



Figure 5 | Dorsoventral patterning in Rotifera and Annelida.

a, Transcription factor *nkx2.1* paralogues, *nkx2.2*, and *pax6* show brain domains (arrowheads); *nkx6* is detected posteriorly (arrowheads). **b**, *E. senta* CNS (green arrowhead indicates the brain; red arrowheads indicate the VNCs, and additional neurites). **c**, Transcription factor *nkx2.2* shows gut expression (arrowhead); *nkx6* is in the ventral midline (arrowheads); *pax6* is in two lateral larval bands (arrowheads), and juvenile head; *pax3/7* is in two ventrolateral larval clusters and midline

(arrowheads), but in two trunk clusters in juveniles (arrowheads); *msx* paralogues are in ventral larval domains and the juvenile VNC (arrowheads). **d**, *O. fusiformis* CNS (green arrowheads indicate the apical larval FMRF-amide cell and juvenile brain; red arrowheads indicate the larval anterior axon and juvenile medial VNC). Abbreviations: ao, apical organ; br, brain; cb, ciliary band; cg, caudal ganglion; ch, chaetae; ln, lateral neurites; mo, mouth; ms, mastax; np, neuropile; vg, vesicle ganglia; vnc, ventral nerve cord. Scale bars, 50 μ m.

in the brachiopod *Novocrania anomala*. In this brachiopod, *nkx2.1* (ref. 30) and *pax6* (ref. 31) are expressed in the apical lobe, and *nkx2.2* and *nkx6* are expressed medially in the trunk (Fig. 3E). As in *T. transversa*, *nkx6* extends more laterally at the anterior trunk, where it co-localizes with *pax3/7* in the early larva, and *msx* is broadly detected in the trunk (Fig. 3E and Extended Data Fig. 7e). Therefore, *N. anomala* also has a medial ventral *nkx2.2*⁺/*nkx6*⁺ domain; remarkably, however, this domain does not co-localize with any serotonergic condensation, which is lacking in the larval CNS of this brachiopod (Fig. 3F). Therefore, the conserved staggered expression of the dorsoventral transcription factors in the anteroventral larval trunk is not necessarily connected to the CNS, suggesting that this system may rather pattern only the ectoderm in Brachiopoda.

Dorsoventral patterning in Nemertea

Similar to brachiopods, some dorsoventral transcription factors show staggered expression along the trunk ventral side of the nemertean *Lineus ruber*. In this worm, dorsoventral transcription factors are first detected in the larval imaginal discs (Extended Data Fig. 8a). In metamorphic and definitive juveniles, *nkx2.1* is expressed in the

head and proboscis, and *pax3/7* is broadly expressed (Fig. 4a and Extended Data Fig. 8a). However, *nkx2.2*, *nkx6*, and *pax6* are detected in isolated ventrolateral cells, as well as in cephalic domains (*nkx2.2*, *nkx6*, *pax6*) and isolated trunk cells (*nkx2.2*) (Fig. 4a and Extended Data Fig. 8a). Remarkably, *nkx2.2* and *nkx6* do not co-localize, but *nkx6* and *pax6* do (Fig. 4b). These staggered domains relate to the disposition of the VNCs of *L. ruber* (Fig. 4c). Furthermore, *nkx2.2*⁺ cells co-express the serotonergic marker *tph*, and *nkx6*⁺ cells express the motor neuron marker *Hb9*, but not *VAcHT* (Fig. 4a, b). Therefore, the staggered expression of the dorsoventral transcription factors *nkx2.2*, *nkx6*, and *pax6* are linked to the ventral trunk CNS and some neuronal cell type markers in *L. ruber*, which is similar to the situation described in vertebrates and *P. dumerilii*^{4,9,12,32}.

Dorsoventral patterning in Rotifera

To explore the conservation of the dorsoventral patterning in Spiralia, we studied the rotifer *Epiphanes senta*, a member of the sister lineage to all remaining Spiralia²⁹. Different from the brachiopod larvae and the nemertean juvenile, *E. senta* juveniles lack a staggered expression of dorsoventral transcription factors along their trunks. The three