CALLS FOR DOCTORAL TOPICS 2020

DOCTORAL SCHOOL COMPETITION ED227 MNHN-SU

Subject of the thesis: **Contribution of marine gregarines to the evolutionary history of Apicomplexa and adaptation to parasitic lifestyle**

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Co-director: Dr PONGER Loïc, loic.ponger@mnhn.fr, UMR 7196 / INSERM U1154, Team ARChE, Structure and instability of genomes, AVIV Department, https://biophysique.mnhn.fr/site/ARChE

Doctoral students currently supervised by the thesis (co) director, specifying the year of enrollment
Julie Boisard, from October 2018, co-director Loic Ponger

Recent publications of the thesis (co) director with former doctoral students

-Imboumy-Limoukou KR, Maghendi-Nzondo S, Kouna CL, Bounaadja L, Mbang S, Biteghe JC, Eboumbou C, Prugnolle F, Florent I, Lekana-Douki JB. 2016. Acta Trop.; 163:149-156.

-Souidenne D, Florent I, Dellinger M, Justine J-L, Romdhane MS, Furuya H, Grellier P. 2016. Parasite 23:33

-Cacheux L, Ponger L, Gerbault-Seureau M, Richard F, Escudé C 2016. BMC Genomics 17:916

-Allain T, Chaouch S, Thomas M, Vallée I, Buret AG, Langella P, Grellier P, Polack B, Bermúdez-Humarán LG, Florent I. 2018. Frontiers in Microbiology 8:2707.

-Boisard J, Florent I. 2020, Biology of the Cell, in press

Description of the thesis subject and envisaged methods

Apicomplexa, unicellular eukaryotes representing ~6,000 species, have all adopted a strict parasitic lifestyle [1]. They include intracellular parasites of vertebrates developing in one to several hosts, responsible for serious pathologies: malaria, toxoplasmosis, cryptosporidiosis. Their genomes have been sequenced and serve as references for comparative genomics and the reconstruction of evolutionary history of Apicomplexa. However, these parasites of vertebrates represent only the tip of the iceberg, since Apicomplexa also include gregarines, parasites of a wide variety of non-vertebrate hosts (polychaetes, crustaceans, insects, etc.) representing ~40% of Apicomplexa biodiversity [1,2]. They are mainly extracellular, monoxenous and non-pathogenic, and are poorly known at genomic level, due to the current impossibility to cultivate them.

Recently, two photo-autotrophic proto-Apicomplexa associated with corals, *Vitrella* and *Chromera*, have been sequenced. The comparative analysis of their genomes with data on *Plasmodium*, *Toxoplasma* and *Cryptosporidium* has revealed groups of genes/functions lost (mainly) and acquired during the transition to the intracellular parasitic lifestyle [3-5]. However, the vision of this transition remains incomplete in the absence of data on gregarines, phylogenetically placed at the base of the apicomplexan speciation, among which archigregarines are considered as having diverged the earliest [2].

The PPL laboratory has been involved since 2017 in the -omic analysis (genome, transcriptome) of marine *(Porospora gigantea*) and terrestrial (*Gregarina acridiorum*) intestinal eugregarines, in closed collaboration with the Bioinformatics Support Unit of the AVIV Department (Evelyne Duvernois-Berthet and Dr. Loic Ponger) and in the framework of Julie Boisard's PhD thesis (ED227, CNRS funding, 2018-2021). This work has made it possible to develop efficient procedures for the genesis and analysis of these original –omic data, despite the intrinsic methodological and analytical difficulties.

Within the framework of this project, we wish to consolidate our expertise by complementing the approach with two new marine gregarine biological models, which are more difficult to study biologically but which are likely to provide different elements of speciation due to their phylogeny or biology: the intestinal archigregarine *Selenidium pendula* and the coelomic eugregarine *Diplauxis hatti* [2].

The study of these two species will include: genome and transcriptome sequencing, genome assembly and gene prediction, and expert data mining. This will allow a comparative analysis with the terrestrial and marine intestinal eugregarines currently studied in the laboratory, in order to better explore the specific traits of this group. It will provide a better understanding of the evolutionary scenarii that have accompanied the establishment of parasitism in Apicomplexa and the progressive conquest of vertebrate hosts, towards polyxenous parasitism and intracellular lifestyle.

Several specific tasks will be addressed:

1- Inventory of the protein heritage deduced from genome analysis, exploration of the molecular architecture of key apicomplexan structures/functions (apical complex, IMC (inner membrane complex), mitochondria, apicoplast, motility) and assessment of the maintenance of metabolic pathways with reference to other (proto)-Apicomplexa and outgroups.

2- Research and reconstruction of potential mitochondrial and apicoplast genomes from genomic data.

3- Identification of genes/functions gains and losses compared to documented occurrences in proto-Apicomplexa and other apicomplexans; establishment of evolutionary scenariii.

4- Phylogenetic studies of preserved components.

The abundant literature available for *Plasmodium*, *Toxoplasma*, *Cryptosporidium*, as well as the genomic and post-genomic data available, will serve as references for digging up these new gregarines data; comparative studies will be based on recent work with *Vitrella* and *Chromera* [3-5] and emerging works on few gregarines [6-7].

The analyses will be mainly carried out bioinformatically. Depending on the results obtained, experiments may be implemented for: 1) experimental validation of certain gene models by RT-PCR/Sanger sequencing, 2) confirmation of the presence of a mitochondrion and/or an apicoplast (transmission electron microscopy).

1. Portman N, Slapeta J. Trends Parasitol, 2014. 30(2): p. 58-64.
2. Desportes I, Schrevel L. Brill, editor, 2013.
3. Janouskovec J et al., PNAS, 2015. 112(33): p. 10200-7.
4. Templeton TJ, Pain A. Parasitology, 2016, 143(1): p. 1-17.
5. Woo YH et al., Elife, 2015, 4: p. e06974.
6. Janouskovec J. et al., eLife, 2019, 8.
7. Mathur, V., et al., Current biology, 2019, 29:2936-2941 e293

Publication strategy

Classical publication strategies for experimental results in: genomics, biology, biochemistry, microbiology, parasitology journals. Special effort in writing reviews on themes related to the thesis topic.

Feasibility in three years with timeline

Year 1: Genesis of the molecular data (genomes, transcriptomes) necessary for the assembly and annotation (gene prediction) of the genomes of the two marine gregarines (in collaboration with CSB). Deduction of theoretical proteomes and expert data mining. Reconstruction of metabolic pathways and identification of molecular components involved in zoite architecture, ultrastructure and dynamics.

Year 2: Reconstruction of organellar genomes. Comparative genomics and inventory of genes/functions gains and losses with respect to documented apicomplexan parasites of vertebrates and available data for proto-apicomplexans. Phylogenetic analyses for conserved components. Characterization of repeated components in genomes (TE, centromeres, telomeres).

Year 3: Subcellular localization of key relevant architectural components by immunofluorescence microscopy (IFA) and of the mitochondrion or apicoplast (genomes) by transmission electron microscop (TEM). Synthesis/writing.

Profile of the desired candidate

A strong background in biology of organisms including eukaryotic microorganisms is required, including a mastery of the literature in the evolutionary history of eukaryotic microorganisms, as well as skills in bioinformatics approaches to: data assembly, annotation, mining and analysis. Ideally the PhD student will also have skills in eukaryotic cell biology, molecular biology and imaging

Non-salary financing (missions, seminars, operations, ...)

The thesis project is part of one of the priority axes of the PPL team for the next quadrennium. An ANR-2020 application (second round selection) is in progress, including the theme of this thesis project but also covering other biological models of marine gregarines. Requests for funding will also be made to the MNHN internal programs (ATM or AVIV Department) as well as to the EMBRC program.

Strong collaborations (ANR) are established with Dr. L. Guillou's team on the comparative biology of Alveolata as well as with Genoscope colleagues involved in the comparative genomics of parasitic protists.

The PhD student will be required to attend national and international congresses which will be covered by the budget of the PPL or ARChE teams.

Availability of necessary biological material/equipment if required

Biological samples are already available for the species *Diplauxis hatti*; biological samples for *Selenidium pendula* will be obtained following a sampling campaign (Brittany, Normandy), or through collaborators at the Roscoff Biological Station. It is also possible to obtain the hosts of these gregarines *via* the “Sea Service” of the Roscoff Biological Station (we have experimented this in the past, for both gregarine species). Sequencing data will be obtained through funding by the PPL team or through dedicated grant calls (internal to the MNHN or external). The PhD student will be able to use the CSB's computer hardware and the Museum's computing cluster.