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Molecular ecology of the sleeper shark subgenus *Somniosus (Somniosus)* reveals genetic homogeneity within species and lack of support for *S. antarcticus*

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Abstract

Inferences made from molecular data support regional stock assessment goals by providing insights into the genetic population dynamics of enigmatic species. Population genomics metrics, such as genetic diversity and population connectivity, serve as useful proxies for species health and stability. Sleeper sharks (genus *Somniosus*) are ecologically important deep-sea predators, estimated to reach ages of 250 to 300 yr and taking decades to reach sexual maturity. The subgenus *Somniosus (Somniosus)* is comprised of 3 species: *S. pacificus*, *S. microcephalus*, and *S. antarcticus*. Given the life history strategy of somniosids, they are vulnerable to overfishing and population declines. Further, data to assess the stocks of these species are limited. To address this deficiency, we used the reduced representation library method Restriction-site Associated DNA sequencing (RADseq) to conduct phylogenomic and population genomics analyses, providing novel information for use in stock assessments. Our results strongly support the species status of *S. microcephalus* ($N = 79$), but recover *S. antarcticus* ($N = 2$) intermixed within the *S. pacificus* ($N = 170$) clade. Population genomics analyses reveal genetic homogeneity within *S. pacificus* and *S. microcephalus*, and estimates of effective population size were in the hundreds for both species. Kinship analysis identified 2 first-degree relative pairs within our dataset (1 within each species). Our results contribute new information for stock assessments of these uniquely long-lived species by providing the strongest molecular evidence to date for the synonymization of *S. antarcticus* and *S. pacificus*, as well as estimating population genomic metrics for each supported species within the *Somniosus (Somniosus)* subgenus.

Key words: phylogenetics, population genetics, RADseq, RRL methods

Introduction

Molecular methods provide a powerful means of increasing our knowledge of the life histories and population structures of enigmatic species that are hard to study directly. Such insights can be crucial for species management, providing estimates of population-level metrics and facilitating stock assessment. While many of these enigmatic species hold ecological importance, their habitat preference, generation times, or scarcity (among, or in combination with, a number of other factors) can make it challenging to directly observe their population dynamics. The rise of reduced representation genomic methods facilitates the collection of data from across the genome without necessitating a high-quality reference genome for the species. Intraspecific population metrics, such as genetic diversity, population connectivity, and estimates of effective population size can be especially informative of species-level characteristics, including health and stability (Hellberg et al. 2002; Hughes and Stachowicz 2004; Danovaro et al. 2008; Cowen and Sponaugle 2009). Advances in technology and bioinformatics are providing unprecedented insights into a number of non-model organisms (Spies et al. 2020; Petrou et al. 2021).

Sleeper sharks (genus *Somniosus* Le Sueur 1818) are long-lived, slow-growing, deep-water predators found throughout the world's oceans, occurring as deep as 2000 m, from the Arctic Circle (Benz et al. 2004) to Antarctica (Francis et al. 1988). In 2004, the 3 extant species constituting the genus *Somniosus*, the Pacific sleeper shark *S. pacificus* Bigelow and Schroeder 1944, the Greenland shark *S. microcephalus* (Bloch and Schneider 1801), and the Little sleeper shark *S. rostratus* (Risso 1827), were rearranged on the basis of morphological characters and geographic distributions

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into 2 subgenera: *Somniosus (Somniosus)* and *Somniosus (Rhinoscymnus)* (Yano et al. 2004). Subgenus *Somniosus* included sleeper shark species characterized by larger body sizes and broader geographic distributions: the Southern sleeper shark *S. antarcticus* Whitley 1939 which had previously been synonymized with *S. microcephalus*, was resurrected and placed within the subgenus *Somniosus* with *S. microcephalus* and *S. pacificus* (Yano et al. 2004).

The 3 species within *Somniosus (Somniosus)* are generally defined by their geographic distribution: *S. antarcticus* is reported throughout the Southern Hemisphere, in the Pacific, Indian, and Atlantic oceans (Yano et al. 2004). *S. pacificus* occupies waters across the North Pacific Ocean: in the eastern Pacific, they may be found from the Chukchi Sea (within the Arctic Circle) to Baja California; in the western Pacific, they have been found as far south as Taiwan (Tanaka 1935; Bigelow and Schroeder 1944; Bright 1959; Lindberg and Legeza 1959; Clemens and Wilby 1961; Gotshall and Jow 1965; Kato et al. 1967; Miller and Lea 1972; Quast and Hall 1972; Hart 1973; Tanaka et al. 1982; Castro 1983; Dolganov 1983; Compagno 1984; Masuda et al. 1984; Borets 1986; Ebert et al. 1987; Last and Stevens 1994; Orlov 1999; Yang 1999; Mecklenburg et al. 2002; Benz et al. 2004; Wang and Yang 2004). The described geographic distribution of *S. microcephalus* is also limited to the Northern Hemisphere. Within the Atlantic Ocean, *S. microcephalus* is described from the Arctic Ocean to the Gulf of Mexico and is generally found at greater depths with lower latitudes (Bigelow and Schroeder 1948; Templeman 1963; Quero et al. 1982; Benz et al. 2007; MacNeil et al. 2012; Deep-C 2013). However, some range overlap with *S. pacificus* has been inferred, especially near the Arctic Circle (Hussey et al. 2015; Walter et al. 2017).

Somniosus (Somniosus) species can grow to lengths of 5 to 7 m (Compagno 1984; Cherel and Duhamel 2004) and are estimated to reach ages of nearly 300 yr, though the standard deviations around these estimates can exceed 100 yr (Nielsen et al. 2016). They are also considered to be extremely slow-growing: females of *S. microcephalus* are estimated to reach sexual maturity at 400 cm, which may take several decades to over a century (Nielsen et al. 2016). Historical fishery pressure on *S. microcephalus* (Jensen 1914, 1948; Dunbar and Hildebrand 1952; Templeman 1963; MacNeil et al. 2012) and current bycatch pressure across all 3 species (Wheeland and Devine 2018; Tribuzio et al. 2020a, 2020b; SIODFA 2020) could leave them vulnerable to loss, especially in light of their life history characteristics. Moreover, large shark species that are long-lived and slow to mature are generally in danger of overexploitation (Dulvy et al. 2014) and recovery can be a long process, if it can be achieved at all (Musick et al. 2000). *S. antarcticus* is designated "Least Concern" by the IUCN Red List (Finucci 2018); though *S. pacificus* was recently deemed "Near Threatened" (Rigby et al. 2021) and *S. microcephalus* has been named a "Vulnerable" species (Kulka et al. 2020). *S. microcephalus*, the *Somniosus (Somniosus)* species which has been studied most intensely, was listed as "Data Deficient" on Norway's Red List (Henriksen and Hilmo 2015) and *S. microcephalus* and *S. pacificus* have declining population trends (Kulka et al. 2020; Rigby et al. 2021). Regionally, the North Pacific Fishery Management Council evaluated the vulnerability of 41 groundfish species, identifying *S. pacificus* as most vulnerable (Ormseth and Spencer 2011).

Despite the ecological importance of *Somniosus (Somniosus)* species as high trophic level generalist predators in the world's deep oceans (Hobson et al. 2002; Hussey et al. 2014), there is a paucity of molecular research focused on these species. In 2008, Murray et al. targeted a single mitochondrial marker (*cytB*) in *S. pacificus*, *S. microcephalus*, and *S. antarcticus*. Aside from the described geographic distributions of *S. pacificus* and *S. antarcticus* (occupying the North and South Pacific Ocean, respectively), little differentiates these 2 species. Parsimony analysis of *cytB* confidently separated *S. microcephalus* from *S. pacificus* + *S. antarcticus*; however, the authors called for further research into the relationship between *S. pacificus* and *S. antarcticus*, which the authors posited may represent a single species or incomplete lineage sorting (Murray et al. 2008). While not designed as a comprehensive population genetics study, Murray et al. (2008) noted a lack of population structure in *S. microcephalus*.

Nearly a decade later, Walter et al. (2017) conducted a more thorough population genetics analysis of *S. pacificus* and *S. microcephalus*, targeting *cytB* as well as 2 nuclear markers (ITS2 and RAG1). Once again, these markers supported genetic differentiation between *S. pacificus* and *S. microcephalus*, although the lack of private alleles in the nuclear markers of *S. pacificus* suggested the possibility of introgression between the 2 species, which the authors estimate diverged at the onset of the Pleistocene glacial oscillations (1 to 2.3 mya). Genetic homogeneity across markers suggests a lack of population structure in either species.

The most recent study of population genetics within *S. microcephalus* generated data for 8 microsatellites (Swintek and Walter 2021) across 259 individuals collected from the Canadian Arctic, Nova Scotia, Iceland, west Greenland, Svalbard, and the Mid-Atlantic Ridge (Swintek et al., in revision). When using location-informed priors, Swintek et al. (in revision) suggested genetic divergence between populations of *S. microcephalus* caught on either side of the Davis Strait. Swintek et al. (in revision) is the first study to identify subtle but significant population genetic structure in the species and posits 2 populations: 1 within Baffin Basin in the Canadian Arctic, and 1 with a much broader geographic distribution outside of Baffin Basin.

Given sleeper sharks' role as vulnerable predators in global oceans, a better understanding of their species-level relationships and genetic structure is needed for effective species management. Our understanding of the subgenus *Somniosus* can be greatly expanded with genome-wide molecular data. Here, we utilize a reduced representation library method to gain insight into the molecular ecology of this taxonomic group. To accomplish this we aimed to: 1) quantify the genetic differentiation between *S. pacificus*, *S. microcephalus*, and *S. antarcticus*, including identifying any potential hybrids; 2) characterize population structure and diversity within each species; and 3) estimate effective population sizes in each species. The research presented here represents the first genomic study of molecular ecology in the *Somniosus* subgenus and the first high-throughput sequencing dataset for the sleeper sharks.

Methods

Sample collection

Sampling occurred opportunistically over 2 decades, 2000 to 2020, and individuals were provisionally identified to species based on collection locality: individuals collected from the Southern hemisphere were identified as *S. antarcticus*; those from the north Pacific and Atlantic oceans

were assigned to *S. pacificus* and *S. microcephalus*, respectively. Individuals of *S. pacificus* were collected from the west coast of North America, from northern Baja California to the Bering Sea, as well as from off the coast of Taiwan (Fig. 1A). Two individuals of *S. antarcticus* were collected from the Southern hemisphere, specifically the South Pacific and the Tasman Sea. Samples of *S. microcephalus* were collected from within Baffin Bay in the North Atlantic (Fig. 1B). All recorded metadata associated with sample collection are detailed in Supplementary Table S1. Throughout the study, maps and plots were generated with the R (R Core Team 2018) packages *ggmap* (Kahle and Wickham 2013) and *ggplot2* (Wickham 2016), respectively.

DNA extraction and RADseq library preparation

Tissue preserved in the field was extracted using the Qiagen DNeasy Blood and Tissue Kits. DNA quantity was ascertained with Quant-it PicoGreen dsDNA assays (Invitrogen, Carlsbad, CA). DNA quality was determined with gel electrophoresis. Any extractions with DNA concentration <5.0 ng/μL or lacking a high molecular weight band following gel electrophoresis were excluded from downstream Restriction-site Associated DNA sequencing (RADseq) library preparation.

In total, 288 samples were prepared for RADseq following the BestRAD protocol (Ali et al. 2016), including 204 representatives of *S. pacificus*, 82 representatives of *S. microcephalus*, and 2 individuals of *S. antarcticus*. Input DNA amounts were normalized to an estimated DNA concentration of 50 ng/μL across samples. DNA was digested with *SbfI* in 2-μL reactions. Following digestion, barcoded adapters were ligated to DNA fragments and these sublibraries, now individually barcoded, were pooled into 3 libraries of 96 samples each. Libraries were sonicated on a Q500 sonicator (Qsonica, Newtown, CT) for 12 to 14 cycles of 30 s each. To remove DNA fragments lacking ligated adapters in each library, fragments containing barcoded adapters were bound to Dynabeads M-280 streptavidin magnetic beads (Invitrogen, Waltham, MA) and nontarget fragments were washed away with buffer. Retained fragments were released from streptavidin beads via incubation and washed with AMPure XP beads (Beckman-Coulter). The cleaned libraries served as input for the NEBNext Ultra DNA Library Prep Kit for Illumina End Prep step, which ligated unique indices to differentiate each library. Indexed libraries were size selected for a 250 bp insert and the size selected product was enriched with a 12-cycle PCR. Successful library preparation was confirmed via visualization on a 2% agarose E-gel (Invitrogen) before a final AMPure bead clean-up and quantification with the Qubit dsDNA High Sensitivity assay (Qubit 2.0 Fluorometer). Final libraries were sent to the University of Oregon Genomics Core Facility for paired end 150 sequencing on the Illumina Novaseq S4 platform. Due to the large genome size estimate for *Somniosus* (up to 12 Gb; Stingo et al. 1980), we sequenced the 288 sharks on 3 Novaseq S4 lanes to achieve sufficient coverage.

Data assembly and filtering

Data were processed and analyzed on SEDNA, the high-performance computing cluster at NOAA's Northwest Fisheries Science Center. We generally followed the analysis methods detailed in Ackiss et al. (2020) to discover single nucleotide polymorphisms (SNPs) and call genotypes. Raw RADseq reads were aligned and assembled in STACKS v2.3 (Rochette et al. 2019). Paired end reads were demultiplexed by barcode with the *process_radtags* function *(-e sbfI --bestrad)*, which also accomplished filtering by enzyme cut-site presence (*-c*) and read quality (*-q --filter_illumina*). Filtered reads and barcodes were "rescued" (*-r*) and remaining reads were truncated to 140 bp (*-t 140*). Demultiplexed, filtered reads were aligned within each individual with *ustacks* to create ungapped stacks of reads within a Hamming distance of 3 (*-disable-gapped -M 3*). Previous literature that evaluated RADseq parameters in organisms with similar genomic structure and ploidy (e.g. Mastretta-Yanes et al. 2015; Paris et al. 2017; Shi et al. 2021) was used to determine parameter thresholds that would likely prevent over- or under-merging reads: ≥3 reads were required to create a new stack (*-m 3*), a single *de novo* locus could not have >4 stacks (*-max_locus_stacks 4*), and the error rate for the bounded model could not exceed 0.05 *(--model_type bounded --bound_high 0.05*). Finally, haplotypes were not called from secondary reads (*-H*).

Initially, to investigate evolutionary relationships between the species within the subgenus, a catalog of consensus loci was built from 5 individuals of each species (except *S. antarcticus*, which was only represented by 2 individuals during this step). To investigate intraspecific population genomics, species-specific catalogs were built from 34 individuals of *S. pacificus* (Aleutian Islands = 5, eastern Bering Sea = 5, US west coast = 5, central Gulf of Alaska = 5, eastern Gulf of Alaska = 5, Taiwan = 4, unreported collection location = 5) and 17 individuals from *S. microcephalus* (Cumberland

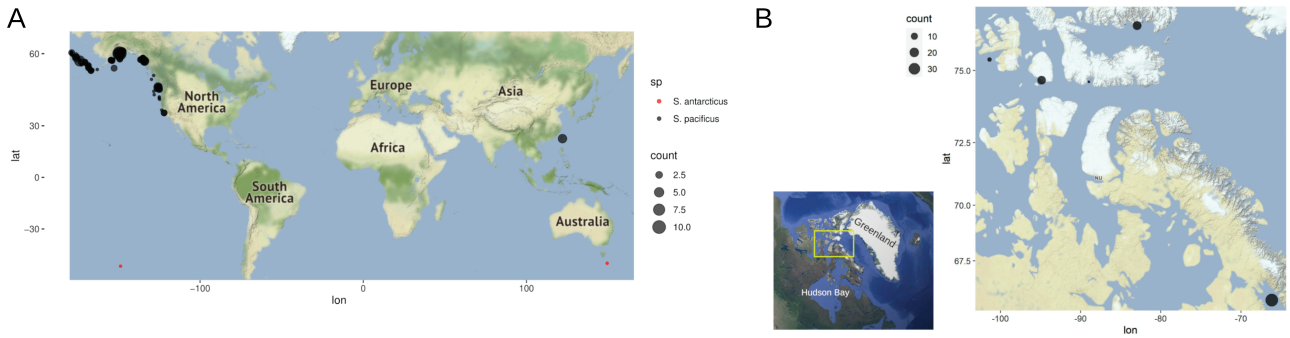

Fig. 1. Collection locations and sample sizes for A) the Pacific sleeper shark (black) and the Southern sleeper shark (red), *Somniosus pacificus* and *S. antarcticus*, respectively, and B) the Greenland shark, *S. microcephalus*.

Sound = 5, Grise Fjord = 5, Maxwell Bay = 2, Scott Inlet = 5). Individuals included in the catalog were chosen to represent all geographic locations, and the individuals with the highest coverage were chosen from each location. Population genomics analyses could not be conducted on the *S. antarcticus* dataset due to low sample size. In each case, a Hamming distance of ≤3 was allowed between sample loci to build a catalog locus and no gaps were allowed in the alignments (*-n 3 --disable_gapped*). *Sstacks* aligned individual locus stacks to the catalog, prohibiting gaps in the alignments (*--disable_gapped*). Finally, read data were oriented by locus with *tsv2bam*, and paired end reads were aligned to loci and SNPs were called with *gstacks*. SNPs found in ≥30% of individuals were written out in vcf format using *populations* (*-R 0.3 --vcf*).

Initial SNP filtering was conducted with VCFtools v0.1.16 (Danecek et al. 2011) using an approach and methodology similar to Shi et al. (2021). Generally, unsalvageable and invariant sites were removed (*--max-missing 0.015 --maf 0.0001*) before removing unsalvageable individual samples (those with missing data >0.95). With very low-quality sites and individuals removed, sites with a minor allele frequency <0.05 and a missingness >0.15 were removed (*--maf 0.05* followed by *--max-missing 0.85*). Initial filtering concluded by removing individuals missing data at >0.50 sites. Following initial filtering, putatively paralogous reads were identified in HDPlot (McKinney et al. 2017) and removed with VCFtools. In the subgenus dataset (containing individuals from all 3 species), loci with heterozygosity >0.45 or a read ratio less than −8 or greater than 8 were removed. Given the computational intensity of phylogenomic methods, the full subgenus dataset was reduced to a representative subgenus dataset, which included individuals from all 3 species, representing each collection location. The species-specific datasets were also examined with HDPlot: in *S. pacificus*, loci with heterozygosity >0.55 or a read ratio below −10 or above 10 were removed; in *S. microcephalus*, a heterozygosity threshold of 0.5 or a read ratio below −8 or above 8 were removed. Finally, SNPs were thinned so that only the SNP with the highest minor allele frequency in each locus was retained. This was accomplished with a custom python script. All scripts associated with this workflow are publicly available at github.com/letimm/*somniosus*RAD.

Phylogenomic analysis

In consideration of previous research, which suggested hybridization between *S. pacificus* and *S. microcephalus*, analyses began with the full subgenus dataset to determine whether formal testing for hybrids was required. This was accomplished with Neighbor Joining in the R package *ape* (Paradis and Schliep 2019). Had hybrid individuals been present in the dataset, we would have expected to see 3 phylogroups, 1 each for the pure species, and 1 containing putative hybrids potentially including representatives from both *S. microcephalus* and *S. pacificus*.

A phylogenetic tree was inferred for the representative subgenus dataset using a maximum likelihood approach, as implemented in RAxML v8.2.12 (Stamatakis 2014), specifically the raxmlHPC-PTHREADS-AVX2 package. Both the bootstrap and parsimony random seeds were set with $RANDOM in bash. The bootstrapping criterion was used for the majority-rule tree-based criterion (*-N autoMRE*). The General Time Reversible (GTR) model of nucleotide substitution was selected with a gamma model of rate heterogeneity (*-m GTRGAMMA*). The resulting ML tree was labeled and color coded in the GNU Image Manipulation Program (The GIMP Team 2020).

Genetic differentiation (F_{ST}) between species was calculated in VCFtools (*--weir-fst-pop*). Once all individual species identifications were confirmed and the possibility of hybrid individuals was excluded, analyses continued on species-specific SNP data.

Population genomics analyses

We conducted separate principal component analyses (PCAs) for each species with the R package *adegenet* (Jombart 2008) to explore genetic relationships among individuals within each species. Initial PCAs displayed a number of outlier individuals, which we thought could potentially be related pairs or be associated with missing data. We tested these hypotheses by 1) exploring patterns of missing data in outlier individuals and 2) conducting relatedness analysis using the KING method (Manichaikul et al. 2010), which rapidly calculates kinship coefficients across large SNP datasets.

Relatedness analysis was conducted in VCFtools (*--relatedness2*). With this method, pairwise kinship estimates >0 indicate that the individuals could be related (i.e. third-degree relatives or closer), while kinship coefficients >0.3 indicate that the pair of individuals are likely first-degree relatives (i.e. full-siblings or parent-offspring) (Manichaikul et al. 2010). Initially, we conducted relatedness analysis on the full datasets for each species. However, this analysis identified 7 pairs of *S. pacificus* with kinship estimates greater than zero and further investigation suggested that some of the pairs may have been caused by high levels of missing data. Based on these observations, we recalculated relatedness with any individuals with a proportion of >0.075 missing data excluded from kinship analysis. We classified related pairs from this analysis as high confidence relationships. We then reran the PCA on a dataset with 1 individual from each of these high confidence pairs removed. However, some outlier clusters still remained in the PCAs. It is possible that some of the putative related pairs revealed in the less stringent analysis could be related, even though kinship coefficients were quite low in some cases (Supplementary Table S2). Therefore, to be as conservative as possible, we removed all but 1 individual from each group of putative related individuals determined by the less stringent relatedness analysis and repeated the PCA on this pruned dataset. All subsequent analyses were conducted on this pruned dataset (i.e. the dataset containing only unrelated individuals based on the less stringent relatedness analysis).

We ran ADMIXTURE (Alexander et al. 2009) to further test for population structure. Cross-validation error (*--cv=5*) was used to determine the optimal number of ancestral populations (K) indicated by the data when K = 1 to 7 were tested. Additionally, a series of summary statistics were calculated in the R package *hierfstat* (Goudet 2005), including observed heterozygosity, expected heterozygosity, and the inbreeding coefficient (F_{IS}). Population differentiation (F_{ST}) was calculated between all collection locations with sufficient sample size ($n \geq 5$).

Effective population sizes (N_E) and parametric 95% confidence intervals were estimated for *S. pacificus* and *S.*

microcephalus under the bias-corrected linkage disequilibrium method, LDNE (Hill 1981; Waples 2006; Waples and Do 2008), with a p-crit (i.e. minor allele frequency cutoff) of 0.05 (Waples et al. 2016), as implemented in NeEstimator v2.1 (Do et al. 2014). Effective population sizes were estimated by species because no population structure within species was documented.

Results

Across 288 individuals, 12,137,097,640 reads were sequenced—an average of 42,142,700 reads per individual. Raw, demultiplexed fastq files are publicly available in the NCBI SRA under BioProject PRJNA858799 (Timm 2022). Four datasets were assembled and filtered for analysis: the full dataset, containing all individuals from all 3 species; a representative subgenus set; and the *S. pacificus*-only set and the *S. microcephalus*-only set. The full dataset was comprised of 30,373 SNPs across 251 samples (79 *S. microcephalus*, 170 *S. pacificus*, and 2 *S. microcephalus*; mean depth per individual = 56.2x). The representative subgenus set included 29,037 SNPs for 22 individuals across all 3 species within the subgenus (2 *S. antarcticus*, 4 *S. microcephalus*, and 16 *S. pacificus*; mean depth per individual = 88.9x). The *S. pacificus*-only set included 52,533 SNPs across 170 samples (mean depth per individual = 57.1x) and the *S. microcephalus*-only set included 39,012 SNPs across 79 individuals (mean depth per individual = 50.2x).

Phylogenomics of the subgenus

Neighbor Joining analysis of the subgenus data did not reveal any hybrids (Fig. 2) and F_{ST} between the 2 species was estimated to be 0.43. Confident that specimens had been accurately identified to species, analysis of the subgenus dataset continued in RAxML, resulting in a midpoint-rooted maximum likelihood tree (Fig. 3). The tree is divided into 2 distinct clades: a *S. microcephalus* clade and a *S. antarcticus* + *S. pacificus* clade.

Somniosus pacificus

In all PCAs, the first 2 principal components (PCs) reveal high genetic similarity among individuals of *S. pacificus* indicating no population structure, and the 2 individuals identified as *S. antarcticus* in the field group with other *S. pacificus* samples (Fig. 4). In the PCA with all data (Fig. 4A), both PCs represent 1.1% of the genetic variance within the dataset and the vast majority of samples fall at the origin. However, 3 peripheral clusters form: a cluster of 2 individuals and 2 clusters of 3 individuals. These 8 individuals form 7 pairs with kinship coefficients greater than zero (Supplementary Table S2). Stringent relatedness analysis retained the 2 individuals from the Bering Sea in the cluster to the left, suggesting they are related (Table 1), but removing one of these individuals still leaves the 2 outlier clusters of 3 individuals (Fig. 4B). These 2 remaining clusters contain potentially related individuals but may be confounded by missing data (Supplementary Table S2). After

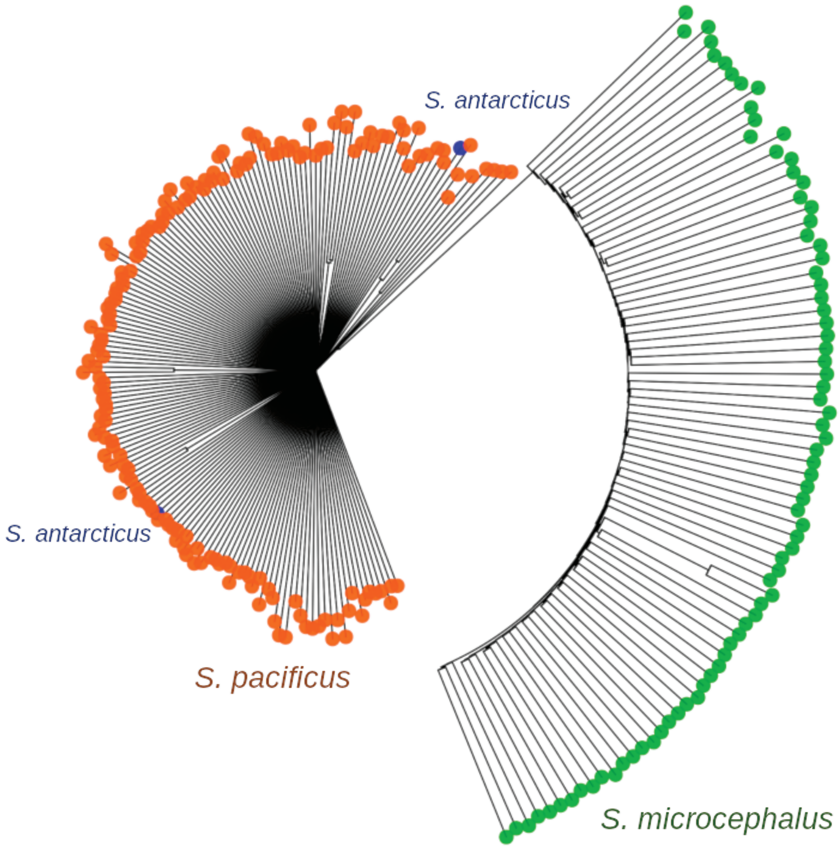

Fig. 2. Neighbor Joining tree built from the full subgenus dataset (30,373 SNPs, 251 individuals) illustrating the divergence between *Somniosus microcephalus* (green) and *S. pacificus* (orange). Note the 2 individuals of *S. antarcticus* (blue) within the *S. pacificus* clade.

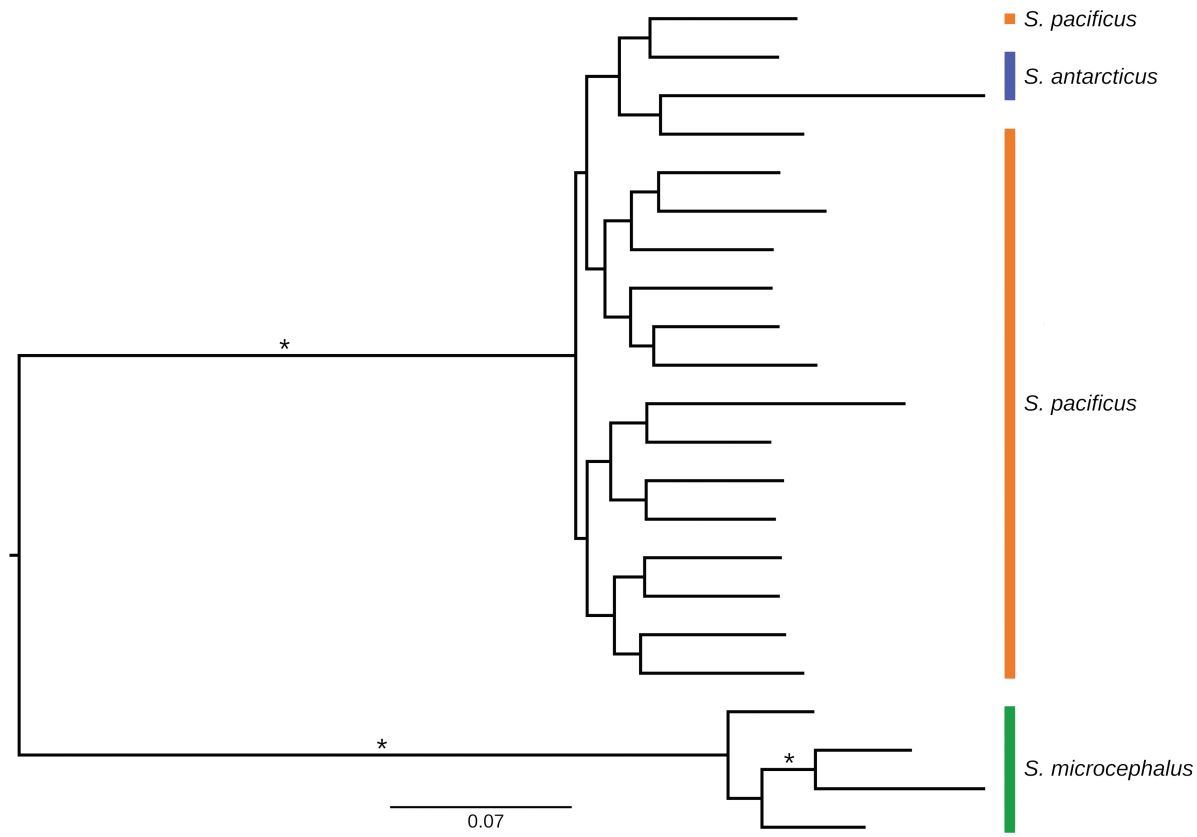

Fig. 3. RAxML tree of the representative subgenus dataset (29,037 SNPs, 22 individuals). While *S. microcephalus* falls into a strongly supported, reciprocally monophyletic clade, *S. pacificus* and *S. antarcticus* occupy the second clade together. * indicates branch support values >70.

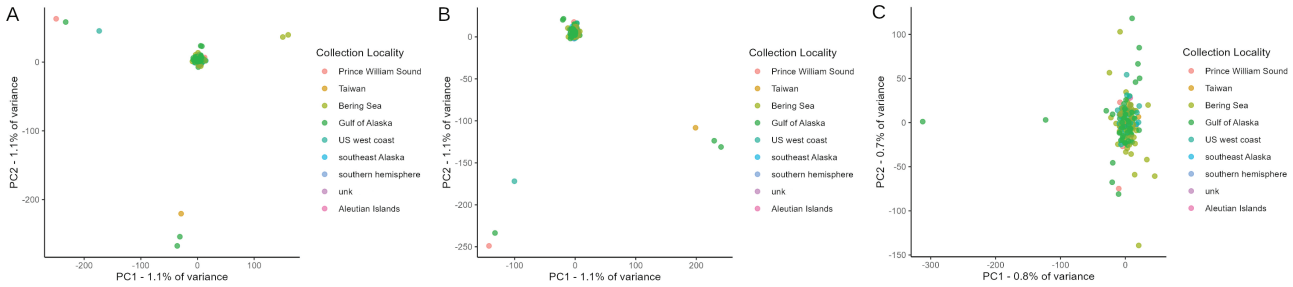

Fig. 4. PCAs of *Somniosus pacificus* and 2 *S. antarcticus* individuals denoted with the "Southern hemisphere" label. PCAs are A) the full dataset (52,533 SNPs, 172 individuals), B) the full dataset except one individual from the high confidence related pair (Table 1; 171 individuals), and C) the full dataset except all but 1 individual from each putatively related group (Supplementary Table S2; 167 individuals).

Table 1. All pairwise relatedness values where kinship ≥0.3 when the proportion of missing data is ≤0.075.

	Ind 1			Ind 2						
Species	ID	Collection location	Collection date	ID	Collection location	Collection date	*k*0	*k*1	Kinship	Hypothesized relationship
S. pacificus	ABLG1384	East Bering Sea	7/9/2010	ABLG1388	East Bering Sea	7/18/2010	6.96E−07	0.189	0.453	Full-siblings
S. microcephalus	ABLG919	Grise Fjord	8/22/2015	ABLG945	Grise Fjord	8/19/2015	8.48E−07	0.177	0.456	Full-siblings

removing all but 1 individual from each of these 3 clusters of potential kin, no outlier clusters containing >1 individual remain (Fig. 4C). However, a few outlier points still remain, and these points do not contain any of the individuals flagged as potentially related. We hypothesize that these are statistical artifacts that arose due to the lack of population structure in this species. Analysis with ADMIXTURE provided further support for our conclusion of no population structure, with cross-validation error estimated to be lowest for $K = 1$, indicating a single ancestral population (Table 2).

Summary and diversity statistics were calculated for the *S. pacificus*-only dataset, with all but 1 individual from each high-kinship cluster indicated by the less stringent relatedness analysis removed (Table 3). Observed heterozygosity is 0.266, expected heterozygosity is 0.322, and F_{IS} for the dataset is 0.176. The N_E for the species is 968.8 (Table 3). Between collection locations, F_{ST} values were low, ranging from 0 (between the Gulf of Alaska and southeast Alaska) to 0.0003 (between the Bering Sea and the Gulf of Alaska; Table 4).

Given the estimated N_E, it was somewhat surprising that we identified a first-degree relationship within the dataset: ABLG1384 and ABLG1388 have a kinship coefficient of 0.453 (Table 1), well above 0.3, the accepted threshold typically cited as indicating a first-degree relationship (Manichaikul et al. 2010). Both individuals were collected from the eastern Bering Sea (54.4° to 166.85° and 54.57° to 166.22°, respectively) within 10 d of each other. A first-degree relationship can indicate parent-offspring or full-siblings and, while we are unable to confidently diagnose this relationship with the data in-hand, a full-sibling relationship is indicated: both individuals are relatively close in size (96 and 111 cm, respectively) and both are well below the estimated minimum length associated with sexual maturity (400 cm in *S. microcephalus*, the only species for which this data are available; Yano et al. 2007; Nielsen et al. 2020).

Somniosus microcephalus

PCA of *S. microcephalus* reveals a tight cluster at the graph origin, containing the vast majority of individuals (Fig. 5A; PC1 = 2.4%, PC2 = 1.5%). A notable pair of outliers, both collected from Grise Fjord, were identified as close kin in the stringent relatedness analysis and subsequently pruned from the dataset. After removing 1 individual from this high-kinship pair, PC1 and PC2 each represent 1.5% of variance and the primary cluster remains (Fig. 5B). As in *S. pacificus*, a few outlier points remained that are likely to be statistical artifacts resulting from low population structure. Our conclusion of a single population was further supported by analysis of the *S. microcephalus*-only dataset with ADMIXTURE, which indicated cross-validation error was lowest when $K = 1$ (Table 2).

Calculating summary and diversity statistics across the *S. microcephalus*-only dataset, with 1 individual from the high-kinship pair removed, we estimated observed heterozygosity is 0.236 and expected heterozygosity is 0.324. The inbreeding coefficient for *S. microcephalus* is 0.275. The estimated 95% confidence interval of the effective population size for the species is 601.1 to 604.9 (Table 3). Population differentiation values between collection locations were nearly zero, with the lowest F_{ST} calculated between Cumberland Sound and Scott Inlet ($F_{ST} = 0$) and the highest F_{ST} between Grice Fjord and Scott Inlet ($F_{ST} = 0.0011$; Table 5).

Despite an effective population size estimate in the high hundreds ($N_E = 603.0$, Table 3), we find a first-degree relationship within the *S. microcephalus*-only data: ABLG919 and ABLG945 have a kinship coefficient of 0.456 (Table 1). This pair of individuals was collected from Grise Fjord in 2015, with only a couple of days separating the collection events. These individuals were 278 and 343 cm, respectively, below the minimum length associated with sexual maturity in the species, likely indicating a full-sibling relationship.

Discussion

The research we report here supports fisheries management goals and represents the first next-generation sequencing study of the sleeper shark genus *Somniosus*. Using a reduced representation approach, we investigated the evolutionary relationships within the subgenus *Somniosus (Somniosus)* and were able to confidently differentiate *S. microcephalus* from *S. pacificus* and *S. antarcticus*. However, we found no molecular evidence to support the validity of separating *S. antarcticus* and *S. pacificus*.

All previous work on the molecular ecology of *Somniosus (Somniosus)*, including the research we present here, has been unable to differentiate *S. antarcticus* from *S. pacificus*

Table 2. Cross-validation error values for ADMIXTURE analysis of *S. pacificus* and *S. microcephalus*.

	Somniosus pacificus	*Somniosus microcephalus*
$K = 1$	0.629	0.667
$K = 2$	0.651	0.736
$K = 3$	0.674	0.820
$K = 4$	0.709	0.885
$K = 5$	0.744	1.026
$K = 6$	0.782	1.105
$K = 7$	0.816	1.162

Table 4. Mean F_{ST} values between collection locations for *S. pacificus* for which $n \geq 5$.

	BS	GOA	PWS	SEAK	USwc
Bering Sea ($n = 68$)	—	—	—	—	—
Gulf of Alaska ($n = 56$)	0.0003	—	—	—	—
Prince William Sound ($n = 10$)	0	0	—	—	—
Southeast Alaska ($n = 6$)	0	0	0	—	—
US west coast ($n = 24$)	0	0	0	0	—

Sample sizes for each collection location are given in parentheses (BS = Bering Sea; GOA = Gulf of Alaska; PWS = Prince William Sound; SEAK = southeast Alaska; USwc = US west coast).

Table 3. Summary and diversity statistics for *Somniosus microcephalus* and *S. pacificus*, including the number of individuals (*N*) and sites (SNPs), mean depth per individual, observed heterozygosity (H_O), expected heterozygosity (H_E), inbreeding coefficients (F_{IS}), and the estimated effective population size with 95% confidence interval (N_E [95% CI]).

Species	*N*	SNPs	Mean depth	H_O	H_E	F_{IS}	N_E [95% CI]
Somniosus pacificus	170	52,533	57.1x	0.266	0.322	0.176	968.8 [967.2 to 970.4]
Somniosus microcephalus	79	39,012	50.2x	0.236	0.324	0.275	603.0 [601.1 to 604.9]

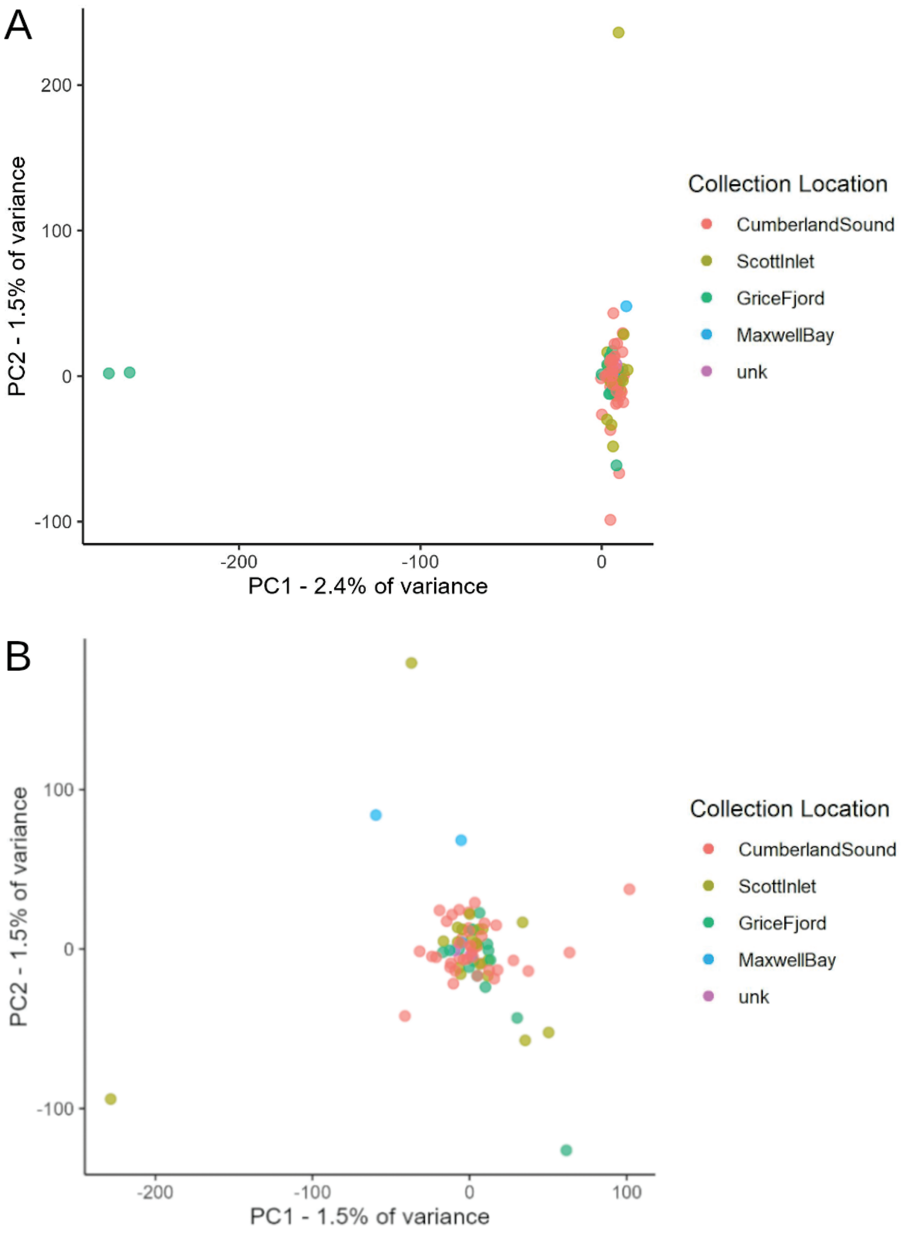

Fig. 5. PCAs of *Somniosus microcephalus* from A) the full dataset (39,012 SNPs, 79 individuals) and B) an all-but-one high-kinship-pruned dataset (78 individuals).

Table 5. Mean F_{ST} values between collection locations for *S. microcephalus* for which $n \geq 5$.

	Cumberland Sound	Grice Fjord	Scott Inlet
Cumberland Sound (n = 38)	—	—	—
Grice Fjord (n = 15)	0.0008	—	—
Scott Inlet (n = 20)	0	0.0011	—

Sample sizes for each collection location are given in parentheses.

(Murray et al. 2008; Figs. 2–4). However, it is worth noting that the few *S. antarcticus* samples included in molecular ecology studies of the subgenus have all originated from the South Pacific, with no *S. antarcticus* representatives from the South Atlantic or Indian oceans. Additional sampling from a broader geographic range is necessary, in the event that *S. antarcticus* contains individuals of both *S. pacificus* and *S. microcephalus*. While a redescription of *S. pacificus* to reflect that the species range extends to the South Pacific is warranted, it is beyond the scope of this work.

Interestingly, *S. antarcticus* was originally described from a single individual, washed ashore on Macquarie Island in the Antarctic (Waite 1916). While morphological characters were noted, differentiating *S. antarcticus* from *S. pacificus* and *S. microcephalus* (Yano et al. 2004), a primary reason for distinguishing this specimen as a new species was the taxon's novelty in the Southern Hemisphere (Whitley 1939, 1940). Thus, sleeper sharks identified in the Southern Hemisphere would be assigned to *S. antarcticus*, even though sketches of the holotype have been described as resembling *S. microcephalus* (Bigelow and Schroeder 1957). Hereafter, we will refer to the

2 species supported by our analyses: *S. microcephalus* and *S. pacificus* (which includes the 2 individuals corresponding to *S. antarcticus*).

Both *S. pacificus* and *S. microcephalus* have estimated effective population sizes in the high hundreds and neither species exhibits population structure (Table 3), suggesting persistent gene flow across the sampled range. Generally, our results agree with previous research, which confidently differentiated between *S. pacificus* and *S. microcephalus* using microsatellites (Swintek and Walter 2021), mitochondrial data (*cytB* in Murray et al. 2008; mt-genomes in Santaquiteria et al. 2017), and multilocus data, including nuclear and mitochondrial markers (Walter et al. 2017). Notably, we did not identify any individuals exhibiting introgression between *S. pacificus* and *S. microcephalus*, as reported by Walter et al. (2017). While we tried to include those introgressed individuals in the dataset presented here, poor sample quality excluded them from RADseq library preparation.

We did not identify genetic structure in either species, indicating both *S. pacificus* and *S. microcephalus* are well mixed and genetically homogeneous. Migration likely plays a role in genetic mixing within each species: tagging studies of *S. pacificus* showed most individuals moved as far as 100 km over the course of 1 yr (Hulbert et al. 2006), and *S. microcephalus* was found to move more than 1,615 km over a similar timescale (Campana et al. 2015). Moreover, *S. microcephalus* individuals were found to traverse more than 1/3 this distance over the course of 38 d (Hussey et al. 2018). This extensive migration could contribute to genetic mixing within the species. However, recent work has indicated some degree of site fidelity (~25%) for *S. microcephalus* within Baffin Bay (Edwards et al. 2022). Additionally, microsatellite data presented in Swintek and Walter (2021) postulated subtle population structure in *S. microcephalus* across a larger geographic range than we sampled. The combination of genetic data from a larger geographic range and tagging data suggests that subtle structure may exist in this species, and that structure may be increasing slowly following the last glacial maxima, but further research would be useful to clarify these patterns.

Notably, our datasets included 1 first-degree relative pair in both *S. pacificus* and *S. microcephalus*, located in the East Bering Sea and Grise Fjord, respectively (Table 1). Data on size of *S. pacificus* are sparse, however, limited weight data reported by at-sea observers suggests that, at least within Alaskan waters, the smallest *S. pacificus* occur most often in the eastern Bering Sea, with few larger animals sampled. The presence of related pairs in the dataset may indicate the eastern Bering Sea plays an important role as a nursery habitat for *S. pacificus* (Tribuzio et al. 2020a). Although little is known about nursery habitat requirements for *S. pacificus*, the Bering Sea is an extremely productive ecosystem and areas near undersea canyons in this region have been identified as nursery habitats for other elasmobranchs, such as skates (*Bathyraja* sp., Hoff 2010). Specifically, a modeling study found that areas of low temperature variability and high oxygen concentrations in Bering Sea canyons were associated with nursery habitat for skates (Rooper et al. 2019). It is possible that suitable nursery areas for *S. pacificus* share similar attributes, but additional data are necessary before any similar conclusions can be made.

While no population genetics studies have been conducted for the *Rhinoscymnus*, the other subgenus within *Somniosus*, several studies exist for species within Order Squaliformes. Within Family Somniosidae, 3 species of *Centroscymnus* have been targeted for population genetics research. Like species of *Somniosus (Somniosus)*, these *Centroscymnus* species also occupy the bathypelagic (100 to 3500 m). Two of these species, *C. coelolepis* and *C. owstoni*, were included in a phylogenetic study with sufficient geographic sampling range to infer a single population for each species (Moura et al. 2008; Veríssimo et al. 2010). Population structure across large spatial scales was identified in *C. crepidater*, with 2 populations found to span both the Atlantic and southern Pacific, though samples in the South Pacific were limited and 1 population had only a single representative from this basin (Cunha et al. 2012).

More taxonomically distant species of sharks have also been analyzed for population structure within *Squalus*: *S. acanthias*, a bottom-dwelling species, typically occupies depths of 50 to 150 m. Multiple studies have described population genetic structure separated by the Equator within the Pacific Ocean (Franks 2006; Ward et al. 2007; Veríssimo et al. 2010). Interestingly, Veríssimo et al. (2010) describe a southern population, occupying the Atlantic and Pacific oceans, and a north Pacific population, which was later formalized as a resurrection of *S. suckleyi* (Ebert et al. 2010). Species of *Squalus* inhabiting deeper bathypelagic waters (down to 1000 m) do not exhibit this differentiation: both *S. blainville* and *S. mitsukurii* show genetic homogeneity across the geographic range of samples (Kousteni et al. 2016 and Daly-Engel et al. 2010, respectively).

Across taxa within Order Squaliformes, our results appear to agree with other deep-sea sharks. The lack of population structure we find is consistent across a number of other species, though notably not *C. crepidater*. The phylogenetic tree built to estimate divergence times within *Centroscymnus* recovered 2 clades within *C. crepidater*, estimated to have diverged ~15 mya (Cunha et al. 2012): 2 populations are described, both spanning the Atlantic and Pacific oceans. The authors hypothesize that this differentiation, and the lack of clear geographic trends, may be the result of vicariance following the closure of the Tethyan corridor. The closure of the Tethys Ocean may also have impacted *Somniosus (Somniosus)*, but species of *Somniosus (Somniosus)* are expected to be much longer lived than *C. crepidater*, which may enable greater mixing over an individual's lifespan, genetically homogenizing the species and preventing population differentiation.

More broadly, our finding of no significant genetic structure in both *S. pacificus* and *S. microcephalus* seems to coincide with expectations from a recent review paper (Hirschfeld et al. 2021), which analyzed potential population genetic barriers for elasmobranchs. Hirschfeld et al. (2021) found that ocean bathymetry (i.e. depth) was the greatest barrier, but that temperate and cold-water species, such as those in our study, often display high connectivity at the scale of a hemisphere. It is likely that our study species do not experience significant barriers to dispersal because they inhabit relatively homogenous cold, deep environments, making our conclusion of no within-species population structure unsurprising. Additionally, the long-lived nature of these species substantially slows genetic drift on a per-year basis, making detection of significant genetic structure even more challenging.

The long generation time of our species also substantially complicates interpretation of N_E estimates (Edwards et al. 2019). In fact, the extremely long lifespan and presumably low fecundity of these species may make N_E estimates more comparable with species such as whales than with other elasmobranchs. Estimates of N_E in whales are highly variable, ranging from the low hundreds in isolated populations (Rivera-León et al. 2019), to thousands (Cypriano-Souza et al. 2018), to tens of thousands (Ruegg et al. 2013). Interestingly, N_E estimates for elasmobranchs are typically smaller, generally ranging from hundreds to a few thousand (reviewed in Domingues et al. 2018). However, many of these estimates are from species with high levels of population structure, making direct comparisons with our study species difficult. These comparisons underlie the challenge of comparing N_E estimates across taxa, as an extremely large number of parameters interact to determine these estimates. In our case, estimates for both study species were in the high hundreds and above the theoretical value of 500 which is hypothesized to maintain evolutionary potential (Jamieson and Allendorf 2012). However, we caution against using these values for conservation purposes, as accurate estimation of N_E is difficult and further complicated in species with such long generation times (Edwards et al. 2019). Instead, we suggest that these estimates be used as a baseline for population monitoring and comparison with future studies.

Given the ecological importance of sleeper sharks as predators in deep-sea environments, molecular studies, like the one presented here, are powerful resources for supporting fisheries management goals and informing stock assessment. These insights are necessary to forecast the future health and stability of the subgenus. Slow-growing, late-maturing shark species are particularly vulnerable to population declines caused by overfishing and species within *Somniosus (Somniosus)* are already listed as Vulnerable and Near Threatened by the IUCN Red List. Our results indicate moderately large population sizes in both species and high levels of intraspecific gene flow, suggesting long-term resilience. These findings have immediate value to stock assessors and fisheries managers, providing valuable information on risk tolerance for these severely data-limited stocks. Additionally, our study provides information on the population genetic characteristics of one of the longest lived vertebrates in the world, providing an important resource for future studies on similarly long-lived species.

Supplementary material

Supplementary material is available at *Journal of Heredity* online.

Supplementary Table S1. All available metadata associated with sample collection, including a unique, internal identifier (ABLG), alternative identifier provided by original collector (alt_id), species identification, and, when available, collection year, region, coordinates, and depth (m). Finally, physical characteristics of the individual, such as length (mm), weight (g), and sex, are reported when known.

Supplementary Table S2. Phi (kinship) estimates for pairs of individuals with initial relatedness values greater than zero. Proportions of missing data for each individual are given in parentheses. Kinship values following more stringent missing data filtering are presented in Table 1. Pairs in bold were also found to be putatively related with more stringent parameters.

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Data availability

The raw, demultiplexed fastqs underlying these analyses are publicly available in the NCBI SRA under BioProject PRJNA858799 (Timm 2022). Scripts associated with data assembly and quality filtering can be found at https://github.com/letimm/somniosusRAD.

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