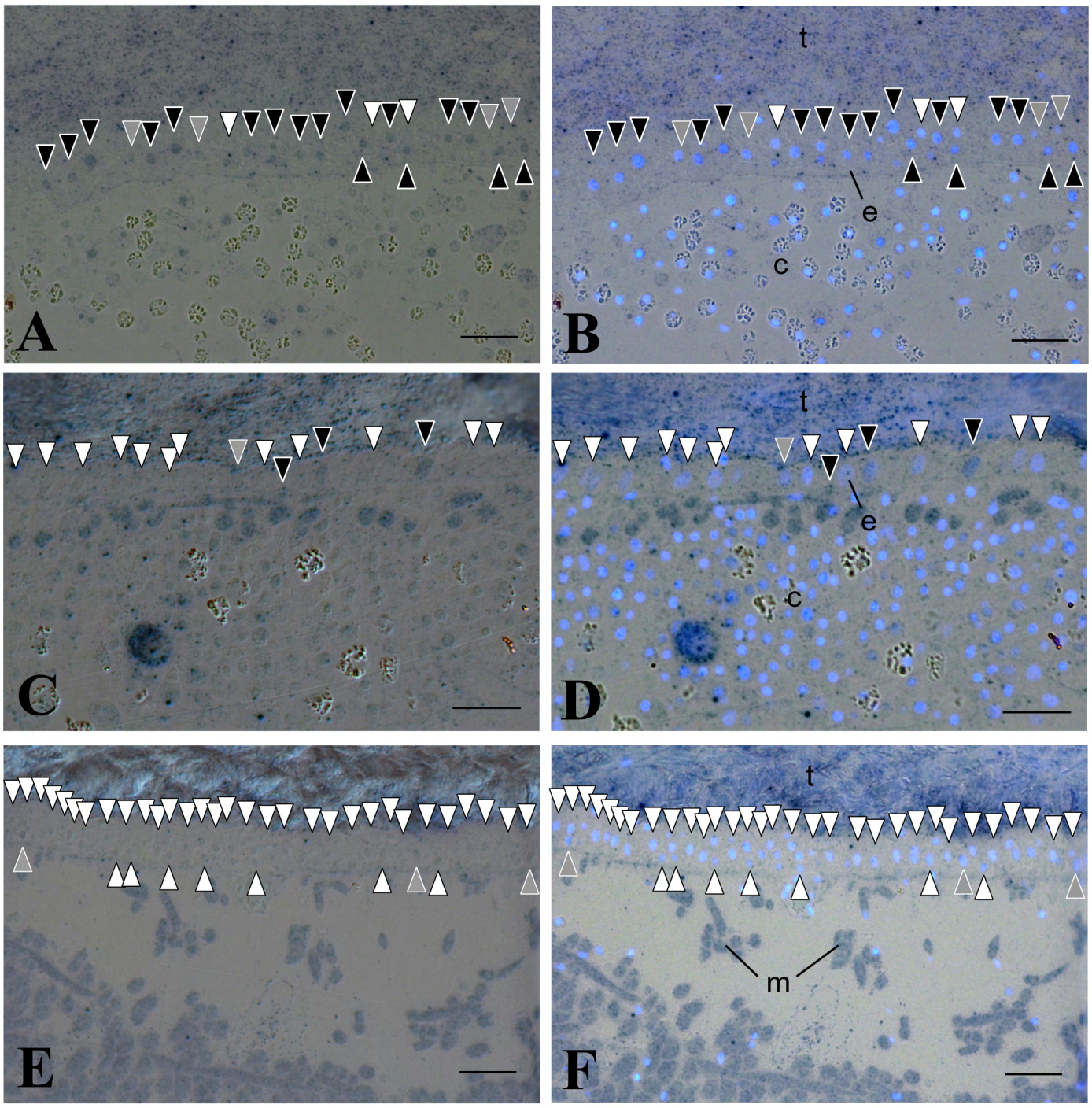

Fig. S1. PCNA expression in epidermal nuclei during budding and zooid senescence. (**A**,**B**) Growing bud. (**C**,**D**) Juvenile zooid. (**E**,**F**) Senescent adult zooid. (A,C,E) Anti-PCNA immunostaining. (B,D,F) Immunostaining merged with DAPI staining. Black and grey arrowheads show strong and moderate PCNA expression, respectively. White arrowheads show no PCNA staining. c, coelomic cell; e, epidermis; m, body muscle cell; t, tunic. Scale bars: 20 µm.

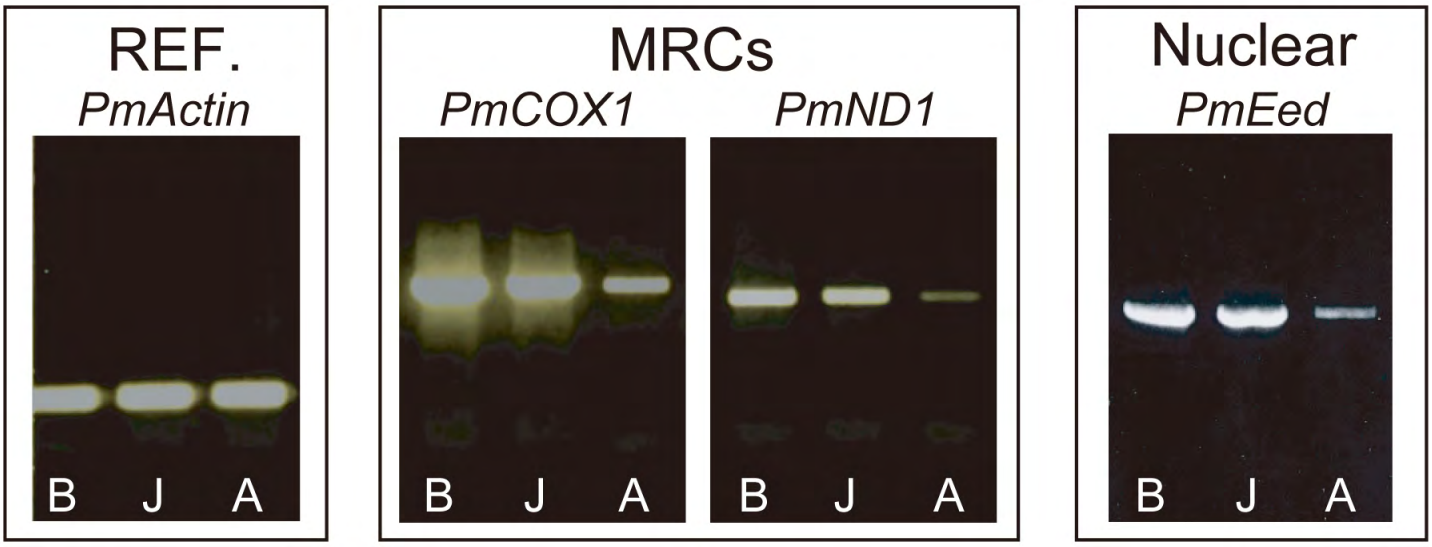

Fig. S2. RT-PCR analysis of the expression of *Polyandrocarpa* MRCs, *PmCOX1* and *PmND1*, and nuclear gene *PmEed* at different ageing stages. mRNAs were extracted from growing buds (B), juvenile zooids (J) and senescent adult zooids (A). Cytoplasmic actin was used as internal standard.

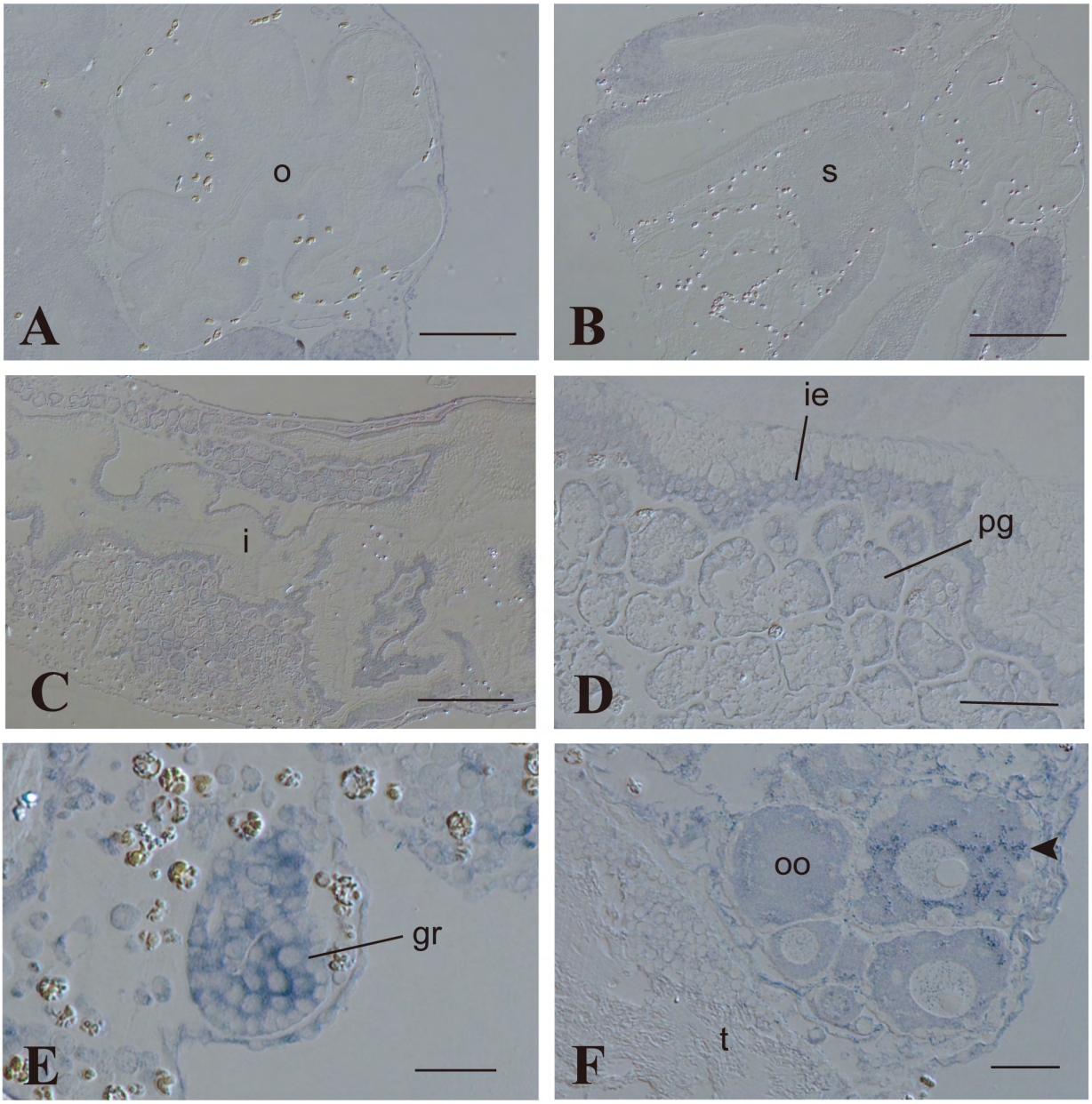

Fig. S3. Expression of *PmCOX1* in differentiated tissues; in situ hybridization. (**A**) Oesophagus. Scale bar: 50 μm. (**B**) Stomach. Scale bar: 50 μm. (**C**) Intestine. Scale bar: 100 μm. (**D**) Higher magnification of (C). Scale bar: 25 μm. (**E**) Gonad rudiment. Scale bar: 20 μm. (**F**) Ovary. Arrowhead shows an aggregate of dotted signals probably from mitochondria. Scale bar: 20 μm. gr, gonad rudiment; i, intestine; ie, intestinal epithelium; o, oesophagus; oo, oocyte; pg, pyloric gland; s, stomach; t, testis.

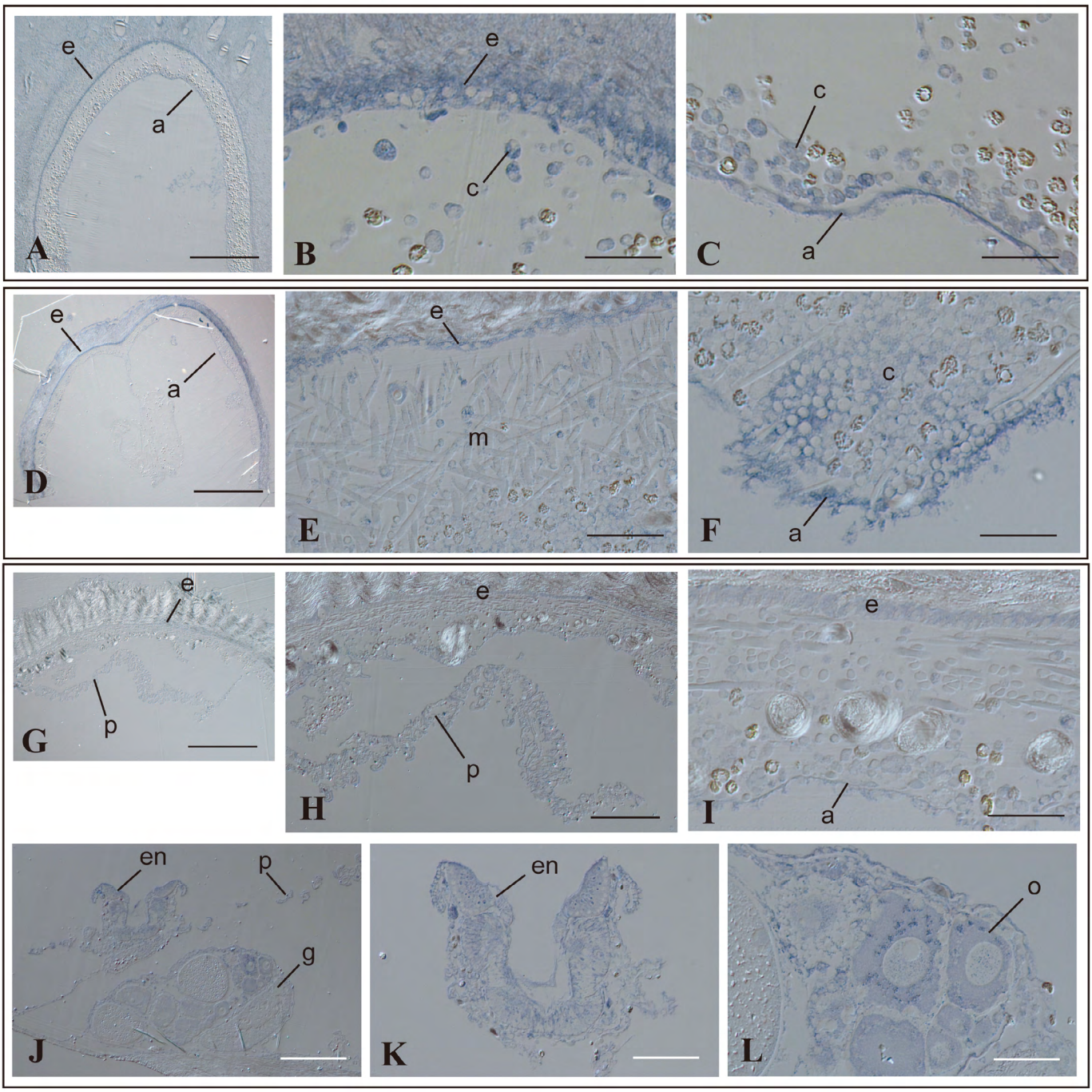

Fig. S4. *PmND1* expression at different ageing stages; in situ hybridization. (**A-C**) Growing buds. (A) Lower magnification. Scale bar: 200 µm. (B) Epidermis and coelomic cells. Scale bars: 20 µm. (C) Atrial epithelium and coelomic cells. Scale bar: 20 µm. (**D-F**) Juvenile zooids. (D) Lower magnification. Scale bar: 300 µm. (E) Epidermis and body muscle tissue. Scale bar: 30 µm. (F) Atrial epithelium and coelomic cells. Scale bar: 20 µm. (**G-L**) Senescent zooids. (G) Lower magnification. Scale bar: 300 µm. (H) Body wall and pharynx. Scale bar: 100 µm. (I) Epidermis, coelomic cells and atrial epithelium. Scale bar: 20 µm. (J) Ventral organs. Scale bar: 100 µm. (K) Endostyle. Scale bar: 50 µm. (L) Gonad. Scale bar: 50 µm. a, atrial epithelium; c, coelomic cell, e, epidermis; en, endostyle; g, gonad; m, body muscle cell; o, oocyte; p, pharynx.

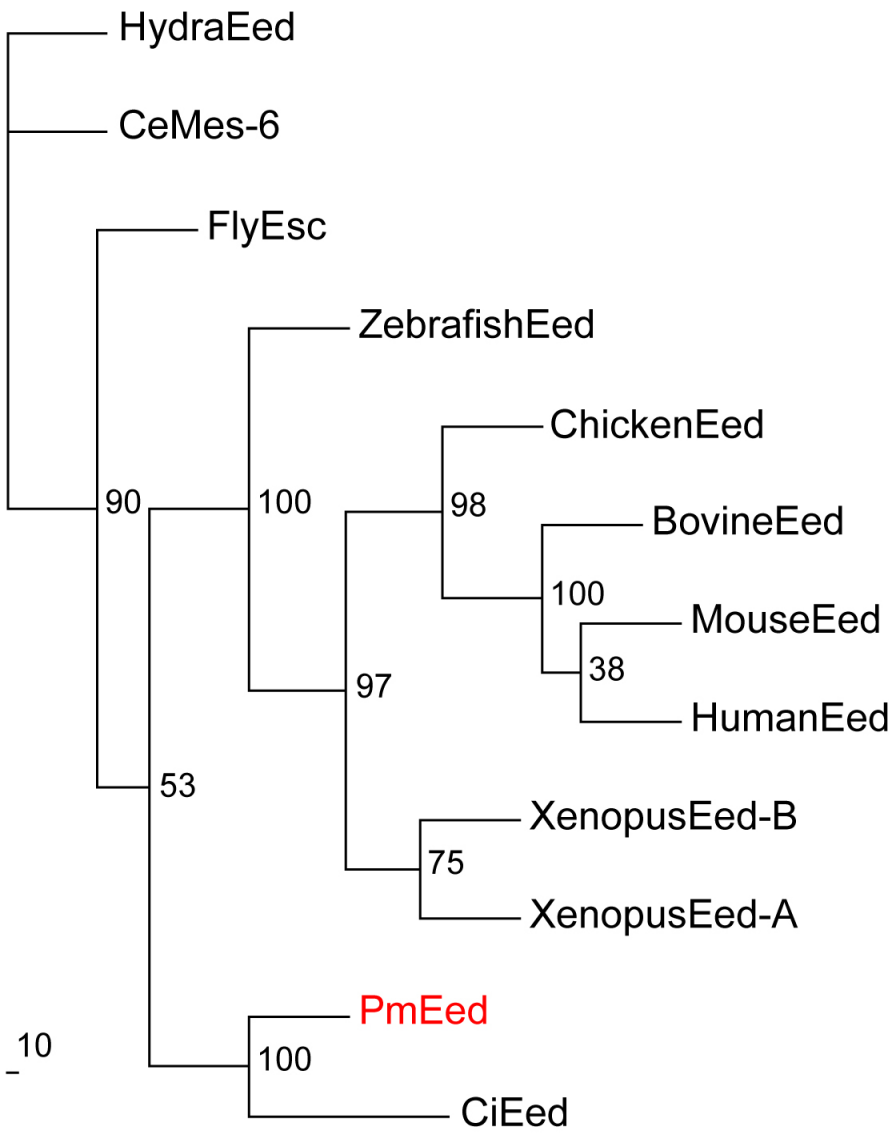

Fig. S5. Molecular phylogeny of the nuclear gene *PmEed*. Phylogenetic relationship among partial amino acid sequence of Eed proteins is shown. The analysis was carried out with the maximum likelihood method. The bootstrap values are shown on each branch. Ce, *Caenohabditis elegans*; Ci, *Ciona intestinalis*; Pm, *Polyandrocarpa misakiensis*.

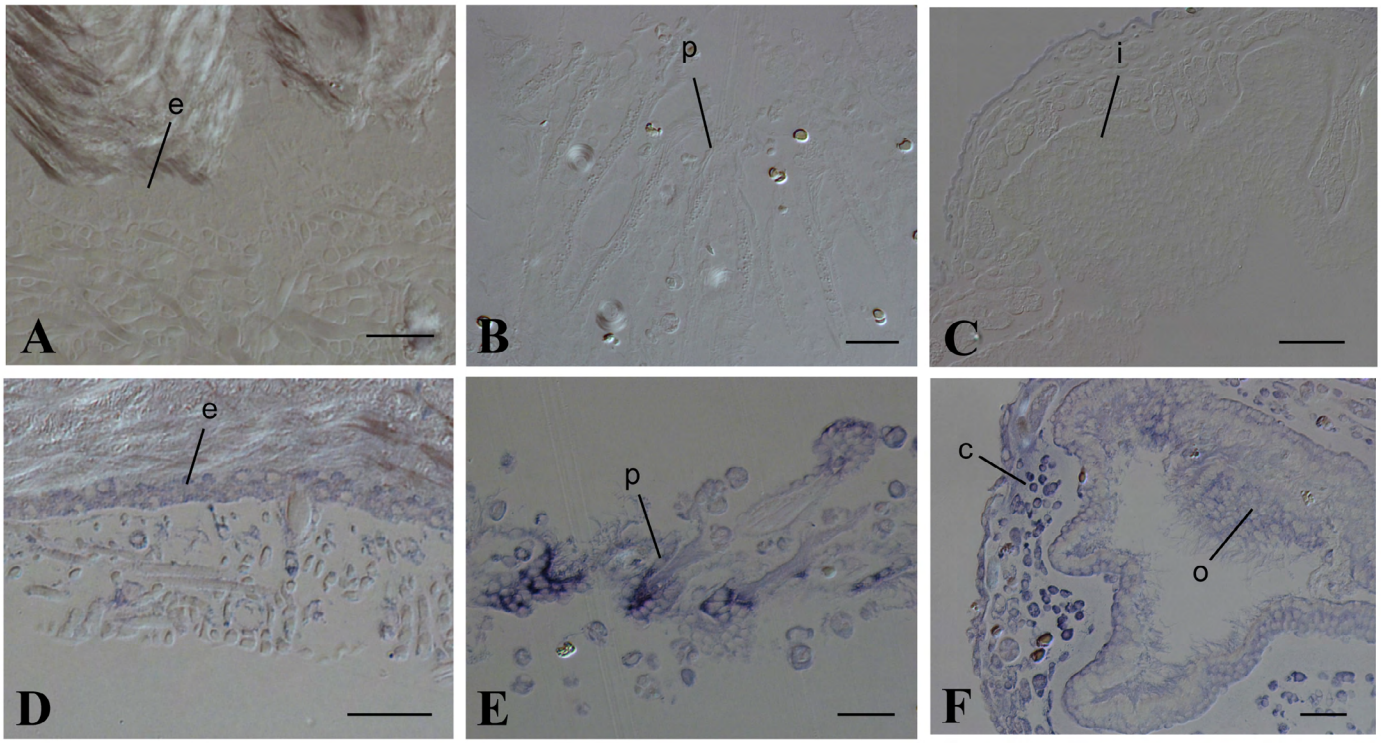

Fig. S6. Effect of TC14-3 on *PmEed* expression in senescent zooid pieces; in situ hybridization. (**A-C**) Negative control. Senescent zooids were only cut into pieces. (A) Epidermis. Scale bars: 20 μm. (B) Pharynx. Scale bars: 20 μm. (C) Digestive tract. Scale bars: 40 μm. (**D-F**) TC14-3-treated zooid pieces. (D) Epidermis and coelomic cells. Scale bars: 20 μm. (E) Pharynx. Scale bars: 20 μm. (F) Digestive tract and coelomic cells. Scale bars: 20 μm. c, coelomic cell; e, epidermis; i, intestine; o, oesophagus; p, pharyngeal epithelium.

Table S1. Number of epidermal mitochondria during senescence

Stage	Number of mitochondria/20 μm^2
Growing bud	8.9±1.4*
Juvenile zooids	6.9±1.9
Senescent zooids	6.2±2.0

*Average±s.d.