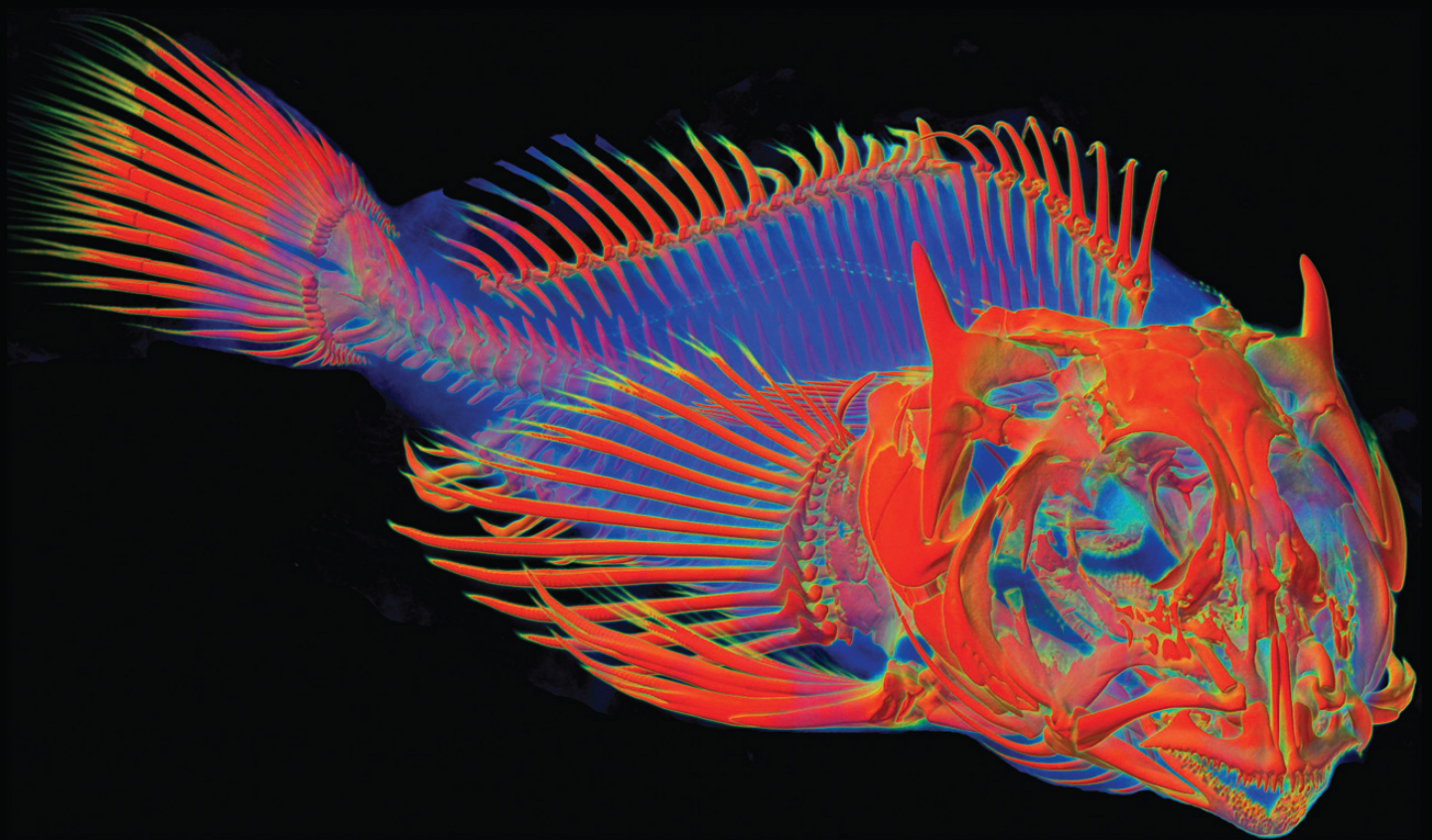


Volume 275, Number 8, August 2014

JOURNAL OF

morphology

ISSN 0362-2525



WILEY Blackwell

Editor: J. Matthias Starck

Divergence in Skeletal Mass and Bone Morphology in Antarctic Notothenioid Fishes

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ABSTRACT Although notothenioid fishes lack swim bladders, some species live temporarily or permanently in the water column. Given its relatively high density, skeletal mass is a key determinant of buoyancy. Notothenioids have reduced skeletal ossification, but there is little quantitative data on the phylogenetic distribution of this trait. We obtained dry skeletal masses for 54 specimens representing 20 species from six notothenioid families. Although comparative data are sparse, notothenioid skeletons comprise a smaller percentage of body mass, <3.5%, than those of three non-notothenioid perciforms. With relatively high skeletal mass, the non-Antarctic *Bovichtus diacanthus* is similar in skeletal mass to some non-notothenioids. *Eleginops maclovinus*, the non-Antarctic sister group of the Antarctic clade, has a relatively light skeleton (<2% of body mass) similar to many species in the Antarctic clade. Low skeletal mass is therefore a synapomorphy shared by *Eleginops* plus the Antarctic clade. We provide gross, histological, and micro-CT documentation of the structure and location of bone and cartilage in skulls, pectoral girdles, and vertebrae, with emphasis on the bovichtid *B. diacanthus*, the eleginopsid *E. maclovinus*, and the channichthyid *Chaenodraco wilsoni*. In *Eleginops* and the Antarctic clade, most bone is spongy and most species have persisting cartilage in the skull and appendicular skeleton. We also measured the relative size of the notochordal canal in adult vertebral centra of 38 species representing all eight families. There is considerable interspecific variation in this pedomorphic trait and all species show an ontogenetic reduction in the relative size of the canal. However, large persisting canals are present in adults of the Antarctic clade, especially in the nototheniids *Pleuragramma* and *Aethotaxis* and in a number of bathydraconid and channichthyid genera. *J. Morphol.* 275:841–861, 2014. © 2014 Wiley Periodicals, Inc.

KEY WORDS: heterochrony; histology; micro-CT scans; Bovichtidae; Eleginopsidae; Channichthyidae

INTRODUCTION

The Notothenioid Radiation in Antarctica

Notothenioids are a monophyletic group of eight families and 134 species of ray-finned fishes confined to the Southern Ocean around Antarctica, its peripheral islands, and the southern extremities of South America, Australia, and New Zealand (East-

man, 1993; Near et al., 2012; Lautrédou et al., 2013). The three non-Antarctic families (Fig. 1) have experienced little speciation or ecological diversification. However, in the cold isolated waters of the Antarctic continental shelf and slope, the Antarctic clade opportunistically expanded from a single lineage into five families and 105 endemic species (Eastman, 2005). Notothenioids are among the numerous examples of the convergent loss of the swim bladder among teleosts, an event that has occurred independently more than 30 times (McCune and Carlson, 2004), and that is an adaptive advantage for negatively buoyant fishes living on the substrate. Paradoxically, Antarctic notothenioids fill most water column and benthic niches on the High Antarctic shelf, a contingency facilitated by the eradication of previous faunas during tectonic, oceanographic, and climatic changes over the last 25 million years (Near et al., 2012).

Although there are relatively recent nonadaptive radiations of exclusively benthic notothenioids such as the 22 species of artedidraconid genus *Pogonophryne* (Eakin et al., 2009; Near et al., 2012), vertical partitioning of the water column accompanied speciation in early notothenioid

Additional Supporting Information may be found in the online version of this article.

Contract grant sponsor: National Science Foundation; Contract grant numbers: ANT 04-36190 (to J.T.E.); IOB 0517257 and IOS-1050154 (to L.M.W. and R.C.R.); Contract grant sponsor: NSF; Contract grant number: OPP 01-32032 (to H.W.D. [Northeastern University]; 2004 ICEFISH cruise on the RVIB Nathaniel B. Palmer).

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Received 14 October 2013; Revised 3 January 2014; Accepted 26 January 2014.

Published online 4 March 2014 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jmor.20258

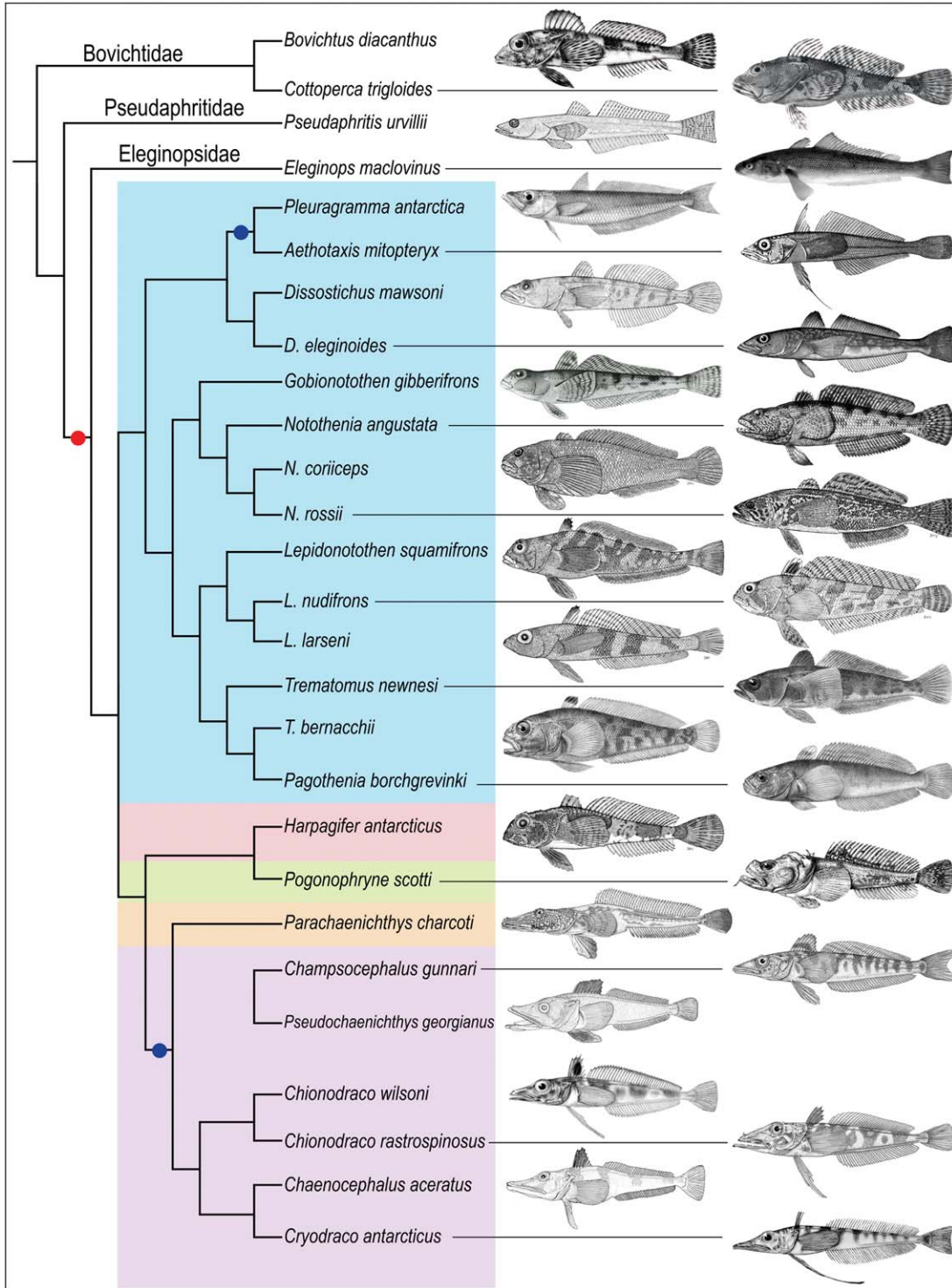


Fig. 1. Cladogram for notothenioids showing diversity in body morphology. Tree topology follows maximum likelihood phylogeny of Near et al. (2012) based on five nuclear and two mitochondrial genes, although paraphyly of Nototheniidae and Bathydraconidae is not shown. This is done to simplify discussion of the Antarctic families, given that the classification of notothenioids, and acanthomorphs in general, is entering a period of flux (Dettai et al., 2012; Betancur-R et al., 2013; Near et al., 2013). The three non-Antarctic families are unshaded and the five Antarctic families are color coded: Nototheniidae (blue), Harpagiferidae (light red), Artedidraconidae (green), Bathydraconidae (orange), and Channichthyidae (purple). Red dot indicates appearance of a relatively light skeleton, usually <2% of body mass (Table 1), although skeletal mass has not been determined for the Pseudaphritidae. Blue dots designate lineages having a relatively large notochordal canal in the vertebral centra. A drawing for *Bovichtus diacanthus* is unavailable; species depicted is the morphologically similar *B. angustifrons*.

diversification, and subsequently differential use of water column and benthic habitats has been an important ecological axis of the notothenioid adaptive radiation (Ekau, 1991; Eastman, 1993, 2005; Klingenberg and Ekau, 1996; Eastman and Barrera-Oro, 2010). This premise has been reinforced by a recent study utilizing stable isotopes of carbon and nitrogen to infer the use of benthic and pelagic resources by notothenioids. Isotopic disparity through time indicates multiple and parallel ecological divergences into overlapping pelagic niches in different families, especially the Nototheniidae and Channichthyidae (Rutschmann et al., 2011). Another study found that notothenioids also show higher than expected morphological disparity in the shape of the opercular bone, with the shape reflecting the benthic-pelagic axis of the species studied (Wilson et al., 2013). The notion that Antarctic notothenioids, or some clades therein, constitute marine species flocks on the Antarctic shelf (Eastman and McCune, 2000) has received support from recent molecular studies using mitochondrial and nuclear markers to examine the complex relationships and history of speciation in various notothenioid groups (Lautrédou et al., 2012), and from a protocol using iterative evaluation of the criteria for recognizing flocks (Lecointre et al., 2013).

Only four or five notothenioid species are neutrally buoyant, but many other species feed in the water column on seasonally abundant items. Living and feeding in a variety of habitats is reflected in the reduced density and divergent buoyancies of various species (Eastman and DeVries, 1982; Eastman and Sidell, 2002; Near et al., 2012), where buoyancy is expressed as percentage buoyancy (%B = weight in water/weight in air \times 100). Notothenioids range in mean percentage buoyancy from 0%, in neutrally buoyant species, to 6% in heavy benthic species, with 68% (or ± 1 SD) of 54 species falling between 2.0 and 4.6% (Near et al., 2012, Supporting Information Table S5).

Skeletal Tissue Density, Skeletal Mass, and Static Buoyancy

The density of teleost bone is relatively high at 1,700–2,000 kg m⁻³ (Alexander, 2003, p. 301). Furthermore, with a density of 1,040–1,092 kg m⁻³, most of the soft tissues composing the body of a marine fish are denser than seawater (1,026 kg m⁻³; Pelster, 1998). The skeletal masses of mammals and other terrestrial vertebrates comprise an increasing proportion of body mass with increasing body size, reaching a maximum of about 25% in the largest terrestrial mammals (Schmidt-Nielsen, 1975). Conversely, teleost skeletons, exemplified by perciforms, compose 3.4–5.1% of the body mass (Reynolds and Karlotski, 1977), and in samples of multiple teleost species the skeleton scales isomet-

rically (Reynolds and Karlotski, 1977; Berrios-Lopez et al., 1996). Although the fish skeleton has a lower mineral content than in terrestrial vertebrates (Dean and Shahar, 2012; Cohen et al., 2012), it is still inordinately influential in determining buoyancy. This is because percentage buoyancy values are a proxy for body density, and this reflects the proportions and densities of the various constituent tissues (Alexander, 1968, p. 186). Because notothenioids are inactive and use primarily anterior–posterior oscillation with the pectoral fins (labriform locomotion), they do not generate lift like continuously swimming fishes. Hence, a combination of static mechanisms is responsible for interspecific differences in density and buoyancy; these include reduction of skeletal mineralization, persistence of cartilage (density = 1,060–1,180 kg m⁻³, Alexander, 2003, p. 301), and accumulation of triglyceride lipids (930 kg m⁻³) in axial musculature and subcutaneous tissue (DeVries and Eastman, 1978; Eastman and DeVries, 1981).

Reduction of Bone in Fishes

Reduction in the size and thickness of bones, as well in as in the mineral content, will minimize skeletal mass (Pelster, 1997). The type of bone is also relevant. Bones in fishes may be spongy (light and porous), compact (heavy and dense), intermediate between bone and cartilage (chondroid bone), or represented by connective tissue or persistent hyaline cartilage rather than osseous tissue (Meunier and Huysseune, 1992). Vertebrate compact bone is fourfold denser than spongy bone (Wainwright et al., 1976, p. 167). Spongy bone is common in teleosts (de Ricqlès et al., 1991, p. 50) and the numerous cavities in bones of the skull and vertebral centra decrease bone mass and overall body density and also provide space for triglyceride lipid, especially in marine fishes (Lee et al., 1975; Phleger, 1975). The morphology of the vertebrae, which comprise 20–30% of the mass of the fish skeleton, is especially important in contributing to bone mass as they contain as much as 26% more calcium than ribs and opercles (Fraser and Harvey, 1982). Reduction in heavy ions significantly reduces bone density (Pelster, 1997, p. 225).

Reduced bone development and skeletal ossification are widespread in fishes (de Beer, 1937; Gossline, 1971; Meunier and Huysseune, 1992), especially within various teleost lineages including notothenioids (Iwami, 1985; Voskoboinikova, 1997, 2001). These are frequently the result of heterochronic processes that balance the opposing demands of buoyancy and mechanical stress (Schaeffer, 1961). In Antarctic notothenioids, pedomorphosis (specifically postdisplacement as defined by Reilly et al., 1997) results in truncation of bone development (Voskoboinikova et al., 1994),

including delayed closure of the notochordal canal in the vertebral centra (Totton, 1914; DeVries and Eastman, 1978), and persistence of cartilage. These traits are apomorphic given the early appearance of bones and rapid rate of bone development in larvae and juveniles of the non-Antarctic *bovichtids* (Voskoboinikova and Bruce, 2001). Recent work using the collagen family of genes as markers for cartilage and bone formation also indicates that the delayed branchial and cranial bone development in low-density pelagic and semipelagic notothenioids is associated with heterochronic shifts in skeletal gene expression, specifically persistence of the chondrogenic program and delay in the osteogenic program during larval development (Albertson et al., 2010; Detrich and Amemiya, 2010). This “adaptive osteopenia” has allowed channichthyids to be advanced as evolutionary-mutant model organisms for investigating osteopenia in humans (Albertson et al., 2009; Maher, 2009).

The literature contains a few descriptions of notothenioid pectoral girdles and skulls (Starks, 1930; Gregory, 1933), and there is a more substantial body of work on descriptive osteology as a component of systematic studies (Eakin, 1981; Voskoboinikova, 1982, 1991, 1993; Andersen, 1984; Balushkin, 1984, 2000; Iwami, 1985). As mentioned previously, there are also numerous contributions by Voskoboinikova and collaborators on skeletal development in larvae from most notothenioid clades. However, this information does not address bone reduction in adult notothenioids, and there is no quantitative data on skeletal mass other than reports that the ashed skeletal masses of 11 species of *Nototheniidae* exhibit sixfold difference between the lightest neutrally buoyant species and the heaviest benthic species (DeVries and Eastman, 1978; Eastman and DeVries, 1981). These ash values for nototheniids are about two to threefold less than ash values for phylogenetically diverse teleosts (Childress and Nygaard, 1973). Moreover, there has been little study of the histology of notothenioid bone and cartilage, especially in the case of the non-Antarctic notothenioids and the some of the phylogenetically derived Antarctic families like the *Channichthyidae*. Therefore, it is not known whether the skeletal reduction in adult Antarctic notothenioids is a phylogenetically persistent condition or whether it appeared later during diversification into the developing Antarctic marine ecosystem. Our specific objectives here are to provide data on 1) the dry mass of skeletons for a sample of 54 specimens from 20 species representing six of eight notothenioid families, including both Antarctic and non-Antarctic species; 2) aspects of gross, microscopic and micro-CT anatomy, emphasizing neurocrania, pectoral girdles, and vertebral centra, with documentation of the distribution of bone and cartilage in skeletons of

three phylogenetically diverse taxa; and 3) interspecific and ontogenetic differences in the relative size of the notochordal canal in specimens from 38 species representing eight families.

MATERIALS AND METHODS

Specimens and Nomenclature

We collected material for this study during fieldwork at shore stations and on cruises to a number of Antarctic and sub-Antarctic localities from 1973 to 2011. As summarized in Tables 1 and 2, we examined a total of 38 species representing all eight notothenioid families. Various components of the study utilized subsets of this total number of species and families. This material is in the personal collection of the senior author. In accordance with protocol L01-14 approved by the Institutional Animal Care and Use Committee at Ohio University, we euthanized all fishes captured through trawling or trapping by severing the spinal cord, or by immersion in a 200-mg l⁻¹ solution of 3-aminobenzoic acid ethyl ester (MS-222, Sigma, St. Louis).

We provide lengths of specimens as standard length (SL) or, when these are undetermined, as total length (TL). Taxonomic nomenclature follows Eastman and Eakin (2000), with subsequent updates from *Catalog of Fishes* (Eschmeyer, 2013) and the list of valid species maintained on Eastman's web site (<http://www.oucom.ohiou.edu/dbms-eastman>). Osteological nomenclature is based on Jollie (1986) and Rojo (1991), and terms relating to heterochrony are those of Reilly et al. (1997).

Preparation of Skeletons for Determination of Dry Weight

We prepared 54 dry skeletons from 1 to 11 specimens of each of 20 notothenioid species (Table 1). We obtained lengths and weights immediately after capture and then froze the specimens at -30°C for periods of 3–6 months. The samples for *Trematomus newnesi* included both large mouth and typical morphs, and these were treated as a single species in the analysis. Values for skeletal masses of three species were obtained during prior studies of buoyancy (Eastman and Barrera-Oro, 2010; Eastman et al. 2011).

Cleaning skeletons involved thawing and then macerating the entire fish in soapy (Ajax Lemon dish washing soap and OxyClean) water for 3–5 days at room temperature (≈23°C), removal of remaining tissue with running water and compressed air, and drying at room temperature to a constant weight for 1–2 months. In the bones of some notothenioids, lipid may account for as much as 15% of the dry weight of the skeleton (Phleger et al., 1999), and this must be removed in order to obtain an accurate dry skeletal weight. In these cases, we extracted the remaining lipid by soaking in acetone in an ultrasonic cleaner with repeated changes until the solvent was clear. We did not determine the mineral content by ashing these skeletons because dry notothenioid skeletons are poorly represented in museum collections, and we wished to preserve these skeletons for future study.

Anatomical and Histological Techniques

For material used in anatomical and histological work, we used a combination of preservation techniques including perfusing specimens with Bouin's solution immediately after capture, immersion of entire specimens in 10% formalin fixative, and immersion of pieces of tissue in either Bouin's or 10% formalin. We also cleared and stained preserved specimens with alizarin red S (Taylor, 1967) dissolved in 75% ethyl alcohol (Springer and Johnson, 2000).

For examination of skeletal tissue histology, we used 1–4 specimens from each of 15 species representing six of the eight notothenioid families. We removed perfusion-fixed and

TABLE 1. Data for sample of 54 specimens from 20 species of notothenioids used to obtain mean percentage masses of dried skeletons

Species	N	Location	Year collected	Range			Mean
				Standard length (mm)	Body mass (g)	Dry skeletal mass (g)	Skeletal mass/body mass $\times 100$ (%)
Bovichtidae							
<i>Bovichtus diacanthus</i> (BDIA)	2	Tristan da Cunha	2004	116–130	31.30–41.50	1.06–1.43	3.42
Eleginopsidae							
<i>Eleginops maclovinus</i> (EMA)	3	Falkland Islands	2004	261–334	273.30–631.30	5.32–11.65	1.91
Nototheniidae							
<i>Dissostichus mawsoni</i> (DMA)	1	McMurdo Sound	1973	880	14,061.40	206.60	1.47
<i>Gobionotothen gibberifrons</i> (GGI)	2	South Orkney Is.	2009	302–314	407.10–454.90	7.94–8.70	1.93
<i>Notothenia angustata</i> (NAN)	1	Portobello, N.Z.	1991	306	716.32	21.56	3.01
<i>N. coriiceps</i> (NCO)	2	Bouvetøya	2004	420–518	1,450.00–2,550.00	45.67–62.59	2.80
<i>N. coriiceps</i>	3	King George Is.	2009	220–372	236.40–1,239.40	5.20–30.97	2.45 ^a
<i>N. rossii</i> (NRO)	3	King George Is.	2009	219–363	204.50–978.90	3.56–15.77	1.65 ^a
<i>Lepidonotothen squamifrons</i> (LSQ)	2	South Orkney Is.	2009	322–325	646.00–654.00	10.24–10.35	1.58
<i>L. nudifrons</i> (LNU)	3	King George Is.	2010	148–180	61.87–114.97	1.19–2.32	2.00
<i>L. larseni</i> (LLA)	2	South Orkney Is.	2009	158–167	42.00–44.80	0.82–0.95	2.04
<i>Trematomus newnesi</i> (TNEtyp)	1	McMurdo Sound	2005	142	45.00	0.64	1.42
typical morph							
<i>T. newnesi</i> typical morph	5	King George Is.	2009	162–179	71.30–112.0	0.90–1.69	1.38 ^b
<i>T. newnesi</i> (TNElm)	5	King George Is.	2009	174–193	115.10–144.00	1.91–2.36	1.68 ^b
large mouth morph							
<i>T. bernacchii</i> (TBE)	3	McMurdo Sound	2005	210–227	225.00–275.00	3.81–4.13	1.59
<i>Pagothenia borchgrevinki</i> (PBO)	3	McMurdo Sound	2005	170–204	88.00–147.00	1.01–1.56	1.12
Artedidraconidae							
<i>Pogonophryne scotti</i> (PSC)	2	South Orkney Is.	2009	147–206	81.40–183.20	2.18–5.16	2.75
Bathydraconidae							
<i>Parachaenichthys charcoti</i> (PCHAR)	2	South Orkney Is.	2009	328–372	232.00–320.00	5.68–7.54	2.40
Channichthyidae							
<i>Champsocephalus gunnari</i> (CGU)	2	South Orkney Is.	2009	426–431	778.00–860.00	8.56–9.27	1.09
<i>Pseudochaenichthys georgianus</i> (PsGEO)	2	South Orkney Is.	2009	435–458	1,200.00–1,480.00	15.65–17.20	1.23
<i>Chionodraco rastrospinosus</i> (CRAST)	2	South Orkney Is.	2009	310–315	354.00–472.00	6.43–6.71	1.62
<i>Chaenocephalus aceratus</i> (CAC)	2	South Orkney Is.	2009	279–294	152.70–173.20	2.81–3.19	1.84
<i>Cryodraco antarcticus</i> (CANT)	1	South Orkney Is.	2009	414	418.00	8.59	2.06
Non-notothenioids							
Percidae							
<i>Perca flavescens</i> (PFL)	4	North America	1977		72.60–92.30	2.90–3.47	3.81 ^c
<i>Stizostedion vitreum</i> (SVI)	1	North America	1977		628.60	23.29	3.71 ^c
Serranidae							
<i>Epinephelus striatus</i> (EST)	3	North America	1977		1,033.00–1,159.60	44.58–58.16	4.72 ^c

The morphs of *Trematomus newnesi* are listed separately but are treated as one species in subsequent analyses. Arrangement of taxa is phylogenetic based on cladogram in Figure 1. Literature values for non-notothenioids are at the end of table. Skeletal masses of individual notothenioid specimens are available in Supporting Information Table S1.

^aData from Eastman et al. (2011).

^bData from Eastman and Barrera-Oro (2010).

^cData from Reynolds and Karlotski (1977).

TABLE 2. Relative percentage diameter of the notochordal canal (horizontal diameter of notochordal canal/horizontal diameter of vertebral centrum \times 100) based on measurements of the first caudal vertebra in each of 130 dry and alizarin stained skeletons from adults of 38 species representing all eight notothenioid families

Species	N	Size		Relative diameter of notochordal canal (%)	
		Maximum known TL or SL (mm) ^a	This sample SL (mm)	Range ^b	Mean
Bovichtidae					
<i>Bovichtus diacanthus</i>	2	250*	116–130	11–10	
<i>B. variegatus</i>	3	250*	125–172	11–7	
<i>Cottoperca trigloides</i>	2	500*	217–240	8	
Pseudaphritidae					
<i>Pseudaphritis urvillii</i>	3	300*	157–182	9–7	
Eleginopsidae					
<i>Eleginops maclovinus</i>	4	1,000*	255–357	10–5	
Nototheniidae					
<i>Pleuragramma antarctica</i>	23	250	105–225	81–50	65
<i>Aethotaxis mitopteryx</i>	1	420*	113	48	
<i>Dissostichus mawsoni</i>	3	2,360*	271–880	17–4	
<i>D. eleginoides</i>	4	2,250*	276–750	17–4	
<i>Gobionotothen gibberifrons</i>	2	550*	302–314	9	
<i>Notothenia angustata</i>	1	410*	306	5	
<i>N. coriiceps</i>	5	620*	220–518	12–5	
<i>N. rossii</i>	3	920*	219–363	14–8	
<i>Lepidonotothen squamifrons</i>	2	500*	322–325	13	
<i>L. nudifrons</i>	4	190*	148–180	22–17	
<i>L. larseni</i>	2	240*	158–167	32–28	
<i>Trematomus newnesi</i> (typical morph)	10	200*	122–179	38–24	30
<i>T. newnesi</i> (large mouth morph)	8		139–193	38–22	28
<i>T. newnesi</i> (all morphs combined)	18		122–193	38–22	29
<i>T. bernacchii</i>	9	350*	93–227	36–15	25
<i>Pagothenia borchgrevinkii</i>	12	280*	121–204	28–19	22
Harpagiferidae					
<i>Harpagifer antarcticus</i>	1	95	92	20	
Artedidraconidae					
<i>Pogonophryne scotti</i>	2	310*	147–206	16–8	
Bathydraconidae					
<i>Gymnodraco acuticeps</i>	1	340	189	19	
<i>Prionodraco evansii</i>	1	150	123	28	
<i>Vomeridens infuscipinnis</i>	1	220	178	40 ^c	
<i>Racovitzia glacialis</i>	1	240	182	35	
<i>Akarotaxis nudiceps</i>	1	130	112	46	
<i>Bathydraco macrolepis</i>	1	250	141	32	
<i>B. marri</i>	1	230	119	43	
<i>Parachaenichthys charcoti</i>	2	420	328–372	28–22	
Channichthyidae					
<i>Pagetopsis macropterus</i>	1	330*	142	36	
<i>P. maculatus</i>	1	250*	193	30	
<i>Champscephalus gunnari</i>	2	660*	426–431	20–19	
<i>Pseudochaenichthys georgianus</i>	2	600*	435–458	17–16	
<i>Chaenodraco wilsoni</i>	3	430*	174–233	46–31	
<i>Chionodraco rastrospinosus</i>	2	520*	310–315	30–28	
<i>Chaenocephalus aceratus</i>	2	750*	279–294	36–35	
<i>Cryodraco antarcticus</i>	1	570*	414	24	
<i>Dacodraco hunteri</i>	1	290*	204	40 ^d	

Arrangement of taxa is phylogenetic based on cladogram in Figure 1.

^aMaximum known sizes from various sources, especially Gon and Heemstra (1990); asterisk * indicates length is total length rather than standard length.

^bArrangement of values is from smallest to largest specimens.

^cData from Kuhn et al. (2011).

^dData from radiograph in Eastman (1999).

immersion-fixed samples of tissue from the skull, pectoral girdle, and vertebral centra in an area of the column centered on the first caudal vertebra. We decalcified tissues for 10–48 h in a

commercial solution containing EDTA in dilute HCl, and post-fixed them in Bouin's solution. We embedded samples in paraffin according to standard procedures. We cut 7- μ m-thick

sections and stained with Gomori's one step trichrome for 30 s, Pollak's trichrome for 7 min (Humason, 1979), or Bodian's Protagol for 24 h at 50°C (Clark, 1981).

We used an ocular micrometer fitted to a dissecting microscope to determine the relative size of the notochordal canal. We measured the diameter of the notochordal canal and the width of vertebral centra. We made these measurements transversally across the posterior aspect of one of the first three caudal vertebrae, usually the first. We took measurements from dry vertebrae or from vertebrae that were removed from alizarin-stained, glycerine-stored specimens.

Imaging Techniques

Micro-CT scanning. We scanned one entire adult specimen of *Bovichtus diacanthus* (SL = 153 mm) and of *Chaenodraco wilsoni* (SL = 203 mm), and the head of an adult *Eleginops maclovinus* (SL = 285 mm). The specimens were fixed in formalin and stored in 70% ethanol. All three specimens were scanned at the Ohio University MicroCT Facility (OU μ CT), using a GE eXplore Locus in vivo Small Animal MicroCT Scanner. Images were acquired at scan resolutions of 45 μ m (isotropic voxel sizes), 80 kVp, 450 μ A, 1,200 views around 360° with seven frames averaged per view, and an integration time of 400 ms. The resulting data volume (in VFF format) were exported from GE Healthcare's MicroView 2.1.2 (<http://sourceforge.net/projects/microview/>) to the DICOM format. These DICOM data were then imported to Avizo 7.1 (FEI Visualization Sciences Group, Burlington, MA) for analysis and visualization.

Soft radiography. We also used soft radiography, i.e., production of X-rays at low voltage with filtration through a beryllium window (Miller, 1957), for visualizing bone and cartilage in some formalin-fixed, alcohol-stored specimens. We produced radiographs with a Hewlett-Packard Faxitron soft X-ray machine. We operated the machine at 30 kVp and 3.0 mA, with an exposure time of 3–5 min. We used Kodak Industrex MX125 film (medium speed, very high contrast, high definition, very fine grain) in lead-backed cardboard cassettes. Film-to-source distance was 122 cm.

RESULTS

Skeletal Mass

In our sample of 20 species (15% of notothenioid diversity), body masses encompass four orders of magnitude with a 454-fold difference between the smallest and largest specimens. A scatter plot of relative skeletal mass (Fig. 2) indicates that notothenioid skeletons compose a smaller percentage of body mass than those of three species of non-notothenioid perciforms, at least as judged by these relatively few perciform measurements available in the literature. The arbitrary value separating the two groups is about 3.5%. With relatively heavy skeletons, the two specimens of the non-Antarctic *B. diacanthus* cluster among the lightest of the non-notothenioids. However, the three specimens of *E. maclovinus*, the non-Antarctic sister group of the Antarctic clade, have relatively light skeletons and cluster among the Antarctic clade. A relatively light skeleton, $\leq 2\%$ of body mass, is therefore a synapomorphy for *Eleginops* plus the Antarctic clade (Fig. 1).

Skeletal System Anatomy and Histology

Generalities. The skulls of teleost fishes, including notothenioids, are composed of a superfi-

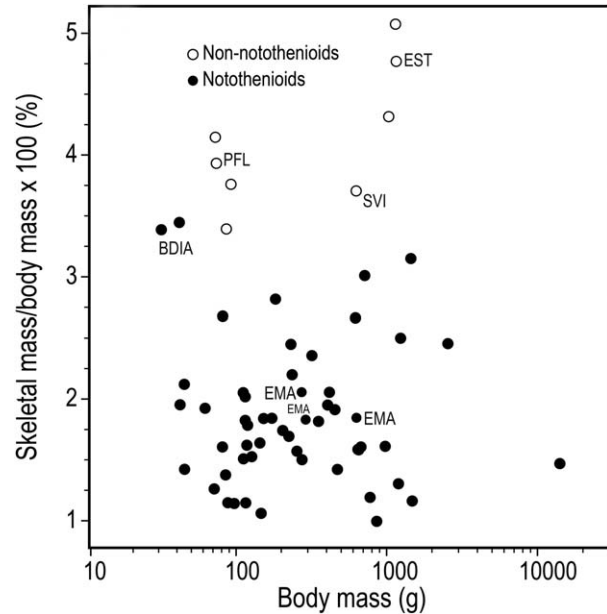


Fig. 2. Scatter plot on semilogarithmic coordinates showing relative skeletal mass. Circles are individual values for 54 notothenioids from 20 species and eight non-notothenioid perciforms (see Table 1, bottom) representing three species (data from Reynolds and Karlotski, 1977). The arbitrary separation between notothenioids and non-notothenioids is about 3.5%. Letter codes for species are given in Table 1.

cial dermal component and a deeper endoskeletal contribution that forms most of the mass of the neurocranium around the brain and special senses. The endoskeletal series, preformed in hyaline cartilage, includes bones such as the ethmoids, sphenoids, otics, and occipitals. The dermal addition invests the endoskeletal elements and is exemplified by the frontals, parietals, prevomer, and parasphenoid (Gregory, 1933, p. 88–90; Harder, 1975; Rojo, 1991). The dermal bones are peripheral to the perichondrium of the endoskeleton and develop via intramembranous ossification.

All notothenioid bone we examined is acellular, as would be expected in an acanthomorph group (Moss, 1961, 1963; Parenti, 1986), and the adaptive significance of this trait is unknown (Horton and Summers, 2009). Actinopterygian fish lack the Haversian organization typically seen in compact bone of other vertebrates (Meunier and Huysseune, 1992; Summers and Long, 2006). We recognize two subtypes of spongy bone: laminar (layers of various thicknesses) and cavitated (i.e., spongy, porous). We refer to small irregular shaped laminae of bone as trabeculae. All cartilage we observed is typical hyaline cartilage, Category 1 under the classification of Witten et al. (2010).

Bone and cartilage in the skull. Among notothenioids, there is variability in the mass and the nature of bone among taxa, and in the amount of cartilage in the skeleton. This is clearly seen in micro-CT scans of species representing families at

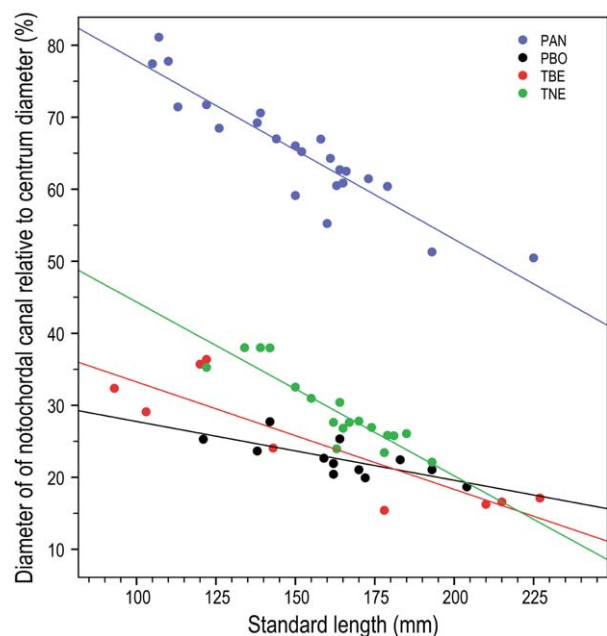


Fig. 3. Scatter plot and trend lines showing interspecific differences and ontogenetic changes in relative diameter of notochordal canal of the first caudal vertebrae with increasing body length in specimens of the nototheniids *Pleuragramma antarctica* ($N = 23$), *Pagothenia borchgrevinki* (12), *Trematomus bernacchii* (9), and *T. newnesi* (18).

the extremes of the phylogeny (Fig. 1). In Figures 4 and 5A,C,E, volumes are rendered in a false-color spectrum in which warmer and cooler colors (reds vs. blues and greens) represent materials of higher and lower density, respectively. The bovidid *B. diacanthus* (Figs. 4A and 5A,B) has a well-ossified skeleton, typical of many acanthomorphs. The red color thus indicates the presence and relative density of bone, and it is evident that all major neurocranial, as well as jaw, branchial, fin girdle, and vertebral elements are present and well ossified. However, this is not the case in the phylogenetically derived channichthyid *C. wilsoni* (Figs. 4B and 5E,F). Based on the limited red signal, there is relatively little bone and considerable cartilage that is not easily differentiated from other soft tissues producing a more widespread lower density blue signal. *Chaenodraco* and other channichthyids do not have a uniformly ossified skull with tight, or even abutting, sutures between bones. Instead, many dermal bones ossify as thin laminae that ensheath persisting cartilage. When a fresh skull dehydrates and the cartilage shrinks greatly in volume, the ensheathing nature of the bones is obvious by comparing Figure 7I versus 7J, and Figure 7K versus 7L. This is also evident in radiography of the dried skull of another channichthyid, *Pseudochaenichthys georgianus* (Fig. 6A). The ensheathing bones impart a predominantly gray tone to the image, although some bones possess foci of heavier ossification (white) that are sometimes

manifest as spinated or crenulated areas. The ethmoids, supraorbital region of frontals, sphenotics, pterotics, and occipital series are the best examples (Fig. 6A).

Persistent cartilage forms most of the roof, floor, and lateral walls of the channichthyid neurocranium (Figs. 4B and 5E,F). Although not evident in micro-CT scans, the prominence of cartilage is highlighted in histological sections. In *C. wilsoni*, the bone to the right of the supraorbital canal is only 68–70- μm thick (Fig. 9D), with considerable cartilage below. In *Chaenocephalus aceratus*, cartilage also dominates the posteriolateral skull; there are two thin (25 and 18 μm) laminae of bone dorsally (not shown) and a thicker lamina ventrally (Fig. 9F). The parasphenoid, ventral to the lens of the eye in Figure 5E, is one of few substantially ossified elements in the floor of the neurocranium. Based on the red signal, other well-ossified bones include the jaws, opercular apparatus with spines, branchiostegal apparatus, cleithral elements, occipital series, pelvic spines, and vertebral centra (Figs. 4B and 5E).

In addition to channichthyids, some nototheniids and most bathydraconids also retain partially cartilaginous neurocrania as adults. Small and large neutrally buoyant species of the family Nototheniidae are notable in this regard. In *Pleuragramma antarctica*, the dorsal neurocranium of a 163 mm SL specimen is largely cartilaginous, with roofing frontal bones that are only 18- μm thick (Fig. 9I). A 1,420-mm SL specimen of *Dissostichus mawsoni* has large areas of persistent cartilage in the ethmoid, frontal, otic, and occipital regions of the neurocranium (Fig. 6B).

Given the considerable differences in the skeletal tissue morphology between *Bovichtus* and channichthyids, what is the situation in *E. maclovinus*, the sister group of the Antarctic clade (Fig. 1)? Micro-CT scanning reveals that *Eleginops* has a head skeleton composed predominantly of spongy bone (Fig. 5C), although the cavitated nature of the bone is best seen in transverse section (Fig. 5D). The only exception in Figure 5D is the radiodense bone of the parasphenoid and branchiostegal rays. Histology provides additional documentation of the nature of the bone porosity. For example, the dorsal skull is a network of thin bony trabeculae surrounded by extensive irregular cavities containing adipocytes and connective tissue of the dermis (Fig. 9B). The bone of *Eleginops* does not give a red signal, as is the case in *Bovichtus* and *Chaenodraco*; the large areas histologically documented as spongy bone are instead green, representing reduced density. This may be an artifact of its preservational history in that this specimen was fixed and stored in unbuffered formalin for 5 months before being transferred to ethanol. Some loss of bone mineral from the thin trabeculae (Fig. 9B) may be responsible for a green rather than a red signal.

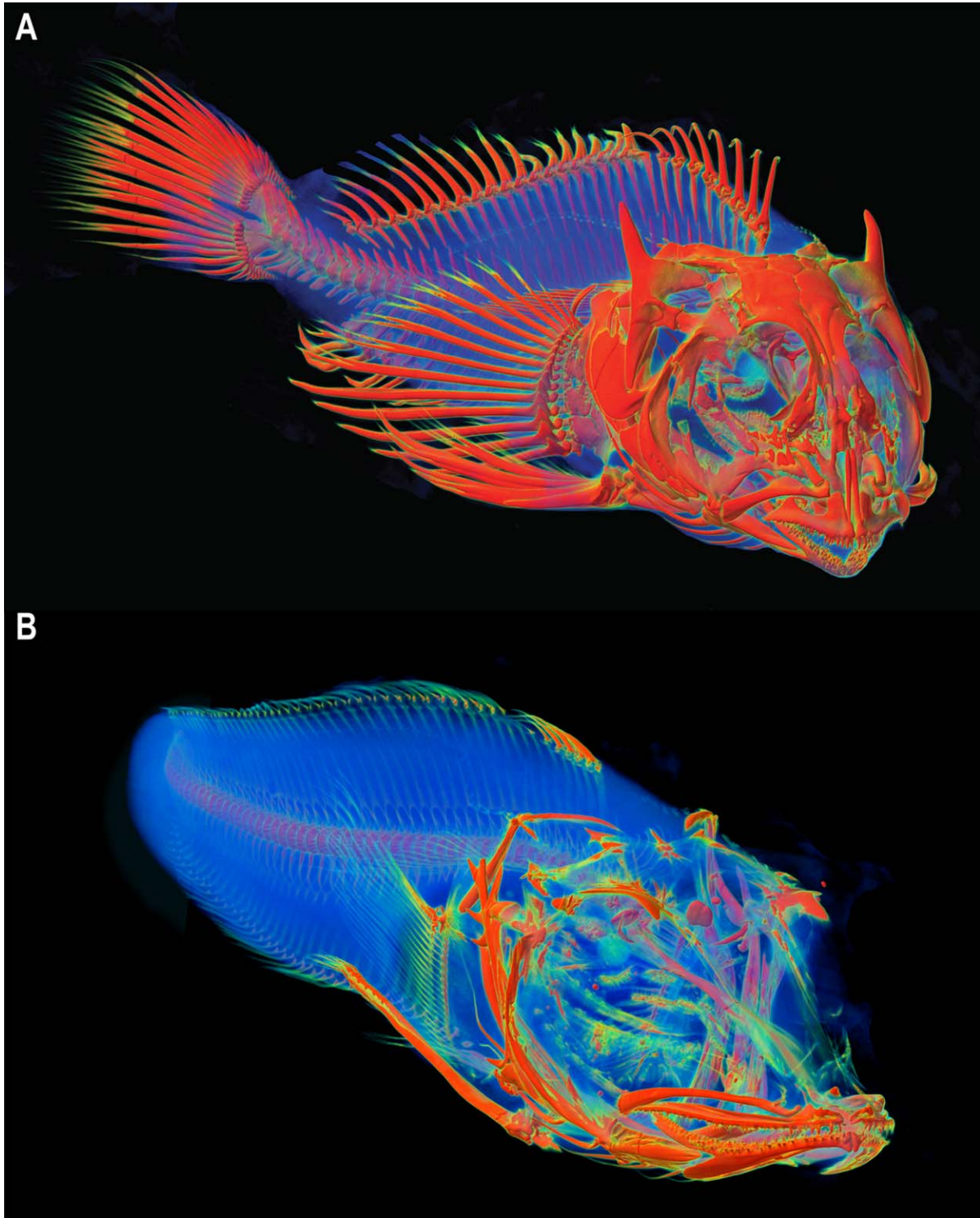


Fig. 4. Volume rendered micro-CT images showing differences in the amount of bone in adult skeletons of (A) non-Antarctic boviichthid *Bovichtus diacanthus* (SL = 153 mm) and (B) Antarctic channichthyid *Chaenodraco wilsoni* (SL = 203 mm). Tissue density is mapped in false color on a spectrum ranging from less dense (cool colors) to more dense (warm colors). Red color is a proxy for the density of bone which is considerably more extensive in *B. diacanthus*. Much of the neurocranium, paired fin girdles and branchial apparatus of *C. wilsoni* consists of cartilage not shown in the image or not discernible from other low-density (blue-colored) soft tissues.

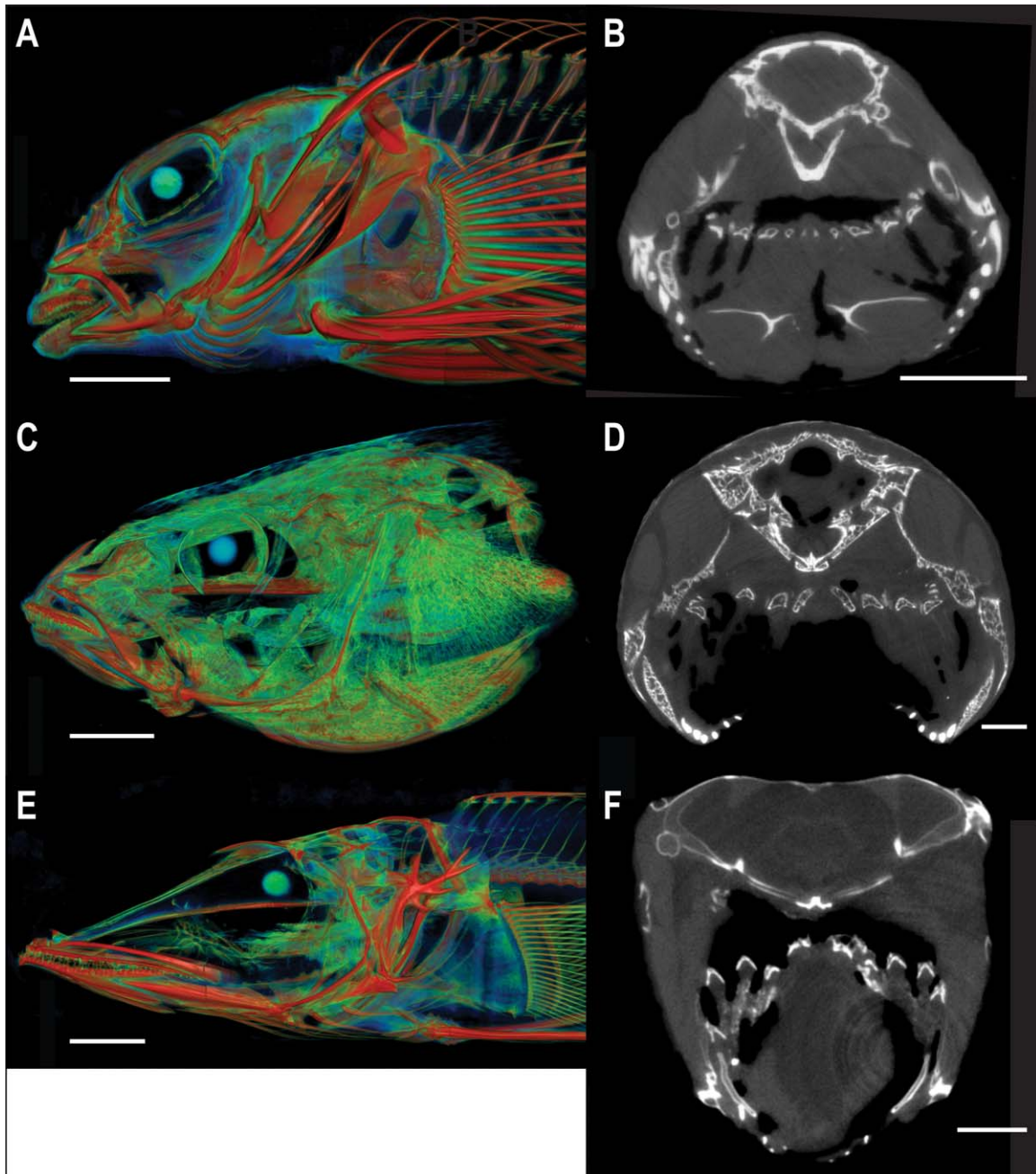


Fig. 5. Left lateral and transverse micro-CT images of adult (A,B) *Bovichtus diacanthus*, (C,D) *Eleginops maclovinus*, and (E,F) *Chaenodraco wilsoni*. A,B and E,F are same specimens as in Figure 4. C,D is eleginopsid (SL = 285 mm; head length = 72 mm), a phylogenetically intermediate species between *Bovichtus* and *Chaenodraco* and the sister group to the Antarctic clade. In lateral views (A,C,E), *Bovichtus* (A) has dense well-ossified bone as indicated by red tone (see caption for Fig. 4 for explanation of the color mapping). In *Eleginops* (C), spongy bone with extensive lipid-filled cavities has lower density and bony mass that collectively register as green (with the possibility of the green attributable to partial demineralization by formalin storage—see text). In *Chaenodraco* (E), red indicates that jaw and opercular elements are ossified but extensive cartilage persists and is visible in pectoral girdle which also shows foci of weak ossification in the radials. Transverse images (B,D,F) show differences in nature and distribution of bone (bright white tones) in equivalent regions of heads posterior to the orbit. Prominent calcified elements visible in transverse sections include, dorsoventrally, in *Bovichtus* (B), dense bone of neurocranium, fifth ceratobranchials, gill arch elements, opercular and cleithral elements, pelvic girdle, and branchiostegal rays; in *Eleginops* (D), spongy bone of neurocranium and most other bones, gill arch elements, opercular and cleithral, and branchiostegal rays and; in *Chaenodraco* (F), thin laminae of bone ensheathing cartilaginous neurocranium with midventral basioccipital most prominent, dentigerous gill rakers, opercular and cleithral elements (cartilage surrounded by thin layer of bone), and branchiostegal rays. Scale bars: (A,B,C,E) 10 mm and (D,F) 5 mm.

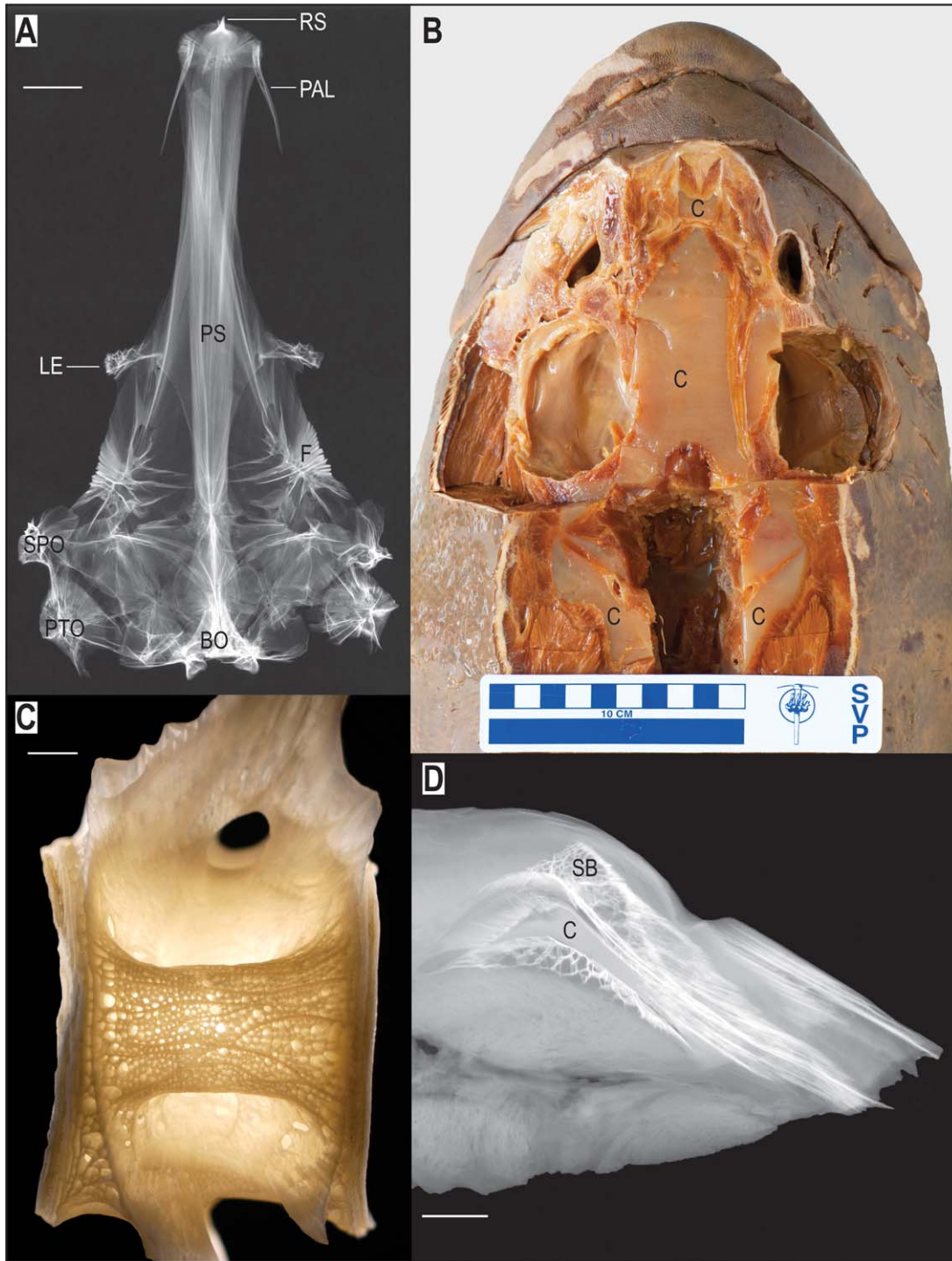


Fig. 6. Aspects of skeletal morphology in adult notothenioids. (A) Channichthyid *Pseudochaenichthys georgianus* and (B–D) nototheniid *Dissostichus mawsoni*. (A) Radiograph of dried skull in Figure 7J showing thin ensheathing bone of neurocranium in general (gray), exemplified by parasphenoid, with foci of heavier ossification (white) in other bones, especially the occipital series. (B) Extensive cartilage in dorsal skull of formalin-preserved alcohol-stored specimen (SL = 1,240 mm). (C) Trans-illumination reveals spongy bone with array of small cavities (lipid-filled in life) in center of fourth caudal vertebra of *D. mawsoni* (SL = 880 mm). (D) Radiograph of transverse section of portion of pectoral fin musculature and girdle of *D. mawsoni* (SL = 1,000 mm) showing spongy bone with variously sized cavities surrounding a core of cartilage. Scale bars: (A,D) 10 mm and (C) 2 mm. Abbreviations: BO, basioccipital; C, cartilage; F, crenulated supraorbital ridge of frontal; LE, lateral ethmoid; PAL, palatine; PS, parasphenoid; PTO, pterotic; RS, rostral spine of ethmoid; SB, spongy bone; and SPO, sphenotic.

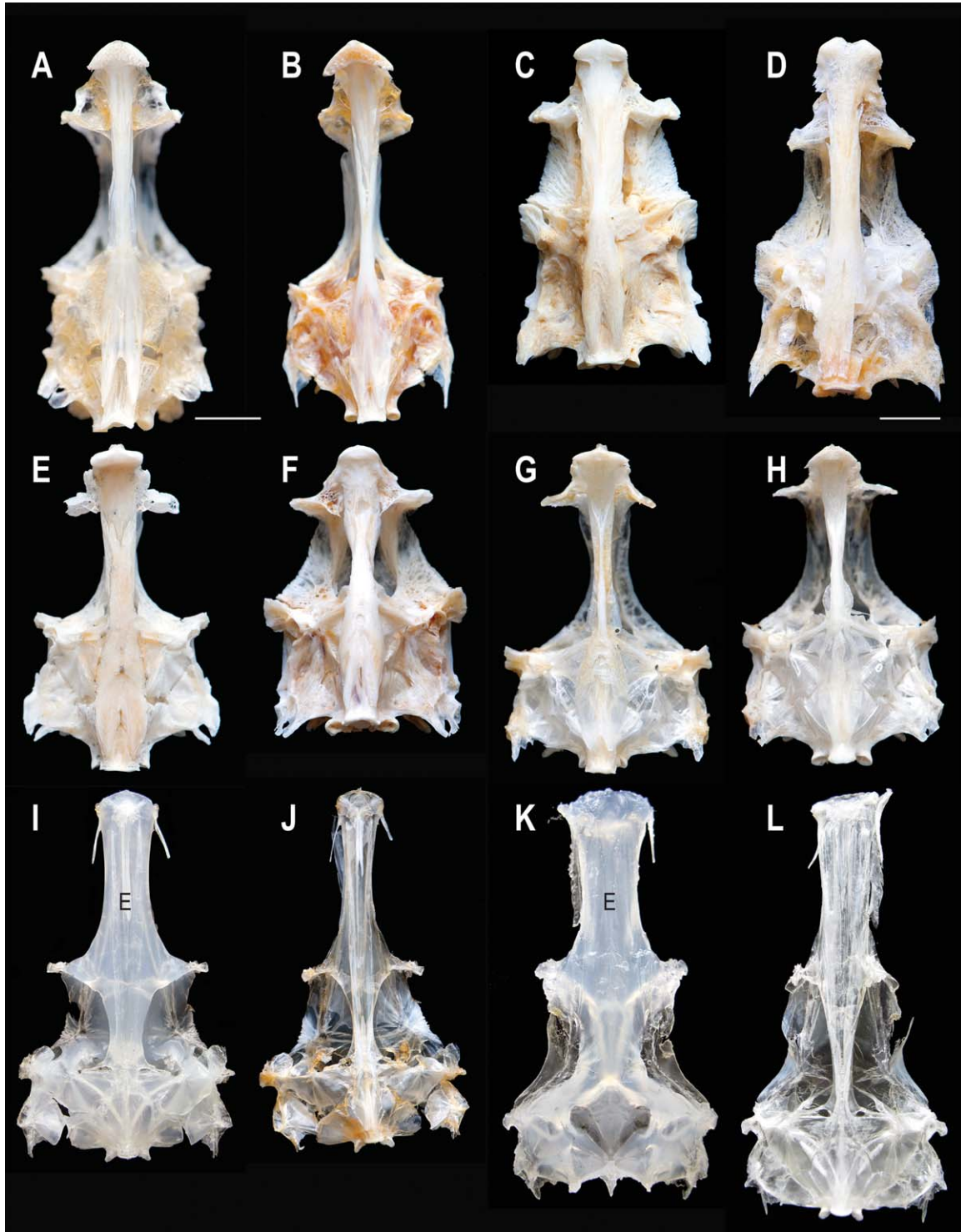


Fig. 7. Ventral view of adult skulls in a phylogenetic series (Fig. 1) representing four of eight notothenioid families: (A) *Bovichtus diacanthus*; (B) *Cottoperca trigloides*; (C) *Eleginops maclovinus*; (D) *Dissostichus eleginoides*; (E) *Gobionotothen gibberifrons*; (F) *Notothenia coriiceps*; (G) *Lepidonotothen squamifrons*; (H) *Trematomus bernacchii*; (I, J) *Pseudochaenichthys georgianus*, I when fresh and wet, showing extensive opaque ethmoid cartilage, and J when dried; and (K, L) *Cryodraco antarcticus*, K when fresh and wet showing cartilage, and L when dried. Smallest skull is *B. diacanthus* (A) at 29 mm long; largest is *D. eleginoides* (D) at 117 mm long. Scale bars: (A) 5 mm and (D) 20 mm. Abbreviation: E, ethmoid cartilage.

The phylogenetic series of skulls in Figure 7, representing four of the eight families, shows that the superficial bone is either cavitated, ridged, or trabeculated or thin smooth-surfaced laminae. In *Eleginops* (Fig. 7C) and some larger and mid-sized nototheniid species (Fig. 7D–G), the bones are pitted by cavities containing adipocytes. The same is true for *Notothenia coriiceps*, a large nototheniid with a heavy skeleton (Fig. 7F). However, other smaller nototheniid species, trematomids for example, have thin and delicate laminar bone (Fig. 7H). The superficial bones in bovichtids (Fig. 7A,B), bathydraconids (not shown), and channichthyids (Fig. 7I–L) are usually smooth-surfaced laminae. The characteristic elongated snout region of channichthyids (Fig. 7I–L) is formed by the thin laminar ethmoids, underlain by cartilage that composes most of the mass of the snout (Fig. 7I,K).

Bone and cartilage in the pectoral girdle.

In notothenioids, as in other acanthomorphs (Koumoundouros et al., 1999), the scapula, coracoid, and radials of the pectoral girdle are endochondral bones. Transverse histological sections of adult pectoral girdles show that skeletal tissue is arranged as a core of cartilage sandwiched between peripheral layers of bone of variable thickness. In a phylogenetic series (Fig. 9A,C,E), cartilage becomes progressively a more dominant component between progressively thinner layers of peripheral bone. For example, at the midpoint of the section (Fig. 9A), *B. diacanthus* has 75- and 200- μm -thick layers of compact bone with a core of persistent cartilage and adipose tissue. In the eleginopsid *E. maclovinus*, peripheral bone is arranged as laminae 13–35- μm -thick, with a core of cartilage and adipose tissue (Fig. 9C). However, in the channichthyid *C. aceratus* (Fig. 9E), most of the mass of the structure of the girdle is formed by a thick layer of cartilage bordered by 15- μm -thick layers of superficial bone. The percentage thickness of the girdle occupied by bone in these three species is 39, 13, and 3%, respectively. Finally, cartilage also persists in the pectoral girdle of most other species, including the neutrally buoyant nototheniids as exemplified by a 1,000 mm SL specimen of *D. mawsoni* (Fig. 6D).

Bone of the vertebral centra. The structure of the vertebral centra of notothenioids conforms to the basic teleost pattern (Ford, 1937; Laerm, 1976). There is no cartilage precursor or persistent cartilage and, instead, the initial mineralization occurs within the notochordal sheath, with a subsequent addition of intramembraneous bone from the somites (François, 1966; Laerm, 1982; Koumoundouros et al., 1999; Bird and Mabee, 2003; Bensimon-Brito et al., 2012). This has been confirmed for nototheniids in the developing urostylel vertebrae of five species, including *P. antarctica* (Voskoboinikova et al., 2004). Centra of adult noto-

thenioids, exemplified by *E. maclovinus*, are cylindrical and amphicelous, with the hourglass shape especially evident in mid sagittal section (Fig. 8A,G). All notothenioids have a notochordal canal at the bony constriction point, but there is considerable interspecific and ontogenetic variation in the patency of the canal (see below). There is a thin, biconid-shaped layer of laminar bone adjacent to the notochordal cavities (Fig. 8A,B). Internally, the bone of the centrum is spongy, with cavities that are extremely variable in size and shape. Cavities are lined by periosteum, filled with adipocytes, and have smooth walls that do not exhibit evidence of erosion by osteoclasts (Fig. 8B,C).

Figure 8D–O is a phylogenetic series showing the lateral aspect of the first caudal centrum from each of 12 species representing seven of the eight nototheniid families. There is considerable variation in the sculpting of the lateral surfaces of the centra, especially in the number and thickness of the longitudinal ridges and in the degree of porosity of the bone (Fig. 8E,I,J,L,N). For example, the bone of the centra shows little porosity in *B. diacanthus* (Fig. 8D). In *E. maclovinus*, the sister group of the Antarctic clade, centra consist of spongy bone (Fig. 8A,B,G). All species in the Antarctic clade also have reduced skeletal mass compared to *Bovichtus* (Table 1, Fig. 2), but this is not necessarily associated with extremely porous bone in the centra. For example, the centra are composed of lamellae and cavities of various sizes in four nototheniids (Fig. 8H–K), an artedidraconid (Fig. 8L), a bathydraconid (Fig. 8M), and two channichthyids (Fig. 8N,O). Another nototheniid, *Notothenia angustata*, a heavy benthic species with largest skeletal mass in this family, has a more massive centrum with a single thick longitudinal ridge and little spongy bone (Fig. 8J).

The three neutrally buoyant nototheniids with weakly ossified skeletons have centra with different morphology and therefore deserve comment. With the exception of *Eleginops*, spongy bone with small cavities is rare in our sample of nototheniid centra, but bone with small cavities is especially pervasive in the centra of *D. mawsoni* (Fig. 6C) and *D. eleginoides* (Fig. 8H). After removal of lipid, transillumination reveals the delicate nature of the lattice and the extreme porosity of the bone in *D. mawsoni* (Fig. 6C). With a percentage skeletal mass of 1.47%, this species has one of the five lightest skeletons of the 20 species for which we have data (Table 1). A different means of reduction of the bone mass is seen in a closely related species, *P. antarctica*. The centra exist as a collar of thin laminar bone, with many small longitudinal ridges, around the notochordal canal and partially persistent notochord (Fig. 9G,H). Some of the bony mass of the centra is therefore absent and the centra are less amphicelous. We do not have skeletal mass data for *Pleuragramma*.

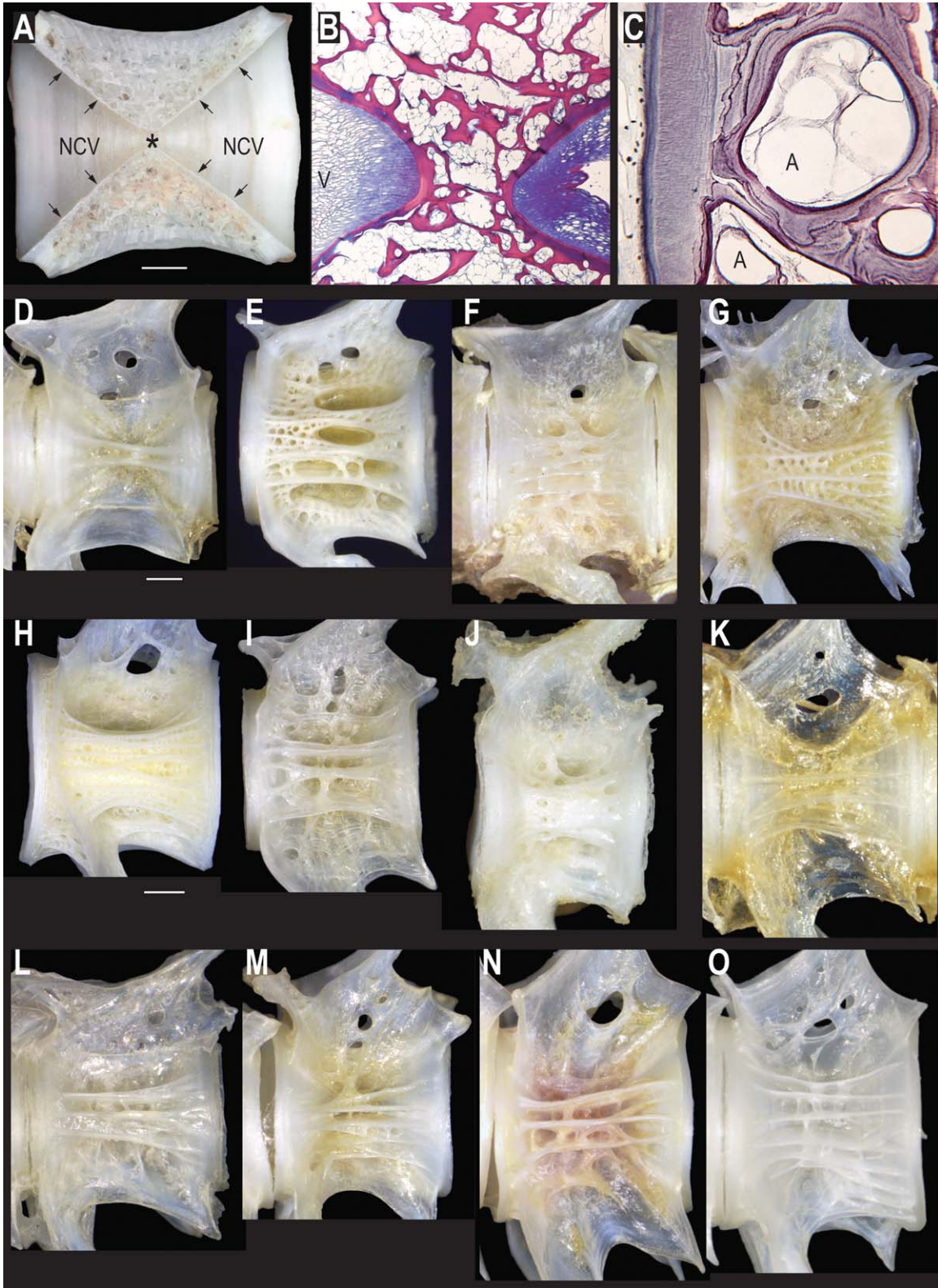


Fig. 8. **A-C.** Anatomy and histology of centrum of first caudal vertebrae of *Eleginops maclovinus*. (A) Sanded midsagittal section showing minimal amount of laminar bone (arrowheads) bordering the large biconcave notochordal cavities, with remainder of centrum consisting of spongy bone. This species has a small notochordal canal (asterisk) in the middle of each centrum. (B,C) Parasagittal sections displaying histological detail of centra. (B) Pattern of red-staining bone and lipid-filled cavities. Vacuolated cells fill notochordal cavities. Width of bone at constriction is 0.6 mm in life and magnification is $\times 27$. Stain: Gomori's trichrome. (C) Nuclear staining and photography at higher magnification indicate bone is acellular. Dark nuclei adjacent to vacuoles are those of prevacuolar cells of notochord. Lipid in cavities of spongy bone is contained in adipocytes. Dark lines in bone matrix are growth checks. Layer of laminar bone is adjacent to notochordal canals and vacuolated cells. Vertical diameter of large cavity is 200 μm in life and magnification is $\times 160$. Stain: Bodian's protargol. **D-O.** Left lateral view of centra of anterior caudal vertebrae in a phylogenetic series (Fig. 1) representing seven of eight notothenioid families: (D) *Bovichtus diacanthus*; (E) *Cottopeca trigloides*; (F) *Pseudaphritis urvillii*; (G) *E. maclovinus*; (H) *Dissostichus eleginoides*; (I) *Gobionotothen gibberifrons*; (J) *Notothenia angustata*; (K) *Pagothenia borchgrevinki*; (L) *Pogonophryne scotti*; (M) *Parachaenichthys charcoti*; (N) *Champscephalus gunnari*; and (O) *Chaenocephalus aceratus*. Vertebra is *B. diacanthus* (D) at 2.5 mm in horizontal length; largest is *D. eleginoides* (H) at 9.4 mm. Scale bars: (D) 0.5 mm and (H) 2 mm. Abbreviations: A, adipocytes; NCV, notochordal cavities; and V, vacuolated cells of notochord.

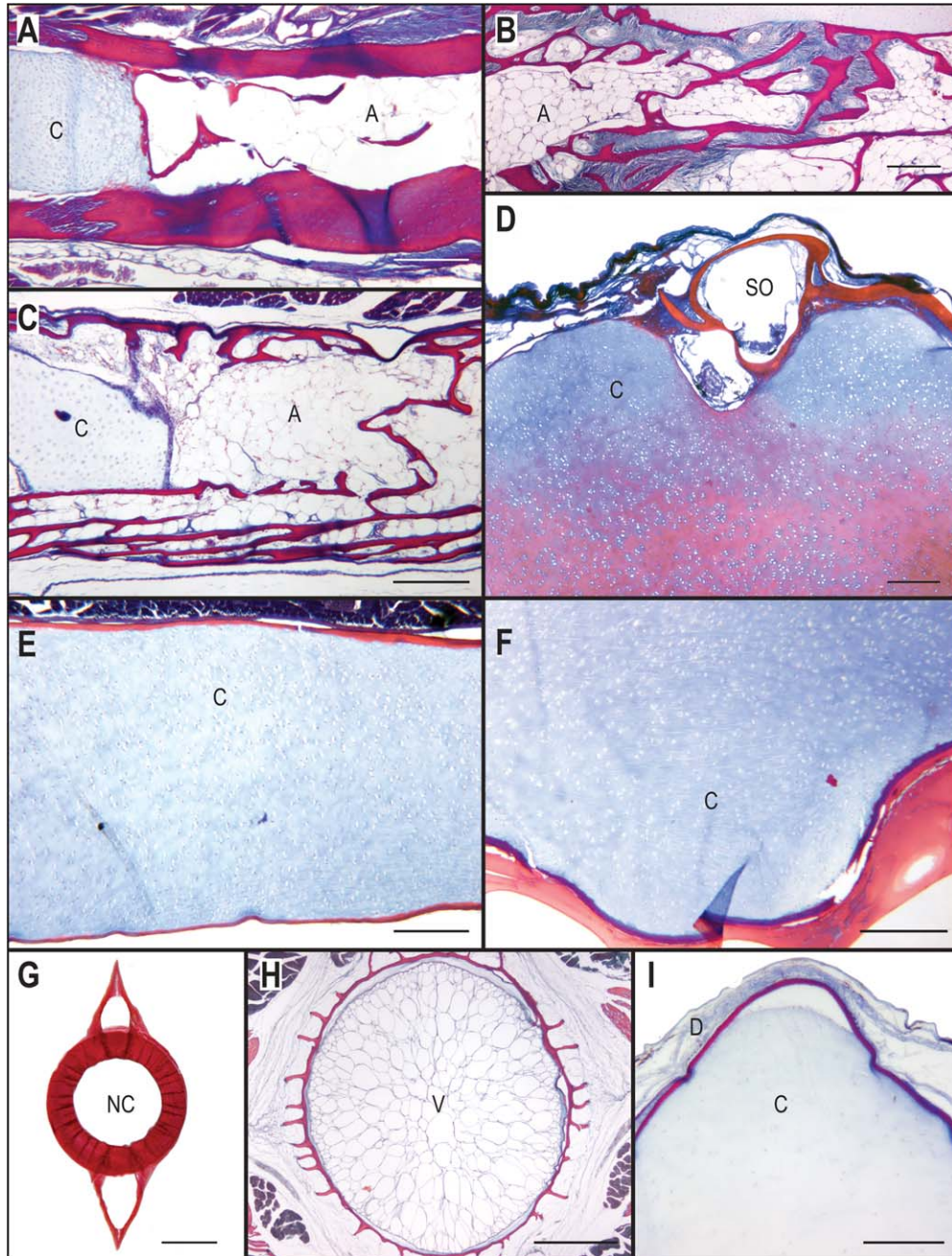


Fig. 9. Variable distribution of bone and cartilage in adult notothenioids. **A, C, E.** Transverse sections of pectoral girdles from (A) *Bovichtus diacanthus*, (C) *Egeinops maclovinus*, and (E) *Chaenocephalus aceratus* showing that in this phylogenetic series there is a decrease in red-staining bone and an increase in blue-staining cartilage. Stain: Gomori's trichrome. **B.** Transverse section of skull dorsal to opercular region of *E. maclovinus* shows spongy bone with large cavities containing adipose tissue and blue-staining dense connective tissue. Stain: Pollak's trichrome. **D.** Transverse section of dorsal skull of channichthyid *Chaenodraco wilsoni* with thin laminae of bone around the supraorbital canal and extensive underlying cartilage. Red staining of matrix does not signify calcified cartilage but is an artifact of staining possibly caused by tissue disruption due to freezing. Stain: Gomori's trichrome. **F.** Transverse section of posterior-lateral skull of channichthyid *C. aceratus* also showing thin bone and preponderance of cartilage. Stain: Gomori's trichrome. **G–I.** Bone anatomy and histology of *Pleuragramma antarctica*. (G) Posterior view of one of the anterior-most caudal vertebrae of 164 mm SL specimen reveals large size (62% of transverse centrum diameter) of notochordal canal. Stain: alizarin red S. (H) Transverse section of caudal peduncle vertebra of 170 mm specimen shows bone of centra as thin collar surrounding vacuolated cells of a partially persistent notochord. Stain: Pollak's trichrome. (I) Transverse section of interorbital region of skull of 163 mm specimen with thin frontal bone underlain by extensive cartilaginous neurocranium with relatively few chondrocytes. Stain: Gomori's trichrome. Abbreviations: A, Adipocytes; C, cartilage; D, dermis of skin; NC, notochordal canal; SO, supraorbital canal; and V, vacuolated cells of notochord. Scale bars: (A–F,I) 250 μ m, (G) 2 mm, and (H) 0.5 mm.

Interspecific and Ontogenetic Variation in the Size of the Notochordal Canal

Table 2 summarizes the extensive interspecific variation that exists in the size of the notochordal canal in a sample of vertebrae of 130 adults representing 38 species (29% of notothenioid diversity) from all eight families. There is also ontogenetic variation, specifically a decrease in relative size of the notochordal canal—meaning that the notochord and canal are larger in smaller specimens and decrease in size with increasing body length. This is graphed for an ontogenetic series in each of four species (Fig. 3), but the trend is also present in all species we examined when individuals were of different sizes (Table 2). As the canals become constricted by bone growth during ontogeny, the centra become more amphicelous.

Examination of Table 2 also indicates that species in the three non-Antarctic families have relatively small canals ($\leq 11\%$), whereas most species in the Antarctic clade have larger canals. Using a value of $\geq 48\%$ as a break point, relatively large canals are present in representatives of three families (Fig. 1). The neutrally buoyant nototheniid species *P. antarctica* (Fig. 9G,H) and *Aethotaxis mitopteryx* have especially large canals and are distinct among notothenioids in the extent of this pedomorphic trait. Consequently, the bone mass of the centra is reduced by the persistence of a partial notochord, and centra are not completely amphicelous. The next largest canals (32–46%) are seen in the bathydraconid genera *Vomeridens*, *Racovitza*, *Akarotaxis*, and *Bathyraco*, and in the channichthyid genera *Pagetopsis*, *Chaenodraco*, *Chaenocephalus*, and *Dacodraco*. Modest sized canals (25–30%) are present in the nototheniid genera *Trematomus* and *Pagothenia*, and in the bathydraconid genus *Prionodraco*. Members of the benthic nototheniid genus *Notothenia* and of the benthic families Harpagiferidae and Artedidraconidae have small canals ($\leq 20\%$, and frequently $< 10\%$ in larger specimens). Although neutrally buoyant and possessing extremely porous bone in their centra, the large heavily muscled, migratory nototheniids *D. mawsoni* and *D. eleginoides* have very small (4%) canals.

DISCUSSION

Skeletal Mass and Life History

Although our data on skeletal mass for 20 species represent only 15% of notothenioid diversity (Table 1, Fig. 2), the higher level taxonomic coverage (six of eight families) provides reasonable confidence in our results concerning skeletal mass within the clade. These are 1) that notothenioid skeletons comprise $< 3.5\%$ of body mass, a lower value than those for three non-notothenioid perciform species; 2) that non-Antarctic *B. diacanthus*

has the highest skeletal mass among the notothenioids sampled; 3) that *E. maclovinus*, the non-Antarctic sister group of the Antarctic clade, has a lighter skeleton than *Bovichtus* and, in a scatter plot, clusters among species of the Antarctic clade; and 4) that a relatively light skeleton, frequently $\leq 2\%$ of body mass, is a synapomorphy for *Eleginops* plus the Antarctic clade (Fig. 1), rather than a trait confined exclusively to the Antarctic clade. Given the sparse data for skeletal mass in non-notothenioid perciforms (see bottom of Table 1), we have no basis for discussing either the frequency or phylogenetic distribution of reduced skeletal mass in other acanthomorphs.

There are several life history factors that may influence skeletal mass in fishes including degree of activity and mode of locomotion. There are no continuously swimming notothenioids—most are sedentary as exemplified by the benthic nototheniid *N. coriiceps*, a species with mean percentage skeletal mass and mean percentage buoyancy of 2.80 and 4.34%, respectively. A 330-mm TL individual monitored with an underwater video camera at Signy Island in the South Orkneys remained within 3 m of a small cave more than 98% of the time, and swam only 1.7% of each day, usually for less than 30 s (North, 1996). For low-speed swimming, most notothenioids use drag-based labriform locomotion, meaning the pectoral fins oscillate in an anterior–posterior fashion, supplemented with occasional bursts of undulatory subcarangiform propulsion (Montgomery and Macdonald, 1984; Archer and Johnston, 1989).

Although a four or five notothenioid species are neutrally buoyant and pelagic, their life histories do not include periods of sustained swimming. For example, the neutrally buoyant *P. antarctica* has respiratory and cardiovascular parameters reflecting a low activity level (Kunzmann, 1990; Tamburini et al., 1997; Wöhrmann et al., 1997), and it hangs in the water column while monitoring zooplankton prey with its well-developed visual and mechanosensory systems (Eastman and Lannoo, 2011). The presence of a weakly ossified skeleton, including a partially hydrostatic vertebral column, is not problematic but advantageous in reducing density. Another neutrally buoyant nototheniid, *D. mawsoni*, a large predator, is migratory and may swim as far as 1,000 km for purposes of reproduction and feeding (Yukhov, 1982; Hanchet et al., 2008). This is accomplished using primarily labriform locomotion (Eastman, 1993, pp. 202–205) to cover distances of at least 6 km/day (Petrov and Tatarnikov, 2010). Although it has a light skeleton with porous bone and persistent cartilage, there is no doubt that the musculoskeletal system has the capacity for sustained labriform locomotion—a vagrant individual of the morphologically identical sister species, *D. eleginoides*, completed a transequatorial migration from the Patagonian shelf to

Greenland, a distance of 10,000 km (Møller et al., 2003).

Diversification in Bone and Cartilage Morphology

General trends. Having established that there is reduced bone mass in *Eleginops* plus the Antarctic clade, how is this manifest morphologically? Gosline (1971, p. 11) observed that among teleosts “the interrelationships between the notochord, cartilage, and bone are highly complex, and the extent of displacement of one skeletal material by another in the different fishes varies greatly.” He also notes that there is a general and repeated phylogenetic trend involving ancestral members of a lineage that are frequently “bonier” than derived members. Gosline’s mention of the notochord also implies an awareness of a role for heterochronic processes in determining skeletal tissue composition in adult fishes.

Gosline’s (1971) statements are consistent with what we have found in notothenioids. Non-Antarctic *B. diacanthus* has a relatively heavy, mostly bony skeleton. Extensive spongy bone appears in *E. maclovinus*, the sister group of the Antarctic clade. In the Antarctic clade, bone is also spongy, with a fine lipid-filled mesh in the two neutrally buoyant species of *Dissostichus*. Other species in the Antarctic clade have thin laminar bone, spongy bone, or a mixture thereof. Persistent cartilage is present in most species, especially in the neurocranium and pectoral girdle, and is prominent in some bathydraconids and all channichthyids where thin laminar bone ensheathes the cartilage. In the vertebral centra, a relatively large notochordal canal associated with a partially persistent notochord is found in various degrees throughout the Antarctic clade but is most prominent in the nototheniid genera *Pleuragramma* and *Aethotaxis* and in some genera of bathydraconids and channichthyids. On the basis of their studies of individual families, Balushkin (1984), Iwami (1985, p. 63), and Voskoboinikova (1993) consider the loss or reduction of bones and teeth to be a recognizable evolutionary trend in notothenioids. Our broader taxonomic sampling here establishes the extremes of nototheniid skeletal morphology as encompassing a typical bony skeleton (Fig. 4A) as well as one with little bone and considerable cartilage (Fig. 4B), as revealed in the micro-CTs of *B. diacanthus* and *C. wilsoni*. This is also supported by our data on percentage skeletal mass—3.42% for *B. diacanthus* and a mean of 1.57% for five channichthyid species.

Our findings are also compatible with Voskoboinikova’s study of the developmental osteology of larval and juvenile notothenioids showing that *Bovichtus angustifrons* has the most rapid rate of skeletal development among notothenioids, with

bones appearing in larvae of relatively small size (Voskoboinikova and Bruce, 2001). Relative to *Bovichtus*, nonbovichtid notothenioids have (Voskoboinikova, 2001) 1) a delay in the appearance (postdisplacement) of numerous bony elements such that the larvae are larger on the initial appearance of a given element and 2) a more pronounced delay in some phylogenetically derived families, especially some nototheniids, many bathydraconids and all channichthyids. For example, the larvae of channichthyids are 2.2–4.7-fold longer (in SL) than those of *B. angustifrons* when the anlagen of most bones appear.

Implications of extensive cartilage in channichthyids. Cartilage is the primary skeletal material in larval actinopterygian fishes (Summers and Long, 2006, p. 55), and studies indicate that most of the cartilage of the developing teleost head serves “as models for ossified elements” (Walter, 2013, p. 90). There is nothing unusual about the retention of cartilage in the adult teleost skeleton; salmonids are a classic example (Gregory, 1933, p. 153; de Beer, 1937, p.115–130; Norden, 1961). What is unusual in channichthyids is the extent of cartilage retained in the head—it is more of a scaffold than a model for the skull bones which are just a veneer. Recent work has revealed the molecular basis for this heterochronic shift in early cranial development, especially in hyal, suspensory, and jaw elements, in benthopelagic channichthyids. Relative to benthic nototheniids and non-notothenioids with more heavily ossified skeletons, channichthyids have the most delayed osteogenic development among notothenioids (Voskoboinikova, 1997; Albertson et al., 2010). This heterochronic shift is attributed to altered gene expression, including delayed expression of the osteogenic markers *coll1a1* and *col10a1*, and prolonged expression of the cartilage differentiation gene *col2a1* (Albertson et al., 2010, p. 2). Albertson et al. (2010) suggest that these changes in collagen gene expression patterns in pelagic notothenioids with lighter skeletons, including *P. antarctica*, could be attributable to mutations in regulatory genes.

One consequence of the minimal bone in the channichthyid head (Iwami, 1985, p.63) is the potential for greater lateral and dorsoventral expansion of the oral and branchial cavities. Loss of rigidity of the head may facilitate feeding on fish and swarms of krill. Channichthyids are ram feeders, swimming to and grasping their prey. They exhibit morphology typical of ram feeders, including an elongated head, an enormous gape and a nonprotractile jaw lacking an ascending process of the premaxilla (Liem, 1993). The jaw teeth are small and conical with a Type 2 mode of attachment, meaning that a collagenous ligament binds the tooth base to the jaw bone, another pedomorphic trait (Fink, 1981). The preferred

diets of many of the 16 species include fish and, as adults, some of the larger channichthyids, such as *C. aceratus* with a maximum TL of 75 cm (Iwami and Kock, 1990), feed almost exclusively on fish (Kock, 2005; Reid et al., 2007; Kock et al., 2013). They routinely engulf other large-headed notothenioids that are 40–50% of their own length, and a *C. aceratus* has been documented as having swallowed a *D. mawsoni* that was 76% of its length and 41% of its weight (Kock et al., 2013). However, pharyngeal gape in fishes is usually smaller than oral gape, and is likely more important in determining what can be swallowed (Wainwright and Richard, 1995), especially when the prey are large or have a rigid exoskeleton. The limiting dimension in the predator is thought to be the distance between the cleithra of the pectoral girdles (Wainwright and Richard, 1995). In comparing the micro-CT scans of *Bovichtus* and *Chaenodraco* in Figures 4 and 5, it can be seen that the right cleithrum in the former is wider, with a medially projecting shelf, whereas in the latter it is less extensive and more planar.

Final Remarks: Adaptation, Heterochrony and Notothenioid Diversification

In both text (Hill et al., 2008) and popular books (Carroll, 2006), notothenioids are highlighted as exemplars of fascinating physiological specializations for life in subzero oxygen-saturated seawater. Some traits are obviously adaptive (antifreeze glycopeptides), whereas others (loss of hemoglobin and ventricular myoglobin) are mutations that would probably have been lethal in any other habitat given the extensive compensation necessary to maintain cardiorespiratory function in the white-blooded channichthyids. However, the appearance of antifreeze preceded most speciation and ecological diversification by at least 10 million years, and therefore it may be viewed as a constitutive adaptation, enabling survival in subzero seawater, rather a key adaptation directly linked to ecological diversification. There are no known adaptations coupled with the three major bursts of relatively recent diversification that resulted in about 50% of modern notothenioid species (Near et al., 2012).

Contrary to the suggestion by Albertson et al. (2010, p. 8), reduced bone mass is not linked to the adaptive radiation of the Antarctic clade but was, as we have shown here, present in their non-Antarctic sister group Eleginopsidae. Given that notothenioids lack a swim bladder, reduction of body density is difficult without some evolutionary modification of dense skeletal tissues. This is accomplished through heterochrony (Gould, 1977), not by novel adult morphology. Pedomorphosis has played an important role in shaping skeletal tissue morphology since early in the history of jawed fishes. For example, notothenioids possess some of

the same pedomorphic traits, such as persistent cartilage in the skull, seen in dipnoan lungfishes (Bemis, 1984).

Like those of the majority of marine fishes (Leis et al., 2011), notothenioid larvae and juveniles are pelagic (Loeb et al., 1993). In the case of the Antarctic notothenioid clade, truncation of bone development in larvae and juveniles by pedomorphosis furnished density-reducing traits that provided potential for niche expansion, adaptive differentiation, and speciation. Unoccupied niche space was available about 22 million years ago because notothenioids were entering a developing Antarctic marine ecosystem where they faced little competition from midwater fishes lacking antifreeze (Near et al., 2012). The Antarctic clade had the relatively low skeletal mass present in their non-Antarctic sister group. Although the weakly ossified skeleton and unconstructed notochord of the nototheniid *P. antarctica* have been known for a century (Totten, 1914), Balushkin (1984, p. 128) was the first to recognize the evolutionary potential of pelagic larvae in nototheniids. Klingenberg and Ekau (1996) subsequently demonstrated the importance of larval growth and morphology in an ecomorphological study of diversification into water column and benthic niches in 10 nototheniid species. They found that morphometric differences in the distinguishing features of pelagic and benthic species develop by divergent growth in larval stages, with growth after metamorphosis accounting for relatively little of the observed interspecific variation in pelagic and benthic traits.

Notothenioids are percomorphs, a group encompassing more than one-third of all fishes (Nelson, 2006), and also exhibiting the most accelerated rate of diversification of the six vertebrate clades with unusually high rates of diversification (Alfaro et al., 2009). Even without the introduction of novel morphology, the percomorph developmental program and body plan have proven to have exceptional potential for adaptation and radiation in many freshwater and littoral marine habitats including some, like the Antarctic, commonly viewed as extreme.

ACKNOWLEDGMENTS

The authors are most grateful to Danette Pratt for assembling the figures and to John Sattler for photography. They also thank Esteban Barrera-Oro for collecting and contributing fish from King George Island, and David Petzel for collecting and contributing specimens from McMurdo Sound. An anonymous reviewer and Steve Reilly provided helpful comments on the manuscript.

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