**Salinity as a driver of speciation   
in free-living dinoflagellates**

**Name:** Nazeefa Fatima

**Field:** Bioinformatics

**Supervisor(s):** Dr. Karin Rengefors, and Dr. Dag Ahrén

**Credits:** 15

**Duration:** 10 weeks between March-May 2018

**Project plan**

Rapid adaptive radiation, i.e. the evolution of phenotypic and ecological diversity in a rapidly multiplying lineage, is proposed to be one of the main mechanisms of speciation (*Schluter 2000*). This process involves differentiation of a single ancestor into multiple species that inhabit a variety of environments and that differ in the traits used to exploit those environments (*Schluter 2000*). Recently, a group of free-living protists belonging to the class Dinophyceae (Phylum Alveolata) was proposed to have undergone recent adaptive radiation (*Annenkova et al. 2015*). This dinoflagellate species flock shows a recent common ancestry, signs of rapid speciation, and diverse phenotypes in different environments. Moreover, there is evidence that morphological and physiological traits associated with a particular environment, enhances survival and performance (*Logares et al. 2007a, 2008, Annenkova et al. 2015*).

The group includes four morphospecies, three found only in freshwater (*Apocalathium aciculiferum*, *A. baicalense*, and *A. euryceps*),whichhave conspicuous morphological differences, and *Apocalathium malmogiense* (previously *Scrippsiella hangoei, (Craveiro et al. 2016*)) consisting of several lineages (possibly, cryptic species) present in marine cold waters, saline Antarctic lakes, and in freshwater Lake Baikal (*Logares et al. 2007a, 2008, Annenkova et al. 2015, Rengefors et al. 2008*). There are differences in salinity tolerance between *A. aciculiferum* (freshwater) and *A. malmogiense* (Baltic Sea) (*Logares et al. 2007a*). Despite having different morphologies, the four species have very small differences in rDNA (up to 0.9% in 772 bp ITS2-LSU rDNA fragments) and mitochondrial DNA sequences (up to 1.7% in 592 bp COB mtDNA fragments) indicating a recent radiation (*Annenkova et al. 2015*).

Salinity is likely a major factor involved in driving the divergence of these species. It is therefore hypothesized that the species should differ in their gene expression at different salinities. Three strains representing *A. aciculiferum* (freshwater lake in Sweden)*, A. malmogiense* (Baltic Sea) *and A*. *aff. malmogiense* (brackish lake in Antarctica) were included in a growth experiment at different salinities (0, 3, and 30). The strains were grown in each salinity except for *A. aciculiferum* that could not be grown at salinity 30. mRNA was extracted and sequenced through the Gordon and Betty Moore Foundation project MMETSP (*Keeling et al. 2014*).

The purpose of the current project is to analyze differential expression among the three species as well as across the different salinities. The hypothesis is that the freshwater and marine/saline species will show differential expression of genes related to salinity.

**Methods**

1. Quality control and assessment of the transcriptome assembly.   
   The data for the 8 samples are publicly available at: <https://www.imicrobe.us/#/investigators/180>
2. The data needs to be downloaded to local compute resources and organized. We will use several tools for assessing the quality of the datasets including FastQC, MultiQC, BUSCO for transcriptome and RSeQC.
3. RNASeq analysis will be performed on the aligned reads using RSEM or similar software such as DESeq2 or EdgeR. Basic sanity checks such as investigating MA-plots and PCA plots will be performed and subsequently the datasets will be normalized in preparation for differential expression analysis.
4. Differential expression analysis to identify significantly up and down regulated genes will be performed. The dinoflagellates are expected to have smaller differences between stages compared to other organisms.
5. Functional analyses of differentially expressed genes will be performed primarily using the publicly available data. The data could be made available using Trinotate to facilitate manual inspection. Possibly enrichment analysis of gene ontology terms could be performed to identify enrichment of functional classes in the datasets especially those that corresponds to the differences in salinity. GSEA or GOSeq could be suitable for such an analysis.

**Time plan**

|  |  |  |
| --- | --- | --- |
| Week | Process | Notes |
| 1-2 | Set-up of project, and quality assessment of current data and annotations | Trinity evaluation, mapping results, QC of reads using FastQC, MultiQC, BUSCO & RSeQC. |
| 3 | RNA-Seq analysis including normalization and sanity checks. | RSEM plus DESeq2 or EdgeR |
| 4 | Differential expression analysis | Same as above |
| 5-8 | Functional Annotation | Sqlite, Trinotate |
| 9-10 | Prepare report & presentation |  |

**References**

* Annenkova, N. V., G. Hansen, Ø Moestrup, and K. Rengefors (2015). *Recent adaptive radiation in a marine and freshwater dinoflagellate species flock*. ISME J. 9 (8):1821-1834.
* Craveiro et al. (2016). *Studies on Peridinium aciculiferum and Peridinium malmogiense (.Scrippsiella hangoei): comparison with Chimonodinium lomnickii and description of Apocalathium gen. nov. (Dinophyceae).* Phycologia Volume 56 (1), 21–35.
* Keeling, P. J., F. Burki, H. M. Wilcox, B. Allam, E. E. Allen, L. A. Amaral-Zettler, E. V. Armbrust, J. M. Archibald, A. K. Bharti, C. J. Bell, et al. (2014). *The Marine Microbial Eukaryote Transcriptome Sequencing Project (MMETSP): Illuminating the Functional Diversity of Eukaryotic Life in the Oceans through Transcriptome Sequencing*. PLoS Biol. 12:e1001889.
* Logares, R., N. Daugbjerg, A. Boltovskoy, A. Kremp, J. Laybourn-Parry J. and K. Rengefors. (2008). *Recent evolutionary diversification of a protist lineage.* Environ. Microbiol. 10:1231-1243.
* Logares, R., K. Rengefors, A. Kremp, K. Shalchian-Tabrizi, A. Boltovskoy , Tengs et al. (2007a). *Phenotypically different microalgal morphospecies with identical ribosomal DNA: A case of rapid adaptive evolution*. Microb. Ecol. 53:549-561.
* Rengefors, K., J. Laybourn-Parry, R. Logares, W. A. Marshall, and G. Hansen. (2008). Marine-derived dinoflagellates in Antarctic saline lakes: annual dynamics and community composition. J. Phycol. 44:592–604.