

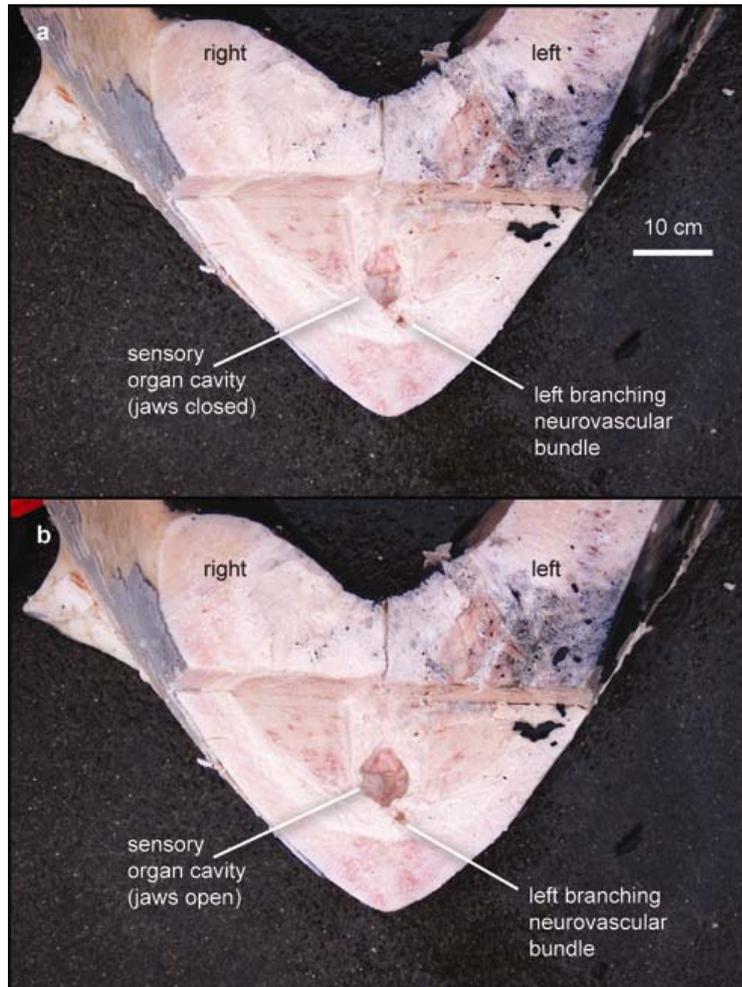
## 1. Study materials

We directly examined material belonging to adult and fetal specimens of *Balaenoptera physalus* (fin whales) and *B. acutorostrata* (minke whales) collected in Iceland (Table S1). We examined tissue samples collected by other researchers from adult specimens of *Balaena mysticetus* (bowhead whales) from Nunavut and *Eubalaena glacialis* (right whales) collected in Nova Scotia, Canada. We also examined digital images collected on-site of an adult *B. edeni* (Bryde's whale) stranded in Washington State and digital images collected for dissections of one immature individual of *Caperea marginata* (pygmy right whales) from New Zealand. Although we examined an adult individual of *Eschrichtius robustus* (gray whales) stranded in Washington State in 2011, we were unable to directly observe a clear sagittal section of the mandibular symphysis because of unyielding field conditions. Later, in 2011, tissue samples were collected by other researchers from a separate gray whale stranding, which we examined in histological section (see section 2).

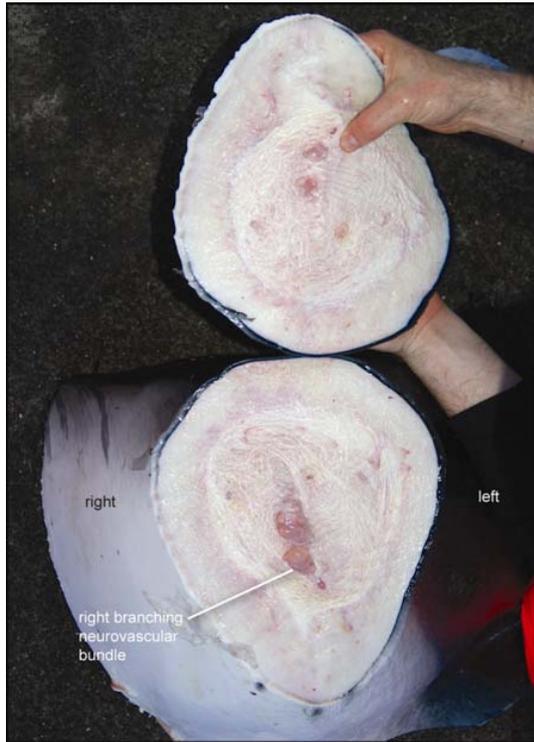
Field season	Taxon	Specimen number	TL	Sex	Neurovascular bundle side
2009	<i>Balaenoptera physalus</i>	H09017	19.08	M	R
2010	<i>Balaenoptera physalus</i>	H10001	18.25	M	L
2010	<i>Balaenoptera physalus</i>	H10002	17.98	M	R
2010	<i>Balaenoptera physalus</i>	H10003	18.49	M	L
2010	<i>Balaenoptera physalus</i>	H10004	17.37	F	R
2010	<i>Balaenoptera physalus</i>	H10005	17.87	M	R
2010	<i>Balaenoptera physalus</i>	H10006	20.14	F	R
2010	<i>Balaenoptera physalus</i>	H10007	20.10	F	R
2010	<i>Balaenoptera physalus</i>	H10008	18.60	M	L
2010	<i>Balaenoptera physalus</i>	H100011	17.35	M	L
2010	<i>Balaenoptera acutorostrata</i>	I031	7.60	M	L
2010	<i>Balaenoptera acutorostrata</i>	I032	7.90	M	R

**Table S1.** Specimen list for fin and minke whales examined for neurovascular bundle asymmetry in Iceland, during 2009 and 2010 field seasons. TL measured according to Mackintosh and Wheeler (1929); sex, male or female; right or left dominance for neurovascular bundle. Note that H09017 was the specimen used for detailed macroscopic anatomical dissections in Fig. 2f,g and Fig. 3b,d-k.

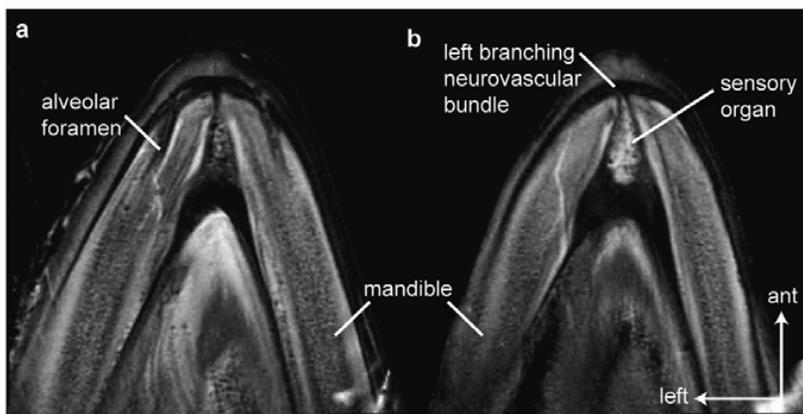
*B. physalus* (fin whales)—We recorded macroscopic observations and collected tissue samples from fresh fin whale (*B. physalus*) carcasses that were collected as part of a commercial catch in Hvalfjörður, Iceland in 2009 and 2010. All tissue samples were less than 24 hours dead, and the carcasses we perfused with 2°C fresh water prior to examination. Large steam-driven winches assisted with specimen manipulation. The sensory organ was patent in all adult fin whale specimens examined. Through direct manipulation of isolated chin tissue, we were able to visually demonstrate how rotation of the jaws can specifically deform the gel-like cavity housing the sensory organ (Fig. S1). Also, each fin whale specimen showed clear asymmetry in the branching of the neurovascular bundle emerging from the midline sensory organ (Table S1), along with fluctuation between left and right dominance (compare Figs. S1 versus S2). We also examined a near-term fetus (Fig. S3) collected late in the 2009 field season in Iceland. All tissue samples from Iceland were transferred and imported to Canada under CITES permits.



**Figure S1.** *B. physalus* (male, total length 18.25 m, specimen H10001 from the 2010 field season in Iceland) showing the mandibular symphysis dissected in a coronal section. The anterior termini of the mandibles are apparent, pointing to the bottom of the image, and enclose the cavity of the sensory organ. Transition between **a** and **b** demonstrates deformation of the sensory organ cavity caused by changes in mandible configuration (closed versus open). Note the left branching neurovascular bundle, and the deformation of the anterior chin surface between both states.

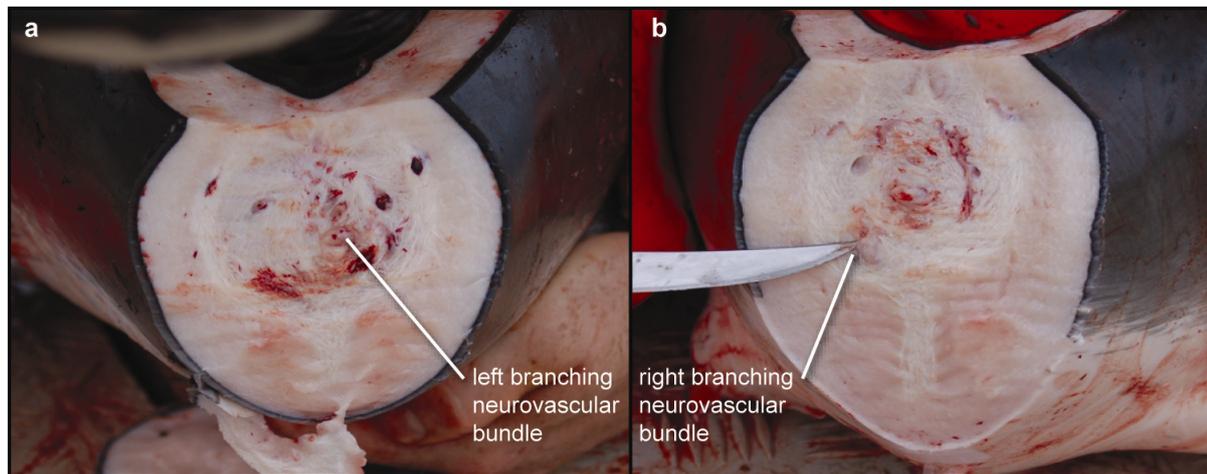


**Figure S2.** *B. physalus* (female, total length 20.14 m, H10006 from 2010 field season in Iceland) showing the mandibular symphysis dissected in transverse section, with the anterior-most section reflected to the top of the image. Note the right branching neurovascular bundle.



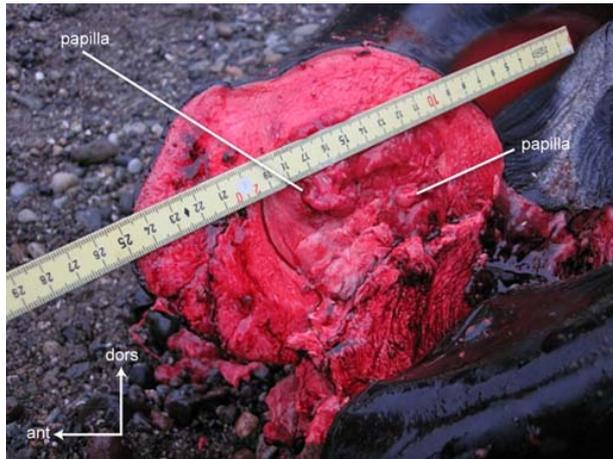
**Figure S3. a-b,** magnetic resonance images (MRI) of a *B. physalus* fetus (male, total length 2.68 m, collected during 2009 field season in Iceland), showing the mandibular symphysis in coronal section, with the anterior directed to the top of the image. Note the left branching neurovascular bundle.

*B. acutorostrata* (minke whales)— We recorded macroscopic observations and collected tissue samples from minke whale carcasses that were collected as part of a commercial catch for Hrefnuveiðimenn Ehf in 2010. All tissue samples were less than 24 hours dead. The sensory organ was patent in both adult minke whale specimens examined (Fig. S4). Through direct manipulation of isolated chin tissue, we were able to similarly verify in minke whales, as with fin whales, that jaw rotation deforms the gel-like cavity housing the sensory organ. Also, the minke whale specimen showed differed in asymmetry in the branching of the neurovascular bundle emerging from the midline sensory organ (Table S1; Fig. S4). All tissue samples from Iceland were transferred and imported to Canada under CITES permits.



**Figure S4.** *B. acutorostrata* (**a**, male, total length 7.60 m, specimen I031; **b**, male, total length 7.90 m, specimen I032; both collected during 2010 field season in Iceland), showing the mandibular symphysis in transverse section, with dorsal directed to the top of the images. Note the alternate branching directions of the neurovascular bundle projecting laterally from the midline sensory organ.

*B. edeni* (Bryde's whales)— We examined photographs collected by staff at Cascadia Research Collective, who dissected the mandibular symphysis of a stranded Bryde's whale in South Puget Sound, Washington State, U.S.A., on 6 December 2010 (male, total length 10.5 m, CRC-1135), freshly dead. These photographs confirmed the presence of a gel-like filled cavity within the mandibular symphysis, along with the presence of papillae of corresponding morphology to *B. physalus* and *B. acutorostrata*.



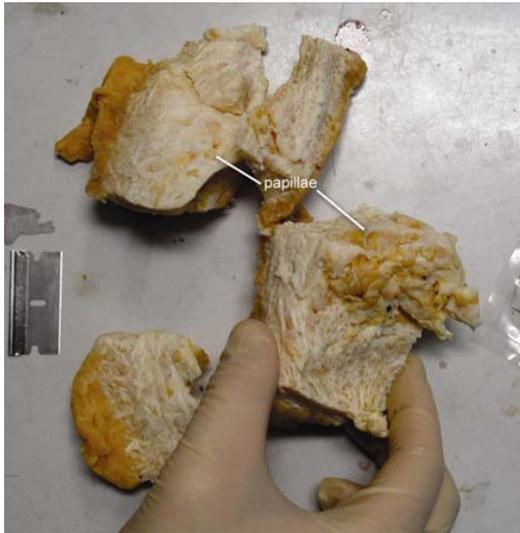
**Figure S4.** *B. edeni* (CRC-1135) showing the mandibular symphysis dissected in a sagittal section, viewed from the left side. Papillae are exposed within the bounded cavity of the sensory organ. Red color reflects hematomae from perimortem trauma. Original photograph by J. Huggins, Cascadia Research Collective. Ant., anterior; dors., dorsal.

*Eschrichtius robustus* (gray whales)—We examined tissue collected within the mandibular symphysis of gray whales by staff at Cascadia Research Collective from a gray whale stranded in Washington State in September, 2011 (male, total length 9.1 m, WDFW2011-090). Histological examination reveals papillae similar in organization to that of balaenopterids described here. Forthcoming work will describe this morphology in gray whales in more detail. We remain equivocal regarding the presence of a gel-like cavity in gray whales because Johnston et al. (2010) did not provide direct evidence for such a structure, and instead described the presence of a mucoid cavity in a decayed neonatal specimen located within a fibrous symphyseal mass (termed “chin strap”), which they noted was similar to that reported in balaenopterids by Pivorunas (1977), among others. Interestingly, Johnston et al. (2010:191) reported unpublished results from histological sections of the “chin strap” that revealed the presence of a nerve, which is consistent with our findings for the alveolar neurovascular bundle in balaenopterids. Johnston et al. (2010) did not report any asymmetry in the “chin strap.”

*Caperea marginata* (pygmy right whales)—We examined digital photographs of an immature specimen (sex unreported, total length 2.9 m, TMP011345) collected by Museum of New Zealand Te Papa Tongarewa in 2010. Photographs by R. E. Fordyce (University of Otago) indicated that *Caperea* does not possess a gel-like filled cavity within the mandibular symphysis. Minute papillae are patent, though in relatively low abundance in the general area of the mandibular symphysis compared with balaenopterids.

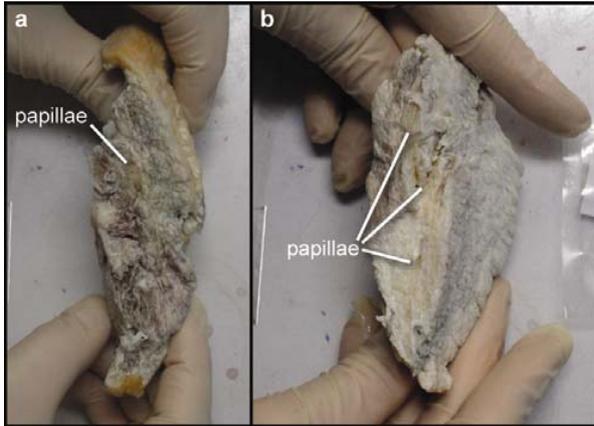
*Balaena mysticetus* (bowhead whale)—We examined formalin-fixed mandibular symphysis tissue from an adult bowhead whale specimen (Fig. S5) collected by Inuit hunters in Nunavut, Canada, approximately 24 hours after death in August, 2010 (AVC/UPEI case X21575-10). Specimens were sent to the University of British Columbia under a Department of Fisheries and Oceans, Canada, permit to Pierre-Yves Daoust (Atlantic Veterinary College, University of Prince Edward Island). We also have

examined digital photographs of the mandibular symphysis in a freshly killed adult bowhead from Nunavut, Canada, in August, 2011. These photographs further confirmed the presence of papillae intercalated within the fibrous connective tissue of the mandibular symphysis, but they also confirmed the absence of a cavity filled with a gel-like matrix in *Balaena*.



**Figure S5.** *Ba. mysticetus* (case X21575-10) tissue showing formalin-fixed samples from the mandibular symphysis. Papillae are exposed, but they are intercalated within the fibrous connective tissue, with no gel-like matrix present.

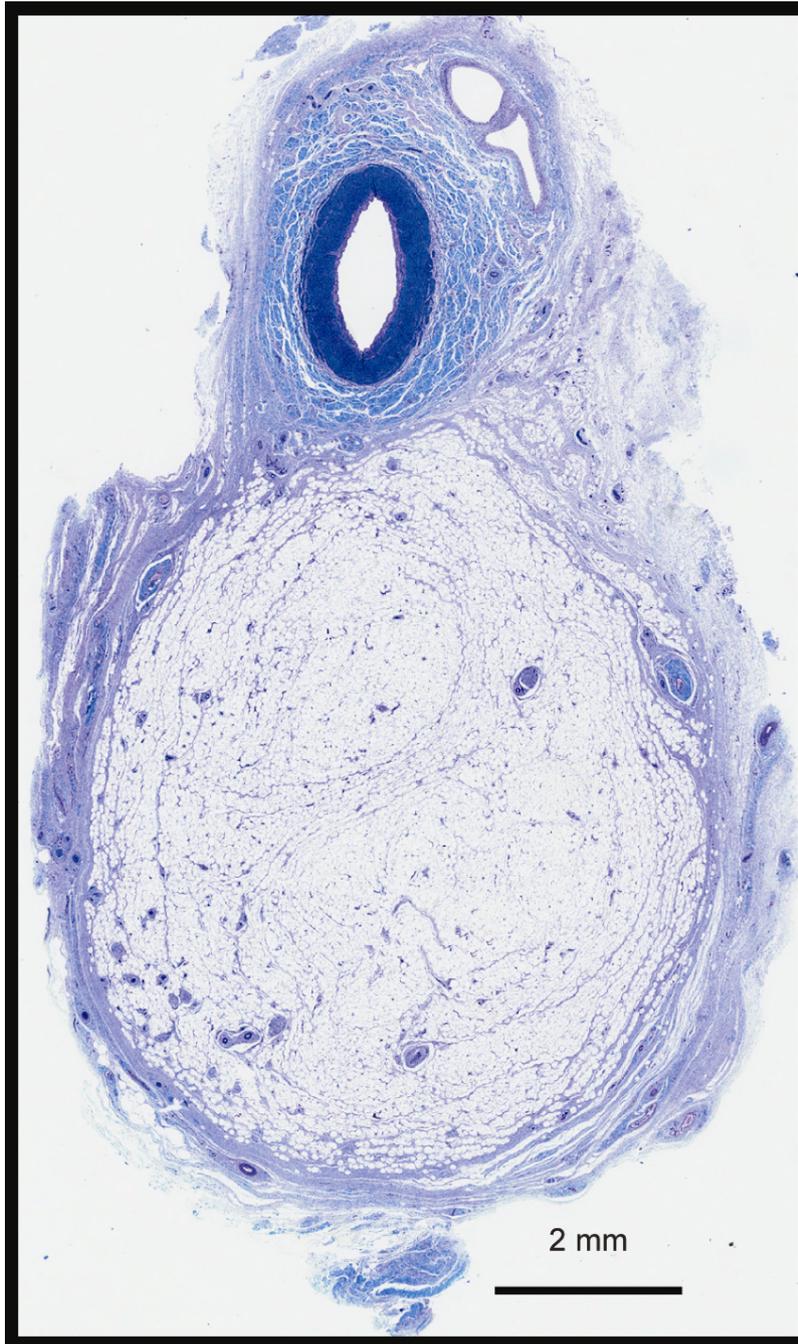
*Eubalaena glacialis* (right whales) — We examined formalin-fixed mandibular symphysis tissue from an adult right whale specimen collected postmortem on a beach near Digby, Nova Scotia, ~1 week after death in 15-16 August, 2010 (AVC/UPEI cas X22447-10). Specimens were sent to the University of British Columbia under a Department of Fisheries and Oceans, Canada, permit to Pierre-Yves Daoust, Atlantic Veterinary College, University of Prince Edward Island. We confirm the presence of papillae intercalated within the fibrous connective tissue of the mandibular symphysis (Fig. S6). P.-Y. Daoust (pers. comm. to N.D.P., 2010), who was present during the right whale necropsy, did not observe any large, gel-like cavity in the mandibular symphysis, although decayed tissue prevented unequivocal confirmation. M. R. Buono (pers. comm., to N.D.P., 2011) reported finding only fibrous connective tissue in a neonatal *Eubalaena australis* specimen from Argentina, and no presence of a gel-like cavity. Thus, based on the available tissue samples, we parsimoniously infer that *Eubalaena* possesses a similar adult mandibular morphology as *Balaena*, and does not possess gel-like cavity.



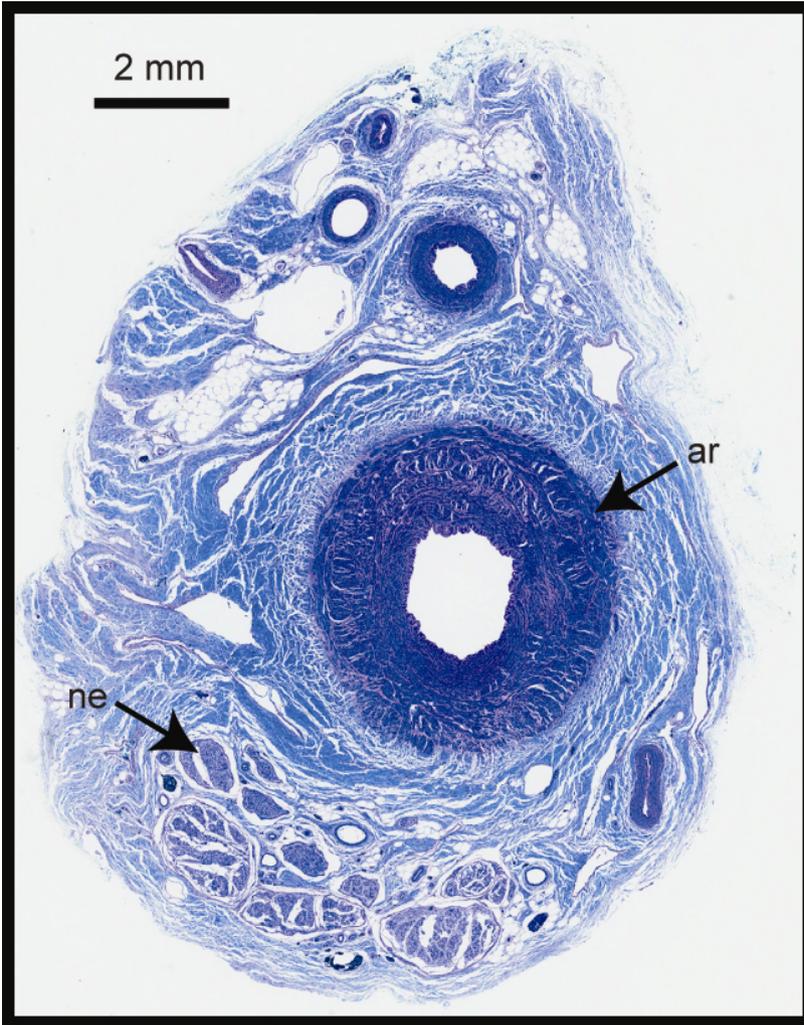
**Figure S6. a-b,** *Ba. mysticetus* (AVC/UPEI case X22447-10) showing formalin-fixed samples from the mandibular symphysis. Papillae are intercalated within the fibrous connective tissue.

## 2. Supplementary histological evidence

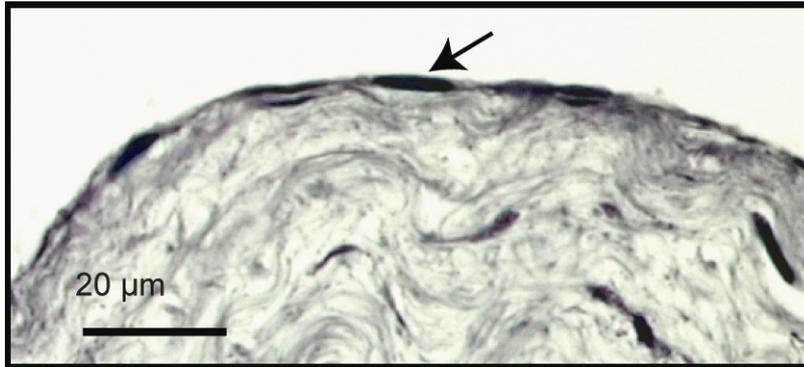
Histological sections from freshly dead fin whale (*B. physalus*) material were first fixed in standard buffered formalin for histology. The tissue sections were then stained for light microscopy using either a generalized hematoxylin and eosin stain (H&E) or a myelin stain (Luxol fast blue), following protocols devised at Wax-it Histology Services Inc., Vancouver, British Columbia, Canada. In initial samples of fresh tissue from the neurovasculature, we attempted Ag (silver) staining, but the results had too much background for the results to be useful. Electron microscopy may be useful for future researchers, but such scanning requires different histological fixatives than the ones we used. (Given the difficulty in obtaining fresh rorqual tissue specimens, such additional samples are not logistically possible for the foreseeable future). Luxol fast blue stains revealed that the vessels exiting the alveolar groove contains both nerves and blood vessels (Fig. S7). Based on their location, this bony feature is homologous with the alveolus of the lower first incisor in “toothed” mysticetes (Deméré et al., 2008), and thus this tissue is innervated by the inferior branch of the alveolar (part of the mandibular division of the trigeminal nerve) and vascularized by the inferior branch of the alveolar artery. Our dissections also revealed the close association of nerves and blood vessels entering the midline sensory organ laterally.



**Figure S6.** Luxol fast blue histological stains of the neurovascular bundle exiting the alveolar groove of an adult fin whale (*B. physalus*) showing an artery (in dark blue, at the top of the image) and connective and fatty tissue containing nerve termini. See Figure 2 and main text for more details.



**Figure S7.** Luxol fast blue histological stains of the neurovascular bundle entering the midline sensory of an adult fin whale (*B. physalus*). Ar., artery; ne., nerve.



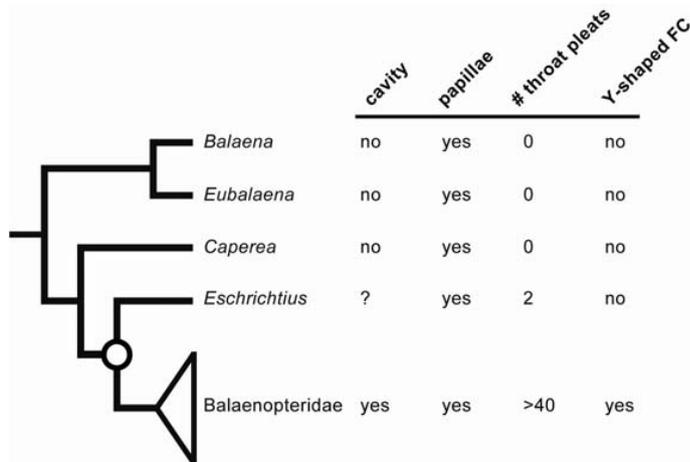
**Figure S8.** Histology of the inner surface of the cavity, featuring a discontinuous squamous layer of cells (arrow) in an adult fin whale (*B. physalus*).

### 3. Supplementary digital scanning information

X-ray CT scanning was conducted on frozen specimens of *B. physalus* and *B. acutorostrata* at the CT Imaging Centre at the FPInnovations Wood Products Division in Vancouver, British Columbia, Canada. The CT Imaging Centre is unique, among non-military instrumentation in the world, for its large scanning envelope (1 m in diameter, and 5 m in height, with a maximum object weight of 2 metric tons), which was built to accommodate large conifer tree trunks. For further details of FPInnovations CT scanner instrumentation, see <http://www.ctimagingcentre.com/index.html>. Consequently, this scanning envelope size was ideal for mysticete tissue. CT images were rendered in three dimensional (3D) images using VGStudioMax 2.0 software from Volume Graphics GmbH. Objects within each DICOM slice were manually separated by visual inspection through the segmentation of the fin whale chin tissue; we manipulated thresholding and colored each object across its range to optimize its visual distinction in the 3D rendering images.

### 4. Supplementary information about evolutionary context

Based on the distribution of mandibular soft tissue traits that we observed in living mysticetes (Fig. S9), we propose that the sensory organ in the mandibular symphysis of rorquals evolved either at the node of Balaenopteroidea (gray whales + rorquals), or along the stem to crown Balaenopteridae (rorquals; *sensu* Deméré, et al. 2008). If the former, then the organ is a pre-adaptation for lunge-feeding; if the latter, the organ evolved in tandem with the ventral groove blubber and specializations of the mandible morphology that are unique to rorquals, despite the fact that gray whales exhibit attenuated degrees of mandible excursion and buccal cavity expansion (Johnston et al. 2010).



**Figure S9.** Extant mysticete phylogeny compared with observed mandibular and soft tissue traits. Molecular phylogeny follows Sasaki et al. (2005). Open circle denotes the clade of Balaenopteroidea (gray whales + rorquals). Y-shaped FC: Y-shaped fibrocartilage, also referred to as YSF in the main text (Pivorunas 1977). Traits reflect observations presented in this work. For further data on number of throat pleats, see Deméré et al. (2008) and references therein for this character state.

## 5. Supplementary references

- Deméré, T.A., McGowen, M. R., Berta, A. & Gatesy, J. Morphological and molecular evidence for a step-wise evolutionary transition from teeth to baleen in mysticete whales. *Syst. Biol.* **57**, 15-37 (2008).
- Johnston, C. et al. Observations on the musculoskeletal anatomy of the head of a neonate gray whale (*Eschrichtius robustus*). *Mar. Mamm. Sci.* **26**, 186-194 (2010).
- Pivorunas, A. Fibrocartilage skeleton and related structures of ventral pouch of balaenopterid whales. *J. Morphol.* **151**, 299–313 (1977).
- Sasaki, T. et al. Mitochondrial phylogenetics and the evolution of mysticete whales. *Syst. Biol.* **54**, 77–90 (2005).