Received: 16 May 2022 | Revised: 17 December 2022 | Accepted: 27 December 2022

DOI: 10.1111/zsc.12580

Zoologica Scripta | WILEY

ORIGINAL ARTICLE

Species identification and population genetics of the Antarctic fish genera *Lepidonotothen* and *Nototheniops* (Perciformes, Notothenioidei)

Luca Schiavon[1] | **Enrico Negrisolo**[2,3] | **Alessandra Battistotti**[1] | **Magnus Lucassen**[4] | **Malte Damerau**[5] | **Lars Harms**[4] | **Emilio Riginella**[6] | **Michael Matschiner**[7] | **Lorenzo Zane**[1,8] | **Mario La Mesa**[9] | **Chiara Papetti**[1,6,8]

[1]Department of Biology, University of Padova, Padova, Italy

[2]Department of Comparative Biomedicine and Food Science, University of Padova, Legnaro, Italy

[3]CRIBI Interdepartmental Research Centre for Innovative Biotechnologies, University of Padova, Padova, Italy

[4]Data Science Support, Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Bremerhaven, Germany

[5]Institute of Fisheries Ecology, Johann Heinrich von Thuenen Institute, Federal Research Institute for Rural Areas, Forestry and Fisheries, Hamburg, Germany

[6]Department of Integrative Marine Ecology, Zoological Station Anton Dohrn, Naples, Italy

[7]Natural History Museum, University of Oslo, Oslo, Norway

[8]Consorzio Nazionale Interuniversitario per le Scienze del Mare (CoNISMa), Rome, Italy

[9]CNR, Institute of Polar Sciences (ISP), c/o Area di Ricerca di Bologna, Bologna, Italy

Correspondence
Mario La Mesa, CNR, Institute of Polar Sciences (ISP), c/o Area di Ricerca di Bologna, via P. Gobetti 101, 40129 Bologna, Italy.
Email: mario.lamesa@cnr.it

Funding information
European Marie Curie Project "Polarexpress", Grant/Award Number: 622320; Italian National Programme of Antarctic Research (PNRA), Grant/Award Number: 2009/A1.07 and 2016_00307; University of Padova BIRD, Grant/Award Number: 164793

Abstract

Accurate species identification is essential to assess biodiversity and species richness in ecosystems threatened by rapid and recent environmental changes, such as warming in most Antarctic waters. The *Lepidonotothen* species complex comprises demersal notothenioid fishes which inhabit the shelf areas of the Antarctic Peninsula, the Scotia Arc and sub-Antarctic islands with a circum-Antarctic distribution. Species determination in this group has often been problematic. In particular, whether *Lepidonotothen squamifrons* and *Lepidonotothen kempi* are valid as separate species has been questioned. In this study, we analysed the genetic variation among four nominal southern polar species within this complex (*L. kempi*, *L. squamifrons*, *Nototheniops larseni*, *Nototheniops nudifrons*) by means of three different markers (*ND2* and tRNA mitochondrial genes and a panel of 16 nuclear microsatellites). We tested whether individuals morphologically assigned to *L. kempi* showed genetic separation from *L. squamifrons*. Our analyses indicated a lack of differentiation between *L. kempi* and *L. squamifrons*. However, a genetically distinct population was found for *L. squamifrons* at the Shag Rocks islands near South Georgia. Antarctic and sub-Antarctic islands are known to be home to many cryptic species and further studies will elucidate if the genetically differentiated population we found potentially originated from this context and can be considered an incipient species. Our analysis contributes to further

characterize the species composition of the most abundant fish suborder in the Southern Ocean, which is among the regions most threatened by climate change.

KEYWORDS

Antarctic Peninsula, *Lepidonotothen kempi*, *Lepidonotothen squamifrons*, microsatellites, ND2, Scotia Arc, Southern Ocean, Shag Rocks, species limits, tRNA

1 | INTRODUCTION

The rapid diversification of notothenioid fishes in Antarctica has led to several instances of morphological similarity and cryptic speciation, which have been brought to light through multidisciplinary approaches (Ceballos et al., 2019; Dornburg, Eytan, et al., 2016; Dornburg, Federman, et al., 2016). On the other hand, genomic investigations have also revealed the lack of genetic separation between some described taxa (Parker et al., 2021). These cases demonstrate that the species richness of notothenioids is not yet fully characterized. Knowing the taxonomic status, the number, abundance and distribution of independent evolutionary units (sensu De Queiroz, 2007, see also Dobzhansky, 1935) inhabiting the continental shelf of Antarctica is important not only for understanding the radiation of notothenioids but also for the conservation and management purposes (Ceballos et al., 2019).

One of the most enigmatic cases of species diversification in Antarctic waters is the *Lepidonotothen* species complex (family Nototheniidae; Balushkin, 1976). The genus *Lepidonotothen* includes some of the most abundant species in the seasonal pack-ice zone and the islands north of it (sensu Kock, 1992; La Mesa et al., 2017), in some cases with circum-Antarctic distributions (La Mesa et al., 2017). Like many other species in the family Nototheniidae, they are benthic or demersal as adults (Eastman, 2020; Gon & Heemstra, 1990) and have a pelagic larval phase that can last up to 1 year (La Mesa et al., 2017). This long larval phase likely increases the potential for dispersal to remote habitats and may be one of the explanations for the large geographic range of distribution of these species (e.g. Dambach et al., 2016; Haye et al., 2014; Papetti et al., 2012; Raupach et al., 2010; Schiavon et al., 2021). This dispersal potential may have been beneficial during glacial events when most Antarctic shelf habitat was inaccessible, allowing these species to reach remote sub-Antarctic islands that could serve as refugia (Díaz et al., 2018).

There is an ongoing debate about species limits and phylogenetic relationships within the genus *Lepidonotothen*, fostered by numerous studies analysing the genetic variability, morphology, physiology and meristic traits of the genus that led to recent taxonomic revisions (Dornburg, Federman, et al., 2016; Near et al., 2012, 2018). Molecular analysis by Dornburg, Federman, et al. (2016) identified two cryptic (morphologically indistinguishable) species of *L. nudifrons* (Lonnberg, 1905) at the islands of the Scotia Arc: *L. nudifrons* from South Georgia and the South Sandwich Islands, and *L.* cf. *nudifrons* from the South Shetland Islands, Elephant Island, and the South Orkney Islands. In this study, we will follow the nomenclature applied by Near et al. (2018) who proposed the new genus *Nototheniops* (Balushkin, 1976) for *L. larseni* and *L. nudifrons*.

While the relationships within the genus *Nototheniops* have been investigated in several studies and are supported by clear diagnostic morphological traits, some uncertainty remains for the so-called '*squamifrons* group' (Gon & Heemstra, 1990), comprising the three nominal species *L. squamifrons*, *L. kempi* and *L. macrophtalma* (Gon & Heemstra, 1990). *L. macrophtalma* is a sub-Antarctic species known only from the Falkland/Malvinas Islands area and the Burdwood Bank off the tip of Patagonia (Gon & Heemstra, 1990) and has been considered a synonym of *L. squamifrons* (Schneppenheim et al., 1994). *Lepidonotothen squamifrons* and *L. kempi* are considered sympatric species in South Georgia and Elephant Island (Figure 1) and have partially overlapping distribution ranges around the Antarctic Peninsula, the Scotia Sea islands, Bouvet Island (Figure 1) and the Kerguelen and Crozet Islands. *Lepidonotothen kempi* is assumed to have a circum-Antarctic distribution (Gon & Heemstra, 1990; Miya et al., 2016). *Lepidonotothen squamifrons* and *L. kempi* are morphologically very similar and differentiated usually by a few characters with overlapping distribution of measures in adults (e.g. interorbital width and the number of vertebrae) as well as by larval pigmentation (Efremenko, 1984; Kellermann, 1990; Koubbi et al., 1990) making the species identification troublesome and leading to the hypothesis that the two species are in fact synonyms (e.g. Near et al., 2018).

Several studies have attempted to assess the species status of *L. squamifrons* and *L. kempi* via molecular methods. Most of these studies have found limited evidence of differences between the two species suggesting that the weak differentiation is indeed indicative of distinct populations over the wide geographical range of the species (Jones et al., 2008; Miya et al., 2016; Schneppenheim et al., 1994).

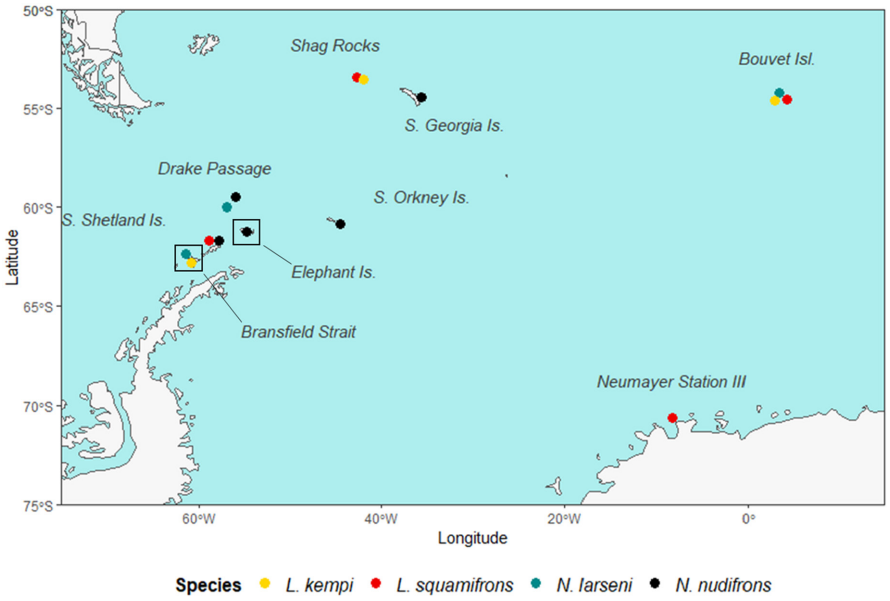

FIGURE 1 Map showing the sampling locations for all *Lepidonotothen* and *Nototheniops* samples analysed in this study. Collection sites are coloured according to the initial morphological identification performed during sample sorting: yellow: *L. kempi*, red: *L. squamifrons*, blue: *N. larseni*, black: *N. nudifrons*. The map was plotted in the R software environment ver. 4.0.0, using packages ggplot2 (Wickham, 2016) and sf (Pebesma, 2018). The R package rnaturalearthdata (South, 2017) was used to access and download the map dataset (http://www.naturalearthdata.com).

More recently, Ceballos et al. (2019) investigated the species differentiation with single nucleotide polymorphisms (SNPs) derived from restriction site-associated DNA sequence (RADseq) markers and determined that samples assigned morphologically to *L. kempi* (five individuals from Shag Rocks) and *L. squamifrons* (one individual from Shag Rocks, two individuals from Bouvet Island, two individuals from South Orkney Islands) formed two reciprocally monophyletic groups (100% bootstrap support, figures 1 and 2 in Ceballos et al., 2019, $F_{ST} = 0.037$). In contrast, the mitochondrial locus *COI* was unable to differentiate the two species ($F_{ST} = 0.085$; Ceballos et al., 2019).

With these conflicting results of past investigations in mind, we aimed at answering the following questions: are *L. squamifrons* and *L. kempi* valid as separate species or should they be considered differentiated populations of the same species? To disentangle this controversy, we based our analyses on the genetic variation of 16 microsatellites, a mitochondrial gene (ND2) and three mitochondrial tRNAs. To increase the power of detecting even subtle differences between closely related individuals, we applied our analyses to a data set of more than 150 individuals, including some larvae morphologically identified as *L. kempi* (based on the ventral melanophore pattern, Efremenko, 1984; Kellermann, 1990; Koubbi et al., 1990). To provide context and comparison, we additionally analysed the genetic variation in *N. nudifrons* (40 individuals) and *N. larseni* (94 individuals), given that these species are closely related and sympatrically distributed with *L. squamifrons* and *L. kempi* but nevertheless clearly divergent from these.

The species assessed in this study are endemic to the Antarctic Peninsula, Scotia Arc and sub-Antarctic islands – that is regions of thermally variable waters (Ceballos et al., 2019) that are particularly exposed to climate-induced sea surface temperature increase (Moffat & Meredith, 2018). The proper identification of the biodiversity of these regions is therefore imperative to monitor changes in spatial distribution and genetic structure that may result from rising water temperatures. Owing to their adaptations to a constant and cold habitat (for a review see Matschiner et al., 2015), Antarctic notothenioid fishes could prove important sentinels of warming (Moffat & Meredith, 2018).

2 | MATERIALS AND METHODS

2.1 | Study areas, samples collection and genomic DNA isolation

The specimens of the genera *Lepidonotothen* and *Nototheniops* analysed in this study were collected during five scientific research cruises (from 2005 to 2012) of the Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research (AWI) and of the U.S. Antarctic Marine Living Resources (AMLR) programme to the Antarctic Peninsula and Scotia Arc islands (Table 1). Specimens

TABLE 1 List of samples analysed in this study. The table reports species names, sampling cruises, general areas of sampling (location), sample acronyms and sample sizes for the microsatellite and mtDNA data sets.

Species	Cruise ID	Location	Location analyses acronym	mtDNA analyses sample size	Microsatellite analyses sample size
Lepidonotothen kempi	ANT-XXIII/2, 2005, AWI	Bouvet Island	ke-BV	3	3
	ANT-XXVII/3, 2011, AWI	Shag Rocks	ke-SR	1[a]	1[a]
	ANT-XXVII/3, 2011, AWI	Bouvet Island	ke-BV	15	17
	AMLR-2011	Bransfield Strait	ke-BS	4	31
	Total			23	52
Lepidonotothen squamifrons	ANT-XXIII/2, 2005, AWI	Bouvet Island	sq-BV	2	2
	ANT-XXIII/2, 2005, AWI	Neumayer Station	sq-NMS	0	1[a]
	ANT-XXVII/3, 2011, AWI	Shag Rocks	sq-SR	41	54
	ANT-XXVIII/4, 2012, AWI	South Shetlands	sq-SS	32	44
	Total			75	101
Nototheniops larseni	ANT-XXIII/2, 2005, AWI	Bouvet Island	la-BV	52	49[b]
	ANT-XXVIII/4, 2012, AWI	South Shetlands	la-SS	24	24
	ANT-XXVIII/4, 2012, AWI	Drake Passage	la-DP	21	21
	Total			97	94
Nototheniops nudifrons	ANT-XXVIII/4, 2012, AWI	Elephant Island	nu-EI	13	13
	ANT-XXVIII/4, 2012, AWI	Drake Passage	nu-DP	1[c]	2[c]
	ANT-XXVIII/4, 2012, AWI	South Shetlands	nu-SS	10	10
	ANT-XXVII/3, 2011, AWI	South Georgia	nu-SG	1[c]	1[c]
	AMLR-2009	South Orkney	nu-SO	14	14
	Total			39	40
Complete dataset				234	287

Abbreviations: AMLR, U.S. Antarctic Marine Living Resources; AWI, Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research.

[a]This individual was included in the haplotype network, in the STRUCTURE analysis and in the calculation of F_{ST} for which all samples for all species were considered. This specimen was omitted from the analyses for which *L. squamifrons* and *L. kempi* were considered a single pool.

[b]Three individuals were excluded from the microsatellite analysis as they could not be amplified in more than 50% of loci. These specimens were used in the *ND2* network.

[c]Individuals used to build the haplotype network, in the STRUCTURE analysis and for the calculation of F_{ST} for which all samples for all species were considered. These specimens were, instead, excluded from the calculation of pairwise population F_{ST} of *N. nudifrons*.

obtained during AWI cruises were all adults and were collected by bottom trawl, weighed, measured and assigned to species according to morphological characteristics given in Gon and Heemstra (1990). A piece of muscle or fin clip was collected from each specimen and preserved in 95% ethanol for the subsequent molecular analysis. Specimens collected during the U.S. AMLR cruise were larvae of the putative species *L. kempi* only (according to Kellermann, 1990) and were preserved whole in 95% ethanol. Sample collection during the AWI and U.S. AMLR cruises was approved by the competent national authorities for Antarctic research. The following nine localities were sampled: Drake Passage, Bransfield Strait (samples collected on the eastern side of Livingston Island), South Shetland Islands (comprising individuals sourced from the western side of King George Island and Livingston Island), Elephant Island, South Orkney Islands, Shag Rocks, South Georgia Islands (one individual of *N. nudifrons*, sensu Dornburg, Federman, et al., 2016), Bouvet Island and Weddell Sea (Neumayer Station, one individual of *L. squamifrons*, the first observation of this species ever reported in the Weddell Sea to our best knowledge; Table 1, Figure 1).

Most of the specimens of *N. nudifrons* were sampled in the areas where *N.* cf. *nudifrons* was described (from the Antarctic Peninsula up to South Orkney Islands), except for one individual from the area of *N. nudifrons* (South Georgia). The two cryptic species *N.* cf. *nudifrons* and *N. nudifrons* will not be recognized until formal description and naming (Eastman & Eakin, 2021) so we will use the traditional species name *N. nudifrons* to indicate both species.

Total DNA was purified from 10 to 20 mg of muscle or fin clip tissue following the standard protocol of the DNeasy Blood and Tissue Kit (Qiagen, Germany). Quality and quantity of DNA extractions were assessed using a NanoDrop™ 2000c spectrophotometer (Thermo Fisher Scientific, USA) before storage at −20°C.

2.2 | Molecular markers and analytical approach

Three types of molecular markers were used to assess genetic variation in the sampled species: the mitochondrial gene *ND2*, three mitochondrial tRNAs (*trnA* – Ala, *trnM* – Met and *trnW* – Trp) and a panel of 16 nuclear microsatellites. The mitochondrial locus *ND2* was used for molecular validation of the morphological species identification, to construct a mitochondrial haplotype network, to perform a phylogenetic analysis and to test for population differentiation for each putative species. This marker has been employed in several studies of notothenioid evolution (e.g. Dornburg, Federman, et al., 2016; Near et al., 2012) and sequences are available in repositories as GenBank (NCBI Resource Coordinators, 2018) for comparative studies. In contrast, tRNA genes have not yet been used in studies on notothenioids. Nonetheless, their sequences and secondary folding structures can prove informative to reconstruct phylogenetic relationships (e.g. in the fish order Myctophiformes, Poulsen et al., 2013). In this study, the tRNAs *trnA*, *trnM* and *trnW* were applied as short molecular markers to delimit species boundaries due to the presence of species-specific patterns of nucleotide substitutions. Microsatellites were used for the species molecular validation in cases where *ND2* amplification failed, the clustering and the population genetics analyses for each target putative species (see the Materials and Methods part on microsatellite markers).

2.3 | Mitochondrial markers

2.3.1 | Analysis of *ND2*

Maximum-likelihood (ML) phylogenetic reconstruction was performed with a multiple sequence alignment (hereafter 230T.ND2.aln, alignment deposited in Dryad repository https://doi.org/10.5061/dryad.wpzgmsbqv, sequences generated in this study are deposited in GenBank, accession numbers: OP561531–OP561676) containing 230 *ND2* sequences, most of which in full length (for methodological information on sequencing and analysis of *ND2* for specimens considered in this study see Supporting Information). The alignment 230T.ND2.aln included all unique haplotypes found in the present study plus 43 sequences available in GenBank for *Lepidonotothen* and *Notothenio*ps species. *ND2* sequences from other species of the sub-family Trematominae as well as more distantly related notothenioids were also included in the 230T.ND2.aln and used as outgroups to improve the resolution of the phylogenetic reconstruction. The *ND2* sequence alignment was produced using the Muscle algorithm implemented in MEGA VER. 5.2.2 (Edgar, 2004; Tamura et al., 2011).

The phylogenetic reconstruction was performed with IQ-TREE VER. 2.1.3 (Minh et al., 2020). The best-fitting evolutionary model (TPM3+F+I+G4) was identified with the ModelFinder algorithm, implemented in IQ-TREE (Kalyaanamoorthy et al., 2017). Fifty independent ML searches were performed to minimize the risk of selecting suboptimal trees, and 10,000 Ultrafast Bootstrap replicates were used to assess the statistical support of the tree topology (Hoang et al., 2018).

To depict relationships of closely related mitochondrial haplotypes and investigate possible geographic patterns of sequence variation, the *ND2* sequences were also used to create a haplotype network with the software POPART VER. 1.7 (Leigh & Bryant, 2015) using the TCS algorithm (Clement et al., 2002). The input file for POPART was generated with ALTER (Glez-Peña et al., 2010). Descriptive statistics (segregating sites, number of haplotypes, haplotype diversity and nucleotide diversity) for each species and population sample were obtained with DNASP VER. 6.12.03 (Rozas et al., 2017). Patterns of population structure and species differentiation were investigated by estimating F_{ST} (Weir & Cockerham, 1984) with ARLEQUIN VER. 3.5.2.2 (Excoffier & Lischer, 2010). A nominal significance threshold before the correction was set to 0.05.

2.3.2 | Analysis of tRNAs

The sequences of the *trnA, trnM* and *trnW* were manually aligned together with all orthologs available for notothenioids in GenBank (Figures S1–S3; sequences deposited in GenBank, accession numbers: OP561531–OP561676, see also Dryad repository https://doi.org/10.5061/dryad.wpzgmsbqv). Secondary structures of *trnA, trnM* and *trnW* were used as a template to improve the alignments and the nucleotide substitution patterns were tracked on the tRNA secondary structures following Montelli et al. (2016) (Figure S4). The orthologous tRNA sequences were arranged in the alignments according to the phylogenetic relationships known for notothenioids (Dornburg et al., 2017; Papetti et al., 2021).

2.4 | Microsatellite markers

2.4.1 | Species assignment and population differentiation

The methodological procedure followed to obtain the final panel of 16 microsatellite loci analysed in this study is described in the Supporting Information, section 'Amplification, genotyping, descriptive statistics and final panel of microsatellite markers'.

To evaluate genetic differentiation between individuals morphologically identified as *L. squamifrons* and *L. kempi*, to determine the number of populations, that is clusters, in our sample of two *Lepidonotothen* and two *Nototheniops* species and to assess the extent of admixture among clusters, we used the model-based method implemented in the software STRUCTURE VER. 2.3.4 (Pritchard et al., 2000). We set a burn-in of 100,000 steps for each run, followed by 1,000,000 MCMC iterations. We used the admixture model with independent allele frequencies for the analysis comprising the entire data set of four putative species. We also performed separate clustering analyses for *N. nudifrons*, *N. larseni* and *L. squamifrons* + *L. kempi*. For these three latter analyses, we applied the admixture model with correlated allele frequencies. We tested *K* values (number of expected clusters) from 2 to 7 in the analyses comprising the entire data set of four putative species, and *K* values from 1 to 7 for *L. squamifrons* + *L. kempi*, from 1 to 6 for *N. larseni* and from 1 to 5 for *N. nudifrons*, following indications from Evanno et al. (2005) (see also Porras-Hurtado et al., 2013, for a review). For each *K*, 10 replicate analyses were performed. We also repeated all analyses applying the LOCPRIOR function in STRUCTURE, to account for the morphological classification or the sampling location of the individuals as prior in the clustering analysis (Hubisiz et al., 2009; Porras-Hurtado et al., 2013). The best-supported value of *K* for each run was determined with the method described in Evanno et al. (2005) and implemented in STRUCTURE HARVESTER VER. 0.6.94 (Earl & vonHoldt, 2012). The different runs for each *K* were averaged using the online tool CLUMPAK (Kopelman et al., 2015) and plotted with the R package 'Pophelper' ver. 2.3.0 (Francis, 2017).

Pairwise and global F_{ST} values (Weir & Cockerham, 1984) and their associated *p* values were estimated with ARLEQUIN VER. 3.5.2.2 (Excoffier & Lischer, 2010), based on 10,000 permutations of the data sets. Individuals morphologically identified as *L. kempi* and *L. squamifrons* and subsequently validated with *ND2* were analysed by geographical location as two single species and as a single unit. Individuals morphologically identified as *N. nudifrons* and *N. larseni* and subsequently validated with *ND2* were analysed by geographical location as two different species removing the single specimen of *N. nudifrons* sampled in South Georgia (see note 1 in Table 1).

3 | RESULTS

3.1 | Mitochondrial markers

3.1.1 | Sequencing and analysis of *ND2*

The fragment containing the entire *ND2* gene was successfully amplified for a total of 234 individuals of the *Lepidonotothen* and *Nototheniops* species (Table 1). It was not possible to successfully amplify and sequence the whole fragment for the remaining 53 individuals, owing to low DNA quality (however, these individuals were successfully genotyped with the microsatellite markers; see below). After comparison to the GenBank database with BLASTN, 97 of our entries were assigned to the species *N. larseni*, 39 to *N. nudifrons* and 75 to *L. squamifrons*. Twenty-three samples morphologically identified as *L. kempi* have the highest levels of sequence similarity with *L. squamifrons* GenBank records. This procedure enabled us to validate the morphological identification for most of the individuals of the four putative species. Among our 234 specimens, 69 individuals were originally identified during field sampling only as belonging to the genus *Lepidonotothen* and not assigned to a specific species. Based on the genetic variability of *ND2*, it was possible to assign unequivocally these specimens to *N. larseni* ($N = 51$), *N. nudifrons* ($N = 15$) and *L. squamifrons* ($N = 3$).

3.1.2 | Phylogenetic reconstruction

The ML tree, obtained from 230T.ND2.aln (Figure 2), showed that *ND2* sequences assigned to *L. kempi* and *L. squamifrons* form a single clade, rendering each of the two species non-monophyletic. Within this clade, sequences attributed to either *L. kempi* or *L. squamifrons* intermix and a species-specific pattern is not recognizable. On the other hand, sequences sampled at Shag Rocks cluster together with full statistical support (bootstrap support, UFTBT = 100%). This clade also contains three GenBank sequences attributed to *L. squamifrons*, one collected in South Georgia and two from Marion Island. The clade combining *L. kempi* and *L. squamifrons* is the sister group of the genus *Patagonotothen* with strong statistical support (UFTBT = 97%), and these two genera result as the sister taxon of the genus *Nototheniops* with full statistical support (UFTBT = 100%). Sequences of *N. nudifrons* form two main clusters. One of these clusters contains the single individual collected by us at South Georgia and

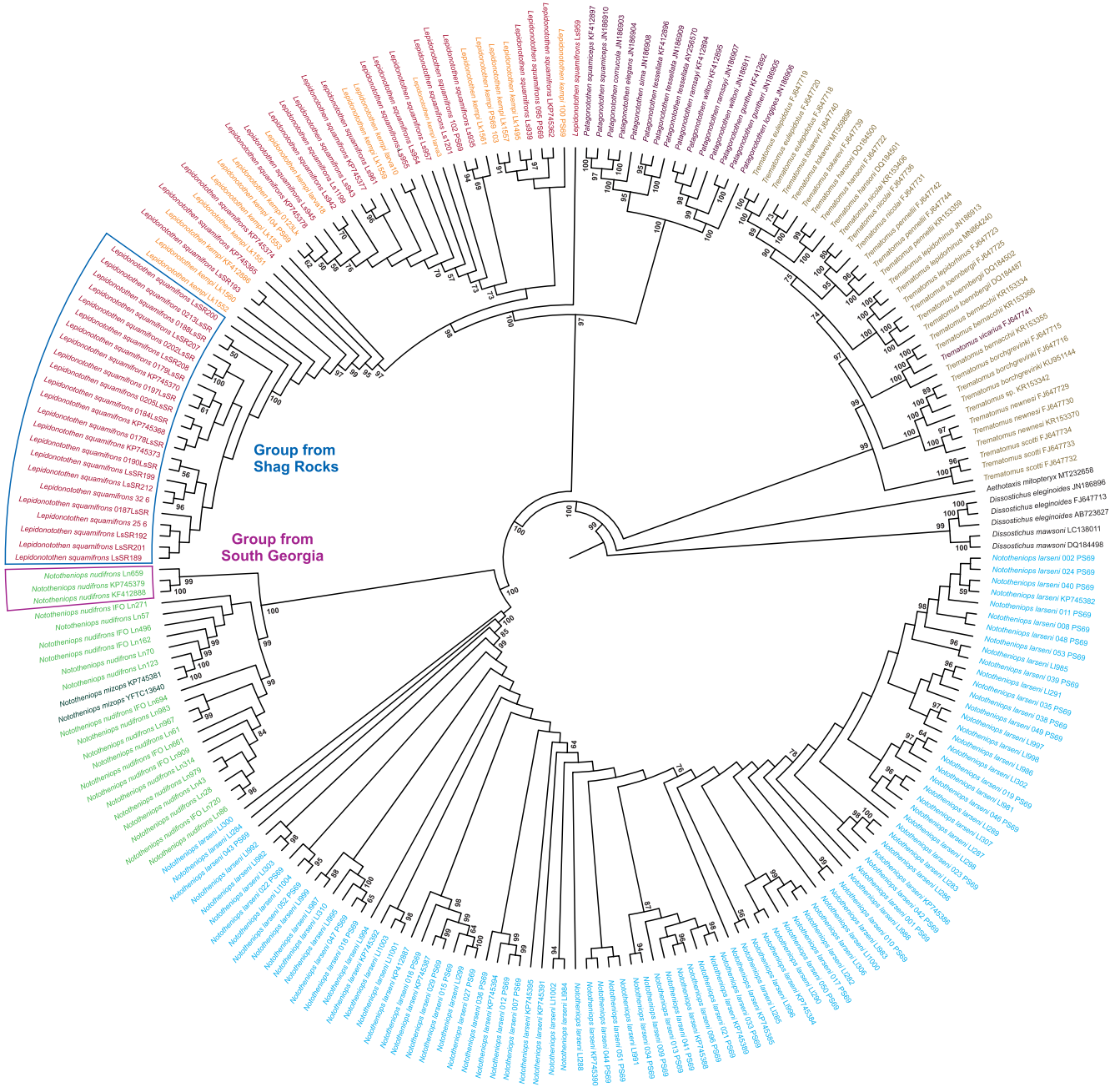

FIGURE 2 Phylogenetic tree based on the analysis of *ND2*. Maximum-likelihood tree (−ln = −11637.5643), computed with the IQ-Tree software by applying the TPM3u + F + I + G4 evolutionary model to 230 T.ND2.aln. The tree shows the phylogenetic relationships among specimens belonging to different species of Notothenioidei. Colours are used to visually indicate different species. For the sake of clarity, only the tree topology is presented. Ultrafast Bootstrap Support (10,000 replicates) to nodes is provided, expressed in percentage, for values ≥50%.

two GenBank sequences also collected at South Georgia. The other cluster contains all the remaining *N. nudifrons* sequences from our samples and two GenBank accessions assigned to *Notothenios mizops*. Within the genus *Trematomus*, sequences of *T. bernacchii* appeared paraphyletic since the clade includes one sequence of *T. vicarius* (GenBank accession number FJ647741).

Fourteen *ND2* haplotypes with 16 segregating sites were identified for individuals morphologically identified as *L. kempi* while 39 haplotypes and 43 segregating sites were found for *L. squamifrons*. The two putative species had four haplotypes in common (Figure 3a). In *N. larseni*, we found 79 haplotypes and 100 segregating sites, whereas *N. nudifrons* had 19 haplotypes with 34 segregating sites. None of these haplotypes was shared among the two *Notothenios* species (Table S1). The haplotype diversity ranged from 0.823 (in *N. nudifrons*) to 0.992 (in *N. larseni*). *Lepidonotothen kempi* and *L. squamifrons* were

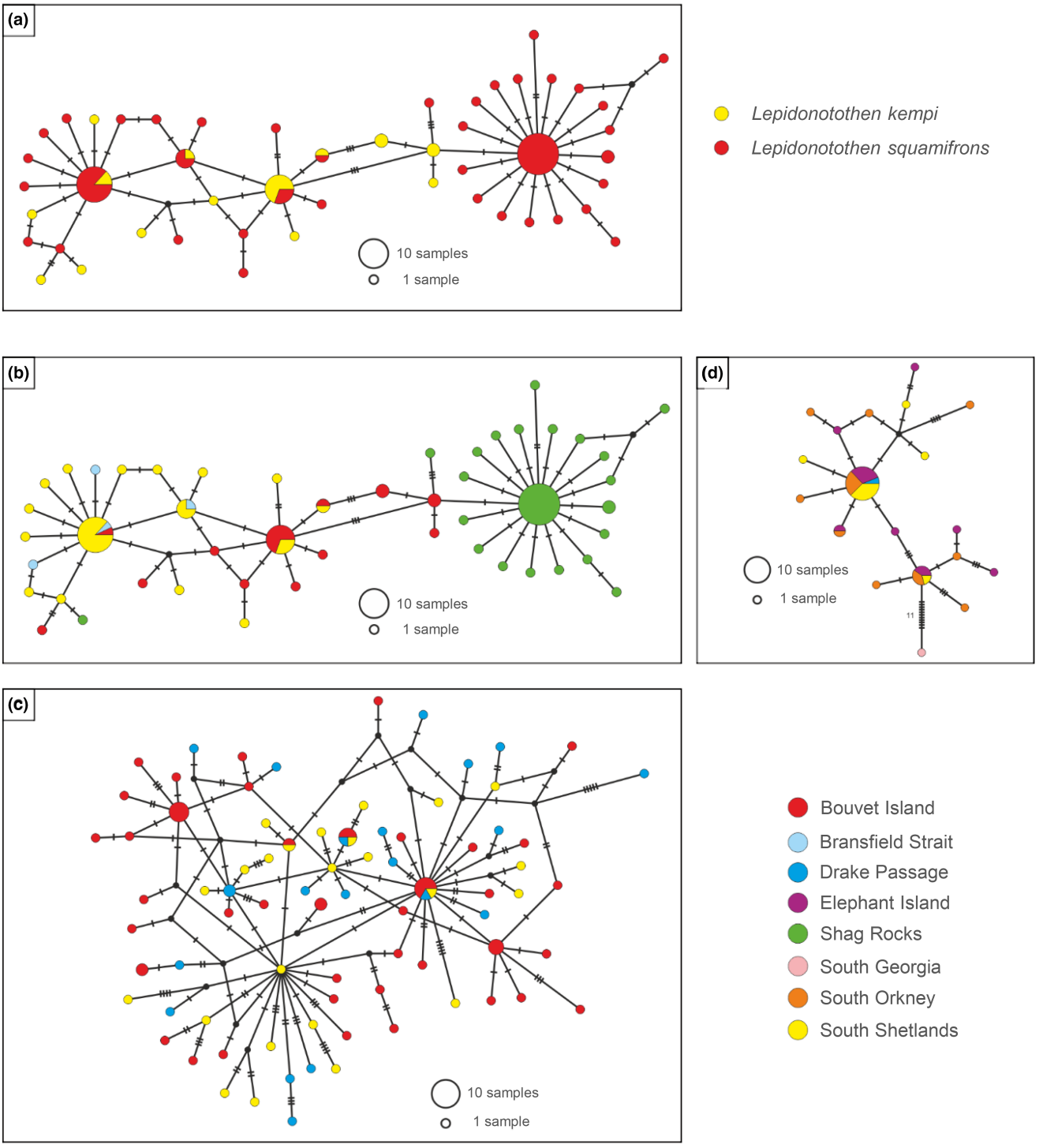

FIGURE 3 Haplotype networks based on the mitochondrial gene *ND2*. Circles represent haplotypes and diameter is proportional to haplotype frequency in the sample. Hatch marks on the edges connecting circles mark the number of mutations between haplotypes. Panel (a) shows the network only for *Lepidonotothen kempi* and *Lepidonotothen squamifrons* and the colours correspond to morphological species assignment during field sampling (yellow: *L. kempi*; red: *L. squamifrons*). Panels (b–d) show the haplotype networks for *L. kempi* and *L. squamifrons* (b), *Nototheniops larseni* (c) and *Nototheniops nudifrons* (d), coloured according to sampling locations (red: Bouvet Island; light blue: Bransfield Strait; blue: Drake Passage; purple: Elephant Island; green: Shag Rocks; pink: South Georgia; orange: South Orkney; yellow: South Shetlands).

characterized by similar values of haplotype diversity (0.905 and 0.901, respectively, Table S1). The nucleotide diversity was similar for all species (0.003 for *L. kempi*, 0.004 for *L. squamifrons* and *N. nudifrons* and 0.005 for *N. larseni*, Table S1). The species differentiation tests performed with ARLEQUIN provided F_{ST} estimates that were significant for all pairwise species comparisons (Table 2), except for the pairwise comparison of *L. kempi* with *L. squamifrons* (Table 2). The most differentiated species according to F_{ST} were *L. kempi* and *N. nudifrons* (0.975, Table 2). Tests of differentiation among geographically distinct populations within each species showed genetic homogeneity in *N. nudifrons* (Table S2) and *N. larseni* (Table S3). When separate populations were defined for *L. kempi* and *L. squamifrons*, highly and significant F_{ST} values up to 0.648 separated the *L. squamifrons* individuals from Shag Rocks from *L. squamifrons* and *L. kempi* individuals from all other localities (Table 3). Additionally, *L. kempi* from Bouvet Island was found to be significantly differentiated from *L. squamifrons* sampled at the South Shetland Islands, with an F_{ST} value of 0.127 (Table 3). When pooling samples of *L. squamifrons* and *L. kempi* by geographic site, F_{ST} values were all significant in all pairwise population comparisons, except for Bransfield Strait vs. South Shetlands and Bransfield Strait vs. Bouvet Island (Table S4). In the network of *ND2* haplotypes, individuals grouped in three clusters in agreement with the morphological species identification and the verification with BLASTN: one for *N. larseni*, one for *N. nudifrons* and a shared one for *L. squamifrons* and *L. kempi* (Figure S5). In the cluster formed by *L. squamifrons* and *L. kempi*, individuals morphologically classified as *L. kempi* did not form a separate group (Figure 3a). However, Shag Rocks individuals (morphologically identified as *L. squamifrons*) were part of a separated star-like haplotype cluster that contains only haplotypes not found elsewhere (Figure 3b). In *N. nudifrons* and *N. larseni*, no clear pattern could be detected by the geographic origin of samples (Figure 3c,d), although in *N. nudifrons*, two main clusters could be identified and the single specimen from South Georgia was clearly very divergent, as expected based on Dornburg, Federman, et al. (2016) (Figure 3d).

3.1.3 | Analysis of tRNAs

Multiple haplotypes were obtained for all tRNAs (Figures S1–S3). Since the intraspecific tRNA sequence variation was negligible, differences within species are not described here. Orthologous tRNA sequences obtained from individuals morphologically identified as *L. squamifrons* and *L. kempi* were always identical. Despite being the least variable marker, *trnA* distinguished individuals of *L. squamifrons* and *L. kempi* from all other notothenioid species in our data set. A unique base mismatch (C I A vs C – G, see Figure S4 for nomenclature used in this section) in the anticodon stem was the molecular signature of *L. squamifrons* and *L. kempi* sequences (Figures S1 and S6). The *trnA* sequences of *N. nudifrons* and *N. larseni* were identical and carried a genus-specific molecular signature in the anticodon stem where a fully compensatory base change (a simultaneous change of both nucleotides of the stem pair, in which a purine–pyrimidine pair (G-C) substitutes a pyrimidine–purine pair (T-A)) distinguishes the two *Nototheniops* species from all other taxa analysed here (Figure S1). This type of base change is rare in the stems of tRNAs because the intermediate step implies a mismatch that disrupts the secondary structure (Figure S4). The *trnM* is the most variable marker, and the four putative species are characterized by unique substitution patterns occurring on both single-strand and double-strand portions of their secondary structure (Figures S2 and S6). The *trnW* sequences carry unique substitution patterns that differentiate *L. squamifrons* and *L. kempi* jointly and *N. larseni* individually from all the other species analysed in this study (Figures S3 and S6).

3.2 | Microsatellite markers

3.2.1 | Amplification and genotyping

A total of 287 specimens were successfully genotyped for the initial panel of 20 loci (Table 1). All markers were polymorphic over the entire data set although some loci were monomorphic in one up to three species (Table S5). Loci Ca48, Ch126, Ln22268 and Ln36100 were monomorphic in *L. kempi*, whereas Ca48, Ch623, Ln22268 and Ln42233 were monomorphic in *L. squamifrons*. In *N. nudifrons*, Ch126, Ch623, Ch3603, Ln22268, Ln36100, Ln41281 and Ln42233 were monomorphic. All loci were polymorphic in *N. larseni*. The microsatellite loci had up to 19, 27, 41 and 23 alleles, with a mean allelic richness of 3.95, 4.27, 3.89 and 6.51 in *L. kempi*, *L. squamifrons*, *N. larseni* and *N. nudifrons*, respectively. Mean observed and expected (under HWE) heterozygosity were 0.23 and 0.25 in *L. kempi*, 0.22 and 0.25 in *L. squamifrons*, 0.28 and 0.3 in *N. larseni* and 0.38 and 0.45 in *N. nudifrons*. The test for HWE and the analysis with MICRO-CHECKER showed that loci Ch1968, Ch5817, Ln40551 and Ln42016 were particularly affected by Hardy–Weinberg disequilibrium or presence of null alleles (Tables S6 and S7). Therefore, we removed these loci from our data set. In addition, only individuals with at least 13 genotyped loci were kept (20% of missing data allowed). The final data set of nuclear markers contained 287 individuals genotyped for 13–16

TABLE 2 Pairwise F_{ST} values based on the mitochondrial *ND2* gene, among *Lepidonotothen* and *Nototheniops* species analysed in this study and grouped according to the morphological species identification.

	Lepidonotothen kempi	*Lepidonotothen squamifrons*	*Nototheniops larseni*	*Nototheniops nudifrons*
Lepidonotothen kempi	*	**.003**	**<.001**	**<.001**
Lepidonotothen squamifrons	**0.104**	*	**<.001**	**<.001**
Nototheniops larseni	**0.967**	**0.966**	*	**<.001**
Nototheniops nudifrons	**0.975**	**0.969**	**0.955**	*

Note: F_{ST} below the diagonal and *p* values above the diagonal. Results significant after correction for multiple tests are highlighted in bold.

TABLE 3 Pairwise F_{ST} values based on the mitochondrial *ND2* gene calculated among populations of *Lepidonotothen kempi* and *L. squamifrons,* identified following the initial morphological characterization.

	ke-BS	ke-BV	sq-SS	sq-SR
ke-BS	*	.214	.705	**<.001**
ke-BV	0.098	*	**.001**	**<.001**
sq-SS	0.000	**0.127**	*	**<.001**
sq-SR	**0.648**	**0.381**	**0.595**	*

Note: F_{ST} values are shown below the diagonal and *p*-values above the diagonal. Negative F_{ST} values were curtailed to zero. Results significant after correction for multiple tests are highlighted in bold. Sample acronyms as in Table 1.

microsatellites (data set deposited in Dryad repository https://doi.org/10.5061/dryad.wpzgmsbqv).

3.2.2 | Species assignment and population differentiation

The clustering analysis performed on the whole data set of 287 individuals of four putative species suggested that specimens could be grouped into three main clusters corresponding to the species *N. larseni*, *N. nudifrons* and the combined *L. squamifrons* and *L. kempi* (Figure 4). Within the combined *L. squamifrons* and *L. kempi* cluster, no clear grouping was evident both without the LOCPRIOR option and with the morphological identification as a prior (Figure 5, top panel). Using the sampling locations as a prior, the number of clusters that best described the subdivision of genetic variation for the group comprising *L. squamifrons* and *L. kempi* was two. One of these clusters included all individuals collected from Shag Rocks, regardless of their morphological identification, and the other contained all remaining individuals of the two species (Figure 5, bottom panel). For *N. larseni*, the clustering analysis with LOCPRIOR again suggested the presence of two clusters, of which one included all individuals from Bouvet Island and the other contained samples from South Shetlands and Drake Passage (Figure S7). Even though the best number of clusters recovered by Evanno's method for *N. nudifrons* was $K = 2$ (with LOCPRIOR) or $K = 3$ (without LOCPRIOR), the CLUMPAK plots (Figures S8 and S9) showed that every individual had an equally subdivided ancestry. When individuals are not clearly assigned to different groups, population structure is probably absent (Porras-Hurtado et al., 2013) and it is known that Evanno's method should not be strictly followed but all K with a biological meaning should be considered (Meirmans, 2015; Porras-Hurtado et al., 2013). Our results suggest that the samples of *N. nudifrons* analysed in this study likely belong to a panmictic population (but note that the data set did not include the two specimens from Drake Passage and the single individual from South Georgia sensu Dornburg, Federman, et al., 2016, Table 1). Pairwise F_{ST} estimates among species were all significant (*p* value <.001, Table 4). The least and most differentiated species pairs were *L. kempi* and *L. squamifrons* ($F_{ST} = 0.013$) and *N. nudifrons* and *L. squamifrons*, in agreement with the mitochondrial analyses. In *N. nudifrons*, no statistically significant subdivision was found (Table S8). In *N. larseni*, the population sample from Bouvet Island was significantly different from the other two samples ($F_{ST} = 0.018$, *p* value = .004 compared to Drake Passage; $F_{ST} = 0.019$, *p* value = .001 compared to South Shetlands; Table S9). Within *L. squamifrons* and *L. kempi*, either considered two species or a single pool, the sample from Shag Rocks always resulted differentiated from all geographic population samples (*p* value <.001, Tables S10 and S11). Differently from the mitochondrial evidence, the comparison between South Shetlands and Bouvet Island for *L. squamifrons* and *L. kempi* was not significant although the *p* value was relatively close to the significance threshold ($F_{ST} = 0.008$, *p* value = .076, Table 5).

4 | DISCUSSION

In this study, we aimed to verify whether *L. squamifrons* and *L. kempi* are divergent species or if they could be considered differentiated populations of the same species. We

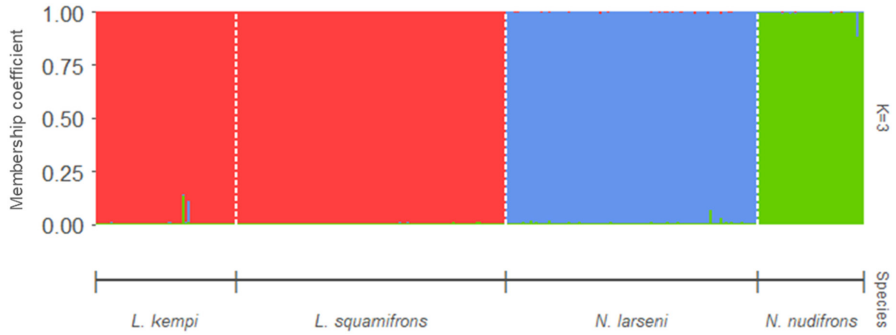

FIGURE 4 Clustering analysis of all *Lepidonotothen* and *Nototheniops* species considered in this study performed with STRUCTURE VER. 2.3.4 (Pritchard et al., 2000) for $K = 3$. Each individual is represented by a bar partitioned into K-coloured segments indicating cluster assignment. The length of the coloured segment is proportional to the estimated percentage of ancestry for each individual (membership coefficient).

analysed markers with different characteristics and mutation rates for a sample including adult and larval specimens. While *N. nudifrons* and *N. larseni*, analysed for a comparative purpose, were easily distinguished with all markers and are highly divergent (F_{ST} = 0.955 for *ND2*, Table 2; F_{ST} = 0.328 for microsatellites, Table 4), genetic distances between *L. squamifrons* and *L. kempi* were very low (F_{ST} = 0.104 for *ND2*, Table 2; F_{ST} = 0.013 for microsatellites, Table 4). All individuals of the two putative species clustered together in the phylogenetic reconstruction (Figure 2) and were undistinguishable based on tRNA sequences (Figures S1–S3). According to these results, we consider that any instance of significant differentiation in our dataset of *L. squamifrons* + *L. kempi* can be attributed to geographic genetic structure within a single species. This is supported by the genetic difference observed in microsatellite data between individuals of *L. squamifrons* and *L. kempi* sampled in Shag Rocks and those collected in the Antarctic Peninsula, Bransfield Strait and Bouvet Island (Tables 3 and 5).

A possible caveat of the study lies in the possibility of incorrect initial morphological identification of the individuals analysed here. There is no general consensus on the actual morphological distinction between *L. kempi* and *L. squamifrons* (Jones et al., 2008) and the lack of clear diagnostic traits with non-overlapping distribution of measures renders species identification challenging. In such cases, species identification often relies more on the reported geographic distribution than on morphology (e.g. the case of *Chionodraco rastrospinosus* in Schiavon et al., 2021). Thus, it may be possible that the samples analysed in this study were identified by applying different criteria based on the operator who handled the specimens during the cruise. This might have led to an incorrect grouping of individuals. However, if this were the case, only the calculation of F_{ST} genetic differentiation indices, that require a priori group definitions, would have been affected but not the phylogenetic reconstruction and clustering analyses with STRUCTURE (in its standard configuration without the use of the LOCPRIOR option).

Despite this potential caveat, our results, based on multiple markers and a large sample size, support the conclusions of Miya et al. (2016), who argued that *L. squamifrons* and *L. kempi* are more likely geographically distinct and genetically differentiated populations of the same species. Although Miya et al. (2016) analysed samples collected in the Pacific sector of the Southern Ocean and not available in our study, similar results for different areas might indicate the existence of differentiated populations at a large geographic scale, possibly circum-Antarctic. In a recent study, Ceballos et al. (2019) analysed RADseq data and sequences of *COI* obtained for the two putative species *L. squamifrons* and *L. kempi*, from Shag Rocks (five and one specimens, respectively), Bouvet Island (two specimens of *L. squamifrons*) and South Shetlands (two specimens of *L. squamifrons*). Ceballos et al. (2019) found that although *COI* did not separate the two putative species, the samples of Shag Rocks clearly clustered by species with STRUCTURE analysis of 999 SNPs. The authors suggested that *L. squamifrons* and *L. kempi* could be two different species that coexist at the Shag Rocks locality (Ceballos et al., 2019). The global genetic distance between *L. squamifrons* and *L. kempi* estimated by Ceballos et al. (2019) on the basis of 9270 RADseq loci (F_{ST} = 0.037) was found to be significant and of the same order of magnitude as our estimates obtained with 16 microsatellite markers (F_{ST} = 0.013, in agreement with mitochondrial *ND2* results). Though significant, we find that these distances are very low compared to F_{ST} values estimated between true and generally accepted different species like *N. larseni* and *N. nudifrons* (F_{ST} = 0.328 for microsatellites in our study). We cannot exclude that the differences between our results and those of Ceballos et al. (2019) are due to a lower resolution power of our data set compared to their RADseq data set. However, in our study, we used many more individuals (N = 150) compared to Ceballos

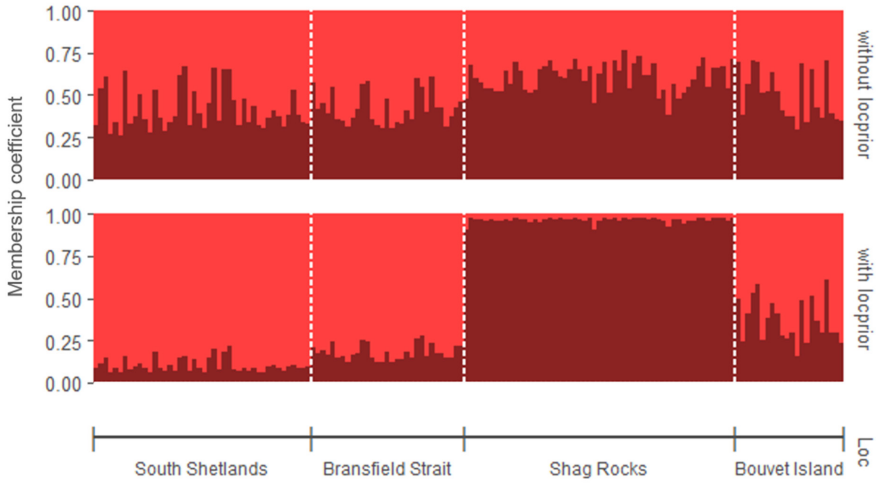

FIGURE 5 Clustering analysis performed with STRUCTURE, considering only the individuals assigned to *Lepidonotothen kempi* and *L. squamifrons* and $K = 2$. The panel on the top shows the results obtained without using the sampling location as a prior. The panel on the bottom presents the analysis using the sampling location as a prior. Each individual is represented by a bar partitioned into *K*-coloured segments indicating cluster assignment. The length of the coloured segment is proportional to the estimated percentage of ancestry for each individual (membership coefficient).

TABLE 4 Interspecific F_{ST} calculated using the panel of 16 microsatellite loci and considering the morphological species identification.

	Lepidonotothen kempi	***Lepidonotothen squamifrons***	***Lepidonotothen larseni***	***Lepidonotothen nudifrons***
Lepidonotothen kempi	*	**<.001**	**<.001**	**<.001**
Lepidonotothen squamifrons	**0.013**	*	**<.001**	**<.001**
Lepidonotothen larseni	**0.502**	**0.535**	*	**<.001**
Lepidonotothen nudifrons	**0.556**	**0.577**	**0.328**	*

Note: F_{ST} values are shown below the diagonal and *p* values above the diagonal. Negative F_{ST} values were curtailed to zero. Results significant after correction for multiple tests are highlighted in bold. Sample acronyms as in Table 1.

et al. (2019) ($N = 10$), making our results more reliable. In fact, the small sample size used by Ceballos et al. (2019) could have led to a wrong clustering by chance. Our analysis benefitted also from the inclusion of an important sample of larvae of *L. kempi*. As previously pointed out, larvae have so far been considered the life stage that most clearly differentiates the two putative species *L. squamifrons* and *L. kempi* morphologically (Efremenko, 1984; Kellermann, 1990; Koubbi et al., 1990). However, our analyses consistently showed that larvae of *L. kempi* (the U.S. AMLR sample from Bransfield Strait, 2011, Table 1) cluster with *L. squamifrons*, corroborating the notion that the two nominal species are not significantly differentiated. Nevertheless, we consider that, altogether, the results available so far point towards the presence of *L. squamifrons*/*L. kempi* individuals inhabiting the continental shelf of Shag Rocks and South Georgia that are genetically differentiated from other areas of the two putative species' distribution. Whether this implies the existence of two true different species with clearly different morphological and/or ecological traits awaits further testing following specific sampling and in situ observations.

Several lines of evidence point towards the existence of differentiated genetic pools for other marine species at Shag Rocks and South Georgia (Ceballos et al., 2019; Damerau, Matschiner, et al., 2014; Damerau, Salzburger, & Hanel, 2014; Kuhn & Gaffney, 2006; Matschiner et al., 2009; Strugnell et al., 2012). Four notothenioid species are considered endemic to South Georgia and the South Sandwich Islands (Duhamel et al., 2014). In addition, the Shag Rocks population of *Patagonotothen guntheri*, which was described as a separate species, *P. shagensis*, by Balushkin (2000), was confirmed to represent a separate unit (but not necessarily a species) in the study of Ceballos et al. (2019). Moreover, as also supported in our study by mitochondrial evidence (Figures 2 and 3; Figure S5), Dornburg, Federman, et al. (2016) identified an additional cryptic species in *N. nudifrons* based on the genetic variability of *ND2* and two nuclear loci. In this case, all markers clearly separated two geographic clusters, one

TABLE 5 Intraspecific F_{ST} calculated for populations of *Lepidonotothen kempi* and *Lepidonotothen squamifrons* using the panel of 16 microsatellite loci and considering the morphological species identification.

	ke-BS	ke-BV	sq-SS	sq-SR
ke-BS	*	.512	.560	**<.001**
ke-BV	0.000	*	.076	**<.001**
sq-SS	0.000	0.008	*	**<.001**
sq-SR	**0.050**	**0.047**	**0.059**	*

Note: F_{ST} values are shown below the diagonal and *p* values above the diagonal. Negative F_{ST} values were curtailed to zero. Significant results after correction for multiple tests are highlighted in bold. Sample acronyms are as in Table 1.

in the Antarctic Peninsula and South Shetland Islands (*N.* cf. *nudifrons*) and one in South Georgia and South Sandwich Islands (*N. nudifrons*). Despite the clear genetic difference, no morphological differences distinguish the two cryptic species (Dornburg, Federman, et al., 2016).

South Georgia and Shag Rocks are considered a distinct biogeographic province and a centre of diversification and speciation (Andriashev, 1965; Clarke, 2008; Hedgpeth, 1970; Linse et al., 2007). This may be explained by their position close to the northern limit of the circum-Antarctic current (ACC; Maldonado et al., 2003). The clockwise flowing ACC is the dominant oceanographic feature along the Antarctic Peninsula and the Scotia Sea. It has often been considered the foremost mechanism for maintaining a degree of connectivity sufficient to prevent genetic differentiation among populations located on the continental shelves in the Scotia Sea and at circum-Antarctic scale (e.g. Díaz et al., 2018; Matschiner et al., 2009; Papetti et al., 2012). However, after passing the Drake Passage between South America and the north-western Antarctic Peninsula (NWAP), the ACC splits into two components: the first of these flows towards the Weddell Sea, and the second enters the western South Atlantic by flowing north between the Falkland Islands and South Georgia through a gap in the North Scotia Ridge known as the 'Shag Rocks Passage' (detailed in Maldonado et al., 2003). The bifurcated path of the ACC in the Scotia Sea may therefore contribute to maintain disjunct geographic species distributions and some degree of isolation of the South Georgia and Shag Rocks marine biodiversity, despite the potential for dispersal owing to long larval phases of notothenioids (e.g. Papetti et al., 2012; Schiavon et al., 2021).

The peculiarity of the biodiversity found on the continental shelves of South Georgia and Shag Rocks may also be explained by the paleoclimatic history of isolation during glacial intervals of this area (Clarke & Crame, 1992; Díaz et al., 2018). The successive ice advances and retreats over the Antarctic and sub-Antarctic shelf areas had a significant impact on marine diversity by generating conditions of repeated isolation and reconnection between faunas (Díaz et al., 2018). The glacial cycles are suggested to be responsible for the diversification of several other marine Antarctic groups like nemerteans, molluscs, echinoderms and crustaceans (Díaz et al., 2018). The role of sub-Antarctic islands as refugia has been indicated by low levels of genetic diversity at these islands, and by star-like haplotype networks with short branches for every single island (Allcock & Strugnell, 2012; Díaz et al., 2018; Provan & Bennett, 2008; Thatje et al., 2005). These refugia usually harbour high numbers of private haplotypes that presumably originated after the colonization process (Allcock & Strugnell, 2012; Díaz et al., 2018; Provan & Bennett, 2008; Thatje et al., 2005). The model by Allcock & Strugnell (2012) posits that the rapid extinction of most populations occurred during the Last Glacial Maximum (LGM, 14–20 k years ago; Anderson et al., 2002), in parallel with a decrease in haplotype diversity in the surviving populations when one or few alleles become fixed by genetic drift. Once the glacial maximum has passed and the ice sheets retreat, individuals rapidly recolonize the other parts of the continental shelf around each island area from refugia and the population expansion generates an increase in genetic diversity (Allcock & Strugnell, 2012; Díaz et al., 2018). In *L. squamifrons* + *L. kempi*, the role of sub-Antarctic islands as refugia is clearly visible for Shag Rocks and partially for the South Shetlands and Bouvet Island (Figure 3). For each of the three areas (South Shetlands, Bouvet Island and Shag Rocks, Figure 3), we detected a dominant haplotype and numerous rare haplotypes differing by one or two substitutions, congruent with the hypothesis of several shelf refugia, one for each island area (Allcock & Strugnell, 2012; Díaz et al., 2018). This pattern is not visible in our data set of *N. nudifrons* owing to the limited geographic sampling, but it was clearly shown for that species by Dornburg, Federman, et al. (2016). The process of cryptic speciation in *N. nudifrons* may also have been facilitated by the depth distribution of the species: *L. squamifrons*/*L. kempi* can be found deeper (up to 570 m for *L. squamifrons* and 900 m for *L. kempi* following Gon & Heemstra, 1990) than *N. nudifrons* (up to 350 m, Gon & Heemstra, 1990). Therefore, differently from *L. squamifrons*/*L. kempi*, it is possible that the limited ability of adult *N. nudifrons* to actively swim across areas of deep sea between distant islands, combined with the circulation pattern that prevents sufficient larval dispersal owing to the ACC bifurcation, may have enabled the diversification of the two cryptic species *N. nudifrons* and *N.* cf. *nudifrons*

under conditions of geographic isolation (Dornburg, Federman, et al., 2016).

The pattern of differentiation we found for *N. larseni* is also in agreement with previous studies. Our analysis of *ND2* sequences suggested a homogeneous distribution of haplotypic variation between the Antarctic Peninsula, Scotia Arc and Bouvet Island, matching the results of Jones et al. (2008) with the same marker and a comparable geographic sampling (Jones et al., 2008). Similarly, based on D-loop sequences, Deli Antoni et al. (2019) found no differentiation in *N. larseni* (Deli Antoni et al., 2019) in the Scotia Arc and the South Shetland Islands. On the other hand, our analysis of the nuclear variability with microsatellites indicated that the population at Bouvet Island is genetically different from the other two at the South Shetlands and Drake Passage. This is in agreement with a study by Damerau, Salzburger, and Hanel (2014) who found population differentiation in *N. larseni* collected from Bouvet Island and the South Orkney Islands based on a mitochondrial marker (*Cytb*) and microsatellites. However, the high number of *ND2* haplotypes found in our study in *N. larseni* (Figure 3) suggests that a different mechanism could have shaped the genetic variability of this species compared to *L. squamifrons*/*L. kempi* and *N. nudifrons*/*N.* cf. *nudifrons*. Since *N. larseni* occurs at depths up to 550 m (Gon & Heemstra, 1990), one possible hypothesis could be that this species has been able to maintain large populations during glacial periods by persisting in a large distribution area, possibly in the deeper part of its bathymetric distributions in less ice-impacted areas, from which it may then have recolonized the shelf during the deglaciation process (Díaz et al., 2018; Lau et al., 2020). In addition, looking at the presence of functional genes and dosage of antifreeze glycoproteins (AFGPs) – the key evolutionary innovation that enabled notothenioids to survive in Antarctic waters (DeVries & Wohlschlag, 1969) – in *Nototheniops* and *Lepidonotothen* could provide further insight into the evolutionary history and distribution of these species. Miya et al. (2016) observed that *L. squamifrons*, unlike *N. nudifrons* and *N. larseni*, does not synthesize AFGPs. The authors hypothesized that, owing to a low- and sub-Antarctic distribution, AFGPs genes in the *L. squamifrons* ancestor have gone through a process of pseudogenization and eventual gene loss in the absence of selective pressures for trait maintenance, resulting in an ineffectual antifreeze protein and non-detectable AFGP activity (Miya et al., 2016). However, *Nototheniops* and *Lepidonotothen* have largely overlapping distributions and are found both in cold and sub-Antarctic waters. This suggests that *L. squamifrons* may apply other strategies, like occupying warmer waters, to avoid freezing and that *N. nudifrons* and *N. larseni*, still carrying functional AFGPs genes, are able to regulate the synthesis of antifreeze proteins in response to different environmental temperatures. The increasing availability of high-quality and well-annotated notothenioid genomes (Bista et al., 2022) will enable to test these hypotheses and to verify whether any population genetic difference may be driven by AFGP functionality.

5 | CONCLUSIONS

In this study, we found that individuals morphologically assigned to two putative species, *L. squamifrons* and *L. kempi*, are genetically similar based on multiple mitochondrial and nuclear markers. Therefore, we suggest that the two species could be considered synonymous, with priority for the taxon name *L. squamifrons*. Nonetheless, we identified differentiated populations of both *L. squamifrons* and *N. larseni*. The differentiated population of *L. squamifrons* from Shag Rocks and *N. larseni* from Bouvet Island are additional examples of a recurrent pattern found in notothenioids, where isolated shelf areas appear prone to promote genetic differentiation (Ceballos et al., 2019; Damerau, Matschiner, et al., 2014; Damerau, Salzburger, & Hanel, 2014; Deli Antoni et al., 2019; Matschiner et al., 2009).

For all species analysed in this study, the distribution of genetic variation appears to have been shaped by climatic history, large geographic distances and the local circulation pattern between the Antarctic Peninsula and remote islands like Bouvet and Shag Rocks. The observed mitochondrial variation suggests that the species analysed in this study were strongly impacted by the Pleistocene Antarctic climatic history while the microsatellite data indicate that present differentiation may be driven by local circulation and geographic distance.

The case of *Lepidonotothen* and *Nototheniops* species also highlights the uniqueness of remote islands of the Scotia Arc as centres of diversification. This study also supports the need to pursue the continuous protection of these islands (e.g. South Georgia and South Sandwich Islands Marine Protected Area, SGSSI MPA; Gregory et al., 2014; Haywood, 2012).

ACKNOWLEDGEMENTS

The authors would like to thank Nils Koschnick (Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, AWI, Bremerhaven, Germany) and the ship crew for their help in collecting samples onboard the R/V Polarstern and during the U.S. AMLR cruise. The authors are grateful to Santiago Ceballos for his insightful comments on the manuscript. We also thank Jutta Jürgens (AWI) for her invaluable help with the laboratory work.

FUNDING INFORMATION

This research was supported by the European Marie Curie Project 'Polarexpress' (grant no. 622320) and the University of Padova BIRD (grant no. 164793 to Chiara Papetti). Lorenzo Zane acknowledges support from the Italian National Programme of Antarctic Research (PNRA) Project 2016_00307. Mario La Mesa acknowledges support from the Italian National Programme of Antarctic Research (PNRA) Project 2009/A1.07. This paper is a contribution to the research programme 'Polar regions and coasts in a changing earth system' (PACES I/II) of the Alfred Wegener Institute for Polar and Marine Research.

ORCID

Chiara Papetti https://orcid.org/0000-0002-4567-459X

REFERENCES

Allcock, A. L., & Strugnell, J. M. (2012). Southern Ocean diversity: New paradigms from molecular ecology. *Trends in Ecology & Evolution*, *27*, 520–528. https://doi.org/10.1016/j.tree.2012.05.009

Anderson, J. B., Shipp, S. S., Lowe, A. L., Wellner, J. S., & Mosola, A. B. (2002). The Antarctic ice sheet during the last glacial maximum and its subsequent retreat history: A review. *Quaternary Science Reviews*, *21*, 49–70. https://doi.org/10.1016/S0277-3791(01)00083-X

Andriashev, A. P. (1965). A general review of the antarctic fish fauna. In J. van Mieghem & P. van Oye (Eds.), *Biogeography and ecology in Antarctica. Monographiae Biologicae* (Vol. *15*). Springer. https://doi.org/10.1007/978-94-015-7204-0_15

Balushkin, A. V. (1976). A review of the "*larseni*" group of the genus *Notothenia* rich. *Voprosy Ikhtiologii*, *16*, 3–15 (In Russian; English translation in *Journal of Ichthyology* 16: 1–12).

Balushkin, A. V. (2000). Morphology, classification, and evolution of notothenioid fishes of the Southern Ocean (Notothenioidei, Perciformes). *Journal of Ichthyology*, *40*, S74–S109.

Bista, I., Wood, J. M. D., Desvignes, T., McCarthy, S. A., Matschiner, M., Ning, Z., Tracey, A., Torrance, J., Sims, Y., Chow, W., Smith, M., Oliver, K., Haggerty, L., Salzburger, W., Postlethwait, J. Q., Howe, K., Clark, M. S., Detrich III, W. H., Cheng, C. H. H., … Durbin, R. (2022). Genomics of cold adaptations in the Antarctic notothenioid fish radiation. *bioRxiv*, https://doi.org/10.1101/2022.06.08.494096

Ceballos, S. G., Roesti, M., Matschiner, M., Fernández, D. A., Damerau, M., Hanel, R., & Salzburger, W. (2019). Phylogenomics of an extra-Antarctic notothenioid radiation reveals a previously unrecognized lineage and diffuse species boundaries. *BMC Evolutionary Biology*, *19*, 13. https://doi.org/10.1186/s12862-019-1345-z

Clarke, A. (2008). Antarctic marine benthic diversity: Patterns and processes. *Journal of Experimental Marine Biology and Ecology*, *366*, 48–55. https://doi.org/10.1016/j.jembe.2008.07.008

Clarke, A., & Crame, J. A. (1992). The Southern Ocean benthic fauna and climate change: A historical perspective. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *338*, 299–309. https://doi.org/10.1098/rstb.1992.0150

Clement, M., Snell, Q., Walke, P., Posada, D., & Crandall, K. (2002). TCS: estimating gene genealogies. *Proceedings - International Parallel and Distributed Processing Symposium, IPDPS*, *2002*, 184. https://doi.org/10.1109/IPDPS.2002.1016585

Dambach, J., Raupach, M. J., Leese, F., Schwarzer, J., & Engler, J. O. (2016). Ocean currents determine functional connectivity in an Antarctic deep-sea shrimp. *Marine Ecology*, *37*, 1336–1344. https://doi.org/10.1111/maec.12343

Damerau, M., Matschiner, M., Salzburger, W., & Hanel, R. (2014). Population divergences despite long pelagic larval stages: Lessons from crocodile icefishes (Channichthyidae). *Molecular Ecology*, *23*, 284–299. https://doi.org/10.1111/mec.12612

Damerau, M., Salzburger, W., & Hanel, R. (2014). Population genetic structure of *Lepidonotothen larseni* revisited: Cyb and microsatellites suggest limited connectivity in the Southern Ocean. *Marine Ecology Progress Series*, *517*, 251–263. https://doi.org/10.3354/meps11061

De Queiroz, K. (2007). Species concepts and species delimitation. *Systematic Biology*, *56*, 879–886. https://doi.org/10.1080/10635150701701083

Deli Antoni, M. Y., Delpiani, S. M., González-Castro, M., Blasina, G. E., Spath, M. C., Depiani, G. E., Ashikaga, F. Y., Cruz, V. P., Oliveira, C., & de Astarloa, J. M. D. (2019). Comparative populational study of *Lepidonotothen larseni* and *L. nudifrons* (Teleostei: Nototheniidae) from the Antarctic peninsula and the South Shetland Islands, Antarctica. *Polar Biology*, *42*, 1537–1547. https://doi.org/10.1007/s00300-019-02540-1

DeVries, A. L., & Wohlschlag, D. E. (1969). Freezing resistance in some Antarctic fishes. *Science*, *163*, 1073–1075. https://doi.org/10.1126/science.163.3871.1073

Díaz, A., Gérard, K., González-Wevar, C., Maturana, C., Féral, J.-P., David, B., Saucède, T., & Poulin, E. (2018). Genetic structure and demographic inference of the regular sea urchin *Sterechinus neumayeri* (Meissner, 1900) in the Southern Ocean: The role of the last glaciation. *PLoS One*, *13*, e0197611. https://doi.org/10.1371/journal.pone.0197611

Dobzhansky, T. (1935). A critique of the species concept in biology. *Philosophy of Science*, *2*, 344–355. https://doi.org/10.1086/286379

Dornburg, A., Eytan, R. I., Federman, S., Pennington, J. N., Stewart, A. L., Jones, C. D., & Near, T. J. (2016). Molecular data support the existence of two species of the Antarctic fish genus *Cryodraco* (Channichthyidae). *Polar Biology*, *39*, 1369–1379. https://doi.org/10.1007/s00300-015-1859-9

Dornburg, A., Federman, S., Eytan, R. I., & Near, T. J. (2016). Cryptic species diversity in sub-Antarctic islands: A case study of *Lepidonotothen*. *Molecular Phylogenetics and Evolution*, *104*, 32–43. https://doi.org/10.1016/j.ympev.2016.07.013

Dornburg, A., Federman, S., Lamb, A. D., Jones, C. D., & Near, T. J. (2017). Cradles and museums of Antarctic teleost biodiversity. *Nature Ecology & Evolution*, *1*, 1379–1384. https://doi.org/10.1038/s41559-017-0239-y

Duhamel, G., Hulley, P.-A., Causse, R., Koubbi, P., Vacchi, M., Pruvost, P., Vigetta, S., Irisson, J.-O., Mormède, S., Belchier, M., Dettai, A., Detrich, H. W., Gutt, J., Jones, C. D., Kock, K.-H., Lopez Abellan, L. J., Dettai, A., & Van de Putte, A. P. (2014). Biogeographic patterns of fish. In C. D. Broyer & P. Koubbi

(Eds.), *Biogeographic atlas of the Southern Ocean* (pp. 327–362). Scientific Committee on Antarctic Research.

Earl, D. A., & vonHoldt, B. M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, *4*, 359–361. https://doi.org/10.1007/s12686-011-9548-7

Eastman, J. T. (2020). The buoyancy-based biotope axis of the evolutionary radiation of Antarctic cryonotothenioid fishes. *Polar Biology*, *43*, 1217–1231. https://doi.org/10.1007/s00300-020-02702-6

Eastman, J. T., & Eakin, R. R. (2021). Checklist of the species of notothenioid fishes. *Antarctic Science*, *33*, 273–280. https://doi.org/10.1017/S0954102020000632

Edgar, R. C. (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, *32*, 1792–1797. https://doi.org/10.1093/nar/gkh340

Efremenko, V. N. (1984). Larvae of the family Nototheniidae from the Soctia Sea. *Journal of Ichthyology*, *24*, 34–43.

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, *14*, 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and windows. *Molecular Ecology Resources*, *10*, 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Francis, R. M. (2017). Pophelper: An R package and web app to analyse and visualize population structure. *Molecular Ecology Resources*, *17*, 27–32. https://doi.org/10.1111/1755-0998.12509

Glez-Peña, D., Gómez-Blanco, D., Reboiro-Jato, M., Fdez-Riverola, F., & Posada, D. (2010). ALTER: Program-oriented conversion of DNA and protein alignments. *Nucleic Acids Research*, *38*, W14–W18. https://doi.org/10.1093/nar/gkq321

Gon, O., & Heemstra, P. C. (1990). *Fishes of the Southern Ocean*. J.L.B. Smith Institute of Ichthyology.

Gregory, S., Brown, J., & Belchier, M. (2014). Ecology and distribution of the grey notothen, *Lepidonotothen squamifrons*, around South Georgia and shag rocks, Southern Ocean. *Antarctic Science*, *26*, 239–249. https://doi.org/10.1017/S0954102013000667

Haye, P. A., Segovia, N. I., Muñoz-Herrera, N. C., Gálvez, F. E., Martínez, A., Meynard, A., Pardo-Gandarillas, M. C., Poulin, E., & Faugeron, S. (2014). Phylogeographic structure in benthic marine invertebrates of the Southeast Pacific coast of Chile with differing dispersal potential. *PLoS One*, *9*, e88613. https://doi.org/10.1371/journal.pone.0088613

Haywood, N. R. (2012). *Wildlife & protected areas. Marine Protected Areas Order 2012 of the Government of South Georgia & the South Sandwich Islands, Gazette 1 (29th February 2012)*. 1–8. https://www.gov.gs/docsarchive/legislation/

Hedgpeth, J. W. (1970). Marine biogeography of the Antarctic regions. In M. W. Holdgate (Ed.), *Antarctic ecology* (pp. 97–104). Academic Press Inc.

Hoang, D. T., Chernomor, O., von Haeseler, A., Minh, B. Q., & Vinh, L. S. (2018). UFBoot2: Improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution*, *35*, 518–522. https://doi.org/10.1093/molbev/msx281

Hubisiz, M. J., Falush, D., Stephens, M., & Pritchard, J. K. (2009). Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources*, *9*, 1322–1332. https://doi.org/10.1111/j.1755-0998.2009.02591.x

Jones, C. D., Eric Anderson, M., Balushkin, A. V., Duhamel, G., Eakin, R. R., Eastman, J. T., Kuhn, K. L., Lecointre, G., Near, T. J., North, A. W., Stein, D. L., Vacchi, M., & William Detrich, H., III. (2008). Diversity, relative abundance, new locality records and population structure of Antarctic demersal fishes from the northern Scotia Arc islands and Bouvetøya. *Polar Biology*, *31*, 1481–1497. https://doi.org/10.1007/s00300-008-0489-x

Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., von Haeseler, A., & Jermiin, L. S. (2017). ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nature Methods*, *14*, 587–589. https://doi.org/10.1038/nmeth.4285

Kellermann, A. (1990). Catalogue of early life stages of Antarctic notothenioid fishes. *Berichte zür Polarforschung*, *67*, 45–136.

Kock, K. H. (1992). *Antarctic fish and fisheries*. Cambridge University Press.

Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., & Mayrose, I. (2015). Clumpak: A program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources*, *15*, 1179–1191. https://doi.org/10.1111/1755-0998.12387

Koubbi, P., Duhamel, G., & Camus, P. (1990). Early life stages of Notothenioidei from the Kerguelen Islands. *Cybium: International Journal of Ichthyology*, *14*, 225–250.

Kuhn, K. L., & Gaffney, P. M. (2006). Preliminary assessment of population structure in the mackerel icefish (*Champsocephalus gunnari*). *Polar Biology*, *29*, 927–935. https://doi.org/10.1007/s00300-006-0134-5

La Mesa, M., Riginella, E., Catalano, B., Jones, C. D., & Mazzoldi, C. (2017). Maternal contribution to spawning and early life-history strategies of the genus *Lepidonotothen* (Nototheniidae, Perciformes) along the southern scotia arc. *Polar Biology*, *40*, 1441–1450. https://doi.org/10.1007/s00300-016-2068-x

Lau, S. C. Y., Wilson, N. G., Silva, C. N. S., & Strugnell, J. M. (2020). Detecting glacial refugia in the Southern Ocean. *Ecography*, *43*, 1639–1656. https://doi.org/10.1111/ecog.04951

Leigh, J. W., & Bryant, D. (2015). POPART: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, *6*, 1110–1116. https://doi.org/10.1111/2041-210X.12410

Linse, K., Cope, T., Lörz, A.-N., & Sands, C. (2007). Is the Scotia Sea a Centre of Antarctic marine diversification? Some evidence of cryptic speciation in the circum-Antarctic bivalve *Lissarca notorcadensis* (Arcoidea: Philobryidae). *Polar Biology*, *30*, 1059–1068. https://doi.org/10.1007/s00300-007-0265-3

Lonnberg, E. (1905). The fishes of the Swedish South polar expedition. *Wissenschaftliche Ergebnisse der Schwedischen Südpolar-Expedition*, *5*, 1–69.

Maldonado, A., Barnolas, A., Bohoyo, F., Galindo-Zaldívar, J., Hernández-Molina, J., Lobo, F., Rodrìguez-Fernández, J., Somoza, L., & Vázquez, J. T. (2003). Contourite deposits in the central Scotia Sea: The importance of the Antarctic circumpolar current and the Weddell gyre flows. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *198*, 187–221. https://doi.org/10.1016/S0031-0182(03)00401-2

Matschiner, M., Colombo, M., Damerau, M., Ceballos, S., Hanel, R., & Salzburger, W. (2015). The adaptive radiation of notothenioid fishes in the waters of Antarctica. In T. Rüdiger, M. Martin, & P. Martin (Eds.), *Extremophile fishes: Ecology, evolution, and*

physiology of Teleosts in extreme environments (pp. 35–57). Springer International Publishing.

Matschiner, M., Hanel, R., & Salzburger, W. (2009). Gene flow by larval dispersal in the Antarctic notothenioid fish *Gobionotothen gibberifrons*. *Molecular Ecology*, *18*, 2574–2587. https://doi.org/10.1111/j.1365-294X.2009.04220.x

Meirmans, P. G. (2015). Seven common mistakes in population genetics and how to avoid them. *Molecular Ecology*, *24*, 3223–3231. https://doi.org/10.1111/mec.13243

Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., von Haeseler, A., & Lanfear, R. (2020). IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution*, *37*, 1530–1534. https://doi.org/10.1093/molbev/msaa015

Miya, T., Gon, O., Mwale, M., & Poulin, E. (2016). Molecular systematics and taxonomic status of three latitudinally widespread notothenиid (Perciformes: Notothenioidei) fishes from the Southern Ocean. *Zootaxa*, *4061*, 381–396. https://doi.org/10.11646/zootaxa.4061.4.4

Moffat, C., & Meredith, M. (2018). Shelf–ocean exchange and hydrography west of the Antarctic peninsula: A review. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, *376*, 20170164. https://doi.org/10.1098/rsta.2017.0164

Montelli, S., Peruffo, A., Patarnello, T., Cozzi, B., & Negrisolo, E. (2016). Back to water: Signature of adaptive evolution in cetacean mitochondrial tRNAs. *PLoS One*, *11*, e0158129. https://doi.org/10.1371/journal.pone.0158129

NCBI Resource Coordinators. (2018). Database resources of the National Center for biotechnology information. *Nucleic Acids Research*, *46*, D8–D13. https://doi.org/10.1093/nar/gkx1095

Near, T. J., Dornburg, A., Kuhn, K. L., Eastman, J. T., Pennington, J. N., Patarnello, T., Zane, L., Fernández, D. A., & Jones, C. D. (2012). Ancient climate change, antifreeze, and the evolutionary diversification of Antarctic fishes. *Proceedings of the National Academy of Sciences of the United States of America*, *109*, 3434–3439. https://doi.org/10.1073/pnas.1115169109

Near, T. J., MacGuigan, D. J., Parker, E., Struthers, C. D., Jones, C. D., & Dornburg, A. (2018). Phylogenetic analysis of Antarctic notothenioids illuminates the utility of RADseq for resolving Cenozoic adaptive radiations. *Molecular Phylogenetics and Evolution*, *129*, 268–279. https://doi.org/10.1016/j.ympev.2018.09.001

Papetti, C., Babbucci, M., Dettai, A., Basso, A., Lucassen, M., Harms, L., Bonillo, C., Heindler, F. M., Patarnello, T., & Negrisolo, E. (2021). Not frozen in the ice: Large and dynamic rearrangements in the mitochondrial genomes of the Antarctic fish. *Genome Biology and Evolution*, *13*, evab017. https://doi.org/10.1093/gbe/evab017

Papetti, C., Pujolar, J. M., Mezzavilla, M., La Mesa, M., Rock, J., Zane, L., & Patarnello, T. (2012). Population genetic structure and gene flow patterns between populations of the Antarctic icefish *Chionodraco rastrospinosus*. *Journal of Biogeography*, *39*, 1361–1372. https://doi.org/10.1111/j.1365-2699.2011.02682.x

Parker, E., Dornburg, A., Struthers, C. D., Jones, C. D., & Near, T. J. (2021). Phylogenomic species delimitation dramatically reduces species diversity in an Antarctic adaptive radiation. *Systematic Biology*, *71*, 58–77. https://doi.org/10.1093/sysbio/syab057

Pebesma, E. (2018). Simple features for R: Standardized support for spatial vector data. *The R Journal*, *10*, 439–446. https://doi.org/10.32614/RJ-2018-009

Porras-Hurtado, L., Ruiz, Y., Santos, C., Phillips, C., Carracedo, Á., & Lareu, M. V. (2013). An overview of STRUCTURE: Applications, parameter settings, and supporting software. *Frontiers in Genetics*, *4*, 98. https://doi.org/10.3389/fgene.2013.00098

Poulsen, J. Y., Byrkjedal, I., Willassen, E., Rees, D., Takeshima, H., Satoh, T. P., Shinohara, G., Nishida, M., & Miya, M. (2013). Mitogenomic sequences and evidence from unique gene rearrangements corroborate evolutionary relationships of myctophiformes (Neoteleostei). *BMC Evolutionary Biology*, *13*, 111. https://doi.org/10.1186/1471-2148-13-111

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*, 945–959. https://doi.org/10.1093/genetics/155.2.945

Provan, J., & Bennett, K. (2008). Phylogeographic insights into cryptic glacial refugia. *Trends in Ecology & Evolution*, *23*, 564–571. https://doi.org/10.1016/j.tree.2008.06.010

Raupach, M. J., Thatje, S., Dambach, J., Rehm, P., Misof, B., & Leese, F. (2010). Genetic homogeneity and circum-Antarctic distribution of two benthic shrimp species of the Southern Ocean, *Chorismus antarcticus* and *Nematocarcinus lanceopes*. *Marine Biology*, *157*, 1783–1797. https://doi.org/10.1007/s00227-010-1451-3

Rozas, J., Ferrer-Mata, A., Sanchez-DelBarrio, J. C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S. E., & Sanchez-Gracia, A. (2017). DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular Biology and Evolution*, *34*, 3299–3302. https://doi.org/10.1093/molbev/msx248

Schiavon, L., Dulière, V., La Mesa, M., Marino, I. A. M., Codogno, G., Riginella, E., Battistotti, A., Lucassen, E., Zane, L., & Papetti, C. (2021). Species distribution, hybridization and connectivity in the genus *Chionodraco*: Unveiling unknown icefish diversity in Antarctica. *Diversity and Distributions*, *27*, 766–783. https://doi.org/10.1111/ddi.13249

Schneppenheim, R., Kock, K. H., Duhamel, G., & Janssen, G. (1994). On the taxonomy of the *Lepidonotothen squamifrons* group (Pisces, Perciformes, Notothenioidei). *Archive of Fishery and Marine Research*, *422*, 137–148.

South, A. (2017). *rnaturalearthdata: World Vector Map Data from Natural Earth Used in "rnaturalearth"*. https://cran.r-project.org/package=rnaturalearthdata

Strugnell, J. M., Watts, P. C., Smith, P. J., & Allcock, A. L. (2012). Persistent genetic signatures of historic climatic events in an Antarctic octopus. *Molecular Ecology*, *21*, 2775–2787. https://doi.org/10.1111/j.1365-294X.2012.05572.x

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, *28*, 2731–2739. https://doi.org/10.1093/molbev/msr121

Thatje, S., Hillenbrand, C. D., & Larter, R. (2005). On the origin of Antarctic marine benthic community structure. *Trends in Ecology & Evolution*, *20*, 534–540. https://doi.org/10.1016/j.tree.2005.07.010

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution*, *38*, 1358–1370. https://doi.org/10.1111/j.1558-5646.1984.tb05657.x

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. https://ggplot2.tidyverse.org

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Schiavon, L., Negrisolo, E., Battistotti, A., Lucassen, M., Damerau, M., Harms, L., Riginella, E., Matschiner, M., Zane, L., La Mesa, M., & Papetti, C. (2023). Species identification and population genetics of the Antarctic fish genera *Lepidonotothen* and *Nototheniops* (Perciformes, Notothenioidei). *Zoologica Scripta*, *52*, 136–153. https://doi.org/10.1111/zsc.12580