Characterization of bacterial ectosymbionts colonizing gills of mangrove crabs (Brachyura) in Guadeloupe

Context
A symbiosis corresponds to an interaction between two organisms, the “host” and the “symbiote,” in which the host harbours the symbiote. A symbiosis can be obligate, implying that the two organisms cannot live independently from each other: this is mutualism. The interaction can also benefit only one organism with no negative impact on the other: this is commensalism. Many symbioses are described in plants, mammals or even invertebrates. Molluscs, for example, are widely studied in this context, unlike crustaceans. In this last group, the most studied interactions concern crustaceans from hydrothermal vents, such as shrimps and crabs living in association with ectosymbiotic bacteria, which participate in the nutrition of their host. Recently, new interactions have been observed in crustaceans that live in coastal environments. Indeed, several species of crabs in Guadeloupe present a bacterial community on their gills. Some of these species are traditionally consumed during Easter celebrations in Guadeloupe, and thus have a high economic value. In addition, crabs have a significant impact on the environment in which they live, in particular through the aeration of the soil and the resuspension of organic matter in the environment. In this context, a better understanding of the interactions between crabs and their symbiotic bacteria may be of ecological as well as economic interest.

Objectives
The main objectives of this thesis project are to:
- Identify crabs associated with bacteria in various environments (marine, terrestrial, semi-marine);
- Identify the bacterial communities living on different species of crabs;
- Characterize the relationship between crabs and their bacteria (obligate or facultative nature, mode of transmission);
- Determine the impact of symbionts on the physiology and the development of crabs.

Methods
Sampling
Crabs from adult and juvenile stages were sampled in different environments. The exclusively marine crabs were harvested by snorkeling in 2-3 meters deep water. Semi-marine crabs were collected with bare hands from the mangroves, while terrestrial crabs were collected from vendors.
Ultra-structural analyses
The gills of the crabs were dissected and specifically prepared for observation under a scanning electron microscope and a transmission electron microscope.

Genetic and metabolomic analyses
DNA from two individuals per species (out of a total of 11 species) was extracted for phylogenetic analysis. Metagenomic analyses were used to identify bacterial communities. Gill samples were also ground in a ball mill for metabolomic analysis, a mass spectrometry-based technique for identifying metabolites present in an organ.

Larval culture
After harvest, females with eggs were placed in saltwater tubs to induce the hatching of the eggs. The larvae obtained were kept and cultivated to obtain different stages of development.

Results
The first results showed that many crabs are associated with bacteria present on their gills. Indeed, out of a total of 16 species observed (including several individuals per species), only four species did not show this bacterial association.

Metagenomic analyses of the bacteria did not lead to the identification of any known human pathogens. These analyses showed that each species of crab harbours a different bacterial community, but that individuals from the same species have similar communities. Other crab species from Africa and Asia also show this association with bacteria on their gills. These results suggest a non-random, but rather specific, association. In general, the same groups of bacteria are found in different species of crabs, although the communities differ, with a majority of Bacteroidetes and Alphaproteobacteria.

Experiments on gonads, eggs, larvae and juveniles conducted on two crab species (the mangrove tree crab, *Aratus pisonii*, and the mudflat fiddler crab, *Minuca rapax*) showed that vertical transmission of bacteria was unlikely.

About the research team
Naëma began her PhD research in 2019 at the Université des Antilles, Guadeloupe. Her doctorate is co-funded by the Guadeloupe Region and Caribaea Initiative, and is co-supervised by Olivier Gros (UMR 7205 MNHN CNRS-Sorbonne) and Sébastien Duperron (UMR 7245 MNHN CNRS). A study is currently underway in collaboration with Gerardo Cebrian-Torrejon from the chemistry department of the Université des Antilles.

Before her doctorate, Naëma completed her academic education at the Université des Antilles. She obtained her Master's degree in Biodiversity, Ecology, Evolution, specializing in Tropical Marine Ecosystems, in 2019.

Publications