

Genomic insights into the shorttail nurse shark and the horn shark, two captive populations.

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Abstract

Genomic insights of captive species are important for informing conservation strategies aimed at sustaining genetically healthy populations, especially when programs aim to support future rewilding. In this study, genetic diversity analyses were done for two shark species: the critically endangered shorttail nurse sharks (*Pseudoginglymostoma brevicaudatum*) and the horn shark (*Heterodontus francisci*), currently listed as Least Concern by IUCN. For the first time ever, the genome of the shorttail nurse shark was assembled using Oxford Nanopore sequencing. Despite high fragmentation, Benchmarking Universal Single-Copy Orthologs (BUSCO) showed good gene space, indicating a high-level draft assembly. Illumina short-read resequencing data of five shorttail nurse sharks were aligned to the genome assembly to estimate nucleotide diversity and inbreeding. Based on the ten longest contigs, the average mean nucleotide diversity was estimated at 0.862 per kb. Runs of Homozygosity (RoH) were used to estimate inbreeding coefficients (F_{RoH}), revealing high inbreeding coefficients in two wild-caught shorttail nurse sharks, likely reflecting small population sizes in the wild. Up until the end of 2025, the horn shark was listed as data deficient on the IUCN Red List. Currently, IUCN lists it as Least Concern, however, little to no information is available regarding the origin and genetic diversity in populations maintained in zoos and aquaria. Therefore, Illumina short-read resequencing data of eight horn sharks was aligned to the reference genome, available at NCBI, to reveal the parents (two female, one male) of five offspring. Also, it was discovered that two parents are distantly related to each other, which was previously unknown. Based on all 51 chromosomes, the average mean nucleotide diversity was estimated at 1.680 per kb. By looking at RoH, it was discovered only one horn shark had a moderately high inbreeding coefficient. These findings underscore the value of genomic data for informing captive breeding management and conservation strategies in sharks.

Introduction

Sharks play vital roles in aquatic ecosystems all over the world. For example, grey reef sharks contribute to nutrient transfer from offshore waters to near-shore reefs, which is important for coral reef health (Williams et al., 2018). Additionally, the absence of predatory sharks from habitats can result in cascading effects on multiple trophic levels (Baum & Worm, 2009; Burkholder et al., 2013; Ferretti et al., 2010; Myers et al., 2007). Regardless of the key roles sharks play in ecosystems, Dulvy et al. (2021) estimated that in 2020, 31.2% of shark species were threatened. The loss of sharks does not only represent a decline in biodiversity but also poses a risk to the stability of marine ecosystems. Population declines are linked to underregulated fisheries and international trade (Marsh et al., 2022; Pacoureaux et al., 2021), rapid expansion of food demand, habitat loss, and climate change (Dulvy et al., 2014). Furthermore, sharks are understudied (Kuraku, 2021; Pearce et al., 2021) which stresses the need for shark conservation research.

Genomic research can provide knowledge about effective population size, population structure, inbreeding, demographic history, and genetic diversity which provide essential knowledge needed for the conservation of wildlife species (Hohenlohe et al., 2021; Pirog et al., 2019; Stanhope et al., 2023). Sharks have slow evolution, low mutation, slow growth, late maturity age and low fecundity (Dulvy et al., 2017; Musick et al., 2000; Sendell-Price et al., 2023) which is linked to low genetic diversity (Barry et al., 2022; Smith, 1986). Additionally, declining population sizes can further decrease the genetic diversity by inbreeding and genetic drift (Frankham et al., 2002; Stanhope et al., 2023). High inbreeding is correlated with an elevated risk of extinction in wild populations (O'Grady et al., 2006; Robinson et al., 2019), highlighting the importance of maintaining genetic diversity for species survival (DeWoody et al., 2021; Domingues et al., 2018). This also applies to zoo populations, where genomic-informed captive breeding can reduce inbreeding and increase genetic diversity (Speak et al., 2024). In collaboration with Artis Royal Zoo, this research aimed to investigate the genetic diversity of five captive shorttail nurse sharks and eight captive horn sharks with the goal to provide evidence-based genetic knowledge to breeding program policy makers.

The shorttail nurse shark (*Pseudoginglymostoma brevicaudatum*) is a critically endangered shark species. They have a flat head, short and rounded snout and are dark brown from above and brownish white from below (Playfair & Günther, 1866) and can reach a total length of 75 cm (Bennett et al., 2021; Ebert et al., 2021). The shorttail nurse shark reproduces by laying eggs, typically two at a time and reaches sexual maturity at around 55 cm total length in females and 59 cm total length in males (Compagno, 2001; Ebert et al., 2021; Pollom et al., 2019). They are valued for local consumption of fins and meat and other products such as jaws and skin (Pollom et al., 2019). These sharks live in the Western Indian Ocean in the shallow coral reefs of Madagascar, Tanzania, and Kenya (Compagno, 2001; Ebert et al., 2021). Due to overexploitation and habitat destruction, the populations are suspected to have declined by more than 80% over the past 35 years (Pollom et al., 2019, 2024). Their exact population numbers, distribution, and interaction between populations have not been researched (Bennett et al., 2021). Additionally, no reference genome is available for this critically endangered species. However, numerous shorttail nurse sharks are present in zoos and aquaria (Janse et al., 2017). In collaboration with Royal Artis Zoo, this research aims to gain genetic knowledge about five shorttail nurse sharks of which three are wild-caught (unknown location), one zoo-bred and one with unknown origin. The findings of this research will support this understudied species by informing breeding management policies that aim to preserve genetic diversity within the captive population.

The horn shark (*Heterodontus francisci*) is native to the west coast of Mexico and the United States, most common at a depth of 2 until 11 meters (Love, 1996). The female horn shark lays two eggs every 11-14 days between February and April (Ebert, 2003). They are caught as bycatch in traps and trawls (Baum et al., 2003; Compagno, 2001; Ebert, 2003). Until the end of 2025, the horn shark was listed by IUCN as Data Deficient, but now IUCN listed the horn shark as Least Concern (Carlisle, 2014). For the horn shark, a reference genome is available at National Center for Biotechnology Information (NCBI) (GCF_036365525.1). This genome has a size of 6 Gb and consists of 51 chromosomes (NCBI, 2023). Canfield et al. (2022) showed that, there is no population structure along the continuous coastline from Santa Barbara to San Diego (USA). A population structure was found between the California coast and the adjacent Channel Islands, indicating that horn sharks disperse along the coastline but not across deep and open water (Frankham, 1997). They also found that genetic diversity was lowest at the most geographically isolated islands which is observed in many other species and often correlated with distance, dispersal capability, and population size (Frankham, 1997). Additionally, Canfield et al. (2022) detected a significant genetic break at Punta Eugenia (Mexico), which is a well-known biogeographic barrier for coastal marine life (Briggs & Bowen, 2012). The study of Canfield et al. (2022) was based on the mitochondrial DNA control regions, which only traces the mother, resulting in an incomplete reflection of demographic history (Kivisild, 2015). Additionally, genetic diversity can differ between wild and captive populations (Jiang et al., 2005). For this research, the collaboration with Artis Royal Zoo aims to investigate the genome-wide genetic diversity of eight captive horn sharks (two females, one male and five offspring). The male and the females are the parents of the offspring; however, it was unknown which mother had which offspring. To prevent breeding of related sharks, this information is essential. Especially since they are part of their species' zoo breeding program.

Main research objective:

Investigate the genetic diversity of five captive shorttail nurse sharks and eight captive horn sharks to provide evidence-based genetic knowledge to the policy makers of breeding programs and rewilding projects and contribute to the understudied critically endangered shorttail nurse sharks.

Shorttail nurse shark (*Pseudoginglymostoma brevicaudatum*)

- Generate the first genome assembly for the shorttail nurse shark.
- Investigate genetic diversity of five shorttail nurse sharks.

Horn shark (*Heterodontus francisci*)

- Conduct a parent analysis of the horn shark of five offspring from two females and one male.
- Investigate genetic diversity of eight horn sharks.

Material and methods

This section explains the methods and tools that were used in this research. For both species, the genetic diversity analyses were conducted in a similar way. Therefore, there are three sub sections: shorttail nurse sharks, horn sharks, genetic diversity analyses. The first two describe information or methods specific to the species and the latter explains the genetic analyses that share most of the methods. The used versions of the tools can also be found in appendix IV. Additionally, a data management plan can be found in appendix VI.

Shorttail nurse sharks

In cooperation with Artis Royal Zoo (Amsterdam, The Netherlands), blood samples of five shorttail nurse sharks were provided. Table 1 shows the sex and DNA sequencing method per sampled shark. More information of the five shorttail nurse sharks can be found in appendix II.

Table 1. Information of the five shorttail nurse sharks.

<i>Shark ID</i>	<i>Sex</i>	<i>DNA sequencing method</i>
Pbrev001	Male	Illumina & Nanopore (10 Gb)
Pbrev002	Female	Illumina & Nanopore (70 Gb)
Pbrev003	Female	Illumina
Pbrev004	Female	Illumina
Pbrev005	Male	Illumina

Genome assembly and BUSCO

For the genome assembly, the Nanopore sequence data of sharks 1 and 2 were combined to obtain around 80 Gb data. After the low-quality reads were filtered (≤ 5000 base pair length), the genome was assembled by using the Nanopore long reads with Flye assembler (Kolmogorov et al., 2019), with an estimated genome size of 4.5 Gb. To assess the genome completeness, Benchmarking Universal Single-Copy Orthologs (BUSCO) vertebrata_odb10 (Tegenfeldt et al., 2025) was used.

Mapping and Variant Calling

The Illumina short reads of all shorttail nurse sharks were mapped with the BWA-MEM algorithm (Li, 2013) to the genome assembly. Mapping quality was investigated with flagstat of SAMtools (Danecek et al., 2021). For the ten longest contigs, variant calling was done with freebayes (Garrison & Marth, 2012). Only single nucleotide polymorphisms (SNPs) were retained and used for genetic analyses.

Horn sharks

In cooperation with Artis Royal Zoo, blood samples of eight horn sharks were provided. These samples were sent to the NovoGene lab to do Illumina whole genome resequencing. Table 2 shows the ID of eight horn sharks, their sex and sequence data. Additionally, the fourth column describes whether the shark is considered mother, father, or offspring. More information about the eight horn sharks can be found in appendix II.

Table 2. Information of the eight horn sharks.

<i>Shark ID</i>	<i>Sex</i>	<i>Data</i>	
Hfran001	Female	Illumina	Offspring
Hfran002	Female	Illumina	Offspring
Hfran003	Female	Illumina	Offspring
Hfran004	Female	Illumina	Offspring
Hfran005	Female	Illumina	Offspring
Hfran006	Male	Illumina	Father
Hfran007	Female	Illumina	Mother
Hfran008	Female	Illumina	Mother

Mapping and Variant Calling (mitochondrial genome)

Because mitochondrial genome is smaller than the nuclear genome (for horn sharks: 16,714 bp) and only passed down by the mother, this was used to quickly find out which mother had which offspring. The Illumina reads of all horn sharks were mapped against the reference mitochondrial genome of the horn sharks (National Center for Biotechnology Information, 2023), using the BWA-MEM algorithm (Li, 2013). After this mapping, variant calling of the mitochondrial genome was done using freebayes (Garrison & Marth, 2012). Finally, Integrative Genomics Viewer (IGV) (Robinson et al., 2011) was used to look at the common variants to determine the mother of each offspring.

Mapping and Variant Calling (nuclear genome)

To investigate the relationship between all sharks, Illumina reads of were mapped to the nuclear reference genome of the horn shark (National Center for Biotechnology Information, 2023), using the BWA-MEM algorithm (Li, 2013). Mapping quality was investigated with flagstat of SAMtools (Danecek et al., 2021). After this, variant calling on all 51 chromosomes was done using freebayes (Garrison & Marth, 2012). Only single nucleotide polymorphisms (SNPs) were retained and used for genetic analyses.

Genetic diversity analyses

Because the genetic diversity analyses methods were similar for both species, this part was combined. The genetic analyses for the shorttail nurse sharks were conducted using SNPs of the ten longest contigs. For the horn sharks, the genetic analyses were conducted using SNPs of all 51 chromosomes. PLINK (Purcell et al., 2007) was used for Identity by Descent (IBD) and the inbreeding coefficient was based on SNP heterozygosity. Runs of Homozygosity (RoH) were found with PLINK (Purcell et al., 2007) and with BCFtools (Danecek et al., 2021). The distribution of RoH was visualised with Matplotlib (Hunter, 2007), NumPy (Harris et al., 2020) and pandas data frame (McKinney, 2010) in Python (Python Software Foundation, 2019). The inbreeding coefficients based on the Runs of homozygosity (F_{RoH}) were visualised with Microsoft Excel (Microsoft Corporation, 2021).

Nucleotide Diversity

The nucleotide diversity (π) across 100 kb windows was calculated by using VCFtools (Danecek et al., 2011), which in single-individual window-based analyses reflects per-site heterozygosity. This was visualised by histograms to investigate the distribution of mean π and the difference of π between when including and excluding runs of homozygosity. This provided an estimated overview between recent inbreeding and older demographic history (Bosse et al., 2012; Ceballos et al., 2018).

Identity by Descent

The Identity by Descent (IBD) tool of PLINK was used to investigate the relatedness among sharks in both species.

Inbreeding coefficient based on SNP heterozygosity

Inbreeding coefficient based on SNP heterozygosity was calculated for the five shorttail nurse sharks and for the eight horn sharks using PLINK. In this analysis, only the biallelic autosomal SNPs were used.

Runs of Homozygosity

To investigate and quantify genetic diversity within each shark, Runs of Homozygosity (RoH) were determined. RoH are continuous regions in the genome where both alleles are identical (homozygous) and are commonly used to detect recent inbreeding and demographic history (Bosse et al., 2012; Stanhope et al., 2023). In this research, RoH were detected using two methods: PLINK and BCFtools. The main difference between PLINK and BCFtools is that PLINK uses a sliding window-based approach and BCFtools uses a Hidden Markov Model to detect RoH; BCFtools is probabilistic and PLINK is based on rules. PLINK has multiple input parameters (thresholds for window size, SNP density, maximum allowable heterozygous sites, and missing data), where with BCFtools only the minimal length threshold for RoH detection can be adjusted.

Inbreeding coefficient based on Runs of Homozygosity

The inbreeding coefficient (F_{RoH}) of each shark was determined using the results of PLINK and BCFtools. The number and length of RoH reflect individual demographic history (Bosse et al., 2012; Ceballos et al., 2018). Therefore, F_{RoH} was calculated for three RoH length thresholds: >100kb length to account for noise ($F_{RoH>100kb}$), >500kb and >1Mb lengths for moderate RoH length ($F_{RoH>500kb}$ and $F_{RoH>1Mb}$). The F_{RoH} of the shorttail nurse sharks was calculated by dividing the sum length of all RoH of the ten longest contigs by the total sum of the ten longest contigs. For the horn sharks, the F_{RoH} was calculated by dividing the sum of all RoH by the total nuclear genome length.

Results

Shorttail nurse shark

Genome assembly

The shorttail nurse shark Nanopore data resulted in an assembly of 9,610 contigs, N50 of 1.68 Mb (1,676,246 base pairs (bp), 502 contigs) and total length of 3.03 Gb (3,030,708,863 bp). The minimal and maximum contig lengths were 105 and 11,228,670 bp with an average contig length of 315,370.3 bp (Q1=8,994 bp; Q2=27,910.5 bp; Q3=188,278 bp). The GC content was 42.94% and the weighted coverage was 14.862x.

BUSCO

Total BUSCO groups searched was 3,354 of which 91.7% complete BUSCOs (single copy: 89.9%; duplicated: 1.8%), 4.5% fragmented and 3.8% missing BUSCOs.

Mapping quality

Table 3 shows the mapping quality of the five shorttail nurse sharks. A total of 0.83-0.93 billion Illumina reads were mapped to the genome assembly of this research. Between 99.59 and 99.81% of the reads were successfully mapped to the genome assembly of which between 99.57 and 99.80% were primarily mapped and between 86.32 and 89.04% were properly paired. Just between 0.09 and 0.14% of the reads were mapped to the genome only once.

Table 3. Mapping quality statistics of the shorttail nurse sharks.

Shark ID	Total reads	Mapped reads (%)	Primary mapped reads (%)	Properly paired reads (%)	Singletons (%)
Pbrev001	933,365,607	99.78	99.77	87.29	0.09
Pbrev002	928,834,507	99.81	99.80	87.16	0.09
Pbrev003	932,780,491	99.81	99.80	86.32	0.09
Pbrev004	865,496,708	99.59	99.57	89.04	0.14
Pbrev005	829,079,056	99.62	99.61	88.98	0.14

Variant calling

Variant calling was done for the ten longest contigs (total sum length = 91,767,680 bp). Out of 212,510 variants, 187,605 single nucleotide polymorphisms (SNPs) were kept after filtering. With a SNP density of 2.04 SNPs per kb, these were used for downstream analyses.

Nucleotide diversity

Figure 3 shows a histogram of the nucleotide diversity (π) in 100kb windows for each of the five shorttail nurse sharks. The x-axis shows the mean π per window, and the y-axis shows the number of windows with this mean π . This was done for the ten longest contigs. The plots of all sharks show two peaks, one shared around a π of 0.0009 and one variable peak around π near zero. Shark 1 shows the highest and shark 4 the lowest abundance of π near zero. Table 4 shows that the average calculated mean π is 0.000809 (0.809/kb) and π for excluding the π near zero is 0.000862 (0.862/kb).

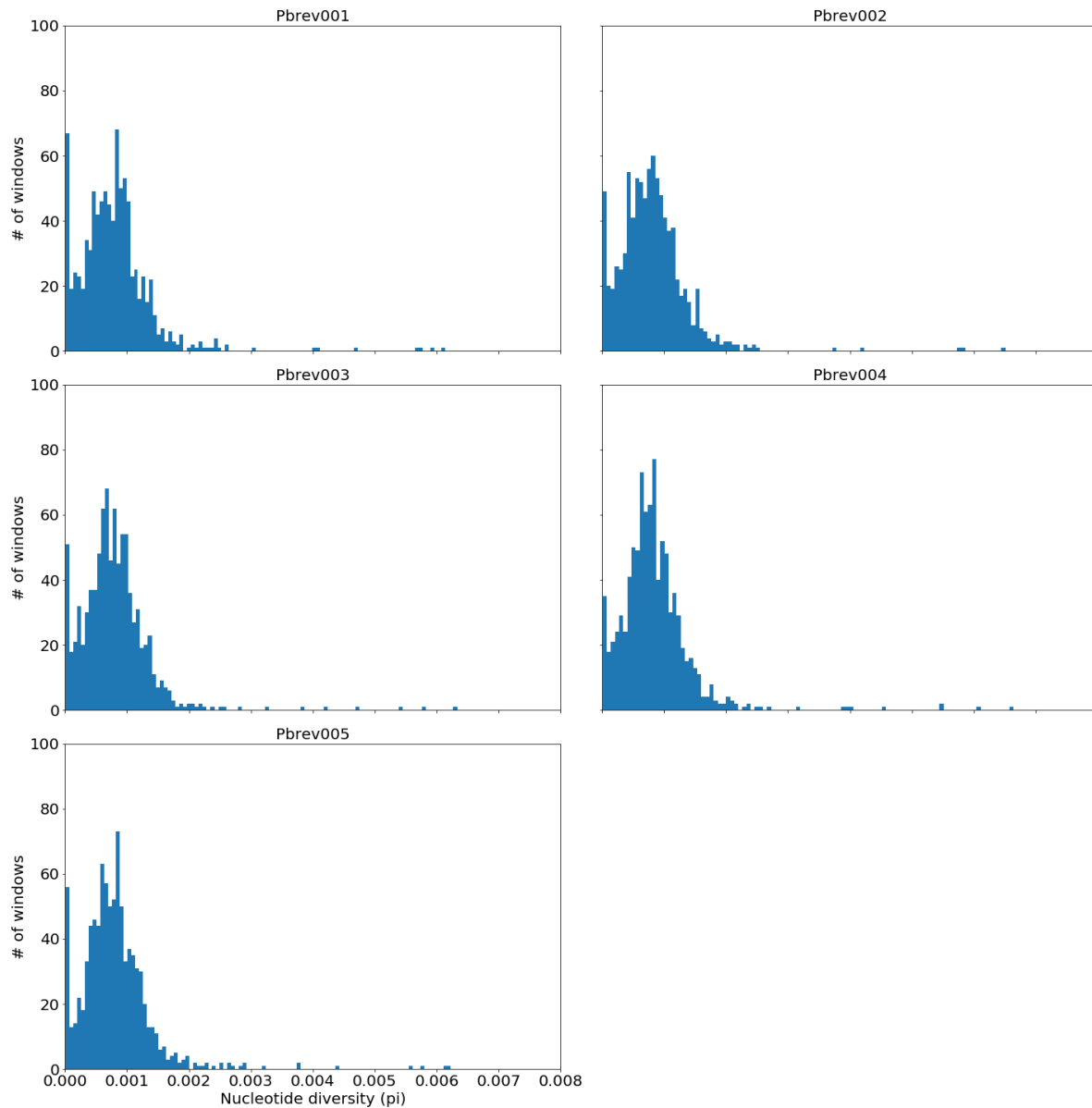


Figure 1. Histogram of mean nucleotide diversity (π) (x-axis) across 100kb for ten longest contigs for five shorttail nurse sharks.

Table 4. Mean nucleotide diversity (π) and mean nucleotide outside regions of near zero π (mean $\pi > 0.0001$) per shorttail nurse shark.

	Pbrev001	Pbrev002	Pbrev003	Pbrev004	Pbrev005	Average π
Mean π	0.000792	0.000804	0.000795	0.000842	0.000814	0.000809
Mean $\pi > 0.0001$	0.000864	0.000856	0.000847	0.000878	0.000867	0.000862

Identity by Descent

The Identity by Descent matrix (table 5) shows that based on the ten longest contigs, sharks 1, 2 and 3 are unrelated to each other, because the estimated relatedness is zero. Shark 4 is the offspring of shark 1 and 2, because the estimated relatedness was 0.500. Finally, shark 5 had an estimated relatedness of around 0.125 with sharks 2, 3 and 4.

Table 5. Identity by Descent matrix with estimated relatedness of five shorttail nurse sharks.

Shark ID	Pbrev005	Pbrev004	Pbrev003	Pbrev002	Pbrev001
Pbrev005		0.113	0.125	0.128	0
Pbrev004			0	0.500	0.500
Pbrev003				0	0
Pbrev002					0
Pbrev001					

Inbreeding coefficients based on SNP heterozygosity

For each of the five shorttail nurse sharks, inbreeding coefficients were calculated using the observed versus the expected homozygous genotypes (table 6). All inbreeding coefficients were negative. Shark 4 had the most negative inbreeding coefficient of -0.2349 and shark 1 had the least negative inbreeding coefficient of -0.1309.

Table 6. Observed vs expected homozygosity and inbreeding coefficient per shorttail nurse sharks.

Shark ID	Observed homozygosity	Expected homozygosity under HWE	Inbreeding coefficient F
Pbrev005	0.596	0.660	-0.1864
Pbrev004	0.580	0.660	-0.2349
Pbrev003	0.609	0.660	-0.1494
Pbrev002	0.608	0.659	-0.1501
Pbrev001	0.615	0.659	-0.1309

Runs of Homozygosity PLINK

PLINK found RoH in all the shorttail nurse sharks (figure 4). The longest RoH were between 1 and 2 million base pairs of length and were found in sharks 1 and 2. Maximum RoH lengths between 500 thousand and 1 million were found in shark 3. Sharks 4 and 5 showed only RoH lengths between 100 and 500 thousand, where shark 5 showed more RoH lengths between 200 and 500 thousand than shark 4.

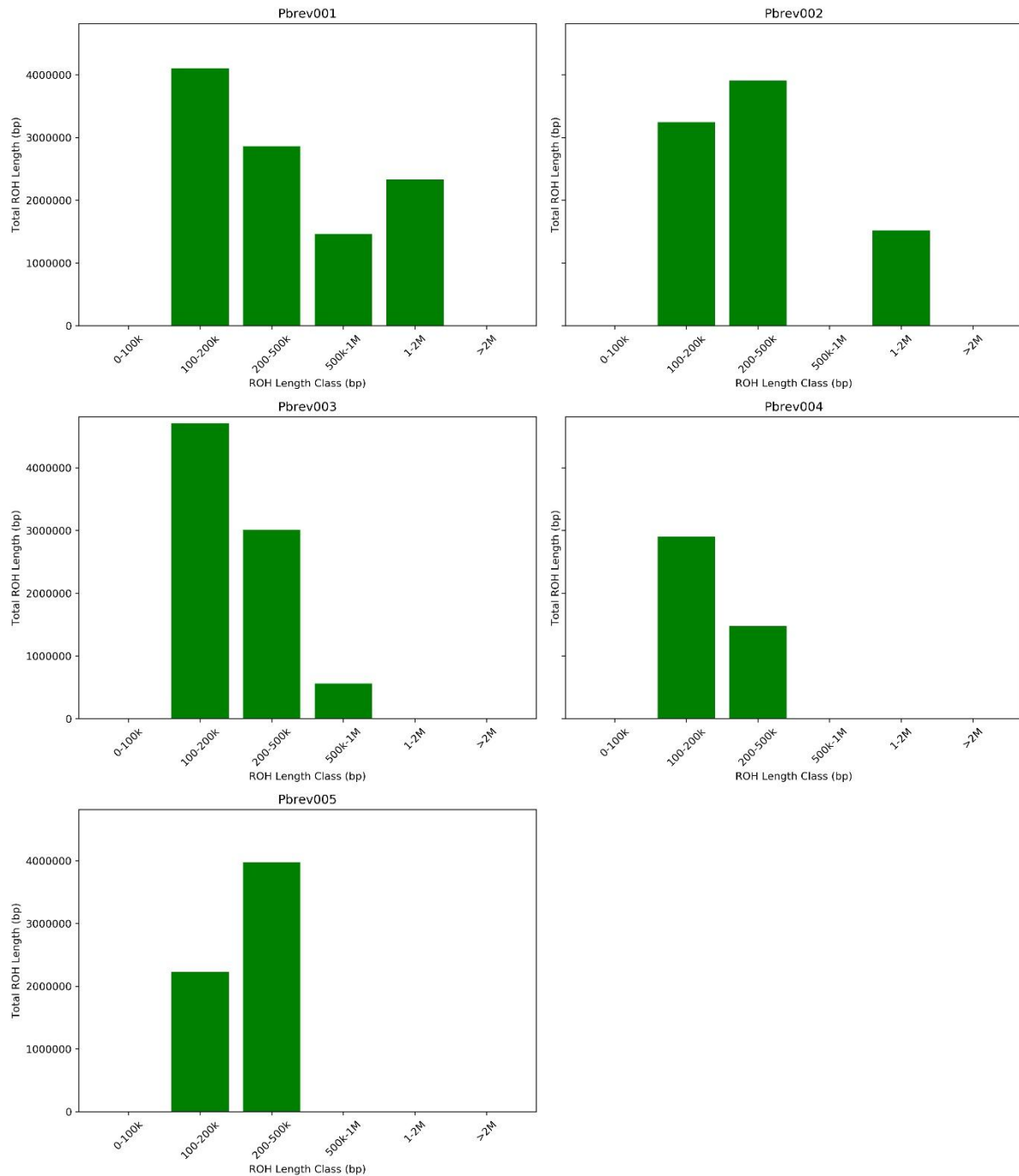


Figure 2. Histogram of RoH length classes for five shorttail nurse sharks (PLINK).

Runs of Homozygosity BCFtools

BCFtools also found RoH in all shorttail nurse sharks (figure 5). Sharks 1 and 2 showed large RoH of between 1 and 2 million base pairs, shark 3 showed a maximum RoH length between 500 thousand and 1 million and sharks 4 and 5 showed a maximum RoH length between 200 and 500 thousand.

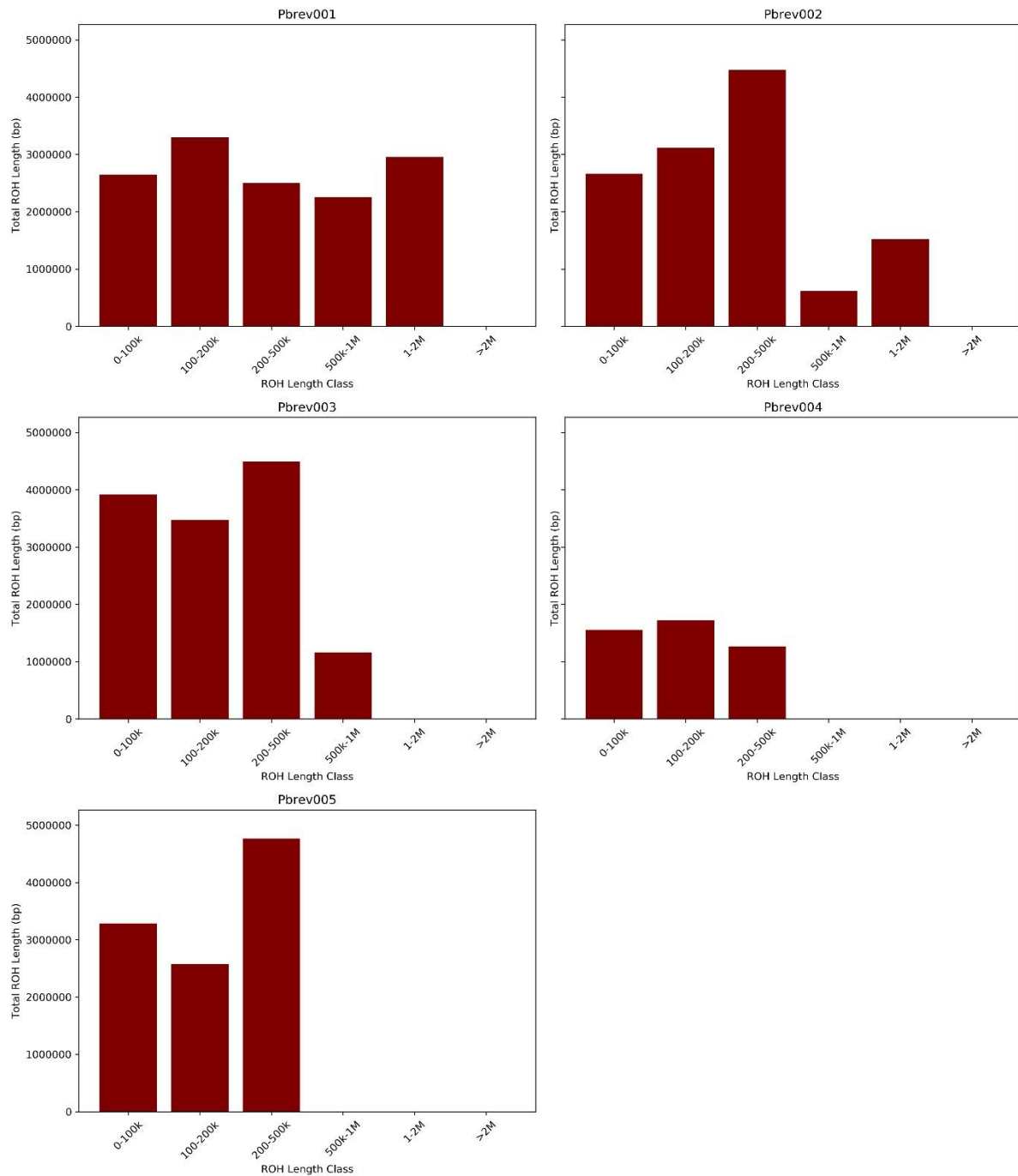


Figure 3. Histogram of RoH length classes for five shorttail nurse sharks (BCFtools).

Inbreeding coefficient based on Runs of Homozygosity (F_{RoH})

The F_{RoH} was calculated with three thresholds for each shark and for PLINK and BCFtools (figure 6). For both methods and with all thresholds, shark 1 showed the highest F_{RoH} , with a $F_{RoH>1Mb}$ of 0.0254 for PLINK and 0.0322 for BCFtools. Shark 2 also showed high F_{RoH} values, with a $F_{RoH>1Mb}$ of 0.0165 for both PLINK and BCFtools. Shark 3 showed moderate F_{RoH} values, with a $F_{RoH>500kb}$ of 0.0061 for PLINK and 0.0127 for BCFtools. For sharks 4 and 5, the $F_{RoH>100kb}$ were 0.0478 and 0.0676 for PLINK and 0.0326 and 0.08 for BCFtools. Sharks 1 & 2 showed the highest inbreeding coefficients and shark 4 the lowest.

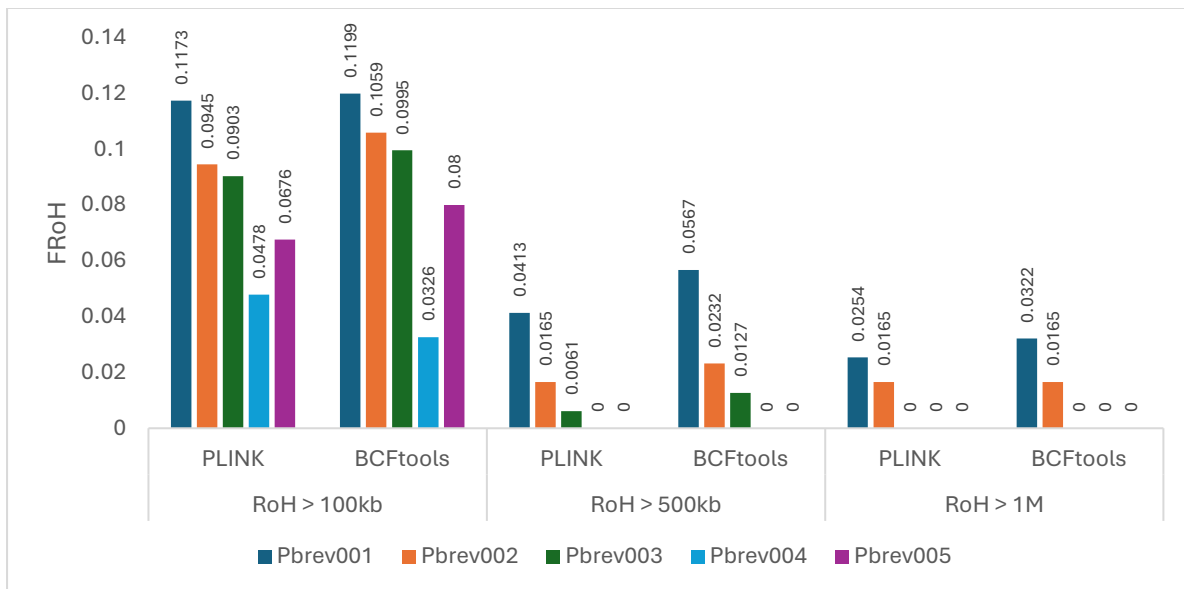


Figure 4. F_{RoH} per RoH length threshold per shorttail nurse shark for PLINK and BCFtools.

Horn sharks

Mapping quality

All horn sharks had at least 99.60% of the reads mapped and at most 0.14% singletons (table 7). Total reads of sharks 1 to 5 are around 300,000,000 whereas for sharks 6, 7 and 8 the total reads were around 1,000,000,000.

Table 7. Mapping quality statistics of the horn sharks.

Shark ID	Total reads	Mapped reads (%)	Primary mapped reads (%)	Properly paired reads (%)	Singletons (%)
Hfran001	296,813,711	99.60	99.60	94.70	0.14
Hfran002	309,134,105	99.60	99.60	94.48	0.14
Hfran003	279,918,655	99.74	99.73	95.64	0.09
Hfran004	342,320,166	99.72	99.72	95.08	0.09
Hfran005	324,709,624	99.72	99.71	95.01	0.09
Hfran006	1,016,453,180	99.69	99.69	95.24	0.09
Hfran007	1,171,956,387	99.72	99.72	95.31	0.08
Hfran008	943,771,943	99.72	99.72	94.96	0.08

Variant calling mitochondrial genome.

The eight horn sharks showed 20 variants over a mitochondrial genome length of 16,714 base pairs. These 20 variants were investigated in IGV to determine the mother of sharks 1 to 5 (figure 5). The genotypes of the mothers (sharks 7 and 8) differed. Because the mitochondrial genome is only inherited maternally, mother and offspring share the same genotype. Based on these variants, it was determined that sharks 3 and 5 are the offspring of shark 6 and 8 and sharks 1, 2 and 4 are the offspring of sharks 6 and 7 (see pedigree in figure 7).

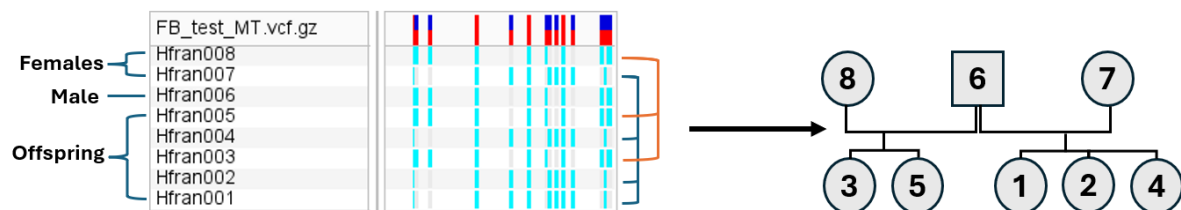


Figure 5. IGV interface with all variants for the eight horn sharks and the pedigree that can be derived from these variants.

Variant calling nuclear genome.

Variant calling was done for all 51 chromosomes, which resulted in 21,942,550 variants. After filtering 17,313,019 SNPs (SNP density of 2.88 SNPs per kb) remained and were used for downstream analyses.

Nucleotide diversity

All eight sharks shared a peak of a nucleotide diversity around 0.0017, this is the nucleotide diversity outside RoH regions (figure 8). Around a nucleotide diversity of zero (RoH regions), sharks showed different peak heights. Shark 8 showed the highest peak at around a nucleotide diversity of zero and shark 6 and 7 the lowest. Table 8 shows that the average calculated mean π is 0.001612 and π for excluding the π near zero is 0.001680.

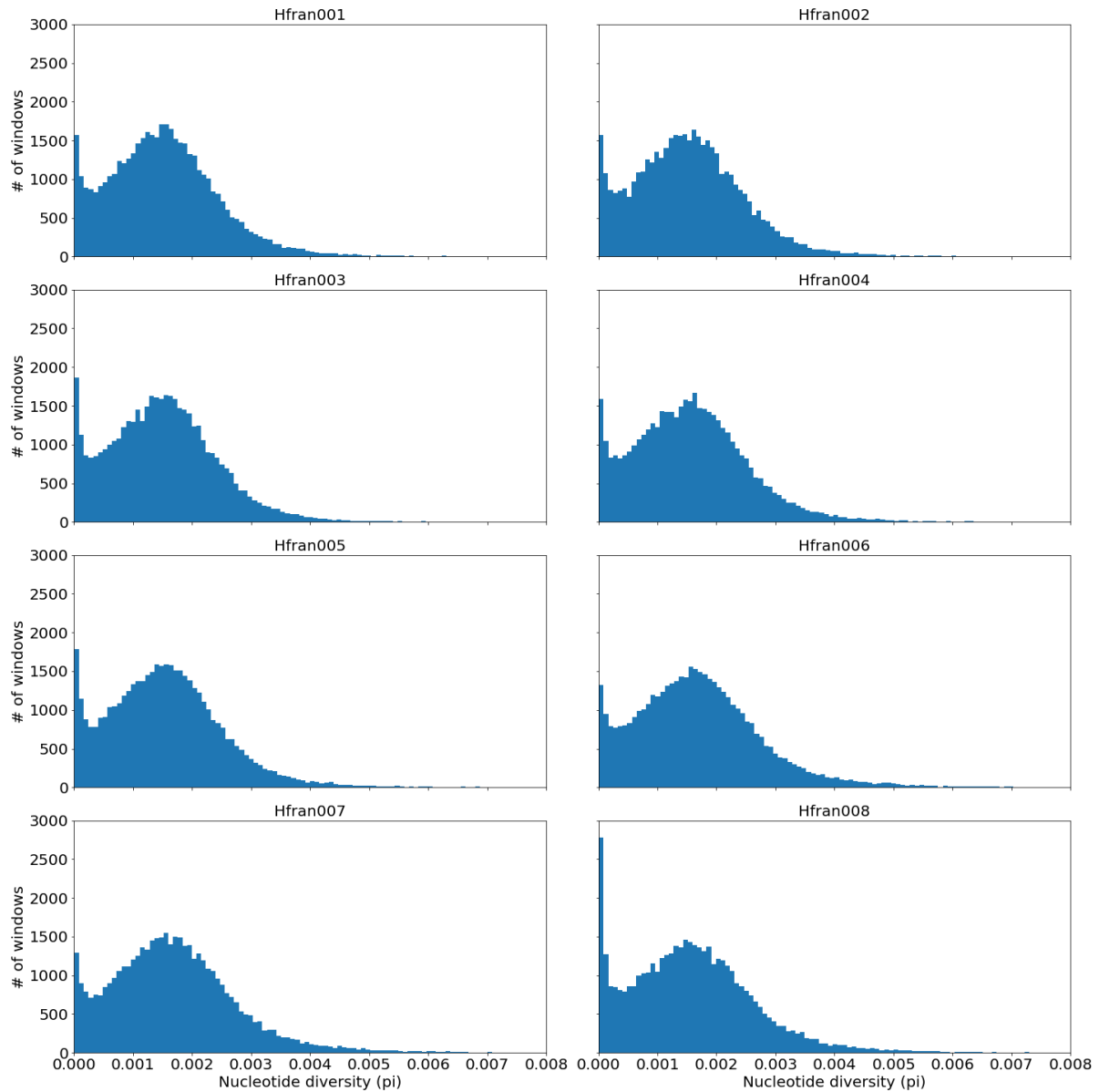


Figure 6. Histogram of nucleotide diversity (π) across 100kb for eight horn sharks.

Table 8. Mean heterozygosity (π) and mean nucleotide outside regions of near zero π (mean $\pi > 0.0001$) per horn shark.

	Hfran 001	Hfran 002	Hfran 003	Hfran 004	Hfran 005	Hfran 006	Hfran 007	Hfran 008	Mean π
Mean π	0.001546	0.001565	0.001533	0.001593	0.001577	0.001730	0.001734	0.001616	0.001612
Mean $\pi > 0.0001$	0.001606	0.001625	0.001603	0.001655	0.001646	0.001786	0.001789	0.001729	0.001680

Identity by Descent

Table 9 shows the estimated genome-wide relatedness between the eight horn sharks. It showed that shark 8 is unrelated to sharks 7, 6, 4, 2 and 1. However, shark 8 showed a relatedness of 0.4128 and 0.3994 with sharks 5 and 3. It also showed that shark 7 is unrelated to sharks 5 and 3, but is related to sharks 4, 2 and 1 with a relatedness of 0.4241, 0.4115 and 0.4042. IBD also showed that shark 6 is indeed the father of all offspring. These results agreed with the results of the variants of the mitochondrial genome of figure 6 on the previous page. However, IBD showed that sharks 6 and 7 have a relatedness of 0.1176 which was not discovered by looking at the mitochondrial genome.

Table 9. Identity by Descent matrix with estimated genome-wide relatedness values of eight horn sharks.

Shark ID	Hfran008	Hfran007	Hfran006	Hfran005	Hfran004	Hfran003	Hfran002	Hfran001
Hfran008		0	0	0.4128	0	0.3994	0	0
Hfran007			0.1176	0	0.4241	0	0.4115	0.4042
Hfran006				0.4151	0.4209	0.4064	0.4105	0.4027
Hfran005					0.1820	0.3883	0.1594	0.1756
Hfran004						0.1587	0.3512	0.2785
Hfran003							0.1921	0.1527
Hfran002								0.3573
Hfran001								

Inbreeding coefficients based on SNP heterozygosity

Based on SNP heterozygosity, the inbreeding coefficient of each shark was negative (table 10). The inbreeding coefficient of shark 7 was the most negative (-0.2761) and the least negative inbreeding coefficient was observed in shark 3 (-0.1544).

Table 10. Observed vs expected homozygosity and inbreeding coefficient per horn sharks.

Shark ID	Observed homozygosity	Expected homozygosity under HWE	Inbreeding coefficient F
Hfran008	0.576	0.642	-0.1852
Hfran007	0.543	0.642	-0.2761
Hfran006	0.544	0.642	-0.2751
Hfran005	0.578	0.643	-0.1800
Hfran004	0.574	0.643	-0.1925
Hfran003	0.587	0.642	-0.1544
Hfran002	0.581	0.643	-0.1736
Hfran001	0.585	0.642	-0.1611

Runs of Homozygosity PLINK

Figure 9 shows that PLINK found RoH in every horn shark. Sharks 4, 5 and 6 had maximum RoH between one and two million base pairs. RoH between two and three million base pairs was found in shark 1 and 3. And in sharks 2, 7 and 8, RoH between 3 and 4 million base pairs were found. It also showed that sharks 1 to 7 share a similar distribution of RoH. However, shark 8 clearly showed a greater abundance of RoH in all length classes. No RoH were found with a length less than 100, because this was put as a threshold of PLINK.

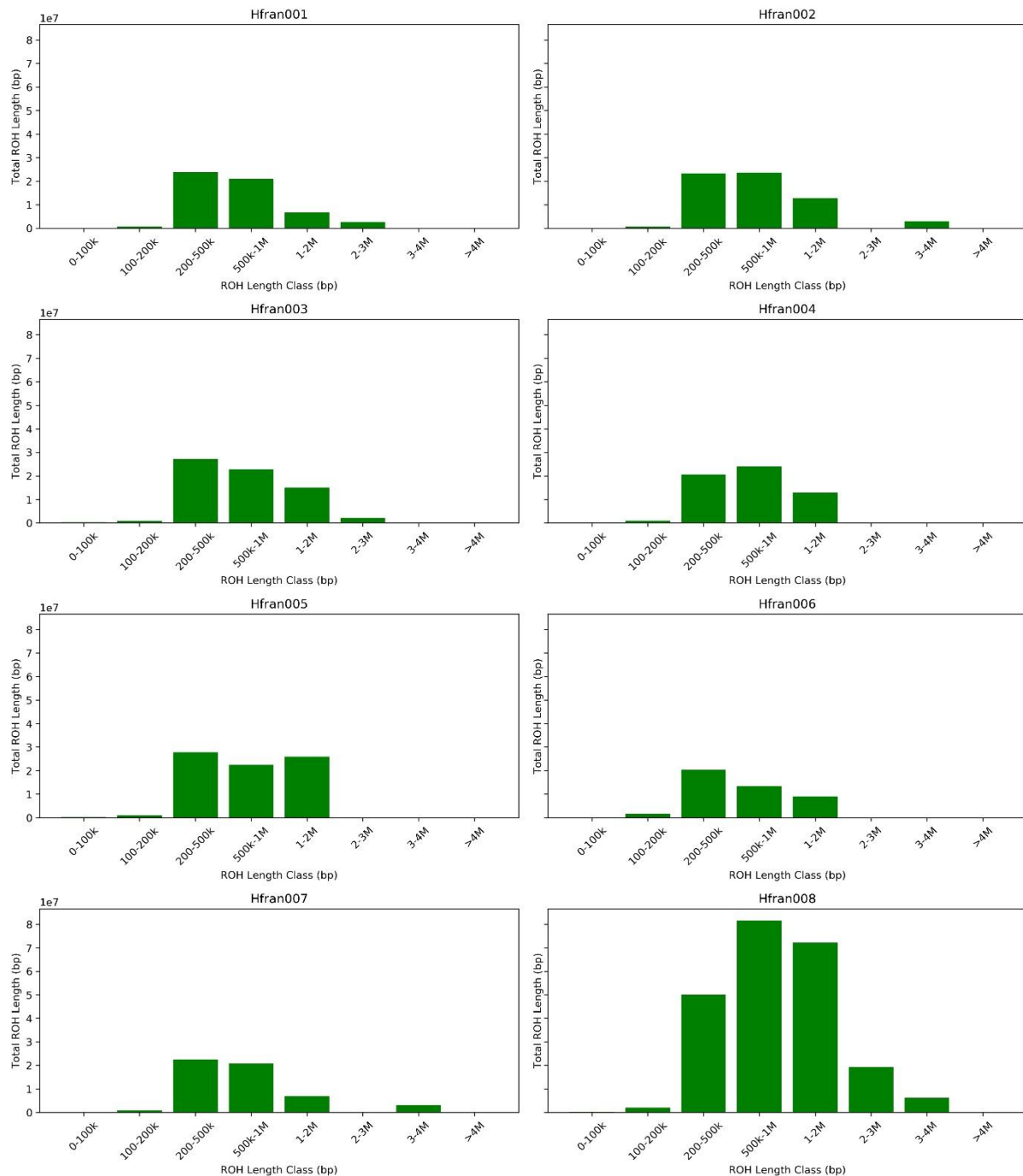


Figure 7. Histograms of RoH length classes for eight horn sharks (PLINK).

Runs of Homozygosity BCFtools

Figure 10 shows that BCFtools found RoH in all sharks up until a RoH length between one and two million base pairs. Sharks 1 to 6 showed a similar RoH length with a maximum RoH length between 1 and 2 million. Shark 7 showed a maximum RoH length between 500 thousand and 1 million and shark 8 showed the highest abundance across the sharks of a RoH length between 1 and 2 million.

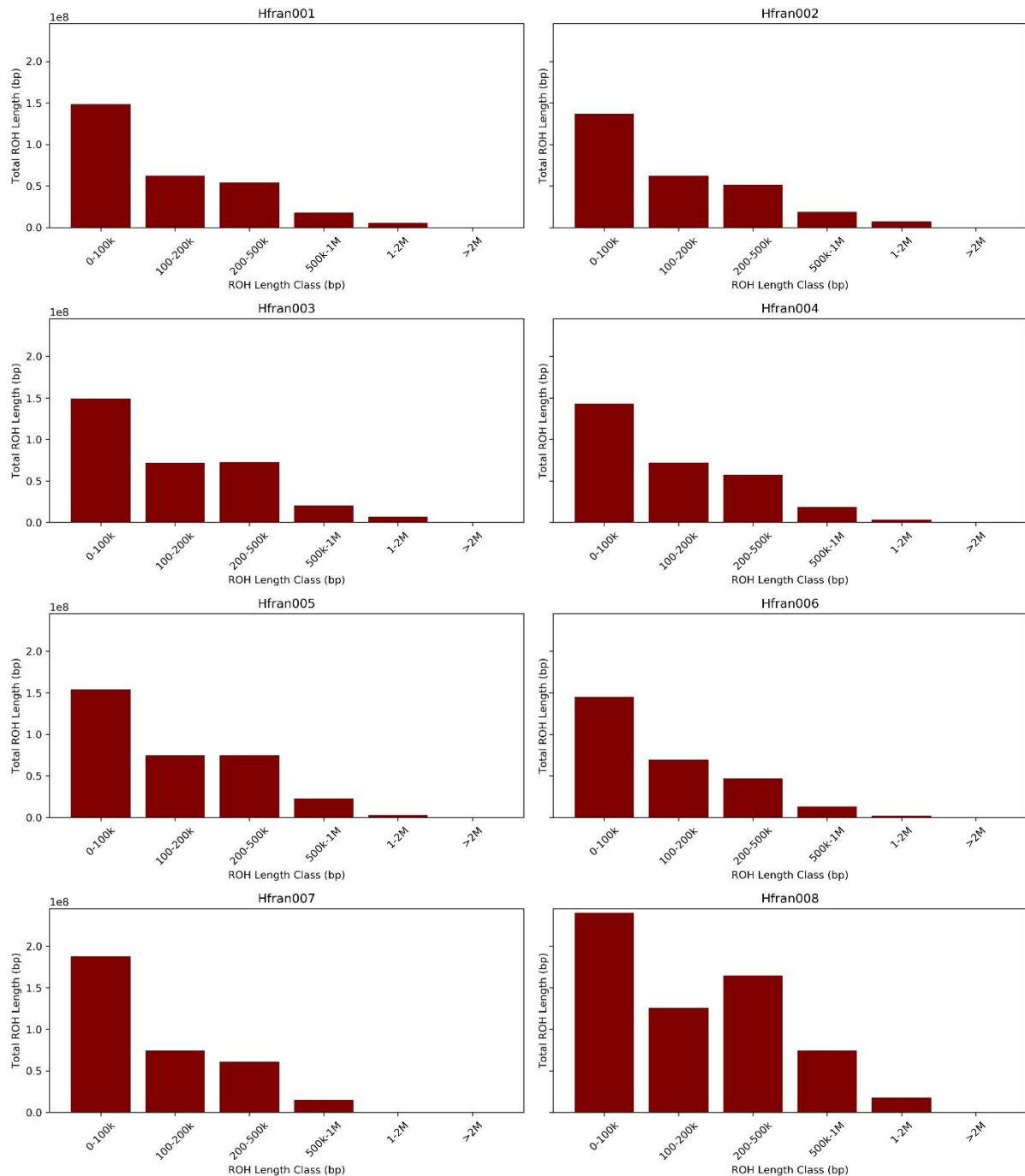


Figure 8. Histogram of RoH length classes for eight horn sharks (BCFtools).

Inbreeding coefficients based on Runs of Homozygosity

Across both methods with all three RoH length thresholds, shark 8 showed the highest and shark 7 showed the lowest F_{RoH} (figure 11). F_{RoH} of sharks 1 to 7 were similar within each method and within each RoH length threshold class.

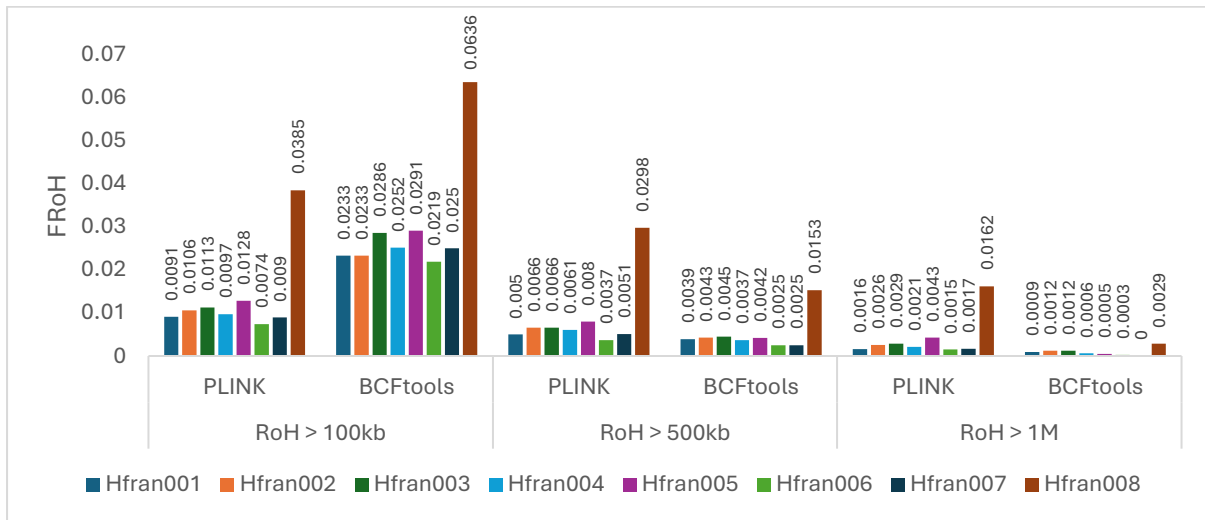


Figure 9. F_{RoH} per RoH length threshold per horn shark for PLINK and BCFtools.

Discussion

Genome assembly shorttail nurse shark

In this research, the first ever genome of the shorttail nurse shark was assembled with a total length of 3.03 Gb, 9,610 contigs, N50 = 1.68 Mb. Even though this assembly was very fragmented, the BUSCO score of 91.7% complete and 3.8% missing showed that the gene space was particularly good despite the fragmentation. Additionally, the BUSCO score is especially good when considering that the vertebrata_odb10 dataset predominantly reflects bony vertebrates rather than cartilaginous fish such as sharks. Because sharks diverged from bony vertebrates 420-425 million years ago (Chen et al., 2012), the BUSCO markers may be very divergent in sharks which affects the BUSCO completeness score. The genome length, however, is likely underestimated because sharks show larger genome sizes (Dufresne & Jeffery, 2011). Additionally, the genome length is underestimated due to the high-level of fragmentation of the assembly. The genome assembly was done by combining Nanopore data of two shorttail nurse sharks, one female (70Gb) and one male (10Gb). Because the female has two copies of the X chromosome, sequencing reads covered regions evenly which ensured a uniform coverage, improved completeness, and a cleaner and more contiguous assembly. The male sequence data can recover the Y-chromosome, however, in this study, the assembly was not at chromosome level and consisted of 9,610 contigs. But when PacBio and Hi-C data is available, the sex chromosomes can be recovered. This can achieve a scaffold or even chromosome-level assembly necessary to conserve this ancient, understudied, yet critically endangered shorttail nurse shark.

Identity by Descent

Shorttail nurse sharks

The Identity by Descent (IBD) analysis of PLINK showed that shorttail nurse sharks 1, 2 and 3 are unrelated to each other. This result justified the assumption that the animals from the wild are unrelated. The IBD analysis also showed that shorttail nurse shark 4 is the offspring of 1 and 2 and shark 5 is related to sharks 2, 3 and 4 with a relatedness of approximately 0.125. This was only based on around 3% of the total genome length, so the shared parts could be coincidence or an inflation of the real relatedness due to no Linkage Disequilibrium (LD) pruning. However, as the origin and pedigree of shorttail nurse shark 5 is unknown, a relatedness between the individuals cannot be excluded. If a scaffold or even chromosome level assembly is available, this would be important to verify and investigate in more detail because it is important for the future breeding program inclusion of this animal.

Horn sharks

The IBD analysis of the horn sharks showed that sharks 1, 2 and 4 are the offspring of sharks 6 and 7 and sharks 3 and 5 are the offspring of sharks 6 and 8. The results of the IBD and the variant calling on the mitochondrial genome resulted in the same pedigree. However, the IBD analysis showed that sharks 6 and 7 have an estimated relatedness of approximately 0.125, this relatedness was not observed in the pedigree of the breeding program. This is often the case, where inbreeding based on the pedigree is lower than the true inbreeding because pedigree based inbreeding misses older shared ancestry (Knief et al., 2015).

SNP density

The ten longest contigs of the shorttail nurse shark genome assembly (total sum length of 91,767,680 bp) had a SNP density of 2.04 SNPs/kb and the horn shark had a SNP density of 2.88 SNPs/kb. These are comparable to what was observed in the white-spotted bamboo shark (2.48 SNPs/kb) (Zhao et al., 2022), much higher than that of the wild-caught common carp (0.11 SNPs/kb) (Yuan et al., 2018) and much lower than that of the rainbow trout (15.6 SNPs/kb) (Gao et al., 2018) and three spine stickleback fish (10 SNPs/kb) (Shanfelter et al., 2019). However, due to assembly fragmentation and repetitive regions, this SNP density of the shorttail nurse shark can be an overestimation of the real diversity (Dallaire et al., 2023). Additionally, no linkage disequilibrium (LD) pruning was done for this research because LD estimation requires many and low related individuals. In both species, small sample sizes with high relatedness were observed. Therefore, it is possible that the heterozygosity estimates are slightly inflated due to linkage among SNPs.

Inbreeding coefficients based on SNP heterozygosity

For a species that is critically endangered, it was expected that the inbreeding coefficients based on the SNP heterozygosity would be positive. However, the calculated inbreeding coefficients were negative for all shorttail nurse shark and horn sharks, suggesting outbreeding. This was likely due to an inflation of expected homozygosity because only five and eight sharks were being used in this research. Furthermore, many sharks within species are related which violated the assumption of PLINK that individuals are independent. This explains why shared rare alleles appear more often than expected. This caused the Hardy-Weinberg Equilibrium expectation to be too homozygous which led to lower observed homozygosity than expected homozygosity. This ultimately resulted in negative inbreeding coefficients which are not indicative for real inbreeding coefficients.

Nucleotide diversity

When looking at the mean nucleotide diversity (π) across 100kb windows, two peaks can be observed in both shark species (figure 3 and 8). One shared peak within species and one peak at π near zero which showed to be very different among sharks within species. The shorttail nurse sharks shared a peak at π around 0.862/kb (based on the ten longest contigs) and the horn sharks shared a peak at π around 1.680/kb (based on all 51 chromosomes). This peak represents the mean π outside regions of π near zero and suggests that, within-species, these animals share a demographic history. The mean π outside regions of π near zero can also be referred to as the genome-wide heterozygosity. In this research, the π of shorttail nurse shark was based on the ten longest contigs so the heterozygosity of 0.862/kb is only an estimate of the genome-wide heterozygosity. In the horn shark, the genome-wide mean heterozygosity was 1.612/kb, which is almost twice as high than for the shorttail nurse sharks. The genome-wide mean heterozygosity of the great hammerhead is comparable to the estimated genome-wide mean heterozygosity of the shorttail nurse sharks (0.528/kb (Stanhope et al., 2023)). However, both the shorttail nurse sharks and horn sharks showed much lower genome-wide mean heterozygosity than the shortfin mako (3.578/kb) (Stanhope et al., 2023) and white-spotted bamboo shark (5.1/kb) (Zhao et al., 2022).

Inbreeding coefficients based on Runs of Homozygosity

The nucleotide diversity plots (figure 3 and 8) showed a peak at a π near zero, which represents recent inbreeding events, and it is different across the individuals. Shorttail nurse sharks 1 and 2 and horn shark 8 showed the highest peak at π near zero and subsequently show the most and longest Runs of Homozygosity (RoH) and highest inbreeding coefficient (F_{RoH}). RoH lengths decrease over each generation if unrelated animals reproduce, creating a relationship between RoH length and population history (Bosse et al., 2012; Kirin et al., 2010). This can explain why horn sharks 3 and 5 still show inbreeding even though the parents (sharks 6 and 8) are unrelated. Similar inbreeding coefficients were observed for horn sharks 1, 2 and 4 where their parents (sharks 6 and 7) showed to be distant relatives.

RoH were identified by using both PLINK and BCFtools, which have different underlying models. PLINK uses a sliding-window approach and marks homozygous SNPs as an RoH if they meet predefined thresholds, such as minimum number of SNPs, minimum RoH length and maximum allowed heterozygosity within a window. In other words, they are based on rules which can be adjusted accordingly. On the other hand, BCFtools uses a Hidden Markov Model to estimate whether regions are homozygous by descent. This makes BCFtools probabilistic and allows to consider genotype uncertainty, making it less sensitive to sequencing errors. Because the two methods differ in underlying models, the location and length of RoH was not the identical. This was visualised in appendix III, where the nucleotide diversity across 100kb windows across some of the chromosomes or contigs were plotted. The BCFtools RoH are visualised in red and the PLINK RoH are visualised in green.

The horn sharks showed a $F_{RoH>100kb}$ between 0.0074 and 0.0385 with PLINK and between 0.0219 and 0.0636 with BCFtools. The shorttail nurse sharks showed more inbreeding with $F_{RoH>100kb}$ between 0.0478 and 0.1173 with PLINK and between 0.0326 and 0.1199 with BCFtools. This is comparable to what is observed in the shortfin mako where the $F_{RoH>100kb}$ was 0.159. In the great hammerhead, even higher $F_{RoH>100kb}$ was observed: 0.744 (Stanhope et al., 2023). For $F_{RoH>1Mb}$, which represents even more recent inbreeding than $F_{RoH>100kb}$, seven of eight horn sharks showed $F_{RoH>1Mb}$ between 0 and 0.0012 (0.0015 and 0.0043) with BCFtools (PLINK) and one of them showed $F_{RoH>1Mb}$ of 0.0029 (0.0162) with BCFtools (PLINK). Shorttail nurse sharks 1 and 2 showed $F_{RoH>1Mb}$ of 0.0322 (0.0254) and 0.0165 (0.0165) with BCFtools (PLINK). The $F_{RoH>1Mb}$ of the shortfin mako (<0.01) (Stanhope et al., 2023) is comparable to that of the horn sharks, but much lower than the $F_{RoH>1Mb}$ of the shorttail nurse shark. In the great hammerhead the $F_{RoH>1Mb}$ of 0.087 was observed (Stanhope et al., 2023), which is much higher than both shark species in this research.

The F_{RoH} of the shorttail nurse shark are based on the RoH on the ten longest contigs with an average contig length of around 9 million base pairs, which is comparable to the smallest chromosome of the horn shark (NC_090420.1; around 10.5 million bp) (National Center for Biotechnology Information, 2023). The length of the RoH of the shorttail nurse shark is likely to be underestimated due to limited coverage and fragmentation of the genome assembly (Formenti et al., 2022; Lan et al., 2025; Shi et al., 2026). It is concerning that the highest F_{RoH} was found in shorttail nurse sharks 1 and 2 because these animals are from the wild. Elevated levels of inbreeding are related to small population sizes and are at risk of the effect of inbreeding depression (Blower et al., 2025; Brzeski et al., 2014; O'Grady et al., 2006). The lowest inbreeding of the shorttail nurse sharks was observed in animal 4, which is the offspring of unrelated sharks 1 and 2. As has been observed in the study of Canfield et al. (2022) and many more (Duncan et al., 2006; Pazmiño et al., 2018; Wagner et al., 2024), there can be a significant mainland and island coastal population structure, suggesting that the horn sharks rarely cross the open water from mainland to island coastal areas and the other way around (Frankham, 1997). This could also be possible for the shorttail nurse shark, where sharks 1 and 2 are from different geographical locations, for example one is from a population of the coastal area of Mozambique and the other from Madagascar, without gene flow between the populations. This could explain that even though high inbreeding was observed in shorttail nurse sharks 1 and 2, their offspring (shorttail nurse shark 4) shows the least inbreeding. In other words, sharks 1 and 2 are inbred due to related mating within their own population. If a chromosome-level assembly and genomic data of shorttail nurse shark from different locations from the wild are available, the mitochondrial control region and/ or microsatellite loci can be used to estimate the degree of population subdivision and gene flow (Canfield et al., 2022; Karl et al., 2012).

Conclusion and future implementations

Genomic research offers important knowledge for species conservation, especially when combined with resources of zoos. This collaboration between zoos and researchers enable genetic monitoring of captive populations, supports research on understudied and endangered species and can even provide essential knowledge for rewilding projects (Shaw et al., 2024). For the horn shark, it was discovered that sharks 6 and 7 are distantly related to each other and shark 8 has high inbreeding. For the shorttail nurse shark, the first ever genome of the species was assembled, and inbreeding was discovered in two out of three wild-caught shorttail nurse sharks. The observed inbreeding, which is likely an underestimation, in the wild-caught shorttail nurse sharks emphasizes that this critically endangered species is facing reduced population sizes which associated risk of loss of heterozygosity, inbreeding depression and genetic drift (Brzeski et al., 2014; O'Grady et al., 2006). These finding are highly relevant for conservation of both captive and wild populations. Zoos can play a significant role in the conservation of endangered species and the preservation of genetic diversity, for example, by providing biological samples, genomic data, and animal's origin information to researchers. This information offers valuable insights into genetic diversity, inbreeding, relatedness among individuals and even geographic dispersal and population numbers in the wild (DeWoody et al., 2021; Hohenlohe et al., 2021; Shaw et al., 2024; Speak et al., 2024). Both the shorttail nurse sharks and the horn sharks of this research are part of breeding programs coordinated by the European Association of Zoos and Aquaria (EAZA) (European Association of Zoos and Aquaria, 2025). The results of this study underscore the value of genomic research as it is essential for careful genetic management in breeding programs. Particularly the high inbreeding observed in two wild-caught shorttail nurse

shark emphasizes the importance of maintaining and documenting genetic diversity within the captive population to avoid further inbreeding. Additionally, the difference in pedigree and IBD relatedness in the horn sharks confirmed the importance of using genomic research in breeding programs of captive populations (Knief et al., 2015). Although horn sharks are not endangered and has an available chromosome-level assembly, doing research into this species can contribute to the general knowledge about shark genetics, which is still greatly understudied (Kuraku, 2021; Pearce et al., 2021). For future research, the need for a scaffold- or chromosome-level assembly of the shorttail nurse shark is high. This will enable more accurate estimates of genetic diversity and historical and recent inbreeding. This can be done by looking at RoH and infer historical changes in effective population size with Pairwise Sequentially Markovian Coalescent approach (PSMC) (Yuan et al., 2018). Additionally, the R package RZooRoH (Druet & Gautier, 2017) can provide the age classes of inbreeding based on RoH. RZooRoH is also probabilistic but goes further than BCFtools, providing inbreeding age classes to identify how many generations ago the inbreeding event occurred. Together with the findings of this research, these tools can be used to support breeding decisions, inform rewilding policies, and increase the role of zoos in the conservation of species.

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Appendices

I. Poster Zoology Conference about the horn sharks

Genomics reveals horn shark families and diversity

Using genomics to discover relatedness and genetic diversity in horn sharks (Heterodontus francisci)

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1. Wageningen University and Research, Animal Breeding and Genomics, Wageningen, The Netherlands 2. ARTIS Amsterdam Royal Zoo, Amsterdam, The Netherlands 3. Vrije Universiteit Amsterdam, The Netherlands 4. Wageningen University and Research, Marine Animal Ecology, Wageningen, The Netherlands

Background

Horn sharks

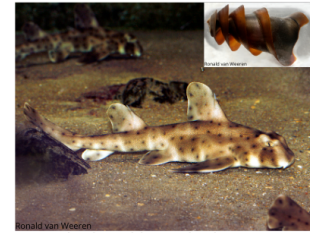
- Native to the coastal reefs of Mexico and the United States^a.
- Up until recently: listed as Data Deficient by the IUCN^b. Currently: listed as Least Concern^c.
- In small zoo populations, a good breeding program is needed to sustain a genetically healthy population.

What is genomics?

- Genomics is an interdisciplinary field of molecular biology focusing on the structure, function, evolution, mapping, and editing of genomes.

Why is genomics useful?

- Genomic research can discover **relatedness** and discover how **genetically healthy** populations/individuals are^{d,e}.



Horn shark and horn shark egg

Aim

To discover the relatedness between 8 horn sharks & investigate their genetic diversity. This enables good decision making for conservation of genetically healthy populations.

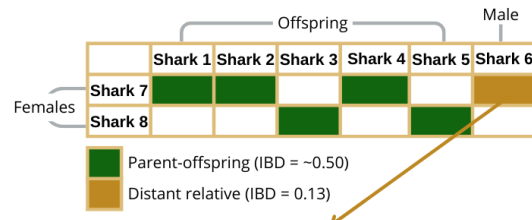
Results relatedness

Mother is determined by using **mitochondrial DNA (mtDNA)**. Mitochondrial DNA is only passed down the maternal line. By comparing mitochondrial DNA variants between animals, the mother of the offspring can be determined.



Relatedness between all sharks is determined by using **whole genome sequencing (WGS)**

Identity by Descent (IBD) measures how much DNA two animals share because it came from the same recent ancestors^{f,g}.



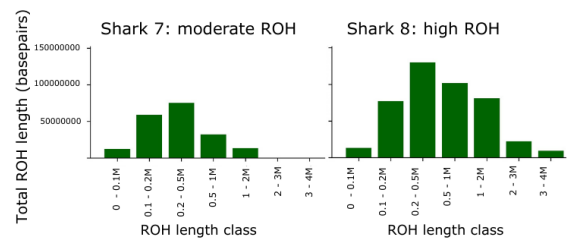
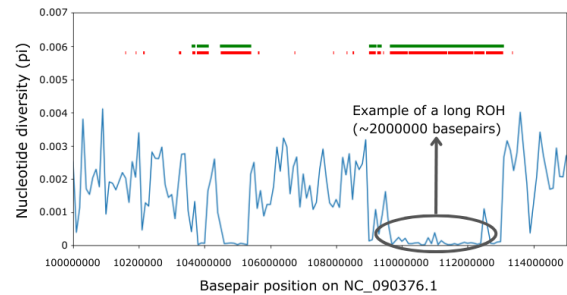
Conclusions relatedness

Both **methods (MtDNA & WGS) agreed** on the same pedigree. However, WGS found that **shark 6 and 7 are distant relatives**.

Results genetic health

Runs of homozygosity (ROH) help detect recent inbreeding and small population size^h

ROH: Long DNA regions where both copies are identical.



Conclusions genetic health

Shark 8 shows substantially higher ROH, across both short and long classes, indicating higher inbreeding.

Key message

Genomic research enables identification of **relatedness, inbreeding, and genetic diversity** needed to **conserve genetically healthy populations**.

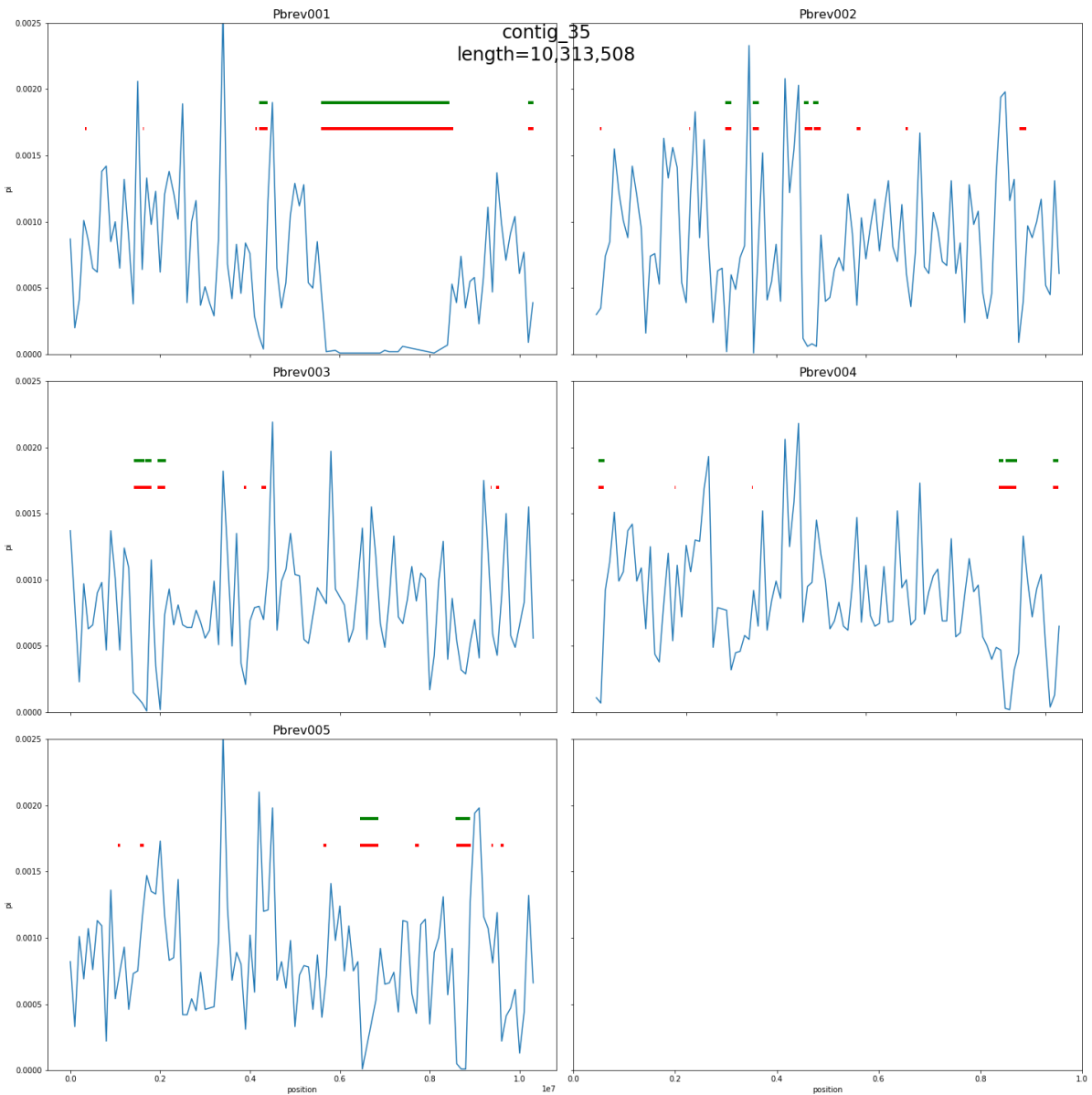
II. Shark information

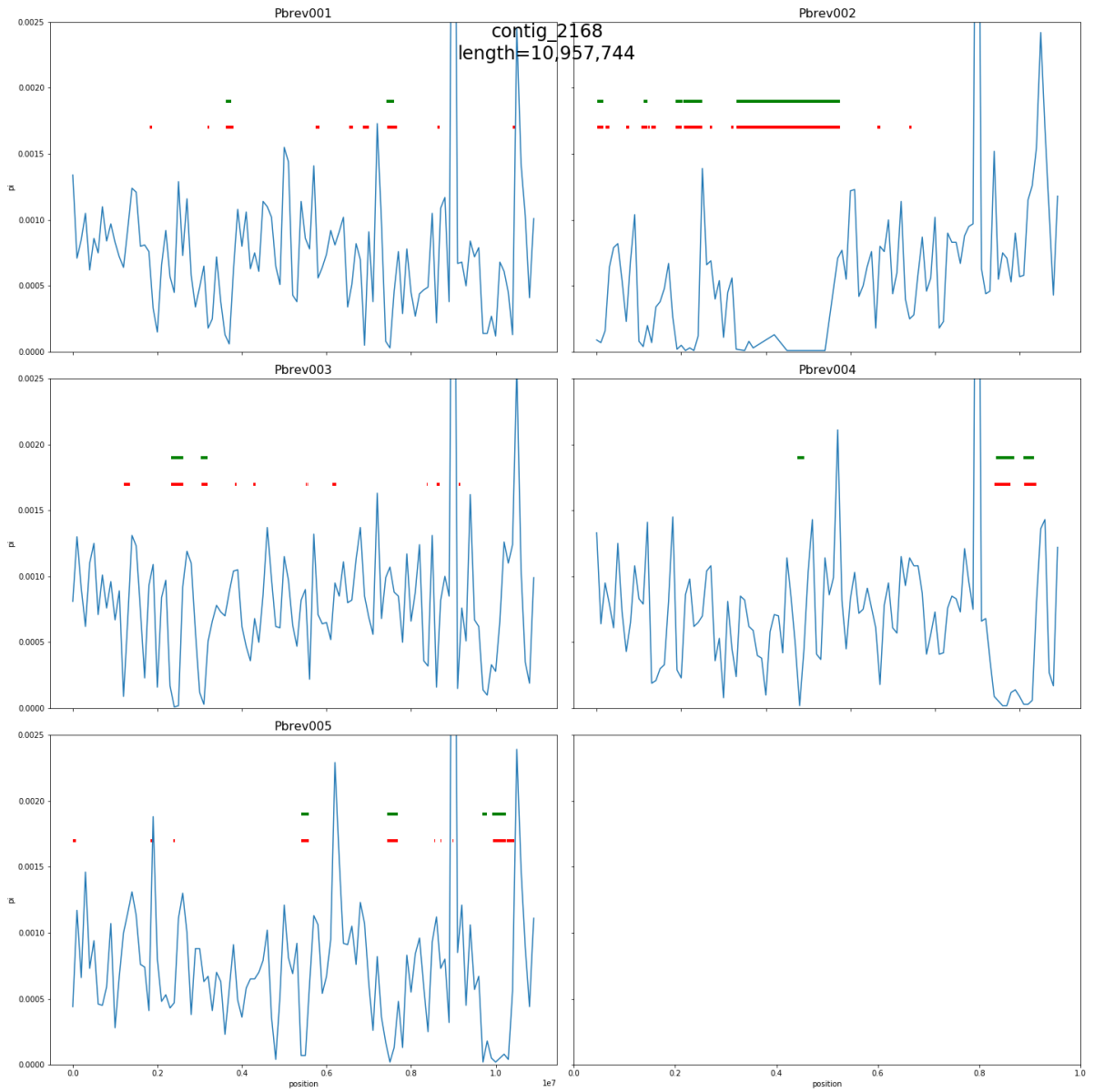
Species	Sample type	Sex number	Sex	Birth date	Origin	Studbook number	ZIMS GAN	Lab number	Zoo	Owned by	Total body length (cm)	Body weight (kg)	Measured on
Shorttail nurse shark (<i>Pseudoginglymostoma brevicaudatum</i>)	1.5 cc blood (EDTA)	1	Male	~ from 01/Jan/1990 to 01/Jan/1992	Wild		2	21020597	1 Burgers' Zoo Artis	Burgers' Zoo Artis	64	2	11/08/2025
Shorttail nurse shark (<i>Pseudoginglymostoma brevicaudatum</i>)	1.5 cc blood (EDTA)	0.1	Female	~< 01/Jan/2001	Wild		4	21025089	2 Burgers' Zoo Artis	Burgers' Zoo Artis	62	2.18	11/12/2024
Shorttail nurse shark (<i>Pseudoginglymostoma brevicaudatum</i>)	1.5 cc blood (EDTA)	0.1	Female	~< 01/Jan/2001	Wild		3	21025088	3 Burgers' Zoo Artis	Burgers' Zoo Artis	69	2.38	11/12/2024
Shorttail nurse shark (<i>Pseudoginglymostoma brevicaudatum</i>)	1 cc blood (EDTA)	0.1	Female	~< 03/Feb/2014	CB Artis		33	PBD14-02812	4 Burgers' Zoo	Burgers' Zoo	67	1.8	25/06/2025
Shorttail nurse shark (<i>Pseudoginglymostoma brevicaudatum</i>)	1 cc blood (EDTA)	1	Male	Undetermined	Undetermined		35	QRT17-05149	5 Burgers' Zoo	Burgers' Zoo	70.5	2.08	25/06/2025

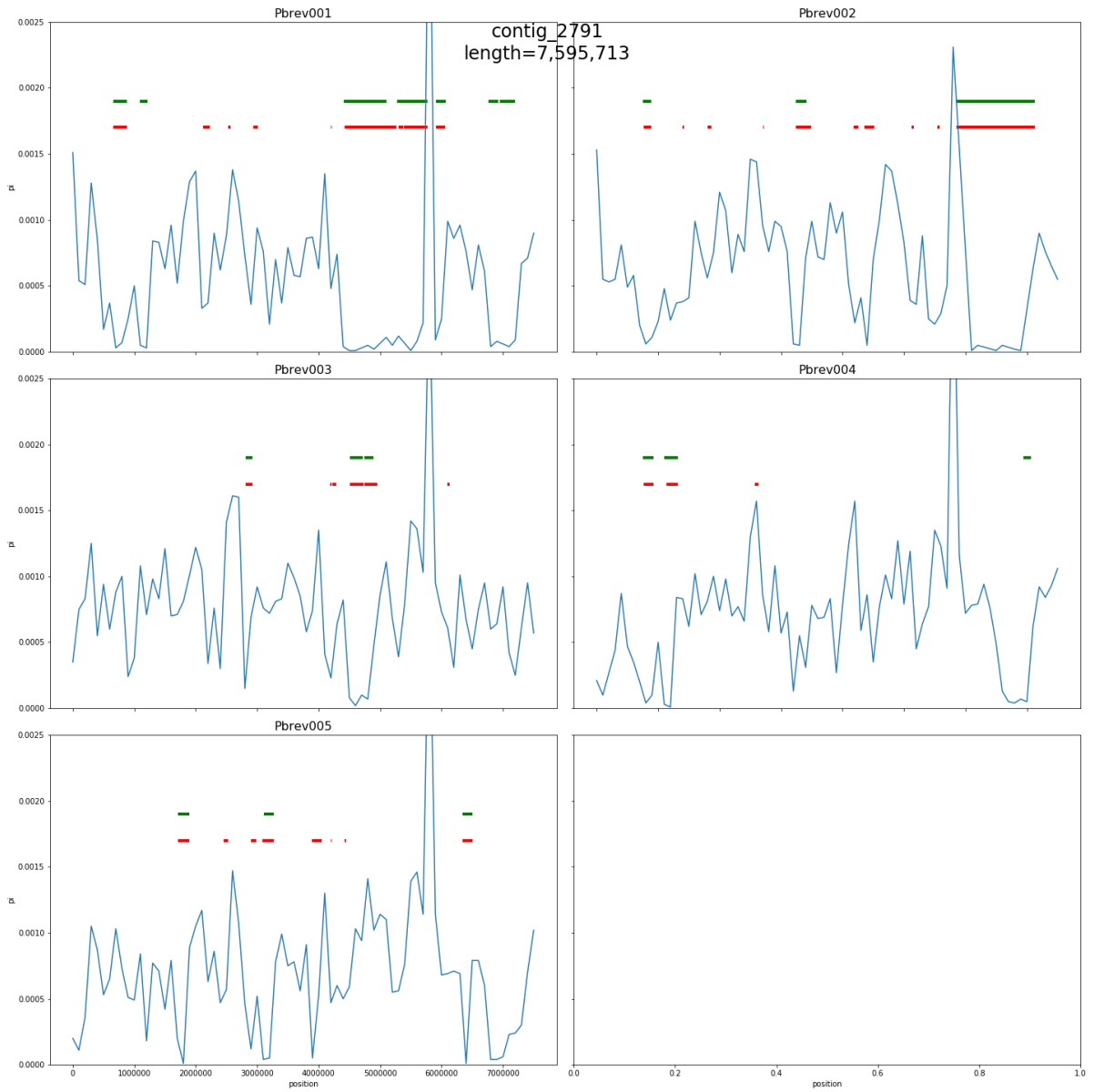
Common Name	Sex Type	Transponder	Age	Location	Remark	SKH
Horn shark	Female	900-110-000-030-589	44Y,9M,1D	Rotterdam (death)	Parent	8
Horn shark	Female	900-110-000-149-939	9Y,1M,27D at the time of death	Basel (death)	Parent	7
Horn shark	Male	900-110-000-149-915	7Y,3M,4D at the time of death	Basel (death)	Parent	6
Horn shark	Female	900-100-000-762-602	4Y,4M,21D	Amsterdam	Offspring	4
Horn shark	Female	900-100-000-762-637	4Y,4M,20D	Amsterdam	Offspring	2
Horn shark	Female	900-100-000-762-638	4Y,3M,24D	Amsterdam	Offspring	3
Horn shark	Female	900-100-000-762-636	4Y,5M,27D	Stralsund	Offspring	5
Horn shark	Female	900-100-000-762-639	3Y,11M,29D	Stralsund	Offspring	1

III. Runs of Homozygosity plots

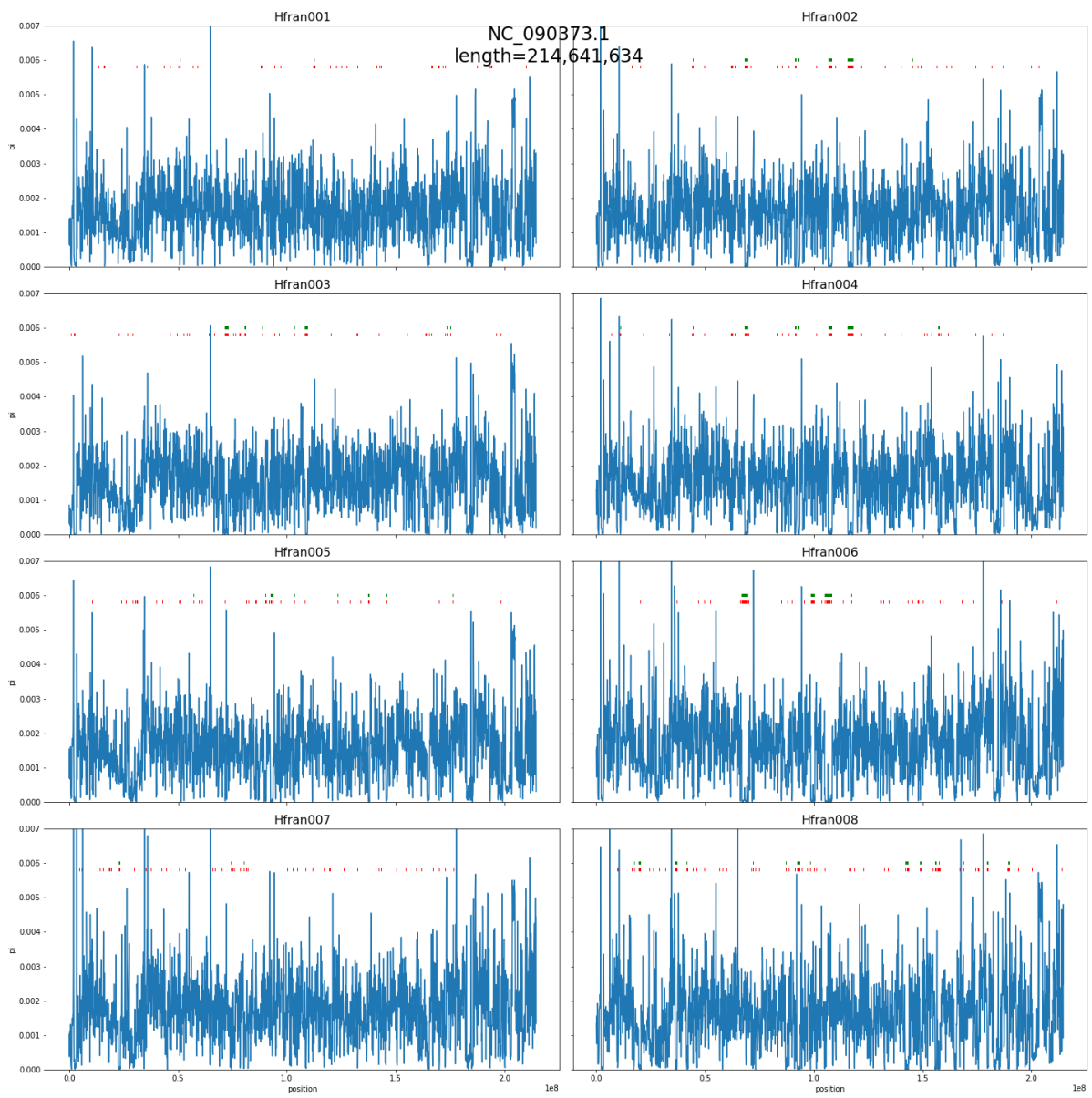
Shorttail nurse shark

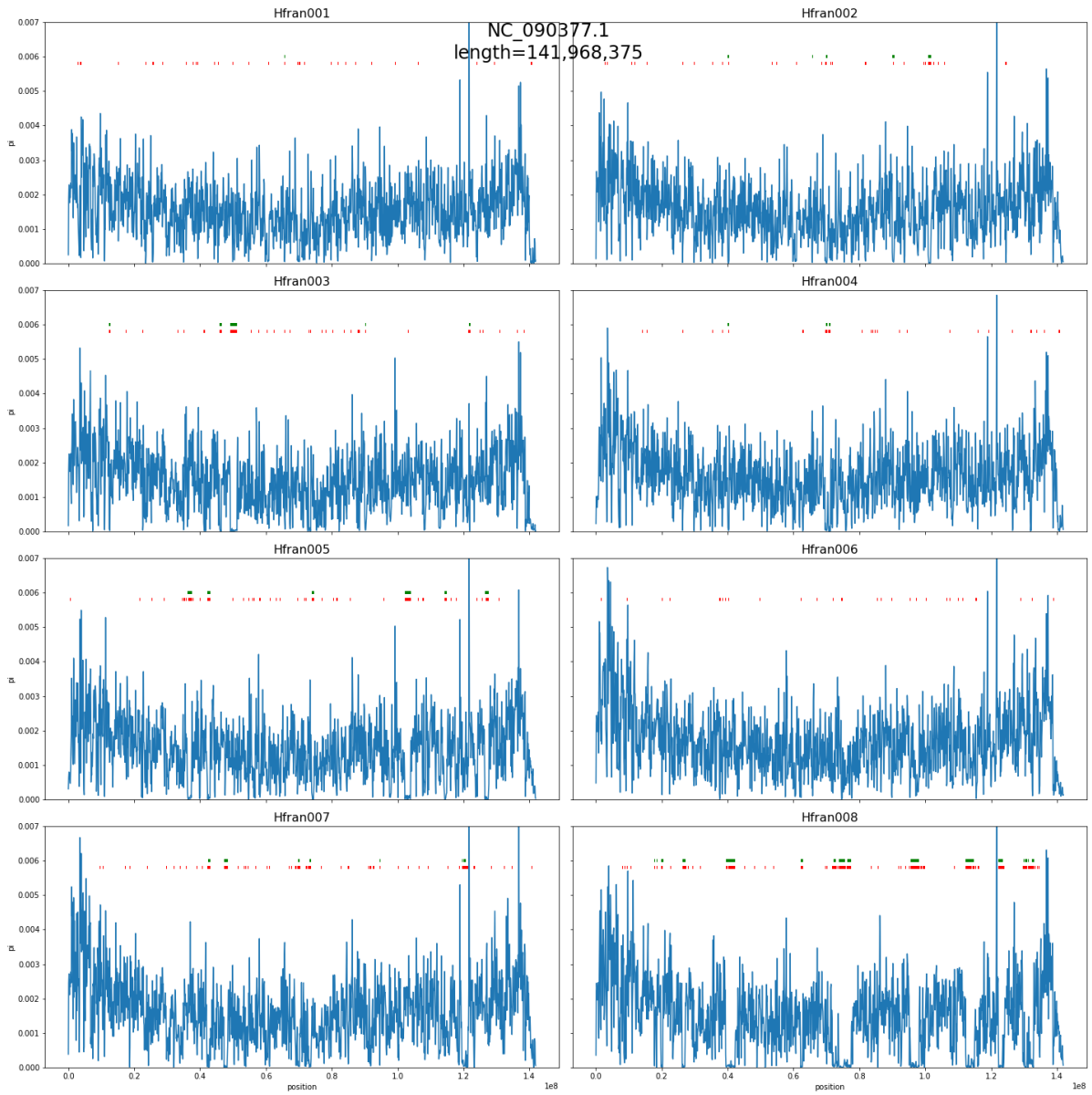


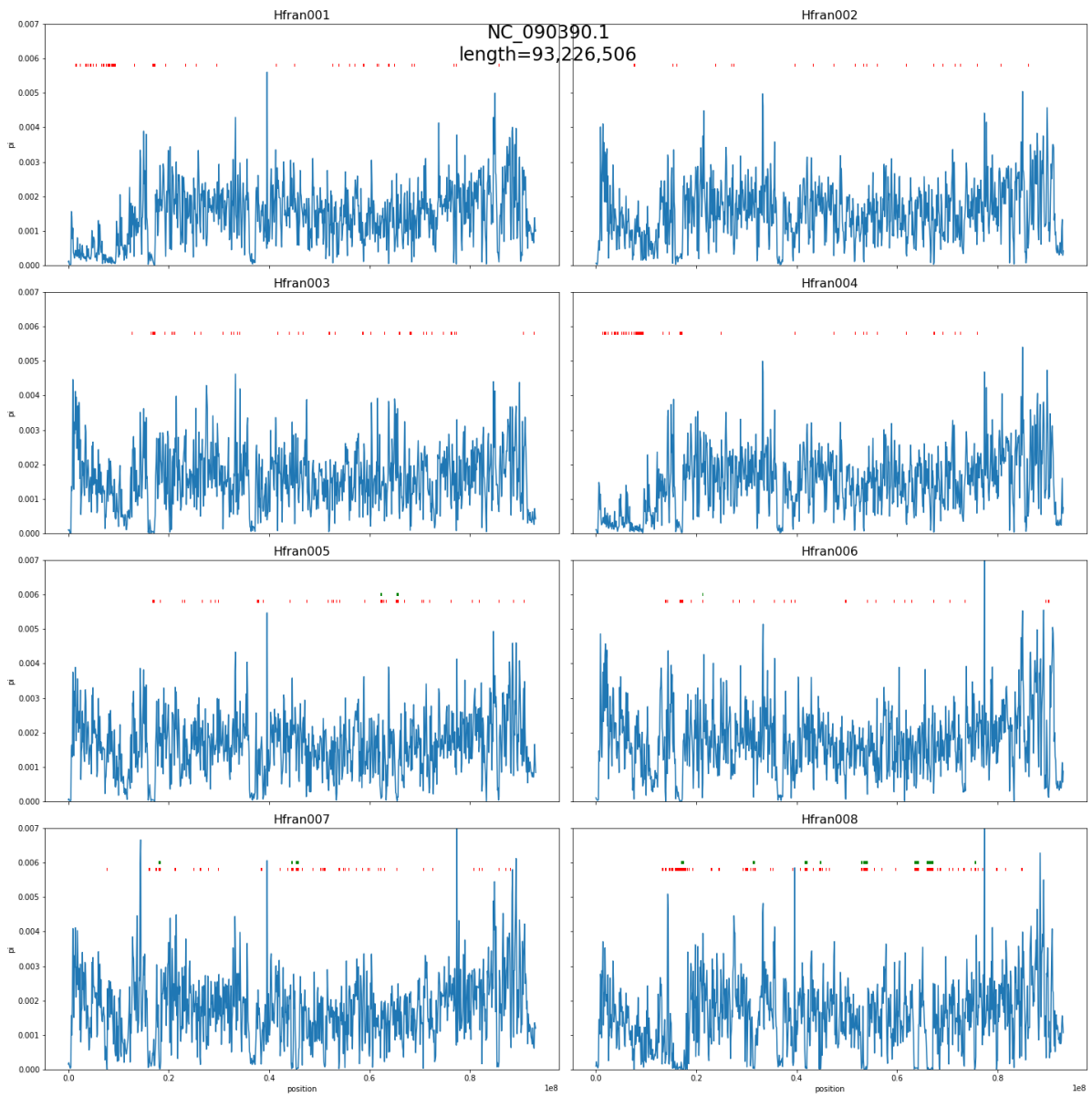


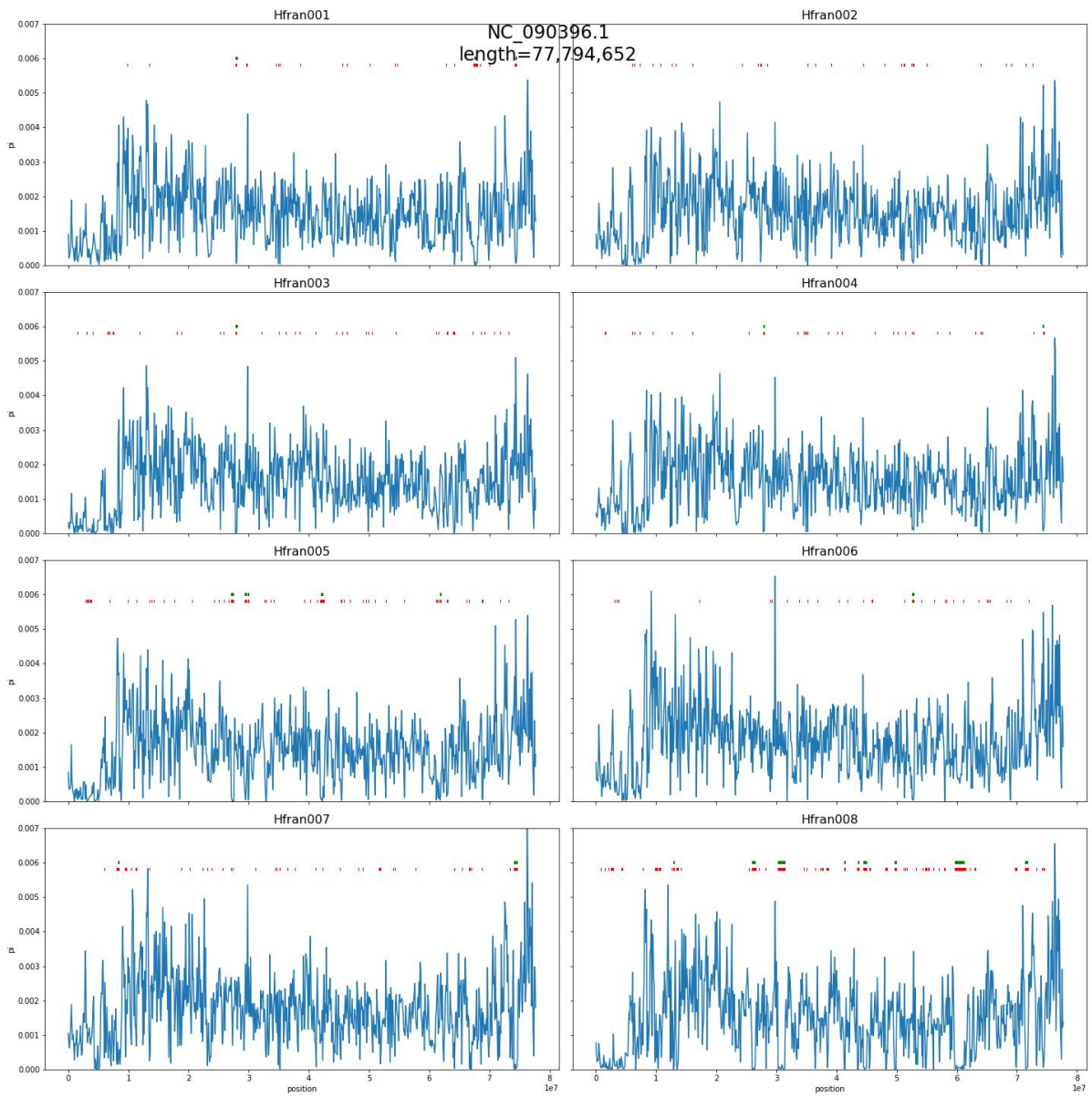


Horn sharks









IV. Tools and software versions

<i>Tool/ software</i>	<i>Version</i>	<i>Reference</i>
Flye assembler	2.9-b1768	(Kolmogorov et al., 2019)
BUSCO	5.2.2_cv1	(Tegenfeldt et al., 2025)
BWA-MEM algorithm	0.7.17-r1188	(Li, 2013)
SAMtools	1.22.1	(Danecek et al., 2021)
Freebayes	v1.3.1-dirty	(Garrison & Marth, 2012)
Integrative Genomics Viewer (IGV)	2.19.2	(Robinson et al., 2011)
VCFtools	0.1.17	(Danecek et al., 2011)
PLINK	v1.9.0-b.7.11	(Purcell et al., 2007)
BCFtools	1.22	(Danecek et al., 2021)
Python	3.7.3	(Python Software Foundation, 2019)
NumPy	1.16.2	(Harris et al., 2020)
Matplotlib	3.0.3	(Hunter, 2007)
Pandas	0.24.2	(McKinney, 2010)

V. DNA isolation

Note: this is the part that I was able to watch, these are not all steps to go from blood samples to sequence data.

DNA was isolated from blood samples of all individuals. This was done according to the Qiagen Genra DNA isolation method (QIAGEN Sample & Assay Technologies, 2011). DNA isolates of every individual were sent to the NovoGene lab to do Illumina whole genome resequencing. DNA isolates from individuals 1 and 2 were used to do Nanopore sequencing (PromethION Flow Cell R10 Version Pk.4) in the laboratory at Wageningen University & Research (Radix building).

The first step is to isolate the white blood cells from the blood sample, because white blood cells nuclei contain DNA. Part of the blood sample (10 µl) is diluted with a phosphate-buffered saline solution (290 µl) and added to a red blood cell lysis solution (900 µl). This solution breaks down the red blood cells while keeping the white blood cells intact. This mix is inverted and shortly incubated (1 min) to ensure uniform lysis of red blood cells while not damaging the white blood cells. After centrifuging (14000g for 1 min), the white blood cells form a pellet, and the lysed red blood cells contents stay in the supernatant. Most of the supernatant is removed, leaving around 10 µl residual liquid and the white blood cell pellet in the tube. The pellet is resuspended in the residual liquid, and the red blood cell lysis solution (900 µl) is added. The steps of inverting, incubating, centrifuging, discarding the supernatant are repeated to ensure clean white blood cell preparation for the next steps.

The second step is to break open the white blood cells and their nuclei to release DNA. A cell lysis solution (300 µl) is added to the white blood cell pellet to break the cell and nuclear membranes. After a short vortex, RNase (0.3 µl; 20 mg/ml) is added to the tube to break down RNA. This solution is inverted (25 times) and incubated (15 min at 37 °C). After that, the RNase activity is stopped by cooling the tube on ice (1 min). Proteinase K (5 µl) is added, shortly vortexed and incubated (1 hour at 55 °C) to digest proteins including histones (DNA-binding proteins) and nucleases (enzymes that can degrade DNA). Because histones and nucleases are removed, DNA is released and protected.

The third step is to remove proteins while leaving the DNA in the solution. The tube was cooled back to room temperature and protein precipitation solution (100 μ l) was added, causing proteins to denature, aggregate or become insoluble. This mixture is vortexed (20 sec) to ensure fully precipitation of the proteins. The mixture is then incubated on ice (10 min) and centrifuged (14000g for 1 min). The pellet was not tight, so the mixture was vortexed, incubated and centrifuged again to ensure maximal protein removal. The pellet contains the proteins that need to be removed, and the supernatant contains the DNA.

The fourth step is to separate the DNA from the solution. The supernatant is poured into a clean tube (1.5 ml) which contains 100% isopropanol (300 μ l). The isopropanol reduces the solubility of DNA. The mixture is gently mixed by inversion (50 times) which prevents shearing long strands of DNA. After centrifuging (14000g for 1 min), the DNA forms a pellet and the supernatant is removed. To wash the DNA, 70% ethanol is added to the tube and inverted several times. The 70% ethanol removes residual salts and isopropanol while keeping the DNA precipitated. After centrifuging (14000g for 1 min) the mixture again, the ethanol is pipetted off, and the pellet is air dried (5 min at room temperature) to let the residual ethanol evaporate.

Finally, the last step is to re-dissolve the DNA into a stable form to store and use it. DNA hydration solution (56 μ l) is added to the DNA pellet and solved by gently flicking the tube. This DNA hydration solution protects the DNA from nucleases. The DNA is rehydrated by incubating the tube (5 min at 55 °C) and finally leaving it overnight at 4 °C to dissolve completely.

VI. Data management plan

Data management plan belonging to the MSc research practice performed at the Animal Breeding and Genomics Group by Aisha Boering, completed in February 2026.

Agreements

1. The data used in this thesis project have been described in this document and have been stored in a systematic manner (at least in separate folders for all sections as described below). Data includes all data as mentioned in the results section of your report.
2. The data management plan has been discussed with the MSc research practice supervisor, and he has agreed on the location for data storage.
3. The data can be found through Hendrik-Jan Megens (hendrik-jan.megens@wur.nl)

Section A – Open-source data

File names	Remarks
Raw Nanopore data of shorttail nurse sharks 1 & 2	https://www.ebi.ac.uk/ena/browser/view/PRJEB106905
Reference genome horn shark	https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_03636525.1/

Section B – Illumina data

Animals	Annuna folder location with multiple raw Illumina sequencing files
Horn shark 1	/lustre/backup/WUR/ABGC/shared/ABGC_datastore/SequenceData/Shark/ARTIS_sharks/ARTIS_data_October2025/X204SC25077422-Z01-F002/01.RawData/SKH_1
Horn shark 2	/lustre/backup/WUR/ABGC/shared/ABGC_datastore/SequenceData/Shark/ARTIS_sharks/ARTIS_data_October2025/X204SC25077422-Z01-F002/01.RawData/SKH_2
Horn shark 3	/lustre/backup/WUR/ABGC/shared/ABGC_datastore/SequenceData/Shark/ARTIS_sharks/ARTIS_data_October2025/X204SC25077422-Z01-F002/01.RawData/SKH_3
Horn shark 4	/lustre/backup/WUR/ABGC/shared/ABGC_datastore/SequenceData/Shark/ARTIS_sharks/ARTIS_data_October2025/X204SC25077422-Z01-F002/01.RawData/SKH_4
Horn shark 5	/lustre/backup/WUR/ABGC/shared/ABGC_datastore/SequenceData/Shark/ARTIS_sharks/ARTIS_data_October2025/X204SC25077422-Z01-F002/01.RawData/SKH_5
Horn shark 6	/lustre/backup/WUR/ABGC/shared/ABGC_datastore/SequenceData/Shark/ARTIS_sharks/ARTIS_data_October2025/X204SC25077422-Z01-F001_02/01.RawData/SKH_6
Horn shark 7	/lustre/backup/WUR/ABGC/shared/ABGC_datastore/SequenceData/Shark/ARTIS_sharks/ARTIS_data_October2025/X204SC25077422-Z01-F001_03/01.RawData/SKH_7
Horn shark 8	/lustre/backup/WUR/ABGC/shared/ABGC_datastore/SequenceData/Shark/ARTIS_sharks/ARTIS_data_October2025/X204SC25077422-Z01-F001_01/01.RawData/SKH_8
Shorttail nurse shark 1	/lustre/backup/WUR/ABGC/shared/ABGC_datastore/SequenceData/Shark/ARTIS_sharks/Pseudoginglymostoma_Batch1March2025/Batch1_25032025/X205SC25016601-Z01-F001_02/01.RawData/SN001
Shorttail nurse shark 2	/lustre/backup/WUR/ABGC/shared/ABGC_datastore/SequenceData/Shark/ARTIS_sharks/Pseudoginglymostoma_Batch1March2025/Batch1_25032025/X205SC25016601-Z01-F001_01/01.RawData/SN002
Shorttail nurse shark 3	/lustre/backup/WUR/ABGC/shared/ABGC_datastore/SequenceData/Shark/ARTIS_sharks/Pseudoginglymostoma_Batch1March2025/Batch1_25032025/X205SC25016601-Z01-F001_03/01.RawData/SN003
Shorttail nurse shark 4	/lustre/backup/WUR/ABGC/shared/ABGC_datastore/SequenceData/Shark/ARTIS_sharks/ARTIS_data_October2025/X204SC25077422-Z01-F001_04/01.RawData/PB_4
Shorttail nurse shark 5	/lustre/backup/WUR/ABGC/shared/ABGC_datastore/SequenceData/Shark/ARTIS_sharks/ARTIS_data_October2025/X204SC25077422-Z01-F001_04/01.RawData/PB_5

Section C – Data analysis

File names	Remarks
Script_Shorttail_Nurse_Shark_Research.txt	Contains all bash command lines with small python scripts that were used in this research for the shorttail nurse sharks
Script_Horn_Shark_Research.txt	Contains all bash command lines with small python scripts that were used in this research for the horn sharks
ROH_plots_Pbrev.ipynb	Used to plot the distribution of RoH of shorttail nurse sharks
ROH_plots_Hfran.ipynb	Used to plot the distribution of RoH of horn sharks

VII. Artis-tijdschrift Stierkophaai onderzoek draft

De Stierkophaai (*Heterodontus francisci*) is een haai van ongeveer 1 meter lang die in het wild leeft in de gematigde en subtropische zeeën van de oostelijke Stille Oceaan aan de kust van Californië tot aan Mexico, Ecuador en Peru. Ze leven op de zeebodem, tussen rotsen en in kelpbossen en leven tot een diepte van 150 meter. Ze voeden zich in de nacht met kleine vissen, zee-egels, schelp- en schaaldieren. In samenwerking met de European Association of Zoos and Aquaria (EAZA) coördineert Artis de Stierkophaaien in Europese dierentuinen en aquaria. Samen zijn ze er verantwoordelijk voor dat de Europese populatie gezond blijft. Hierbij is het nauw bijhouden van de stamboom heel belangrijk. Als dit niet gebeurt kan het zijn dat twee verwante dieren samen nakomelingen krijgen. Deze inteelt kan invloed hebben op de gezondheid en zelf leiden tot erfelijke aandoeningen. Twee vrouwtjes en één mannetje hebben vijf nakomelingen gekregen, maar het was onbekend van welk vrouwtje. In samenwerking met Wageningen Universiteit is niet alleen dit raadsel opgelost, maar ook is er ontdekt dat de stamboom niet alles weet...

In elke cel van je lichaam zit DNA, zo ook zit er DNA in elke cel van de stierkophaaien. Dit DNA komt half van je moeder en half van je vader en vormen de bouwstenen van wie je bent en hoe je eruit zit. Met de huidige technologieën kunnen onderzoekers heel veel aflezen uit je DNA. Ieders totale pakketje aan DNA is uniek, maar ook is veel van het DNA hetzelfde. Bijvoorbeeld het stukje DNA wat verantwoordelijk is voor celdeling en haargroei. Door deze verschillen en overeenkomsten was het mogelijk om te achterhalen wat de verwantschappen zijn tussen de acht Stierkophaaien. Hierbij werd bekend dat het ene vrouwtje drie en het andere vrouwtje twee nakomelingen had. Door dit onderzoek werd ook bekend dat één van de vrouwtjes deels aan het mannetje verwant is, waardoor hun nakomelingen te maken hebben met een kleine hoeveelheid inteelt. Ook werd ontdekt dat het andere vrouwtje een redelijke wat inteelt liet zien op basis van het DNA. Geen van de Stierkophaaien hebben erfelijke aandoeningen gelukkig, maar deze informatie is heel belangrijk om te bepalen welke dieren er mee mogen doen met het Europese fokprogramma. Dit om ervoor te zorgen dat de populaties in dierentuinen en aquaria zo gezond mogelijk blijven.

VIII. Shorttail nurse shark exhibit signage draft

De Kleine Verpleegsterhaai (*Pseudoginglymostoma brevicaudatum*) is een kleine haai (75 cm) die in het wild voorkomt in de riffen van de Westelijke Indische Oceaan bij Oost-Afrika. Hij leeft op de zeebodem en voedt zich met kleine vissen, schaaldieren en krabben. Ze worden door mensen gevangen voor hun vlees en huid en de riffen waar ze leven wordt kapot gemaakt door menselijke activiteiten. Hierdoor zijn de populaties over de afgelopen 35 jaar met 80% afgenomen en is de soort nu kritisch bedreigd. Er is weinig bekend over de huidige populatie aantallen, leefgebieden en interactie tussen populaties. In het Aquarium van Artis leven momenteel drie Kleine Verpleegsterhaaien die een belangrijke rol hebben gespeeld in het onderzoek doen naar deze bedreigde haaiensoort.

The shorttail nurse shark (*Pseudoginglymostoma brevicaudatum*) is a small shark (75 cm) that lives in the reefs of the West Indian Ocean near the east coast of Africa. It lives on the seabed and eats small fish, crustaceans, and crabs. They are caught by humans for their meat and skin and due to human activities, their habitat is destructed. This resulted in populations decline of 80% over the last 35 years and they are listed as Critically Endangered. Artis Aquarium currently house three shorttail nurse sharks which played a key role in researching this critically endangered species.

IX. Zoology conference abstract

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Using genomics to discover relatedness and genetic diversity in horn sharks.

Genomic insights are important for informing conservation strategies aimed at sustaining genetically healthy populations, especially when programs aim to support future rewilding. In this study, genomics are used to discover relationships and genetic diversity to help improve management of the horn shark (*Heterodontus francisci*). The species is listed as data deficient on the IUCN Red List, and moreover little to no information is available regarding the origin and genetic diversity in populations maintained in zoos and aquariums. For the horn shark, a re-sequencing approach was applied, revealing millions of variants in the genome. From this information, family relationships between individuals were inferred, resulting in recommendations for future breeding to preserve genetic diversity. Additionally, genome-based indicators of inbreeding, such as the occurrence of Runs of Homozygosity were computed. providing key information for informing current breeding programs and, and the potential for rewilding.