Symbiosis
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SHORT COMMUNICATIONS

Bacterial ectosymbionts colonizing gills of two Caribbean mangrove crabs

Naëma S. Béziat[1,2] · Sébastien Duperron[3] · Sébastien Halary[3] · Catherine Azede[1] · Olivier Gros[1,4]

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Abstract

We describe here the interactions between bacterial ectosymbionts and two Caribbean mangrove crabs: *Aratus pisonii* (Sesarmidae) and *Minuca rapax* (Ocypodidae). Specimens of *A. pisonii* and *M. rapax* were collected in Guadeloupe from mangrove trees (*Rhizophora mangle)* and from the mangrove mud, respectively. Ectosymbionts colonizing gills in all host individuals were observed using scanning and transmission electron microscopy (SEM and TEM). No intracellular bacteria were observed in gills cells suggesting that the biofilm only occurs on the surface of the gills. For *A. pisonii* and *M. rapax,* four different bacterial morphotypes were distributed throughout the surface of gill lamellae. Different sizes and lengths were observed in the bacterial population colonizing *A. pisonii* and *M. rapax*. Either symbionts cover the entire surface of the gills, or they formed irregularly distributed patches. Molecular analyses (high-throughput amplicon sequencing of bacterial 16S rRNA-encoding genes) confirmed the occurrence of multiple bacterial taxonomic units, with dominance of Alphaproteobacteria and Bacteroidetes in both host species. However, dominant bacterial phylotypes were not shared between *A. pisonii* and *M. rapax*. This suggests that each species of these semiterrestrial crabs may harbor a specific and distinct bacterial community despite living in the same mangroves. The discussion compares the bacterial compositions of the two species and their potential functions are hypothesized. Further investigations are needed to confirm the specificity and nature of the symbiosis, including potential exchanges occurring between the partners.

Keywords Bacterial symbiosis · Crustacea · Metabarcoding · Ultrastructural analysis

1 Introduction

Symbiotic relationships between Prokaryotes and Eukaryotes are widespread in terrestrial and aquatic ecosystems. Many organisms establish symbiotic relationship in which partners together can achieve new functions. In marine environments, well-documented examples include annelids, bivalves, crustaceans, and nematodes associated with chemoautotrophic bacteria in reducing environments (Dubilier et al. 2008). There, symbionts often provide a substantial supply of nutrients to the hosts as well as protection from toxic compounds, as exemplified by various marine invertebrates that shelter sulfur-oxidizing autotrophic bacteria (Nyholm and McFall-Ngai 2004 Dubilier et al. 2008 Sharma et al. 2013).

In mangroves, comparable associations occur in many organisms that live in contact with the reduced sediment (Goffredi et al. 2004; Dubilier et al. 2008; Brissac et al. 2011). Mangroves are highly productive coastal ecosystems distributed in the intertidal zone of tropical and subtropical regions, and harbor a diverse marine and terrestrial fauna. Bird, reptiles, mammals, crustaceans, mollusks, sponges, echinoderms are usually observed (Rützler and Feller 1988). Organisms have an essential role in mangrove ecosystems structure and functioning. Animal bioturbation for example contributes to oxygen

✉ Naëma S. Béziat
beziat.naema@gmail.com

[1] Institut de Systématique, Evolution, Biodiversité (ISYEB), Muséum national d'Histoire naturelle, CNRS, Sorbonne Université, EPHE, Université des Antilles, Campus de Fouillole, 97110 Pointe-à-Pitre, France

[2] Caribaea Initiative, Université des Antilles, Pointe-à-Pitre, Guadeloupe

[3] Molécules de Communication et Adaptation des Microorganismes (MCAM), UMR 7245 CNRS, Muséum national d'Histoire naturelle, 57 rue Cuvier (CP54), 75005 Paris, France

[4] C3MAG, UFR des Sciences Exactes et Naturelles, Université des Antilles, BP 592 - 97159, Pointe-à-Pitre, France

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penetration into the coastal sediment, and activity of crabs was shown to have a greater influence on the structure and composition of microbial communities than environmental conditions (Booth et al. 2019). Microbial communities play an essential role in the degradation of litter, which ultimately results in the presence of reduced compounds and increased availability of associated nutrients (Liang et al. 2007). Some mangrove organisms establish symbiotic associations with chemosynthetic bacteria, as documented for bivalves (Frenkiel et al. 1996), nematodes (Himmel et al. 2009), and medusozoans (Abouna et al. 2015).

Symbiosis is less documented in crustacean taxa. Recently though, some crab species living in mangrove environments from Saudi Arabia and South Africa were reported to have gill-associated bacteria (Booth 2018). These crabs belong to the families Dotillidae, Grapsidae, Ocypodidae, Portunidae, and Sesarmidae, and harbor rod-shaped bacteria and cocci of different sizes covering gills lamellae vertically or horizontally depending on crab family based on SEM observations. Most of these ectosymbionts were identified using 16S rRNA sequencing as Alphaproteobacteria and Acidimicrobiia, but no information is available regarding their metabolisms. Several crabs from hydrothermal vents have been more thoroughly investigated, including members of the Kiwaidae and Galatheidae families (Superfamily: Galatheoidea). These were shown to harbor bacteria on their body surface, attached to the outer part of their cuticle (Goffredi et al. 2008; Tsuchida et al. 2011). This epibiotic community is dominated by chemosynthetic Campylobacterota and Gammaproteobacteria, as well as by Bacteroidetes (Goffredi et al. 2008). The galatheid crab *Shinkaia crosnieri* from the Pacific–Antarctic Ridge also possesses setae on its entire body and shelters filamentous bacteria on the ventral setae (Tsuchida et al. 2011) belonging to the same bacterial taxa as above.

In this study, we investigated potential bacterial symbioses in two brachyuran crabs colonizing the fringe mangrove forest of Guadeloupe (Lesser Antilles in the Caribbean), namely *Aratus pisonii* (Milne Edwards 1837), an arboreal crab living on the mangrove tree *Rhizophora mangle*, and *Minuca rapax* (Smith 1870), the fiddler crab living on mangrove mud (see online resource 1). These crabs are semi-aquatic, and depend on sea-water for their reproduction (Warner 1967; Christy 1978).

The aim was (i) to test for the presence of bacterial symbionts in these species using ultrastructural investigation, and (ii) to characterize the symbiotic community using a comparative 16S rRNA gene amplicon sequencing approach in order to compare it with other mangrove crabs as well as with deep-sea species. We provide the first investigation of symbioses in mangrove crabs from the Caribbean arc.

2 Materials and methods

2.1 Sampling site

Thirty adult individuals of each species were collected manually during one year, 12 females and 18 males of *Aratus pisonii* (carapace length [CL] = 21.48 ± 3.4 mm) and 9 females and 21 males of *Minuca rapax* (CL = 20 ± 1.4 mm). They were collected from the marine fringe of the mangrove under a *Rhizophora mangle* canopy in Guadeloupe (French West Indies) directly on trees (around ten sampled) for *A. pisonii* or running on the sediment for *M. rapax*. Samples were collected at two main sites "Manche à Eau" and "Rivière Salée" (located 16°16′22°N/61°33'22 W" and 16°15′11°N/61°32′58°W), and three secondary sites ("Canal des Rotours": 16°21′12°N/61°29'34 W, "Marina du Gosier": 16°13′06°N/61°31′20°W, and "Sablière" 16°14′2°N/61°33′06°W). Dissections were performed in the laboratory, after a cold anesthesia, using forceps to open the carapace. The gill filaments were retrieved from the gill cavity and placed either directly in the fixative solution or used for DNA extraction. In accordance with Article 17, paragraph 2, of the Nagoya Protocol on Access and Benefit-sharing, a sampling permit was issued and published in the number APA 2298730.

2.2 Scanning electron microscopy preparation

Immediately after dissection, gill samples were fixed at 4 °C in 2.5% glutaraldehyde in 0.8x PBS buffer (pH 7.2). They were then dehydrated in series of acetone solutions of increasing concentration (30°,50°,70°,90° and 3 times 100°), dried to critical point in CO_2 at 31 °C and 74 bars and sputter-coated with gold before observation with a FEI Quanta 250 electron microscope at 20 kV.

2.3 Transmission Electron microscopy preparation

After dissection, gill filaments from adult crabs were prefixed for 1 h at 4 °C in 2.5% glutaraldehyde in 0.8x PBS buffer (pH 7.2). After a wash in the same buffer, they were fixed for 45 min at Room temperature (RT) in 1% osmium tetroxide and post-fixed with 2% aqueous uranyl acetate for 1 h at RT. Subsequently, gills were embedded in London Resin White resin. Ultrathin sections (60 nm thick) were observed under Tecnai G20 (FEI) microscope at 200 kV. A total of 30 specimens from each species (*A. pisoni* and *M. rapax*) were analyzed under the electron microscope.

2.4 Composition of gill-associated bacterial communities

Whole DNA from two adult individuals per species, in which the presence of symbionts was first checked by

SEM, was extracted from gills using the DNeasy Blood and Tissue kit (Qiagen) according to the manufacturer's instructions. PCR using universal primers to amplify the V4-V5 region of the 16S rRNA-encoding gene were performed as described in Duperron et al. 2019. A ~ 400 bp fragment of the rRNA-encoding gene corresponding to the V4-V5 variable region of *Escherichia coli* was amplified using 515F and 926R primers (Parada et al. 2016) and sequenced on an Illumina MiSeq platform (2 X 300 bp, paired-end sequencing, Genoscreen, France). Company-provided mock communities of known composition were used as an internal control for the whole sequencing process. Raw reads were deposited into the GENBANK Sequence Read Archive (SRA, Bioproject PRJNA638519) database under accession numbers SAMN15196320–1 (*A. pisonii* specimen #1 and #2) and SAMN15196324–5 (*M. rapax* specimen #1 and #2).

Sequence analysis was performed using QIIME2 (Hall and Beiko 2018). Amplicon Sequence Variants (ASVs (Callahan et al. 2017)) were identified using DEBLUR (Amir et al. 2017) using default parameters, i.e. a maximal probability for indels of 0.01 and mean read error rate of 0.5% for normalization. Chimeric sequences were identified using UCHIME (de novo chimera detection) and then removed (Edgar et al. 2011). Taxonomic affiliations were obtained by the sklearn-based classifier (GreenGenes 13–8-99 release). Sequences matching "Eukaryota", "Chloroplast" and "Mitochondria" were discarded. Venn diagrams were drawn using the web-based software available at http://bioinformatics.psb.ugent.be/webtools/Venn/.

2.5 Phylogenetic analysis of dominant ASVs

A dataset was assembled including sequences of dominant ASVs that represented at least 10% of reads in at least one specimen, their most similar sequences according to BLAST, and sequences representative of relevant bacterial clades (Alphaproteobacteria, Actinobacteria and Bacteroidetes). Sequences were aligned using MUSCLE (358 nucleotide positions), and the alignment was checked visually. Phylogenetic relationships were inferred using a Maximum likelihood approach. A General Time Reversible model with Gamma-distributed rates (5 categories and invariant positions) was selected based on the Akaike Information Criterion (AIC) using the SMS model selection tool (Lefort et al. 2017). Bootstrap values were calculated based on 1000 independent replicates analyzed using the same approach and model. Actinobacteria were used as an outgroup in the tree. Analyses were performed using the software MEGAX (Kumar et al. 2018).

3 Results

3.1 Ultrastructural analysis

All 30 individuals of each of the 2 species examined throughout one year of collection using SEM showed a bacterial community associated with gills, at all sampling sites. These were all adults and all of them displayed bacteria on gills. Males and females were observed randomly. The morphology of the gill is identical for both sexes within the same species. Gill's morphology changes according to the taxonomic group.

Brachyuran crabs are characterized by a phyllobranchia type of gills with lamellae positioned in pairs and attached to a gill axis. Generally, this type of gill has a very thin tip at its end (Fig. 1a). Phyllobranchia gills length depends on the individual size. *M. rapax* gills have triangular branches (Fig. 1a) while *A. pisonii* gills seem to have more rounded branches (Fig. 1b). On all samples examined, both edges and surface of gill lamellae were colonized, either by some irregularly distributed patches of bacteria or by a regular bacterial biofilm (Figs. 1c, d). For both species, several different bacterial morphotypes (cocci, rods, but no filaments) were observed throughout the surface of gill lamellae by SEM (Figs. 1e, f). Four morphotypes (rod-shaped bacteria) are present on *M. rapax* (Fig.1e), size is similar but bacteria differ by their thickness. First morphotype (white arrow) measures 1.69 ± 0.33 μm with a 0.21 ± 0.03 μm thickness. Second morphotype (black arrow) measures 1.16 ± 0.22 μm × 0.26 ± 0.04 μm while the third morphotype (dotted black arrow) measures 1.54 ± 0.42 μm × 0.18 ± 0.03 μm. The last one (white curved arrow), possesses outgrowth at its surface and measures 0.93 ± 0.27 μm × 0.24 ± 0.04 μm (Fig. 1e). Also, four morphotypes are observed on *A. pisonii*, thickness seems similar but bacteria differ by their size. Two long rod-shaped morphotypes are present (Fig. 1f). The long straight morphotype (black arrow) measures 2.26 ± 0.76 μm while the long curved morphotype (white arrow) measures 1.68 ± 0.48 μm. Two short morphotypes are also present (Fig. 1f): a cocci-like morphotype (black dotted arrow) measuring 0.73 ± 0.19 μm and short rods measuring 0.98 ± 0.26 μm.

Observations were homogeneous between males and female specimens. Even between the different sites sampled, no differences could be observed regarding the bacterial morphotypes present and/or their distribution on the gill cells. (Online resource 2).

The TEM observations from ultrathin sections showed that *A. pisonii* specimens harbored up to two layers of bacteria on their gill's cuticle (Fig. 2a) while *M. rapax* presented a single layer (Fig. 2b). Both TEM and SEM observations confirmed the ectosymbiotic status of the relationship, as no bacterium was detected within host cells (Figs. 1, 2).

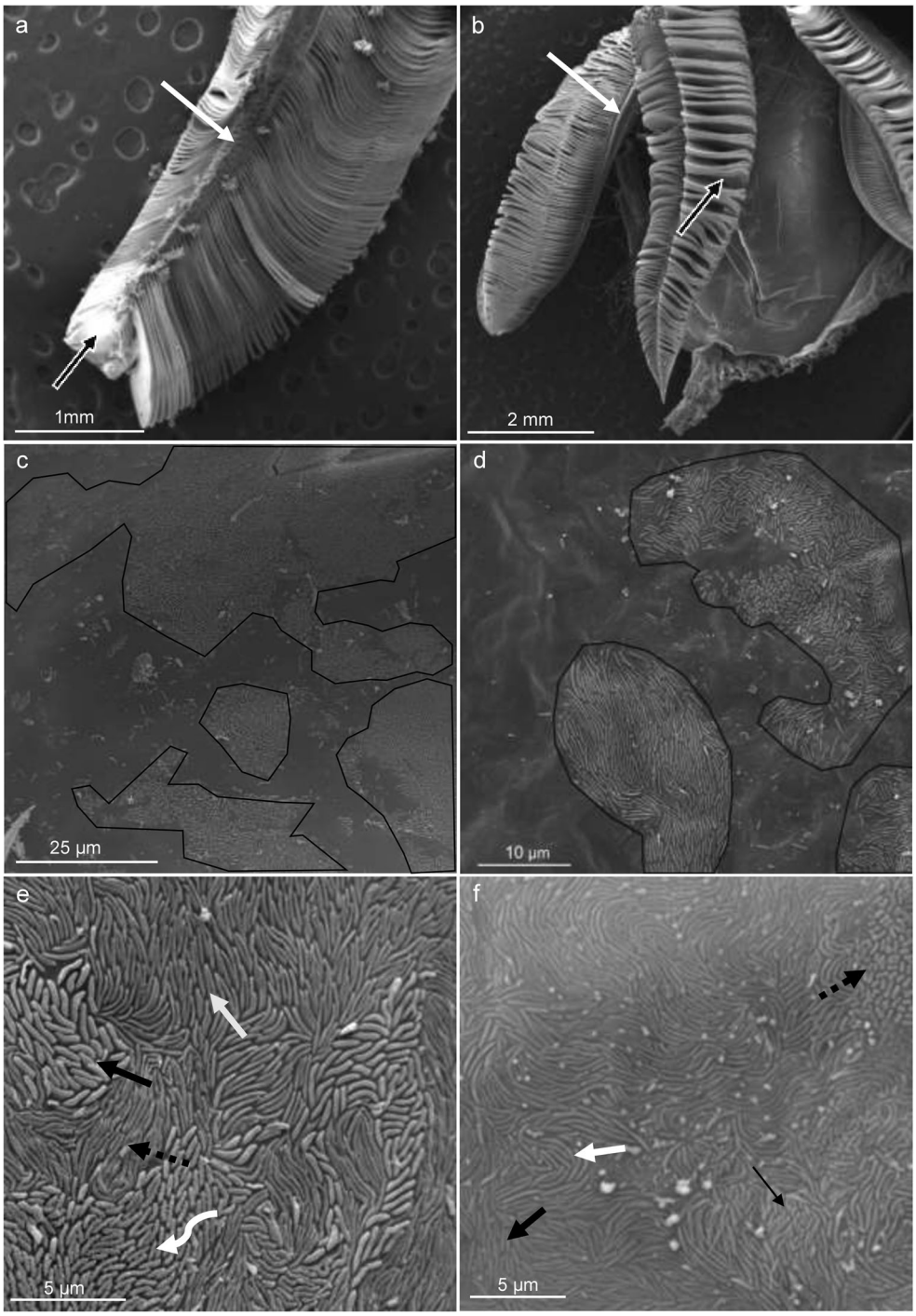

Fig. 1 Structural analysis of gill filaments from two mangrove crabs according to SEM views. (a) Overview of the phyllobranchia of *Minuca rapax*. Each filament is composed of several lamellae (black arrow) present in pair attached to a central axis (white arrow) (a-b). Gill lamellae can have several forms depending on crab's species. *Aratus pisonii* presents a very rounded shape of the gill lamellae (b) while *M. rapax* has a more triangular shape (a). Bacterial cover does not appear as uniform on all part of gill lamellae (c) and (d). The limits of the bacterial patches are highlighted in black to show their distribution on gill lamellae. These lamellae are colonized by different bacterial morphotypes covering the cuticle of each gill cells. In *M. rapax* (e), bacterial populations are composed by a large morphotype (black arrow), a medium and long morphotype (white arrow), a thin form (black dotted arrow), and a morphotype with little outgrowth on the surface (white curved arrow). On *A. pisonii* gills (f), four bacterial morphotypes are obviously observed as indicated by various arrows

3.2 Diversity of gill-associated bacteria

In the two *Aratus pisonii* specimens analyzed, 24,009 and 21,241 quality-filtered reads were obtained clustering into 42 and 35 ASVs, respectively. The two *Minuca rapax* specimens yielded 20,749 and 22,289 quality-filtered reads representing 111 and 87 ASVs, respectively (Online resource 3). Together, Bacteroidetes and Proteobacteria (mostly Alphaproteobacteria) represented 76.5 to 97.1% of reads in the four specimens (Fig. 3). Actinobacteria represented 16.7 and 17.7% of reads in the *M. rapax* specimens, while they were below 6% in *A. pisonii*.

Four and three of the *A. pisonii* ASVs displayed abundances greater than 10% of reads in the two specimens, respectively summing to 84.5 and 66.5% of reads (Online resource 3). Two of these dominant ASVs were dominant in both specimens. Additional five and eight ASVs were between 1 and 10% and were thus considered

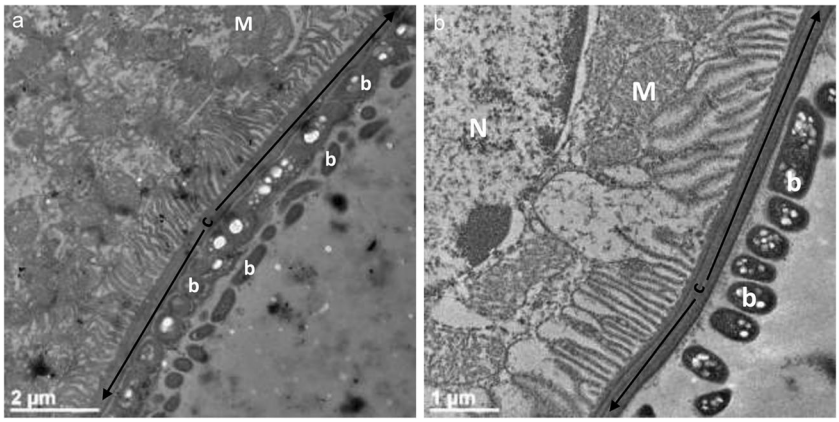

Fig. 2 Ultrastructural analysis of the gill cells with TEM. (a) Transversal section of *Aratus pisonii* gill lamellae showing the lack of intracellular bacteria. Moreover, in some areas, two layers of bacteria are superposed at the surface of cuticle (c). In *Minuca rapax* (b) only a single layer of bacteria (b) covering the cuticle (c) was detected from the gill cells. The cuticle is thick on this species, no intracellular bacteria were observed within the cytoplasm of the gill cells. The cytoplasm volume of some bacteria appears filled with vesicles corresponding to internal former sites of sulfur granules lost during the dehydration and embedding processes (b: bacteria, M: Mitochondria, N: Nucleus, I: Ionophore)

abundant in the two specimens, respectively. Among the 12 dominant and abundant ASVs, 11 were present in both specimens (Fig. 4). Three and three of the ASVs present in *M. rapax* were above 10% in the two specimens, respectively, summing up to 53.2 and 51.8% of reads (Online resource 3). A single ASV was dominant (>10%) in both specimens, while another was dominant in specimen 1 (12.8%) and not detected from specimen 2. Additional 11 and 13 ASVs were between 1 and 10% and were thus considered abundant in the two specimens, respectively. Among the 23 dominant and abundant ASVs identified, 21 were present in both specimens (Fig. 4).

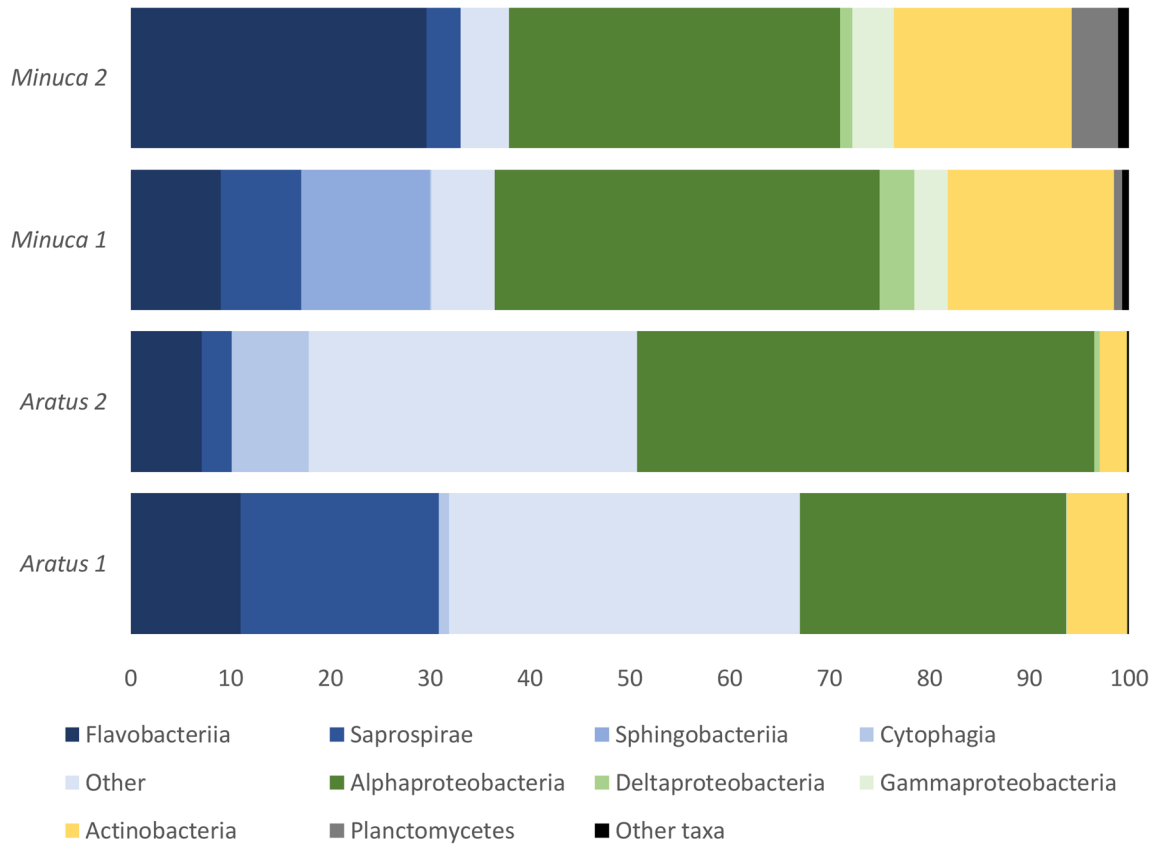

Fig. 3 Percentage relative abundances of major bacterial classes in the two *Aratus pisonii* and two *Minuca rapax* specimens. Classes within the Bacteroidetes are colored in shades of blue; classes within the Proteobacteria are in shades of green

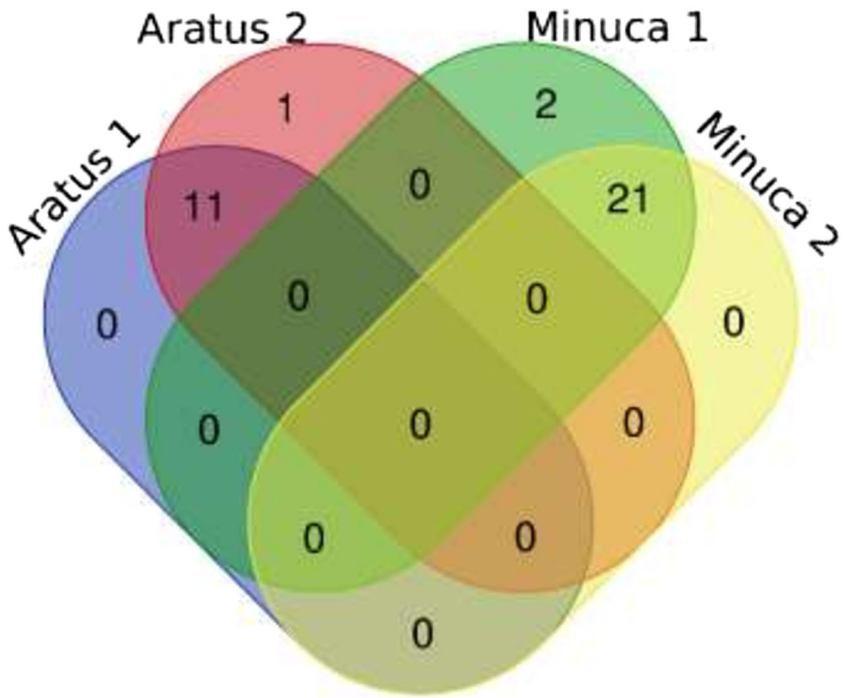

Fig. 4 Venn Diagram displaying the number of abundant (i.e. >1%) gill bacterial ASVs shared among the two *Aratus pisonii* and the two *Minuca rapax* specimens

Despite overall similarity between composition of bacterial communities in *A. pisonii* and *M rapax* at the class level (Fig. 3), AVSs in the two species were distinct. Overall, 117 ASVs were specific for *M. rapax* and 39 for *A. pisonii*, while only 12 were shared between the two species, none of these shared ASVs being abundant (i.e. >1%) in both species (Fig. 4 and Online resource 4).

3.3 Phylogenetic relationships of dominant ASVs

Four of the 5 dominant ASVs found in *Aratus pisonii* clustered within the Bacteroidetes (Fig. 5). One ASV (ASV-Ara - 5) was closely related to a dominant sequence from *Minuca rapax* (ASV – Min - 2, 4.0% divergence (Online resource 3)), and these were most similar (yet with >6.8% difference) to a sequence identified from the gill of the mangrove crab *Uca urvillei* from Kenya (Marasco et al., unpublished). Two ASVs were closely related (ASV-Ara – 2 and ASV-Ara - 4, 4.9% divergence) and displayed a clone from pig litter as their closest relative. The fourth Bacteroidetes ASV, ASV-Ara – 3, was related to sequences from a soda lake and from the heterotroph *Membranicola marinus*. The fifth dominant ASV, ASV-Ara – 1, belonged to the Alphaproteobacteria and was identical to bacterial sequences from the intestine of the Chinese mitten crab *Eriocheir sinensis* (Li et al. 2007) and gills of mangrove crabs *Perisesarmea guttatum* from South Africa (Marasco et al., unpublished).

Two of the 5 dominant ASVs in *Minuca rapax* were Bacteroidetes. Besides ASV – Min −2 resembling *A. pisonii* ASV-Ara – 5 and discussed above, another ASV clustered within the Bacteroidetes and was similar to sequences from seawater bacteria (ASV – Min – 5). Two ASVs were Alphaproteobacteria. ASV Min - 4 was closely related to various *Roseovarius* species (which are strict aerobic marine bacteria), while the second, ASV Min - 1, was almost identical (1 bp difference out of 350) to gill bacteria from the mangrove crabs *Perisesarma guttatum* and *Uca urvillei* from Kenya and South Africa (Marasco et al, unpublished). The last dominant ASV, ASV Min – 3, was a member of the Actinobacteria, related to a sequence from a hydrate ridge.

4 Discussion

4.1 Symbiont distribution and composition

Bacteria were found coating the gills of *Aratus pisonii* and *Minuca rapax*. This distribution is comparable to that recently documented in the gills of several mangrove crab species presented in a PhD thesis (Booth 2018), and different from that reported in hydrothermal crustaceans. Hydrothermal shrimps such as *Rimicaris exoculata* indeed display symbionts on the inner surface of the carapace (branchiostegites) and hypertrophied mouthparts (Zbinden et al. 2004), while galatheid crabs present symbionts on the external cuticle surface (Goffredi et al. 2008; Tsuchida et al. 2011). Mangrove crabs studied here on the other hand harbor their ectosymbionts inside the carapace, on gill lamellae, while the carapace is mostly devoid of bacterial biofilms. Booth (2018) reported similar localization in gills for fourteen crabs

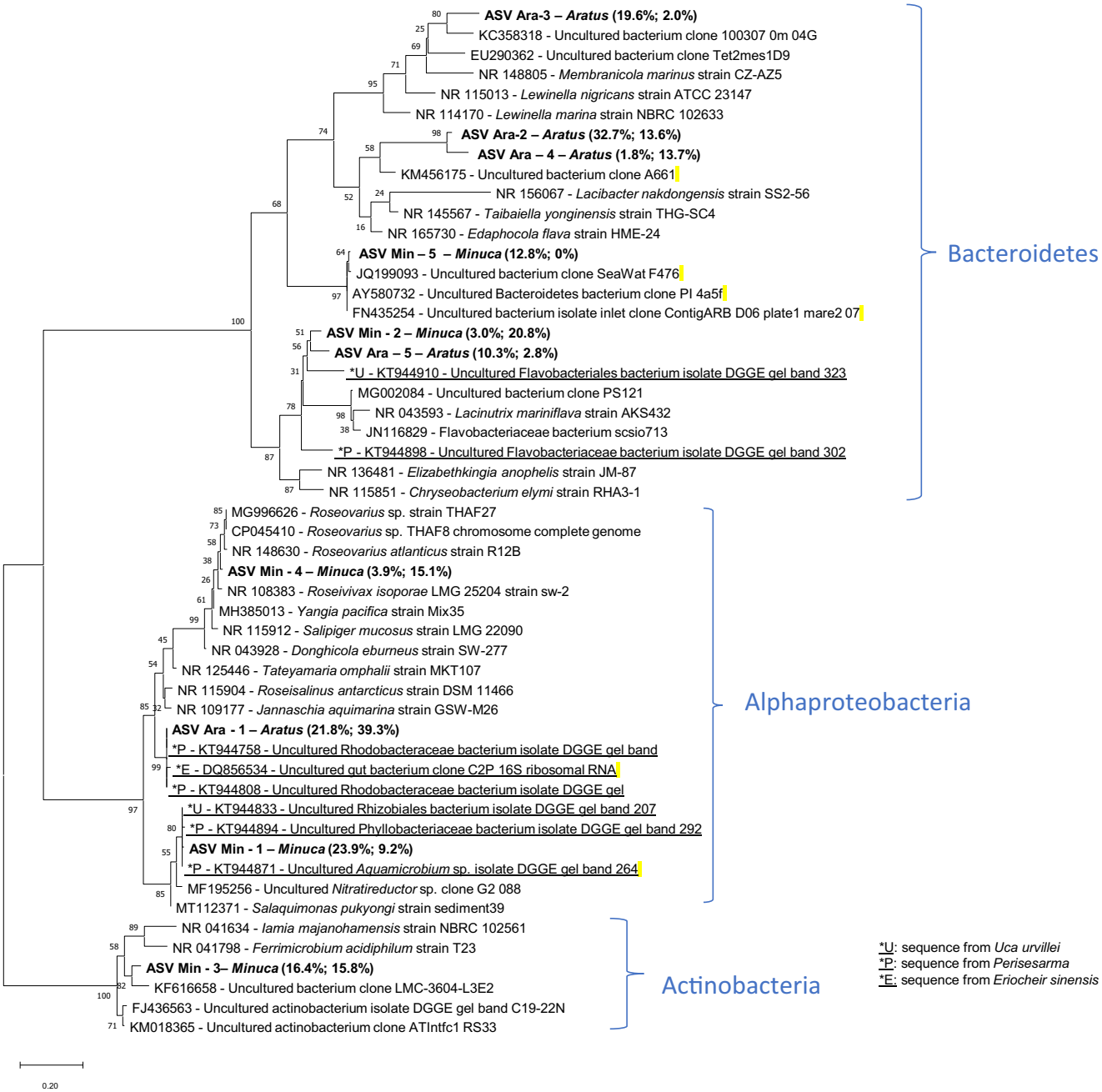

Fig. 5 Phylogenetic relationships of the 10 dominant ASVs occurring in *Aratus pisonii* and *Minuca rapax* (in bold). Sequences obtained from other crab genera (*Eriocheir, Perisesarma,* and *Uca*,) are underlined. See material and methods for description. Percentages between parentheses after ASV names represent abundance in specimens 1 and 2 of the species, respectively. The scale bar represents 20% estimated sequence divergence; percentages at nodes were calculated based on 1000 bootstrap replicates

from several ecological niches in the mangrove ecosystems of Saudi Arabia and South Africa. Marine, semi-terrestrial, and terrestrial crabs displayed bacteria covering the entire gill lamellae. The characteristics of the mangrove environment could thus influence the distribution of symbionts. Crabs studied here and those analyzed by Booth (2018) indeed display similar symbioses despite being from very different localities. No ectosymbiont is described to date from gill tissues of hydrothermal crabs or shrimps, while gill ectosymbioses have been repeatedly reported, for example in Mollusks found at wood falls including bivalves, chitons, and gastropods (Gros and Gaill 2007; Duperron et al. 2008, 2013; Zbinden et al. 2010; Brissac et al. 2011). Regarding symbiont identification, four of the 10 dominant ASVs identified in either *A. pisonii* or *M. rapax* are closely related or identical to sequences obtained from terrestrial mangrove crabs distributed worldwide, namely *Perisesarma gutattum* from Kenya, *Uca urvillei* from South Africa, and the catadromous crab *Eriocheir sinensis* from

China. Some of these crabs belong to the same family (the genera *Minuca* and *Uca* belong to the family Ocypodidae, while *Aratus* and *Perisesarma* belong to the family Sesarmidae) and share closely related bacteria. Unfortunately, apart from sequence DQ856534 from *Eriocheir sinensis*, these sequences are only available from databases and are not presented in a published paper.

Nevertheless, this suggests that both mangrove crabs investigated here harbor a bacterial community on their gills, different both morphologically as well as taxonomically from ectosymbioses found in other crustaceans. Despite the low number of replicates, molecular results are very homogenous among the two specimens from each species. The fact that these specimens of a given host share most of their abundant ASVs, while they share none with the other host species suggests a non-random association of hosts and bacteria. This could be either because of species-specificity in the association, or because of slightly different habitats which expose each species to different pools of environmental bacteria since *A. pisonii* often occurs on *R. mangle* roots and branches, while *M. rapax* is found on mangrove mud.

None of the dominant ASVs is related to a known bacterial pathogen of crustaceans or invertebrates, and no lesions were visible on the tissue carrying bacteria, suggesting that the interaction may not be parasitic. Bacteria could thus be commensals, or the symbiotic relationship could be beneficial to either mangrove crabs, bacteria, or both. More work will be needed to test these hypotheses. According to Zhang et al. (2016), despite the constant exposure of gills to surrounding water, the Chinese mitten crab *Eriocheir sinensis* harbors different bacterial communities in its gut and gills compared to that from surrounding water. Similar host influence on gill community compositions may thus be expected in mangrove crabs. Tsuchida et al. (2011) have shown that the bacterial community of the hydrothermal vent crab *Shinkaia crosnieri* (consisting of ectosymbionts covering the body) is more diverse than the bacterial community of the shrimp *Rimicaris exoculata* (symbionts located in the gill chamber) because this crab is directly exposed to high concentrations of reduced chemical compounds from the environment, supporting that habitat differences may lead to major differences in crab symbiont communities. In the present study, *Aratus pisonii* and *Minuca rapax* do not share identical bacterial communities on their gills, likely because they are not exposed to the exact same environment. Adults of *Aratus pisonii* live mainly on mangrove trees using the long aerial roots to occasionally wander into the marine water meanwhile adults of *M. rapax* live exclusively on the sediment and never climb trees or roots.

4.2 Hypothetical role of symbionts

Despite that mangrove habitats can be rich in sulfides and that several examples of symbioses involving sulfur-oxidizing bacteria were documented, none of the dominant ASVs found in *A. pisonii* and *M. rapax* is closely related to a known sulfur-oxidizing chemoautotrophic bacterium. In this study, Alphaproteobacteria and Bacteroidetes are the most abundant bacterial groups colonizing gills of *Aratus pisonii* and *Minuca rapax*. In crabs from Asian and African mangroves, the most abundant bacterial groups were Acidimicrobiia and Alphaproteobacteria, (Booth 2018). One dominant ASV found on *A. pisonii* is for example related to *Membranicola marinus* (Li et al. 2016), an aerobic heterotroph. Many Alphaproteobacteria also consume dissolved organic matter as Bacteroidetes do (Cottrell and Kirchman 2000). Based on their taxonomic affinities, dominant bacteria colonizing the gills of mangrove crabs are thus most likely heterotrophs rather than chemoautotrophs. A nutritional role of bacteria could be possible despite their localization on gills. Gill-located bacteria are well documented to contribute host nutrition in various invertebrates including bivalve mollusks in which bacterial gill-associated endosymbionts transfer fixed carbon through different tissues (Fisher and Childress 1986). Wood-boring teredinid bivalves are associated with endosymbionts present in specialized gill cells called bacteriocytes. There, bacteria are thought to be secreting cellulolytic enzymes that degrade plant material in the pallial cavity, helping in wood digestion, and perform nitrogen fixation that supplements the hosts nutrition (Distel 2003). Overall, localization of symbionts in the gills does not preclude their contribution to host nutrition, although details need to be explored in order to evaluate this potential role.

5 Conclusion

Our study shows that mangrove crabs *Aratus pisonii* and *Minuca rapax* harbor bacterial communities on their gills, composed of rod- and cocci-shaped bacteria mostly belonging to the Bacteroidetes and Alpha-proteobacteria. While sympatric in the same mangrove, the bacterial community is different between the two species and none of the main bacterial symbionts is shared. The role of this interaction remains to be elucidated, but the fact that highly similar bacterial sequences were reported from mangrove crabs on other continents indicates that bacterial symbiosis may be a common feature of mangrove crabs worldwide. This supports that this symbiosis may be an adaptation to the mangrove habitat. Future work should investigate the nature of the symbiotic relationship by addressing the role of epibiotic bacteria using functional approaches (metagenomics, metabolome analysis, experiments, etc.), in order to reveal its eventual adaptive significance to

mangrove ecosystems, in which crabs are major ecosystems engineers (Booth et al. 2019).

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All the authors contributed to the writing and editing the manuscript. All authors have read and agreed to the published version of the manuscript.

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Code availability Not applicable.

Declarations

Conflicts of interest/competing interests There is no conflicts of interest or competing interests.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

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