The Adaptive Radiation of Notothenioid Fishes in the Waters of Antarctica

Michael Matschiner, Marco Colombo, Malte Damerau, Santiago Ceballos, Reinhold Hanel, and Walter Salzburger

Abstract Fishes of the perciform suborder Notothenioidei, which dominate the ichthyofauna in the freezing waters surrounding the Antarctic continent, represent one of the prime examples of adaptive radiation in a marine environment. Driven by unique adaptations, such as antifreeze glycoproteins that lower their internal freezing point, notothenioids have not only managed to adapt to sub-zero temperatures and the presence of sea ice, but also diversified into over 130 species. We here review the current knowledge about the most prominent notothenioid characteristics, how these evolved during the evolutionary history of the suborder, how they compare between Antarctic and non-Antarctic groups of notothenioids, and how they could relate to speciation processes.

1 Antarctic Waters: An Extreme Environment

Antarctica represents an isolated "continental island," separated from other continental shelves by the Antarctic Circumpolar Current (ACC) that reaches the ocean floor (Foster 1984) and transports more water than any other ocean current on Earth (Tomczak and Godfrey 2003). The Antarctic Polar Front (APF), located between 50 and 60°S, thermally isolates the continent (Gordon 1971) and poses an

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M. Matschiner (✉) • W. Salzburger (✉)
Zoological Institute, University of Basel, Vesalgasse 1, 4051 Basel, Switzerland

Centre for Ecological and Evolutionary Synthesis (CEES), Department of Biosciences, University of Oslo, Oslo, Norway
e-mail: michaelmatschiner@mac.com; walter.salzburger@unibas.ch

M. Colombo
Zoological Institute, University of Basel, Vesalgasse 1, 4051 Basel, Switzerland

M. Damerau • R. Hanel
Thünen Institute of Fisheries Ecology, Palmaille 9, 22767 Hamburg, Germany

S. Ceballos
Zoological Institute, University of Basel, Vesalgasse 1, 4051 Basel, Switzerland

Centro Austral de Investigaciones Científicas (CADIC), Bernardo A. Houssay 200, cp 9410 Ushuaia, Tierra Del Fuego, Argentina

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additional physical barrier to marine organisms (Shaw et al. 2004). As a result, Antarctic waters are unique marine environments, characterized by sub-zero temperatures and the widespread presence of sea ice. At high latitudes, temperatures remain close to the freezing point of seawater at -1.86 °C throughout the year (Eastman 1993). Due to the weight of the continental ice cap, the Antarctic shelf is eight times deeper than the world average (Anderson 1999). Many potential shallow water habitats are covered by ice foots and anchor ice, and gigantic icebergs regularly rework the bottom topography as deep as 550 m below sea level, so that these habitats are constantly in a state of change or recovery (Barnes and Conlan 2007). Even at depths below 400 m, water temperatures can remain near the freezing point throughout the year (Cheng and Detrich 2007). As a consequence, Antarctic waters are among the thermally most stable habitats on Earth. Nevertheless, they are subject to strong seasonality in light conditions, which in turn influences primary production and nutrient availability (Clarke 1988). Taken together, sub-zero temperatures, the continuous presence of sea ice, and extreme seasonality pose great ecophysiological challenges for marine organisms living in Antarctic waters.

Due to the harsh environment and the isolation by the APF, only a few groups of teleost fishes have managed to successfully colonize Antarctic waters. Out of a diversity of about 28,000 teleost species worldwide (Nelson 2006), less than 400 are known to occur in Antarctica (Eastman 2005). The bulk of the Antarctic fish diversity (~90 %; Eastman 2005) belongs to three different taxonomic groups, which have all been assigned to the recently redefined order Perciformes (Betancur-R et al. 2013): the suborder Notothenioidei (107 species; see below), the family Liparidae (~150 species; Stein 2012), and family Zoarcidae (28 species; Matallanas 2008). The two largest of these groups (Notothenioidei and Liparidae) occupy mostly non-overlapping habitats, as liparids are almost exclusively found in the deep sea below ~800 m depth and are of low abundance (Stein 2012), whereas Antarctic notothenioids dominate the continental shelf and upper slope in terms of vertebrate species number (~50 %) and biomass (90–95 %) (Eastman and Clarke 1998). As most scientific sampling to date has focused on depths shallower than 1,000 m, the Antarctic liparid diversity is greatly understudied, and new species are still frequently described (Stein 2012). Nevertheless, it seems clear that Antarctic Liparidae represent a polyphyletic group resulting from multiple independent invasions from the north (Balushkin 2012), so that they are considered a secondary Antarctic group (Stein 2012). In contrast, the similarly species-rich Antarctic notothenioids apparently evolved in situ on the continental shelf and have been described as a rare example of a marine "species flock."

The species flock concept was developed more than 100 years ago by botanists to describe assemblages of closely related taxa that "flock together," i.e., coexist in the same area, and later adopted by ichthyologists for the particularly diverse cichlid fishes of the East African Great Lakes and other lacustrine evolutionary radiations (Salzburger et al. 2014). The key features of a species flock are thus, besides species richness, the common ancestry of its members, a clear-cut geographic circumscription, and, hence, high levels of endemicity. Most, if not all, species flocks are the product of adaptive radiations (Eastman and McCune 2000; Salzburger and Meyer 2004), and as we will describe below in more detail, Antarctic notothenioid fishes represent what is arguably the most spectacular example of an extant adaptive radiation in the marine realm.

2 Taxonomy of Notothenioids

The Notothenioidei have been taxonomically classified into 8 different families and 136 species (Eastman and Eakin 2000; Table 1, continuously updated by Eastman and Eakin and available at http://www.oucom.ohiou.edu/dbms-eastman/; version Oct. 18, 2013). Five families are predominantly Antarctic and three occur in the coastal waters of New Zealand, Australia, South America, and subantarctic islands (Fig. 1a). The most widely distributed family is Bovichtidae, consisting of nine

Table 1 All non-Antarctic notothenioids with presumed Antarctic ancestry and presence of AFGP

Family/genus and species	Occurrence	AFGP
Nototheniidae		
Dissostichus eleginoides	SA, NZ, SG	No
Notothenia angustata	NZ	Yes
Notothenia microlepidota	NZ	Yes
Paranotothenia magellanica	SA	Yes
Lepidonotothen macrophthalma	SA	?
Patagonotothen brevicauda	SA	?
Patagonotothen canina	SA	?
Patagonotothen cornucola	SA	?
Patagonotothen elegans	SA	?
Patagonotothen guntheri	SA, SG	No
Patagonotothen jordani	SA	?
Patagonotothen kreffti	SA	?
Patagonotothen longipes	SA	?
Patagonotothen ramsayi	SA	No
Patagonotothen sima	SA	?
Patagonotothen squamiceps	SA	?
Patagonotothen tessellata	SA	No
Patagonotothen trigramma	SA	?
Patagonotothen thompsoni	SA	?
Patagonotothen wiltoni	SA	?
Harpagiferidae		
Harpagifer bispinis	SA	?
Channichthyidae		
Champsocephalus esox	SA	Yes

SA South America, *NZ* New Zealand, *SG* South Georgia, ? AFG possession unknown species in three genera. Only one of these, *Bovichtus elongatus*, is found in Antarctica at the tip of the Antarctic Peninsula, while all other bovichtid species occur north of the ACC. Two notothenioid families are monotypic, and limited to temperate habitats. *Pseudaphritis urvillii* is the only member of the family Pseudaphritidae and occurs in southeast Australia and Tasmania. It is one of very few species of notothenioids that inhabits freshwaters such as slow-flowing streams and estuaries. The second monotypic family, Eleginopsidae, is represented by *Eleginops maclovinus*, which is distributed on the shelf areas of Patagonia and the Falkland Islands and commonly fished commercially in Chile and Argentina, where it is known as "róbalo." The remaining five families Nototheniidae, Bathydraconidae, Harpagiferidae, Artedidraconidae, and Channichthyidae represent the bulk of the notothenioid species diversity, including at least 125 species, most of which occur only in Antarctic waters. The exception to this are 22 non-Antarctic species of the genera *Lepidonotothen*, *Notothenia*, *Paranotothenia*, *Champsocephalus*, *Harpagifer*, *Dissostichus,* and *Patagonotothen* (see Table 1), which secondarily escaped the Southern Ocean to colonize the coastal waters of New Zealand and South America (Cheng et al. 2003).

3 Characteristics of Notothenioids

The diversification of Notothenioidei has been accompanied by a number of physiological innovations. In their ice-laden environment, the greatest ecophysiological challenge for ectotherm organisms is to prevent freezing of blood and body tissue. As marine teleost fishes have a higher colligative freezing point than seawater, contact with sea ice would lead to rapid freezing of body fluids (Cheng and Detrich 2007), which is lethal for almost all vertebrates. Thus, arguably the most important innovation of Antarctic notothenioids are antifreeze glycoproteins (AFGPs) that effectively lower their freezing point and thus prevent freezing upon contact with sea ice. AFGPs are present in all notothenioids of the five predominantly Antarctic families, with the exception of the nototheniid genus *Patagonotothen* that secondarily escaped to continental shelves of South America (Near et al. 2012). The AFGPs evolved from a pancreatic trypsinogen gene and are usually composed of 4–56 repeats of a threonine–alanine–alanine tripeptide, with threonine residues being O-glycosylated by disaccharides (Hsiao et al. 1990; Chen et al. 1997a). According to size differences, AFGPs are grouped into eight distinct types, with molecular weights between 2.6 and 33.7 kDa (DeVries and Cheng 2005). They are synthesized in the exocrine pancreas as large polyprotein precursors that are cleaved post-translationally to produce the eight different types of AFGPs (Hsiao et al. 1990; Evans et al. 2012). From the exocrine pancreas, AFGPs are discharged into the gastrointestinal tract (Cheng et al. 2006), where they bind to ice crystals ingested with food or water, and inhibit their growth until they are excreted along with feces (see Fig. 1b). Free AFGPs are resorbed via the rectal epithelium and enter the blood and the interstitial fluid. Blood-borne AFGPs reach

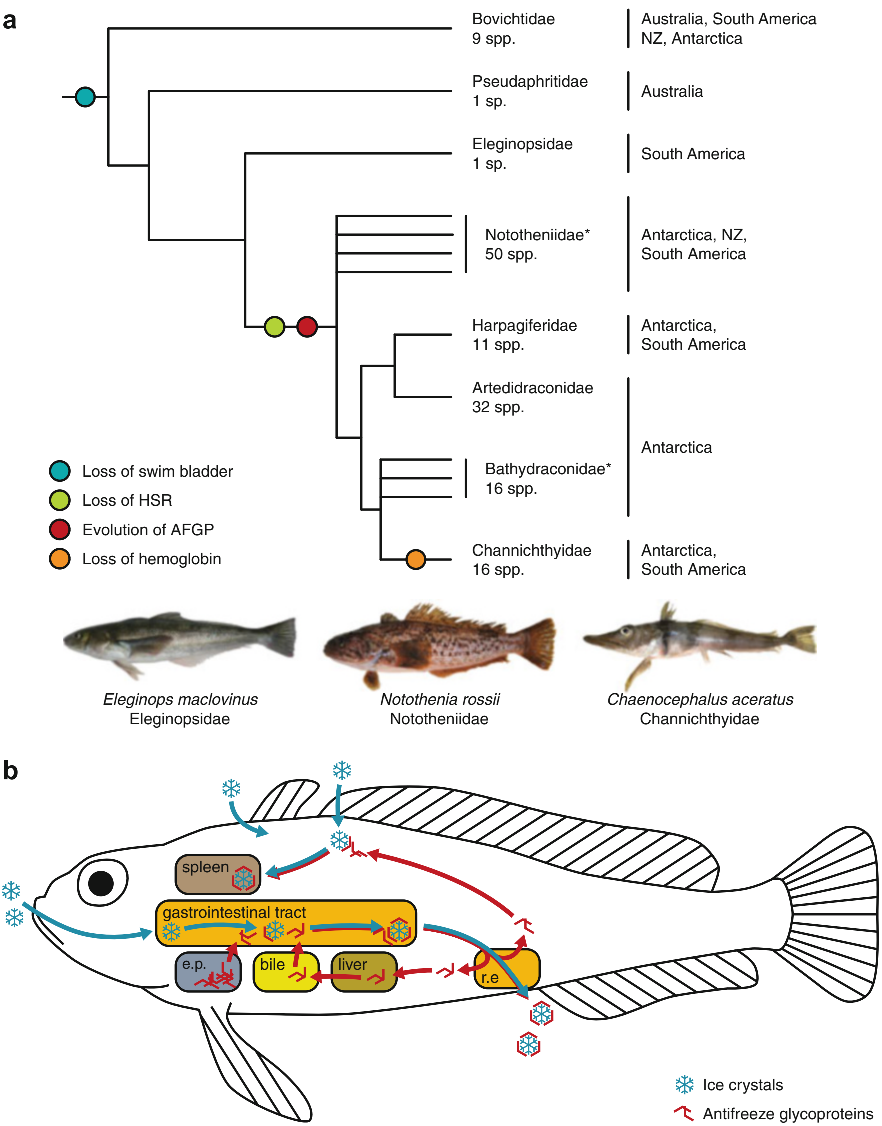

Fig. 1 (**a**) A simplified cladogram showing relations between notothenioid families, their species richness, and distribution. Major evolutionary innovations and losses (see main text) are marked by *circles* (*Asterisks* indicate presumably non-monophyletic families. *HSR* heat-shock response, *AFGP* antifreeze glycoproteins). (**b**) Schematic representation of the function of AFGP in notothenioids (*Blue-green arrows* indicate points of entry and transport of ice crystals, *red arrows* show AFGP pathways. For details see main text)

the bile via the liver and are discharged again into the gastrointestinal tract. Ice crystals present in the circulatory system usually enter the body through the epithelium, as endogenous ice nucleators are apparently absent in notothenioid fishes (Evans et al. 2011). It has been shown that larvae with low levels of AFGPs survive in ice-laden waters as long as the epithelium is intact, thus highlighting the important role of the epithelium as a protection against freezing (Cziko et al. 2006). In addition, external mucus of adult notothenioids contains AFGPs, which inhibit ice growth on the body surface, and thus prevent injury of the epithelium (albeit the mechanisms by which AFGPs are incorporated in the mucus are still unknown; Evans et al. 2011). If, despite these protective mechanisms, ice enters through the body surface, it is adsorbed by AFGPs of the blood and the interstitial fluid and transported to the spleen, where it is stored in ellipsoidal macrophages (Præbel et al. 2009; Evans et al. 2012). As no mechanism is known for the disposal of AFGP-bound ice from the spleen, it is assumed that ice accumulates in the spleen until seasonal warming events allow melting (Evans et al. 2011).

In a remarkable case of convergent evolution, near-identical AFGPs have independently emerged in at least seven Arctic species of the family Gadidae (Chen et al. 1997b; Zhuang 2013). As in notothenioids, gadid AFGP contains a large number of threonine–alanine–alanine tripeptide repeats, but apparently evolved from noncoding DNA through recruitment of an upstream regulatory sequence, rather than from a precursor gene as in notothenioids (Zhuang 2013). Different types of non-glycosylated antifreeze proteins (AFP) are known from distantly related Arctic and Antarctic fish groups, such as Zoarcidae (eelpouts), Labridae (cunner), Cottidae (sculpins), Hemitripteridae (sea ravens), Osmeridae (smelt), and Clupeidae (herring) (Cheng and DeVries 1989; Fletcher et al. 2001). The latter three lineages possess highly conserved sequences in both exons and introns of AFP genes despite an evolutionary distance of ~250 million years (Ma) (Betancur-R et al. 2013), which led Graham et al. (2008) to suggest sperm-mediated lateral gene flow as the mean of AFP acquisition. In this scenario, fish sperm would absorb foreign DNA from seawater, followed by partial integration into the sperm nucleus. Regardless of the mode of transfer, the presence of highly conserved AFP genes in distantly related lineages highlights the strong natural selection for freeze protection in sub-zero environments.

Besides AFGPs, another general feature found in all notothenioids is the lack of a swim bladder. For this reason, most notothenioids are heavier than seawater and dwell on or near the seafloor. However, several notothenioid lineages, including the genera *Aethotaxis*, *Pleuragramma*, and *Dissostichus*, have independently colonized the water column in a trend termed pelagization (Klingenberg and Ekau 1996; Rutschmann et al. 2011). If these fishes were not neutrally buoyant, continuous investment of muscular energy would be required to provide hydrodynamic uplift. Therefore, these species evolved a plethora of morphological adaptations to compensate for the lack of a swim bladder and attain neutral buoyancy (see below). To name but a few of these adaptations, some pelagic species have reduced ossification of the vertebral column and other body components, the scales of *Pleuragramma* and *Dissostichus* are only weakly mineralized in order to save weight, and *Pleuragramma* further deposits lipids in large assemblages of adipose cells to decrease overall density and to produce static uplift (Eastman 1993; Fernández et al. 2012).

In addition to the loss of the swim bladder, at least some notothenioid species have lost a second trait that is otherwise ubiquitous: The expression of heat-shock proteins (HSPs) as a response to elevated temperatures is regarded a universal characteristic of nearly all organisms, but is absent in the highly cold-adapted stenothermal nototheniid *Trematomus bernacchii* (Hofmann et al. 2000). Further research revealed that the absence of the heat-shock response (HSR) in *T. bernacchii*, as well as in a second member of the same genus, *T. borchgrevinki*, is not due to a loss of HSPs itself, but, on the contrary, due to a constitutive upregulation of Hsp70, which is attributed to permanent cold-stress conditions (Place et al. 2004; Place and Hofmann 2005). Subsequently, this finding has been extended to a representative of the Harpagiferidae, *Harpagifer antarcticus*, suggesting that the loss of the HSR affects most notothenioids and occurred just once during their diversification (Clark et al. 2008). Despite the lack of the classic heat-shock response, notothenioids have recently been shown to retain the ability to acclimatize to elevated temperatures of up to 13–18 °C, yet the molecular mechanisms of this heat hardening remain unknown (Bilyk and DeVries 2011; Bilyk et al. 2012).

Another exceptional loss affecting part of the notothenioid radiation, namely the members of the most derived family, the Channichthyidae, is the lack of the ability to synthesize hemoglobin (Ruud 1954; Eastman 1993). The Channichthyidae are thus the only vertebrate group without oxygen-bearing blood pigments. While the absence of hemoglobin apparently results from the loss of the β-globin subunit gene due to a single deletion event (di Prisco et al. 2002), truncated and inactive remnants of the α-globin gene are retained in the channichthyid genomes (Cocca et al. 1995; Near et al. 2006). Since the oxygen-carrying capacity of the hemoglobin-less phenotype is reduced by a factor of 10, the Channichthyidae evolved compensational features such as an increased blood volume that is 2–4 times that of comparable teleosts, a large stroke volume and cardiac output, and relatively large diameters of arteries and capillaries (Eastman 1993). The adaptive value and evolutionary cause of the loss of hemoglobin remain uncertain (Sidell and O'Brien 2006), but could potentially be related to low iron availability in the Southern Ocean (von der Heyden et al. 2012).

4 Notothenioid Phylogeography

The sister lineages of Notothenioidei have long been uncertain (Dettaï and Lecointre 2004), but molecular phylogenetic analyses that have recently become available support the placement of notothenioids within a redefined order of Perciformes that also contains the suborders Serranoidei, Percoidei, Scorpaenoidei, Triglioidei, Cottioidei, and the two families Percophidae and Platycephalidae (Betancur-R et al. 2013; Lautrédou et al. 2013). Within this order, relationships remain poorly resolved, but close affinities of Notothenioidei with Percophidae, Trachinidae, and Percidae have repeatedly been suggested (Matschiner et al. 2011; Lautrédou et al. 2013). Of the three families, Trachinidae and some members of Percidae are also characterized by the lack of a swim bladder (Lewis 1976; Evans and Page 2003), which could thus represent a shared loss between notothenioids and their sister lineage(s), depending on the precise interrelationships of these groups.

Within notothenioids, all molecular phylogenies to date agree on the sequence of the basal splits: the basal Bovichtidae are the sister group of all other notothenioid families, and the monotypic families; Pseudaphritidae and Eleginopsidae diverged before the diversification of the five predominantly Antarctic families (Balushkin 1992; Bargelloni et al. 2000; Near et al. 2004; Near and Cheng 2008; Matschiner et al. 2011; Rutschmann et al. 2011; Near et al. 2012; Betancur-R et al. 2013). Uncertainty remains only regarding the placement of the monotypic genus *Halaphritis*, which appears to be endemic to southeastern Australia and Tasmania. Only seven specimens are known of *H. platycephala*, and DNA could not be extracted from this species (Last et al. 2002). Morphologically, *H. platycephala* superficially resembles the sympatrically occurring pseudaphritid *Pseudaphritis urvillii*, but was provisionally assigned to the Bovichtidae, as it shares almost all diagnostic characters defining this family (Last et al. 2002).

Regardless of the exact affinities of *Halaphritis* with Bovichtidae and Pseudaphritidae, three out of the four most ancestral genera of notothenioids (the monotypic bovichtid genus *Cottoperca* being the exception) occur in, or are even endemic to Australian waters, suggesting that the initial diversification of the suborder took place in this region (Balushkin 2000; Matschiner et al. 2011). This scenario was supported by the time-calibrated molecular phylogeny of Matschiner et al. (2011), who found that the separation of bovichtid and pseudaphritid ancestors may have coincided with shelf area fragmentation between Australia and New Zealand around 70 Ma ago, and that the divergence between Pseudaphritidae and more derived Notothenioidei could have been caused by the breakup of Australia and Antarctica that became complete only around 32 Ma (Barker et al. 2007). According to this hypothesis, individual bovichtid lineages that occur in South America and the island of Tristan da Cunha could have arrived with paleogene currents, owing to their extended pelagic larval durations (Balushkin 2000; Matschiner et al. 2011). The same time-calibration further supports a vicariant separation of the South American Eleginopsidae from the five predominantly Antarctic families in the Eocene, before the opening of the Drake Passage around 41 Ma (Scher and Martin 2006).

Subsequent to the opening of both the Tasman Gateway and the Drake Passage, the onset of the ACC led to thermal isolation of the Antarctic continent and, in combination with declining atmospheric carbon dioxide (DeConto and Pollard 2003; Scher and Martin 2006), to a decrease in water temperatures by up to 4 °C (Nong et al. 2000), resulting in widespread Antarctic continental glaciation at the time of the Eocene–Oligocene transition 34–33 Ma. Whereas the drop to sub-zero temperatures may have been delayed in the marine environment compared to continental Antarctica, there is evidence for sea ice since the early Oligocene. Deposits in offshore drill cores show that since that time, glaciers have repeatedly extended well onto the continental shelf (Cape Roberts Science Team 2000). Sea ice-dependent diatoms have been found in Oligocene sediments (Olney et al. 2009), and widespread ice-rafting occurred as early as 33.6 Ma (Zachos et al. 1996). Freezing conditions in Antarctic waters have been episodic before the middle Miocene climate transition (MMCT) around 14 Ma (Shevenell et al. 2004); however, even seasonal presence of sea ice during cold events of the Oligocene and early Miocene (Naish et al. 2001) must have had a strong impact on the marine fauna of Antarctica.

Fossil evidence from the La Meseta Formation of Seymour Island, off the Antarctic Peninsula, shows that a diverse temperate ichthyofauna existed in the Late Eocene, when Antarctic water temperatures ranged between 10 and15 °C (Eastman 1993; Claeson et al. 2012). Even though ancestral notothenioid lineages were probably present in Antarctic waters during the Eocene, only a single putative notothenioid fossil is known from the La Meseta Formation. *Proeleginops grandeastmanorum* has originally been described as a gadiform (Eastman and Grande 1991), but was subsequently claimed to represent an early member of Eleginopsidae (Balushkin 1994). The fossil has been used to time-calibrate the molecular phylogeny of Near (2004); however, its taxonomic assignment remains questionable. The type locality is specified as RV-8200 and reported to be about 40 Ma (Eastman and Grande 1991). However, according to Long (1992), RV-8200 lies in the lower section of "Tertiary Eocene La Meseta" (Telm) 4, the age of which has recently been reevaluated and is now considered to be 52.5–51.0 Ma (Ivany et al. 2008). This age is substantially older than the mean molecular date estimate for the origin of Eleginopsidae (42.9 Ma) in the study of Matschiner et al. (2011). In their molecular analysis, Matschiner et al. (2011) deliberately excluded *P. grandeastmanorum* as a time constraint due to its debated taxonomic assignment. The presumed fit of their results with the fossil's age (there assumed to be 40 Ma) supported the interpretation of *P. grandeastmanorum* as a notothenioid; however, this does not hold if the fossil is in fact 52.5–51 Ma old. Thus, Notothenioidei may not be represented at all in the Eocene fossil record of the La Meseta Formation, even though a large number of other fishes are found at the same location.

According to the time-calibrated molecular phylogeny of Matschiner et al. (2011), the diversification of the five predominantly Antarctic notothenioid families began near the Oligocene–Miocene boundary, about 24 Ma. Their study agrees with almost all other molecular phylogenies of notothenioids in finding the most basal divergences of Antarctic notothenioids within a paraphyletic family Nototheniidae (Bargelloni et al. 2000; Near and Cheng 2008; Rutschmann et al. 2011; Near et al. 2012; Dettai et al. 2012; Betancur-R et al. 2013). However, uncertainty remains regarding the sister group of all other Antarctic notothenioids, with different analyses recovering either the genus *Gobionotothen* (Matschiner et al. 2011; Near and Cheng 2008; Near et al. 2012), *Aethotaxis* (Rutschmann et al. 2011; Betancur-R et al. 2013), a clade composed of *Aethotaxis* and *Dissostichus* (Near and Cheng 2008; Near et al. 2012; Dettai et al. 2012), or the combined genera *Pleuragramma*, *Aethotaxis*, and *Dissostichus*, in this position (Near et al. 2012).

Individual groups within Nototheniidae receive overwhelming support from molecular analyses, such as the species-rich Trematominae that are composed of the genera *Trematomus*, *Lepidonotothen*, *Patagonotothen*, *Pagothenia* (now included in *Trematomus*; Near et al. 2012), and *Cryothenia* (Janko et al. 2011; Lautrédou et al. 2012), or the clade combining *Notothenia* and *Paranotothenia* (Dettai et al. 2012). Similarly, the more derived families Artedidraconidae, Harpagiferidae, and Channichthyidae appear nested within the paraphyletic Nototheniidae, but are themselves strongly supported to be monophyletic (Derome et al. 2002; Johnston et al. 2003; Rutschmann et al. 2011; Near et al. 2012; Dettai et al. 2012). The same cannot be claimed for the family Bathydraconidae. Monophyly of a clade combining Bathydraconidae and Channichthyidae has not been questioned; however, most analyses recover Channichthyidae nested within Bathydraconidae, thus rendering the latter family paraphyletic (Derome et al. 2002; Near et al. 2012; Dettai et al. 2012; Betancur-R et al. 2013).

5 The Adaptive Character of the Notothenioid Radiation

Adaptive radiation is the rapid origin of an array of morphologically and ecologically distinct species from a common ancestor, as a consequence of the adaptation to distinct ecological niches (Schluter 2000; Gavrilets and Losos 2009). Adaptive radiations typically occur after an ancestral species conquers a new, island-type environment with many open niches (“ecological opportunity”), after the extinction of antagonists, liberating previously occupied niches (another form of opportunity), or following the evolution of a novel trait (a so-called key innovation) allowing to effectively exploit new niches (Schluter 2000; Gavrilets and Vose 2005; Yoder et al. 2010). Schluter (2000) defined four main criteria of an adaptive radiation: common ancestry, rapid diversification, phenotype-environment correlation, and trait utility. In the following, we discuss these criteria with respect to the notothenioid species flock:

The first two criteria, common ancestry and rapid diversification, were highlighted by several studies investigating notothenioid phylogeny and diversification rates (Eastman 2005; Matschiner et al. 2011; Near et al. 2012). However, diversification rates seem to be lower in notothenioids compared to other adaptive radiations like the East African cichlid fishes (Rutschmann et al. 2011). It has been suggested that this inequality is due to the lack of habitat heterogeneity, the absence of certain prime inshore habitats in the Antarctic shelf area, enhanced long-range migration ability of pelagic larval stages (Damerau et al. 2014), the absence of genetic population structuring over large distances (see below), and that the notothenioid radiation may not yet have reached its final stage (see Rutschmann et al. 2011, and references therein).

Phenotype–environment correlation and trait utility in notothenioids are best understood with regard to pelagization (the shift from a benthic to a pelagic lifestyle) that has arisen independently in several notothenioid clades (Klingenberg and Ekau 1996; Rutschmann et al. 2011). This shift, referred to as “the hallmark of the notothenioid radiation” (Eastman 2000), was facilitated by adaptations enabling various species to exploit previously unoccupied niches in the water column. Starting from a benthic ancestor, substantial morphological diversification led to phenotypes suited for foraging modes of pelagic or partially pelagic zooplanktivory and piscivory (Eastman 2000). Notothenioids diversified to fill these niches while at the same time also remaining the dominant benthic group of vertebrates (Eastman 2000).

Various morphological and physiological adjustments were needed for species to be able to colonize the water column, mainly to achieve effective swimming performance and to compensate the lack of a swim bladder that most other teleosts use to regulate their buoyancy (Klingenberg and Ekau 1996). Several notothenioid species achieved neutral buoyancy by reducing the mineralization of the skeleton and scales (a pedomorphic trait; Balushkin 2000; Eastman 2000), and by the accumulation of lipid deposits (Eastman 2000). While pelagization has occurred independently in several notothenioid clades (see e.g. Rutschmann et al. 2011), the most complete examples can be found within the family Nototheniidae, where about half of the species occupy the ancestral benthic habitat, whereas the other half adopted a semipelagic, epibenthic, cryopelagic, or pelagic lifestyle (Eastman 2005). Pelagization may be best depicted by *Pleuragramma antarctica,* a sardine-like zooplankton feeder. Morphological adaptations to a life in the water column are highly pronounced in this species, and it evolved to become the dominant species in the water column and the key species in the high-Antarctic food web (Eastman 2005), with several species of channichthyids (*Chionodraco hamatus, Chionodraco myersi, Dacodraco hunteri, Neopagetopsis ionah*) feeding almost exclusively on this species (La Mesa et al. 2004). On the High Antarctic shelf, *Pleuragramma antarctica* is the most important prey item for *Dissostichus mawsoni*, and top predators like penguins, Weddell seals (*Leptonychotes weddellii*), and minke whales (*Balaenoptera bonaerensis*) also greatly rely on *Pleuragramma antarctica* as a food source (Eastman 1985; La Mesa et al. 2004). *Pleuragramma antarctica* has become the dominant species of the midwater fish fauna, with over 90 % both in abundance and biomass (La Mesa et al. 2004), and this dominance can be attributed to a wide range of highly specialized morphological adaptations.

Pleuragramma antarctica evolved neutral buoyancy by driving the abovementioned adaptations (reduced mineralization of the skeleton and lipid deposits) to a degree of completion unreached by any other notothenioid species. Lipid, more precisely triglyceride, is stored in intermuscular and subcutaneous sacs. Translucent sacs containing lipid are present between the muscle masses at the bases of the dorsal and anal fins. Furthermore, smaller subcutaneous sacs can be found at the sides of the body (DeVries and Eastman 1978; Eastman 1993).

The skeleton of *P. antarctica* is pedomorphic and reduced, including the reduction of vertebrae and the persistence of the notochord in adult specimens (Eastman 1993).

Adaptations for a life in the water column other than the reduction of buoyancy include morphological changes to alter feeding and swimming performance. *Pleuragramma antarctica* possesses short, protractile jaws featuring a single row with few but large oral teeth suited for suction feeding on planktonic prey (Albertson et al. 2010). Notothenioids living in the water column generally tend to have more elongated, slimmer bodies, but smaller heads than benthic feeders (Klingenberg and Ekau 1996)—the latter probably due to planktonic prey generally being smaller than benthic prey (Klingenberg and Ekau 1996, and references therein). Analyses of the shape of the operculum (Wilson et al. 2013) have furthermore shown that members of the Channichthyidae and Nototheniidae evolved broadly similar opercle shapes in relation to their position along the benthic-pelagic axis and that benthic species generally have an extended posterior margin of the opercle compared to pelagic species, probably reflecting the generally larger head width of benthic notothenioids.

Ecological diversification along the benthic–pelagic axis is also reflected in carbon isotope levels, which can be used to approximate the habitat type. The lowest ∂^{13}C values are found in more pelagic species like *Chaenodraco wilsoni*, *Champsocephalus gunnari*, and *Pleuragramma antarctica*, while strictly benthic notothenioids like *Gobionotothen gibberifrons*, *Lepidonotothen nudifrons*, and *Pogonophryne scotti* occupy the upper end of the range (Rutschmann et al. 2011). Carbon isotopic levels further correlate with nitrogen isotope amounts in notothenioids, indicating a connection between habitat and trophic levels. With the exception of the pelagic top predator *Dissostichus mawsoni*, the highest ∂^{15}N values are found almost exclusively in benthic species. Remarkably, very similar ranges of isotope signatures are present in at least two notothenioid families, Nototheniidae and Channichthyidae, suggesting convergent ecological evolution along habitat and trophic axes, which is considered characteristic for adaptive radiation (Muschick et al. 2012).

Other than buoyancy adaptations, a second trait that serves well to illustrate both phenotype–environment correlation and trait utility in notothenioids are AFGPs. As these proteins are present in all Antarctic notothenioid clades they are commonly thought to have evolved only once prior to the notothenioid radiation (Chen et al. 1997a; Cheng et al. 2003). The utility of AFGPs in the Antarctic environment is obvious, as these proteins are essential to prevent the formation of ice crystals within the fish's body, and thus are needed for the survival of notothenioids in sub-zero waters (as described above). A correlation between the phenotype and the environment could also be demonstrated in a case study of 11 channichthyid species, where freeze avoidance due to AFGP expression was found to be greater in species occurring at higher latitudes (and thus at colder water temperatures; Bilyk and DeVries 2010). Thus, the four criteria outlined by Schluter (2000) for the detection of adaptive radiation are all fulfilled by Antarctic notothenioids, whereby the latter two criteria (phenotype–environment correlation and trait utility) apply to even more than one notothenioid characteristic.

The evolution of AFGPs is often viewed as a "key-innovation" (see Schluter 2000 for more details on the term), meaning that the emergence of this trait allowed notothenioids to effectively exploit new niches and therefore triggered the notothenioid adaptive radiation (Matschiner et al. 2011). It has been hypothesized that the drop to sub-zero water temperatures around Antarctica led to the extinction of most of the previously existing ichthyofauna (Eastman 1993), which enabled notothenioids to diversify and occupy the subsequently vacant niches (Matschiner et al. 2011). However, diversification rate analyses have recently suggested that major pulses of lineage diversification within notothenioid clades, responsible for a large share of the notothenioid species richness, occurred substantially later than the origin of AFGPs, thus suggesting that the key innovation of AFGPs may not have been the only driver of the notothenioid radiation (Near et al. 2012).

6 Non-Antarctic Notothenioids

The non-Antarctic notothenioids comprise two main groups of fishes: basal lineages that diverged before the isolation of Antarctica (families Eleginopsidae, Pseudaphritidae, and Bovichtidae), which therefore never experienced the "Antarctic permanent cold conditions" during their evolutionary history, and a more derived group that presumably originated from northward dispersal events of Antarctic ancestors (belonging to families Nototheniidae, Harpagiferidae, and Channichthyidae). The comparison between Antarctic and non-Antarctic notothenioids may be important to better understand the numerous unique traits that notothenioids have evolved in Antarctic waters. In addition, the specific comparison with non-Antarctic notothenioids of Antarctic ancestry may allow the identification of features that allow them to inhabit cold-temperate waters outside the APF. The knowledge about these latter traits may be a key to better understand how evolution in the stable cold waters of Antarctica has constrained the ability of Antarctic notothenioids to deal with environmental changes and global warming.

So far, phylogenetic analyses have identified seven genera (represented by 22 species) that are nested within the Antarctic notothenioid clade, but occur north of the border drawn by the APF (Table 1). There are still many uncertainties about the phylogenetic relationships between and within these seven genera, though. Nonetheless, there is some evidence that supports monophyly of a clade combining *Paranotothenia magellanica* and the two non-Antarctic *Notothenia* species (Cheng et al. 2003). Likewise, *Lepidonotothen macrophthalma*, the only non-Antarctic representative of the genus, has never been included in a molecular phylogeny, but is morphologically closely related to *L. squamifrons* (Balushkin 2000; Pequeño 2000), which was found to be the sister taxon of the genus *Patagonotothen* (Dettai et al. 2012; Near et al. 2012). Thus, there is the possibility that *L. macrophthalma* and the genus *Patagonotothen* form a monophyletic group as well. The remaining components of the group of non-Antarctic notothenioids are strongly supported as non-monophyletic (Rutschmann et al. 2011; Dettai et al. 2012; Near et al. 2012). Therefore, the most parsimonious explanation would involve at least five putative ancestors that dispersed northward across the APF. Three would belong to the family Nototheniidae, the most basal one of the five Antarctic notothenioids families, and the other two are members of the more derived high-Antarctic families Harpagiferidae and Channichthyidae.

Some authors have suggested that the “escapes” of these ancestors from the Antarctic waters may be linked to temporally northwards movements of the APF (Bargelloni et al. 2000; Cheng et al. 2003; Coppes Petricorena and Somero 2007). If the five putative escapes are linked to paleoceanographic events, it seems that at least two different events would be involved. The divergence between the Antarctic and non-Antarctic sister lineages of the family Nototheniidae apparently took place more than 7 Ma (Near 2004; Near et al. 2012), whereas the divergence of *Champsocephalus esox* and its Antarctic sister taxon *C. gunnari* was estimated at around 4–1.7 Ma (Near et al. 2004; Stankovic et al. 2002). An estimation of the divergence time between the South American *Harpagifer bispinis* and its closest relative from Antarctica, *H. antarcticus,* is still lacking, albeit their very similar morphology (Gon and Heemstra 1990) may suggest a recent divergence, probably closer in time to the separation of non-Antarctic Channichthyidae than to that of non-Antarctic nototheniids.

The Antarctic ancestry of these non-Antarctic notothenioids led to the prediction that these species might have AFGP genes or at least its remnants in their genomes. The occurrence of AFGP in non-Antarctic notothenioids from South America and New Zealand waters has already been examined in eight species (Cheng and Detrich 2007), confirming its presence in four of them (Table 1). The most parsimonious explanation for the apparent absence of AFGP in *Dissostichus eleginoides* and three species of *Patagonotothen* involves at least two independent losses or severe mutations of this gene. On the other hand, whereas the Antarctic notothenioids lost the HSR, the New Zealand notothenioid *Notothenia angustata* is able to upregulate the transcription of hsp70 in response to heat shock (Hofmann et al. 2005).

One of the main differences in the evolutionary history between non-Antarctic and Antarctic notothenioids is that the former evolved in the presence of fish groups that are absent or uncommon in Antarctic waters. Therefore, these lineages likely experienced more competition compared with the Antarctic notothenioids, limiting the occupation of niches distinct from the original benthic one. In agreement with this idea, no evidence for diverse static buoyancy values was found in non-Antarctic notothenioids that would allow them to occupy different areas in the water column in the same way as Antarctic notothenioids (Fernández et al. 2012). Comparison of Antarctic and non-Antarctic sister taxa with modern genomic technology may help to identify the genetic changes underlying the transition across the APF, and reveal whether or not they led to adaptations in a similar fashion in different notothenioid families.

The main radiation of notothenioids occurred in an Antarctic environment, and thus the bulk of notothenioids species inhabit the Southern Ocean within the APF. Nonetheless, the second-most species-rich genus is found almost exclusively in non-Antarctic waters: the genus *Patagonotothen* with so far 15 described species is only surpassed in diversity by the Antarctic genus *Pogonophryne* that contains 24 species. The 15 *Patagonotothen* species occur in southern South America with the only exception being *P. guntheri,* which has a trans-APF distribution and it is also found in South Georgia. Morphological analysis suggests that *P. guntheri* may be considered as a derived species within the genus (Balushkin 1992). Thus its presence within the APF is probably a derived character rather than an ancestral one.

The age of the most recent common ancestor of the *Patagonotothen* genus was estimated to be around 5 Ma (Near et al. 2012). This implies a rather rapid radiation of the 15 species, whereby the drivers of this radiation remain unknown but are likely unrelated to the key innovation hypothesis for AFGP. The inshore fish fauna of southern South America seems to be characterized by generally low diversity (Ojeda et al. 2000), which could have facilitated the *Patagonotothen* expansion. A similar radiation in the same region is the one exhibited by the species of the mollusc genus *Nacella*. In this case it has been proposed that the currently overlapping distributions of *Nacella* species and their close genetic relationships could be explained by allopatric speciation, or at least incipient separation, in separate refugia during glaciations, followed by geographical re-expansion and ecological separation (González-Wevar et al. 2011). A similar scenario could explain the *Patagonotothen* radiation; however, more research on this group will be needed to support this hypothesis.

7 Demography and Population Structure in Antarctic Notothenioids

Whereas phylogenies can inform about the macroevolutionary history of Antarctic notothenioids, the underlying forces of speciation processes are commonly linked to ecological factors that often act on a far more microevolutionary timescale (Nosil 2012). Understanding the population dynamics of species, especially the factors modulating demography and gene flow among populations, is therefore crucial for the understanding of the adaptive radiation of notothenioids. Molecular genetic signatures left by past and present demographic events, such as population size changes or migration, allow us to disentangle the importance of biotic and abiotic factors that influence differentiation processes on the population level.

For polar organisms including notothenioid fishes, it has often been hypothesized that population size changes are driven by glaciation cycles associated with severe implications for species' survival and distribution (Kennett 1982; Eastman 1993; Petit et al. 1999). During major glacial periods, the Antarctic ice sheet extended as far as the edge of the continental shelf (Ingólfsson 2004; Gersonde et al. 2005), sometimes eradicating Antarctic marine bottom communities on the large scale (Thatje et al. 2005) and "bulldozing the surviving fauna to the deep continental margin" (Barnes and Conlan 2007). As a result, populations were periodically isolated in remaining ice-free refugia (Barnes et al. 2006), which was suggested as a key mechanism for allopatric speciation (Hewitt 1996; Rogers 2007), and is expected to result in population expansions subsequent to glacial retreat. The use of population level molecular data allows the investigation of past population size changes, and in fact has provided evidence for demographic expansions in multiple notothenioid fishes (Zane et al. 2006; Janko et al. 2007; Matschiner et al. 2009), which highlights the impact of glacial cycles on notothenioid populations.

The extent to which population fragmentation leads to differentiation and allopatric speciation in notothenioid fishes remains unclear, but is strongly linked to their potential for long-distance gene flow. While distances between isolated notothenioid populations are on the order of thousands of kilometers (Matschiner et al. 2009), notothenioid fishes are characterized by extended pelagic larval stages that may last between a few months and more than 1 year (Loeb et al. 1993; La Mesa and Ashford 2008). This, in combination with the strong current of the ACC endows the propagules of many species with a great potential for long-distance dispersal (Damerau et al. 2014). Hence, high levels of gene flow could be expected between distant notothenioid populations, which might counteract differentiation and allopatric speciation events.

Since the advent of DNA sequencing and genotyping technologies, estimates of population connectivity have been inferred based on population genetic tools, which measure the distribution of genetic variation among populations. To date, at least 29 population genetic studies have been published for 22 notothenioid species (see references in Volckaert et al. 2012, as well as Carvalho and Warren 1991; Smith and Gaffney 2000; Damerau et al. 2012; Agostini et al. 2013; Damerau et al. 2014). The results of these studies were highly variable and depended clearly on sampling designs and applied marker types (see Table 3 in Matschiner et al. 2009). Nonetheless, an overall trend uncovered by these studies is the decrease of genetic homogeneity among populations with distance, indicating an isolation-by-distance relationship. On a regional scale (within a few hundred kilometers), the vast majority of species showed genetic homogeneity. Even population differentiations within ocean sectors are predominantly insignificant, sometimes over several thousand kilometers. Although a marginal majority of studies revealed significant population differentiations between ocean sectors, many populations of species with circum-Antarctic distributions showed no significant differentiation, as, for example, in the bentho-pelagic Antarctic toothfish *Dissostichus mawsoni* (Smith and Gaffney 2005) or the truly pelagic Antarctic silverfish *Pleuragramma antarctica* (Zane et al. 2006). Moreover, populations of strictly benthic species, such as *Gobionotothen gibberifrons*, which is confined to shelf areas as adults, were not significantly differentiated over their distribution range on subantarctic islands (Matschiner et al. 2009).

The seemingly high levels of gene flow among populations separated by deep ocean over large geographic scales, but connected by currents like the ACC, regardless of the adult life strategy, suggest that gene flow is mediated via dispersal of pelagic developmental stages such as eggs, larvae, or juveniles. This finding is corroborated by genetic breaks that have been found over much shorter geographic distances, where oceanographic barriers exist. For example, populations of *D. eleginoides* are not significantly differentiated over large parts of its circumpolar distribution range, whereas populations that are geographically close but separated by the APF show little connectivity (Shaw et al. 2004; Rogers et al. 2006). Hence, oceanographic features are an important factor regulating population connectivity of notothenioids by either enhancing or attenuating larval dispersal, as has also been shown in species from warmer waters with distinctly shorter pelagic larval stages (e.g. Taylor and Hellberg 2003; Bay et al. 2006; Cowen and Sponaugle 2009). However, the general validity of the observed patterns is limited by varying sampling designs, genetic marker types, and species' biogeography, what makes general inferences about gene flow by larval dispersal a challenging task.

8 Conclusions and Outlook

It has been 60 years since notothenioid fishes were first brought to the attention of a broader scientific community, with Johan T. Ruud's (1954) publication in *Nature* demonstrating the loss of hemoglobin as the cause of the colorlessness of the blood of channichthyids. Our knowledge about the nature of the notothenioid evolution has greatly increased over the recent decades, especially since the advent of molecular sequencing technology, but important questions of the notothenioid radiation remain to be answered. While recent phylogenetic work (Rutschmann et al. 2011; Near et al. 2012; Lautrédou et al. 2012) helped to identify multiple well-supported clades such as Trematominae, Artedidraconidae, and Channichthyidae, the same studies also disagreed with respect to more basal notothenioid relationships and thus highlight the need for more comprehensive sequence data sets. Due to ongoing sampling efforts in combination with rapid improvements in sequencing technologies and methodological advances, we may soon be able to address these questions. Through combination of population level and species level sequence data, approaches like the multi-marker coalescent model implemented in *BEAST (Heled and Drummond 2010) are able to account for incomplete lineage sorting, which is common in rapidly diversifying clades (Koblmüller et al. 2010), and could be the cause of incompatibilities between published phylogenies. To date, family-level relationships within Bovichtidae and Harpagiferidae have not been investigated in detail, but could provide valuable insights into the geographic origin and the early phylogeography of the notothenioid radiation. Finally, thanks to the rapidly decreasing cost of next generation sequencing, genome-size data sets may soon be available for notothenioid fishes and permit investigations into the molecular basis of notothenioid adaptations.

Other than molecular data, recent studies have begun to systematically quantify morphological and physiological characteristics (Rutschmann et al. 2011; Near et al. 2012; Wilson et al. 2013), a trend that will continue to give us increasingly well-resolved descriptions of ecological niches occupied by notothenioid taxa. In addition, more behavioral data can be acquired through continuing field expeditions equipped with remotely operated underwater vehicles. In combination, these data will allow us to better understand the axes along which the notothenioid radiation has proceeded (and continues to proceed), as well as the molecular adaptations that enabled their tremendous evolutionary success.

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