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Phylogeny Reconciles Classification in Antarctic Plunderfishes

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The resolution of phylogenetic relationships within rapid radiations poses a significant challenge in systematic biology. However, the integration of genome-scale DNA data with multispecies coalescent-based tree inference methods offers a strategy to resolve historically recalcitrant nodes within radiations of closely related species. Here, we analyze a dataset of over 60,000 loci captured via double digest restriction site-associated DNA sequencing (ddRADseq) using both concatenation- and coalescent-based approaches to infer the phylogenetic relationships of the Antarctic notothenioid lineage Artedidraconinae. Previous studies identify artedidraconines as the most rapidly diversifying subclade of notothenioids, but evolutionary studies of the clade are stymied by pervasive phylogenetic and taxonomic uncertainty. The results of our phylogenomic analyses provide clarity to several long-standing challenges in the systematics of artedidraconines, including the deep paraphyly of *Artedidraco*. Our findings enable the construction of a classification that reflects phylogenetic relationships, including the description of a new genus and the resurrection of a classification of Notothenioidei that places Artedidraconinae as a subfamily of Harpagiferidae. This work provides a phylogenetic perspective for investigations of the tempo and mode of diversification in artedidraconines, which is likely to provide new insights on the dynamics of the notothenioid adaptive radiation as a whole.

EVOLUTIONARY radiations captivate the fascination of evolutionary biologists, as evidenced by the decades of research devoted to understanding the factors and circumstances that trigger these extraordinary diversification events (e.g., Simpson, 1953; Grant, 1986; Schluter, 2000). The study of evolutionary radiations relies crucially on the availability of robust phylogenetic hypotheses and taxonomic frameworks for clades of interest; however, disentangling the earliest divergence events within rapid radiations represents an obstacle to the inference of phylogenetic frameworks (Rokas et al., 2005; Alda et al., 2018). Characteristic of many species radiations is a sequence of rapid divergence events that occurs early in their evolutionary history, producing a phylogenetic signature of short internodes near the root that provide insufficient time for fixation of phylogenetically informative character state changes between divergence events. Short internodes are also prone to the effects of incomplete lineage sorting, where the stochastic sorting of alleles into diverging lineages produces individual gene trees with evolutionary histories that differ from the species phylogeny (McCormack et al., 2013; Suh et al., 2015).

The analysis of both molecular and morphological data has brought into sharper focus the evolutionary history of a remarkable radiation of Antarctic notothenioid fishes (Bargelloni et al., 1994; Balushkin, 2000; Eastman, 2000; Near et al., 2018). Antarctic notothenioids are a lineage of ~80 species that dominate the diversity, abundance, and biomass of the teleost fish fauna of the Southern Ocean surrounding Antarctica and represent a rare example of an adaptive radiation in a marine environment (Clarke and Johnston, 1996; Rutschmann et al., 2011; Near et al., 2012; Daane et al., 2019). Given the unique features of their physiology (e.g., Ruud, 1954; Chen et al., 1997), their central role in the Antarctic marine food web (e.g., La Mesa et al., 2004), and the high economic importance of their fisheries (e.g., Constable et al., 2000), the phylogenetics and classification of notothenioid fishes have been an area of extensive research for more than a century (Dollo, 1904; Regan, 1914; Norman, 1938; Balushkin, 2000; Near et al., 2018).

Despite progress in the phylogenetics and classification of notothenioids, there remain several areas where inferred phylogenetic relationships are incongruent with the accepted taxonomy. Persistent challenges to the systematics of notothenioids are evident in the Antarctic plunderfishes of the clades Artedidraconidae and Harpagiferidae. The artedidraconids include ~15 species classified into four genera: *Artedidraco* (7 species), *Dolloidraco longedorsalis*, *Histiodraco velifer*, and *Pogonophryne* (5–6 species; Eastman and Eakin, 2021; Parker et al., 2021). Harpagiferidae consists of 11 species classified in a single genus, *Harpagifer* (Eastman and Eakin, 2021), and it is hypothesized to be the sister lineage of Artedidraconidae (e.g., Bargelloni et al., 2000; Near et al., 2012, 2018). In the description of *Artedidraco*, the type genus of Artedidraconidae, Lönnberg (1905) noted strong similarities with *Harpagifer* but did not place these two genera into one taxonomic family. Regan's (1913) first classification of notothenioids placed both *Harpagifer* and *Artedidraco* into Nototheniidae. In the following year, Regan (1914: 6) placed *Harpagifer*, *Artedidraco*, *Dolloidraco*, *Histiodraco*, and *Pogonophryne* in Harpagiferinae, which was treated as a subfamily of Nototheniidae. In an extensive revision of notothenioids, Norman (1938) recognized Harpagiferidae as a family, which was identical in composition to Regan's (1914) Harpagiferinae. Andriashev (1965, 1967) recognized two groups in Harpagiferidae, with species of *Harpagifer* in Harpagiferinae and species of *Artedidraco*, *Dolloidraco longedorsalis*, *Histiodraco velifer*, and species of *Pogonophryne* placed in a group he named Artedidraconinae. In the 1980s, two morphological phylogenetic studies resolved a clade containing Harpagiferidae, Bathydraconidae, and Channichthyidae to the exclusion of Nototheniidae (Eakin, 1981; Iwami, 1985). Subsequent morphological phylogenetic studies were congruent with these earlier investigations but included the appropriate taxon sampling to support the monophyly of Harpagiferidae as a clade containing *Harpagifer* and Artedi-

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draconinae (Balushkin, 1992, 2000; Hastings, 1993). Hureau (1985) elevated Artedidraconidae to a family distinct from Harpagiferidae in a taxonomic key and regional species list, later providing the justification by arguing the presence of a chin barbel in the artedidraconines was sufficient for the elevation of the clade to a taxonomic family (Hureau, 1986).

While the elevation of Artedidraconidae was adopted without inquiry in important summaries of Antarctic fish biodiversity and biology (Eakin, 1990; Hureau, 1990; Eastman, 1993), the third edition of *Fishes of the World* retained the more inclusive Harpagiferidae because *Harpagifer* and Artedidraconinae "form a monophyletic group" (Nelson, 1994: 394). Molecular phylogenetic studies based on mitochondrial DNA sequences (Bargelloni et al., 1994, 2000; Ritchie et al., 1997; Derome et al., 2002; Near et al., 2004), DNA sequences from mitochondrial and nuclear genes (Near and Cheng, 2008; Matschiner et al., 2011; Dettaï et al., 2012; Near et al., 2012; Colombo et al., 2015; Dornburg et al., 2017), and genome-scale phylogenetic data (Near et al., 2018; Daane et al., 2019) all resolve *Harpagifer* and species of Artedidraconinae as a monophyletic group with strong support. A monogeneric Harpagiferidae as delimited by Hureau (1985, 1986, 1990) communicates nothing about the evolutionary relationship of *Harpagifer* to other notothenioid lineages, as the two names delimit the same set of species. Thus, a taxonomic revision reflecting the consistently supported monophyly of species of *Harpagifer* and species of Artedidraconinae is needed.

Phylogenetic and taxonomic uncertainty is also apparent within the clade Artedidraconinae. Molecular phylogenetic analyses consistently resolve the genus *Artedidraco* as paraphyletic, with *A. skottsbergi* placed as the sister lineage of all other species of Artedidraconinae (Derome et al., 2002; Lecointre et al., 2011; Near et al., 2012, 2018). However, no phylogenetic analysis has included all species of *Artedidraco*, forestalling a revision of notothenioid classification that reflects evolutionary history. In addition, it remains unclear if morphological traits traditionally used to delimit genera of Artedidraconinae exhibit patterns of variation consistent with the paraphyly of *Artedidraco* resolved in molecular phylogenies. Finally, application of molecular data to species delimitation within the lineage *Pogonophryne* resulted in the dramatic reduction of 29 previously recognized species to five described species (Parker et al., 2021). Species delimitation in *Pogonophryne* underscores findings of previous studies demonstrating that variation in the mental barbel, which is frequently used as an important diagnostic character in Artedidraconinae, exhibits a high degree of intraspecific variation and is not a reliable character for species delimitation (Eakin et al., 2001, 2006; Eastman and Eakin, 2001; Parker et al., 2021). Within *Artedidraco*, the two most recently described species, *A. glareobarbatus* (Eastman and Eakin, 1999) and *A. longibarbatus* (Eakin et al., 2015), are distinguished primarily by variation in the mental barbel, suggesting that the morphological delimitation of currently recognized species of *Artedidraco* warrants evaluation.

Here, we use genome-scale sequence data captured via double digest restriction site-associated DNA sequencing (ddRADseq) to infer phylogenetic relationships among the Antarctic Plunderfishes. Results of both concatenation- and coalescent-based phylogenetic analyses corroborate previous studies in resolving *Artedidraco* as paraphyletic. Analysis of morphological traits reveals differences among the lineages currently delimited as species of *Artedidraco*, and we describe a new genus to accommodate the genetically and morphologically distinct lineage identified in this study. In addition, we find a lack of support for genetic or morphological distinctiveness of *A. glareobarbatus* relative to *A. shackletoni*, suggesting that species diversity in *Artedidraco* is overestimated on the basis of variation in mental barbel morphology. Finally, we provide a new family-level taxonomic rank classification for Plunderfishes, resurrecting the classification presented by Norman (1938: 43) that groups *Artedidraco*, *Dolloidraco*, *Histiodraco*, *Pogonophryne*, the new genus, and *Harpagifer* in the Harpagiferidae and that places all of these lineages to the exclusion of *Harpagifer* in Artedidraconinae (Andriashev, 1967; Eakin, 1981). The phylogenomic analyses presented here provide a basis for future studies examining mechanisms responsible for the Antarctic notothenioid adaptive radiation.

MATERIALS AND METHODS

Taxon sampling and ddRAD sequencing.—We used ddRADseq to capture loci from across the genomes of 148 individuals representing 14 of the 15 currently recognized species of Artedidraconinae following a protocol modified from Peterson et al. (2012). Taxon sampling included 20 specimens of *Harpagifer antarcticus*, which served as an outgroup in the phylogenomic analyses (Supplemental Table S1; see Data Accessibility). The recently described species *Artedidraco longibarbatus* is known only from two specimens, one of which was lost aboard the expedition vessel following collection (Eakin et al., 2015), and tissue samples for this species are unavailable. Specimens for this study were collected between 2001 and 2019 from locations spanning a nearly circum-Antarctic distribution (Supplemental Table S1; see Data Accessibility). Specimens from the Antarctic Peninsula, South Shetland Islands, Elephant Island, and the South Orkney Islands were collected during Antarctic Marine Living Resources (AMLR) expeditions conducted in the austral summers of 2001, 2003, 2006, and 2009. Benthic trawls were used to collect all specimens, and muscle tissue samples were taken and stored in 95% ethanol. Tissue samples and their associated voucher specimens were provided to the Yale Peabody Museum of Natural History. Samples from the Ross Sea and Wilkes Land were available through loans provided by the Museum of New Zealand Te Papa Tongarewa, and samples from the Weddell Sea were furnished by gifts from the University of Padova, Padova, Italy (Supplemental Table S1; see Data Accessibility).

We extracted whole genomic DNA from tissues using the Qiagen DNeasy Kit (Qiagen Inc., Valencia, CA) following manufacturer protocol. DNA concentration was quantified using a Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA), and the quality of DNA extractions was checked visually using a 1% agarose gel. Ethanol precipitation of DNA was performed to concentrate whole genomic DNA and to remove contaminants which could interfere with enzymatic digestion of the DNA. Library preparation of ddRADseq loci began with double digestion of 200 ng of DNA from each sample using the PstI and MspI restriction enzymes for 16 hours. Digestion of all samples was confirmed using a 1% agarose gel, and common MspI adapters and sample-specific barcoded PstI adapters were ligated to the digested fragments. Equimolar amounts of each sample were then combined into

pools containing four unique barcoded samples each, which were then cleaned using the QIAquick Purification Kit (Qiagen Inc., Valencia, CA) following manufacturer protocol. Polymerase chain reaction (PCR) was used to amplify the cleaned libraries in 50.5 μL reactions consisting of 10 μL 5x Phusion Buffer HF, 1 μL 10mM DNTPs, 1.5 μL DMSO, 1 μL each of 10 μM PstI and 10 μM MspI primers, 1 μL Phusion High Fidelity DNA Polymerase, 6 μL DNA library template, and 29 μL DNase-free water. Following PCR, libraries were again combined into pools containing 24 unique barcoded samples each and were cleaned using the QIAquick Purification Kit. An Agilent 2100 Bioanalyzer Instrument (Agilent Technologies, Santa Clara, CA) was used to assess the size distribution, quantity, and purity of the DNA in each of our indexed libraries. We then pooled equimolar amounts of each our libraries into two 95-sample multiplexed libraries. The pooled libraries were size selected for fragments between 300–500 bp on a 2% agarose gel using the Blue Pippin DNA Size Selection System (Sage Science, Beverly, MA) according to manufacturer protocol. Size-selected libraries were again checked for appropriate fragment length distribution and DNA quality using the Agilent 2100 Bioanalyzer, and a Qubit fluorometer was used to quantify DNA concentrations. Each of the final ddRAD libraries was then sequenced on an Illumina HiSeq 2000 using single-end sequencing at the University of Oregon GC3F facility (https://gc3f.uoregon.edu/).

Bioinformatics.—The software ipyrad v0.9.50 (https://github.com/dereneaton/ipyrad/) was used to assemble phylogenomic datasets from the sequenced ddRAD libraries (Eaton, 2014). Raw sequence reads were first demultiplexed using sample-specific barcodes. The software cutadapt, which is implemented within the ipyrad bioinformatic pipeline, was then used to filter out Illumina adapters by setting the filter_adapters parameter to 2, and reads with more than five bases with a phred Q-score <20 were excluded from downstream processing. Reads were then clustered *de novo* within samples based on a quantitatively optimized sequence similarity threshold using the vsearch tool, and the resulting clusters were aligned using the MUSCLE algorithm (Edgar, 2004). Clusters with a sequencing depth of less than six reads were excluded from downstream processing, and consensus allele sequences estimated from clustered reads were discarded if they contained more than 5% ambiguous sites ("N"s) or if they contained more than 5% heterozygous bases. The remaining consensus sequences were then clustered as homologous loci across samples using the optimal sequence similarity threshold, and loci shared by fewer than four individuals were excluded from the final dataset. For *de novo* clustering of reads within samples (step 3 of ipyrad) and between samples (step 6 of ipyrad), we identified the optimal sequence similarly threshold by comparing a set of metrics calculated for ddRAD assemblies generated under threshold values ranging from 88% to 95%. These metrics included: (1) per-individual percent heterozygosity; (2) total number of variable sites (SNPs); (3) cumulative variance explained by the first eight principal components retained from a principal components analysis (PCA) of the genetic data; and (4) Pearson's correlation coefficient between pairwise genetic dissimilarity and data missingness (McCartney-Melstad et al., 2019). We used scripts available from McCartney-Melstad et al. (2019) to calculate metrics 3 and 4 and to generate heatmaps visualizing pairwise data missingness (Zheng et al., 2012; Galili, 2015; Mastretta-Yanes et al., 2015; Kolde, 2018; Potter, 2018). We identified a clustering threshold of 88% as optimal for our dataset, as this value maximized per-individual heterozygosity and cumulative variance explained by the first eight PCs retained from PCA of the genetic data. Furthermore, the choice of a lower sequence similarity threshold satisfies the recommendation of O'Leary et al. (2018) to select a clustering threshold that minimizes the chances of over-splitting naturally occurring allelic variation into separate loci.

The ddRAD assembly generated with the 88% sequence similarity threshold included 293,755 loci shared across at least four individuals (hereafter referred to as the "min4" dataset). In order to test the effects of missing data on downstream phylogenetic analyses, we generated a set of assemblies including 50%, 25%, and 15% missing data: (1) a dataset including only loci shared across at least 84 individuals (min84, 64,980 loci); (2) a dataset including only loci shared across at least 126 individuals (min126, 19,042 loci); and (3) a dataset including only loci shared across at least 144 individuals (min144, 1,331 loci).

Phylogenomic analyses.—The phylogenetic relationships among species of Artedidraconinae were inferred using separate maximum likelihood (ML) analyses of the concatenated min84, min126, and min144 datasets as implemented in IQ-TREE v1.6.12 (Nguyen et al., 2015). The best-fit nucleotide substitution model for each dataset was determined using the program ModelFinder (Kalyaanamoorthy et al., 2017). Node support was assessed using an ultrafast bootstrap approximation (UFboot) with 1,000 replicates (Minh et al., 2013; Hoang et al., 2018). We also inferred a species tree for Artedidraconinae under the multispecies coalescent using tetrad v0.9.10, an implementation of the program SVD-quartets (Chifman and Kubatko, 2014) in ipyrad, which takes as input an alignment of unlinked single nucleotide polymorphisms (SNPs). For each bootstrap replicate conducted over the course of the analyses, a single SNP was randomly selected from each locus for each quartet inference in the analysis. A total of 1,788,088 random quartets were inferred, and the individual quartet trees were joined into a single supertree using the wQMC algorithm (Snir and Rao, 2012). Nonparametric bootstrapping was used to assess node support, and a 50% majority rule consensus tree was generated from 1,000 bootstrap replicates.

Morphology.—To aid with taxonomic revision and species delimitation within *Artedidraco*, we compiled data on five meristic traits for a total of 90 specimens representing all seven currently recognized species of *Artedidraco* (Supplemental Table S2; see Data Accessibility). Focal traits included: (1) number of spines in the first dorsal fin, (2) number of rays in the second dorsal fin, (3) number of rays in the anal fin, (4) number of rays in the pectoral fin, and (5) number of tubular scales in the upper lateral line. Meristic data was collected from specimens in the Peabody Museum of Natural History, Yale University, following Eastman and Eakin (1999). Meristic data for *Artedidraco glareobarbatus* were taken from La Mesa and Vacchi (2005), and counts for the only two known specimens of *A. longibarbatus* were taken from the species description (Eakin et al., 2015). We conducted a principal component analysis (PCA) of the meristic data

Fig. 1. *Neodraco skottsbergi* from the South Orkney Islands (–61.213304, –45.935358), 7.1 cm standard length. YPM ICH 022463.

using the 'prcomp' function in R. The Kruskal-Wallis and pairwise rank sum Wilcoxon tests were then used to evaluate the significance of differences of the mean meristic trait values among the species of *Artedidraco* and the new genus.

RESULTS

Systematic account

Neodraco, new genus

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Type species.—*Artedidraco skottsbergi* (Lönnberg, 1905: 48–49; Fig. 1).

Definition.—The least inclusive clade that includes *Neodraco skottsbergi* (Lönnberg, 1905) and *Neodraco loennbergi* (Roule, 1913). The reference phylogeny is one inferred from the min84 dataset (including DNA sequences of 64,980 ddRAD loci) presented in Figure 2.

Diagnosis.—First dorsal fin with two to three spines, second dorsal fin with 24 to 28 rays, anal fin with 14 to 17 rays, and pectoral fin with 17 to 21 rays. The upper lateral line has 2 to 9 anterior tubular scales and 1 to 16 posterior disc-shaped scales. Number of vertebrae ranges between 36 and 40. In the caudal skeleton, the parhypural and lower hypural plate are fused with the urostyle (Eakin, 1981). The depth of the head is similar to the body depth to the caudal peduncle. The diameter of the orbit exceeds the length of the snout. The interorbital width is narrow, ranging 13 to 24 times smaller than the head length. The mental barbel is short, ranging between 3.8 to 11.0 times smaller than the head length. There are 6 to 8 dark patches of pigment at the bases of the two dorsal fins that form saddles when viewed from above (Eakin, 1990).

Comparisons.—*Neodraco* is distinguished from species of *Artedidraco* in having 2 to 9 tubular scales in the upper lateral line (more than 6 in the latter), with the tubular scale row not extending beyond the first ray of the second dorsal fin (upper lateral line extends well under the dorsal fin in *Artedidraco*), 17 or 18 gill rakers on the first gill arch (13 to 16 in *Artedidraco*), and two to three spines in the first dorsal fin (three to five in *Artedidraco*; Eakin, 1981, 1990; Eakin et al., 2015). *Neodraco* differs from *Dolloidraco longedorsalis* in having fewer than 8 tubular scales in the upper lateral line and the first dorsal fin is positioned above the base of the pectoral fin versus 8 to 18 tubular upper lateral-line scales and the placement of the first dorsal fin above the operculum in *D. longedorsalis* (Norman, 1938; Eakin, 1990). *Neodraco* is distinguished from *Histiodraco velifer* and species of *Pogonophryne* by the absence of post-temporal ridges (Norman, 1938; Eakin, 1990).

Etymology.—From the Ancient Greek words νέος (neos) meaning new and δράκων (dracon) meaning dragon. The name *Neodraco* highlights that this is a newly-described lineage of Artedidraconinae identified through the application of molecular phylogenetics (Derome et al., 2002; Lecointre et al., 2011; Near et al., 2012, 2018).

Artedidraconinae A. P. Andriashev 1967: 403

Type species.—*Artedidraco mirus* Lönnberg (1905: 40–41).

Definition.—The least inclusive clade that includes *Artedidraco mirus* Lönnberg (1905: 40–41), *Neodraco skottsbergi* (Lönnberg, 1905: 48–49), and *Dolloidraco longedorsalis* Roule (1913: 16), but not *Harpagifer bispinis* (Forster in Bloch and Schneider, 1801: 45).

Morphological apomorphies.—(1) Presence of a mental barbel (Eakin, 1981; Balushkin, 2000), (2) five branchiostegal rays (Eakin, 1981; Balushkin, 2000), (3) opercle with a flattened hook (Eakin, 1981), (4) floating pleural ribs starting on 5th to 8th vertebrae, or absent (Eakin, 1981; Balushkin, 2000), and (5) four or five hypurals (Eakin, 1981).

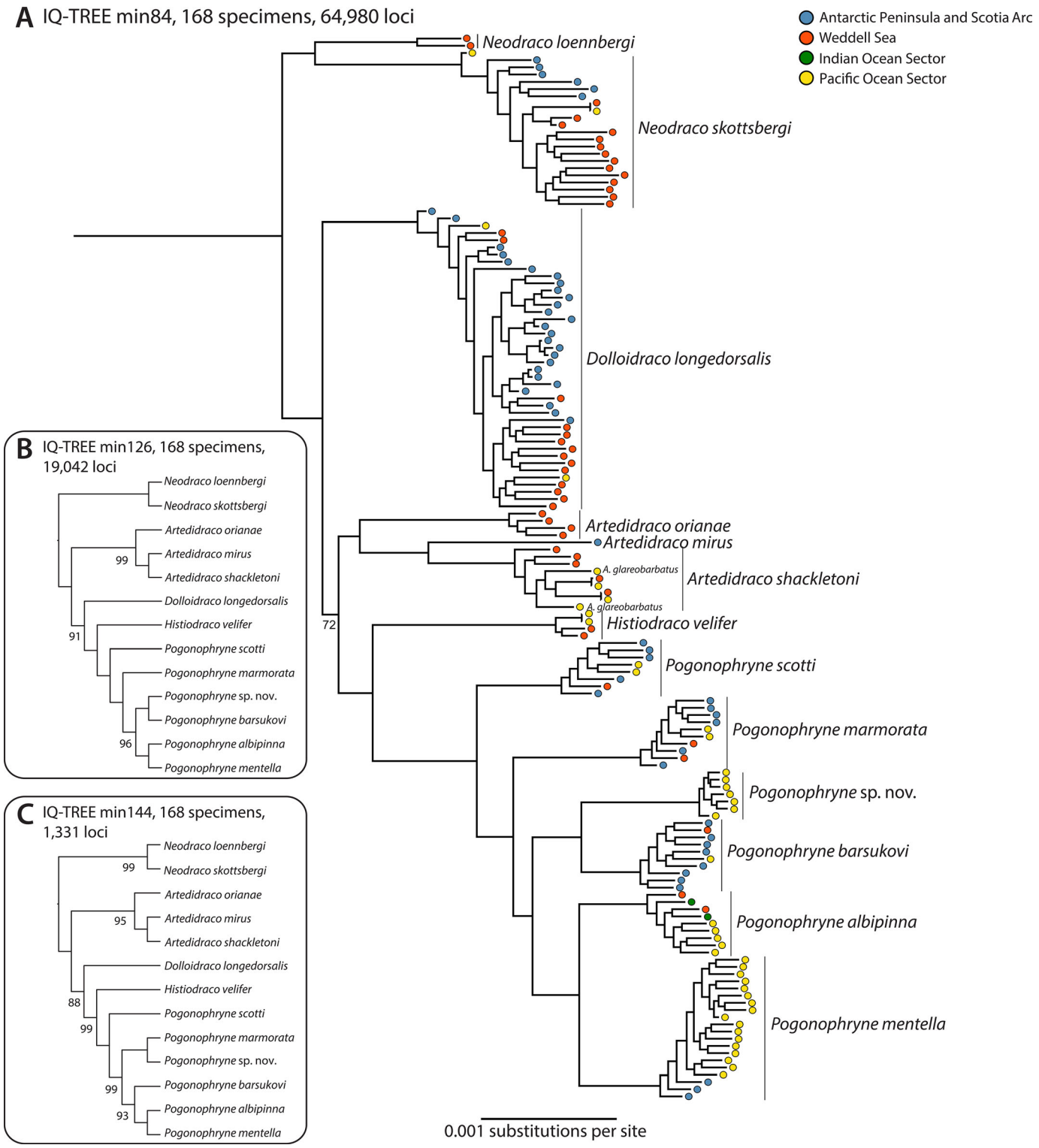

Fig. 2. Phylogenetic relationships of Artedidraconinae based on maximum likelihood analyses of concatenated ddRAD datasets using IQ-TREE. Bootstrap support values for nodes less than 100% are shown. (A) Phylogeny resulting from analysis of the dataset that includes 64,980 ddRAD loci. The DNA sequence alignments include data for at least 84 of the 168 sampled specimens (min84). The sectors of the Southern Ocean where specimens were collected are indicated with colored circles at the tips of the phylogeny. (B) Phylogeny resulting from analysis of the dataset that includes 19,042 ddRAD loci. The DNA sequence alignments include data for at least 126 of the 168 sampled specimens (min126). (C) Phylogeny resulting from analysis of the dataset that includes 1,331 ddRAD loci. The DNA sequence alignments include data for at least 144 of the 168 sampled specimens (min144). See Data Accessibility for tree file.

Composition.—There are 12 valid and distinct species of Artedidraconinae with three species of *Artedidraco*, two species of *Neodraco*, five species of *Pogonophryne*, *Dolloidraco longedorsalis*, and *Histiodraco velifer*. Eastman and Eakin (2021) list 27 species of *Pogonophryne*, but analysis of morphology and DNA sequences of ddRAD loci lead to the delimitation of only five described species in the clade (Parker et al., 2021). Based on analyses of morphology and DNA sequences of ddRAD loci presented in this study, we recognized three species of *Artedidraco*. We treat *Artedidraco glareobarbatus* (Eastman and Eakin, 1999) as a junior synonym of *A. shackletoni* (Waite, 1911). Pending additional morphological and molecular analyses, we suggest that the recognition of *A. longibarbatus* (Eakin et al., 2015) as a distinct species is unwarranted.

Harpagiferidae T. Gill 1861: 510

Type species.—*Harpagifer bispinis* (Forster in Bloch and Schneider, 1801: 45).

Definition.—The least inclusive clade that includes *Harpagifer bispinis* (Forster) and *Artedidraco mirus* Lönnberg (1905: 40–41). The reference phylogeny is one inferred from a Sanger sequenced dataset comprising two mitochondrial gene regions and seven nuclear genes (Dornburg et al., 2017: fig. 2).

Morphological apomorphies.—(1) Gill membranes are united and joined at the isthmus but do not form a fold (Eakin, 1981; Balushkin, 2000) and (2) the presence of one or two epurals (Eakin, 1981).

Composition.—There are 18 valid and distinct species of Harpagiferidae with six species of *Harpagifer* and 12 species of Artedidraconinae. Duhamel et al. (2005: 328, 358) and Eastman and Eakin (2021) call into question the distinctiveness of the five additional species of *Harpagifer* described by V. P. Prirodina and A. V. Neyelov (*H. andirashevi* Prirodina 2000, *H. nybelini* Prirodina 2002, *H. crozetensis* Prirodina 2004, *H. macquariensis* Prirodina 2000, and *H. permitini* Neyelov and Prirodina 2006). Duhamel et al. (2005) warn that these species are diagnosed primarily by the degree of development of the supraorbital protuberance, which is known to vary widely within a single species (Eastman and Eakin, 2021). Given these concerns, we conservatively recognize six species of *Harpagifer* and suggest that species delimitation analyses based on morphological and molecular data are needed to confirm the distinctiveness of the five species described by V. P. Prirodina and A. V. Neyelov.

Phylogenetic analyses.—Consistent with previous molecular phylogenetic studies (Derome et al., 2002; Lecointre et al., 2011; Near et al., 2012, 2018), *Artedidraco sensu lato* (s.l.) is paraphyletic in the ddRAD phylogenies inferred from both concatenated data and species tree analyses (Figs. 2, 3). A clade containing *Neodraco skottsbergi* and *N. loennbergi* is resolved as the sister lineage of all other species of Artedidraconinae. All other species of *Artedidraco sensu stricto* (s.s.) included in this study (*A. orianae*, *A. mirus*, *A. glareobarbatus*, and *A. shackletoni*) resolve as a monophyletic group with strong node support (Figs. 2, 3). Relationships among species of *Artedidraco* are consistent and strongly supported across all analyses: specimens of *A. shackletoni* do not resolve as a monophyletic group because specimens of *A. glareobarbatus* are nested within the species (Figs. 2A, 3A). A clade containing *A. shackletoni*, specimens of *A. glareobarbatus*, and *A. mirus* is resolved as the sister lineage of *A. orianae* (Figs. 2, 3).

In both the concatenated and species tree analyses of the min84 dataset, *Artedidraco* is resolved as sister to a clade containing *Histiodraco velifer* and *Pogonophryne* (Figs. 2A, 3A). This clade, inclusive of *Artedidraco*, *H. velifer*, and *Pogonophryne*, is resolved as the sister lineage of *Dolloidraco longedorsalis* (Figs. 2A, 3A). These relationships among the major artedidraconine lineages are also resolved in the species tree analysis of the min126 dataset (Fig. 3B); however, the concatenated analyses of the min126 and min144 datasets as well as the species tree analysis of the min144 dataset result in slightly different topologies. In each of these analyses, *D. longedorsalis* is resolved as the sister lineage of the clade containing *H. velifer* and *Pogonophryne*, and this clade including *D. longedorsalis*, *H. velifer*, and *Pogonophryne* is resolved as the sister lineage of *Artedidraco* (Figs. 2B, C, 3C). These alternative phylogenetic hypotheses likely emerge from differences in the phylogenetic information content across our analyzed datasets. Specifically, the hypothesized placement of *D. longedorsalis* as sister to *H. velifer* and *Pogonophryne* is resolved only in datasets which contain fewer missing data and therefore also include fewer loci (Figs. 2, 3). It has been demonstrated that stricter thresholds on missing data may result in the filtering out of loci with the highest mutation rates, thereby producing datasets with lower phylogenetic information content (Huang and Knowles, 2016). The reduction of phylogenetic informativeness in the datasets with fewer loci is evident by the decreasing node support observed for bipartitions in the phylogenies as the number of loci in a dataset is reduced (Figs. 2, 3).

Morphology.—There are significant differences among species of *Artedidraco* and *Neodraco* in several meristic traits (Fig. 4; Tables 1–4; Supplemental Tables S3–4; see Data Accessibility). *Neodraco loennbergi* and *N. skottsbergi* exhibit a significantly lower mean number of tubular upper lateral-line scales compared with species of *Artedidraco* (pairwise rank sum Wilcoxon test: $P < 0.05$ for comparisons with all species except *A. longibarbatus*; Table 1, Supplemental Table S4f; see Data Accessibility). The mean number of spines in the first dorsal fin exhibited by *A. shackletoni* and *A. glareobarbatus* is significantly higher than that of species of *Neodraco* and *A. mirus* ($P < 0.05$ for all comparisons; Table 2; Supplemental Table S4b; see Data Accessibility). *Artedidraco mirus*, *A. orianae*, and *A. longibarbatus* exhibit fewer anal-fin rays than observed in other species of *Artedidraco* and the two species of *Neodraco* (Table 3). In addition, *Artedidraco mirus* and *A. orianae* exhibit a lower mean number of second dorsal-fin rays (Table 4).

The disparity in the meristic traits is reflected in the results of the PCA (Fig. 4). The first three PC axes account for 93.4% of the variance in meristic traits. The first PC axis (51.2% of the variation) mostly represents variation in the first dorsal-fin spines and the number of tubular scales in the upper lateral line, the second PC axis (30.2%) mostly describes variation in the number of second dorsal-fin rays and anal-fin rays, and the third PC axis (12.0%) mostly represents

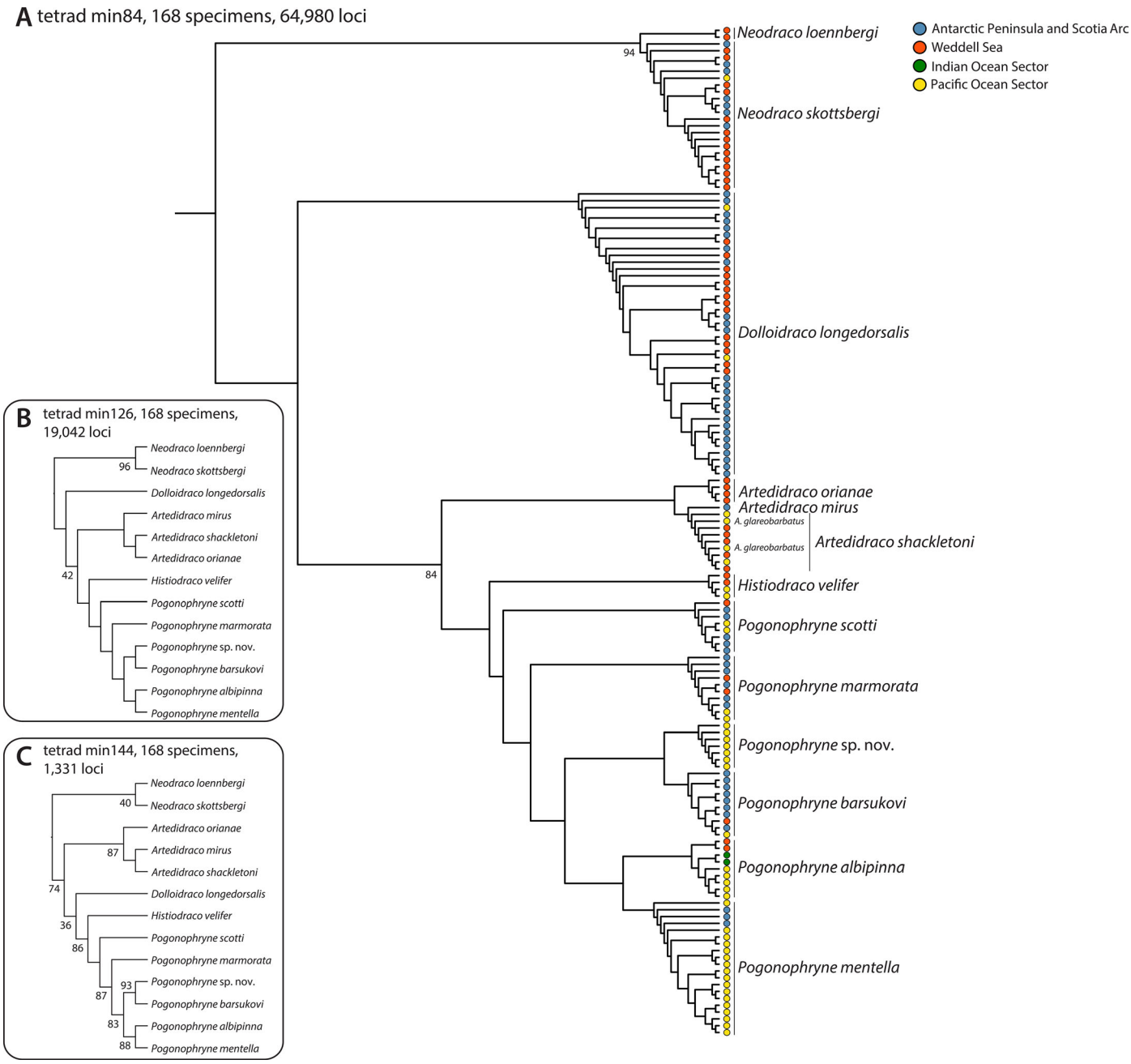

Fig. 3. Phylogenetic relationships of Artedidraconinae based on multispecies coalescent analyses of SNPs extracted from ddRAD datasets using tetrad. Bootstrap support values for nodes less than 100% are shown. (A) Phylogeny resulting from analysis of the dataset that includes 64,980 ddRAD loci. The DNA sequence alignments include data for at least 84 of the 168 sampled specimens (min84). The sectors of the Southern Ocean where specimens were collected are indicated with colored circles at the tips of the phylogeny. (B) Phylogeny resulting from analysis of the dataset that includes 19,042 ddRAD loci. The DNA sequence alignments include data for at least 126 of the 168 sampled specimens (min126). (C) Phylogeny resulting from analysis of the dataset that includes 1,331 ddRAD loci. The DNA sequence alignments include data for at least 144 of the 168 sampled specimens (min144). See Data Accessibility for tree file.

variation in the number of pectoral-fin rays. Plotting PC2 against PC1 reveals separation of *Neodraco skottsbergi* and *N. loennbergi* from all species of *Artedidraco* along both PC2 and PC1 (Fig. 4). The distribution of specimens in the PC meristic morphospace is consistent with the diagnosis of *Neodraco* by a lower number of tubular scales in the upper lateral line as well two or three spines in the first dorsal fin (Fig. 4; Tables 1, 2). The PC plot shows almost no separation of *A. shackletoni* and *A. glareobarbatus* (Fig. 4).

DISCUSSION

Despite over a century of research in the systematics of notothenioids, there still remains uncertainty in various aspects of phylogenetic relationships in the clade. Here, we apply a dataset of over 60,000 loci captured using ddRADseq to infer phylogenetic relationships within Artedidraconinae, a lineage identified as the fastest-diversifying lineage of notothenioids and which has historically represented a hotbed of taxonomic uncertainty (Near et al., 2012; Parker

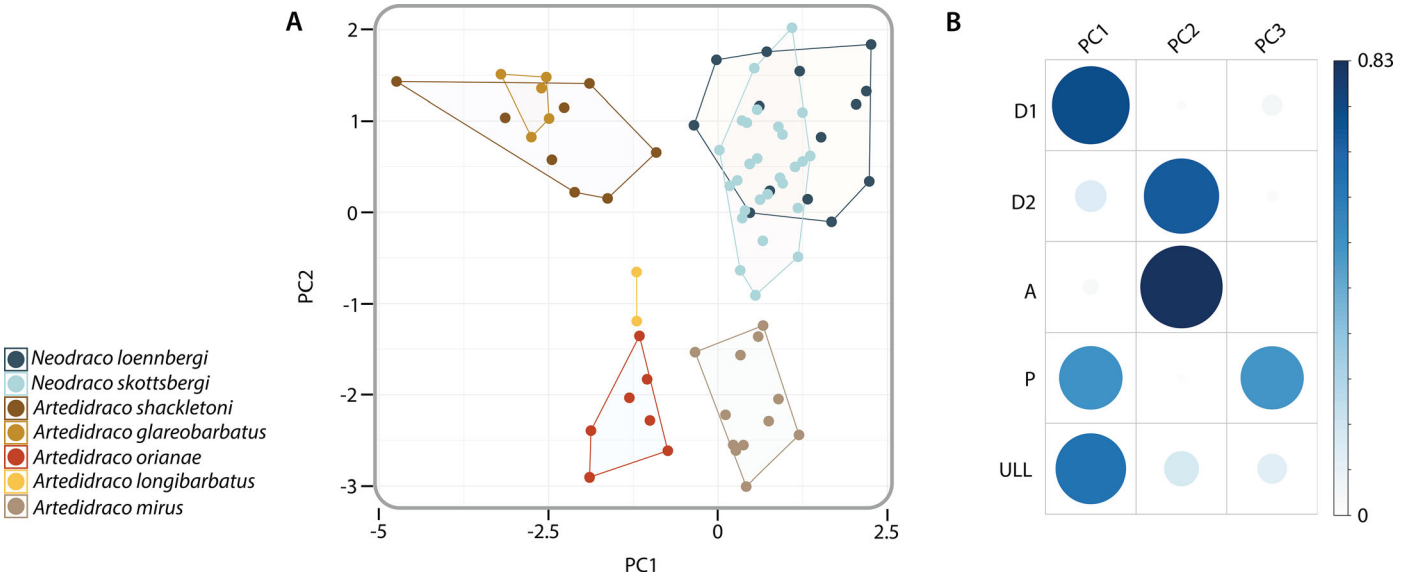

Fig. 4. Results of principal component analysis (PCA) of meristic trait data for five species of *Artedidraco* and two species of *Neodraco*. (A) Biplot visualization of PC axes 1 and 2. (B) Visualization of variable loadings for all PC axes 1, 2, and 3. Points and convex hulls in biplots are colored according to species. In panel B, D1 = first dorsal-fin spines, D2 = second dorsal-fin rays, A = anal-fin rays, P = pectoral-fin rays, and ULL = tubular scales in upper lateral line.

et al., 2021). Our results are consistent with previous molecular phylogenetic studies based on both legacy markers (Derome et al., 2002; Lecointre et al., 2011; Near et al., 2012) and genome-wide sequence data (Near et al., 2018) in resolving the deep paraphyly of *Artedidraco* s.l. The results of our study present several novel phylogenetic hypotheses, including the resolution of a strongly supported clade containing *Neodraco skottsbergi* and *N. loennbergi* and a lack of support for reciprocal monophyly of the species *Artedidraco shackletoni* and *A. glareobarbatus*. Based on these results, we describe the genus *Neodraco* and suggest that species diversity in *Artedidraco* is overestimated as a result of overreliance on the morphology of the mental barbel. Specifically, we argue that the species *A. glareobarbatus* (Eastman and Eakin, 1999) and *A. longibarbatus* (Eakin et al., 2015), which are both diagnosed primarily on the basis of mental barbel morphology, are likely not distinct species. We discuss the remaining uncertainty in the phylogenetic relationships of Artedidraconinae and the implications of this uncertainty for understanding patterns of diversification within the clade.

Reducing redundancy and conveying relationships in the classification of notothenioids.—From 1938 to 1985, Harpagiferidae included *Harpagifer* and the lineages classified as Artedidraconinae. This delimitation of Harpagiferidae was motivated by the observation that *Artedidraco mirus* closely resembled *Harpagifer* (Lönnberg, 1905), particularly in the shared possession of a sculpin-like body shape, a naked body except for lateral-line scales, and gill membranes that are broadly united, but which do not form a fold across the isthmus (Eakin, 1981). *Harpagifer* was clearly distinguished from all other lineages of Harpagiferidae in possessing a spinate rather than hooked opercle and by the absence of a mental barbel (Lönnberg, 1905; Norman, 1938; Andriashev, 1965). A subsequent morphological study of Harpagiferidae revealed several additional osteological characters that distinguish *Harpagifer* from Artedidraconinae, including the presence of well-developed epipleural ribs, a complete supratemporal canal with three pores, and a medial extrascapular that is fused to the parietal (Eakin, 1981). Hureau (1985, 1986) elevated Artedidraconidae, while Harpagiferidae was limited to *Harpagifer* (Hureau, 1990). A delimitation of Harpagiferidae that contains only *Harpagifer* results in redundant names that both delimit the exact same set of species. Also, several morphological and molecular analyses support monophyly of the group containing *Harpagifer* and

Table 1. Counts of tubular upper lateral line scales in species of *Neodraco* and *Artedidraco*. *n* refers to total number of specimens examined and SD refers to the standard deviation.

	Number of tubular upper lateral line scales																							
Species	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	*n*	Mean	SD
Neodraco loennbergi	1	3	6	4	3																	17	4.29	1.16
Neodraco skottsbergi	2	4	13	9	3	4																35	4.54	1.31
Artedidraco shackletoni										4	2		1	2			1					10	13.00	2.40
Artedidraco glareobarbatus									1			1	3									5	13.00	1.73
Artedidraco mirus					1	4	4		1	2												12	8.33	1.97
Artedidraco orianae																	3	1	1	1	1	7	19.43	1.62
Artedidraco longibarbatus						1	1															2	7.50	NA

Table 2. Counts of first dorsal-fin spines in species of *Neodraco* and *Artedidraco*. *n* refers to total number of specimens examined and SD refers to the standard deviation.

	Number of dorsal-fin spines						
Species	2	3	4	5	*n*	Mean	SD
Neodraco loennbergi	6	11			17	2.65	0.49
Neodraco skottsbergi	1	35			36	2.97	0.17
Artedidraco shackletoni		1	7	2	10	4.10	0.57
Artedidraco glareobarbatus			5		5	4.00	0.00
Artedidraco mirus		13			13	3.00	0.00
Artedidraco orianae		6	1		7	3.14	0.38
Artedidraco longibarbatus		2			2	3.00	NA

Artedidraconinae (Balushkin, 2000; Bargelloni et al., 2000; Derome et al., 2002; Near et al., 2018). In an effort to minimize monogeneric family-level group names and ensure that the classification of notothenioids conveys information on phylogenetic relationships, we delimit Harpagiferidae as containing the species of *Harpagifer*, the species of *Artedidraco*, the species of *Neodraco*, *Dolloidraco longedorsalis*, *Histiodraco velifer*, and the species of *Pogonophryne*.

Remaining phylogenetic and taxonomic uncertainty in Artedidraconinae.—While molecular phylogenetics have clarified the relationships of *Artedidraco* and *Neodraco*, there remains uncertainty in the phylogenetic relationships among the major lineages of Artedidraconinae (Figs. 2, 3). In the ddRAD-inferred phylogenies, the new genus *Neodraco* is confidently resolved as the sister lineage of all other artedidraconines, and *Histiodraco velifer* and *Pogonophryne* are consistently resolved as a monophyletic group (Figs. 2, 3). However, *Dolloidraco longedorsalis* resolves either as the sister lineage of a clade containing *H. velifer* and *Pogonophryne* or as the sister lineage to a larger clade inclusive of *H. velifer*, *Pogonophryne*, and *Artedidraco* (Figs. 2, 3). Furthermore, *Artedidraco* is resolved as the sister lineage of either a clade containing *H. velifer* and *Pogonophryne* or a larger clade containing *H. velifer*, *Pogonophryne*, and *D. longedorsalis* (Figs. 2, 3). Previous phylogenomic analyses resolved a clade containing *D. longedorsalis* and *Artedidraco* (Near et al., 2018), but this relationship is not supported in any of our phylogenetic analyses (Figs. 2, 3). The uncertainty in the phylogenetic relationships among lineages of Artedidraconinae is likely due to a history of rapid diversification and the resultant short internodes that characterize the backbone of the phylogeny (Figs. 2, 3). Short internodes provide limited intervals of time for accumulation of phylogenetically informative character state changes and are particularly susceptible to incomplete lineage sorting owing to the limited number of generations between diversification events (McCormack et al., 2013; Suh et al., 2015).

Molecular phylogenetics and morphological traits also reveal uncertainty in species delimitation within *Artedidraco*. The phylogenomic analyses reveal that *A. shackletoni* and *A. glareobarbatus* are not reciprocally monophyletic (Figs. 2, 3), suggesting that *A. glareobarbatus* does not represent an evolutionarily distinct lineage. *Artedidraco shackletoni* and *A. glareobarbatus* exhibit complete overlap in the ranges of meristic traits, including the number of second dorsal-fin rays and number of vertebrae (Eastman and Eakin, 1999). The only trait that distinguishes *A. glareobarbatus* from *A. shackletoni* is the morphology of the mental barbel. Investigations of mental barbel variation in three different artedidraconine species, *A. mirus*, *Dolloidraco longedorsalis*, and *Pogonophryne scotti*, reveal that the length of the mental barbel and the ornamentation of its terminal expansion exhibit substantial intraspecific variation (Eakin et al., 2001, 2006; Eastman and Eakin, 2001). Phylogenomic species delimitation within *Pogonophryne* reveals that species delimited on the basis of mental barbel morphology do not represent distinct evolutionary lineages, suggesting that mental barbel traits are not appropriate for delimiting and diagnosing species of Harpagiferidae (Parker et al., 2021). Given that *A. glareobarbatus* is not genetically distinct from *A. shackletoni* and is primarily distinguished from other species of *Artedidraco* by a character known to exhibit extensive intraspecific variation, we suggest that *A. glareobarbatus* does not represent an independently evolving lineage and is best considered a junior synonym of *A. shackletoni*. Future morphological and molecular analyses will likely result in a similar conclusion regarding the distinctiveness of the

Table 3. Counts of anal-fin rays in species of *Neodraco* and *Artedidraco*. *n* refers to total number of specimens examined and SD refers to the standard deviation.

	Number of anal-fin rays								
Species	16	17	18	19	20	21	*n*	Mean	SD
Neodraco loennbergi			4	8	5		17	19.06	0.75
Neodraco skottsbergi			5	24	6	1	36	19.08	0.65
Artedidraco shackletoni			2	7	1		10	18.90	0.57
Artedidraco glareobarbatus			1	4			5	18.80	0.45
Artedidraco mirus	6	5	2				13	16.69	0.75
Artedidraco orianae	2	4	1				7	16.86	0.69
Artedidraco longibarbatus	1	1					2	16.50	NA

Table 4. Counts of second dorsal-fin rays in species of *Neodraco* and *Artedidraco*. *n* refers to total number of specimens examined and SD refers to the standard deviation.

Species	Number of dorsal-fin rays										
	22	23	24	25	26	27	28	29	*n*	Mean	SD
Neodraco loennbergi					6	7	4		17	26.88	0.78
Neodraco skottsbergi			3	14	16	3			36	25.53	0.77
Artedidraco shackletoni					1	5	3	1	10	27.40	0.84
Artedidraco glareobarbatus							2	3	5	28.60	0.55
Artedidraco mirus	3	4	6						13	23.23	0.83
Artedidraco orianae		1	3	2	1				7	24.43	1.62
Artedidraco longibarbatus					2				2	26.00	NA

recently described *Artedidraco longibarbatus* (Eakin et al., 2015), which is distinguished from all other species of *Artedidraco* by a proportionally longer mental barbel. However, *A. longibarbatus* is known only from a juvenile specimen of indeterminate sex and an immature female that was lost aboard the ship during the sampling expedition. The description of *A. longibarbatus* reports mitochondrial DNA sequences from the 16S rRNA and ND2 genes collected from the lost specimen (GenBank accessions KR088973 and KR088974; Eakin et al., 2015), but these two sequences are identical to haplotypes sampled from *Histiodraco velifer*. Precluding more comprehensive morphological and genetic analyses, we suggest that *A. longibarbatus* is likely not a distinct species of *Artedidraco*.

The evolutionary diversification of Artedidraconinae.—Analyses of diversification rates in the notothenioid radiation identified Artedidraconinae as the fastest-diversifying lineage of notothenioids (Near et al., 2012). In contrast with other rapidly radiating notothenioid lineages such as the notoperches (Trematominae) and crocodile icefishes (Channichthyidae), the rapid diversification observed in Artedidraconinae does not appear to have been accompanied by significant ecological divergence (Rutschmann et al., 2011). Indeed, all species of Artedidraconinae are benthic sit-and-wait predators that exhibit broad dietary overlap, feeding primarily on gammarid amphipods, isopods, mysids, and errant polychaetes found on or just above the substrate (Olaso et al., 2000; Lombarte et al., 2003). This suggests factors other than ecological opportunity drove the rapid lineage diversification observed in Artedidraconinae, but investigations of evolutionary mechanisms were stymied by pervasive phylogenetic and taxonomic uncertainty within the clade. The results of our phylogenetic analyses of ddRAD loci provide at least two alternative phylogenetic hypotheses for Artedidraconinae and clarification of species delimitations, providing a basis to investigate the factors that catalyzed diversification in this lineage of Antarctic notothenioids.

DATA ACCESSIBILITY

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.280gb5mr2. Raw sequence data are available for download from the NCBI Sequence Read Archive (SRA; BioProjects PRJNA777599 and PRJNA701357). Unless an alternative copyright or statement noting that a figure is reprinted from a previous source is noted in a figure caption, the published images and illustrations in this article are licensed by the American Society of Ichthyologists and Herpetologists for use if the use includes a citation to the original source (American Society of Ichthyologists and Herpetologists, the DOI of the *Ichthyology & Herpetology* article, and any individual image credits listed in the figure caption) in accordance with the Creative Commons Attribution CC BY License. ZooBank publication urn:lsid:zoobank.org:pub:8CE4A8C6-1C11-4C2E-A6FD-CE38238864C0.

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