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SUPPLEMENTARY MATERIAL

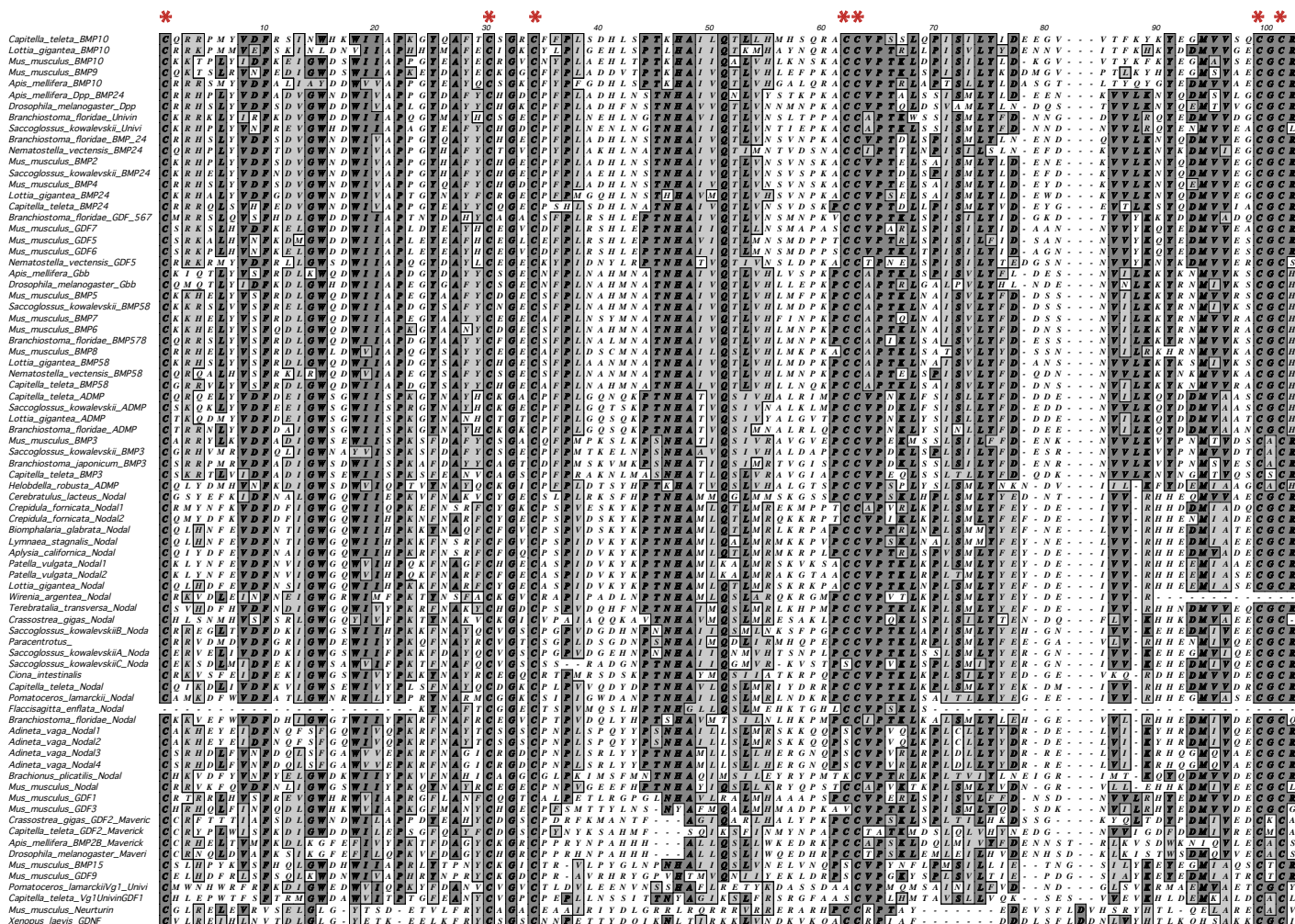
corresponding to:

Evolution, divergence and loss of the Nodal signalling pathway: new data and a synthesis across the Bilateria

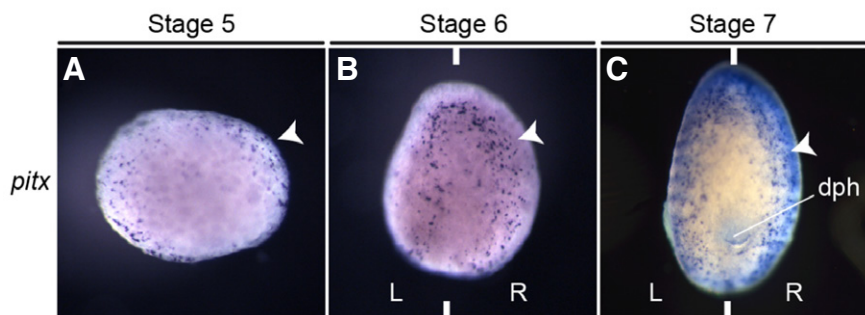
CRISTINA GRANDE, JOSÉ MARÍA MARTÍN-DURÁN, NATHAN J. KENNY,
MARTA TRUCHADO-GARCÍA and ANDREAS HEJNOL

***Address correspondence to:** Cristina Grande. Laboratorio 421, Centro de Biología Molecular Severo Ochoa, C/ Nicolás Cabrera, 1, Universidad Autónoma de Madrid, 28049 Madrid, Spain. Tel: +34-91-196-4703. Fax: +34-91-196-4698.

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Supp. Fig. 1. Alignment of the carboxy-terminal region of the newly determined amino acid sequences of Nodal as well as the known Nodal sequences of other spiraliens and deuterostomes and other TGF- β superfamily members available from GenBank (Accession numbers in Supp. Table 2). Identical amino acids are on the shaded background. Red asterisks mark the seven cysteine residues that are conserved in most of the members of the TGF- β superfamily. Nodal sequence of *F. enflata* corresponds to a small fragment of the carboxy-terminal region that could be amplified with our degenerate primers. A small fragment in the 3' of the carboxy-terminal region is missing for the Nodal sequence of *W. argentea*.



Supp. Fig. 2. Expression of *Pitx* in planarian embryos. (A) Single colorimetric in situ hybridization for *Pitx* in *S. polychroa* embryos. *Pitx* is first detected in early stage 5 embryos, as isolated cells distributed in the embryonic margins (white arrowhead in A). As the embryo adopts the bilateral symmetry by stage 6 (B), *Pitx* is mostly expressed in cells corresponding to the developing central nervous system (anterior brain and the pair of ventral nerve cords; arrowhead in B). The distribution in the nervous system observed in stage 6 embryos is maintained until the end of development (stage 7, arrowhead in C) and matches the expression described in adult planarians. *Dph*, definitive pharynx; R, right; L, left.

Supplementary Tables can be downloaded from the "Supplementary Material" link at: <http://dx.doi.org/10.1387/ijdb.140133cg>

Supp. Table 1. *Nodal* and *Pitx* presence/absence and amino acid sequences in several examined metazoan species, as determined in the present manuscript or documented in previous studies. *Sequences were retrieved from genomic sources or by direct amplification with degenerate primers for both genes.*

Supp. Table 2. TGF- β and paired-class amino acid sequences of the different metazoan Phyla used in the phylogenetic analyses.