



**Annalisa Zuccarotto (Ms.c)**

**Molecular Evolution and Biochemical Characterization of  
Ovothiol Biosynthesis in Cnidaria**

*Doctor of Philosophy*

**The Open University, UK  
School of Life, Health and Chemical Sciences**

**Stazione Zoologica Anton Dohrn, IT  
Department of Biology and Evolution of Marine Organisms**

**April 2024**

**Director of Studies:**

**Dr. Serena Leone**

Department of Biology and Evolution of Marine Organisms  
Stazione Zoologica Anton Dohrn, Naples, Italy

**Internal Supervisors:**

**Prof. Immacolata Castellano**

Department of Molecular Medicine and Medical Biotechnology  
University of Naples Federico II, Naples, Italy

Department of Biology and Evolution of Marine Organisms  
Stazione Zoologica Anton Dohrn, Naples, Italy

**Dr. Mariella Ferrante**

Department of Integrated Marine Ecology  
Stazione Zoologica Anton Dohrn, Naples, Italy

**External Supervisor:**

**Prof. Marco Gerdol**

Department of Life Sciences  
University of Trieste, Italy

## Abstract

Ovothiols are 5-thiohistidine derivatives produced by several marine invertebrates, protists, and bacteria, which have recently attracted research community interest for their peculiar antioxidant properties and anti-inflammatory activities. In nature, ovothiol biosynthesis is catalyzed by two enzymes: 5-histidylcysteine sulfoxide synthase (OvoA) and pyridoxal phosphate-dependent lyase (OvoB). Bioinformatics analyses have highlighted that OvoA is highly conserved in Metazoa, from Porifera to Echinodermata, despite the loss of the *ovoA* gene in Ecdysozoa and Urochordata. In addition, two horizontal gene transfer events have been identified in Rotifera Bdelloidea and Hydrozoa. The aim of my PhD project was to deepen the evolutionary history and molecular diversification of ovothiol biosynthesis with a special focus on the Cnidaria phylum. Cnidarians are particularly intriguing for this investigation due to their early branching position in the animal kingdom and their close relationship with bilaterians. Moreover, they represent an extraordinary example of molecular diversification of ovothiol biosynthesis, as Anthozoa conserve the canonical OvoA structure, Medusozoa lost OvoA, and Hydrozoa reacquired it through a horizontal gene transfer event. In this study, a deep phylogenetic analysis of OvoA in cnidarian species confirmed and enriched previous findings of Gerdol and colleagues (2019), revealing a new hydrozoan species, *Clytia hemisphaerica*, as the first case in marine organisms displaying a single transcript that combines *ovoB* and *ovoA* coding regions, probably acquired through horizontal gene transfer from closely related photosynthetic symbionts which display a similar genomic organization. Subsequently, in order to gain insights into the biological roles of ovothiols, the expression levels of *ovoA* during embryo and larval development of several cnidarian and other metazoan species were compared, by *in silico* analyses of publicly available transcriptomes. This analysis revealed a consistent pattern of *ovoA* overexpression in specific life cycle stages of these animals, but also upon exposure of different environmental stressors. Additionally, by HPLC and LC-MS techniques, the occurrence of cellular thiols was traced in key model species of Anthozoa, Medusozoa, and Hydrozoa in order to begin a survey of ovothiols distribution in these basal Metazoa, thus shedding light on the

biodiversity of ovothiol biosynthesis in this phylum. Finally, *ovoB* and *ovoA* coding sequences from the hydrozoan *C. hemisphaerica* were cloned and the recombinant proteins were produced in order to reconstruct the enzymatic activities necessary for ovothiols biosynthesis *in vitro* and gather information about the mechanism of reactions. In conclusion, the results of this PhD proposal shed light on the evolutionary origin and molecular diversification of ovothiol biosynthesis and allow us to make hypotheses on the role of this secondary metabolic pathway in the alternation of animal life cycle and in the complex relationships of marine organisms with their environment.