Supplementary Material for

Ancestry of motor innervation to pectoral fin and forelimb

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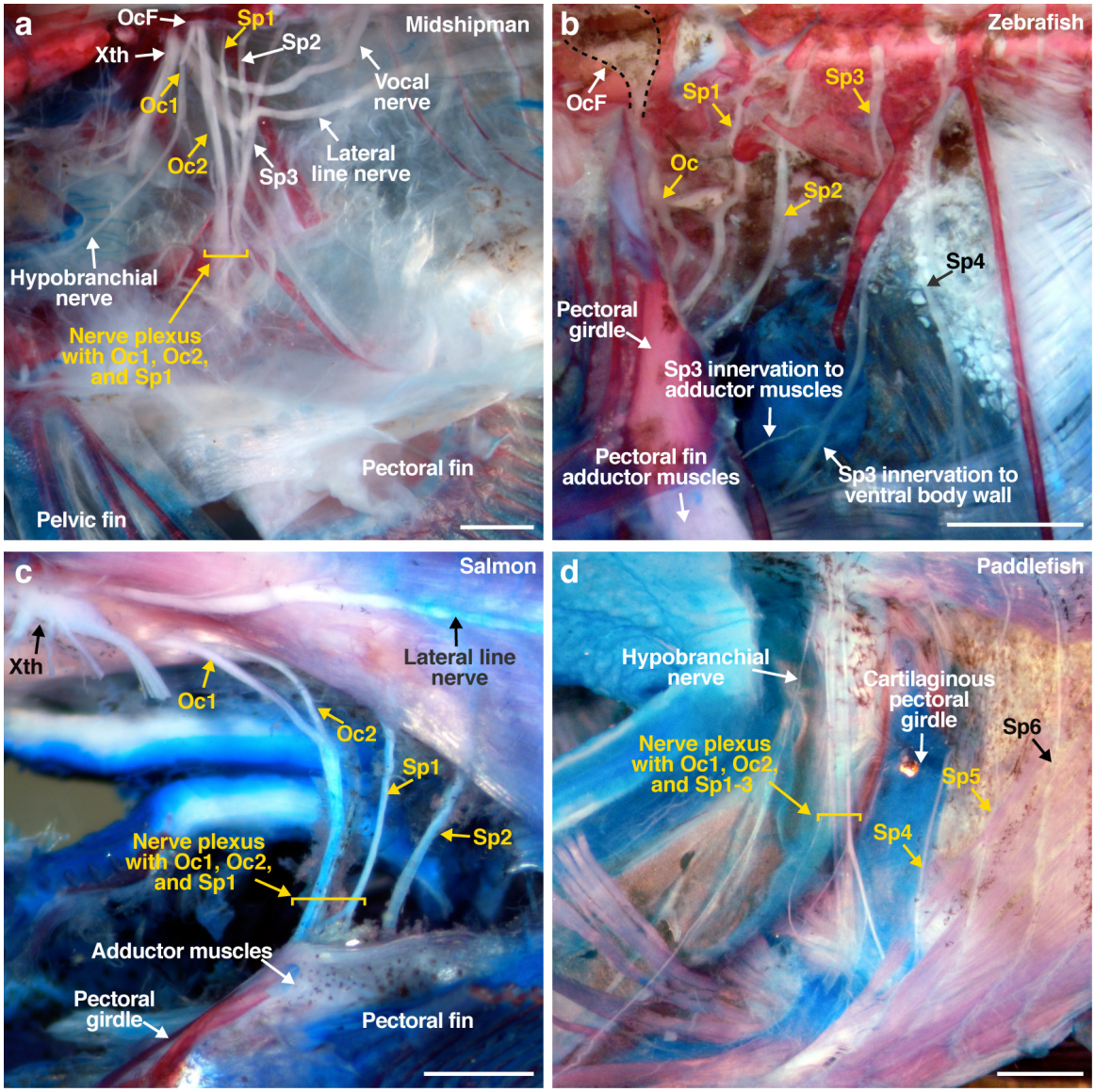
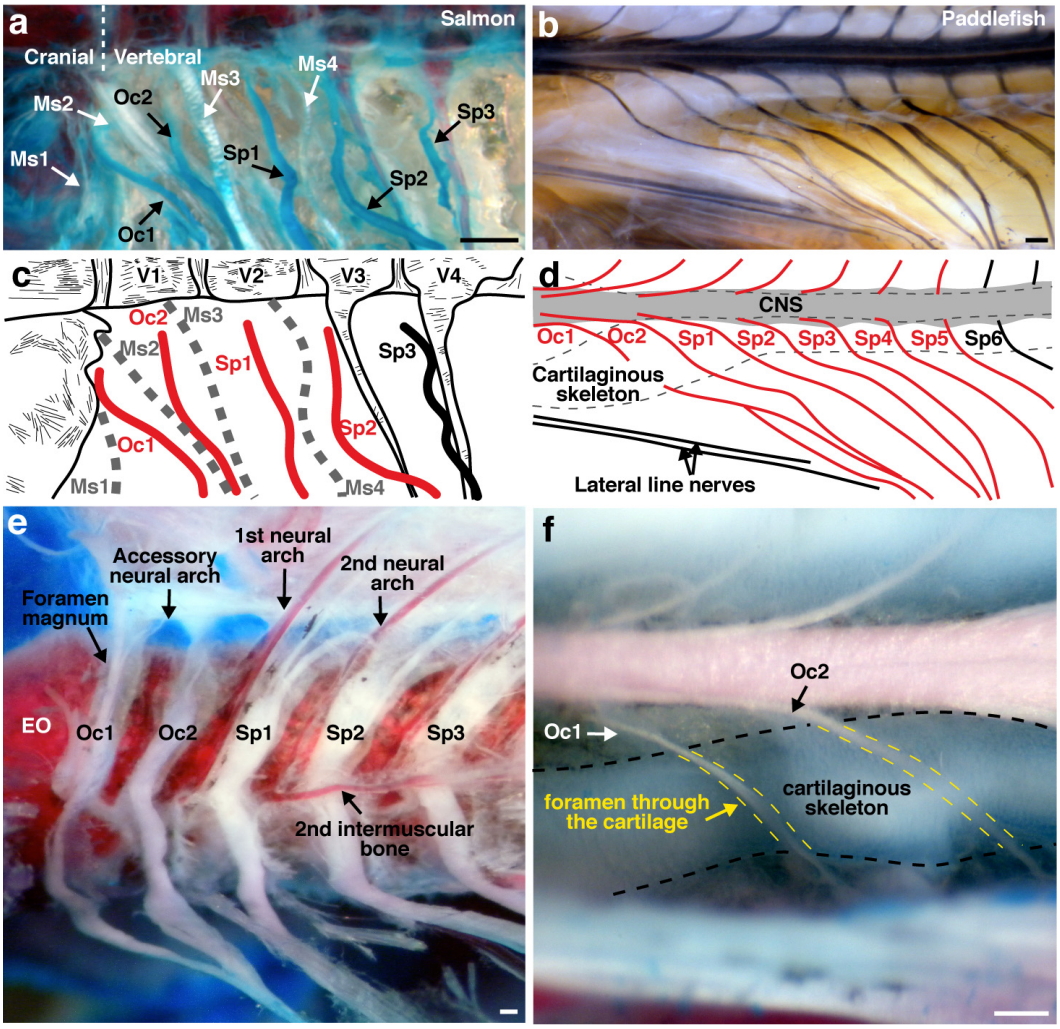
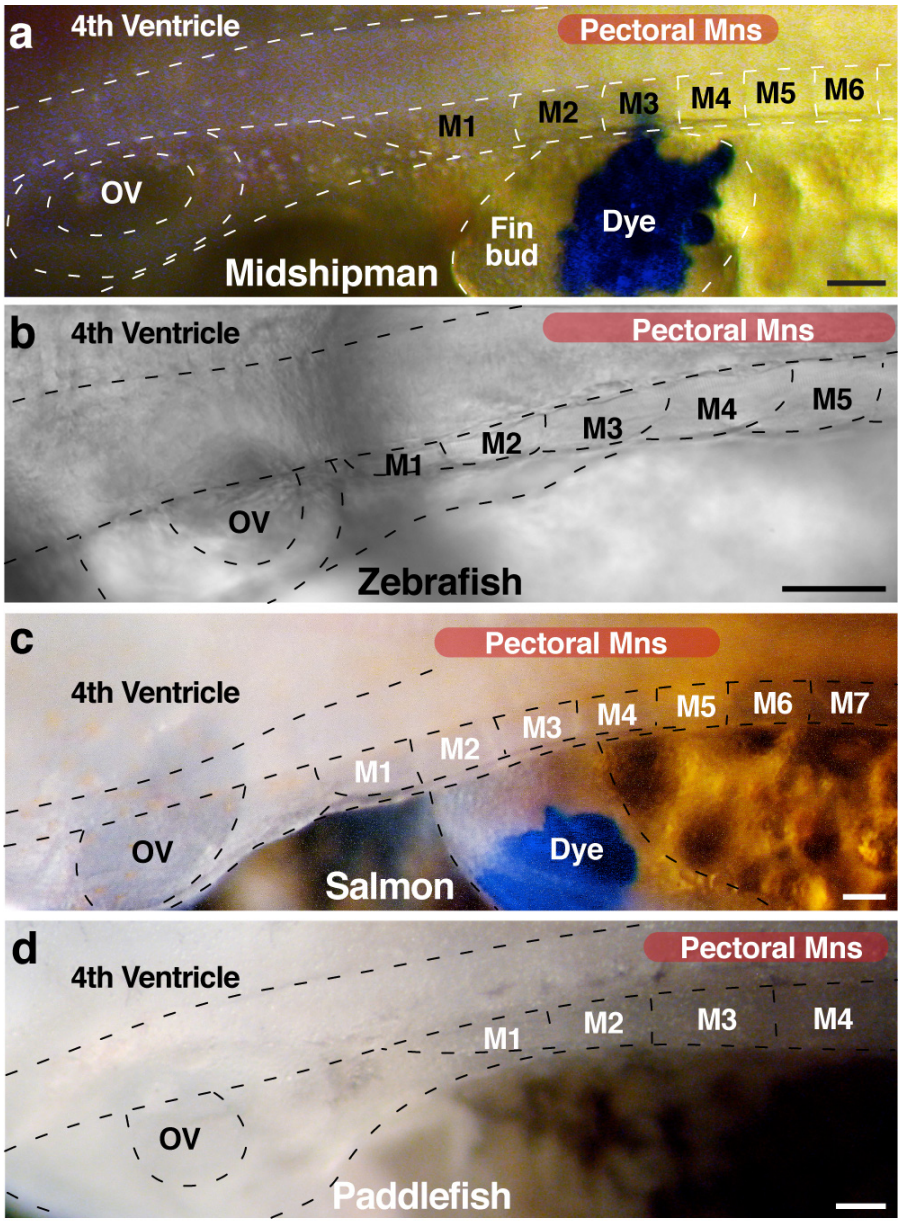
Supplementary Figures S1-5



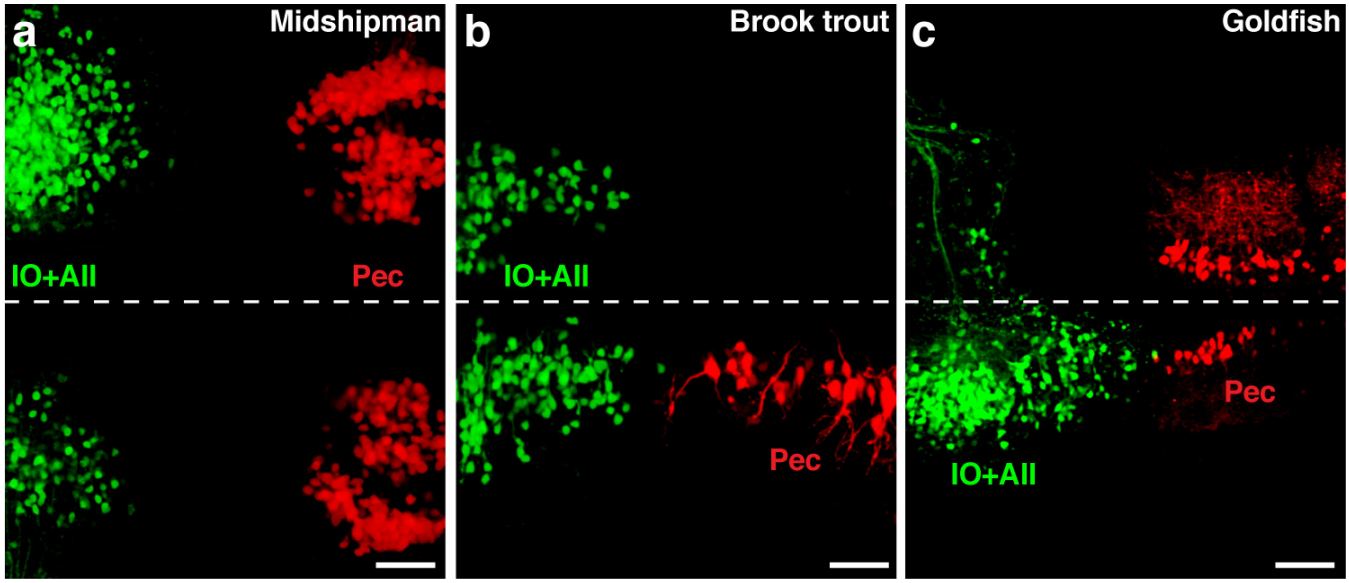
Supplementary Figure S1. Pectoral innervation among juvenile actinopterygians. Dissections showing pectoral fin innervation by occipital (Oc) and spinal (Sp) nerves in (**a**) midshipman, (**b**) zebrafish, (**c**) salmon and (**d**) paddlefish previously stained with alcian blue and alizarin red. In all cases, pectoral nerves formed a plexus near the pectoral girdle before innervating the fin musculature (most obvious in paddlefish; **d**). Occipital nerves emerged from the hindbrain through either an occipital foramen (OcF) in midshipman (**a**) and zebrafish(**b**) or the foramen magnum (see Suppl. Fig. S2 for salmon and paddlefish). Oc1 also gave rise to the hypobranchial nerve (clearest in **a** and **d**). Images shown are ventral (**a**) and lateral (**b-d**) views with anterior to the left. Scale bar is 1 mm. Xth, vagus nerve. Specimen sizes: **a** (~7 cm), **b** (~3.5 cm), **c** (~7 cm), **d** (~10 cm).



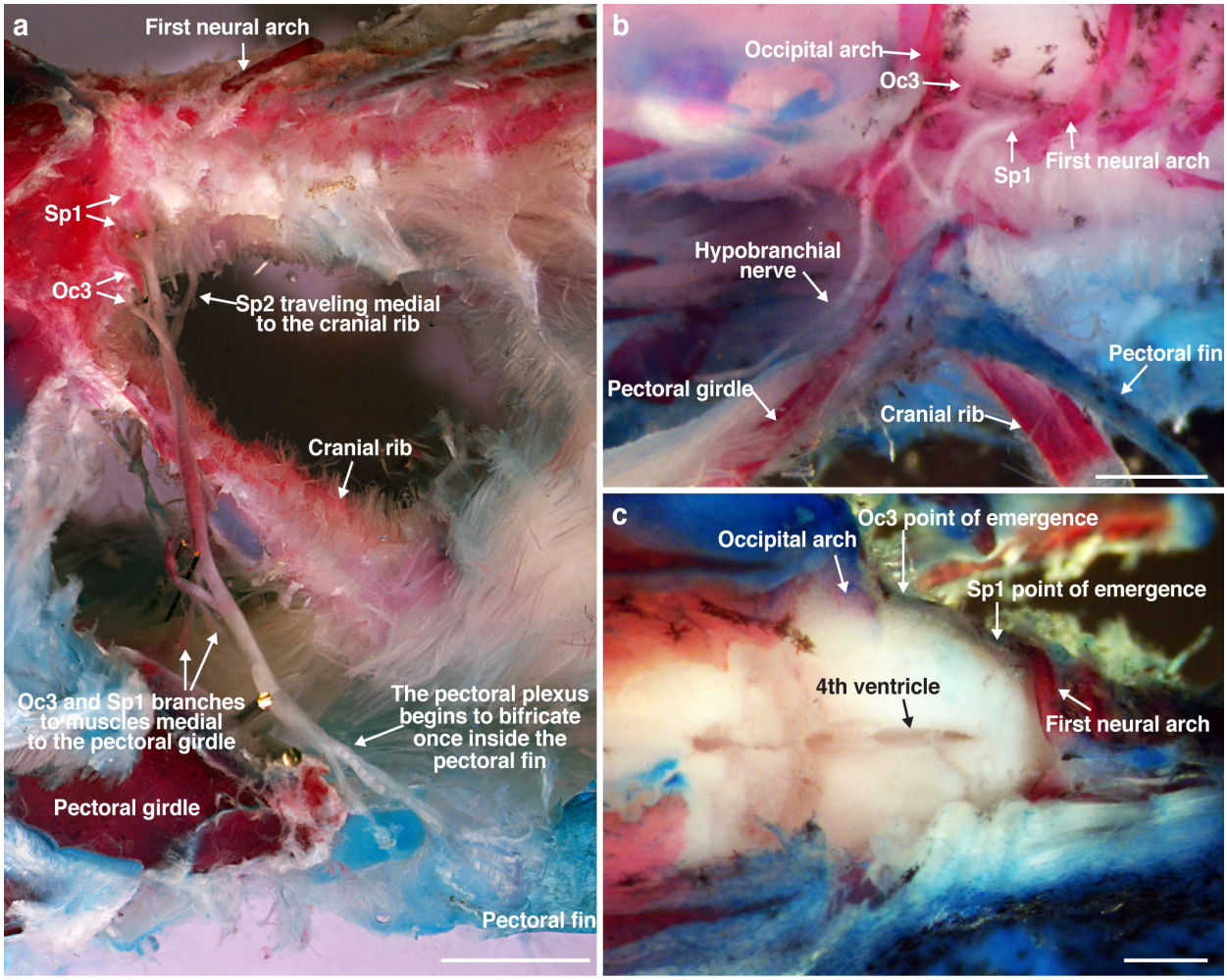
Supplementary Figure S2. Occipital region of juvenile salmon and paddlefish. **a-d,** Occipital hindbrain and rostral spinal cord in (**a**) salmon and (**b**) paddlefish. Dissections of juveniles confirmed location of the cranio-vertebral junction relative to myotomes/myosepta (most evident in salmon; **a**). Pectoral (red) and non-pectoral (black) nerves are indicated in the corresponding schematic (**c-d**). **e,** Lateral view of a salmon showing occipital (Oc) and spinal (Sp) nerve alignment with skeletal elements (neural arches). Occipital nerves emerged from the hindbrain through the foramen magnum with an accessory neural arch separating Oc1 and Oc2. **f,** Occipital nerves in paddlefish exited the cartilaginous skull via occipital foramina. Ventral (**a, c**), dorsal (**b, d, f**) and lateral (**e**) views are shown with anterior to the left. Specimens were stained with alcian blue and alizarin red prior to dissection (**a, e, f**) and in (**b**) with osmium tetroxide. Ms, myoseptum; V, vertebra.Scale bars: 1 mm (**a-d**), 200 µm (**e-f**). Specimen sizes: salmon (~7 cm), paddlefish (~8 cm).



Supplementary Figure S3. Embryonic blueprint of hindbrain and peripheral structures in actinopterygian fishes. (**a-d**) Brightfield images of midshipman (**a**), zebrafish (**b**), salmon (**c**) and paddlefish (**d**) embryos showed a similar alignment of neuroepithelium, otic vesicle (OV) and myotomes (M) (also see Fig. 1). Pectoral motoneuron location (mns; red bar) is also indicated (see Fig. 2). **a, c,** The blue lipophilic dye DiD was injected into the fin bud for retrograde labelling of motoneurons. Scale bars: 100 µm. Specimen sizes: **a** (~5 mm), **b** (~4 mm), **c** (~10 mm), **d** (~10 mm).



Supplementary Figure S4. Comparative alignment of pectoral motoneurons with precerebellar inferior olive and Area II. **a-c,** Pectoral motoneurons (Pec, red) and inferior olive/Area II (IO+AII, green) retrogradely labelled, respectively, from fin buds and cerebellum using fluorescent dextran. **a,** Pectoral motoneurons in larval midshipman were located ~150 μm caudal to the inferior olive and Area II nuclei. Pectoral motoneurons in both brook trout (**b**) and goldfish (**c**) were located immediately caudal to the inferior olive/Area II, as in zebrafish (see Fig. 3). All images are ventral view with anterior to the left. White hatching indicates midline. Pectoral motoneurons were labelled bilaterally in midshipman and goldfish, and ipsilaterally in trout; IO+AII were bilaterally labelled in midshipman and trout, and mainly ipsilaterally in goldfish. Scale bars: 50 μm. Specimen sizes: **a** (~11 mm), **b** (~14 mm), **c** (~7 mm).



Supplementary Figure S5. Pectoral innervation in Dipnoi. **a,** In adult spotted African lungfish (*Protopterus dolloi*), dorsal and ventral roots of Oc3 and Sp1 emerged through 4 foramina, fused together and travelled lateral to the cranial rib. Sp2 and Sp3 (damaged in this specimen) travelled medial to the cranial rib and joined Oc3 and Sp1 to form the pectoral plexus. **b,** In juvenile South American lungfish (*Lepidosiren paradoxa*), Oc3 joined Sp1 and travelled long the lateral side of the cranial rib as in spotted African lungfish. Oc 3 in this species also contributed to the hypobrachial nerve (**b**). **c,** Dorsal view of the same specimen showed that Oc3 emerged from the hindbrain, anterior to the end of the fourth ventricle. All specimens were stained with alcian blue and alizarin red prior to dissection. Images are lateral (**a-b**) and dorsal (**c**) views with anterior to the left. Scale bars: **a** (5 mm), **b-c** (1 mm).