



Stazione
Zoologica
Anton Dohrn
Napoli

School of Life, Health and Chemical Sciences

Doctor of Philosophy (Ph.D.)

Evolution of the sex determination mechanisms in the diatom genus *Pseudo-nitzschia*

Andrea Broccoli

Director of Studies

Dr. Maria Immacolata Ferrante
Stazione Zoologica Anton Dorhn, IT

Internal Supervisor

Dr. Diana Sarno
Stazione Zoologica Anton Dorhn, IT

External Supervisor

Prof. Peter von Dassow
*Pontificia Universidad Católica de Chile,
Santiago, CL
Stazione Zoologica Anton Dorhn, IT*

Advisor

Dr. Monia Teresa Russo
Stazione Zoologica Anton Dorhn, IT

September 2025

EXAMINATION PANEL

External examiner

Prof. Dr. David G. Mann

Royal Botanic Garden Edinburgh, UK

External examiner

Dr. Agnieszka Lipinska

Max Planck Institute for Biology, DE

Internal examiner

Dr. Maria Immacolata Arnone

Stazione Zoologica Anton Dorhn, IT

Chair

Dr. Giovanna Romano

Stazione Zoologica Anton Dorhn, IT

Abstract

Various sex determination systems have emerged during the evolution of eukaryotes. In unicellular organisms, sexes are termed Mating Types (MT). In 2018, the first diatom MT-determining gene was identified in the pennate species *Pseudo-nitzschia multistriata*: the *MRP3* gene defines the MT+, regulating the expression of four downstream genes. We investigated the conservation of this mechanism, focusing on species representing two clades distant from *P. multistriata* but within *Pseudo-nitzschia*, *P. arenysensis* and *P. calliantha*. RNA-seq data analysis of these two species revealed partial conservation of the MT-related genes expression pattern, with *MRP3* exclusively expressed in the MT+, suggesting conservation of its role as the sex-determining gene. Exploring the MT locus with long-read genome sequencing and PCR amplifications revealed that *MRP3* is absent from the genome of MT- in *P. arenysensis*, in contrast to *P. calliantha*, where MT- strains either lack *MRP3* or produce a truncated transcript variant, leading to an inactive MRP3 protein. *MRP3* and the other MT-related genes showed conservation within the genus in genomes and transcriptomes available for other *Pseudo-nitzschia* species. Comparative analyses of seven MRP3 protein 3D structures predicted with high confidence from different *Pseudo-nitzschia* species revealed a shared structural motif, currently with unknown function. Collectively, these results suggest that the role of *MRP3* as a master sex-determining gene is preserved throughout the *Pseudo-nitzschia* genus, while the downstream genes differ, and the MT locus exhibits variation. Moreover, tracking gene expression during sexual reproduction in *P. arenysensis* revealed the induction of several genes, including some of the MR genes, supporting their involvement in the process. Other highly regulated genes may act as markers of specific sexual stages. These findings contribute to the understanding the molecular regulation of key phases and transitions in diatom life cycle, and broaden our knowledge on the evolution of sex determination mechanisms.