

Developmental and molecular studies of the arms formation in *Octopus minor*.

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ABSTRACT

Cephalopods (e.g. cuttlefish, nautilus, octopus, squid) belong to a class of the phylum Mollusca. In general, cephalopods as advanced invertebrates have interesting biological characteristics such as extraordinary rapid growth, short lifespan, large brain, and sophisticated sense organs with complex nervous system. *Octopus minor*, commonly called long arm octopus has eight arms, each of which also carry out common function and its own role. In this study, we performed the transcriptome analysis on the embryo of the *Octopus minor* in the development stage. We selected genes with very high expression level in each arm of the analysis. Based on gene data, we conducted molecular biological experiments such as in situ Hybridization, Immunohistochemistry and Histological analysis. These results are the first step to understanding the complex functions of the *Octopus minor*'s arms and will valuable resource for analyzing the functions of gene repertoires in various developmental phases.

Morphological analysis of the arms in early stages of *Octopus minor* using Scanning Electron Microscopy (SEM)

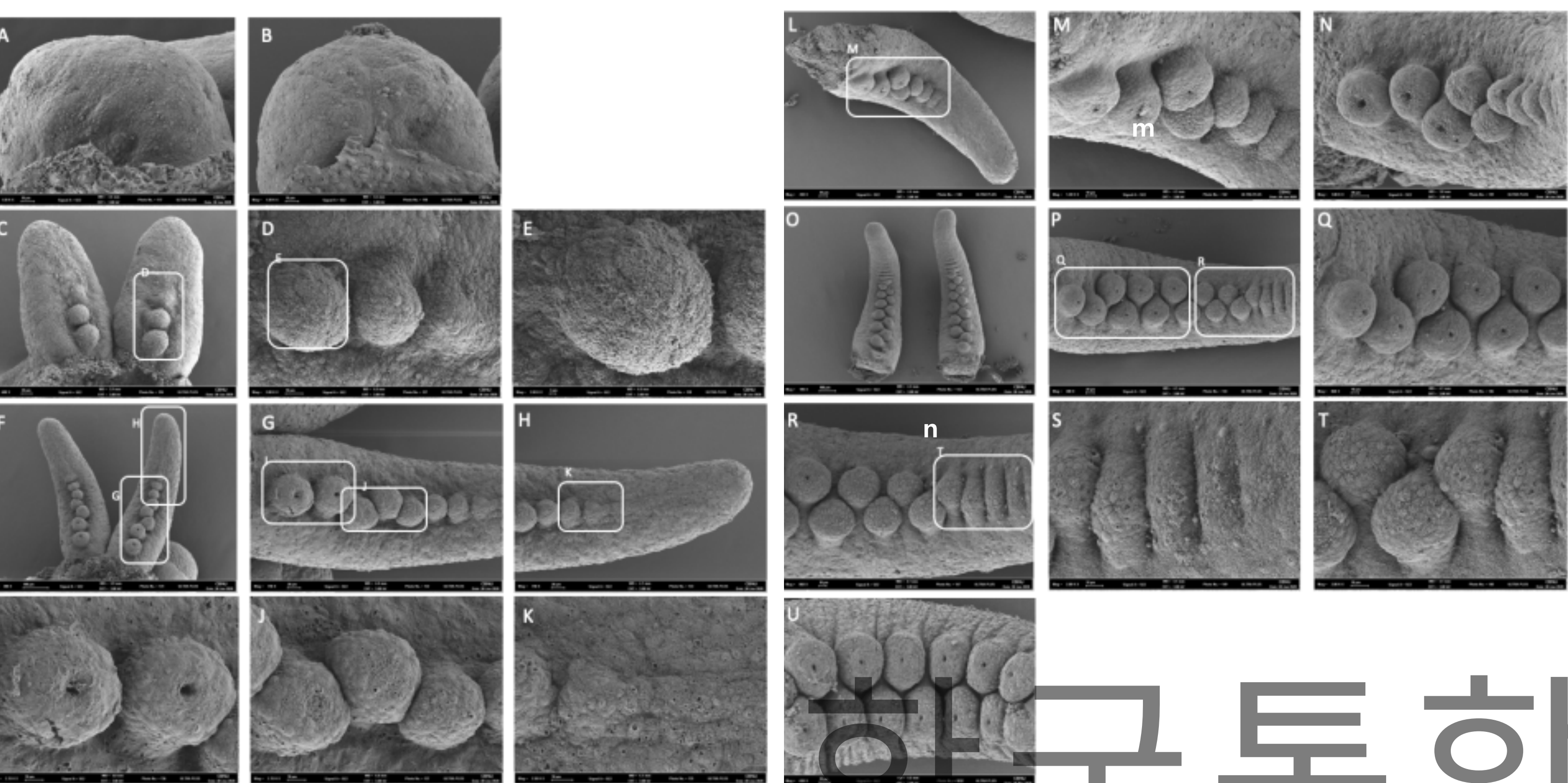


Fig.1. Scanning Electron Microscopy (SEM) images of the arms.
O. minor have eight bilaterally symmetric limb pairs. Morphogenesis of the arm is first observed as a slight swelling and then turned into proximo-distal outgrowth progress at stage 10 (A, B). The formation of suckers begins at stage 12 (C-E). Starting sucker invagination and arrangement changes to asymmetrically at stage 14 (F-K). The number of suckers with invagination increased and the arrangement of suckers became more asymmetric at stage 16-18 (L-T). Most of suckers were invaginated and the arrangement was completely asymmetric at stage 20 (U).

EXPRESSION PATTERN OF FLUORESCENT IMMUNOHISTOCHEMISTRY IN EARLY *Octopus minor*

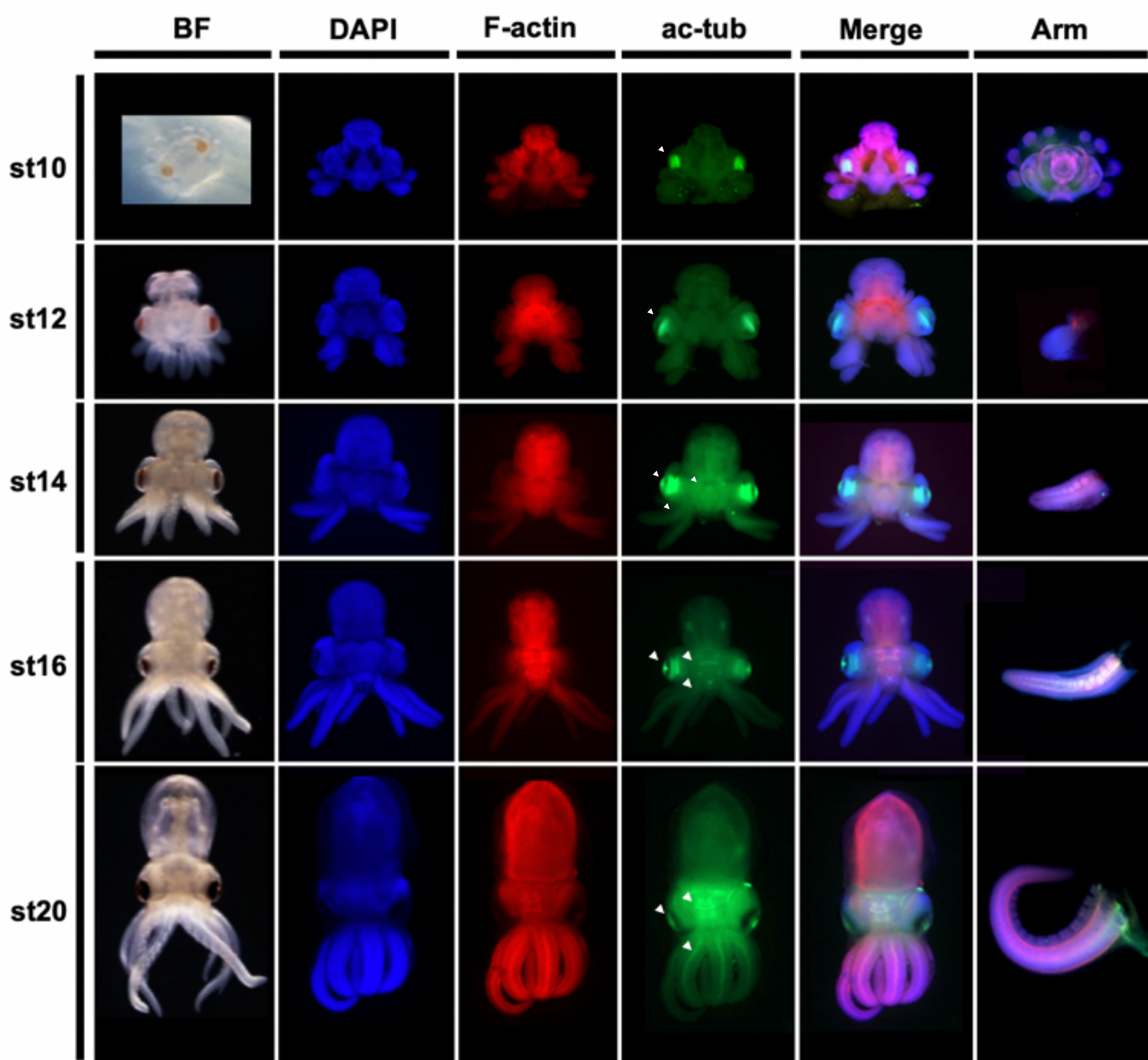


Fig.2. Images of Whole mount immunostaining in *O. minor*.
The picture from left to right shows Bright field, DAPI staining, F-actin, α -acetylated tubulin and merge. In the developmental process, the expression pattern of nerves in the eyes is clearly observed. Neuronal expression of the arms gradually increases the arms become longer. Each arrow represents nerves in the eyes, brain and arms. Fluorescent images were taken with an Zeiss AXIO Zoom.V16

RNA-Sequence Analysis to find the key of the arm and sucker formation in *Octopus minor*

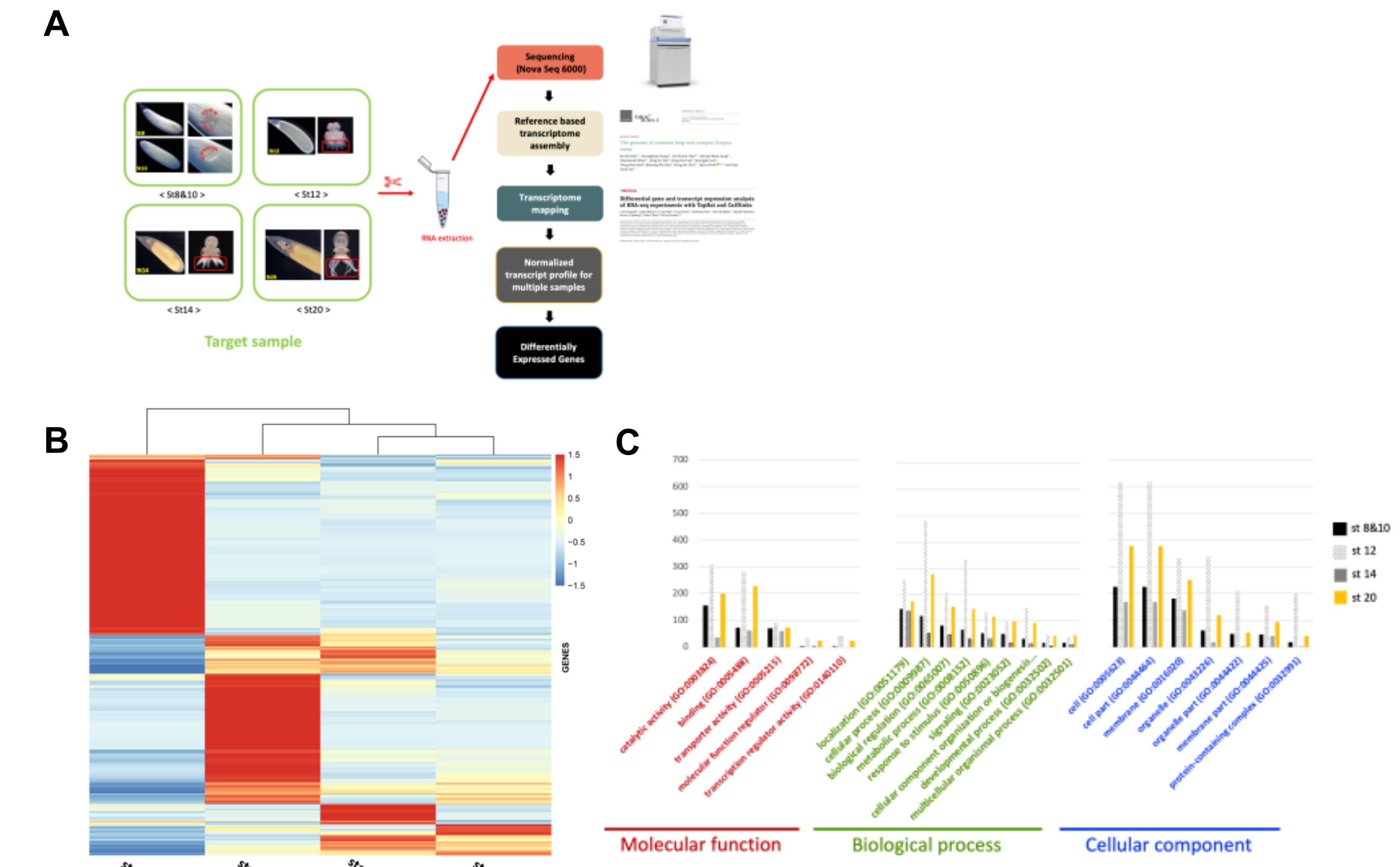


Fig.3 The stage-specific RNA transcriptome profiles of embryogenesis in *O. minor*.
Schematic of RNA-SEQ analysis to find the key of sucker formation in *O. minor* (A). Heat-map of the Differential expression genes (DEGs) during the arms and sucker development in *O. minor*. Heat-map data in stage 8 and 10, stage 12, stage 14, stage 20. Genes with z-score of 1 or more were clearly confirmed from the Heat-map data of the four stages of development (B). Genes with z-score of 1 or more in *O. minor*. Heat-map was analyzed using PANTHER. GO functional annotations are summarized in three main categories: biological process, cellular component and molecular function (C).

In situ Hybridization of genes for arms formation in *Octopus minor*

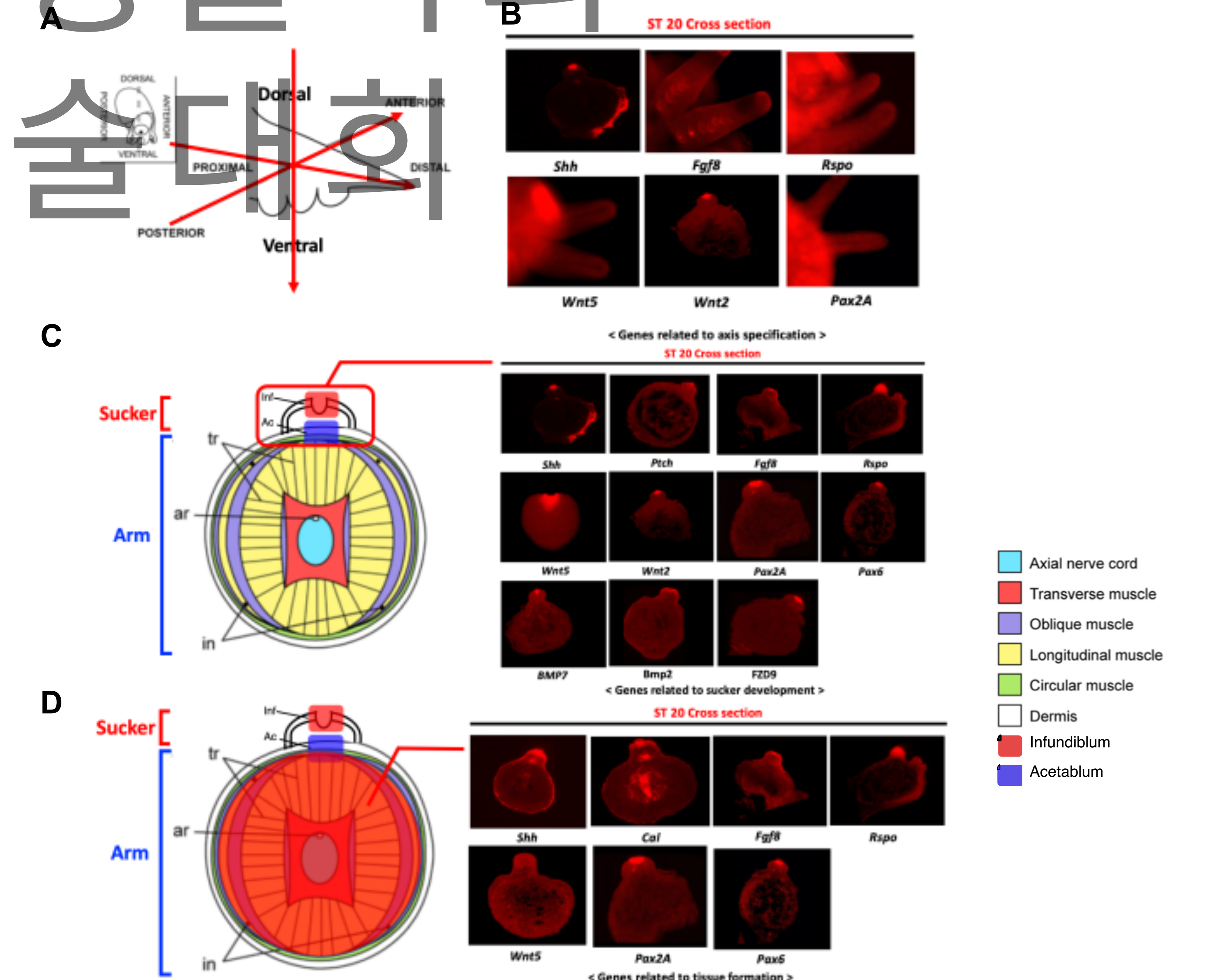


Fig.4. Summary of gene expressions involved in arm formation in *O. minor*.
Schematic diagram of the arm axis in *O. minor* (A). Cross-section view showing the expression of genes related to axis specification in *O. minor* (B). Schematic representation of the arm and sucker anatomy and cross-section view showing the expression of genes related to sucker development in *O. minor* (C). Schematic representation of the arm and sucker anatomy and cross-section view showing the expression of genes related to tissue(ex. nerve, muscle) formation in *O. minor* (D).

DISCUSSION

In this study, We established the early embryonic stages of *O. minor* and confirmed the distribution of nerves and muscles in early *O. minor*. To find out the differences of gene expression during developmental stages, we performed transcriptome analysis of *O. minor* according to developmental stages. In addition, we analyzed DEGs data of 8&10, 12, 14, 20 stages arms. As a results, several genes related to arm development have been found. Based on the analysis results, PD axis-forming factors, transcription factors, indirect influence factors and factors related to muscle composition were confirmed spatial expression patterns. As a result, it was confirmed that the *shh*, *fgf8*, *rspo*, *wnt5*, *wnt2* and *pax2a* genes are involved in axis formation during the process of developing the arm. Most of genes related to axis formation and sensory cell development were found to be involved in the formation of suckers. It was also confirmed that *shh*, *calponin*, *fgf8*, *rspo*, *wnt5*, *pax2A*, and *pax6* genes are involved in the development of nerves and muscles of the arm. These data are the first step for understanding the complex developmental gene regulatory networks in the arm of *O. minor* and will be valuable resources for analyzing the functions of gene repertoires in various developmental phases.

ACKNOWLEDGMENT

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