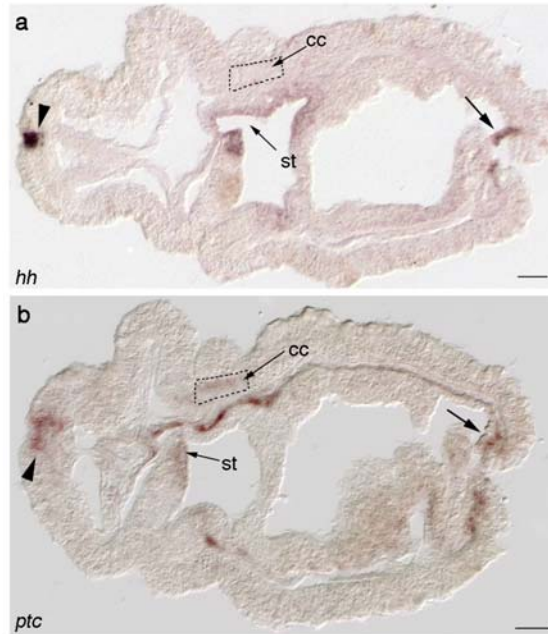


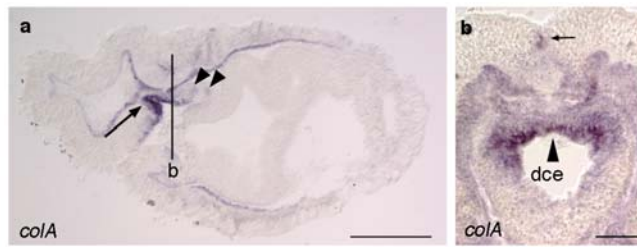
Supplementary Figure S1. *BsimPax6* expression in one-week old juvenile.

(a) *BsimPax6* was expressed in the epidermis of the proboscis stem and the posterior part of the collar. **(b)** A cross section through b in a showing that *BsimPax6* was expressed in the epidermis (arrow), but not in the collar cord. cc, collar cord. Scale bars: a, 200 μ m; b, 50 μ m.



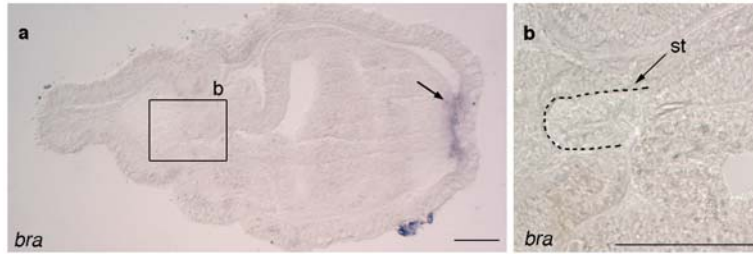
Supplementary Figure S2. *BsimHh* and *BsimPtc* expression in juveniles.

(a) A longitudinal section of 2 day juvenile showing *BsimHh* expression in the stomochord and dorsal endoderm of the buccal tube. In addition, *BsimHh* expression was detected at the anterior tip of the proboscis (arrowhead) and the posterior endoderm (arrow). **(b)** A longitudinal section of 2 day juvenile showing *BsimPtc* expression in the stomochord, collar cord and the collar mesoderm. Expression was also detected at the anterior tip of the proboscis (arrowhead) and the posterior mesoderm (arrow). st, stomochord. Scale bars: 100 μm .



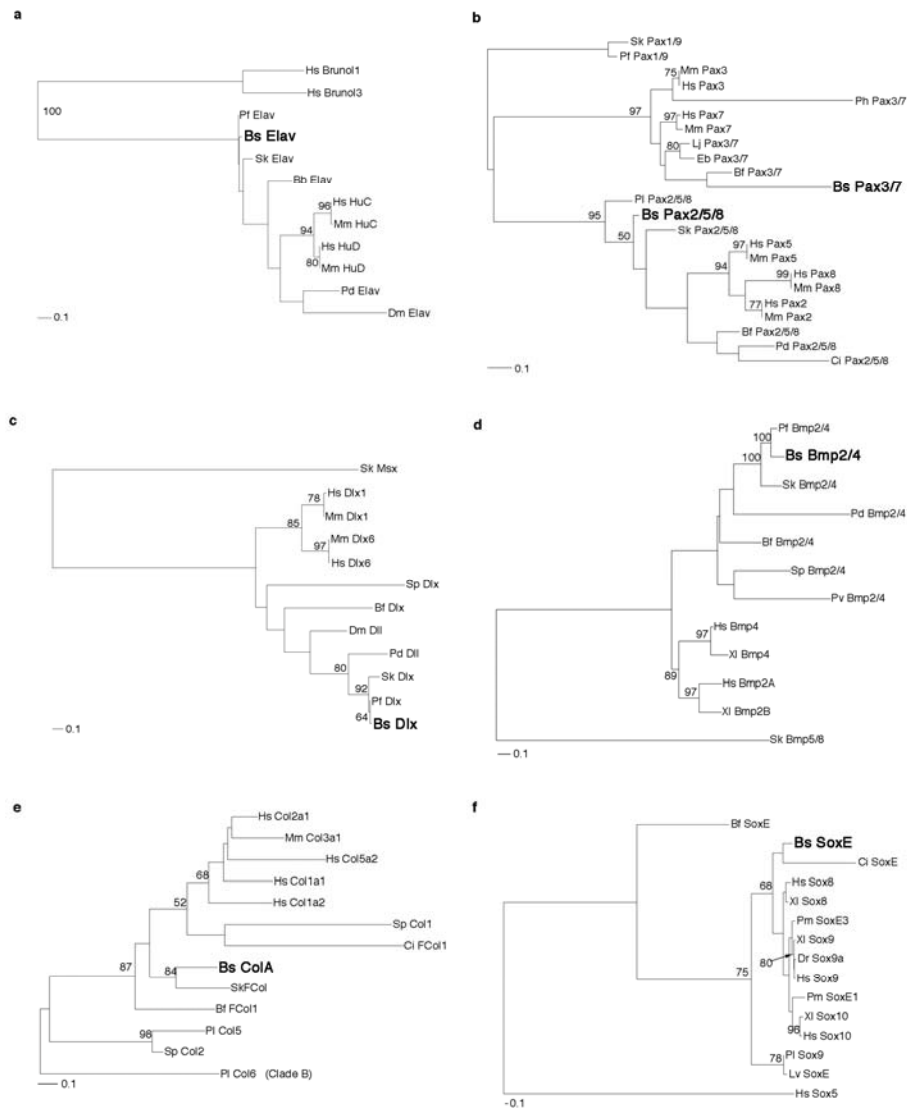
Supplementary Figure S3. *BsimColA* expression during metamorphosis.

(a) A longitudinal section of a 2 days juvenile showing *BsimColA* expression in the stomochord (arrow), gill slits (arrowheads) and mesodermal cells. **(b)** A cross section through b in **a** showing *BsimColA* expression in the dorsal collar endoderm (arrowhead). Arrow indicates the expression in the developing collar cord. dce, dorsal collar endoderm. Scale bars: a, 200 μ m; b, 50 μ m.



Supplementary Figure S4. *BsimBra* expression in juveniles.

(a) A longitudinal section of 2 day juvenile showing *BsimBra* expression in endodermal cells around the anus (arrow). **(b)** The boxed area in A showing no *BsimBra* expression in the stomochord. st, stomochord. Scale bars: 100 μ m.



Supplementary Figure S5. Analysis of gene orthology.

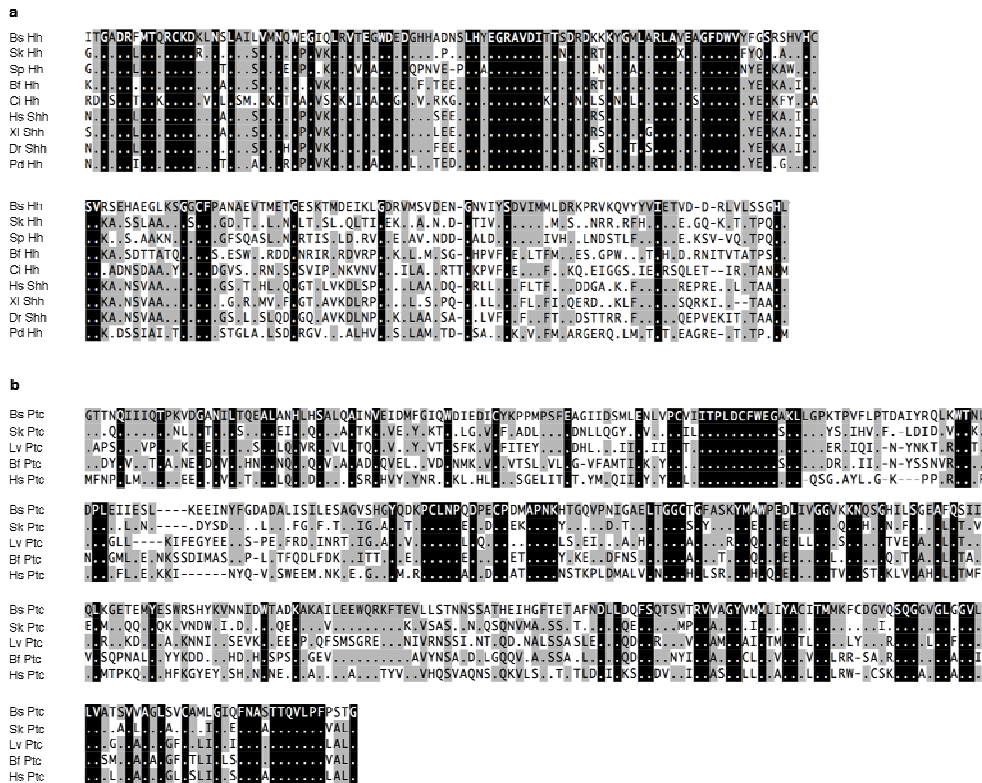
Bb, *Branchiostoma belcheri*; Bf, *Branchiostoma floridae*; Bs, *Balanoglossus simodensis*; Ci, *Ciona intestinalis*; Dm, *Drosophila melanogaster*; Dr, *Danio rerio*; Eb, *Eptatretus burgeri*; Hp, *Hemicentrotus pulcherrimus*; Hs, *Homo sapiens*; Lj, *Lethenteron japonicum*; Lv,

Lytechinus variegatus; Mm, *Mus musculus*; Pd, *Platynereis dumerilii*; Pf, *Ptychodera flava*;

Ph, *Parhyale hawaiiensis*; Pl, *Paracentrotus lividus*; Pm, *Petromyzon marinus*; Pv, *Patella*

vulgata; Sk, *Saccoglossus kowalevskii*; Sp, *Strongylocentrotus purpuratus*; Xl, *Xenopus*

laevis.



Supplementary Figure S6. Alignments of the partial amino acid sequences of Hedgehog and Patched proteins in metazoans.

Bf, *Branchiostoma floridae*; Bs, *Balanoglossus simodensis*; Ci, *Ciona intestinalis*; Dr, *Danio rerio*; Hs, *Homo sapiens*; Lv, *Lytechinus variegatus*; Pd, *Platynereis dumerilii*; Sk, *Saccoglossus kowalevskii*; Sp, *Strongylocentrotus purpuratus*; Xl, *Xenopus laevis*.

Supplementary Table S1. Primers Used for Gene Amplification and Accession

Numbers

<i>colA</i>	Forward	5' AARAAAYCCAGCNAGRCANTGYCGNGAC 3'
(AB642245)	Reverse	5' TTCTTNGTRTCGTATTTCNAANACNGT 3'
<i>elav</i>	Forward	5' AARGCNATNAAYACNCTNAAYGGTTTA 3'
(AB642246)	Nested Forward	5' GGTTTACGNTTACAAGCCAARAC 3'
	Reverse	5' CATGGTNACAAANCCNAAGCCTTTACA 3'
<i>hedgehog</i>	Forward	5' ACNNGNGCNGAYMGNYTNATGAC 3'
(AB642247)	Reverse	5' ACCCARTCRAANCCNGCYTC 3'
	3' RACE1	5' TCACTACATTACGAGGGTCGTGCAG 3'
	3' RACE2	5' TTACGAGGGTCGTGCAGTGGACATTACC 3'
<i>patched</i>	Forward	5' AAYGTNCCNAARTGYTGGGARGT 3'
(AB642248)	Reverse	5' ATNGTNACRTTNGCYTCRCANAC 3'
<i>soxE</i>	Forward	5' AARGGNTAYGAYTGGACNYTNGTNCC 3'
(AB642249)	Nested Forward	5' CCNATGAAYGCNTTYATGGTNGG 3'
	Reverse	5' TTNGGNGGNARRTAYTGNTCRAAYCT 3'

<i>pax3/7</i>	Forward	5' GGNGGNGTNTTYATHAAYGG 3'
(AB642250)	Reverse	5' GTRTADATRTCNGGRTARTGNGT 3'
<i>dlx</i>	Forward	5' TAATTGTCCGCTGATGTCKTTAATGACA 3'
(AB642251)	Reverse	5' ATTGTCTTGRTGATACCAGGAATA 3'
<i>brachyury</i>	Forward	5' CGCCGTKCAGASCGAGATGAACGACTG 3'
(AB642252)	Reverse	5' AGGTGGCGTSAGGGGGYTCCA 3'
<i>bmp2/4</i>	Forward	5' GAGTCCAGYCTSYTSAGTATGTTTGG 3'
(AB842169)	Reverse	5' AAGGCATCTTTGYGGTTTAGGCACAAG 3'
<i>pax2/5/8</i>	Forward	5' TCAATGGTCGCCCGTTAC 3'
(AB842170)	Reverse	5' GTTTGGTCCCGGGTGGTAT 3'
<i>pax6</i>	Forward	5' GGNCAYWSNGGNGTNAAYCARYTNGG 3'
(AB842171)	Nested Forward	5' GGNGCNMGNCNTGYGAYAT 3'
	Reverse	5' TTYTCYTCNCKNCKCCAYTTNGC 3'
	Nested Reverse	5' AANACRTCNGGRTARTGNGT 3'