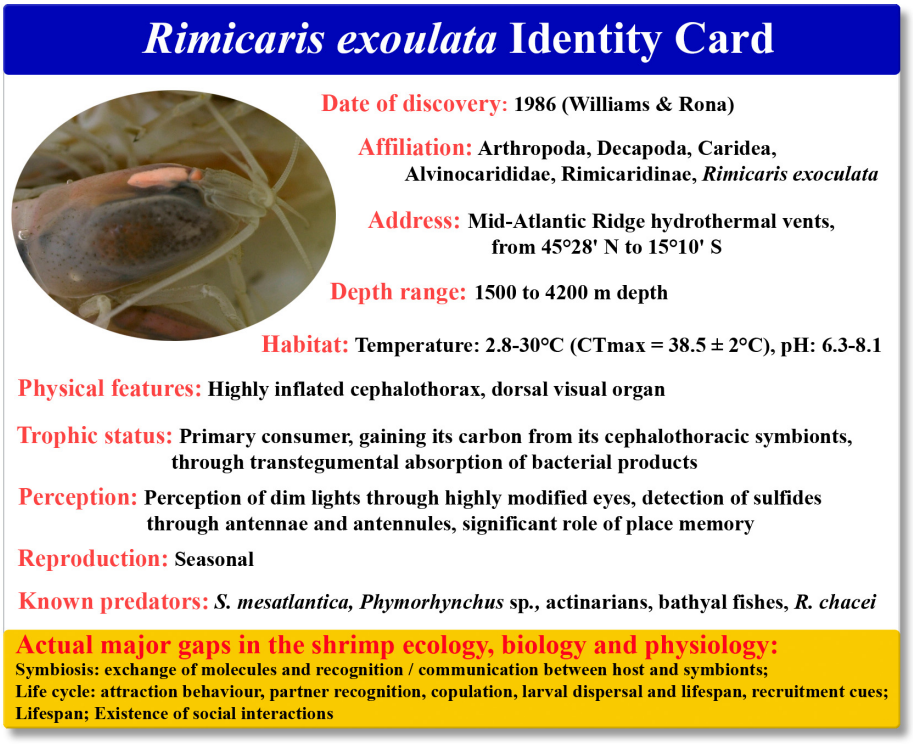

Fig. 10. *Rimicaris exoculata* identity card

cations, whether of natural (tectonic event, volcanic eruption) or anthropogenic origin (global warming, ocean acidification, mining) can impact hydrothermal ecosystems. Although difficult to reach because of their depth and globally less affected than coastal or terrestrial ecosystems, deep-sea hydrothermal vents are under a growing threat because of the strategic metals and minerals they contain (Van Dover 2011, Miller et al. 2018). Despite 40 yr of scientific investigations, our knowledge of the resilience of these ecosystems (i.e. the ability to cope with the modifications and to rebuild after disturbances) is still too incomplete to assess the risks associated with this activity, or to predict the impacts of a possible exploitation. There are still too many crucial gaps regarding biodiversity or functioning of hydrothermal ecosystems (Boschen et al. 2013, Orcutt et al. 2020).

Mining procedures in the deep sea are still in their infancy, so it is difficult to predict if mining rejections would occur at the surface, in the water column or on the sea bottom. If close to hydrothermal sites, the mining rejections in the water could impact vent communities, first, through the appearance of microbial blooms that would use these mineral wastes. That would modify the microbial diversity and thus potentially affect the entire food chain that depends on it, as well as the horizontally transmitted symbiotic associations, such as observed in *R. exoculata*. Second, mineral deposition on faunistic assemblages could result in niche destruction, or aberrant recruitment signals compromising colonization by juveniles. *R. exoculata* is a keystone species, living in symbiosis with 2 symbiotic communities, which dominates and probably shapes the faunal assemblages of the sites that it colonizes. Impacts on shrimp aggregations could destabilize the entire faunal assemblages and thus also strongly impact all other species. Even if *R. exoculata* does not, at first glance, meet the criteria to be considered a vulnerable species because of its life traits (high population density, high reproduction, significant migration capacity), its patchy habitat is nonetheless likely to be impacted by exploitation mining, thereby putting the shrimp itself at risk. Since little is currently known about the source–sink dynamics of *R. exoculata* between sites, the metapopulation stability or the resilience of the shrimp, it is difficult to assess the consequences of the destruction of one or more sites hosting this species on the long-term persistence of the species at hydrothermal sites. Apart from their ecological and cultural value, isolated ecosystems house a huge and unpredictable genetic reservoir, with potential new industrial, biotechnological and medical applications. Filling the many gaps in our knowledge of the biology and ecology of this major species of the MAR is therefore more important than ever to establish appropriate conservation strategies for these ecosystems.

7. PERSPECTIVES

Our knowledge of *Rimicaris exoculata* ecology has increased greatly since its first description (Williams & Rona 1986). However, studies on hydrothermal fauna have long remained, and still are, limited due to the remoteness of the sites, the difficulty to perform *in vivo* experiments on animals shocked by decompression during ascent to the surface, as well as the low frequency of cruises, usually scheduled during spring/summer due to weather conditions. These impediments are now overcome by the development of pressurized equipment, which allows recovery (Shillito et al. 2008) and maintenance (Shillito et al. 2014) of faunal species at native pressure to tackle physiological issues by *in vivo* experiments on animals in good physiological condition. Furthermore, long-term rearing of some deep-sea fauna is now possible thanks to aquariums like the 'AbyssBox,' which allows studies of species interactions and behavior, sensorial adaptations, reproduction and larval development (Shillito et al. 2015). From an ecological point of view, studies on deep-sea currents, habitat description and population distribution at a finer scale, as well as *R. exoculata* biotic interactions with other hydrothermal species are needed. For symbiotic species, such as *R. exoculata* and its endosymbionts, a gap is still to be filled regarding our ability to reproduce *in situ* life conditions in pressurized aquaria to allow the study of symbiosis dynamics. An integrated study of the holobiont (i.e. both the symbionts and host) genome metatranscriptome and metaproteome (as done on the siboglinid *Paraescarpia echinospica*, Yang et al. 2020) would provide important information on host and symbionts (inter)dependence on nutrient provisions and how they regulate each other to maintain a stable symbiotic relationship. For example, in recent years, cruises scheduled outside summer months allowed sampling during the shrimp breeding season, providing precious samples of the first eggs and early larval stages. Nevertheless, larval dispersal remains a clear knowledge gap to fully understand the ecology of this holobiont and its colonization success in so many vent sites along the MAR.

In the future, technological developments will undoubtedly help to answer the still unresolved questions about the biology and physiology of this shrimp, such as the regulation of the different metabolic pathways within the epibiosis according to the harvested site and the possible synthrophy between symbiont lineages; the nature of the molecules exchanged between symbionts and hosts; the establishment of symbiosis and host–symbiont recognition/communication mechanisms; the life span of shrimp and their full life cycle or the way these shrimp locate in their environment and communicate with each other. This holobiont is one of the dominant species along the MAR, showing an apparent huge potential of dispersion and adaptation. In the frame of the mining licenses implying a threat to deep-sea environments, this species is a model still to be studied to better understand life in extreme deep-sea ecosystems at the global scale of an ocean.

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