Head patterning and Ho*x* gene expression in an onychophoran and its implications for the arthropod head problem

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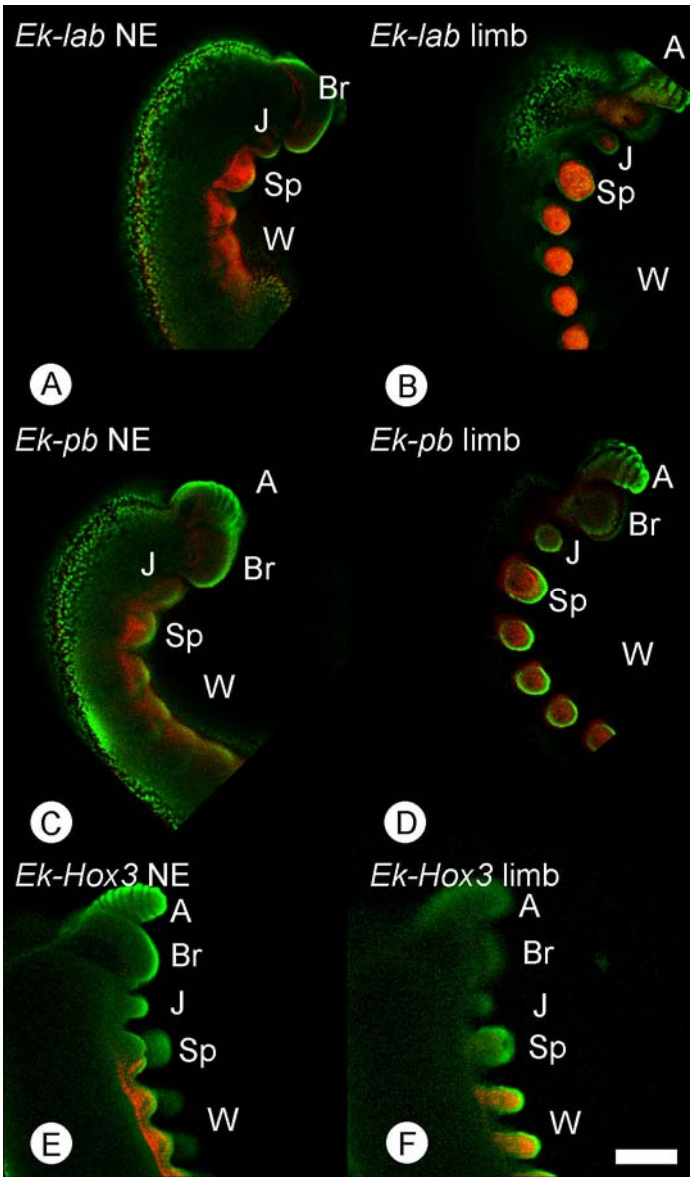
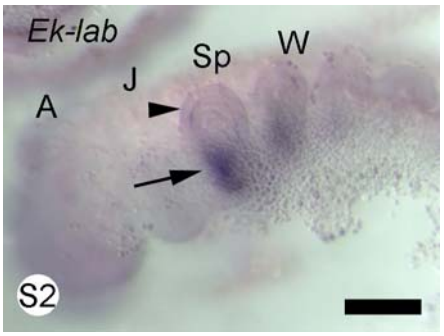
Development Genes and evolution, supplementary data

Supplementary figures

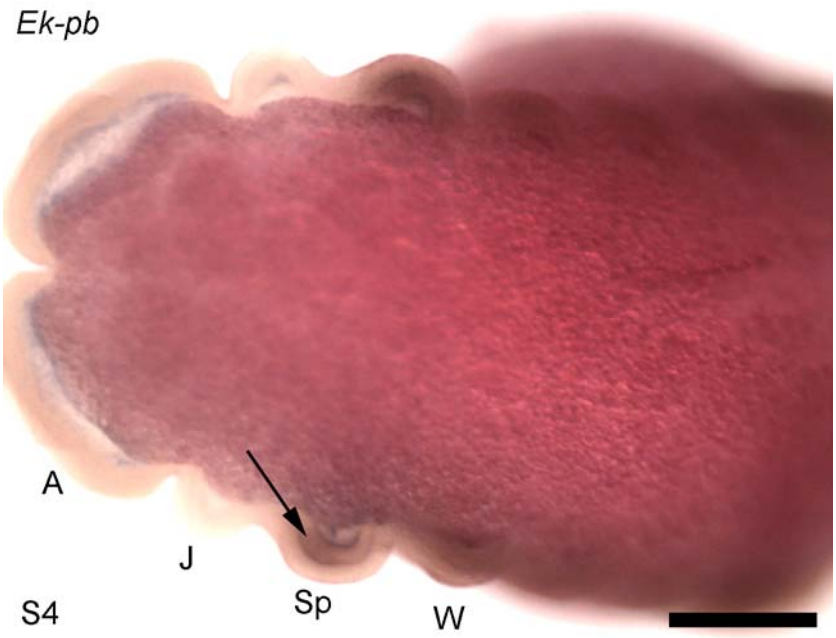


S. 1 Optical sections from CLSM showing embryos of *Euperipatoides kanangrensis* at stage V stained for: *lab*, *pb* and *Hox3*. Lateral view, anterior is up. A, Section through the neuroectoderm showing *lab* expression in the mesoderm beneath as well as the basal and apical ectoderm of segments from the slime papilla (Sp) and continuing posteriorly. B, Same embryo as in A with a section through the limbs showing expression of *lab* in the mesoderm as well as basal and apical ectoderm of segments from the slime papilla and continuing posteriorly. C, Section through the neuroectoderm showing expression of *pb* in the mesoderm and basal ectoderm but lacking in the apical ectoderm of segments from the slime papilla and continuing posteriorly. D, Same embryo as in C with a section through the limbs showing expression of *pb* in the mesoderm and basal ectoderm but lacking in the apical ectodermal layer of segments from the slime papilla and continuing posteriorly. E, section through the neuroectoderm showing expression of *Hox3* in the mesoderm and basal ectoderm of the posterior half of the slime papilla segment and continuing posteriorly. F, Same embryo as in C with a section through the limbs showing expression of *Hox3* in the interior starting with the first walking (W) leg and continuing posteriorly. A = antenna, Br = brain rudiment, J = jaw, scale bar = 500 µm.

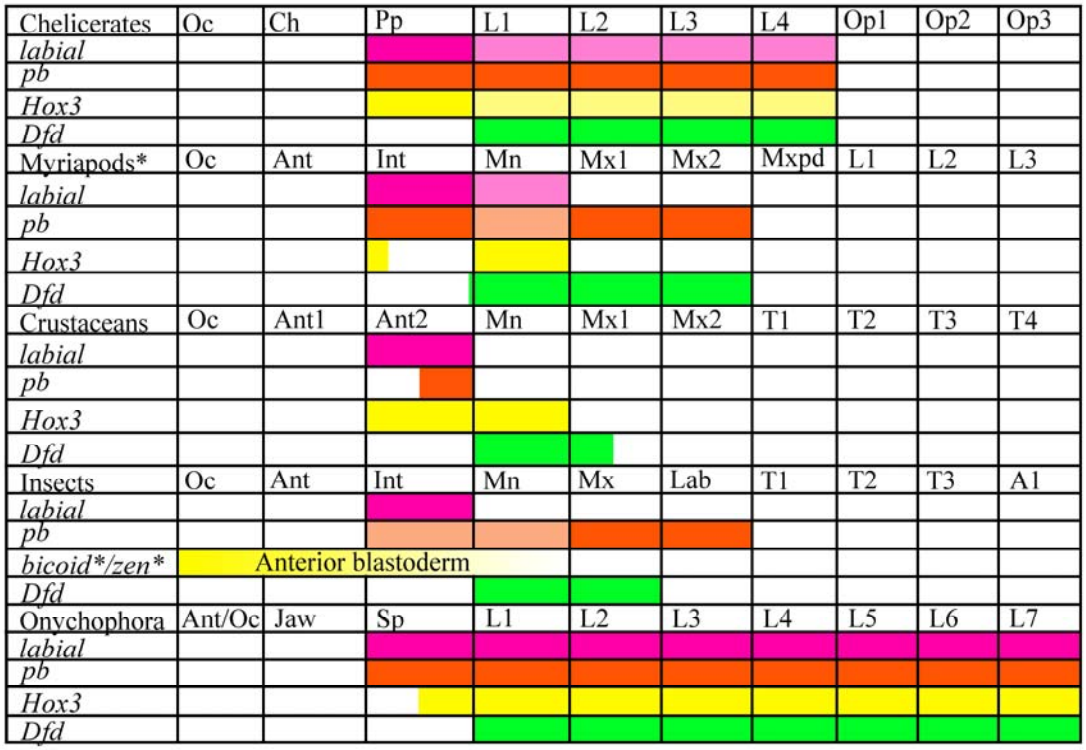


S. 2 The anterior of a whole mount of *Euperipatoides kanangrensis* embryo at stage III stained for the presence of *lab* mRNA. Lateral view, anterior is to the left. Arrow points at the neuroectoderm of the slime papilla segment (Sp) and the arrowhead indicates the expression in the limb. A = antenna, W = walking leg, scale bar = 200 µm.

S. 3 Whole mount of *Euperipatoides kanangrensis* embryo at stage V stained for the presence of *lab* mRNA. Lateral view, anterior is to the left. The mRNA of *lab* can be seen from the slime papilla (Sp) segment and posteriorly to the proctodeum (P). A = antenna, J = jaw, scale bar = 500 µm.



S. 4 Whole mount of *Euperipatoides kanangrensis* embryo at stage III stained for the presence of *pb* mRNA. Dorsal view, anterior is to the left. The expression has its anterior border in the slime papilla segment (sp) and the arrow indicates its presence in the mesoderm of the limb bud. A = antenna, J = jaw, scale bar = 200 µm.



S. 5 Scheme of the expression of the anterior *Hox* genes *lab* (purple), *pb* (orange), *Hox3* (yellow) and *Dfd* (green) in relation to segment borders in different arthropod groups and onychophorans. The anterior expression borders of the four anterior *Hox* genes are conserved between onychophorans and arthropods suggesting that the head segments can be aligned from anterior to posterior, see main text. The star at Myriapods indicates that a more recent investigation in myriapods show a slightly different expression pattern, however, the anterior expression border is the same (Janssen and Damen 2006). The star at bicoid/zen indicates that these genes have gained different functions in insects. For some of the original data on the expression of *Hox* genes and other genes relevant for the question of head segmentation in arthropods see: Abzhanov A, Kaufman T C (1999); Damen et al. (1998); Haas et al. (2001); Hughes and Kaufman (2002); Jager et al. (2006); Kimm and Prpic (2006); Posnien et al. (2009); Rogers and Kaufman (1996); Rogers et al. (2002); Schmidt-Ott and Technau (1992); Schmidt-Ott et al. (1994); Telford and Thomas (1998).

Suplementary material no. 6

The orthology assignment of the anterior Hox genes was based on phylogenetic analysis. The phylogenetic analyses were carried out using amino acid sequences. We compiled a Hox gene alignment including anterior Hox genes of representatives of bilaterians. The species names and their abbreviations used in phylogenetic analysis are provided in Table S 1. Sequences were aligned using the program ClustalX v.2.0.10. First the homeobox region was aligned, and then using the homeobox as anchor, the flanking regions were aligned with subsequent trimming carried out manually. Bayesian likelihood analyses were conducted using MrBayes version 3.1.1 with four simultaneous Markov chains per run and two independent runs per analysis. Chains were run for one million generations with a sampling frequency of 1000 generations and burnin of 1000. The clear classification of the *E. kanangrensis Hox* genes into

their orthology groups is apparent from phylogenetic analyses (S 6). Although our phylogenetic analysis clearly assigns the *E. kanangrensis Hox* genes to their paralog class with high support values, the internal grouping remains unclear.

Table S. 1 List of the species used in phylogenetic analysis

Abbreviation	Species name	Common name	phylum or class
Bfl	*Branchiostoma floridae*	amphioxus	Cephalochordata
Btu	*Bugula turrita*	bryozoan	Bryozoa
Cel	*Caenorhabditis elegans*	nematode	Nematoda
Cin	*Ciona intestinalis*	tunicate	Tunicata
Dja	*Dugesia japonica*	flatworm	Platyhelminthes
Dme	*Drosophila melanogaster*	fruit fly	Insecta
Esc	*Euprymna scolopes*	squid	Cephalopoda
Eka	*Euperipatoides kanangrensis*	velvet worm	Onychophora
Fen	*Flaccisagitta enflata*	arrow worm	Chaetognatha
Lan	*Lingula anatina*	brachiopod	Brachiopoda
Lat	*Lithobius atkinsoni*	centiped	Myriapoda
Lgi	*Lottia gigantea*	limpet	Gastropoda
Lsa	*Lineus sanguineus*	ribbon worms	Nemertea
Mmu	*Mus musculus*	house mouse	Vertebrata
Ngr	*Nymphon gracile*	sea spider	Pycnogonida
Nvi	*Nereis virens*	segmented worm	Polychaeta
Pca	*Priapulus caudatus*	Priapulid	Priapulida
Pdu	*Platynereis dumerilii*	segmented worms	Polychaeta
Sca	*Sacculina carcini*	crustacean	Cirripedia
Sko	*Saccoglossus kowalevskii]*	hemichordate	Hemichordata
Sro	*Symsagittifera roscoffensis*	flatworm	Acoelomorpha
Tca	*Tribolium castaneum*	beetle	Insecta

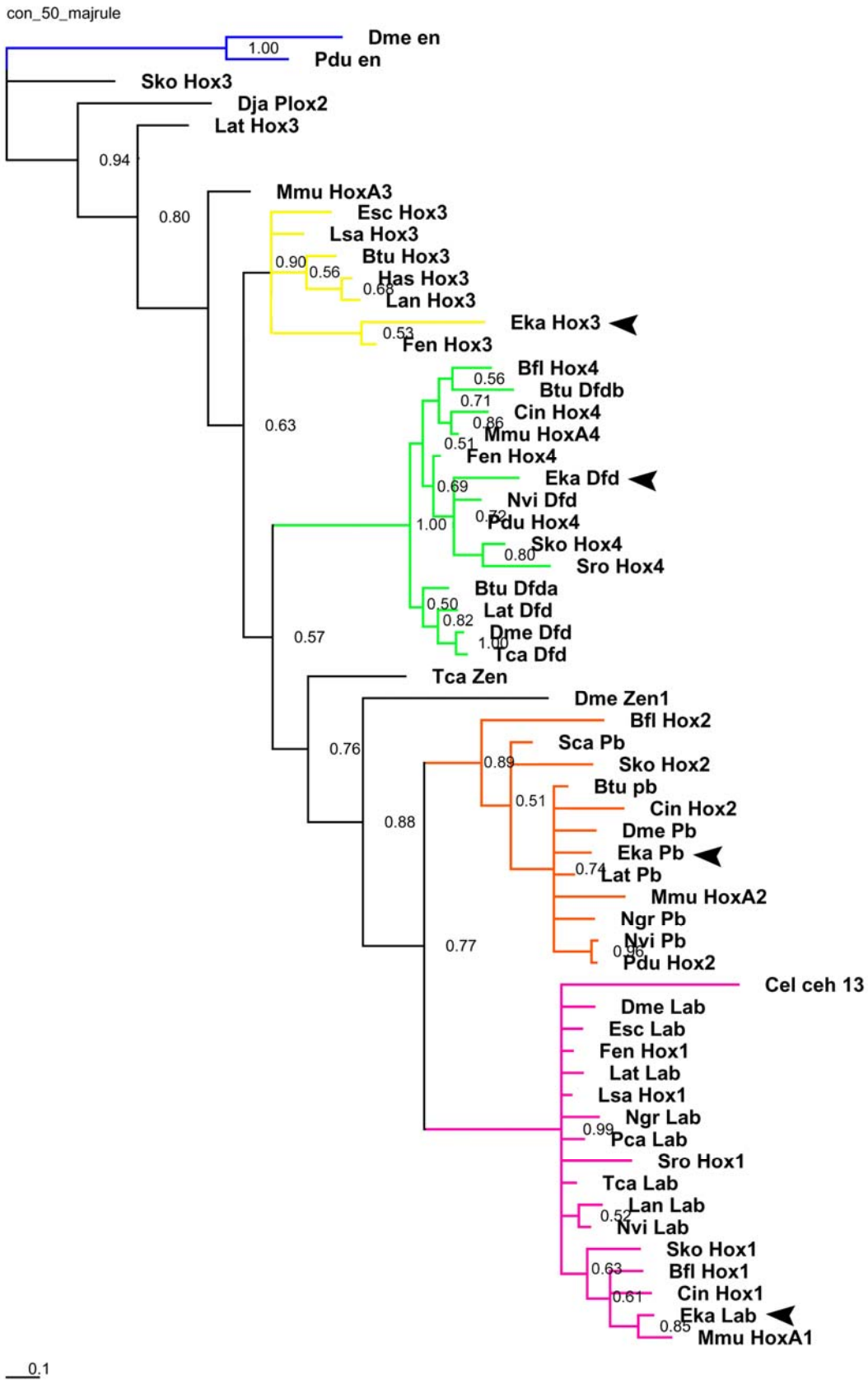


Figure S 6 Phylogenetic reconstruction of Hox paralog group 1 (purple), paralog group 2 (orange), and paralog group 3 (yellow), and paralog group 4 (green) relationships. *Engrailed* (*en*) is used as outgroup (blue). Arrow heads point *E. kanangrensis Hox* sequences.

Supplementary references

Abzhanov A, Kaufman T C (1999) Homeotic genes and the arthropod head: Expression patterns of the *labial*, *proboscipedia*, and *Deformed* genes in crustaceans and insects. Proc Natl Acad Sci USA 96:10224-10229

Haas S M, Brown S J, Beeman R W (2001a) Homeotic evidence for the appendicular origin of the labrum in *Tribolium castaneum*. Dev Genes Evol 211:96-102

Hughes C L, Kaufman T C (2002) Exploring the myriapod body plan: expression patterns of the ten *Hox* genes in a centipede. Development 129:1225-1228

Jager M, Murienne J, Clabaut C, Deutsch J, Le Guyader H, Manuel M (2006) Homology of arthropod anterior appendages revealed by *Hox* gene expression in a sea spider. Nature 441:506-508

Rogers B T, Kaufman T C (1996) Structure of the insect head as revealed by EN protein pattern in developing embryos. Development 122:3419-3432

Rogers B T, Peterson M D, Kaufman T C (2002) The development and evolution of insect mouthparts as revealed by the expression patterns of gnathocephalic genes. Evol Dev 4:96-110

Schmidt-Ott U, Technau G M (1992) Expression of *en* and *wg* in the embryonic head and brain of *Drosophila* indicates a refolded band of seven segment remnants. Development 116:111-125

Schmidt-Ott U, Gonzáles-Gaitán M, Jäckle H, Technau G M (1994) Number, identity, and sequence of the *Drosophila* head segments as revealed by neural elements and their deletion patterns in mutants. Proc Natl Acad Sci USA 91:8363-8367