Emerging of unconventional model organism - *Corallochytrium limacisporum* - to study origin of animal multicellularity

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Abstract: The Holozoa clade emerges as an important group for comparative cell biology analyses among eukaryotes. In addition to a well-studied Metazoa group, it includes four different unicellular lineages (Choanoflagellata, Filasterea, Ichthyosporea and Corallochytrea). Interestingly, these unicellular lineages are highly heterogeneous in their ecological distribution and have diverse developmental modes, cell morphologies, and life stages. Importantly, some species in each of the unicellular holozoan clades can transiently form multicellular structures resembling those in animals. The best way to understand in depth their biology, in particular their complex life cycles is by performing functional analysis. To do so we need to have genetic tools available in each of the four lineages.

So far, genetic tools have been recently established in all of those holozoan lineages, except for Corallochytrea, a clade that occupy a key phylogenetic position as sister-group to Ichthyosporea. To have the complete approach for comparative cell biology among this relevant eukaryotic group, we need to develop genetic tools, ideally stable transfection, in the remaining lineage of Corallochytrea.

Corallochytrea includes two taxa described so far, *Corallochytrium limacisporum* and *Syssomonas multiformis*. *C. limacisporum* is an understudied marine free-living walled saprotroph, that in addition to its key phylogenetic position, has features that make it relevant to be developed as a model organism: a peculiar and still uncharacterized life cycle. *C. limacisporum* has also a well-annotated genome which contains several conserved homolog genes with animals. It grows fast and it can be cultured in axenic conditions, in both, liquid and solid medium facilitating the isolation of clonal lines.

We here report a set of genetic tools that allow stable transfection in *C. limacisporum*. We have also developed a battery of cassettes tagging key cellular components, such as nucleus, plasma membrane, cytoplasm and actin filaments, that can serve for a better understanding of life cycle of *C. limacisporum*. Using nuclear labelling we have already gained some insights into particularities of *C. limacisporum* cell biology. We discovered that *C. limacisporum* is bi-nucleate for the majority of its life cycle. Interestingly, unlike most studied eukaryotes, the nuclear division is decoupled from the cellular division. We could also identify that *C. limacisporum* can go through multinucleate stage, hence the life cycle is non-linear. Progress and the potential implications of our research will be presented and further discussed.