INVESTIGATING THE LARVAL/JUVENILE NOTOTHENIOID FISH SPECIES ASSEMBLAGE IN MCMURDO SOUND, ANTARCTICA USING PHYLOGENETIC RECONSTRUCTION

BY

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THESIS

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ABSTRACT

Aim

To investigate and identify the species found within the little-known larval and juvenile notothenioid fish assemblage of McMurdo Sound, Antarctica, and to compare this assemblage to the well-studied local adult community.

Location

McMurdo Sound, Antarctica.

Methods

We extracted genomic DNA from larval and juvenile notothenioid fishes collected from McMurdo Sound during the austral summer and used mitochondrial ND2 gene sequencing with phylogenetic reconstruction to make definitive species identifications. We then surveyed the current literature to determine the adult notothenioid communities of McMurdo Sound, Terra Nova Bay, and the Ross Sea, and subsequently compared them to the species identified in our larval/juvenile specimens.

Results

Of our 151 larval and juvenile fishes, 142 specimens or 94.0% represented seven species from family Nototheniidae. Only one specimen was not matched directly to a reference sequence but instead was placed as sister taxon to *Pagothenia borchgrevinki* with a bootstrap value of 100 and posterior probability of 1.0. The nine non-nototheniid specimens represented the following six

species: *Pogonophryne scotti*, *Pagetopsis maculatus*, *Chionodraco myersi*, *Chionodraco hamatus*, *Neopagetopsis ionah*, and *Psilodraco breviceps*.

Main conclusions

All of our specimens (100%) were identified as notothenioids, closely matching the adult fauna which is 91% notothenioid. Our specimens were overwhelming nototheniid (94% abundance), compared to the 50% nototheniid abundance that is seen in adults, indicating that our data suffer from sampling bias – a common problem in larval studies. Surprising results included a specimen that we hypothesize to be *Pagothenia brachysoma* as it was placed as sister taxon to *P. borchgrevinki*. Additionally, *C. hamatus*, *C. myersi*, *N. ionah*, *P. maculatus*, and *P. scotti* have never yet been documented in McMurdo Sound though they are found in the nearby Ross Sea. Finally, *P. breviceps* is of note as this larval fish was collected approximately 7,000 miles away from any adult specimen of this species. This study also provides the framework for future studies of gene flow and population connectivity both within McMurdo Sound and with the much larger, nearby Ross Sea and presents the first investigation into the larval fish diversity of McMurdo Sound.

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INTRODUCTION

Antarctic waters are home to a largely benthic and highly endemic ichthyofauna, dominated by the members of the perciform suborder Notothenioidei, the result of an adaptive radiation within the isolated Southern Ocean (Eastman, 2005). There are 107 recognized Antarctic notothenioid species comprising five families: Artedidraconidae, Bathydraconidae, Channichthyidae, Harpagiferidae, and Nototheniidae (Eastman & Eakin, 2015). In coastal waters, notothenioids make up as much as 71% of the species diversity and 91% of the biomass (Eastman & Hubold, 1999; Eastman, 2005). In the near-shore waters of McMurdo Sound (77°S), members of the genus *Trematomus* (including *Pagothenia*; family Nototheniidae) are particularly prevalent (Eastman & Devries, 1982; Buckley, 2013), with a few species from the other families present as well (Eastman & Devries, 1982).

While nearly all notothenioid species have benthic lifestyles as adults, many species have pelagic eggs and most have extended pelagic larval stages (Kock, 1992; Loeb *et al.*, 1993). Other than this basic knowledge, the pelagic larval assemblage is poorly studied at present. Collection of larval fish is inherently logistically difficult, especially so at high latitudes such as McMurdo Sound that is chronically ice covered and frequented by severe weather conditions. Another major difficulty is larval species identification. Unlike adults with distinctive external morphological differences that readily enable species identification, larval and very young juvenile fishes of different species often look similar superficially, making visual identification unreliable or impossible (Cziko *et al.*, 2006). The rarity of larval specimens being collected and in good physical condition to inspect and document perpetuates the inability to gain visual familiarity, making identification of larval and juvenile specimens by sight an enduring

challenge. For the same reason, there is a paucity of larval identification guides. Available larval identification keys (Kellerman, 1990; North & Kellerman, 1990) are also fraught with uncertainties because the common distinguishing characters used such as pigmentation patterns vary with developmental stages.

Despite existing difficulties, understanding the Antarctic larval fish distribution is and must be an integral component in understanding Antarctic fish biodiversity and biogeography. As adults, notothenioid fishes are largely benthic and often described as sedentary (Eastman, 1993) with limited dispersal. For example, experiments on *Trematomus bernacchii* Boulenger, 1902 have demonstrated that adults of this species only traveled up to 14 m over an eight-day period (Miyamoto & Tanimura, 1999), and that individuals could be recovered within 500 m of their original capture point after a period of seven months (Kawaguchi *et al.*, 1989). Conversely, notothenioid larvae are highly pelagic and might readily be dispersed by the strong Antarctic current systems (Eastman, 1993; Loeb *et al.*, 1993; Kuhn *et al.*, 2009; Matschiner *et al.*, 2009; Damerau *et al.*, 2012; Volckaert *et al.*, 2012), therefore likely playing an important contributing role in species distributions and gene flow among populations around the Southern Ocean (Kuhn *et al.*, 2009; Matschiner *et al.*, 2009; Damerau *et al.*, 2012; Volckaert *et al.*, 2012). With pelagic larval stages that last from months to a year or more (Kellerman, 1990; Kock, 1992), larvae entrained by these currents may potentially be moved to distant habitats from their hatching sites (Kuhn *et al.*, 2009; Matschiner *et al.*, 2009; Volckaert *et al.*, 2012). Comparing the taxon composition of both larval and adult species within the same site would help address the possibility of this important dispersal mechanism.

The adult fish fauna of McMurdo Sound, the southernmost component of the Ross Sea system, is well documented as a result of decades of biological research supported by the US

Antarctic Program. However, very few studies involved larval fish. Only two studies utilized larval *Gymnodraco acuticeps* Boulenger, 1902 and *Pagothenia borchgrevinki* (Boulenger, 1902) from McMurdo Sound, as well as *Pleuragramma antarctica* Boulenger, 1902 from Terra Nova Bay (74.8°S, 164.5°E) further north (Cziko *et al.*, 2006; Evans *et al.*, 2006). The studies were not on investigating species diversity of larval assemblages, but physiological studies on freeze avoidance and energy metabolism in early development utilizing species whose embryos were discovered to be available in relatively large numbers. The only dedicated studies of high-latitude larval diversity were in the nearby western Ross Sea and Terra Nova Bay (Guglielmo *et al.*, 1998; Vacchi *et al.*, 1999; Granata *et al.*, 2002), not McMurdo Sound. Vacchi *et al.* (1999) identified 21 species of larval and juvenile fishes collected by midwater trawls, with nototheniids representing 94.3% of all individuals caught. Guglielmo *et al.* (1998) collected 34,436 fish larvae from Terra Nova Bay, though they focused primarily on *P. antarctica* within their collection as this species represented approximately 98% of their catch. The largest study was by Granata *et al.* (2002) when they collected over 390,000 larvae from the Ross Sea and identified 46 species based on morphology. One must note that the presence and abundance of larval species from these studies do not necessarily correspond to that of the Ross Sea adult abundance reported by Eastman and Hubold (1999), nor to adults reported from the shallow areas of McMurdo Sound by Buckley (2013) which is part of the Ross Sea system. While this discrepancy is intriguing, it may reflect sampling bias due to the exact fishing locations, fishing method, the time of year, and how these factors correspond to the hatching times and larval development of the various notothenioid species.

During various austral summer field seasons in McMurdo Sound, we collected a number of larval and juvenile fish from McMurdo Sound by scuba diving, dip net, plankton tow or from

gut content of adult fishes. Juveniles were readily obvious as either red-blooded species or hemoglobin-less icefish but not the small larval specimens, and definitive species identification by appearance was not possible for any. Therefore, in this study we obtained mitochondrial DNA sequences (complete ND2 gene) of these larval specimens and, along with known adult sequences, used phylogenetic reconstruction to determine species identity. The extensive ND2 gene sequences of adult notothenioids from our prior studies (Cheng *et al.*, 2003; Near *et al.*, 2003; Cziko *et al.*, 2006) and others available in the GenBank database made this approach readily feasible. Additionally, we surveyed the literature and compiled catch data on the adult communities of McMurdo Sound as well as the nearby Terra Nova Bay and Ross Sea. Our results allow for the first comparisons between the larval/juvenile and adult fish communities in McMurdo Sound. Moreover, the species identities are paired with photographic images of the corresponding larval/juvenile fish we have taken. This will serve as a beginning resource, to be added to with future captures of additional species, which will be useful for the Antarctic notothenioid fish community at large.

MATERIALS AND METHODS

Specimen collection

Juvenile and larval fishes were collected from McMurdo Sound (approximately 77°S, 165°E), Antarctica during the austral summer seasons from 2002 through 2012. For our purposes, "larval" refers to specimens that are in either the prolarva or postlarva stages as defined by Hubbs (1943). Specimens were primarily collected alive by divers, though some were recovered from plankton tows, dip nets, or gut content of adult fishes. Of 151 total samples, 111 were collected in 2012 and consisted entirely of juvenile fishes. The remaining 40 samples were caught from 2002 to 2004 and consisted of both larval and juvenile specimens. Specimens were tagged, photographed, preserved in 90% ethanol and stored at -20°C. Locations and numbers of fishes collected from each site are given in Table 1.

ND2 amplification and sequencing

Genomic DNA was isolated from ethanol-preserved fin clippings or a small bit of pectoral muscle using a DNeasy Blood and Tissue kit (Qiagen, Valencia, CA, USA), and subsequently used to PCR amplify the complete mitochondrial-encoded NADH dehydrogenase subunit 2 (ND2) gene. ND2 has been utilized in previous phylogenetic investigations of Antarctic fishes, including the closely related trematomid species, and proved useful in the taxonomic discrimination of these samples (Near & Cheng, 2008). The entire 1047-base long ND2 gene was amplified using previously published primers (Kocher *et al.*, 1995) designed from cichlid sequence data. The resulting ND2 PCR products were treated to degrade residual primers and single-stranded DNA using 5.0 units of Antarctic and 1.0 unit of Exonuclease I (New

England BioLabs, Ipswich, MA, USA). The treated products were directly sequenced using ABI BigDye Terminator v3.1 chemistry (Applied Biosystems, Grand Island, NY, USA) and run on an ABI capillary sequencer at the University of Illinois' W. M. Keck Center for Comparative and Functional Genomics (Urbana, IL, USA). ND2 sequences were edited and full length ND2 gene contigs assembled using CHROMASPRO version 1.5 (Technelysium, South Brisbane, AUS). In the case of larval species for whom adults have not been reported to occur in the Ross Sea region, inclusive of McMurdo Sound, we extracted DNA and sequenced the ND2 gene from an available preserved adult specimen to include as an additional reference source.

Sequence data reported by this study have been deposited with GenBank under accession numbers KR153329 to KR153480.

Species identifications of larval/juvenile specimens via phylogenetic reconstruction

To make species identifications, we obtained ND2 reference sequences for 71 notothenioid species and two basal non-Antarctic notothenioid outgroups from GenBank (Table A1) and aligned them to our larval/juvenile sequences using MUSCLE (Edgar, 2004). As some of the GenBank sequences were missing the final 3 bases of the 3' end, all sequences within the alignment were trimmed to the respective 1044 bases using MESQUITE (Maddison & Maddison, 2011) to ensure the correct comparison.

Next, we used JMODELTEST version 2.1.6 (Guindon & Gascuel, 2003; Darriba *et al.*, 2012) with the following parameters to determine which model provided the best fit to the data: three substitution schemes, ML for the base tree likelihood calculations, nearest neighbor interchange (NNI) for the tree topology search operation, and inclusion of both invariable sites (I) and rate variation about sites (gamma, or G). This tested all 24 models that could be used in a

Bayesian analysis. Analyses of the negative log likelihood, Akaike Information Criterion (AIC), and Bayesian Information Criterion (BIC) within JMODELTEST all agreed that the best model was GTR+I+G, a general time reversible model that includes invariable sites and rate variation about sites.

Following this, phylogenetic trees were constructed using both maximum likelihood and Bayesian analyses, with two basal non-Antarctic notothenioids, *Pseudaphritis urvilii* (Valenciennes, 1832) and *Eleginops maclovinus* (Cuvier, 1830), serving as outgroups. The maximum likelihood phylogenetic tree was constructed via RAXML version 8 (Stamatakis, 2014) using the GTR+I+G model and 500 bootstrap replicates. The Bayesian analysis was run on MRBAYES version 3.2.2 (Ronquist *et al.*, 2012) with 10,000,000 generations, sampling every 1000 generations, burn-in set to 25%, and the same model as with RAXML. The final trees were visualized with FIGTREE version 1.4.0. Species identifications were then confirmed based on the position of larval specimens relative to the reference sequences on both trees.

Identifying the adult communities of McMurdo Sound, Terra Nova Bay, and the Ross Sea

In order to fully assess what species are known to the adult notothenioid community of McMurdo Sound, we completed an in-depth review of the current literature and compiled catch data. We gathered additional information for the nearby Terra Nova Bay and Ross Sea, allowing us to have a basis for comparison against our larval/juvenile specimens.

RESULTS

Species identifications of larval/juvenile specimens via phylogenetic reconstruction

Both the maximum likelihood and the Bayesian analyses of larval and known adult ND2 sequences yielded definitive identifications for 150 of the 151 larval specimens, all with bootstrap support of 91-100 (Fig. 1) and posterior probabilities of 0.9999-1.0 (Fig. 2). Species identifications were consistent between analyses. Photographs of the larval/juvenile specimens were subsequently paired with photos of corresponding adult specimens (Fig. 3).

Of the 151 specimens, 138 specimens were identified as trematomids (the subfamily Trematominae; family Nototheniidae); this equates to 91.4% of abundance (Table 2; Fig. 1; Fig. 2). Four additional specimens were identified as *P. antarctica*, for a total of 142 samples in family Nototheniidae; this corresponded to 94.0% of the total abundance. The most prevalent species was *T. bernacchii* with a total of 106 specimens or 70.2% abundance. The one unidentified specimen (labeled "03unk01"; Table 2) was placed as sister taxon to the clade that contained adult *P. borchgrevinki* and 23 larval specimens; this placement was supported by a bootstrap support of 100 (Fig 1) and posterior probability of 1.0 (Fig. 2). The remaining nine samples included the following six species: *Psilodraco breviceps* Norman, 1937 (family Bathydraconidae), *Pogonophryne scotti* Regan, 1914 (family Artedidraconidae), and *Neopagetopsis ionah* Nybelin, 1947, *Chionodraco myersi* DeWitt & Tyler, 1960, *Chionodraco hamatus* (Lönnberg, 1905), and *Pagetopsis maculatus* Barsukov & Permitin, 1958 (all of family Channichthyidae) (Table 2; Fig. 1; Fig. 2).

Specimens collected by diver or dip net at the Jetty, Arrival Heights, Turtle Rock, Cape Evan, and Cape Evans Wall were generally trematomid species: *T. bernacchii*, *Trematomus*

nicolai (Boulenger, 1902), *Trematomus pennellii* Regan, 1914, *P. borchgrevinki*, and our unknown specimen ("03unk01"); the exception was our *N. ionah* specimen (Table 1; Table 2). Any other non-trematomid specimen was primarily collected from the gut of an adult fish such as *Dissostichus mawsoni* Norman, 1937, *P. borchgrevinki*, or another trematomid, by plankton tow, or – in the case of our Winter Quarters Bay specimens – regurgitated by an adult *Trematomus hansoni* Boulenger, 1902.

Based on both the maximumum likelihood and Bayesian analyses the two families Nototheniidae and Bathydraconidae each showed paraphyletic relationships, while the other families were monophyletic (Fig. 1; Fig. 2). However while all families were not resolved as monophyletic, relationships between the larval specimens and their corresponding reference sequences were always fully resolved with strong support (bootstrap ≥ 91; posterior probability ≥ 0.9999).

Comparing our larval/juvenile specimens to the adult communities of McMurdo Sound, Terra Nova Bay, and the Ross Sea

Our literature search identified 15 notothenioid species known to be caught as adults from McMurdo Sound, 24 species from Terra Nova Bay, and 53 species from the Ross Sea (Table 3). The adult communities of McMurdo Sound and Terra Nova Bay represent non-identical but overlapping subsets of the species known to the larger Ross Sea. The one exception is *Cryothenia amphitreta*, a new species discovered in McMurdo Sound (Cziko & Cheng, 2006), as thus far the holotype is the only individual that has been caught (Table 3).

Out of our 151 larval/juvenile specimens, 141 were of species that are known to occur in McMurdo Sound as adults (Table 2; Table 3). The ten other specimens represented species

unknown as adults to McMurdo Sound but known instead to the Ross Sea: *C. hamatus*, *C. myersi*, *N. ionah*, *P. maculatus*, *P. breviceps*, *P. scotti*, and our one unknown "03unk01".

DISCUSSION

From decades of research on the fishes of McMurdo Sound and the Ross Sea we know that notothenioids dominate the high-latitude Antarctic shelf waters by making up roughly 91% of the adult biomass (Eastman & Hubold, 1999; Eastman, 2005). Nototheniidae is by far the most prevalent family and comprises approximately 50% of fish abundance (Eastman & Hubold, 1999; Eastman, 2005), with fishes of the genus *Trematomus* known to be particularly abundant (Eastman & Devries, 1982; Buckley, 2013). A handful of additional species are present from the families Artedidraconidae, Bathydraconidae, and Channichthyidae as well (Table 3). In fact, the notothenioid fishes of McMurdo Sound appear to represent a subset of those found in the nearby Ross Sea (Table 3), with the exception of *C. amphitreta* which is only known from McMurdo Sound.

Our results reflect this Notothenioidei dominance, as 100% of our specimens were notothenioids. On the other hand, our specimens were overwhelming nototheniids – 94% abundance, compared to the 50% abundance in adults. We can conclude that our data likely suffer from sampling bias, as our 2012 collection from the Jetty clearly hit a cohort of young *T. bernacchii*. This is a frequent problem with larval studies, such as with the study by Guglielmo *et al*. (1998) whose catch was overwhelmingly *P. antarctica* – 98% of their 34,436 fishes. The larval fishes that researchers are able to catch on a given day are dependent upon the exact location, depth, season, fishing method, and a multitude of other factors. Unlike the notothenioid adults that are frequently considered sluggish and sedentary (Eastman, 1993), the larval and juvenile assemblage may be highly transient and changeable due to their pelagic nature. In order

to combat this difficulty, sampling size must be large and should ideally occur at multiple sites throughout the year as well as over the course of multiple years.

Unfortunately, sampling larval fishes is not so easy at the high-latitude location of McMurdo Sound, thus resulting in a poorly studied larval assemblage. McMurdo Sound is covered by sea ice throughout most of the year and frequently experiences extreme weather, making trawling or any form of fishing by boat unfeasible. Larval fishes are also too small to be caught by traditional hook-and-line fishing. This means that these fishes can generally only be caught by scuba diver, by retrieval from the gut of an adult fish, or by small plankton tows deployed by hand through a hole drilled in the sea ice; this severely limits the number of fishes that can be caught compared to large-scale trawling. Additionally, the extreme cold and 24-hour darkness of winter means that fishing can only be attempting during the austral summer at McMurdo Sound.

While the larval assemblage of McMurdo Sound has consequently remained understudied, there have been more comprehensive studies on the larval fishes in both the lower latitudes waters of the Antarctic Peninsula (Kellerman, 1990; Loeb *et al.*, 1993; Morales-Nin *et al.*, 1995; Matschiner *et al.*, 2009) as well as in the high-latitude waters of the Ross Sea and Terra Nova Bay (Guglielmo *et al.*, 1998; Vacchi *et al.*, 1999; Granata *et al.*, 2002). These locations have open water that allows for trawling by research vessel. Over the course of ten years in the Ross Sea, Granata *et al.* (2002) collected nearly 395,000 fish larvae by multinet and midwater trawling. Similar to the problem faced by Guglielmo et al. (1998), the collection by Granata *et al.* (2002) was overwhelmed by cohorts of *P. antarctica* in three of their four cruises. This problem was partially alleviated by their massive sample size, and they were therefore able to catch larvae of notothenioid species not known to the Ross Sea as adults: *Lepidonotothen*

nudifrons (Lönnberg, 1905), *Chionodraco rastrospinosus* DeWitt & Hureau, 1979, *Pseudochaenichthys georgianus* Norman, 1937, *Parachaenichthys charcoti* (Vaillant, 1906), and *Artedidraco mirus* Lönnberg, 1905, plus additional unidentified specimens. However, these identifications were based solely on morphology, which as we have established is not always reliable. These findings should ideally be confirmed by genetic analyses, which may also allow for the identification of their unknown specimens.

In a different study near the Antarctic Peninsula that instead focused on how ocean currents may shape gene flow and population connectivity, Matschiner *et al.* (2009) modeled the influence of ocean currents in the Scotia Sea on *Gobionotothen gibberifrons* (Lönnberg, 1905) during their four-month larval stage. Examining the population genetics from six locations, they determined that the populations were not significantly differentiated from one another. This was explained by the eastward movement of larvae driven by the Antarctic Circumpolar Current (ACC), connecting the populations of the far less mobile adults and providing adequate gene flow to avoid differentiation.

As the notothenioid species found in McMurdo Sound and the Ross Sea are also known to have extended pelagic larval stages (Kock, 1992; Loeb *et al.*, 1993), their populations might therefore be heavily influenced by the strong local currents as well. Near the Ross Sea, the most prominent currents are the Antarctic Circumpolar Current that moves clockwise around Antarctica and is driven by western winds, and the much narrower Antarctic Coastal Current driven by eastern winds that moves counterclockwise along the coastline (Loeb *et al.*, 1993; Rintoul, 2010). North of McMurdo Sound, the Ross Sea Gyre drives the water clockwise with its eastern boundary near the Antarctic Peninsula and at its south providing a major inflow to the Ross Sea continental shelf (Assmann & Timmermann, 2005). McMurdo Sound itself generally

experiences a southward flow originating from the Ross Sea along its eastern side, followed by a northward flow along the western edge of the Sound (Barry & Dayton, 1988). This current pattern may explain our unknown specimen (Table 2) that was placed as sister taxon to *P. borchgrevinki* in our analyses, but clearly was a different species as indicated by strong bootstrap support (Fig. 1) and posterior probability (Fig. 2). We hypothesize that this specimen is in fact *Pagothenia brachysoma* (Pappenheim, 1912), a rarely caught species known to the Ross Sea (Table 3) and that is accepted as sister taxon to *P. borchgrevinki*. Unfortunately, at this time we have no adult specimens of *P. brachysoma* nor are there ND2 sequences (or any molecular sequences at all) available on the NCBI database with which we could compare.

The current patterns surrounding McMurdo Sound may also explain our nine non-nototheniid larval and juvenile specimens. Eight specimens were identified as *N. ionah, C. myersi, C. hamatus, P. maculatus* and *P. scotti;* while these are all species that have circum-Antarctic distributions (Eakin, 1990; Iwami & Kock, 1990) and are known to inhabit the high-latitude waters of the Ross Sea (Table 3), they have never been caught as adults in McMurdo Sound. However, given the currents that run down from the Ross Sea along the eastern edge of McMurdo Sound (Barry & Dayton, 1988), it seems highly likely for some larvae to get pushed into this area despite the lack of known local adults. Additionally, one *C. myersi* sample came from the gut of an adult *D. mawsoni*, and all three *P. maculatus* specimens came from the gut of *P. borchgrevinki* adults. Both *D. mawsoni* and *P. borchgrevinki* are pelagic species in these high-latitude waters – unlike most other species which are benthic – and are known to feed in the water column. This pelagic lifestyle may have thus led them towards the Ross Sea and into contact with a greater variety of larval species.

The final, least-expected and especially intriguing discovery was the identification of a larval specimen as *P. breviceps*. This specimen was collected from Winter Quarters Bay, a site directly adjacent to McMurdo Station, when it was regurgitated from an adult *T. hansoni* that our team caught while hook-and-line fishing. This identification was fully supported by a bootstrap value of 100 (Fig. 1) and posterior probability of 1.0 (Fig. 2), leaving no question that the sample truly is *P. breviceps.* This is intriguing because this species of dragonfish is currently considered to be endemic as adults to the far more northerly and milder waters surrounding South Georgia Island (54°S, 36°W) (Gon, 1990). That places our larval specimen at approximately 7,000 miles away from the known adult populations, given that a larvae would have to travel eastward from South Georgia due to the ACC. However, there is at least one other documented case of *P. breviceps* larvae being caught as far south as Terra Nova Bay in the Ross Sea, and in fact that study caught seven larvae (Guglielmo *et al.*, 1998). Given that there are now at least eight *P. breviceps* larvae that have been caught in high-latitude waters, it seem unlikely that they are strictly outliers that were pushed to an extreme distance by the ocean currents. This therefore lends credence to the idea that there are likely as-yet-unknown adult populations located closer to McMurdo Sound than is South Georgia Island, but future research is needed to verify this supposition. This is also supported by data from the study by Matschiner *et al.* (2009), who simulated larval dispersal by ocean currents near the Antarctic Peninsula and Scotia Sea. In their models larvae could travel hundreds of kilometers eastward from the Peninsula and subantarctic islands due to the ACC, but still nowhere near a distance that would allow them to reach McMurdo Sound if they started from South Georgia, unless this species experiences a heavily extended larval stage. Regardless, it does seem unlikely that these larval specimens would have survived to adulthood given that the adults have thus far been found solely in relatively milder

environments, perhaps explaining why this species has never yet been seen in the harsh high-latitude waters of McMurdo Sound.

While this study's phylogenetic trees did not yield strictly monophyletic groupings at the family level, unlike the commonly accepted phylogeny based on morphological data (Balushkin, 1992), we successfully identified the larval specimens. In particular, the families Nototheniidae and Bathydraconidae were both paraphyletic, though all other families occurred as monophyletic. This paraphyly is a common problem that has been noted by other studies (Near *et al.*, 2004; Near & Cheng, 2008), and still remains a problem even with the incorporation of multiple genes in a single analysis (Dettai *et al.*, 2012). It has been suggested that the phylogeny should undergo a reclassification that would reclassify the current families of Channichthyidae, Bathydraconidae, Artedidraconidae, and Harpagiferidae as instead subfamilies within the family Nototheniidae (Dettai *et al.*, 2012). Regardless of this potential reclassification, in our case this paraphyly does not pose a problem as the main focus of this study was species identification and all specimens were resolved with strong bootstrap and posterior probability support.

The completion of these species identifications marks one of the first investigations into the larval and juvenile fish diversity of McMurdo Sound and provides the framework for future studies on gene flow and population connectivity of the McMurdo fishes. Ideally, more larval and juvenile fishes will be collected from more locations around McMurdo Sound. Without more data on the larval community, there is no ability to fully ask and answer questions about gene flow and population structure, or to understand how changes in the local larval assemblage may impact the adult community (and vice-versa). Basic species identifications from ND2 gene sequences must be combined with other, more variable genes in order to get a measure of intraspecies diversity. Subsequently increasing our knowledge of the larval assemblage will

likewise allow us to appropriately adapt our current definitions for species ranges. Additionally, new species of fishes continue to be identified in Antarctic waters. Recent examples of newly discovered species even include one from the considerably well-studied adult fish community of McMurdo Sound (Cziko & Cheng, 2006). The relatively unstudied larval assemblage, including members not found in McMurdo Sound as adults, may therefore have a greater chance of harboring undescribed species.

Despite the comparatively well-studied adult notothenioid communities of McMurdo Sound, Terra Nova Bay, and the Ross Sea, no resource prior to this study summarized all of the species known to these areas. Individual studies tended to focus on a select subset of species or on species caught within a short timeframe, and thus never provided a complete picture of the adult notothenioid communities. Our study therefore provides a valuable resource to the scientific community by compiling a full list of notothenioids currently known as adults to these three locations.

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TABLES

Table 1. Collection locations in McMurdo Sound, Antarctica with the total number of larval/juvenile specimens collected at each site (N) given. Note that the "gut content" location refers larval/juvenile fishes that were removed from the stomach of adult notothenioid fishes. Exact catch sites for these adult fishes are unrecorded. WQB specimens were regurgitated onsite by adult trematomids. "Unknown" or "unrecorded" indicates that no record exists for catch method or location.

Location	Latitude	Longitude	Year	Catch method	N
Granite Harbor	76°57'55"S	163°03'48"E	2012	unknown	1
Jetty	77°51'05"S	166°39'39"E	2003, 2012	diver	91
Winter Quarters Bay (WQB)	77°50'00"S	166°39'1"E	2012	gut content	2
Arrival heights (AH)	77°49'00"S	166°39'00"E	2012	diver	16
Turtle Rock (TR)	77°44'00"S	166°46'00"E	2004	diver	15
Cape Evans Wall (CEW)	77°38'29"S	166°31'00"E	2003	dip net or diver	5
Cape Evans (CE)	77°38'10"S	166°24'34"E	2003	dip net or diver	1
gut content of *Dissostichus mawsoni*	n/a	n/a	2012	gut content	1
gut content of *Pagothenia borchgrevinki*	n/a	n/a	2002-2003	gut content	6
gut content of a trematomid	n/a	n/a	2012	gut content	1
unrecorded	n/a	n/a	2002-2003	unknown	12

Table 2. A) Summary of total notothenioid larval and juvenile species identifications from McMurdo Sound, Antarctica based on ND2 gene sequences; B) larval fish species identifications, separated by each collection site. N indicates the number of specimens collected for each species.

A)

Family	Species	N
Nototheniidae	*Pleuragramma antarctica*	4
Nototheniidae	unknown ("03unk01")	1
Nototheniidae	*Pagothenia borchgrevinki*	23
Nototheniidae	*Trematomus bernacchii*	106
Nototheniidae	*Trematomus newnesi*	1
Nototheniidae	*Trematomus nicolai*	1
Nototheniidae	*Trematomus pennellii*	6
Bathydraconidae	*Psilodraco breviceps*	1
Channichthyidae	*Chionodraco hamatus*	1
Channichthyidae	*Chionodraco myersi*	2
Channichthyidae	*Pagetopsis maculatus*	3
Channichthyidae	*Neopagetopsis ionah*	1
Artedidraconidae	*Pogonophryne scotti*	1
total		**151**

B)

Location	Species	N	Year of Catch
Jetty	unknown	1	2003
	T. bernacchii	87	2012
	T. nicolai	1	2012
	T. pennellii	2	2012
Arrival Heights	*P. borchgrevinki*	2	2012
	T. bernacchii	10	2012
	T. pennellii	4	2012
Winter Quarters Bay	*T. bernacchii*	1	2012
	P. breviceps	1	2012
Turtle Rock	*P. borchgrevinki*	15	2004
Granite Harbor	*T. bernacchii*	1	2012
Cape Evans	*P. borchgrevinki*	5	2003
Cape Evans Wall	*P. borchgrevinki*	1	2003
gut content of *D. mawsoni*	*C. myersi*	1	2012
gut content of *P. borchgrevinki*	*P. antarctica*	3	2002, 2003
	P. maculatus	3	2002, 2003
gut content of a trematomid	*T. newnesi*	1	2012
unrecorded	*T. bernacchii*	8	2002
	C. hamatus	1	2003
	C. myersi	1	2003
	N. ionah	1	2003
	P. scotti	1	2003

Table 3. Adult notothenioid species distributions in McMurdo Sound, Terra Nova Bay, and the Ross Sea, Antarctica (Dewitt & Tyler, 1960; Takahashi & Nemoto, 1984; Eastman, 1985; Eakin & Eastman, 1998; Eastman & Hubold, 1999; Vacchi *et al.*, 1999; Moylan & Sidell, 2000; Vacchi *et al.*, 2000; Cheng *et al.*, 2003; Donnelly *et al.*, 2004; Near *et al.*, 2004; Cziko & Cheng, 2006; La Mesa *et al.*, 2006; Balushkin *et al.*, 2010; Clark *et al.*, 2010; Balushkin & Spodareva, 2013; Buckley, 2013; Shandikov & Eakin, 2013; Shandikov *et al.*, 2013). An x indicates that the species is found in that location.

Taxa	McMurdo Sound	Terra Nova Bay	Ross Sea
Family Nototheniidae			
Aethotaxis mitopteryx DeWitt, 1962			x
Cryothenia amphitreta Cziko & Cheng, 2006	x		
Dissostichus mawsoni Norman, 1937	x	x	x
Lepidonotothen squamifrons (Günther, 1880)			x
Notothenia coriiceps Richardson, 1844		x	x
Pagothenia borchgrevinki (Boulenger, 1902)	x	x	x
Pagothenia brachysoma (Pappenheim, 1912)			x
Pleuragramma antarctica Boulenger, 1902	x	x	x
Trematomus bernacchii Boulenger, 1902	x	x	x
Trematomus eulepidotus Regan, 1914		x	x
Trematomus hansoni Boulenger, 1902	x	x	x
Trematomus lepidorhinus (Pappenheim, 1911)		x	x
Trematomus loennbergii Regan, 1913	x	x	x
Trematomus newnesi Boulenger, 1902	x	x	x
Trematomus nicolai (Boulenger, 1902)	x	x	x
Trematomus pennellii Regan, 1914	x	x	x
Trematomus scotti (Boulenger, 1907)		x	x
Trematomus tokarevi Andriashev, 1978			x
Family Artedidraconidae			
Artedidraco glareobarbatus Eastman & Eakin, 1999			x
Artedidraco loennbergi Roule, 1913		x	x
Artedidraco orianae Regan, 1914			x
Artedidraco shackletoni Waite, 1911			x
Artedidraco skottsbergi Lönnberg, 1905			x
Dolloidraco longedorsalis Roule, 1913			x
Histiodraco velifer (Regan, 1914)	x	x	x
Pogonophryne barsukovi Andriashev, 1967			x
Pogonophryne brevibarbata Balushkin, Petrov & Prutko, 2011			x
Pogonophryne cerebropogon Eakin & Eastman, 1998			x
Pogonophryne immaculata Eakin, 1981			x
Pogonophryne marmorata Norman, 1938			x
Pogonophryne mentella Andriashev, 1967			x
Pogonophryne neyelovi Shandikov & Eakin, 2013			x
Pogonophryne sarmentifera Balushkin & Spodareva, 2013			x
Pogonophryne scotti Regan, 1914		x	x
Pogonophryne tronio Shandikov, Eakin & Usachev, 2013			x
Family Bathydraconidae			
Akarotaxis nudiceps (Waite, 1916)			x
Bathydraco macrolepis Boulenger, 1907			x
Bathydraco marri Norman, 1938			x
Cygnodraco mawsoni Waite, 1916		x	x

Table 3 (continued)

Taxa	McMurdo Sound	Terra Nova Bay	Ross Sea
Gerlachea australis Dollo, 1900			x
Gymnodraco acuticeps Boulenger, 1902	x	x	x
Prionodraco evansii Regan, 1914		x	x
Racovitzia glacialis Dollo, 1900			x
Vomeridens infuscipinnis (DeWitt, 1964)			x
Family Channichthyidae			
Chaenodraco wilsoni Regan, 1914		x	x
Chionobathyscus dewitti Andriashev & Neyelov, 1978			x
Chionodraco hamatus (Lönnberg, 1905)		x	x
Chionodraco myersi DeWitt & Tyler, 1960		x	x
Cryodraco antarcticus Dollo, 1900	x		x
Cryodraco atkinsoni Regan, 1914			x
Dacodraco hunteri Waite, 1916	x		x
Neopagetopsis ionah Nybelin, 1947		x	x
Pagetopsis macropterus (Boulenger, 1907)	x	x	x
Pagetopsis maculatus Barsukov & Permitin, 1958			x
Total number of species	**15**	**24**	**53**

FIGURES

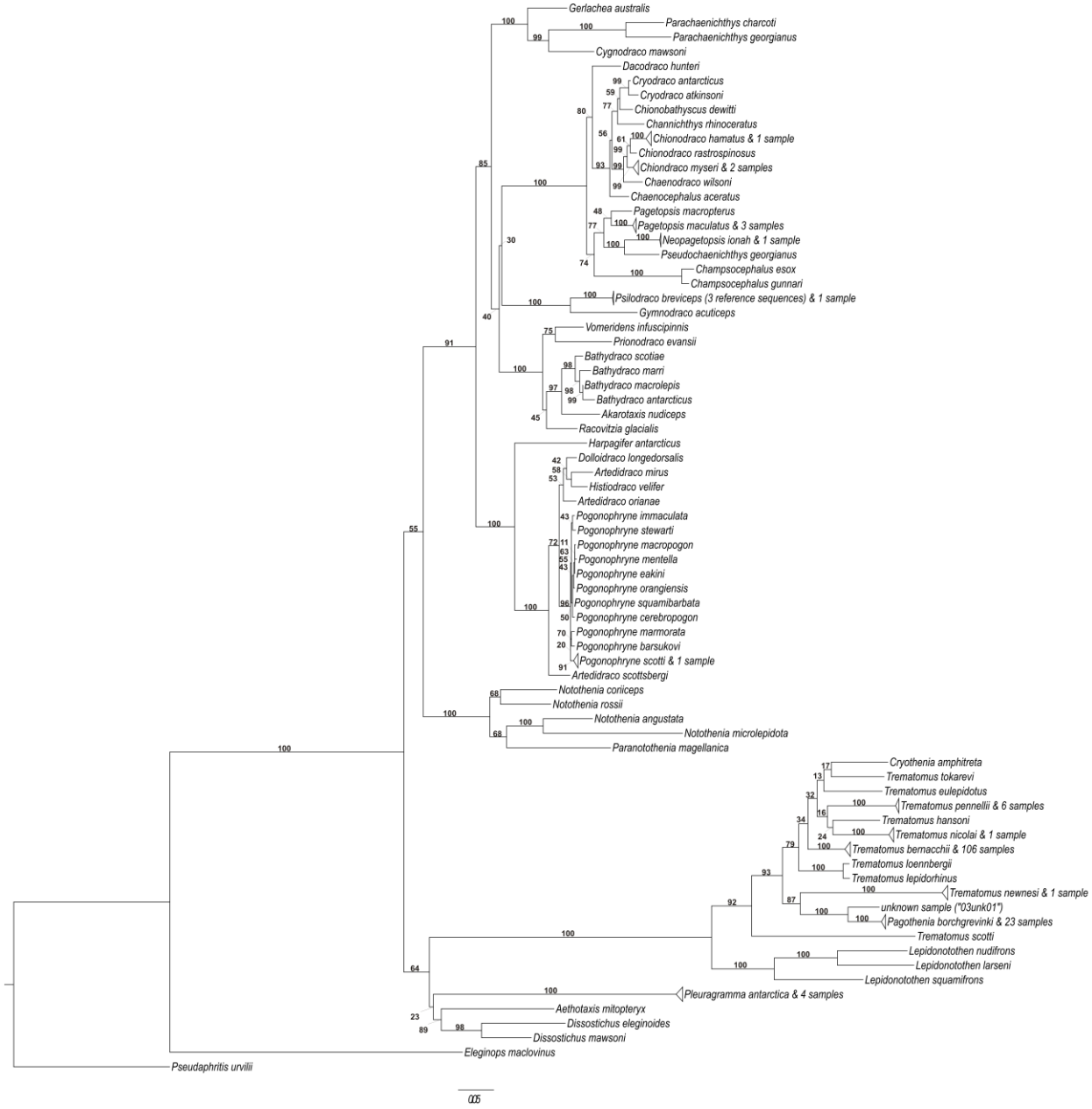


Figure 1. Maximum likelihood phylogenetic tree for 151 unknown notothenioid larval/juvenile fish specimens, 71 reference species and two outgroups. The tree was constructed using RAxML version 8 (Stamatakis, 2014) with the model GTR+I+G and 500 bootstrap replicates. *Pseudaphritis urvilii* and *Eleginops maclovinus* were used as outgroups. Nodes containing larval/juvenile samples and their respective reference species have been collapsed for viewing purposes, as indicated by triangles on the tree above. Bootstrap values are indicated along the branches. Branches are scaled by the number of substitutions per site.

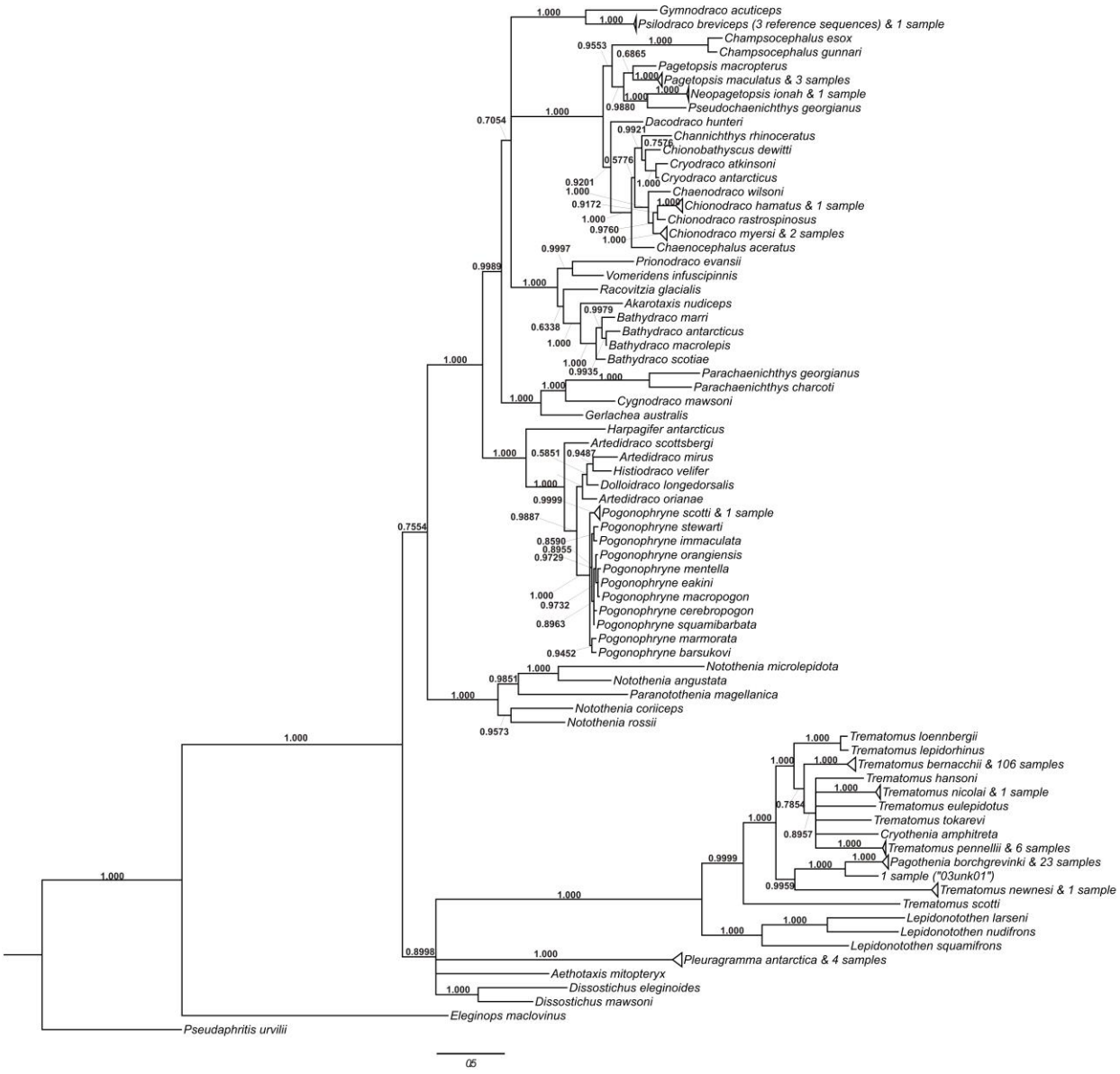


Figure 2. Bayesian phylogenetic tree for 151 unknown notothenioid larval/juvenile fish specimens, 71 reference species and two outgroups. The tree was constructed using MrBayes version 3.2.2 (Ronquist *et al.*, 2012) with the model GTR+I+G, 10,000,000 generations, and a relative burn-in of 0.25. *Pseudaphritis urvilii* and *Eleginops maclovinus* were used as outgroups. Posterior probabilities are included. Nodes containing the larval/juvenile specimens and their respective species have been collapsed for viewing purposes, represented by triangles above. Branches are scaled by the expected number of substitutions per site.



Figure 3. Select photographs of larval and juvenile specimens collected for this study from McMurdo Sound, Antarctica, paired with corresponding photos of adult specimens where possible.

APPENDIX: ACCESSION NUMBERS FOR DNA REFERENCE SEQUENCES

Table A1. List of GenBank accession numbers for reference sequences used in this study. An asterisk indicates non-Antarctic species.

Taxa	Accession Number
Family Nototheniidae	
Aethotaxis mitopteryx DeWitt, 1962	DQ367401
Cryothenia amphitreta Cziko & Cheng, 2006	DQ367400
Dissostichus eleginoides Smitt, 1898	FJ647713
Dissostichus mawsoni Norman, 1937	AY256561
Lepidonotothen larseni (Lönnberg, 1905)	JN186900
Lepidonotothen nudifrons (Lönnberg, 1905)	JN186901
Lepidonotothen squamifrons (Günther, 1880)	JN186902
Notothenia angustata Hutton, 1875*	AY256562
Notothenia coriiceps Richardson, 1844	AY256563
Notothenia microlepidota Hutton, 1875*	AY256565
Notothenia rossii Richardson, 1844	AY256566
Pagothenia borchgrevinki (Boulenger, 1902)	FJ647715
Paranotothenia magellanica (Forster, 1801)	AY256568
Pleuragramma antarctica Boulenger, 1902	DQ184495
Trematomus bernacchii Boulenger, 1902	AY256569
Trematomus eulepidotus Regan, 1914	FJ647718
Trematomus hansoni Boulenger, 1902	FJ647721
Trematomus lepidorhinus (Pappenheim, 1911)	FJ647723
Trematomus loennbergii Regan, 1913	FJ647726
Trematomus tokarevi Andriashev, 1978	FJ647730
Trematomus nicolai (Boulenger, 1902)	FJ650508
Trematomus pennellii Regan, 1914	FJ647744
Trematomus scotti (Boulenger, 1907)	CFJ647734
Trematomus tokarevi Andriashev, 1978	FJ647740
Family Artedidraconidae	
Artedidraco mirus Lönnberg, 1905	KF412876
Artedidraco orianae Regan, 1914	JN186888
Artedidraco skottsbergi Lönnberg, 1905	FJ973333
Dolloidraco longedorsalis Roule, 1913	HQ169654
Histiodraco velifer (Regan, 1914)	FJ973335
Pogonophryne barsukovi Andriashev, 1967	FJ973340
Pogonophryne cerebropogon Eakin & Eastman, 1998	FJ973341
Pogonophryne eakini Balushkin, 1999	FJ973342
Pogonophryne immaculata Eakin, 1981	FJ973343
Pogonophryne macropogon Eakin, 1981	FJ973349
Pogonophryne marmorata Norman, 1938	FJ973352
Pogonophryne mentella Andriashev, 1967	FJ973353
Pogonophryne orangiensis Eakin & Balushkin, 1998	FJ973354
Pogonophryne scotti Regan, 1914	FJ973357
Pogonophryne stewarti Eakin, Eastman & Near 2009	FJ973358
Pogonophryne squamibarbata Eakin & Balushkin, 2000	FJ973360
Family Bathydraconidae	
Akarotaxis nudiceps (Waite, 1916)	HQ170109
Bathydraco antarcticus Günther, 1878	HQ170114
Bathydraco macrolepis Boulenger, 1907	HQ170110

Table A1 (continued)

Taxa	Accession Number
Bathydraco marri Norman, 1938	AY249487
Bathydraco scotiae Dollo, 1906	HQ170115
Cygnodraco mawsoni Waite, 1916	HQ170117
Gerlachea australis Dollo, 1900	HQ170119
Gymnodraco acuticeps Boulenger, 1902	HQ170120
Parachaenichthys charcoti (Vaillant, 1906)	HQ170122
Parachaenichthys georgianus (Fischer, 1885)	HQ170124
Gymnodraco acuticeps Boulenger, 1902	HQ170127
Psilodraco breviceps Norman, 1937	HQ170128, HQ170129
Racovitzia glacialis Dollo, 1900	HQ170131
Vomeridens infuscipinnis (DeWitt, 1964)	HQ170133
Family Channichthyidae	
Chaenocephalus aceratus (Lönnberg, 1906)	HM166185
Chaenodraco wilsoni Regan, 1914	HM165854
Champsocephalus esox (Günther, 1861)	HQ170096
Champsocephalus gunnari Lönnberg, 1905	HQ170097
Channichthys rhinoceratus Richardson, 1844	AY249503
Chionobathyscus dewitti Andriashev & Neyelov, 1978	AY249496
Chionodraco hamatus (Lönnberg, 1905)	AY249500
Chionodraco myersi DeWitt & Tyler, 1960	HQ170103
Chionodraco rastrospinosus DeWitt & Hureau, 1979	HM165958
Cryodraco antarcticus Dollo, 1900	HM166088
Cryodraco atkinsoni Regan, 1914	HQ170105
Dacodraco hunteri Waite, 1916	AY249507
Neopagetopsis ionah Nybelin, 1947	HM165754
Pagetopsis macropterus (Boulenger, 1907)	HM165572
Pagetopsis maculatus Barsukov & Permitin, 1958	HQ170107
Pseudochaenichthys georgianus Norman, 1937	HM165672
Family Harpagiferidae	
Harpagifer antarcticus Nybelin, 1947	HQ170094
Outgroups	
Family Pseudaphritidae	
Pseudaphritis urvillii (Valenciennes, 1832)*	JN186885
Family Eleginopidae	
Eleginops maclovinus (Cuvier, 1830)*	JN186886