ABSTRACT

Polychaetes in the suborder Aphroditiformia are commonly called scale worms because they are characterized by having dorsal elytra or scales. Scale worms are a large group of polychaetes and play an important role in marine ecosystems. They are widely distributed in the sea, from the tropics to polar regions, and from the shallow water to the deep sea. Since the first discovery of hydrothermal vents in 1977, many deep-sea scale worms, most of which belong to the largest scale-worm family Polynoidae, have been reported. Yet little is known about the phylogeny and genetic adaptation of deep-sea polynoids to the extreme deep-sea environment. The identification of these scale worms was solely based on morphology. However, the morphological character states for the delimitation of some of these scale worms were ambiguous. In addition, due to the lack of molecular information, phylogenetic relationships among several groups of scale worms are unclear. Thus, the aim of my study was to improve our knowledge about the phylogeny and evolution of Aphroditiformia with a focus on deep-sea polynoids.

My thesis is divided into five chapters. The first chapter is an introduction of the status of polychaete taxonomy, with an emphasis of Aphroditiformia. Among the three experimental chapters, Chapter 2 is a systematics study of deep-sea scale worms collected from the Okinawa Trough. In this chapter, I described four species of polynoids, including a new species of Levensteiniella, a new species of Branchinotogluma, a male morphotype of Lepidonotopodium okinawae, and a female morphotype of Branchinotogluma japonicus. Moreover, a fragment of the COI gene for selected specimens of these species were amplified to determine phylogenetic relationships with other deep-sea polynoids.

In Chapter 3, I applied low-coverage DNA sequencing to obtain 18S, 28S rRNA and mitochondrial genomes from 16 species representing 7 families in Aphroditiformia. Two phylogenetic trees were constructed: the first based on the concatenated sequences of four partial genes (cox1, 16S, 18S and 28S rRNA), and the second using mitochondrial protein-coding genes, rRNA genes and two nuclear rRNA genes (18S and 28S rRNA). The results showed that Aphroditiformia is monophyletic. Eulepethidae and Aphroditidae for the sister group of the other scale-worm families. Acoetidae is sister to Iphionidae. Polynoidae is monophyletic, but within this family, deep-sea subfamilies Branchinotogluminiae and Macellicephalinae are paraphyletic. There are two large gene order rearrangements in the mitochondrial genomes of deep-sea polynoids, and
substitution rates of mitochondrial protein-coding genes in deep-sea species are much higher than those in shallow-water species, which indicate that the extreme environment in deep sea may have affected the evolution rate and gene order of the mitochondrial genomes of deep-sea polynoids. The large mitochondrial gene order rearrangements have provided evidence to refute the assumption that mitochondrial gene order is conserved in Errantia.

In Chapter 4, I reported two transcriptomes of deep-sea polynoids (*Branchipolynoe pettiboneae* and *Lepidonotopodium okinawae*) newly obtained by next generation sequencing, and compared them with the transcriptome of a shallow-water polynoid (*Harmothoe imbricata*). The results showed that among the highly expressed genes, duplicated genes related to DNA recombination and metabolism were only enriched in the deep-sea species, indicating that the deep-sea species have a more complex DNA repair system which could prevent harmful DNA mutation caused by DNA damage. Compared to the shallow-water polynoid, the deep-sea species have more hemoglobin genes and their expression levels were higher than those of the shallow-water species, indicating high expression of more hemoglobin genes is crucial for deep-sea scale worms to adapt to the hypoxic environment.

Overall, my empirical systematics study and molecular phylogenetic study have improved our understanding about the systematics and phylogeny of Aphroditiformia, and my transcriptomic comparison between the deep-sea and shallow-water polynoids has enhanced our understanding about the adaptation of deep-sea scale worms to the extreme environmental conditions in hydrothermal vents and methane seeps.
Table of contents

Chapter 1 General introduction ........................................................................................................... 1

1.1 Brief introduction of Polychaeta ................................................................................................. 1

1.2 General introduction of scale worms (Suborder Aphroditiformia, Order Phyllodocidae) ................................................................................................................................. 2

1.3 Deep-sea environment and deep-sea scale worms ..................................................................... 5

1.4 Systematics and phylogenetic analysis ...................................................................................... 8

1.5 Phylogeny of scale worms .......................................................................................................... 11

1.6 Overview of the thesis ................................................................................................................. 13

Chapter 2 Deep-sea polynoids (Polynoidae: Polychaeta) from the Okinawa Trough .............. 17

2.1 Introduction .................................................................................................................................. 17

2.2 Materials and methods ............................................................................................................... 18

2.2.1 Sample collection and morphological observation .................................................................. 18

2.2.2 Cox1 amplification and phylogeny .......................................................................................... 19

2.3 Results ......................................................................................................................................... 19

2.3.1 Genus Lepidonotopodium Pettibone, 1983 ............................................................................. 19

2.3.2 Genus Levensteiniella Pettibone, 1985 .................................................................................... 22

2.3.3 Genus Branchinotogluma Pettibone, 1985 .............................................................................. 25

2.3.4 Phylogenetic tree based on molecular data ............................................................................ 31

2.4 Discussion ..................................................................................................................................... 31

Chapter 3 Phylogeny and evolution of scale worms (Aphroditiformia, Annelida): Insights from analysis of mitochondrial genomes and ribosomal sequences .................. 48

3.1 Introduction .................................................................................................................................. 48

3.2 Material and methods .................................................................................................................. 51

3.2.1 Collection the specimens of scale worms ............................................................................... 51

3.2.2 Genome sequencing and assembly .......................................................................................... 52

3.2.3 Mitochondrial genome annotation .......................................................................................... 52

3.2.4 Phylogenetic analysis .............................................................................................................. 52

3.2.5 Comparisons of mitochondrial gene order .............................................................................. 53

3.2.6 Substitution rates of mitochondrial genes .............................................................................. 53

3.3 Results ......................................................................................................................................... 54