

## DOCTORAL THESIS

### Characterization of the egg and embryonic proteome of *Pomacea canaliculata*, and responses of the proteome to environmental stressors

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**Characterization of the Egg and Embryonic Proteome of *Pomacea  
canaliculata*, and Responses of the Proteome to Environmental  
Stressors**

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**A thesis submitted in partial fulfillment of the requirements  
for the degree of  
Doctor of Philosophy**

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## ABSTRACT

The apple snail *Pomacea canaliculata* is an invasive gastropod introduced from Argentina now widely distributed in Asia including Hong Kong. It has been suggested as a model animal for toxicological and evolutionary studies due to several advantages including ease of laboratory rearing. In this study, I used both transcriptomic sequencing and proteomics to provide genomic resources for this species and demonstrate the applicability of this species to answer fundamental questions, such as the roles of various proteins in egg defense, embryonic development, and discovery of molecular markers of exposure to pollutants and abiotic stressors. During the embryonic development, stages II and III are crucial to the organogenesis. Thus, I first constructed a reference map of the apple snail embryos and was able to identify 52% of the 125 protein spots by using cross-species identification strategy. The identified proteins exhibited multiple functions, including utilization of the perivitelline fluid for energy production, shell formation, protein synthesis and folding, and cell cycle and cell fate determination.

However, due to the complementary role of proteomics and genomics, a comprehensive genomic resource is required for the improvement of the protein identification. I thus conducted a *de novo* assembly of the transcriptome for this species and applied this transcriptome in several downstream proteomic applications involving protein identification. The transcriptome was sequenced using Illumina “paired-end” sequencing of cDNA short reads, and the sequences were assembled *de novo*. This transcriptome contained 128,436 unigenes with an average length of 419 bp (range: 150 to 8,556 bp). Further analysis revealed 2,439 putative transposable elements, 3,196 microsatellites and 31,710 SNPs. To demonstrate the application of this transcriptome in protein identification, I profiled a juvenile snail using LC-MS/MS, resulting in 878 identified proteins. These genomic resources can facilitate various molecular ecological studies, such as stress physiology and range expansion.

*Pomacea canaliculata* is a freshwater gastropod but adopts a unique aerial reproduction strategy. Proteins in the perivitelline fluid (PVF) must play an important role in nurturing and protecting the embryo. I therefore profiled the PVF of this gastropod. There were 59 proteins, among them 19 are novel. KEGG analysis showed that the functions of the majority of these proteins are "unknown" (n = 34), "environmental information processing" (10), 9 of which are related to innate immunity, and "metabolism" (7). In addition, there were 19 novel proteins, some of which might be involved in adaptation to aerial reproduction but require further characterization. Toxicological test using copper and cadmium revealed that apple snail juveniles are sensitive to metal pollution, and copper is more toxic than cadmium to the juveniles. The sequential 2DE based proteomics revealed several

unique biomarkers of exposure to the metals. In addition, I examined the molecular mechanism of how apple snail responded to desiccation using iTRAQ proteomics and found a metabolic shift and the up-regulation of proteins involved in the tissue protection during desiccation. Overall, my study has demonstrated great potential of proteomics in understanding basic questions about early development and responses to environmental stressors in a non-model organism, and rapid generation of genomic resources using second generation sequencing is a key to realize such potential.

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