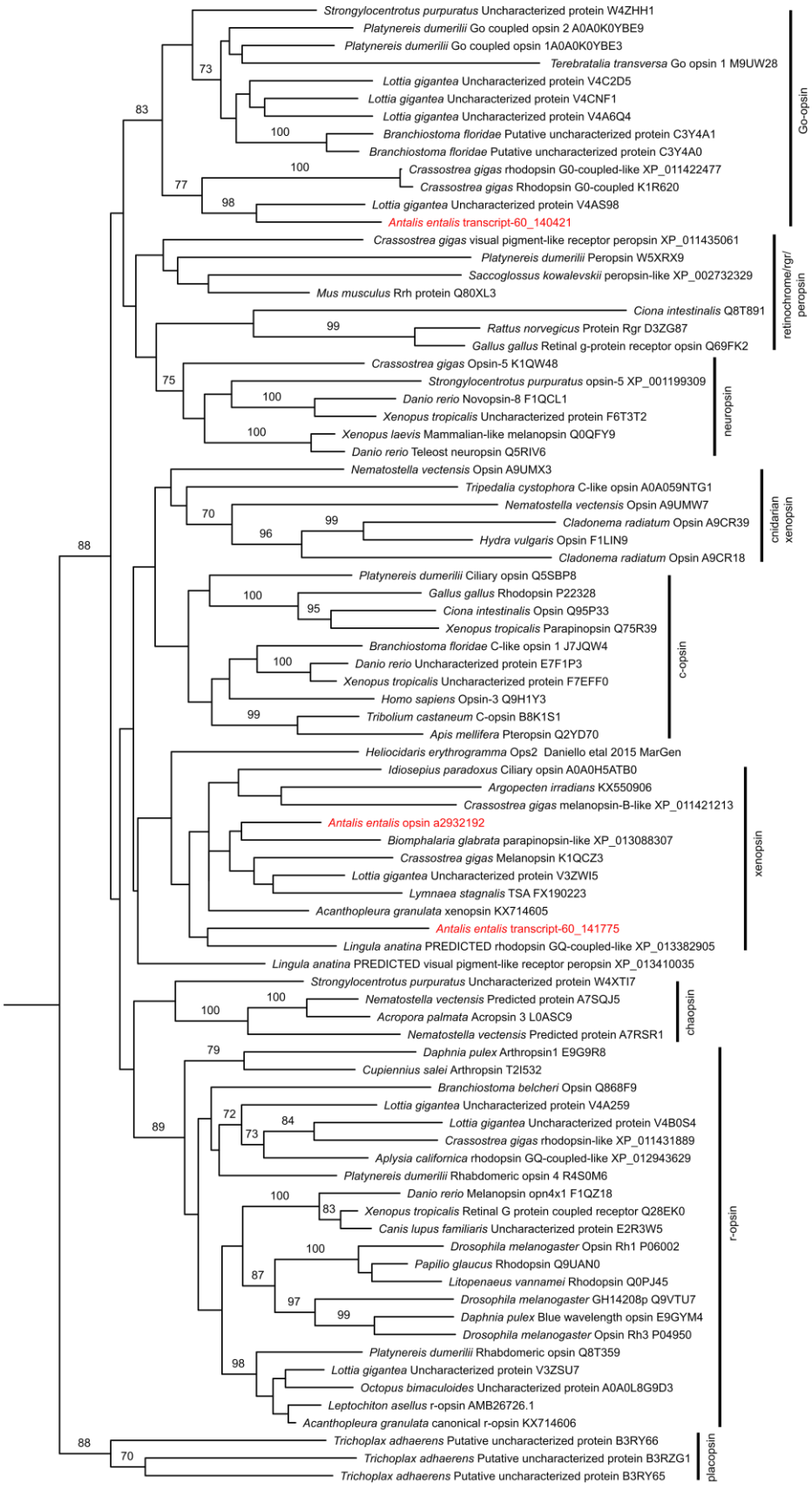
Additional file 1. Phylogenetic analyses (Figures S1a-h), additional gene expression patterns (Figures S2-9), Table S1, primer and Go-opsin sequences.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Mus musculus Rrh protein Q80XL3
Ciona intestinalis Q8T891
Rattus norvegicus Protein Rgr D3ZG87
Gallus gallus Retinal g-protein receptor opsin Q69FK2
retinochrome/rgr/ peropsin
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c-opsin
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Papilio glaucus Rhodopsin Q9UAN0
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Drosophila melanogaster GH14208p Q9VTU7
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Trichoplax adhaerens Putative uncharacterized protein B3RY65
placopsin
0.4

2

Figure S1a. Phylogenetic analyses on metazoan opsins.

Maximum likelihood phylogenetic analysis of the *Antalis entalis* opsin proteins (red). NCBI accession numbers are provided for each sequence (accession number for *aen-xenopsin*: MK934770). Tree is based upon that presented in Ramirez et al. 2016. Percent bootstrap values over 70 are displayed, and the scale bar indicates the branch length for 0.4 amino acid substitutions. *Trichoplax* opsin proteins (placopsins) are included as an outgroup.

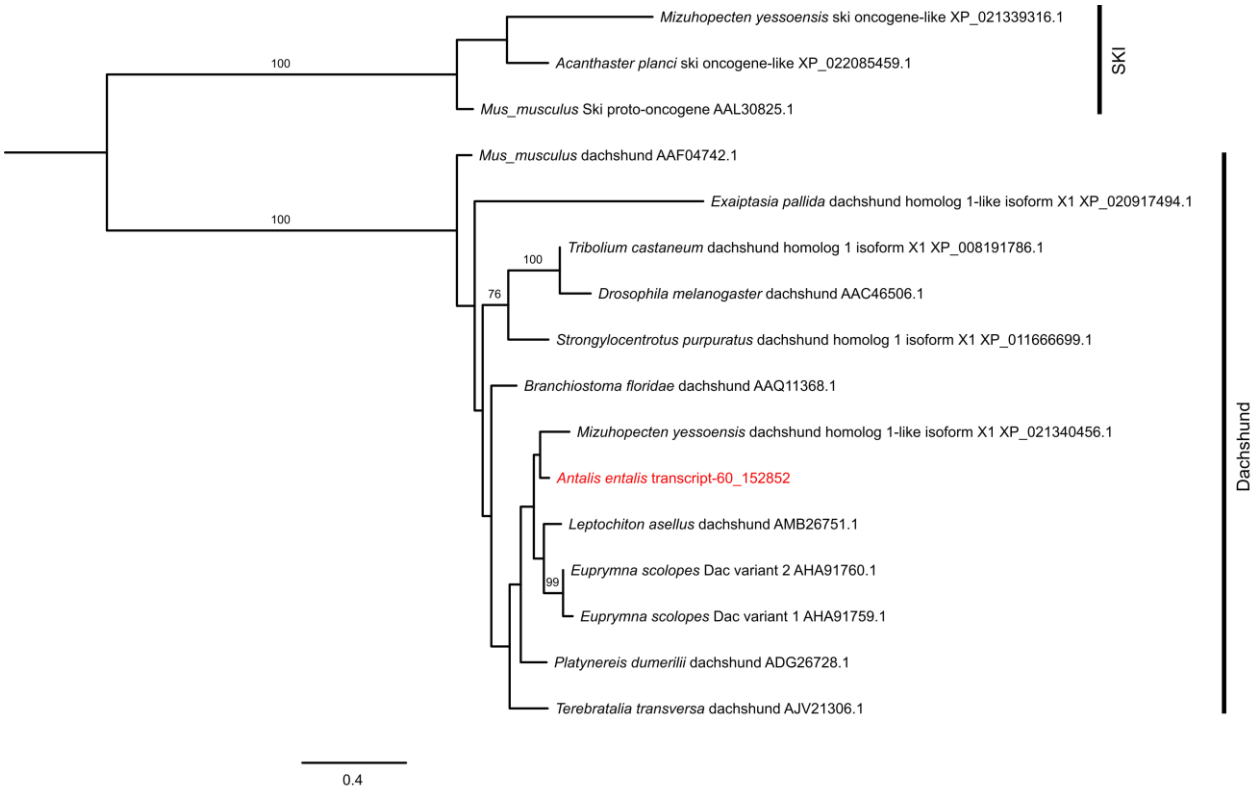


Figure S1b. Maximum likelihood phylogenetic analysis of the *Antalis entalis* Dachshund protein (red). NCBI accession numbers are provided for each sequence. Percent bootstrap values over 70 are displayed, and the scale bar indicates the branch length for 0.4 amino acid substitutions. Related ski proteins are included as an outgroup.

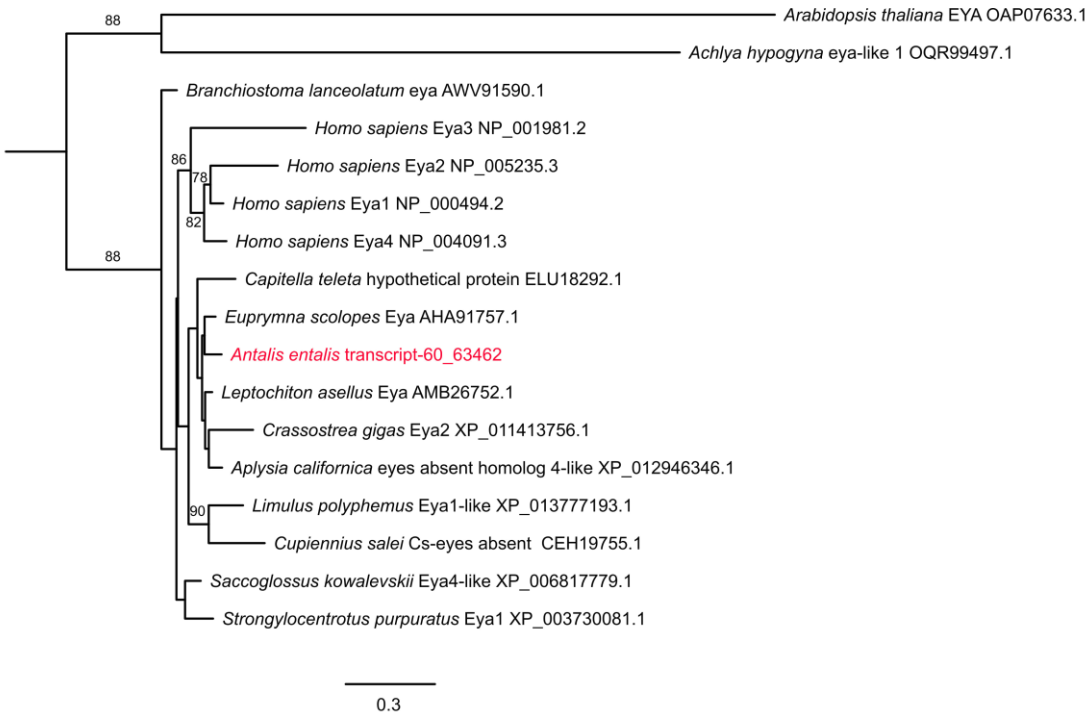


Figure S1c. Maximum likelihood phylogenetic analysis of the *Antalis entalis* Eya protein (red). NCBI accession numbers are provided for each sequence. Percent bootstrap values over 70 are displayed, and the scale bar indicates the branch length for 0.3 amino acid substitutions. Non-metazoan Eya sequences are included as an outgroup.

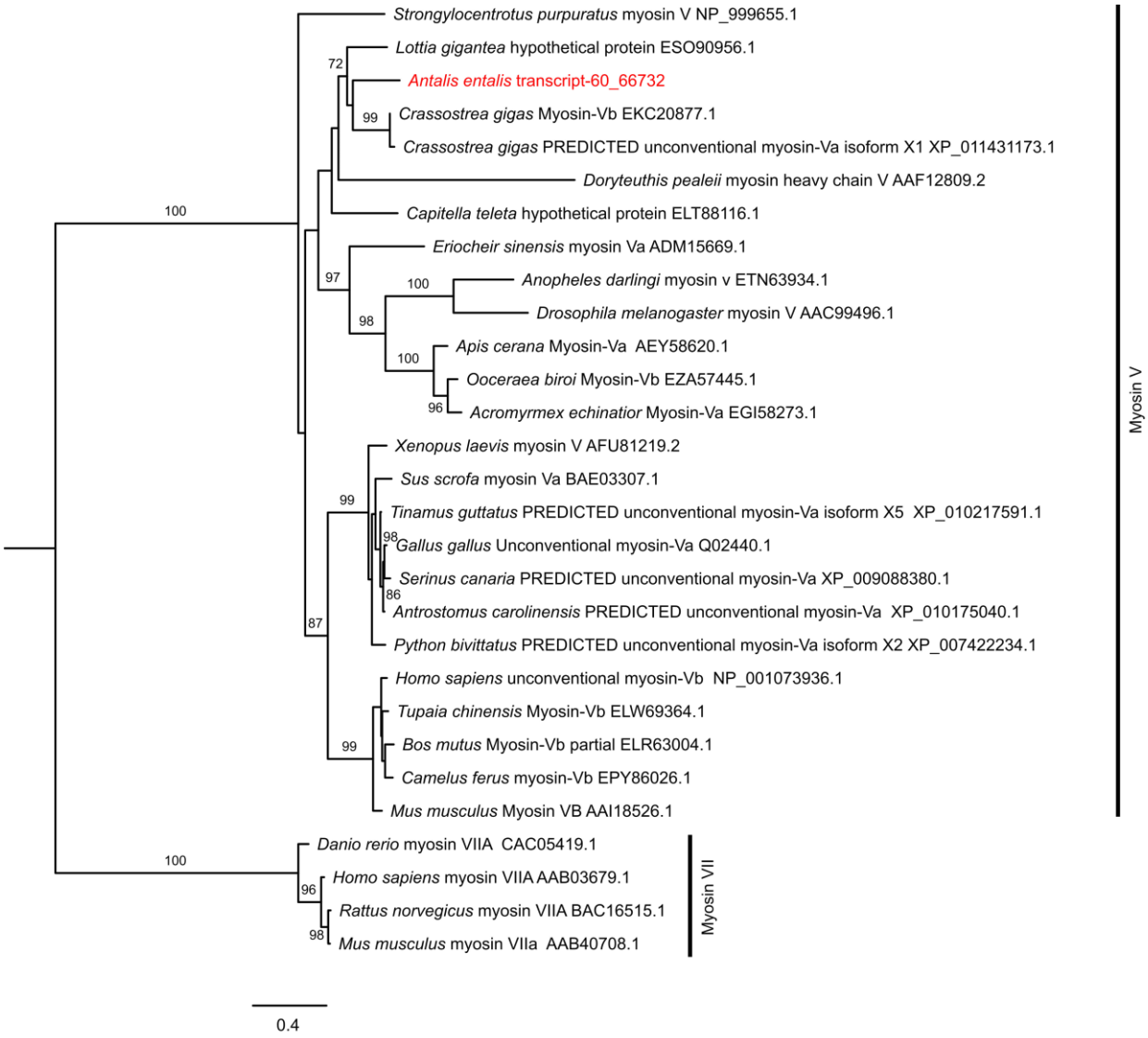
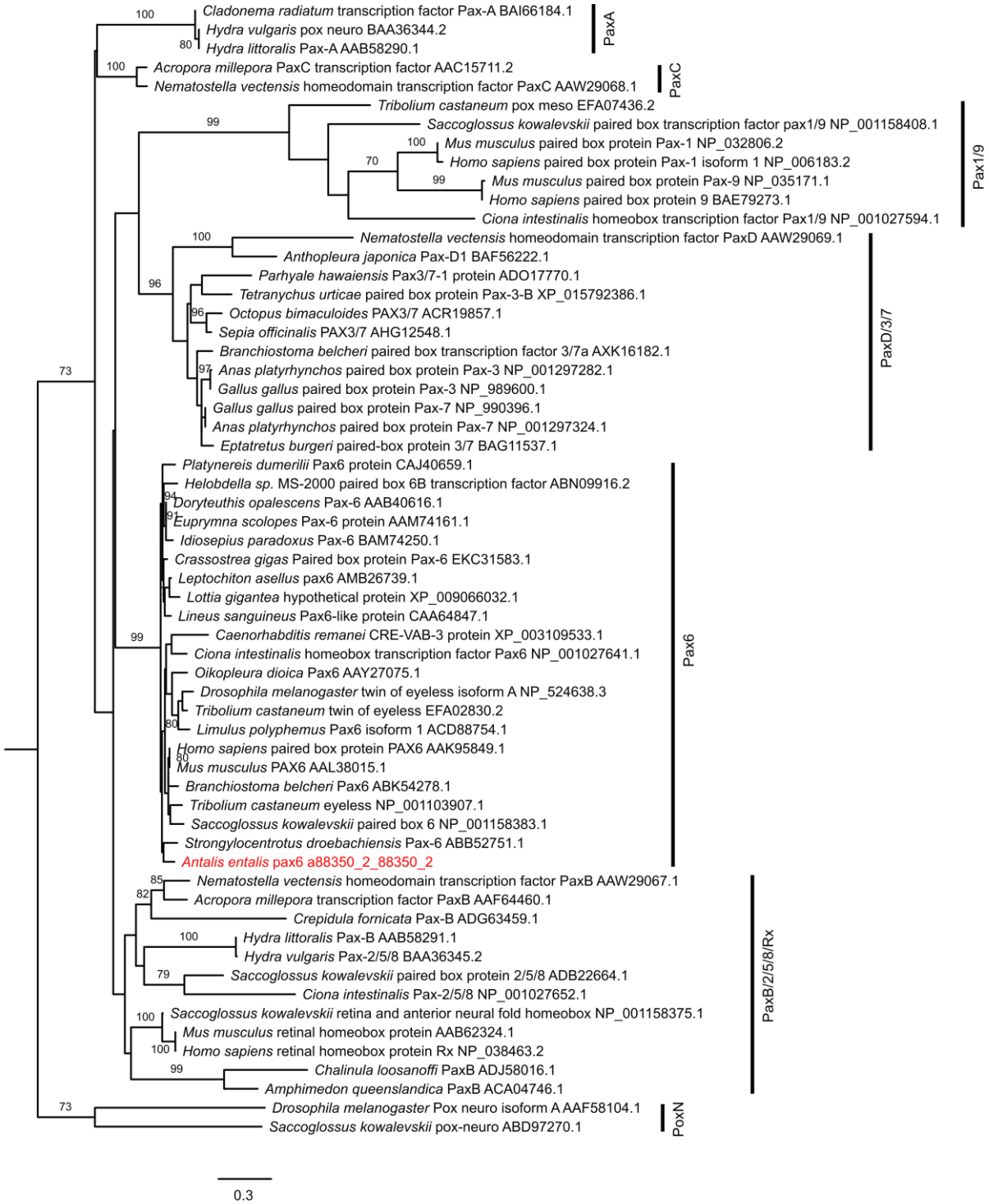


Figure S1d. Maximum likelihood phylogenetic analysis of the *Antalis entalis* Myosin V protein (red). NCBI accession numbers are provided for each sequence. Tree is based upon that presented in Vöcking et al. (2015). Percent bootstrap values over 70 are displayed, and the scale bar indicates the branch length for 0.4 amino acid substitutions. Myosin VII proteins are included as an outgroup.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Nematostella vectensis homeodomain transcription factor PaxC AAW29068.1
PaxC
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Homo sapiens paired box protein Pax-1 isoform 1 NP_006183.2
Mus musculus paired box protein Pax-9 NP_035171.1
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Ciona intestinalis homeobox transcription factor Pax1/9 NP_001027594.1
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Nematostella vectensis homeodomain transcription factor PaxD AAW29069.1
Anthopleura japonica Pax-D1 BAF56222.1
Parhyale hawaiensis Pax3/7-1 protein ADO17770.1
Tetranychus urticae paired box protein Pax-3-B XP_015792386.1
Octopus bimaculoides PAX3/7 ACR19857.1
Sepia officinalis PAX3/7 AHG12548.1
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PaxD/3/7
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Idiosepius paradoxus Pax-6 BAM74250.1
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Oikopleura dioica Pax6 AAY27075.1
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PoxN
0.3

Figure S1e. Maximum likelihood phylogenetic analysis of the *Antalis entalis* Pax6 protein (red). NCBI accession numbers are provided for each sequence (accession number for *aen-pax6*: MK934769). Tree is based upon that presented in Vöcking et al. (2015). Percent bootstrap values over 70 are displayed, and the scale bar indicates the branch length for 0.3 amino acid substitutions. Pox neuro proteins are included as an outgroup.

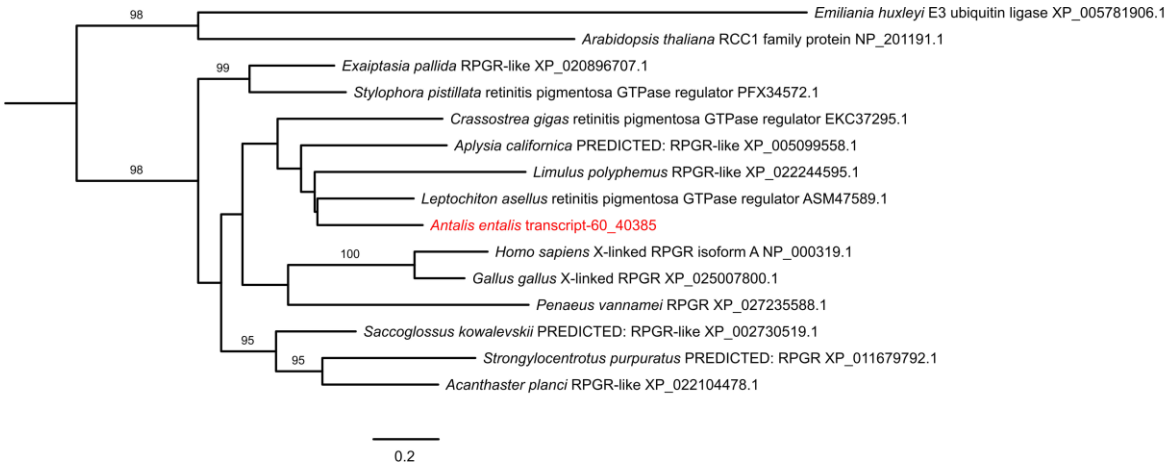

Figure 1f. Maximum likelihood phylogenetic analysis of the *Antalis entalis* Rpgr protein (red). NCBI accession numbers are provided for each sequence. Percent bootstrap values over 70 are displayed, and the scale bar indicates the branch length for 0.2 amino acid substitutions. Related RCC1-containing proteins are included as an outgroup.

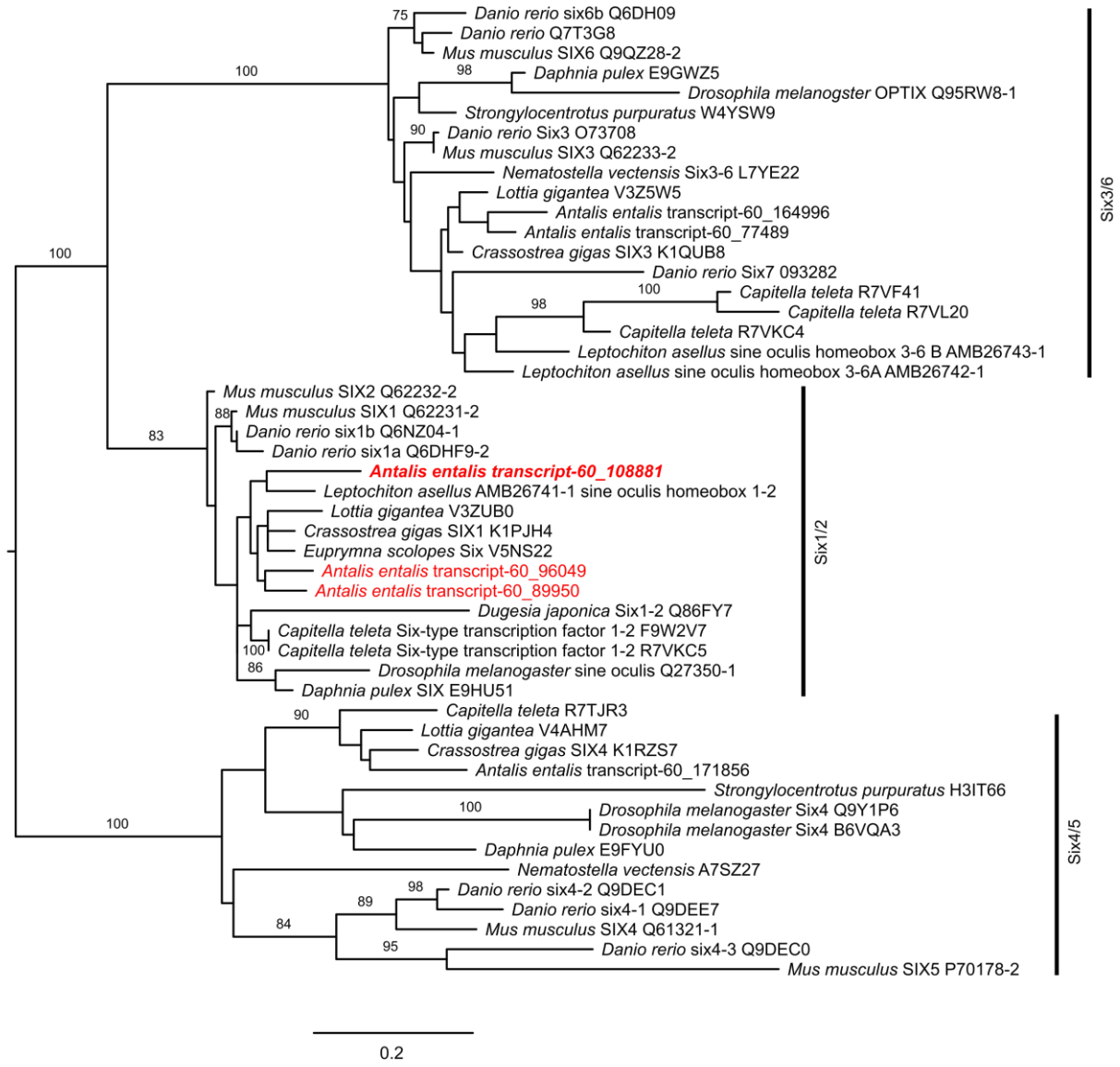

Figure S1g. Maximum likelihood phylogenetic analysis of the *Antalis entalis* Six1/2 proteins (red). Three transcripts encode proteins that fall within the Six1/2 clade, the sequence with the highest similarity to *L. asellus* Six1/2 is bold. NCBI or UniProt accession numbers are provided for all sequences. Tree is based upon that presented in Vöcking et al. (2015). Percent bootstrap values over 70 are displayed, and the scale bar indicates the branch length for 0.2 amino acid substitutions. Midpoint rooting.

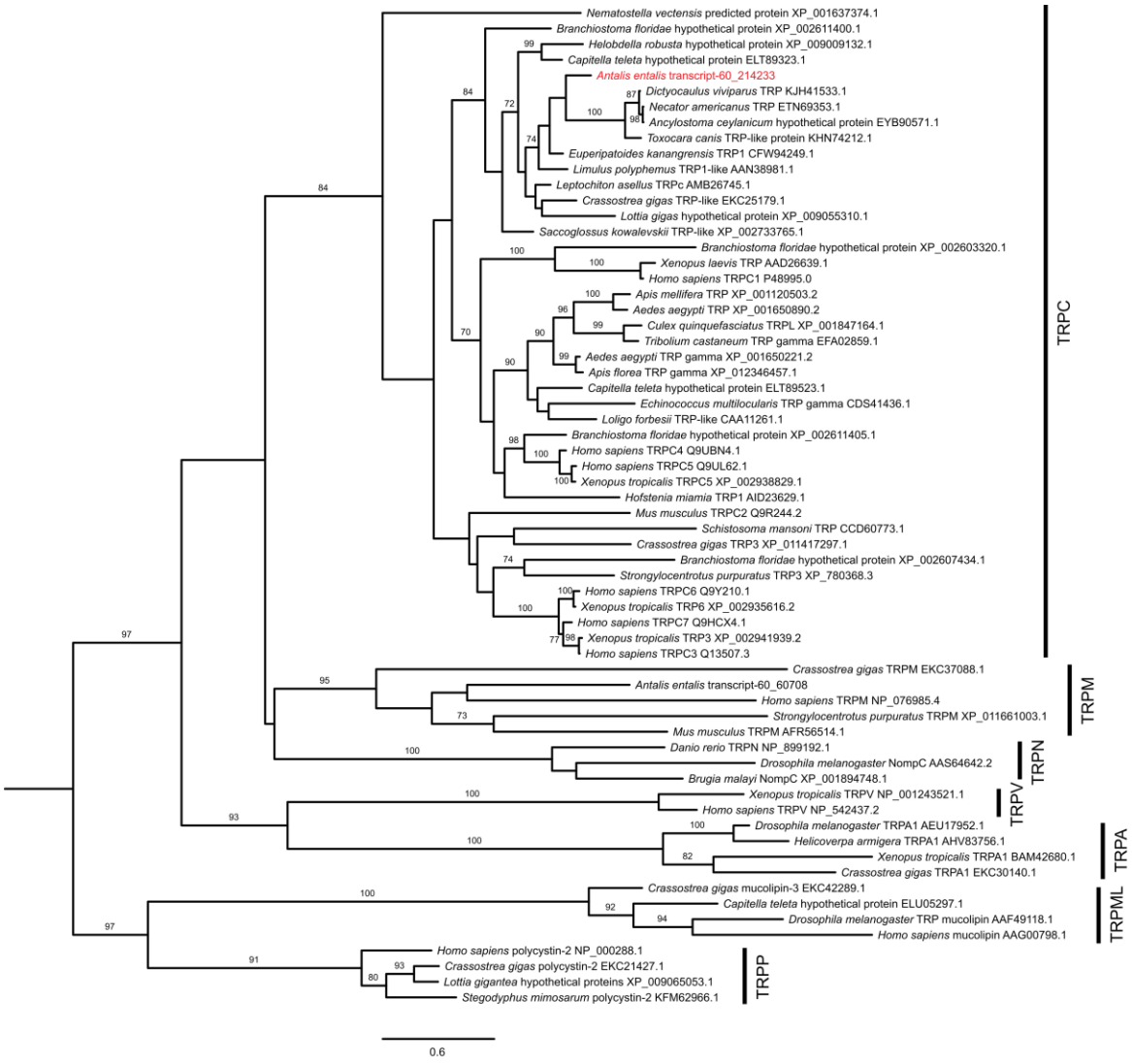

Figure S1h. Maximum likelihood phylogenetic analysis of the *Antalis entalis* TRPC protein (red). NCBI accession numbers are provided for each sequence. Tree is based upon that represented in Vöcking et al. (2015). Percent bootstrap values over 70 are displayed, and the scale care indicates the branch length for 0.6 amino acid substitutions. Midpoint rooting.

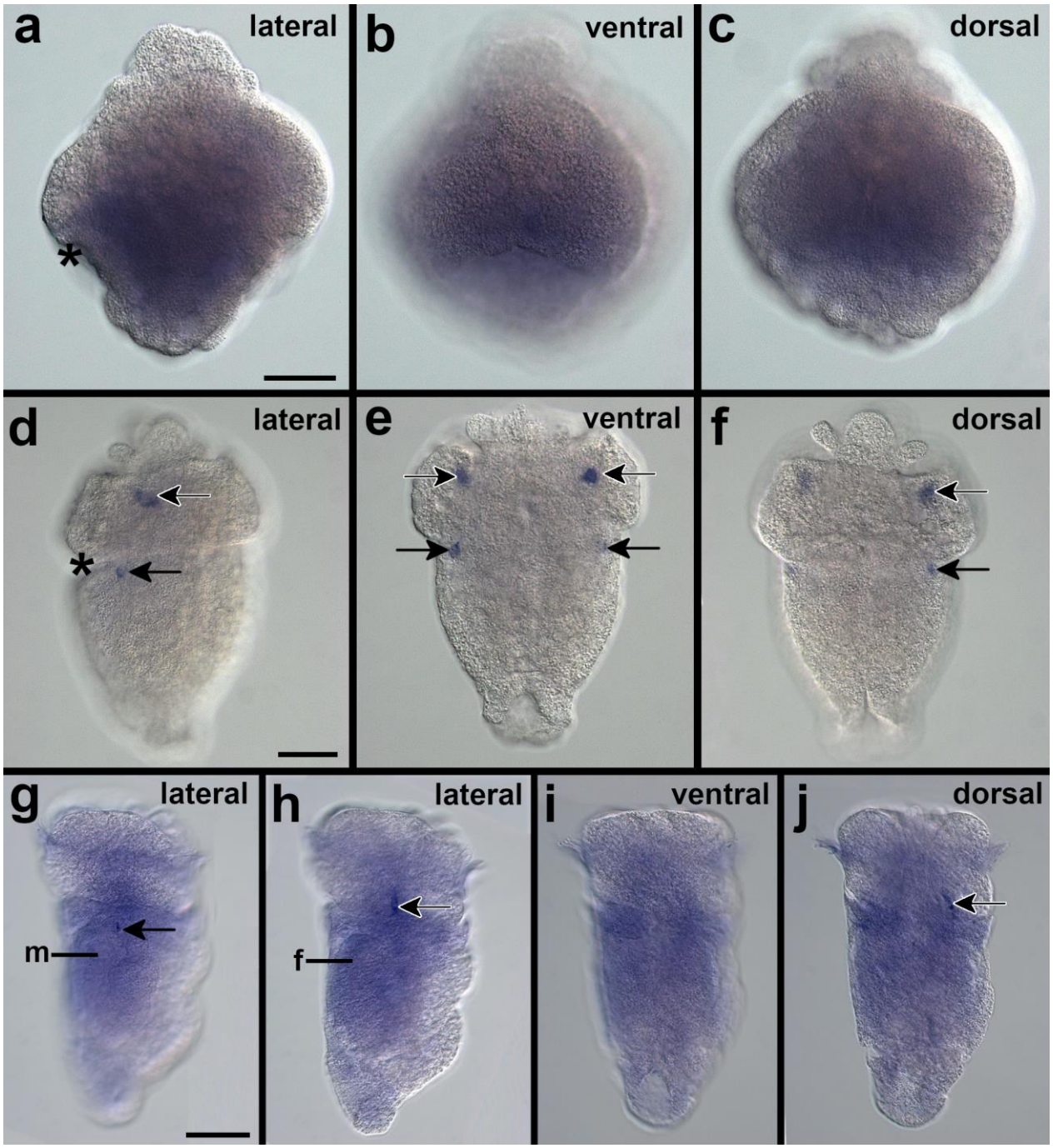

Figure S2. *Go-opsin* expression during the development of the scaphopod *Antalis entalis*. Anterior faces up in all panels and dorsal faces to the right in lateral views. Asterisk labels the mouth. (**a-c)** Early trochophore larvae express *go-opsin* in the region around the mouth. **(d-f)** Early mid-stage trochophores express *go-opsin* in two cells adjacent to the apical organ (white-lined arrows) and two cells in the anterior inner mantle (black arrows). **(g-j)** Latero-distal view

(g), latero-proximal view **(h)**, ventral view **(i)**, and dorsal view **(j)** of mid-stage trochophore larvae that express *go-opsin* in cells migrating to the posterior pole of the trochophore larvae. Both other cells embedded in the antero-lateral mantle margin do still exist (black arrow). Abbreviations: f foot; m mantle, pt prototroch. Scale bars: 50 µm.

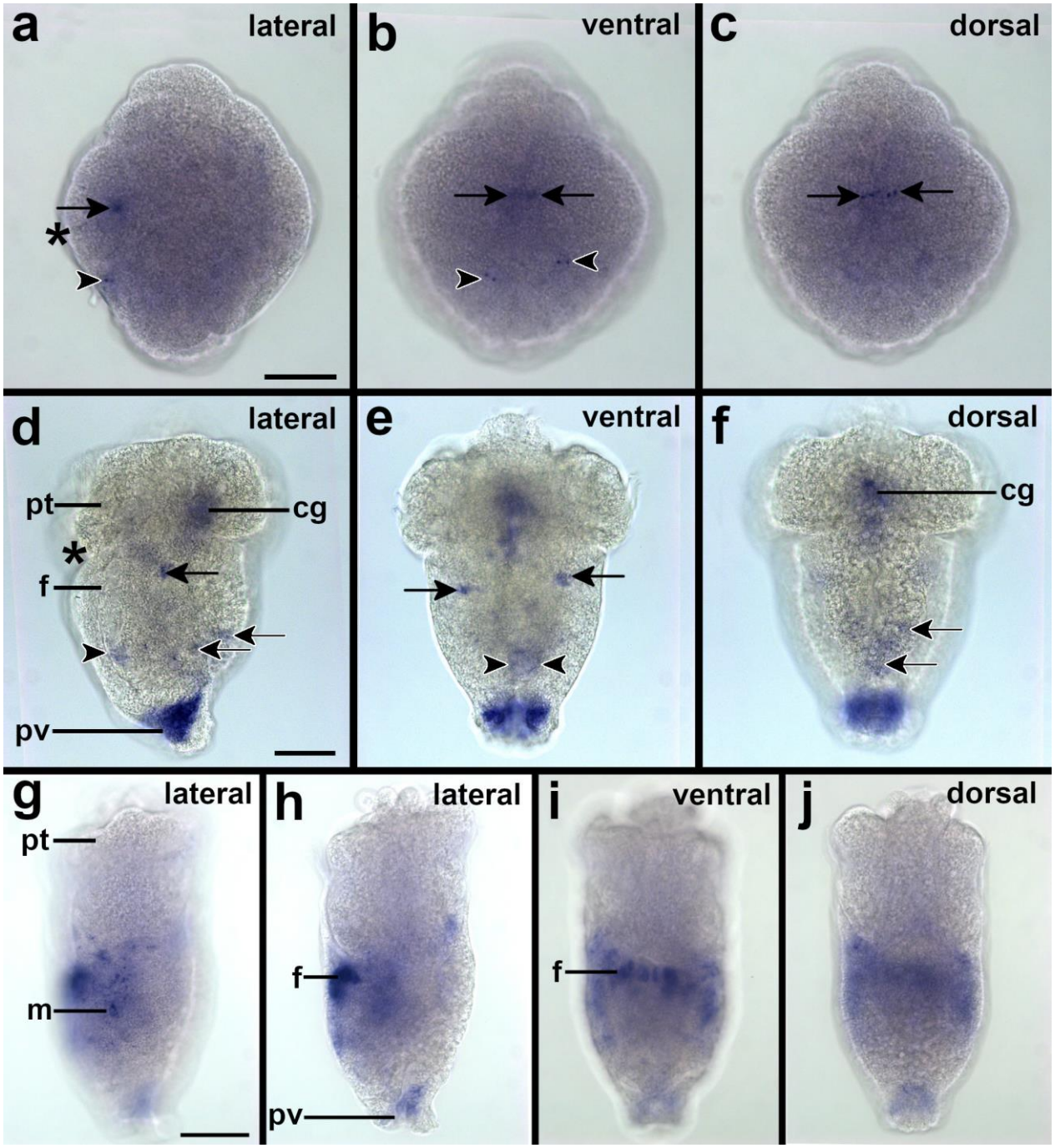

Figure S3. *Dach* expression during the development of the scaphopod *Antalis entalis*. Anterior faces up in all panels and dorsal faces to the right in lateral views. Asterisk labels the mouth. (**a-c)** Early trochophore larvae express *dach* in each two bilateral cells (white-lined arrowheads) embedded in the epidermis posterior to the mouth and in two cells close to the foregut (black arrows). **(d-f)** Early mid-stage trochophores express *dach* in the region of the cerebral ganglia, the pavilion, the antero-lateral and posterior foot, the posterior dorsal mantle region, and the lateral foot. **(g-j)** Latero-distal view **(g)**, latero-proximal view **(h)**, ventral view **(i)**, and dorsal view **(j)** of mid-stage trochophore larvae that express *dach* in cells of the mantle, the foot, and pavilion. Abbreviations: cg cerebral ganglion, f foot, m mantle, pt prototroch, pv pavilion. Scale bars: 50 µm.

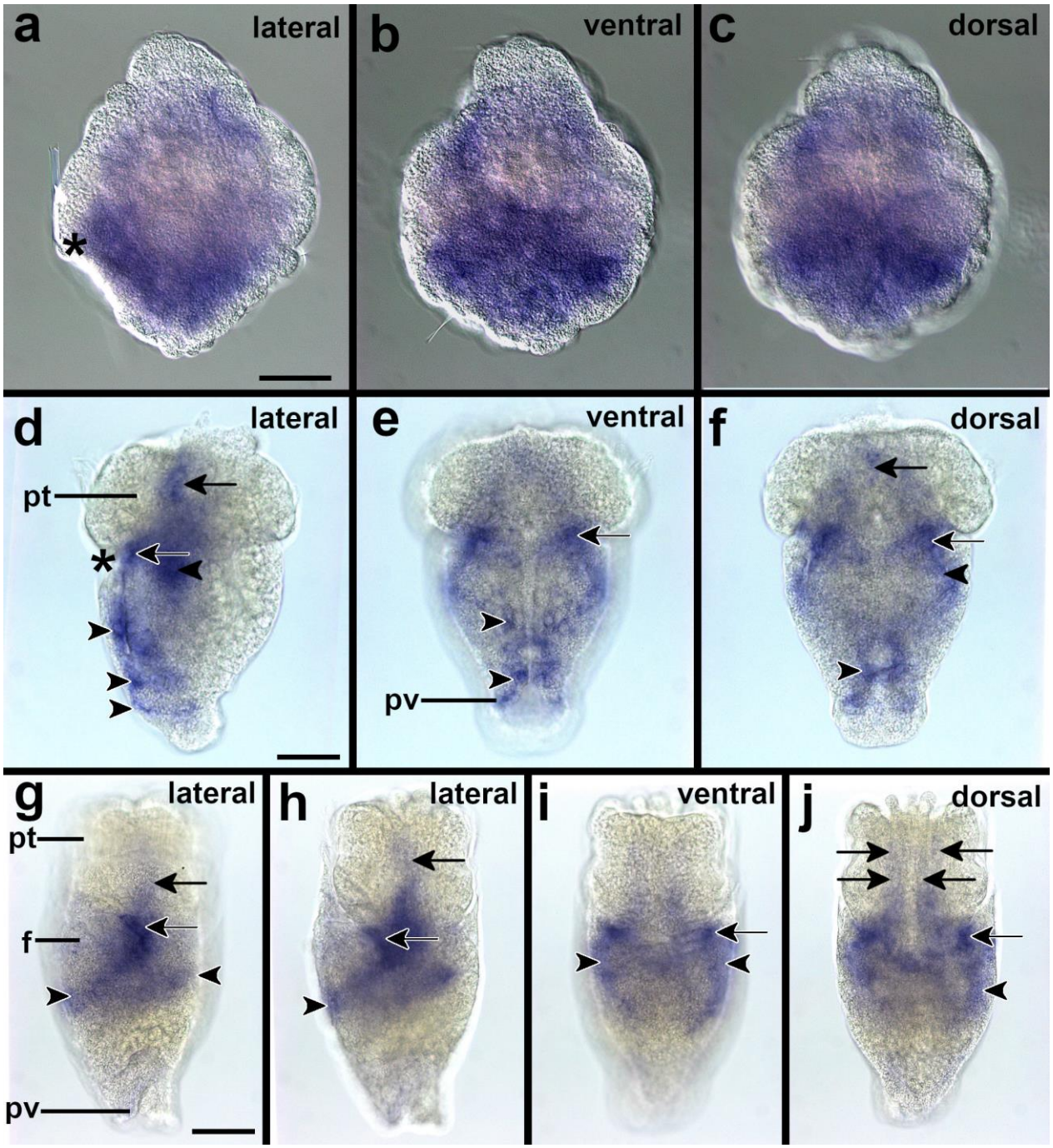

Figure S4. *Eya* expression during the development of the scaphopod *Antalis entalis*. Anterior faces up in all panels and dorsal faces to the right in lateral views. Asterisk labels the mouth. **(a-c)** Early trochophore larvae express *eya* globally in the interior of the larva. **(d-f)** Early mid-stage trochophores express *eya* in cells of the apical organ (arrowheads), in the region connecting the hyposphere with the invaginating episphere and the prototroch (white-lined arrows), and in

individual cells of the ventral mantle (white-lined arrowheads). **(g-j)** Latero-distal view **(g)**, latero-proximal view **(h)**, ventral view **(i)**, and dorsal view **(j)** of mid-stage trochophore larvae that express *eya* in apical organ cells (arrows), the mantle (white-lined arrowheads), the region connecting trunk with episphere and prototroch (white-lined arrows), and the pavilion. Abbreviations: f foot, pt prototroch, pv pavilion. Scale bars: 50 µm.

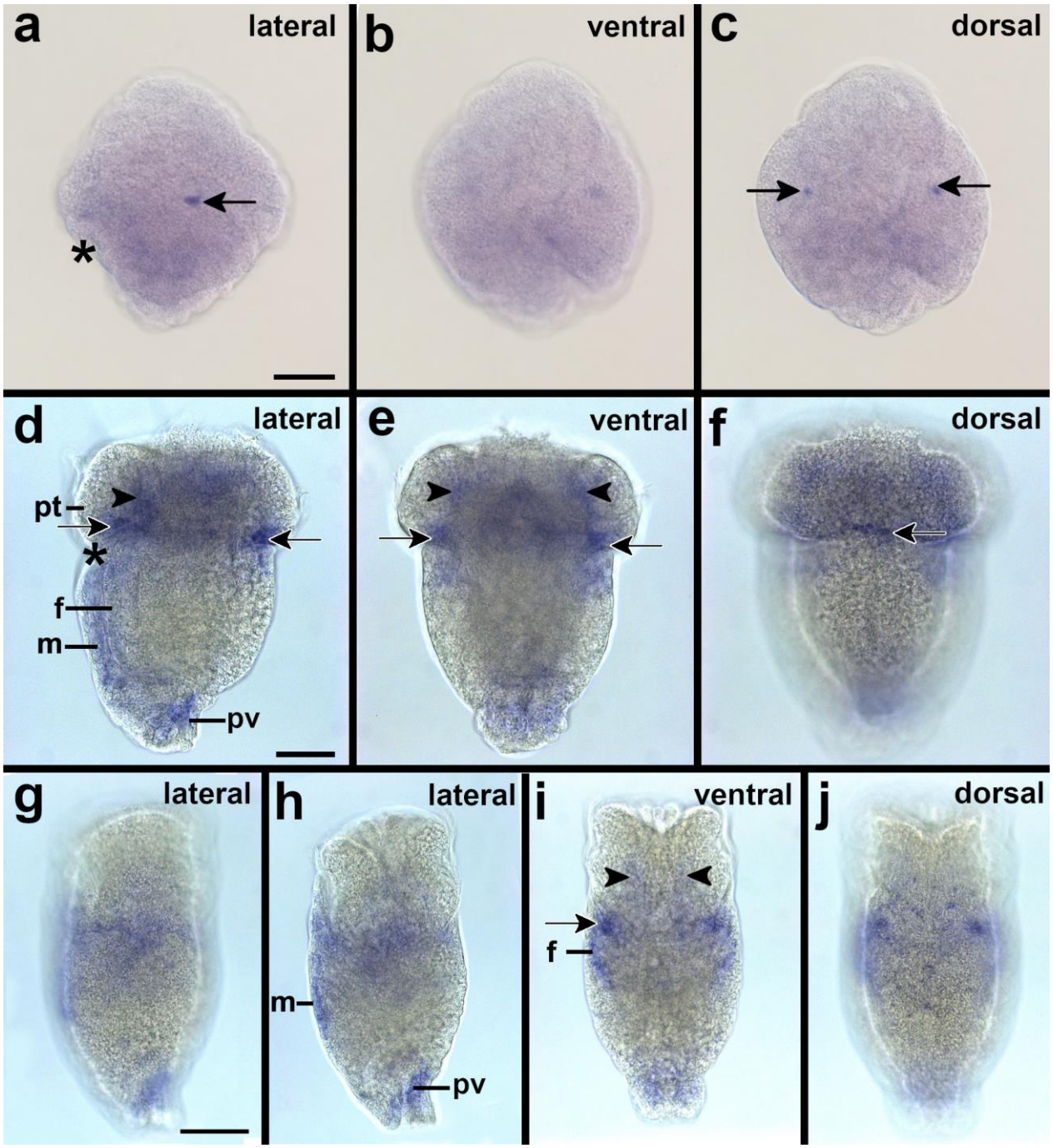

Figure S5. *MyoV* expression during the development of the scaphopod *Antalis entalis*.

Anterior faces up in all panels and dorsal faces to the right in lateral views. Asterisk labels the mouth. (**a-c)** Early trochophore larvae express *myoV*+ in two cells that are located on the level of the prototroch inside the early trochophore larva. **(d-f)** In early mid-stage trochophores *myoV*+ cells line the mantle and the foot and are present in the pavilion. *MyoV* is strongly expressed in the dorsal and ventral anterior mantle margins (white-lined arrows). Several cells of the episphere including the apical organ express *myoV*. *MyoV*+ cells are present in the apical organ (black arrowheads). **(g-j)** Latero-distal view **(g)**, latero-proximal view **(h)**, ventral view **(i)**, and dorsal view **(j)** of mid-stage trochophore larvae that expresses *myoV*+ in cells in similar location as early mid-stage trochophores, however, apical *myoV*+ cells migrate into the interior of the trochophore larvae (s. above). Abbreviations: f foot, m mantle, pt prototroch, pv pavilion. Scale bars: 50 µm.

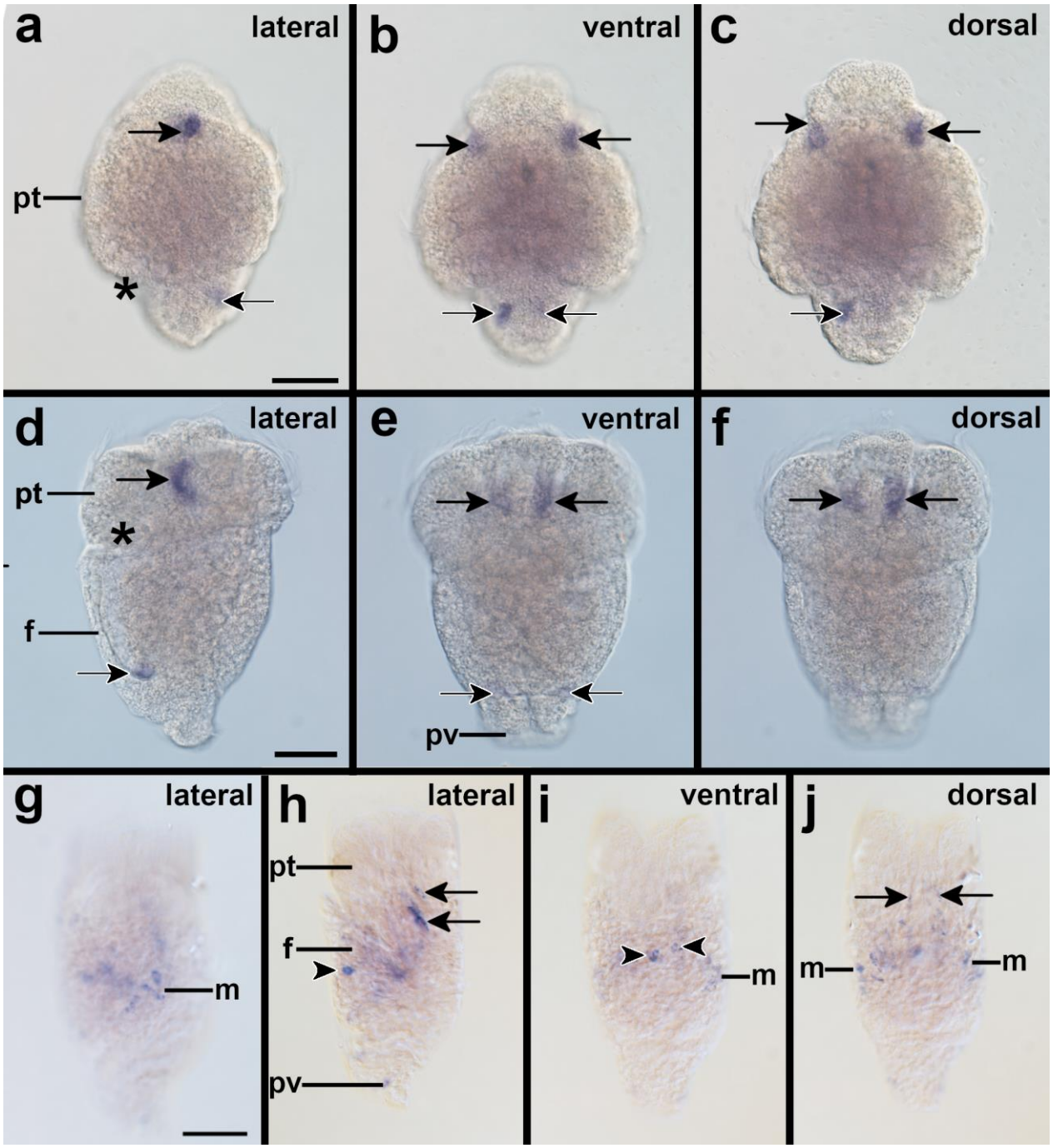

Figure S6. *Pax6* expression during the development of the scaphopod *Antalis entalis*.

Anterior faces up in all panels and dorsal faces to the right in lateral views. Asterisk labels the mouth. (**a-c)** Early trochophore larvae exhibit *pax6*+ cells in the region of the prospective foot (white-lined arrows). Another pair of *pax6*+ cells is situated in the cerebral pits, invaginations that have been documented to give rise to the cerebral ganglia precursors (black arrows). **(d-f)**

Early mid-stage trochophores exhibit two *pax6*+ cells in the posterior foot (white-lined arrows) and each two *pax6*+ flask-shaped cells in the lateral apical organ (arrows). **(g-j)** Latero-distal view **(g)**, latero-proximal view **(h)**, ventral view **(i)**, and dorsal view **(j)** of mid-stage trochophore larvae that expresses several *pax6*+ cells in the mantle, two *pax6*+ cells in the pavilion, two *pax6*+ cells in the anterior foot (white-lined arrowheads), and four *pax6*+ cells in the apical organ. The latter migrate in posterior direction into the trochophore larva (black arrows). Abbreviations: f foot, m mantle, pt prototroch, pv pavilion. Scale bars: 50 µm.

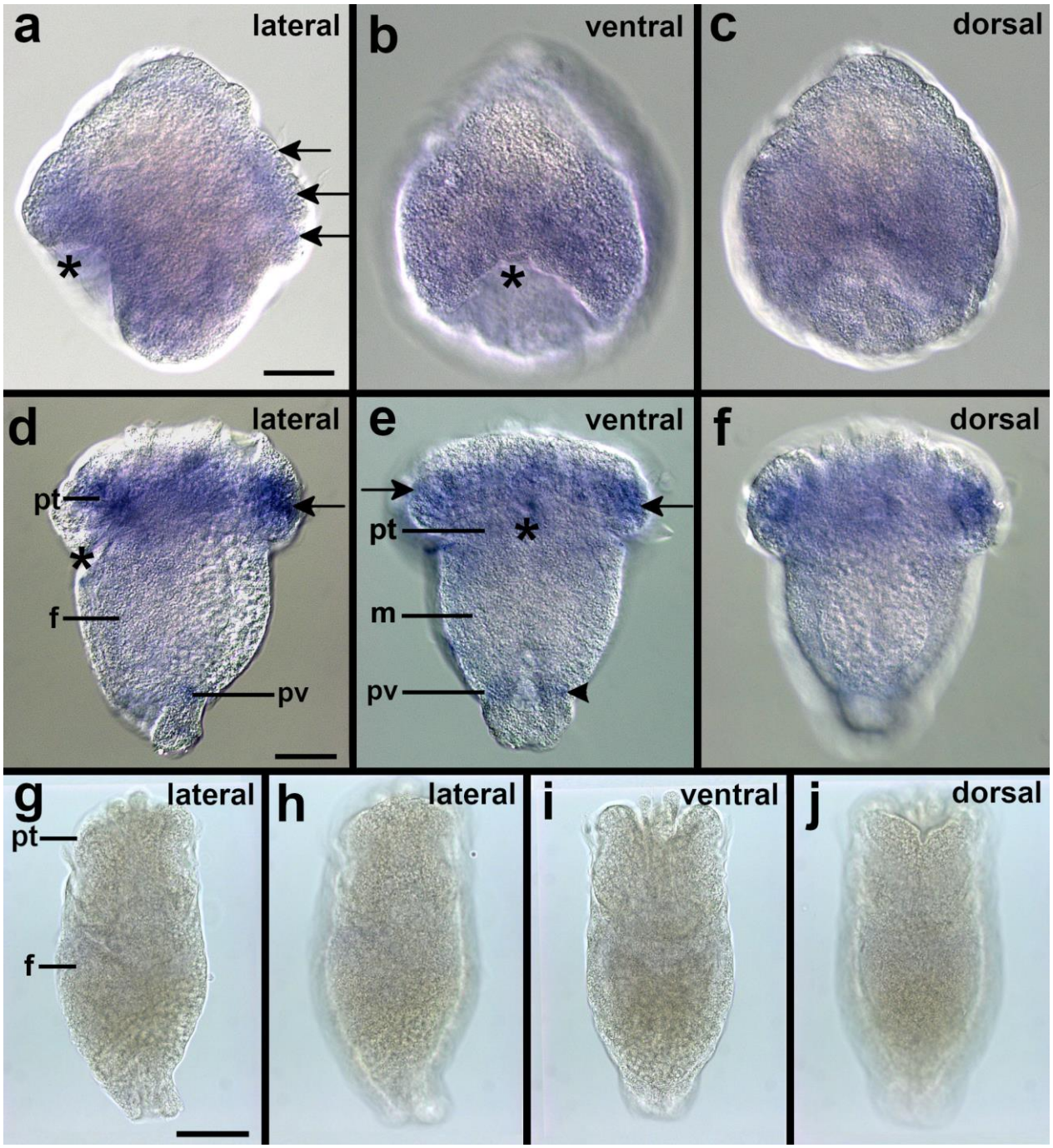

Figure S7. *Rpgr* expression during the development of the scaphopod *Antalis entalis*.

Anterior faces up in all panels and dorsal faces to the right in lateral views. Asterisk labels the mouth. (**a-c)** Early trochophore larvae express *rpgr* in the trochoblasts (arrows). **(d-f)** Early mid-stage trochophores express rpgr in the trochoblasts (arrows) and in the pavilion (arrowhead). **(g-j)** Latero-distal view **(g)**, latero-proximal view **(h)**, ventral view **(i)**, and dorsal view **(j)** of mid-stage

trochophore larvae that possess no *rpgr*+ cells. Abbreviations: f foot, m mantle, pt prototroch, pv pavilion. Scale bars: 50 µm.

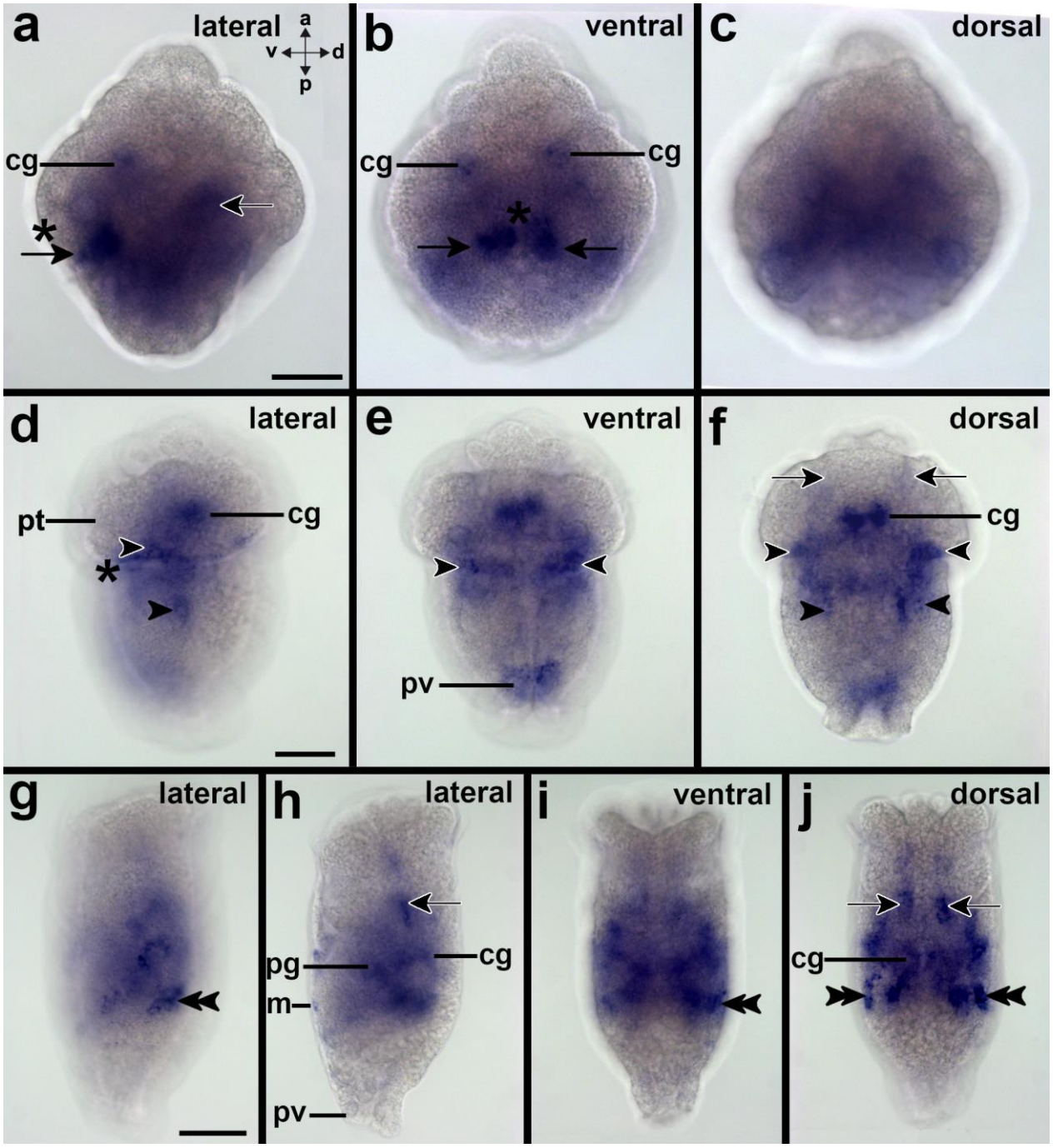

Figure S8. *Six1/2* expression during the development of the scaphopod *Antalis entalis*.

Anterior faces up in all panels and dorsal faces to the right in lateral views. Asterisk labels the

mouth. (**a-c)** Early trochophore larvae express *six1/2* in two domains (black arrows) located in the region of the prospective foot. Additional expression is found in the tissue around the foregut in a region that could correspond to the anlagen of the cerebral ganglia and in two other cells that are located more posterior to the latter ones (white-lined arrow). **(d-f)** Early mid-stage trochophores express *six1/2* in the cerebral ganglia, on a low level in two cells in the apical organ (white-lined arrows), in cells in the mantle (black arrowheads), and in the region that connects the hyposphere with the episphere (white-lined arrowheads). **(g-j)** Latero-distal view **(g)**, latero-proximal view **(h)**, ventral view **(i)**, and dorsal view **(j)** of mid-stage trochophore larvae that expresses *six1/2* in two cells of the apical organ (white-lined arrows), in epidermal cells that are located dorso-laterally to the midgut gland (double arrowheads), in individual cells of the ventral mantle (m), and in cells of the prospective central nervous system, i.e. the cerebral ganglia (cg) and the pedal ganglia (pg). Abbreviations: cg cerebral ganglion, m mantle, pg pedal ganglion, pt prototroch, pv pavilion. Scale bars: 50 µm.

Table S1: Ancestral state reconstruction of larval apical and post-trochal photoreceptors suggesting that apical and post-trochal photoreceptors are molluscan synapomorphies.

	Apical photore-ceptors	***Go-opsin/ Xenopsin***	**Post-trochal photoreceptors**	***Go-opsin/ Xenopsin***	**Reference**
Caudofoveata	no evi-dence	Data missing	Data missing	Data miss-ing	Sigwart and Sumner-Rooney 2015
Solenogastres	no evi-dence	Data missing	No evidence	Data miss-ing	Todt and Wanninger 2010
Poly-placophora	Present	*Xenopsin*	present	*xenopsin*	Vöcking et al. 2015
Cephalopoda	no true larval stage (direct development)				
Mono-placophora	Data missing	Data missing	Data missing	Data miss-ing	
Scaphopoda	present	*go-opsin*	present	*go-opsin*	Present study
Gastropoda	present	Data missing	No evidence	Data miss-ing	Nielsen 2004
Bivalvia	present	Data missing	present	Data miss-ing	Nielsen 2004

No evidence: Taxon has been studied histologically and immunochemically but no photoreceptors were found.

References

Nielsen C. Trochophore larvae: cell-lineages, ciliary bands, and body regions. 1. Annelida and Mollusca.J Exp Zool.2004;302B:35-68.

Sigwart JD, Sumner-Rooney. Mollusca: Caudofoveata, Monoplacophora, Polyplacophora, Scaphopoda. In: Structure and evolution of invertebrate nervous systems. 2015. Eds. Schmidt-Rhaesa A, Harzsch S, Purschke G. Oxford University Press, Oxford.

Todt C, Wanninger A. Of test, trochs, shells, and spicules: Development of the basal mollusk *Wirenia argentea* (Solenogastres) and its bearing on the evolution of trochozoan larval key features.Front Zool.2010;7:6.

Vöcking O, Kourtesis I, Hausen H. Posterior eyespots in larval chitons have a molecular identity similar to anterior cerebral eyes in other bilaterians. EvoDevo.2015;6:40.

Sanger-sequenced nucleotide read of *go-opsin*:

TTCNTGTGTCCAATAGCATAGATAATTGGATTGTATGCACTTTGGGCCGTGGCAATTGC

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ATCAACCCGTATGGTATCCAAGCTATCACGTATACGACAATGGTAACCAGTATGATCTT

TGTAGCCCTTTTCCCAATATCTGGNATGTAGCTAACACCATGTCTTGAGTCGGTGATCT

GTGCCTTTGTTTGCGTGATACCATGGGACACTCTGACATGCNNNNTAACAAGGATCTT

CACGTACCAATATATCATAACGGTGAGTGGTAGGAACAATATGAAGATGACAACGCAT

ATTATGTAAGATACAGCTCCCTTGGNATGACCGTGCCAATCAATACTGCATGTCGTGCC

AAATGG

Predicted amino acid sequence (position 296 is in bold):

PFGTTCSIDWHGH?KGAVSYIICVVIFILFLPLTVMIYWYVKILV??HVRVSHGITQTKAQIT

DSRHGVSY?PDIGKRATKIILVTIVVYVIAWIPYGLIFLASAAGGYIPLGLSLVPTAIA**T**AQS

AYNPIIYAIGH

Methods

***Aen* Primer sequences used in the present study**

Primers used to amplify the region that translates into a T296 instead of a K296 residue.

Go-opsin forward primer: GAGGAATGGGGCTGCTCCAT

Go-opsin reverse primer: GACCTTGAACTTCATGTGTCCAATAGCATAG

Primers used to amplify template for riboprobe synthesis:

Go-opsin forward primer: CTATCACGTATACGACAATGGTAAC

Go-opsin reverse primer: CAATAAGTATGTACAGATACGCCGTG

Dach forward primer: ACAACTCATCCGTCTACAGAACTT

Dach reverse primer: CTGATCGTCATCTTCATCCTCTGT

Eya forward primer: ACAGAGGTTGGCATTCGTTGCTG

Eya reverse primer: CATATACATTAGATTTACAGCCCGCTGC

MyoV forward primer: CTTTCCCATTTGATAATCATATCCATGC

MyoV reverse primer: GATGCTCTTGATTTAATTGCTGTTAAAC

Pax6 forward primer: AAGGTCACAAAGATTTGCTACCGGAGGGC

Pax6 reverse primer: CGAAACAGATGAACAGATGCGCATGCGTC

RPGR forward primer: CTCAAATGATTTGATAACTTTTTATGATCTTCATC

RPGR reverse primer: GAGAGGGTAATAATGGTCAGCTTG

Six1/2 forward primer: GACGATGGCAGGGTACATG

Six1/2 reverse primer: GTCTGGTAACTACTAAGAGCTGAC

TrypC forward primer: CTGTACCAGACCAGGACCAATAC

TrypC reverse primer: CATGACAGTGTCTCTGTAAACATCTG

Xenopsin forward primer: GTTCCAGAGAGGGTACTGGAATG

Xenopsin reverse primer: CTTGGACGCCGTATGCAATAG

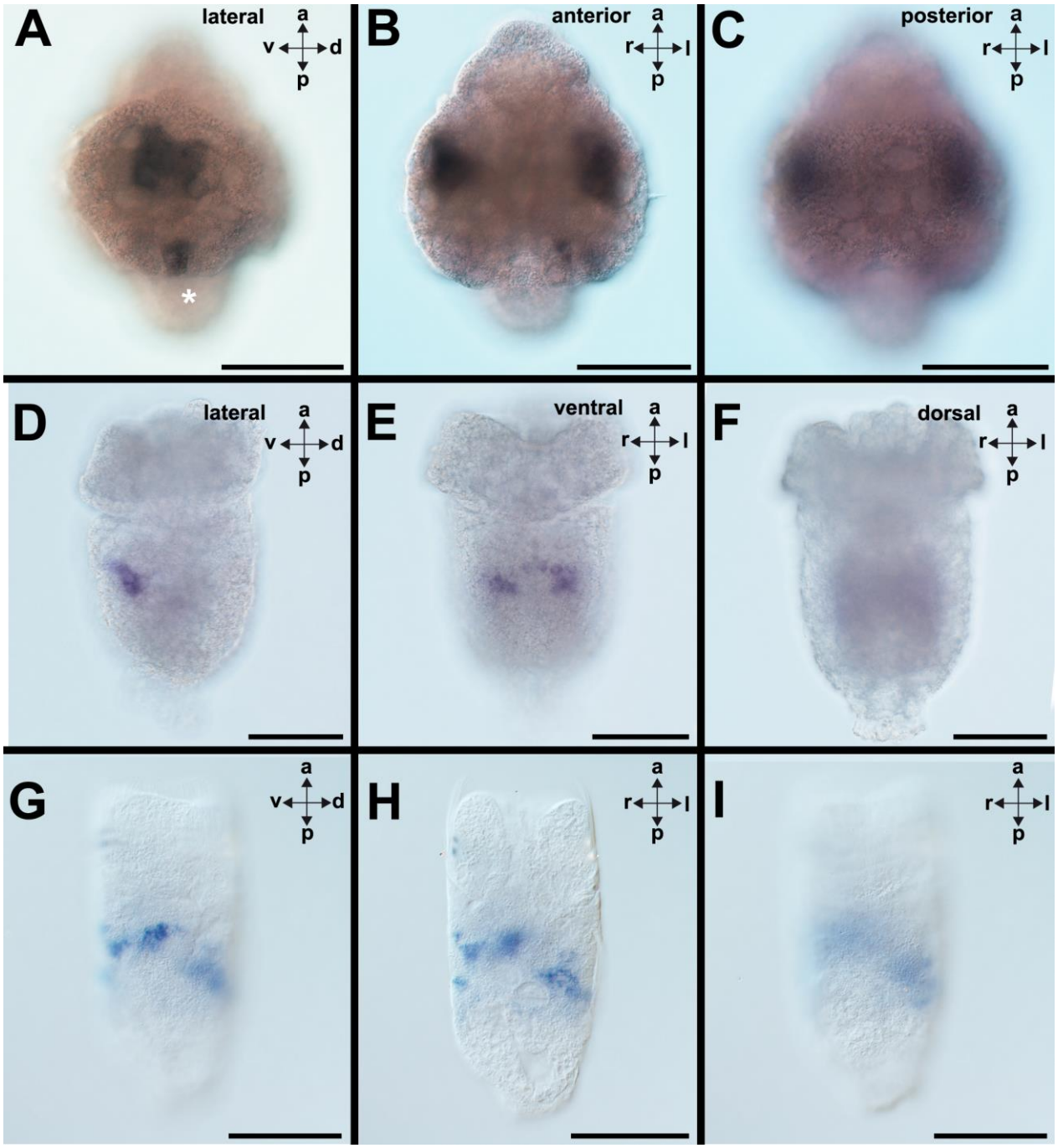

Figure S9. *Hox4* expression during the development of the scaphopod *Antalis entalis* as off-target control for the *in situ* hybridization experiments. Anterior faces up in all panels and dorsal faces to the right in lateral views. **(a-c)** Early trochophore larvae. **(d-f)** Early mid-stage trochophores **(g-j)** Mid-stage trochophores. Scale bars: 100 µm.