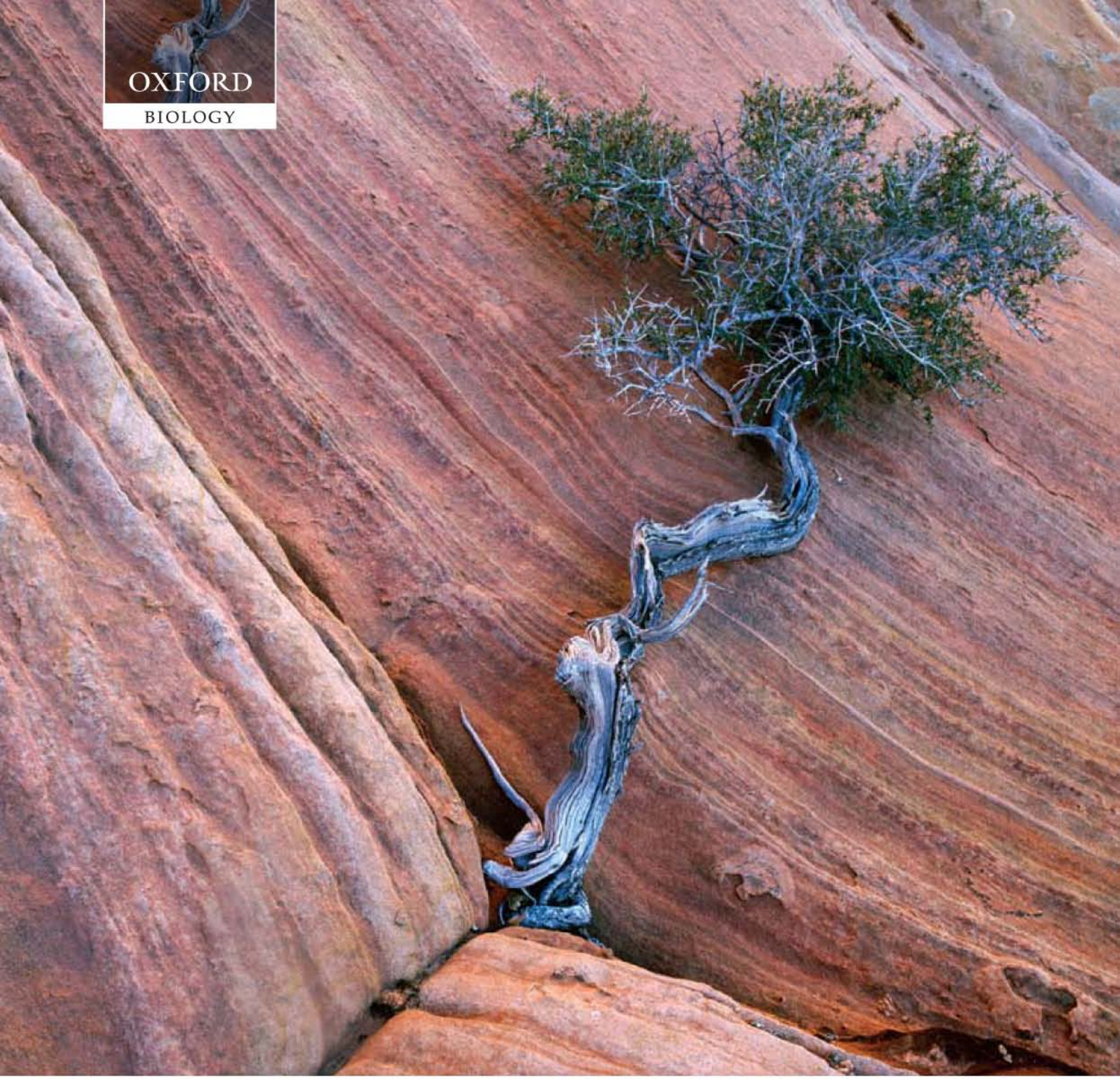

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Notothenioid fishes (Notothenioidei)

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Abstract

Notothenioids are a clade of acanthomorph teleosts that represent a rare example of adaptive radiation among marine fishes. The notothenioid Antarctic Clade is characterized by extensive morphological and ecological variation and adaptations to avoid freezing in the ice-laden water of Southern Ocean marine habitats. A recent analysis of notothenioid divergence times indicates that the clade dates to the Cretaceous (125 million years ago, Ma), but the Antarctic Clade diversified near the Oligocene–Miocene boundary (23 Ma). These age estimates are consistent with paleogeographic events in the Southern Ocean that drove climate change from temperate to the polar conditions observed today.

Notothenioids represent an adaptive radiation of teleost fishes in the frigid waters of the Southern Ocean surrounding Antarctica (*1*). Of the ~129 recognized species, 101 are found in marine costal habitats of Antarctica (Fig. 1), and the remaining species are distributed along costal areas of southern South America, the Falkland Islands, southern New Zealand, southern Australia, and Tasmania (*2*). In addition to a diverse array of adaptations to survive in the freezing Antarctic marine habitats, notothenioids are unique in that they completely dominate the fish fauna of the Southern Ocean. Among benthic fish samples taken on the Antarctic shelf, notothenioids comprise nearly 77% of the species diversity, more than 91% of the species abundance, and ~91% of the biomass (*3*).

A hypothesized key innovation that facilitated the diversification of Antarctic notothenioids is the origin of an antifreeze glycoprotein from a tyripsinogen-like ancestral gene that confers protection from freezing in the subzero Southern Ocean waters (*4*). The ecological and morphological diversity of Antarctic notothenioids is extensive and it is thought that the clade diversified and filled vacant niches after the onset of polar conditions ~35 Ma (*2*). The fossil fishes preserved in the Eocene La Meseta Formation on Seymour Island at the tip of the Antarctic Peninsula indicate that before the development of polar conditions the nearshore fish fauna of Antarctica was diverse, cosmopolitan, and not dominated by notothenioids (*5*). The only documented notothenioid fossil is a well-preserved neurocranium of the extinct species *Proeleginops grandeastmanorum* from the La Meseta Formation that is dated to ~40 Ma (*6–10*).

Ecologically, Antarctic notothenioids have diversified into both benthic and water column habitats (*2*). Several lineages are able to utilize water column habitats despite lacking a swim bladder by modification of buoyancy through the reduction of ossification and the evolution of intra- and intermuscular lipid deposits (*11, 12*). A notable group of notothenioid species is the Channichthyidae, or icefishes (Fig. 1). Species in this clade are also called the "white-blooded" fishes, because of the absence of the oxygen-transporting molecule hemoglobin, which is the result of deleted globin loci possibly initiated by interspecific hybridization and subsequent introgression (*13*). These are the only vertebrates that exhibit this bizarre phenotype and it is thought that the persistence of this apparently deleterious trait is due to the cold oxygen-saturated water that provides adequate oxygen via passive diffusion into the body (*14*).

Fig. 1 A 29.2 cm long (standard length) channichthyid notothenioid (*Chionodraco myersi*: YPM 16533) sampled from the Bransfield Strait. Credit: T. J. Near.

T. J. Near. Notothenioid fishes (Notothenioidei). Pp. 339–343 in *The Timetree of Life*, S. B. Hedges and S. Kumar, Eds. (Oxford University Press, 2009).

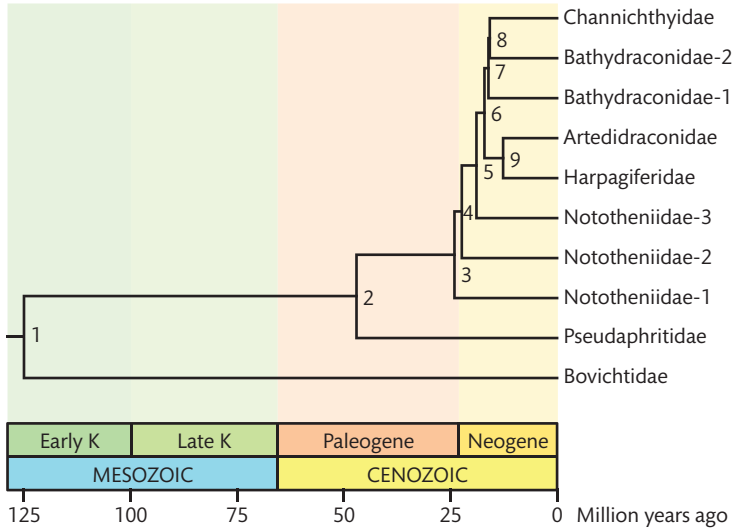

Fig. 2 A timetree of notothenioid fishes (Notothenioidei). Divergence times are shown in Table 1. *Abbreviation*: K (Cretaceous).

The monophyly of Notothenioidei has been supported in phylogenetic analyses of morphological characters (*15, 16*) and DNA sequences from mitochondrial and nuclear genes (*17–20*). Phylogenetic relationships among lineages within Notothenioidei inferred from analyses of morphological and molecular data sets are generally consistent with traditional taxonomic hypotheses developed during the time of great Antarctic exploration in the early twentieth century. Taxonomically, eight families are recognized in the Notothenioidei and all but the Bathydraconidae (Dragonfishes) and Nototheniidae were resolved as monophyletic groups in molecular phylogenetic analyses (*21–28*). Monophyly of Nototheniidae was supported in morphological phylogenetic analyses (*29*), and phylogenetic analyses of complete mtDNA *16S rRNA* (*23*). Other phylogenetic analyses have focused on specific notothenioid subclades, including the Channichthyidae (*30–33*), Bathydraconidae (*22*), Artedidraconidae (*15*), and Nototheniidae (*24, 34*). One important result from these phylogenetic investigations is the consistent monophyly of the Antarctic Clade (Nototheniidae, Harpagiferidae, Artedidraconidae, Bathydraconidae, and Channichthyidae) that comprises the major lineages of notothenioids that are found in the Southern Ocean south of the Antarctic Polar Front (*25, 26*).

Eleven published studies have presented molecular divergence time estimates for notothenioid clades. The ages estimated from these molecular analyses were quite broad, but fairly similar across studies. One study estimated the divergence time of the entire notothenioid radiation at 57–45 Ma (*28*). The range of estimated divergence times for the common ancestor of the Antarctic Clade was 21–5 Ma (*4, 21, 26–28, 35*). Several of these studies also estimated the divergence times of particular lineages within the Antarctic Clade. Age estimates for the Channichthyidae ranged between 23.3 and 2 Ma (*26, 31, 36*), and molecular age estimates for the notothenioid clade *Trematomus* (with and without *Trematomus scotti*) were 3.4–2.8 Ma (*26, 34*).

Any effort that attempts to estimate divergence times using molecular data will face a specter of uncertainty and ever present confounding variables, and all of these notothenioid molecular divergence time studies suffer minimally from one of three severe methodological problems. First, calibration is a major concern as most estimates of notothenioid divergence times rely on external or "universal" rates of DNA sequence evolution estimated for other clades and applied to notothenioids (*4, 27, 31, 34–37*). The extreme example of this strategy was the application of the rate of mtDNA evolution in trout and salmon to estimate the age of the Antarctic Clade from pairwise genetic distances calculated from a nuclear-encoded intron (*4*). This strategy is problematic and ill-advised, because animal mtDNA genes have a much higher nucleotide substitution rate than any sampled nuclear gene, including introns (*38*).

Even when paleogeographic calibrations have been used, they do not represent current hypotheses in the geological literature. For example, the separation of New Zealand from East Gondwana is given as 57 Ma in ref.

Table 1. Divergence times (Ma) and their credibility/confidence intervals (CI) among notothenioid fishes (Notothenioidei).

Timetree		Estimates							
Node	Time	Ref. (*4*)	Ref. (*21*)	Ref. (*26*)	Ref. (*27*)	Ref. (*28*)	Ref. (*35*)	Ref. (*42*)	
		Time	Time	Time	Time	Time	Time	Time	CI
1	125.0	–	–	–	–	57–45	–	125.0	129–121
2	47.0	–	–	–	–	–	–	47.0	48.4–45.6
3	24.1	14–5	12–8	21	16–10	15–11	15–7	24.1	24.6–23.6
4	22.4	–	–	–	–	–	–	22.4	22.9–21.9
5	18.9	–	–	–	–	–	–	18.9	19.3–18.5
6	17.0	–	–	–	–	–	–	17.0	17.4–16.6
7	16.1	–	35–15	–	–	–	–	16.1	16.4–15.8
8	15.8	–	–	–	–	–	–	15.8	16.1–15.5
9	12.7	–	–	–	–	–	–	12.7	–

Note: Node times in the timetree are from ref. (*42*).

(*28*) and used to calibrate the divergence of Bovichtidae from other notothenioids. However, it appears that 80 Ma is a more appropriate age for this event (*39, 40*). In another study, the fragmentation of Australia and Antarctica is presented as occurring 38 Ma and used to calibrate the divergence of Pseudaphritidae and all other notothenioids *(26)*. However, a range of 52–35 Ma is the more appropriate age for this paleogeographic event (*39, 40*). Second, most molecular estimates of divergence times in notothenioids have ignored heterogeneity of molecular evolutionary rates among lineages. However, a few studies have used relative rate tests, where species exhibiting departure from rate heterogeneity were excluded from the analysis (*26–28*). Relative rate tests are problematic, because they measure the substitution rate in only a small portion of the phylogeny and the statistical significance of relative rate tests must be corrected when multiple tests are used (*41*). Third, divergence time estimates have often been presented without confidence or credibility intervals.

The collective problems exhibited among these notothenioid divergence time estimates were directly addressed in a study that used a fossil calibration and a tree-based method to account for rate heterogeneity (*42*). A molecular phylogeny and branch lengths were estimated from mtDNA gene sequences sampled from the *12S* and *16S rRNA* genes using maximum likelihood. The fossil *P. grandeastmanorum* was used to provide a fixed minimal age estimate of 40 Ma for the node that represents the most recent common ancestor of Eleginopidae and the Antarctic Clade. Penalized likelihood was used to correct for molecular evolutionary rate heterogeneity among lineages and confidence intervals were calculated with a bootstrapping method. The time-calibrated phylogeny is presented in Fig. 2 and the divergence times are shown in Table 1. The clade Nototheniidae is not monophyletic in this analysis, and the timetree in Fig. 2 contains three nototheniid clades: clade 1 containing all the species sampled from *Dissostichus*, *Notothenia*, *Aethotaxis*, *Lepidonotothen*, *Patagonotothen*, *Trematomus*, and *Indonotothen*; clade 2 containing *Gobionotothen gibberifrons* and *Gobionotothen acuta*; and clade 3 containing *Pleuragramma antarcticum*. Bathydraconidae is also not monophyletic and the species are distributed between two clades: clade 1 contains *Gymnodraco acuticeps*, and clade 2 contains *Cygnodraco mawsoni* and *Parachaenichthys charcoti*.

Most of the estimated divergence times from this penalized likelihood rate smoothed molecular phylogeny are older than age estimates resulting from analyses of pairwise genetic distances. For example, the molecular divergence time estimate for the common ancestor of Notothenioidei is ~125 Ma, more than double the single previous molecular estimate (*28*). One study used the fragmentation of Antarctica and Australia as a calibration set at 38 Ma for the common ancestor of Pseudaphritidae and the remaining notothenioids (*26*); however, the penalized likelihood estimated age for this node is substantially older (Fig. 2; Table 1). Perhaps of greatest interest to comparative biologists is the age of

the common ancestor of the Antarctic Clade, since this is the lineage that exhibits adaptations to polar conditions such as the presence of antifreeze glycoproteins. Previous estimates using pairwise genetic distances had estimated this clade diversified between 21 and 5 Ma (*4, 35*) (Table 1). The penalized likelihood estimate for the age of this clade is older and close to the Oligocene–Miocene boundary at 23 Ma (Fig. 2; Table 1). This indicates that the Antarctic Clade diversified after the development of the unrestricted Antarctic Circumpolar Current and Antarctic Polar Front, which formed after the opening of the Drake Passage between South America and Antarctica in the late Eocene (*43*).

The available divergence time estimates in notothenioids are far from providing the last word on the topic. Molecular age estimates for notothenioids need exploration with data sets that are much larger in terms of the number of genes and species sampled, relative to the partial mtDNA gene sequences used in all previous studies. In addition, the consistency of the *P. grandeastmanorum* fossil calibration should be assessed in a cross-validation analysis with external fossil calibrations sampled from other acanthomorph teleost clades. These future investigations also need to utilize strategies of divergence time estimation that account for heterogeneity of molecular evolutionary rates, as well as uncertainty in the fossil calibrations. Such analyses will provide reliable credibility intervals for the molecular age estimates. The nonparametric bootstrap procedure used in the penalized likelihood estimate of notothenioid divergence times does not account for uncertainties in the fossil calibrations and likely results in misleadingly narrow confidence intervals (*44, 45*). Robust divergence time estimates for notothenioids will facilitate investigations of the role of climate change and adaptive evolution in the diversification of this Antarctic adaptive radiation.

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