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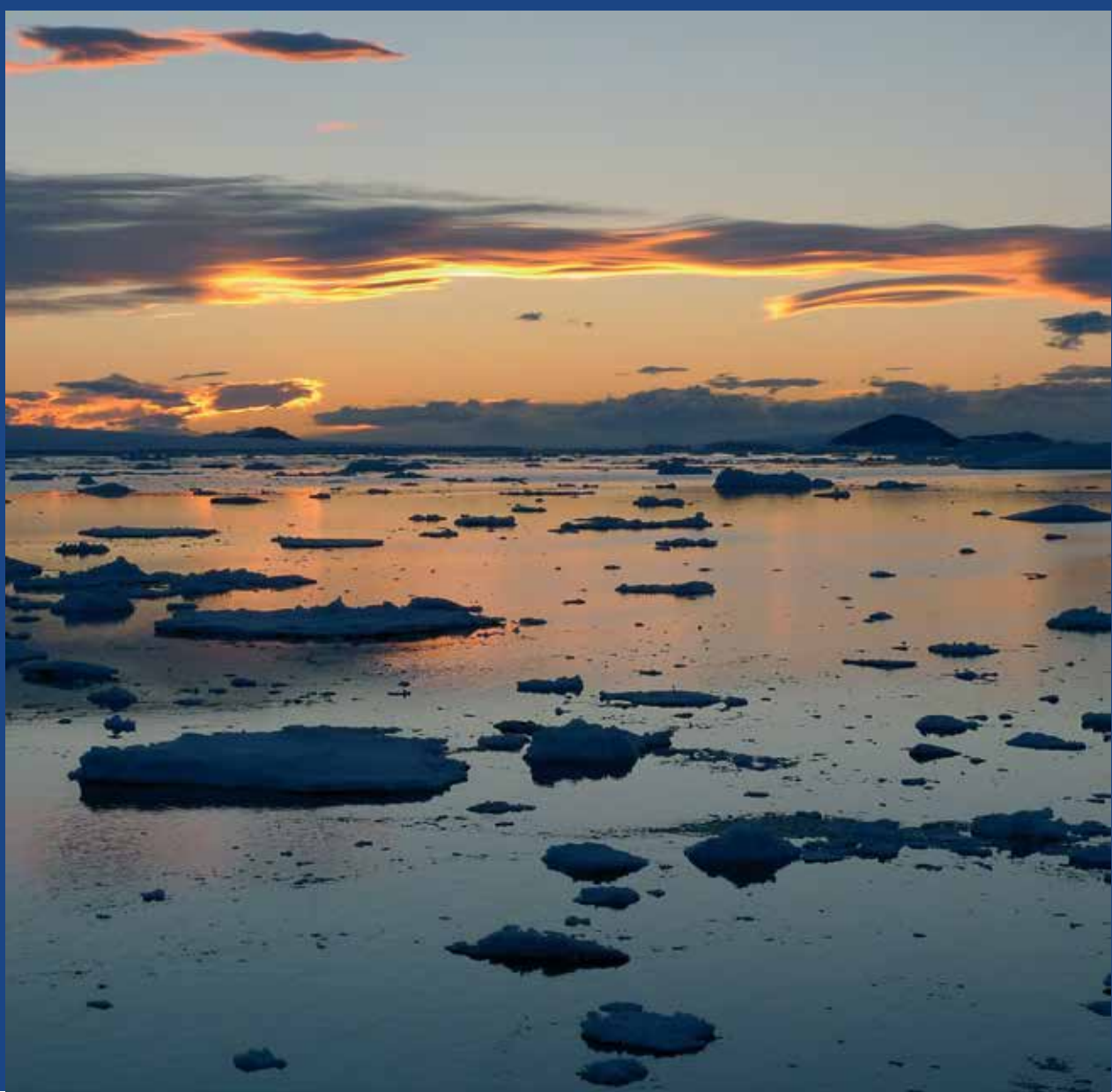

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Parallel ecological diversification in Antarctic notothenioid fishes as evidence for adaptive radiation

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Abstract

Antarctic notothenioid fishes represent a rare example of a marine species flock. They evolved special adaptations to the extreme environment of the Southern Ocean including antifreeze glycoproteins. Although lacking a swim bladder, notothenioids have diversified from their benthic ancestor into a wide array of water column niches, such as epibenthic, semipelagic, cryopelagic and pelagic habitats. Applying stable carbon (C) and nitrogen (N) isotope analyses to gain information on feeding ecology and foraging habitats, we tested whether ecological diversification along the benthic–pelagic axis followed a single directional trend in notothenioids, or whether it evolved independently in several lineages. Population samples of 25 different notothenioid species were collected around the Antarctic Peninsula, the South Orkneys and the South Sandwich Islands. The C and N stable isotope signatures span a broad range (mean $\delta^{13}C$ and $\delta^{15}N$ values between −25.4‰ and −21.9‰ and between 8.5‰ and 13.8‰, respectively), and pairwise niche overlap between four notothenioid families was highly significant. Analysis of isotopic disparity-through-time on the basis of Bayesian inference and maximum-likelihood phylogenies, performed on a concatenated mitochondrial (cyt *b*) and nuclear gene (*myh6*, *Ptr* and *tbr1*) data set (3148 bp), showed that ecological diversification into overlapping feeding niches has occurred multiple times in parallel in different notothenioid families. This convergent diversification in habitat and trophic ecology is a sign of interspecific competition and characteristic for adaptive radiations.

Keywords: disparity-through-time, marine speciation, niche overlap, pelagization, phylogeny, stable nitrogen and carbon isotopes

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Introduction

Adaptive radiation, the evolution of ecological and phenotypic diversity within a rapidly multiplying lineage, is thought to be responsible for a great portion of the diversity of life (Simpson 1953; Schluter 2000). The most famous examples of adaptive radiations are the Darwin's finches on Galápagos, the Caribbean *Anolis* lizards and the East African cichlid fishes. One of the key features of an adaptive radiation is the correlation between the morphologically diverse phenotypes of the 'participating' species and the various habitats that these occupy (Schluter 2000). While it is conceivable how such an 'adaptive disparity' is fulfilled by the paradigmatic Darwin's finches, anoles and cichlids with their characteristic adaptations in beaks, limbs and trophic structures, respectively, the inference of phenotype-environment correlation remains a challenge in other cases of adaptive radiation (Schluter 2000; Gavrilets & Losos 2009).

In fishes, most studies on adaptive radiation focus on freshwater systems, with the cichlid species flocks of

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the East African Great Lakes being the prime examples (Salzburger 2008, 2009). The Antarctic notothenioids represent a marine species flock that evolved under extreme environmental conditions (Eastman & Clarke 1998; Eastman 2000). The perciform suborder Notothenioidei diversified into at least 130 species in eight families, encompassing over 100 Antarctic species (Eastman 2005; Eakin *et al.* 2009). Three ancestral families, Bovichtidae, Pseudaphritidae and Eleginopidae, comprise eleven primarily non-Antarctic species, distributed around southern South America, the Falkland Islands, southern New Zealand and southeastern Australia (Eastman 1993). The remaining families Artedidraconidae, Bathydraconidae, Channichthyidae, Harpagiferidae and Nototheniidae are, with few exceptions, endemic to Antarctic waters and are usually referred to as the 'Antarctic clade' (e.g. Eastman 1993). Notothenioids dominate the Antarctic continental shelf and upper slope, accounting for approximately 46% of the species diversity and over 90% of the fish biomass (Eastman & Clarke 1998; Eastman 2005).

Antarctic waters are constrained by the Antarctic Circumpolar Current (ACC). The Antarctic Polar Front, the northern boundary of the ACC between 50°S and 60°S, acts as major oceanographic barrier, effectively isolating the Southern Ocean faunal assemblages from those of the Indian, Pacific and Atlantic oceans. Through the establishment of a thermally and oceanographically isolated area and the inhibition of faunal admixture, the Antarctic Polar Front is, hence, a likely driver of notothenioid evolution (Coppes Petricorena & Somero 2007). As a means to adapt to Southern Ocean environmental conditions, the Antarctic notothenioids evolved special anatomical and physiological features and, at the same time, lost traits no longer 'needed' in permanently cold waters: (i) The evolution of antifreeze glycoproteins is regarded as an evolutionary key innovation of notothenioids (Eastman 1993; Matschiner *et al.* 2011), facilitating permanent life in subzero temperate waters. (ii) All notothenioids lack a functional swim bladder. Several pelagic species, however, have evolved neutral buoyancy by a combination of skeletal mineralization and the accumulation of lipid deposits (Eastman 1993; Klingenberg & Ekau 1996). (iii) Some notothenioids have lost the classical heat-shock protein response (Place & Hofmann 2005; Clark *et al.* 2008). (iv) The Channichthyidae represent the only known vertebrate group that lacks erythrocytes in the adult state and that is unable to synthesize a functional version of the respiratory oxygen transporter haemoglobin (Ruud 1954; Near *et al.* 2006).

Here, we investigate niche evolution in notothenioids, using a set of 25 representative species (and 365 individuals) that belong to four of the five notothenioid families in the exceptionally species-rich Antarctic clade. Apparently, Antarctic notothenioids diversified along the benthic-pelagic axis in the absence of competition from other fish taxa (Eastman 1993, 2005). From a morphological perspective, this process termed 'pelagization' appears to have occurred independently in several clades (Klingenberg & Ekau 1996; Bargelloni *et al.* 2000).

We used isotopic signatures as indicators for ecological specialization to assess the diversity of lifestyles and feeding strategies/habits of the Antarctic clade, as has been done for adaptively radiating rockfishes (Ingram 2011), and to further test whether these strategies/habits evolved clade-specifically and unidirectionally or independently in several lineages. Stable isotope analysis (SIA) makes use of the fact that the C and N stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) of organisms are directly related to their diet. In general, the ratio of the heavier over the lighter stable isotope is greater in consumers than in food material and thus continuously increases with trophic level (TL; e.g. Hobson & Welch 1992; Hobson *et al.* 1994). This is particularly true for nitrogen, where N isotope fractionation leads to trophic shifts of 3–5‰ (DeNiro & Epstein 1978; Minagawa & Wada 1984; Post 2002). The C isotope fractionation is less pronounced during food chain processing, with a typical 1‰ increase per TL (Hobson & Welch 1992). Yet, carbon isotopic values can often be used to assess constraints on the primary carbon source, which can vary strongly between different feeding grounds (e.g. inshore vs. offshore and pelagic vs. benthic). Thus, while N isotope ratios can be used to predict the relative TL of an organism, its C isotopic composition yields valuable information with regard to its habitat (e.g. Hobson *et al.* 1994).

To reconstruct the evolution of ecological specialization in notothenioids, which has not been studied in detail, we established a new phylogeny of the studied species based on mitochondrial and nuclear markers [3148 base pairs (bp) in total]. This phylogeny extends previous work (e.g. Near & Cheng 2008) by the use of multiple nuclear markers and by the longest total sequence length used in notothenioid phylogenetics to date. Phylogeny and time estimation were fully integrated with SIA by the application of a disparity-through-time (DTT) analysis.

According to the results of earlier studies (Klingenberg & Ekau 1996; Eastman & McCune 2000), we expected to find evidence for independent colonization of ecological niches in different lineages. Furthermore, should previous descriptions of the notothenioid diversification as an adaptive radiation be appropriate, the pattern of average subclade disparity throughout the radiation could be expected to resemble those found in

other adaptive radiations like *Liolaemus* lizards (Harmon *et al.* 2003) or Tanganyikan cichlid fishes (Gonzalez-Voyer *et al.* 2009) and to be different from patterns observed in putative non-adaptive radiations, such as rats (Rowe *et al.* 2011).

Materials and methods

Sample collection

Sampling took place during three expeditions in the austral summer to the Scotia Sea: The ICEFISH 2004 cruise with RV Nathaniel B. Palmer (Jones *et al.* 2008), cruise ANT-XXIII/8 with RV Polarstern, and the 2008/09 US AMLR Survey with RV Yuzhmorgeologiya (Jones *et al.* 2009) (Fig. 1 and Table 1, Tables S1 and S2, Supporting information). White muscle tissue samples were preserved in 95% ethanol and stored at −20 °C for subsequent investigations. A total of 365 adult individuals of 25 Antarctic notothenioid species were processed for SIA. Molecular analyses were performed with 39 individuals of the same 25 species and three representatives of non-Antarctic notothenioid families serving as outgroups (Table 1).

DNA extraction, amplification, sequencing and alignment

Genomic DNA from approx. 10 mm^3 white muscle tissues was extracted by proteinase K digestion, followed by sodium chloride extraction and ethanol precipitation. Marker selection was based on the genome-wide marker comparison of Li *et al.* (2007). We included a fast-evolving gene (*myh6*), a gene evolving at intermediate rates (*Ptr*) and a slowly evolving gene (*tbr1*). As a representative mitochondrial marker (mtDNA), we used cytochrome *b* (cyt *b*), which had previously been proven suitable for phylogenetic analyses in notothenioids (Chen *et al.* 1998; Matschiner *et al.* 2011). Nuclear markers were amplified with the following primer pairs: myh6_F507/myh6_R1325, Ptr_F458/Ptr_R1248 and tbr1_F86/tbr1_R820 (Li *et al.* 2007); the amplification of cyt *b* was performed using the primers NotCytBf and H15915n (Matschiner *et al.* 2011). Sequences of the three outgroup species and *Pogonophryne scotti*, as well as *Ptr* sequences of *Notothenia coriiceps* and *Trematomus newnesi* were obtained from GenBank (see Data accessibility and Table S4, Supporting information).

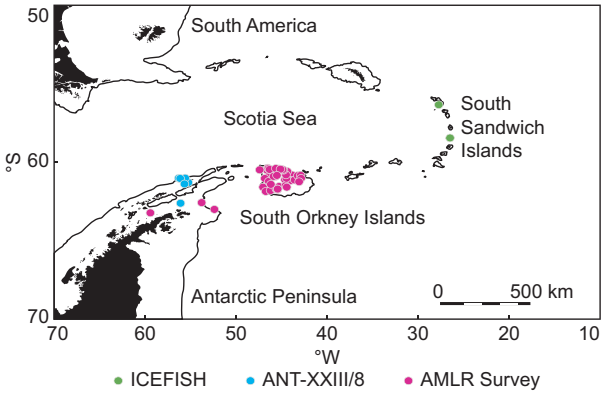

Fig. 1 Sampling sites off the northern Antarctic Peninsula, the South Orkney Islands and the South Sandwich Islands. The solid line indicates the 1000 m depth contour.

The gene fragments were amplified using different polymerase chain reaction (PCR) protocols. Cyt *b*, *myh6* and *Ptr* PCR products were achieved using the Finnzymes' Phusion® High-Fidelity DNA Polymerase (Finnzymes). Individual reaction volumes contained 8.6 μL ddH_20, 10.0 μL 2 × Phusion® Master Mix with HF Buffer [containing 0.04 U/μL Phusion® DNA Polymerase, 2 × Phusion® HF Buffer, 400 μM of each deoxynucleotides (dNTP)], 0.2 μL forward primer, 0.2 μL reverse primer and 1.0 μL DNA template. The PCR profiles included initial denaturation (30 s, 98 °C), followed by 30 (cyt *b*) or 40 cycles (*myh6*, *Ptr*) of denaturation (10 s, 98 °C), annealing (30 s, 56 °C) (53 °C for *Ptr*), extension (30 s, 72 °C) and a final extension phase (10 min, 72 °C). *Tbr1* amplification was achieved using REDTaq® DNA Polymerase (Sigma-Aldrich). The PCR mixes contained 5.5 μL ddH_2O, 1.25 μL 10× Taq buffer (Sigma-Aldrich), 1.0 μL $MgCl_2$, 1.25 μL dNTP mix, 1.0 μL forward primer, 1.0 μL reverse primer, 0.5 μL REDTaq® DNA Polymerase (Sigma-Aldrich) and 1.0 μL DNA template. Amplifications of *tbr1* were carried out using the following temperature profile: initial denaturation (2 min, 94 °C) followed by 32 thermocycles of denaturation (30 s, 94 °C), annealing (30 s, 57 °C), extension (1 min, 72 °C) and a final extension phase (7 min, 72 °C). All amplification products were purified using the ExoSAP-IT (USB) standard protocol, adding 0.5 μL ExoSAP-IT and 3.5 μL ddH_2O to 2.5 μL PCR templates, incubating (15 min, 37 °C; 15 min, 80 °C) and, in some cases, using the GenElute™ Gel Extraction Kit (Sigma-Aldrich). The purified PCR products were used as templates for cycle sequencing reactions with the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), following the manufacturer's instructions. The reaction volumes included 0.5 μL primer, 1.0 μL BigDye® Terminator Reaction Mix (Applied Biosystems) and 3.0–6.5 μL purified DNA in a total volume of 8 μL. The nuclear markers were sequenced with one forward and reverse primer each. Sequencing of cyt *b* was additionally performed with two different forward primers: NotCytBf (Matschiner *et al.* 2011) and

Table 1 Sampled species with collection site, sample size for stable isotope analysis (*n*) and lifestyle of adult individuals. Lifestyle descriptions are often based on trawl depth and may not be definite.

Sample	Location (*n*)	Lifestyle of adults
Bovichtidae		
Bovichtus diacanthus	Tristan da Cunha	
Pseudaphritidae		
Pseudaphritis urvillii	Victoria, Australia	
Eleginopidae		
Eleginops maclovinus	South America	
Nototheniidae		
Aethotaxis mitopteryx	AP (4), SO (7)	Pelagic[*,†,‡,§], benthopelagic[¶]
Dissostichus mawsoni	AP (2), SO (5)	Pelagic[†,§]
Gobionotothen gibberifrons	AP (10), SO (10)	Benthic[†,‡]
Lepidonotothen larseni	SO (10), SSI (10)	Semipelagic[†]
Lepidonotothen nudifrons	SO (10)	Benthic[†,§]
Lepidonotothen squamifrons	AP (10), SO (10)	Benthic[†]
Notothenia coriiceps	AP (10), SO (11)	Benthic[§]
Notothenia rossii	SO (11)	Semipelagic[†]
Pleuragramma antarcticum	AP (10), SO (10)	Pelagic[*,†,§]
Trematomus eulepidotus	AP (10), SO (10)	Epibenthic[*,†,‡]
Trematomus hansoni	SO (11)	Benthic[†,‡]
Trematomus newnesi	AP (10), SO (10)	Cryopelagic[†]
Trematomus nicolai	SO (6)	Benthic[*,†,‡,**,††], benthopelagic[‡‡]
Trematomus tokarevi	SO (11)	Benthic[††]
Artedidraconidae		
Pogonophryne barsukovi	SO (8)	Benthic[§§]
Pogonophryne scotti	SO (10)	Benthic[†,§§]
Bathydraconidae		
Gymnodraco acuticeps	AP (15)	Benthic[†]
Parachaenichthys charcoti	SO (11)	Benthic[†]
Channichthyidae		
Chaenocephalus aceratus	AP (10), SO (10)	Benthic[†,¶¶]
Chaenodraco wilsoni	AP (10)	Pelagic[***]
Champsocephalus gunnari	AP (11), SO (10)	Pelagic[†,¶¶]
Chionodraco rastrospinosus	AP (10), SO (10)	Benthic[†], benthopelagic[†††]
Cryodraco antarcticus	AP (10), SO (10)	Pelagic[†], benthic[¶¶]
Neopagetopsis ionah	AP (6), SO (6)	Pelagic[¶¶]
Pseudochaenichthys georgianus	SO (10)	Pelagic[†,¶¶], semipelagic[†]

[*]DeWitt *et al.* (1990); [†]Eastman (1993); [‡]Klingenberg & Ekau (1996); [§]Kock (1992); [¶]Kunzmann & Zimmermann (1992); [**]Kuhn *et al.* (2009); [††]La Mesa *et al.* (2004); [‡‡]Brenner *et al.* (2001); [§§]Lombarte *et al.* (2003); [¶¶]Kock (2005); [***]Kock *et al.* (2008); [†††]Hureau (1985b).
AP, Antarctic Peninsula, SO, South Orkney Islands, SSI, South Sandwich Islands.

cyt*b*centralF (5′- CYA CCC TNA CYC GYT TCT TTG C -3′), which was newly designed to bind at a central position of cyt *b* (bases 518–539 in cyt *b* of *Chionodraco rastrospinosus*). The reaction conditions were as follows: initial denaturation (1 min, 94 °C) followed by 25 cycles of denaturation (10 s, 94 °C), annealing (20 s, 52 °C) and elongation phase (4 min, 60 °C). Unincorporated BigDye® terminators were removed with the BigDye® XTerminator™ Purification Kit (Applied Biosystems). To this end, 14.5 μL ddH_2O, 22.5 μL SAM™ solution and 5.0 μL XTerminator™ beads were added to the sequencing products, then shaken (30 min, 2000 rpm), and finally centrifuged (2 min, 211 *g*). All sequences were read with an ABI3130*xl* Capillary Sequencer (Applied Biosystems). Sequence reads were verified by eye, and forward and reverse fragments were assembled using CodonCode Aligner v.3.5.6 (CodonCode Corporation).

All sequences were aligned per locus with the multiple sequence alignment program mafft v.6.717b (Katoh & Toh 2008). The alignments were trimmed in Mesquite v.2.72 (Maddison & Maddison 2009) so that each alignment started and ended with codon triplets, and we also checked for stop codons. Alignments were concatenated and partitioned by molecule type and codon position to account for heterogeneity in evolutionary rates and substitution patterns. Thus, the first and second codon positions of mitochondrial cyt *b* (‘mit12’), the third codon positions of mitochondrial cyt *b* (‘mit3’), the

first and second codon positions of nuclear genes ('nuc12') and the third positions of nuclear genes ('nuc3') were used as separate partitions. In a second partitioning scheme, the data set was partitioned with respect to the four genes. The best-fitting models of molecular evolution for each of the eight partitions were estimated with the computer program jModelTest v.0.1.1 (Posada 2008), using the Bayesian information criterion (BIC; Schwarz 1978). Selected models were TPM2uf+G (*myh6*), K80+G (*Ptr*), HKY+I (*tbr1*), TrN+G+I (cyt *b*), HKY+I+G (mit12), K80+I (nuc12) and TrN+G (mit3, nuc3).

Phylogenetic analysis

Phylogenetic tree reconstructions were carried out using maximum-likelihood (ML) and Bayesian inference (BI) approaches. Maximum-likelihood phylogenetic inference was performed with both partitioning schemes, applying the respective models of molecular evolution for each partition, in a partition-enabled version of garli, garli-part v.0.97 (Zwickl 2006). Heuristic searches were used to find the topology with the best likelihood score. The searches were conducted using automatic termination, after a maximum of 5 million generations, or, alternatively, after 10 000 generations without significant ($P < 0.01$) improvement in scoring topology. Bootstrap (BS) analysis was performed with 100 BS replicates, which were summarized using PAUP* v.4.0a110 (Swofford 2003). The non-Antarctic notothenioid species *Bovichtus diacanthus* was defined as outgroup on the basis of well-supported phylogenetic information (e.g. Near & Cheng 2008; Matschiner *et al.* 2011).

Bayesian phylogenetic analyses were performed with the software beast v.1.5.3 (Drummond & Rambaut 2007). For divergence date estimation, the separation of Bovichtidae, Pseudaphritidae and Eleginopidae from the Antarctic lineage (nodes A, B, and C in Fig. 3), as well as the initial diversification of the Antarctic clade (node D) were temporally constrained according to the results of Matschiner *et al.* (2011). Specifically, normal prior distributions were used for each of these splits to approximate highest posterior density (HPD) intervals found by Matschiner *et al.* (2011). Thus, the root of Notothenioidei (node A) was constrained with a mean divergence prior to 71.4 million years ago (Ma; 2.5% quantile: 89.1 Ma, 97.5% quantile: 53.8 Ma), and nodes B-D were constrained at 63.0 (79.5–46.6) Ma, 42.9 (56.5–29.4) Ma and 23.9 (31.3–16.4) Ma, respectively. While these time constraints generally agree with the interpretation of *Proeleginops grandeastmanorum* from the La Meseta Formation on Seymour Island (~40 Ma; Eastman & Grande 1991) as an early representative of the eleginopid lineage (Balushkin 1994), we deliberately avoided using it as a time constraint owing to its debated taxonomical assignment (Near 2004). With the exception of outgroup relationships, which were used for time calibration, no topological constraints were applied. Divergence dates were estimated using the uncorrelated lognormal relaxed molecular clock and the reconstructed birth-death process as a tree prior (Gernhard 2008). Following Shapiro *et al.* (2006), we implemented the codon position-specific model of sequence evolution $HKY_{112} + CP_{112} + \Gamma_{112}$, but we furthermore tested $GTR_{112} + CP_{112} + \Gamma_{112}$ and the model combination selected by BIC for codon-specific partitions. For each of the three combinations, 10 independent analyses were performed with 20 million generations each. Replicates were combined in LogCombiner v.1.5.3 (Drummond & Rambaut 2007) after removing the first 2 million generations of each run as burn-in. Convergence of run replicates was verified by effective sample sizes > 1200 for all parameters and by comparison of traces within and between replicates in Tracer v.1.5 (Rambaut & Drummond 2007). The three settings were compared with Bayes factors (BF), using the harmonic mean approach as implemented in Tracer. While we acknowledge that the harmonic mean estimator may be biased towards more parameter-rich models (Lartillot & Hervé 2006), we chose this approach owing to the lack of suitable alternatives. As the inclusion of multiple individuals per species may violate assumptions of constant diversification implicit in the birth–death tree prior, BI analyses were repeated with a reduced data set containing only one individual of each species.

Stable isotope analysis

In this study, approximately 10 mm^3 of white muscle tissue was used for the SIA. White muscle tissue is less variable with regard to the carbon and nitrogen isotope composition and has a longer retention time than other tissue types (Pinnegar & Polunin 1999; Quevedo *et al.* 2009). Samples were dried (24 h, 60 °C) and then ground in a Zirconia bead mill (30 min, 1800 bpm). Then, the sample powder was rinsed from the beads using 1 mL 99% ethanol, and the supernatant was evaporated (24 h, 60 °C). The ethanol treatment had no effect on subsequent carbon isotope analyses (e.g. Syväranta *et al.* 2008). For C and N isotope measurements, between 0.5 and 0.8 mg sample powder was filled into 5×9 mm tin capsules and introduced into an elemental analyser (Thermo Finnigan) coupled to a Finnigan Delta V Advantage Isotope Ratio Mass Spectrometer, with standard setup for N_2 and CO_2 analysis. Measurements were replicated for about 10% of the samples (42 samples). The isotopic composition is expressed in the

conventional delta notation as permil (‰) deviation vs. atmospheric N_2 (AIR) and carbonate standards (V-PDB): $\delta = [(R_{sample}/R_{standard}) - 1] \times 1000$, with R representing the ratio of the heavy to the light isotope (i.e. $^{13}C/^{12}C$ and $^{15}N/^{14}N$) in the sample and in the standard material, respectively. EDTA ($\delta^{13}C = -30.25‰$, $\delta^{15}N = -1.1‰$) and ammonium oxalate ($\delta^{13}C = -17.02‰$, $\delta^{15}N = 32.7‰$) were used as internal standards, calibrated against international nitrogen (IAEA-N1, IAEA-N2) and carbon (NBS22) standards. The analytical reproducibility based on replicate sample and standard measurements was better than 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$. Isotope values are presented as mean ± standard deviation (SD). Variable lipid content can have a biasing effect on the interpretation of bulk C and N stable isotope data. In marine fish samples, this effect seems to be minor (Kiljunen *et al.* 2006; Logan *et al.* 2008), and hence, we did not perform a lipid removal step. Nevertheless, we performed a posteriori 'mathematical lipid correction' after the study of Logan *et al.* (2008). The correction, however, did not affect the species distribution pattern, and thus, only the uncorrected values are presented in this study. (The corrected data set is available upon request.)

Statistical analysis

The correlation of $\delta^{13}C$ and $\delta^{15}N$ was tested with a Pearson correlation, whereby we accounted for phylogenetic non-independence using phylogenetic independent contrast ('pic' function in the R package 'ape'; Paradis *et al.* 2004; R Development Core Team 2009). We tested for the effect of geographic sites on isotopic signatures by comparison of pooled $\delta^{13}C$ and $\delta^{15}N$ values between AP and SO (*t*-test). Here, only values from species with similar sample sizes at both locations were considered. Pairwise niche overlap between all families and additional comparisons of the notothenid *Lepidonotothen–Trematomus* clade with the other families were tested with a multivariate analysis of variance (MANOVA). To assess the group overlap in isotopic signatures, we calculated Wilk's lambda (Wilk's λ) for each comparison.

We analysed the subdivision of ecological niche space throughout the radiation using the BI phylogeny (Fig. 3) and the averaged stable isotope data for each species. Average subclade disparity was calculated at each splitting event and plotted against time. A Brownian motion (BM) model of trait evolution was employed for comparison. Disparity-through-time analyses were conducted in R using the package 'geiger' (Harmon *et al.* 2008). Using 475 trees drawn from the posterior distribution of the BI analysis and 500 permutations of the stable isotope data, we assessed the robustness of the observed pattern against phylogenetic uncertainty and intraspecific variation.

Results

Phylogenetic analysis

The alignments had lengths of 1099 bp (cyt *b*), 705 bp (*myh6*), 702 bp (*Ptr*) and 642 bp (*tbr1*), resulting in a total of 3148 bp with only 0.3% missing data. The *myh6* alignment contained a short insertion (6 bp) in the non-Antarctic outgroup *B. diacanthus*; these 6 bp were excluded from the following phylogenetic analyses. Sequences are available at GenBank under the accession numbers JF264479–JF264629. Bayes factors provided 'very strong' (Kass & Raftery 1995) evidence that the codon position-specific combination of substitution models selected by BIC yielded a better fit than both the $HKY_{112} + CP_{112} + \Gamma_{112}$ (log 10 BF 6.215) and $GTR_{112} + CP_{112} + \Gamma_{112}$ (log 10 BF 19.19) models.

Our ML and BI phylogenetic analyses produced identical topologies and confirmed the monophyly of the Antarctic clade with high support values (BS 100%; Fig. 2, Fig. S1, Supporting information). Yet, BS support and Bayesian posterior probability (BPP) were low at the base of the diversification of the Antarctic clade (but high at species-level relationships). In all cases, clustering of individuals from different populations of the same species was strongly supported (BS ≥ 93% and BPP = 1.00). The three families Artedidraconidae, Bathydraconidae and Channichthyidae were recovered as monophyletic, while the Nototheniidae appeared paraphyletic. An ancestral position was assigned to *Aethotaxis mitopteryx*. The monophyly of a clade containing *Lepidonotothen* and *Trematomus* was highly supported (BS 100% and BPP 1.00), and *Notothenia* appeared as the sister group to the more derived 'high-Antarctic clade', comprising the families Artedidraconidae, Bathydraconidae and Channichthyidae. Both the high-Antarctic clade and the channichthyid family were found monophyletic with BS 100% and BPP 1.00. The two artedidraconids, *P. barsukovi* and *P. scotti*, grouped together in all analyses (with high support values). Monophyly of the two bathydraconid representatives was weakly supported (BS 35% and BPP 0.67). Within the family of Channichthyidae, *Champsocephalus gunnari* was placed as sister species of all other representatives followed by a clade containing *Pseudochaenichthys georgianus* and *Neopagetopsis ionah* and a clade containing the four genera *Chionodraco*, *Chaenodraco*, *Chaenocephalus* and *Cryodraco*. The ML reconstruction with gene-specific partitions resulted in minor topological differences (Fig. S1, Supporting information). Reduction in the data set to one individual per species did not change the tree

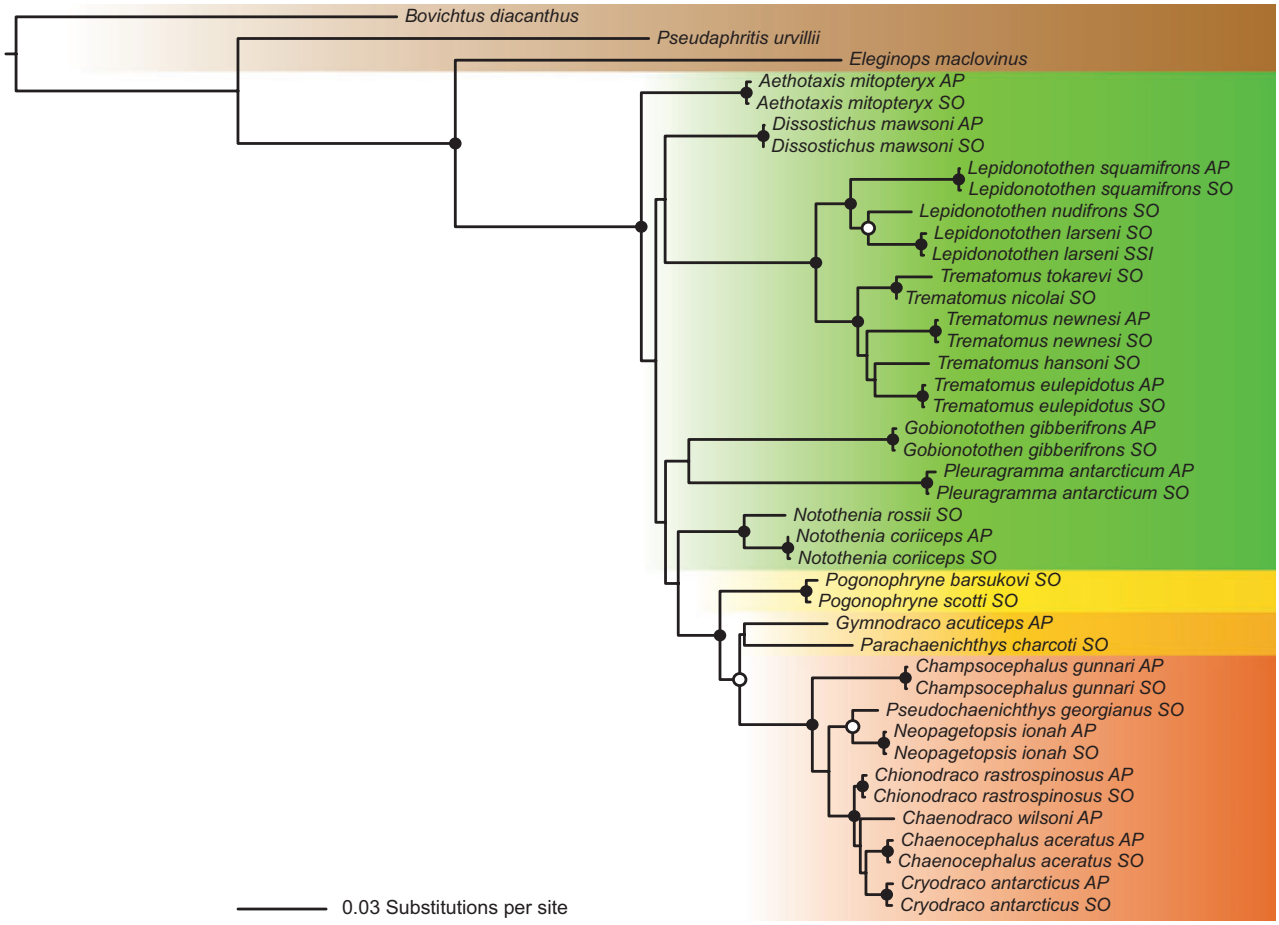

Fig. 2 Maximum-likelihood tree of the notothenioid phylogeny based on the codon position–specific partitioning scheme. Filled circles indicate strongly supported nodes, and moderately supported nodes are marked by open circles Bootstrap (BS ≥ 95 and BS ≥ 70). All species are coloured according to family: brown = non-Antarctic species, green = Nototheniidae, yellow = Artedidraconidae, orange = Bathydraconidae and red = Channichthyidae.

topology with the exception of *Dissostichus mawsoni*, which appeared basal to a group containing the high-Antarctic clade as well as *Nototheniia*, *Pleuragramma* and *Gobionotothen* and the relationships within the *Trematomus* genus (Fig. S1, Supporting information).

According to our time-calibrated phylogeny, diversification of the well-supported nototheniid clade combining *Lepidonotothen* and *Trematomus* began 12.0 Ma (95% HPD 16.4–7.9 Ma; node H) (Fig. 3). The high-Antarctic clade separated from the Nototheniidae around 18.6 Ma (95% HPD 24.0–13.4 Ma; node E). Within the high-Antarctic clade, artedidraconids separated from bathydraconids and channichthyids around 14.6 Ma (95% HPD 15.5–7.0 Ma; node F). The split between Bathydraconidae and Channichthyidae occurred around 2 million years later (12.5 Ma; 95% HPD 16.7–8.5 Ma; node G). The radiation of Channichthyidae, the most derived notothenioid family, began 7.7 Ma (95% HPD 10.6–5.0 Ma; node I).

Stable C and N isotope ratios

The stable carbon and nitrogen isotope composition for the 25 notothenioid species exhibited a comparatively large variability, with values between −27.8‰ and −19.7‰ for $\delta^{13}C$ and between 7.3‰ and 15.6‰ for $\delta^{15}N$ (Fig. 3). Mean values ranged between −25.4‰ and −21.9‰ for $\delta^{13}C$ (SD: 0.3‰ to 1.8‰) and 8.5‰ to 13.8‰ for $\delta^{15}N$ (SD: 0.2‰ to 1.7‰; Fig. 4). Intraspecific ranges of isotopic signatures span from 1.0‰ to 8.1‰ for $\delta^{13}C$ and from 0.4‰ to 5.7‰ for $\delta^{15}N$. Overall, mean intraspecific ranges ($\delta^{13}C$: 2.79‰, $\delta^{15}N$: 2.80‰) were small compared to interspecific ranges of isotopic signatures ($\delta^{13}C$: 8.12‰, $\delta^{15}N$: 8.29‰). The isotopic signatures of $\delta^{13}C$ and $\delta^{15}N$ correlated significantly (0.69; $P < 0.001$), and the correlation remained significant ($P < 0.01$) after correcting for phylogenetic non-independence. No significant difference between values from AP and SO locations was found ($P > 0.16$; *t*-test), even though the

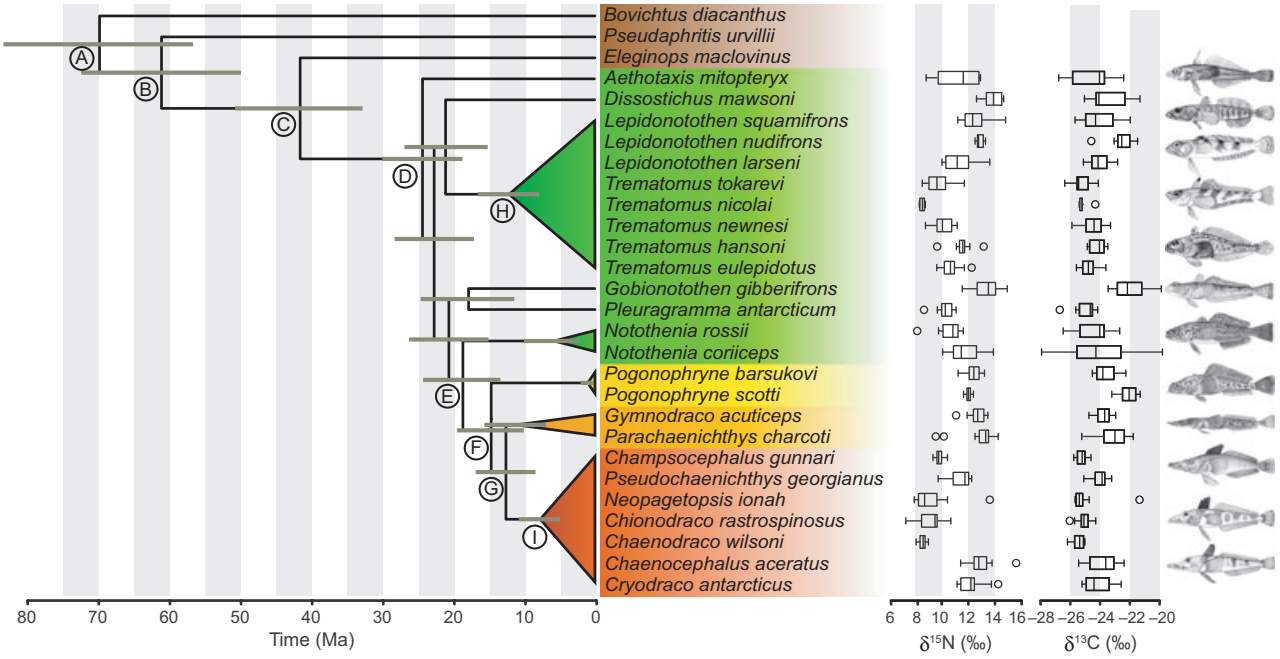

Fig. 3 Left: Time-calibrated phylogeny based on codon-specific partition, inferred with Bayesian inference. Time axis is given in million years ago and nodes labelled A-I are mentioned in the text. Grey node bars indicate upper and lower 95% HPD. All species are coloured according to family: brown = non-Antarctic species, green = Notothenioidae, yellow = Artedidraconidae, orange = Bathydraconidae and red = Channichthyidae. Right: Boxplot of stable isotope values of all included notothenioids. Representative habitus are illustrated at the right, from top to bottom: *Aethotaxis mitopteryx*[d], *Dissostichus mawsoni*[d], *Lepidonotothen nudifrons*[d], *Lepidonotothen larseni*[d], *Trematomus tokarevi*[d], *Gobionotothen gibberifrons*[d], *Notothenia rossii*[b], *Pogonophryne barsukovi*[c], *Gymnodraco acuticeps*[a], *Pseudochaenichthys georgianus*[e], *Chionodraco rastrospinosus*[e] and *Chaenocephalus aceratus*[e]. [a]Boulenger (1902); [b]DeWitt *et al.* (1990); [c]Eakin (1990); [d]Hureau (1985a); [e]Hureau (1985b).

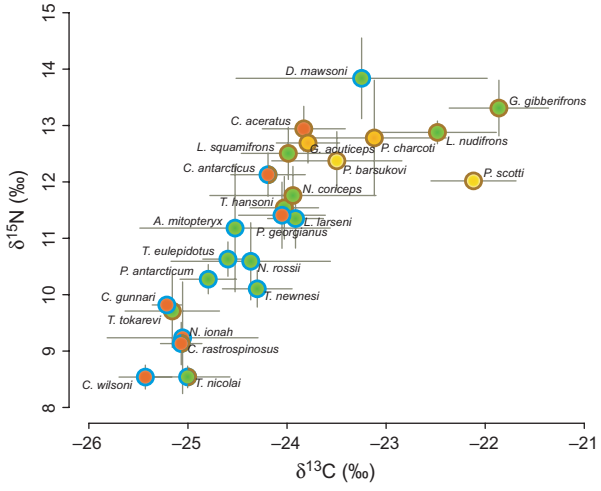

Fig. 4 Scatter plot of carbon and nitrogen isotopic values. Grey bars indicate 95% confidence intervals. All species are coloured according to family (brown: non-Antarctic species, green: Notothenioidae, yellow: Artedidraconidae, orange: Bathydraconidae, red: Channichthyidae), and strokes indicate corresponding lifestyle [blue = pelagic, benthopelagic, semipelagic and epibenthic; brown = benthic; and semicircles when references (Table 1) disagree].

mean values differed slightly (AP $\delta^{13}C$: −24.37‰, SO $\delta^{13}C$: −24.13‰; AP $\delta^{15}N$: 11.30‰, SO $\delta^{15}N$: 10.99‰).

With regard to inferred lifestyle patterns, our SIA data are consistent with previous studies (Hobson *et al.* 1994; Post 2002) in that species that are commonly classified as pelagic clustered around lower $\delta^{13}C$ values, while benthic species possessed relatively higher $\delta^{13}C$ signatures. However, there are notable exceptions to this: *D. mawsoni*, *C. rastrospinosus*, *Trematomus nicolai* and *T. tokarevi* (Fig. 4, Table 1 and Data S1, Supporting information). Most species had relatively high $\delta^{15}N$ signatures, indicating feeding at upper TL. The two well-represented families Nototheniidae and Channichthyidae covered a wide range of isotopic signatures, while bathydraconids and artedidraconids displayed a relatively low variability in both $\delta^{13}C$ and $\delta^{15}N$ (although the number of individuals was significantly lower). Overlap of the C and N isotope compositions as proxies for niche space was found in all pairwise comparisons (MANOVA) of the four Antarctic notothenioid families (Table 2). Wilk's λ was largest for comparisons of Nototheniidae with all other families ($\lambda > 0.91$; Table 2), and lower values were found for comparisons

Family 1	Family 2	Wilk's λ
Artedidraconidae	Nototheniidae	0.936
	Lepidonotothen–Trematomus clade	0.791
Bathydraconidae	Nototheniidae	0.913
	Lepidonotothen–Trematomus clade	0.818
Channichthyidae	Nototheniidae	0.930
	Lepidonotothen–Trematomus clade	0.932
Artedidraconidae	Bathydraconidae	0.681
Artedidraconidae	Channichthyidae	0.629
Bathydraconidae	Channichthyidae	0.781

Table 2 Pairwise niche overlap comparisons for the four Antarctic notothenioid families, performed with MANOVA (Wilk's λ)

including the lesser-represented families Artedidraconidae and Bathydraconidae ($\lambda > 0.68$). Notably, within-family variation resulted mostly from interspecific variation, instead of intraspecific variation, and closely related species with small intraspecific variation could be found at both ends of the ranges (e.g. *T. nicolai* and *Lepidonotothen nudifrons*; Fig. 3).

Using the DTT method, we assessed how the stable isotope space (as a proxy for ecological niche space) used by the whole clade was subdivided by smaller and smaller subclades as the radiation proceeded. We find positive deviations from the averaged neutral-evolution BM model, indicating larger overlap in niche space between subclades than would be expected if evolution proceeded neutrally (Fig. 5). This result was found to be robust against phylogenetic uncertainty and intraspecific variation by visual inspection of repeated DTT analyses.

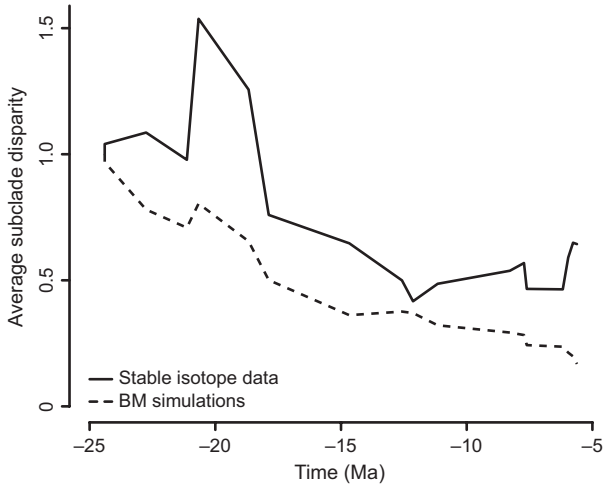

Fig. 5 Disparity-through-time plot for the stable isotopic signatures of Antarctic notothenioid fishes and Brownian motion simulations of character evolution. Time axis is given in million years ago.

Discussion

Phylogenetic relationships

Previous molecular phylogenetic analyses of notothenioids were based on mitochondrial DNA sequences (Bargelloni *et al.* 2000; Stankovic *et al.* 2002; Near 2004; Near *et al.* 2004), on a combination of mtDNA with a single nuclear gene (Near & Cheng 2008) or on morphological characters in addition to molecular data (Derome *et al.* 2002; Sanchez *et al.* 2007). The family-level phylogeny of notothenioids is thus relatively well established. Several questions remain, however, such as the position of the genus *Gobionotothen* (Near *et al.* 2004; Sanchez *et al.* 2007; Near & Cheng 2008) or whether Bathydraconidae are mono- or paraphyletic (e.g. Derome *et al.* 2002; Near & Cheng 2008).

In agreement with most previous studies (e.g. Near 2004; Near & Cheng 2008), our results support paraphyly of the family Nototheniidae. The low support values at the beginning of the Antarctic diversification are characteristic for rapid diversifications. Consequently, the basal position of *D. mawsoni* and the sister species relationships of *G. gibberifrons* and *Pleuragramma antarcticum* remain questionable. As in previous studies (Near 2004; Near & Cheng 2008), the three neutrally buoyant species *A. mitopteryx*, *D. mawsoni* and *P. antarcticum* diverged early within the Antarctic clade but did not cluster together. Phylogenetic relationships of the two genera *Notothenia* and *Lepidonotothen* are consistent with former studies (Bargelloni *et al.* 2000; Near & Cheng 2008). Also, the topology of the nototheniid subfamily Trematominae agrees with previous findings (Sanchez *et al.* 2007; Kuhn & Near 2009), except for *T. tokarevi* and *T. nicolai*, which appeared at basal positions in the phylogeny based on codon position–specific substitution models (Fig. 2, Fig. S1, Supporting information). The early split of the two included bathydraconid species relative to the divergence between Bathydraconidae and Channichthyidae

could indicate paraphyly of the former, as was concluded in previous studies (e.g. Derome *et al.* 2002; Near *et al.* 2004; Near & Cheng 2008). Resulting support values within the channichthyids were high, and the recovered topology was in complete agreement with the study of Derome *et al.* (2002). The three genera *Champsocephalus*, *Neopagetopsis* and *Pseudochaenichthys* seem to be well established as the most basal channichthyids (Chen *et al.* 1998; Near *et al.* 2003). In disagreement with former findings, *C. rastrospinosus* and *Chaenodraco wilsoni* did not cluster monophyletically (Chen *et al.* 1998). Near *et al.* (2003) also recovered these two species as paraphyletic but placed *Chaenocephalus aceratus* as the sister taxon to the genera *Cryodraco*, *Chaenodraco* and *Chionodraco,* which disagrees with our findings. Near & Cheng (2008) determined *C. aceratus* as the closest related species of *C. rastrospinosus*.

Inferred split dates (Fig. 3) roughly agree with those found by Near (2004) and Matschiner *et al.* (2011): Divergence estimates for the *Lepidonotothen–Trematomus* clade and the high-Antarctic clade were 12.0 (95% HPD 16.4–7.9) Ma and 18.6 (95% HPD 24.0–13.4) Ma, respectively, while Near (2004) reported them to be 14 ± 0.4 Ma and Matschiner *et al.* (2011) found these splits at 10.3 (95% HPD 15.2–6.1) Ma and 14.7 (95% HPD 20.0–9.9) Ma. According to our estimates, the radiation of the Channichthyidae began 7.7 (95% HPD 10.6–5.0) Ma ago, in good agreement with the estimates of Near (2004) (8.5 ± 0.3 Ma) and Matschiner *et al.* (2011) (6.2 Ma; 95% HPD 9.4–3.4 Ma).

Foraging ecology of notothenioids

So far, it has been shown that some particular feeding strategies are poorly represented or even absent in notothenioids, such as active skeleton-breaking predation (Clarke *et al.* 2004) or planktivory (Eastman & Grande 1989; Eastman 1993). The latter is probably due to restricted phytoplankton production during the austral winter (Clarke *et al.* 2004). The drawback of traditional dietary proxies (stomach content analyses and foraging observations) is that they only captures a snapshot of food uptake. Contrarily, SIA provides time-integrated information on the feeding 'ecology' for a period of weeks to years (McIntyre & Flecker 2006). Isotopic signatures could theoretically be influenced by geographic differences, sampling season and the age of sampled individuals, especially when ontogenic shifts occur in the investigated species. However, our sampling design accounted for these potential problems, as only adult specimens were collected, and all expeditions took place during austral summers. Also, most species were collected at the same two sampling locations, AP and SO, and populations from these two sites did not differ in isotopic signatures. Thus, the observed interspecific differences suggest ecological specialization rather than effects of geographical distribution or life history traits.

Our SIA data confirm that notothenioids occupy a wide variety of ecological niches (Figs 3 and 4). Comparatively high $\delta^{15}N$ values suggest that most investigated species reside at a high TL and may be considered tertiary consumers (see also Dunton 2001; Pakhomov *et al.* 2006). The wide range of the carbon stable isotope signatures reflects the notothenioids' variety in habitats along the benthic-pelagic axis (Fig. 4). However, our results are only partly congruent with the lifestyles and feeding reports based on stomach content analyses (Fig. 4, Table 1, Table S3 and Data S1, Supporting information).

At the family level, Nototheniidae are – in terms of habitat and feeding strategies – the most diverse clade among Antarctic notothenioids (La Mesa *et al.* 2004; this study) and include plankton, nekton and benthos feeders, as well as species that combine several feeding modes (Gröhsler 1994). The five included *Trematomus* species were differentiated in both isotopic signatures, thus indicating trophic niche separation (see also Brenner *et al.* 2001). Artedidraconids and bathydraconids represent the most benthic families among notothenioids (Fig. 4; Olaso *et al.* 2000; La Mesa *et al.* 2004). Their $\delta^{15}N$ values suggest feeding habits at higher TL (Olaso *et al.* 2000; Jones *et al.* 2009). The well-studied channichthyids clustered into three groups according to their diet (Fig. 4: *C. wilsoni*, *N. ionah*, *C. rastrospinosus* and *C. gunnari* at low TL; *P. georgianus* and *Cryodraco antarcticus* at intermediate TL; and *C. aceratus* at high TL; see also Kock 2005). Carbon signatures indicated a rather pelagic lifestyle for most channichthyid species, with the exception for *C. aceratus*, which we can classify as benthic top predator, in agreement with previous findings (Kock 2005; Reid *et al.* 2007).

The DTT plot (Fig. 5) indicates larger overlap of subclades in niche use than expected from a model of neutral evolution. This is characteristic for adaptive radiations (Harmon *et al.* 2003; Gonzalez-Voyer *et al.* 2009) and differs from patterns of putative nonadaptive radiations, which show a negative deviation from the averaged neutral-evolution BM model (e.g. Rowe *et al.* 2011). Taking into account the considerable variation in stable isotope signatures found in notothenioids as a whole (Fig. 4) – basically ruling out stasis in the evolution of niche use – as well as the robustness of this pattern against intraspecific variation, these results suggest convergent evolution in niche use between species of notothenioid subclades, especially between those clades separating around 20 Ma (Figs 3 and 5). This emphasizes the importance of ecological niche differentiation in the adaptive radiation of notothenioids.

Adaptive radiation and ecological diversification in notothenioids

Our integrative analyses, combining both the phylogenetic relationships and the isotopic signatures of 25 notothenioid species, reveal that ecological diversification into overlapping feeding niches has occurred multiple times in parallel in different notothenioid families (Figs 3 and 5). Using carbon and nitrogen stable isotope ratios as indicators of TL, feeding strategy and macrohabitat, we find great variation within, and substantial overlap between the more basal nototheniids and the derived channichthyids. The representatives of the benthic artedidraconids and bathydraconids also overlap and cluster at high TLs and $\delta^{13}C$ values. Our results further confirm partitioning of habitat and trophic resources within notothenioid fishes, indicating that diversification along the benthic–pelagic axis and to different TLs took place independently in at least two of five notothenioid families of the Antarctic clade (Notothenioidae and Channichthyidae; Fig. 3 and Table 2).

Convergent diversification in habitat and trophic ecology suggests interspecific competition and is a characteristic of adaptive radiations (e.g. Losos 1995; Schluter 2000). For example, *Anolis* lizards of the Caribbean have independently evolved four to six so-called ecomorphs on each of the four large islands of the Greater Antilles, including species specialized to live on grass, twigs, trunks and tree crowns. Variation in limb lengths of anole ecomorphs supports these different lifestyles, so that e.g. the trunk-ground ecomorph possesses relatively long legs adapted to running and jumping on broad surfaces, while the twig ecomorph has short legs and moves slowly on narrow surfaces (Losos 2009). In this context, diversification of notothenioids along the benthic-pelagic axis, as evidenced by their isotopic composition, and the respective adaptations in buoyancy (Eastman 1993) can be considered analogous to the *Anolis* diversification along the ground-tree axis. The notothenioid adaptive radiation shows further analogies to that of Caribbean anoles in terms of species richness (both around 120 species) and age (about 24 and 15–66 Ma, respectively) (Fig. 3; Eastman 2005; Nicholson *et al.* 2005; Losos 2009; Matschiner *et al.* 2011). Not all descendents of the *Anolis* radiation remained within the confined area of the radiation (Nicholson *et al.* 2005), and neither did the notothenioids: *Notothenia angustata*, *N. microlepidota* and the genus *Patagonotothen* secondarily escaped Antarctic waters and occur in New Zealand and South America (Eastman 2005). Moreover, both radiations were probably triggered by key innovations: subdigital toepads support the particular arboreality of *Anolis* lizards, whereas antifreeze glycoproteins in blood and tissues allow notothenioid survival in ice-laden Antarctic waters (Chen *et al.* 1997; Losos 2009; Matschiner *et al.* 2011).

Compared to another well-studied adaptive radiation, that of cichlid fishes in East African lakes, the rate at which lineage formation seems to have occurred is much smaller in Antarctic notothenioids. In the Great Lakes of East Africa, cichlid fishes have diversified into at least 1500 species that differ greatly in naturally and sexually selected traits, including body shape, mouth morphology and colouration (Salzburger 2009). Comparison of cichlid species flocks between East African lakes, as well as mathematical models, have shown that larger habitats effectuate higher diversification rates, as they provide greater habitat heterogeneity and facilitate isolation by distance ('area effect'; Salzburger & Meyer 2004; Gavrilets & Vose 2005; Seehausen 2006). Different adaptive radiations may not be directly comparable as they depend on many ecological, genetic and developmental factors, with an important contribution of historical contingencies (Gavrilets & Losos 2009). Cichlids are known for their philopatry and low dispersal abilities (Danley & Kocher 2001; Salzburger & Meyer 2004), whereas most notothenioids have prolonged pelagic larval stages, enhancing long-range migration (Eastman 1993). Notothenioid populations are characterized by fragmented habitat, historical demographic fluctuations (Patarnello *et al.* 2011) and the absence of genetic structuring over large distances (Matschiner *et al.* 2009; and references therein), whereas many cichlid species posses significant population structuring even on extremely small scales (e.g. Arnegard *et al.* 1999; Rico & Turner 2002). Genetic differentiation over small scales has rarely been found in notothenioids (but see Clement *et al.* 1998). Eastman & McCune (2000) suggested that the smaller species number of notothenioids, compared with cichlid species flocks, could be explained by the absence of certain prime inshore habitats in the Southern Ocean. Alternatively, the notothenioid adaptive radiation may not yet have entered its final stage, namely the diversification with respect to communication. Streelman & Danley (2003) suggested a three-stage model of adaptive radiation (see also Danley & Kocher 2001), in which diversification first occurs with respect to macrohabitats, then with respect to microhabitats and finally with respect to communication (e.g. mating traits such as colouration; see also Gavrilets & Losos 2009). Full species richness would only be achieved through this final step. Streelman & Danley (2003) further suggested that divergence of habitat and trophic morphology is driven by natural selection, whereas diversification along the axis of communication is forced by sexual selection. It is as of yet unclear whether the radiation of notothenioids followed discrete stages. Here, we provide conclusive evidence that the

species are separated along the benthic-pelagic axis (i.e. according to macrohabitats; Figs 3 and 4) and probably also as a function of bottom topography and sediment types (Kock & Stransky 2000). Much less is known about microhabitat diversification, although our data suggest that closely related species do differ with respect to foraging strategies (e.g. genera *Lepidonotothen* and *Trematomus*; Figs 3 and 4). Recent evidence further indicates the possibility of divergence along Streelman and Danley's axis of communication, as egg guarding and parental care were observed in all major notothenioid lineages except within the Artedidraconidae (Kock *et al.* 2006; Barrera-Oro & Lagger 2010 and references therein).

On the other hand, because of the paucity of the Antarctic fossil record, it cannot be excluded that the notothenioid radiation has already surpassed its maximum species richness. It is an important characteristic that young adaptive radiations often 'overshoot' in terms of species number and that, generally, niche filling causes declining speciation rates (e.g. Seehausen 2006; Gavrilets & Losos 2009; Meyer *et al.* 2011). That notothenioids already underwent periods of 'overshooting' and niche filling could possibly explain the smaller diversity of Notothenioidei compared to the younger cichlid radiation in the East African Lakes. However, in this case, an early burst of diversification should have left its footprint in a 'bottom-heavy' phylogeny (Gavrilets & Vose 2005). A more extensive study, including many more representatives of the nototheniods, would be necessary to reconstruct the succession of their adaptive radiation.

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S.R. is interested in the diversity of animals, including their evolution and adaptations to the environment. M.Ma. works on the molecular processes underlying adaptive radiation. M.D. is interested in population ecology and evolutionary biology. M.F.L. is specialized on stable isotope biogeochemistry in aquatic environments. R.H. is interested in causes and pathways of adaptation and speciation in the sea. W.S. and M.Mu.'s research focuses on the understanding of the genetic basis of adaptation, evolutionary innovation and animal diversification, using the East Africa' cichlid radiations as main model system. The laboratory's homepage at http://www.evolution.unibas.ch/salzburger provides further details on the group's (research) activities.

Data accessibility

All DNA sequences from this study are available under GenBank accessions: JF264479–JF264516 (cyt *b*); JF264517–JF264554 (*myh6*); JF264555–JF264590 (*Ptr*); and JF264591–JF264629 (*tbr1*). GenBank accession numbers for sequences of other studies are the following: *B. diacanthus* (HM049936; HM050034; HM050153; HM050214); *Eleginops maclovinus* (DQ526429; HM050045; HM050163; HM050225); *N. coriiceps* (HM050183); *P. urvillii* (HM049963; HM050074; HM050195; HM050258); *P. scotti* (HM049962; HM050072; HM050193); and *T. newnesi* (HM050204) (see Table S4, Supporting information). All stable isotope values are given in Table S5, (Supporting information).

Supporting information

Additional supporting information may be found in the online version of this article:

Fig. S1 Maximum-likelihood tree based on the codon position-specific partitioning with numbered nodes (1–19).

Table S1 Antarctic notothenioid samples with corresponding collection id (Table S2) and sample size (*n*) for stable isotope analysis.

Table S2 Collection id for all Antarctic notothenioid samples.

Table S3 Lifestyle and feeding for all included Antarctic notothenioid species.

Table S4 GenBank accession numbers for all used samples.

Table S5 Stable isotope values of all investigated species.

Data S1 Discussion of stable isotope analysis results of individual species.

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