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Integrating genomic tools into ex situ conservation management of caribou (Rangifer tarandus) in Canada

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Abstract

The erosion of genetic diversity represents one of the most pervasive yet underappreciated threats to the long-term persistence of wildlife populations, particularly for species at risk. While conservation genomics research has predominantly focused on wild populations, characterizing the genomic status of individuals in managed care is equally critical for informing effective breeding programs and supporting future reintroduction efforts. Large-scale genotyping platforms provide an opportunity to generate the population genetic metrics necessary to guide breeding management, minimize inbreeding, and identify candidates for genetic rescue. Here, we applied a 63K single nucleotide polymorphism (SNP) array developed for caribou (*Rangifer tarandus*) to genotype 49 individuals housed across accredited Canadian zoological facilities. Samples included both whole blood and non-invasive sources, demonstrating the applicability of molecular tools even for fragmented DNA of low quality. Using these data, we characterized population structure, ecotype, sex, and pairwise relatedness among individuals, producing the first molecular characterization for the managed *Rangifer tarandus* population in Canada. Our results demonstrate that high-resolution genomic tools can meaningfully improve ex situ breeding management by preserving genetic diversity and ecotype integrity, enabling targeted assisted reproduction for genetic rescue, and strengthening long-term recovery strategies for wild caribou populations.

Introduction

Global biodiversity is declining at an unprecedented rate, with wildlife populations facing severe demographic losses driven by habitat destruction, overexploitation, pollution, invasive species, and climate change[1]. These demographic declines often lead to the erosion of genetic diversity – a consequence of small population size and increased fragmentation, which intensify genetic drift and inbreeding, ultimately reducing genetic variation and constraining a population's adaptive potential[2, 3]. A recent global meta-analysis across major taxonomic groups demonstrated that declines in genetic diversity are statistically significant for mammals and birds[4], suggesting that genetic variability declines in parallel with species abundance. This is particularly concerning because, in contrast to demographic recovery, the restoration of genetic diversity is a slow process that is difficult to reverse once lost[5]. Maintaining genetic variability is therefore critical for populations to respond to environmental change, emerging disease, and shifting ecological pressures. As conservation efforts increasingly face small, isolated, and genetically impoverished populations, genomic tools that enable precise characterization and monitoring of genetic diversity are becoming essential for informing management decisions.

Recent advances in molecular techniques have fundamentally transformed the capacity to characterize genetic diversity and inform conservation management. Early approaches relied on allozymes and mitochondrial DNA sequencing, followed by the widespread adoption of microsatellite markers, which offered improved resolution of population structure, kinship, and genetic diversity[6]. While next-generation sequencing technologies have made it possible to apply genome-wide approaches that

provide high resolution insights into demographic history, local adaptation and individual-level inbreeding[7], whole-genome sequencing remains logistically and financially challenging for large-scale population monitoring, particularly when population sizes are large or DNA quality is variable[8]. Single nucleotide polymorphism (SNP) arrays offer a practical middle ground, by targeting a fixed set of highly informative loci distributed across the genome to deliver genome-wide resolution at reduced cost and with relaxed DNA quality requirements[9]. SNP arrays, therefore, could be advantageous for non-invasive sampling, where DNA is often degraded or available only in limited quantities. Likewise, SNP arrays generate reproducible, standardized genotypes across laboratories and sample types[10], a property that is particularly valuable for comparative studies spanning multiple populations or facilities. The development of species-specific arrays for wildlife taxa has therefore opened new avenues for integrating genomic data into applied conservation frameworks.

Caribou (*Rangifer tarandus*), known as reindeer across Eurasia, is an example of the conservation challenges facing large mammals in the northern hemisphere. Distributed across the Arctic tundra and the boreal forests, caribou populations have diverged into ecologically and morphologically distinct ecotypes, each adapted to local environmental conditions[11]. The loss of any ecotype or population, therefore, could negatively impact the evolutionary significance of the species as a whole by reducing its adaptive breadth. Ongoing habitat degradation driven by industrial development, wildfires, and climate change has led to severe demographic declines in several caribou populations across Canada, resulting in their designation as threatened under the Species at Risk Act[12]. Thanks to a growing investment in population genomics research, wild caribou is now among the most thoroughly genomically characterized wild ungulates in North America[13–18]. These studies have provided detailed insights into population structure, demographic history, local adaptation, and the consequences of habitat fragmentation across wild herds. However, comparable genomic baselines for managed and captive caribou populations remain largely undeveloped, limiting the integration of genomic data into a unified recovery framework.

As threatened populations continue to decline, managing individuals outside their natural range has become an increasingly important component of comprehensive conservation strategies. Ex situ populations represent a safeguard against stochastic environmental events, predation and other external threats, while providing demographic and genetic reservoirs that can support reintroduction and reinforcement of wild populations[19]. Traditionally, ex situ management has relied on studbook records and pedigree-based analyses to implement minimum-kinship breeding strategies, which aim to maximise the retention of genetic diversity by pairing the least related individuals in each successive generation[20]. This approach, however, assumes that founder individuals are unrelated – an assumption that is often difficult to meet in practice, as wild-caught individuals frequently originate from structured or fragmented populations. Molecular markers, in contrast, overcome this limitation by providing empirical and more reliable estimates of relatedness, thereby improving the identification of suitable breeding pairs[21]. Unfortunately, genomic approaches remain largely underutilized in ex situ conservation programs. The recent development of species-specific SNP arrays for wildlife taxa now

provides a practical opportunity to address this gap, offering ex situ programs a standardized and cost-effective framework to establish the genomic baselines needed to integrate managed populations into a unified conservation framework.

The *Rangifer* SNP array[22], developed from whole-genome sequencing data of wild caribou populations in Canada, provides a cost-effective platform for high-throughput genotyping. Comprising thousands of SNPs distributed across the genome, the array allows a reliable characterization of important population genetic parameters, such as heterozygosity levels, population structure, and relatedness – even from non-invasive samples (e.g., feces, hair, or environmental DNA). Here, we use the *Rangifer* SNP array to characterize the genomic profiles of the managed caribou population housed in zoological institutions across Canada, with the dual aim of establishing molecular baselines for ex situ management and bridging the genomic gap between wild and managed populations. Specifically, we: (1) assess genotyping performance across non-invasive sample types relative to whole blood; (2) characterize sex, ecotype, population structure, and pairwise relatedness across individuals; and (3) provide the genomic foundation for a molecular studbook to support kinship-based breeding decisions and long-term genetic management of ex situ caribou in Canada.

Results

Variant calling and sample filtering

A total of 55 samples met the minimum quality call rate threshold (>0.15) and were retained for downstream analyses. Samples with high-quality genotype data (call rates >0.90) were predominantly obtained from gDNA extracted from whole blood samples (Fig. 1). The contrast between the number of high-quality SNPs and those with missing calls of failed samples (n=15) was remarkable (Fig. S2) and, therefore, were removed from further analyses. As a result, the number of paired blood and fecal samples decreased from 19 to 9 (Fig. S3).

Sex assignment

Observed heterozygosity (H_O) of SNPs located on the X chromosome accurately assigned sex for all but three male samples (Fig. 2). Sex assignment for individuals with paired samples (n=9), was accurate for genotype data obtained from both blood and fecal samples (Fig. S4).

Population structure

After filtering, a total of 39,716 SNPs were used to determine genetic clusters in the managed caribou population. To assign population groups, all PCs (>50) were retained and chose 4 as the optimal number of genetic clusters, corresponding to the lowest Bayesian Information Criterion (BIC) value (Fig. S5).

DAPC analysis revealed that population variation is mostly explained by the first 35 PCs (Fig. S6) and 3 discriminant functions (Fig. S7). The first discriminant captured 73% of the variation, the second

captured 20.3%, and the third 6.7%. Figure 3 shows results of genetic clusters for the first two discriminants. A total of 9 samples from 9 individuals were assigned to cluster (1), all originating from the Yukon Wildlife Preserve. Cluster (2) included 21 samples from 18 individuals. Cluster (3) comprised 7 samples from 4 individuals, from the Riverview Park Zoo and the Toronto Zoo, which are known to belong to the same herd. Finally, 18 samples from 15 individuals were assigned to cluster (4), all of which appear to be reindeer according to ZIMS records. Refer to Table S1 in Supplementary Information for raw clustering results. Individuals with both blood and fecal samples were consistently clustered into the same genetic groups (Fig. S8). Refer to Fig. S9 for a 3D view genetic clusters with 3 discriminant functions.

Genetic relatedness

Hierarchical clustering based on genetic distances derived from kinship estimates revealed several genetically similar groups of individuals (Fig. 4), suggesting that genetic structuring could be consistent with herd or institutional origin. Several clusters displayed short branch lengths, indicating closely related individuals, whereas longer internal branches separating clusters suggest lower relatedness among groups and the presence of multiple genetic lineages within the dataset. A heatmap showcasing pairwise kinship estimates is provided in Fig. S10.

When considering only genotype data from fecal samples of individuals with paired blood-fecal samples, the overall relationships among individuals show only minor shifts (Fig. S11). These slight differences are likely an artifact of the lower quality of fecal-derived genotypes, which can increase uncertainty in branch placements compared to blood-derived data (12,161 SNPs with >0.20 missingness were excluded). Nevertheless, the major clades remain largely intact, indicating that fecal samples still reliably capture the primary genetic relationships.

Ecotype assignment

The model that yielded the best balanced accuracy was a multinomial NaiveBayes algorithm with a very low smoothing parameter (α=10-8) and prior class probabilities learned from the proportion of observations (=samples) in each class in the learning set. The model yielded an accuracy of 1.0 in the test set of 60 individuals representing the same class proportions as the learning set (Fig. S12). All samples from 12 accredited Canadian zoological institutions were categorized as migratory ecotype (Fig. 5).

Discussion

Conservation genomics has transformed our understanding of wild population dynamics, providing detailed insights into kinship, dispersal, gene flow, and demographic history across a wide range of taxa. Yet despite this progress, genomic approaches have been rarely extended to managed populations, leaving a critical gap in our capacity to coordinate evidence-based conservation strategies. Here, we present the first genomic characterization of the managed *Rangifer tarandus* population in Canada. We

used a recently developed species-specific SNP array to assess genotyping performance across sample types, infer population structure, estimate pairwise relatedness, and assign ecotype. Our results demonstrate that high-resolution genomic tools can be effectively implemented in ex situ conservation contexts, providing the molecular baseline necessary to inform evidence-based breeding management and support long-term recovery strategies for wild caribou populations.

Genotyping performance was consistently high for whole blood samples, whereas approximately half of fecal-derived samples failed to meet minimum call rate thresholds (Fig. 1). This result contrasts with a recent validation study of the *Rangifer* SNP array, which reported high genotyping call rates for fecal samples collected under controlled field conditions[23], and likely reflects the cumulative effects of opportunistic sample collection in managed facilities. In contrast to freshly collected field samples, fecal material obtained in zoological settings may be exposed to enclosure substrates and variable environmental conditions prior to freezing. These factors could exacerbate DNA fragmentation, bacterial contamination, and degradation, all of which reduce the proportion of recoverable host DNA and compromise downstream genotyping success[24, 25]. Despite this, fecal samples meeting minimum quality thresholds performed reliably across all downstream analyses. Individuals with paired blood and fecal samples were consistently assigned to the same genetic clusters (Fig. S8) and produced concordant relatedness estimates (Table S1). Likewise, all fecal samples of paired individuals accurately assigned sex based on observed heterozygosity at X-linked SNPs (Fig. S4B). Across the full dataset, only three male individuals returned ambiguous X-chromosome heterozygosity values (Fig. 2), likely reflecting genotyping uncertainty arising from low call rates rather than a true biological signal. Together, these results indicate that non-invasive fecal sampling still represents a viable alternative when tissue or blood sample collection is challenging, provided that standardized collection protocols are followed – including minimizing the time elapsed between defecation and sample collection.

We report genetic structure among managed *Rangifer tarandus* individuals in Canada, which likely reflect differences in founder origin, historical translocation events, and the maintenance of separate breeding groups across institutions. For example, individuals originating from the Yukon Wildlife Preserve (YWP) formed a distinct and cohesive cluster, as did individuals from both Riverview Park Zoo (RPZ) and the Toronto Zoo (TZ) – which are known to share herd origin. Some individuals recorded as reindeer in ZIMS records formed a cluster clearly differentiated from all caribou clusters (Fig. 3, Table S1), highlighting the utility of SNP arrays for resolving taxonomic identity at subspecies level, which is relevant for management decisions to conserve genetic integrity. These findings are consistent with studies demonstrating that institutional boundaries and sourcing decisions leave detectable genomic signatures in ex situ populations[26].

Kinship estimates were overall concordant with genetic structuring and institutional origin (Fig. S10). In several cases, closely related individuals co-occurred within the same DAPC cluster, which indicate shared ancestry. Individuals from YWP, for instance, formed two closely related but distinct sub-clades, suggesting the presence of at least two genealogical lineages or reflecting the management of distinct breeding groups over time. A number of individual pairs had short branch lengths, indicative of first- or

second-degree relationships such as parent-offspring or full-sibling pairs (Fig. 4). Regardless of their institutional origin or ZIMS records, all individuals included in the ecotype prediction analysis were assigned a migratory. This result should nonetheless be interpreted with caution, as the original dataset used to build the model was heavily weighted toward samples from Eastern Canada and may not fully represent the diversity of western populations. However, the dataset did include samples from Western Canada from both migratory and forest-dwelling ecotypes, and the model predicted ecotype assignments with perfect accuracy. This high accuracy is likely due to the high number of features (= SNPs) that were included in the model, allowing to discriminate ecotypes from Western to Eastern Canada. Additional genotyping of samples from other Canada regions would allow to corroborate the prediction accuracy or updating the predictive model to improve its generalization.

The dataset generated in this work provides the foundation for a genomic studbook for the ex situ *Rangifer tarandus* population in Canada, representing a valuable resource to complement and enhance traditional pedigree-based management. Conservation breeding programs are increasingly integrating molecular data with pedigree analyses to address incomplete knowledge of founder genetic diversity and relatedness[27–31]. In this context, our empirical estimates of relatedness can directly inform breeding decisions that preserve lineage integrity while minimizing inbreeding. Beyond immediate breeding management, these data could support broader conservation objectives, including the prevention of unintended hybridization or introgression between genetically distinct groups, and the identification of suitable candidates for genetic rescue of declining wild herds[32].

Conclusions

This study provides the first genomic baseline for the managed *Rangifer tarandus* population in Canada, demonstrating that a species-specific SNP array can generate robust genomic profiles from both whole blood and non-invasive fecal samples. Our analyses resolved population structure, revealed close familial relationships, and confirm consistent ecotype assignments within the managed *Rangifer tarandus* population in Canada. Establishing this genomic baseline is a critical step toward aligning ex situ management with in situ conservation strategies, positioning managed individuals not only as demographic reservoirs, but as genetically informed contributors to the long-term recovery of wild caribou populations. This work contributes to the growing body of evidence that genome-based approaches are transforming the management of ex situ populations, strengthening their role as active contributors to in situ conservation and long-term species sustainability. Future work employing whole-genome sequencing could build on these findings to reconstruct individual inbreeding histories at finer resolution, detect signatures of adaptation to ex situ environments, and identify candidate loci underlying ecotype-specific traits relevant to reintroduction success.

Methods

Sample collection

A total of 70 blood and/or fecal samples were obtained from 49 *Rangifer tarandus* individuals housed at 12 accredited Canadian zoological institutions (Fig. S1 in Supplementary Information), including 19 individuals for which paired blood and fecal samples were available. Samples were opportunistically collected from live animals during planned veterinary examinations or routine husbandry procedures at the host institutions, in compliance with the ethical standards of the Association of Zoos and Aquariums (AZA) and/or the Canadian Association of Zoos and Aquariums (CAZA). All procedures were conducted under the oversight and approval of each institution's animal care and use committee (IACUC or equivalent), and no animals were subjected to additional interventions beyond those planned as part of standard zoological care. Where chemical restraint or sedation was required, anesthetic agents were administered by qualified veterinary staff as part of standard clinical practice at each host institution, and not as a research intervention. Specific anesthetic protocols varied by species and institution in accordance with established veterinary guidelines. Whole blood samples were collected into K_2EDTA *vacutainer* tubes, with a minimum volume of 1.4 mL. Fecal samples were collected as sets of approximately 7–10 pellets and stored in 50 mL Falcon tubes. Both, blood and fecal samples were immediately frozen after collection and shipped frozen, in dry ice, for further processing.

DNA extraction and genotyping

DNA extractions from fecal samples were performed using the DNeasy Blood & Tissue Kit (Qiagen; #69504), with the following modifications to the manufacturer's protocol. Fecal samples were thawed in sets of 5–7 pellets by placing them into 50 mL Falcon tubes containing 30 mL of 1× PBS (pH 7.4) for 10 min. Individual pellets were transferred to 50 mL Falcon tubes containing 1 mL of Buffer ATL for 30 seconds. The outer mucosal layer of each pellet was then collected using a sterile polyester swab, soaked with Buffer ATL, and placed into a 1.5 microcentrifuge tube containing 1 mL Buffer ATL. The swab handle was gently rotated while submerged in the tube to release mucosal material. A single swab was used for all pellets per individual. After collection, 20 µL Proteinase K was added. Samples were incubated at 62°C for 2 h, vortexing every 30 min. Following incubation, an additional 10 µL Proteinase K was added, and samples were left incubating at 37°C overnight. Next, 4 µL RNase A (100 mg/mL) was added. Steps 2–7 of the manufacturer's protocol were followed, with the modification of doubling the volume of Buffer AL. DNA was eluted in 60 µL Buffer AE preheated to 56°C. DNA concentration was quantified using a Qubit dsDNA Quantification Assay Kit (Thermo Fisher Scientific; # Q32851) according to the manufacturer's instructions. A minimum gDNA concentration of 20 ng/µL was targeted for downstream genotyping. Whole blood DNA extraction was performed by Centre d'Expertise et de Services of Génome Québec (Montréal, QC, Canada) as well as the genotyping of all samples (feces and blood) using the *Rangifer* SNP array[22].

Genotype data analyses

Variant calling and filtering

Sample call rates – the percentage of SNP loci that a genotype was called per sample, and call frequency – the percentage of samples that a genotype was called per SNP, were calculated from

intensity files in the Illumina's GenomeStudio software. A total of 55,199 informative variants were obtained across individuals per array. Samples with call rates < 0.15 were excluded from downstream analyses.

Sex assignment

Sex assignment was performed by calculating observed heterozygosity (H_O) levels for each individual in a subset of SNPs (n = 1,626) mapping to the X chromosome. These values were calculated as the proportion of heterozygous genotype calls (e.g., "0/1" or "1/0") relative to the total number of non-missing loci for that individual. While males, with a single X chromosome, should have observed heterozygosity values close to zero, females should have observed heterozygosity values fluctuating between 0.05–0.5[22].

Population structure

Genetic clustering was assessed using Discriminant Analysis of Principal Components (DAPC), implemented by the *dapc* function in the R package *adegenet*[33]. Unlike Principal Component Analysis (PCA) or Principal Coordinates Analysis (PCoA), which describe overall patterns of genetic variation, DAPC infer differences between groups while minimizing variation within groups[33]. Prior to analysis, SNPs with > 20% missing genotype calls were removed. The optimal number of genetic clusters was determined using the *find.clusters* function, which identifies the *k* value that minimizes the Bayesian Information Criterion (BIC). The maximum number of clusters was set to six, reflecting the five caribou population types represented in the array design – migratory, mountain, western boreal, central boreal, and eastern boreal[22] – with one additional cluster included to account for the presence of reindeer in the dataset.

Genetic relatedness

Pairwise relatedness analyses were restricted to individuals with high-quality genotype data (n = 44). For individuals with paired blood and fecal samples, only blood-derived genotypes were retained to maximize data accuracy. SNPs with > 20% missing data were removed using the function *snpgdsSelectSNP* in the R package *SNPRelate*[34], which excluded 5,130 SNPs. Pairwise kinship coefficients were estimated using the *KING-robust* method implemented in the *snpgdsIBDKING* function, which is appropriate for datasets with underlying population structure, as indicated by DAPC results (see below). Under this framework, kinship coefficients near zero indicate unrelated individuals, values around 0.125 correspond to second-degree relationships (e.g., half-siblings), and values around and above 0.25 correspond to first-degree relationships (e.g., full siblings or parent–offspring).

Genetic distances among individuals were calculated from pairwise kinship coefficients using the transformation distance = 0.5 − kinship. Under this metric, genetically identical samples or duplicates (kinship ≈ 0.5) yield distances close to 0, whereas unrelated individuals (kinship ≈ 0) yield distances close to 0.5. The resulting distance matrix was used to perform hierarchical clustering using the unweighted pair group method with arithmetic mean (UPGMA) implemented in the *hclust* function in R

with the "average" linkage method. The clustering object was then converted to a phylogenetic tree format using the *as.phylo* function from the *ape* package[35] for visualization.

Ecotype assignment

Ecotype assignment was performed using a machine-learning approach developed from data gathered in previous work[22], complemented with data coming from additional sampling for population diversity surveillance. The overall data set included 1,326 *R. tarandus caribou* samples representing three different ecotypes – migratory, forest-dweller and Gaspesian – from east Canada and Northwest Territories. While a previous ecotype-assignment method was developed from a subset of diagnostic SNPs (N = 5,195) for anti-poaching purposes in the Quebec province[23], a new machine-learning model was developed to more generally predict the ecotype of caribou from the maximum set of SNPs available from the *Rangifer* SNP array. Thus, the total of 1,326 samples was genotypes for 45,419 SNPs and used to build a predictive model using a supervised classification approach with a “one-vs.-rest” strategy. The dataset was filtered for features (= SNPs) and observations (= samples) with low missing data proportion and stratified split into a learning set of 1,266 samples and a test set of 60 samples. Model optimization was performed searching across four algorithms (MultinomialNaiveBayes, RandomForest, K-Nearest-Neighbors, and GradientBoosting) and large distributions of hyperparameters using the “balanced accuracy” optimization and the *gridsearch* function from the Python library *scikit-learn* version 1.7.2[36].

Declarations

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Author contribution

VLA, PM, GM, and CR conceptualized the study; VLA and PM obtained samples; VLA and JP analyzed genotype data; VLA wrote the first draft of the manuscript; JP wrote methods for ecotype assignment; GM provided funding and supervision; all authors substantially contributed to revisions.

Competing interests

The authors declare no competing interests.

Data availability statement

Genotype data have been deposited in NCBI's Gene Expression Omnibus (GEO) database (Edgar et al., 2002) and will become publicly available upon acceptance of the full manuscript version for publication (accession number: GSE325110, reviewer token: cvwtooiihjallgp; https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE325110). Pipelines for genotype data analyses in RStudio are available in GitHub: https://github.com/VLuzuriaga/pop_gen_managed_caribou

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Figures

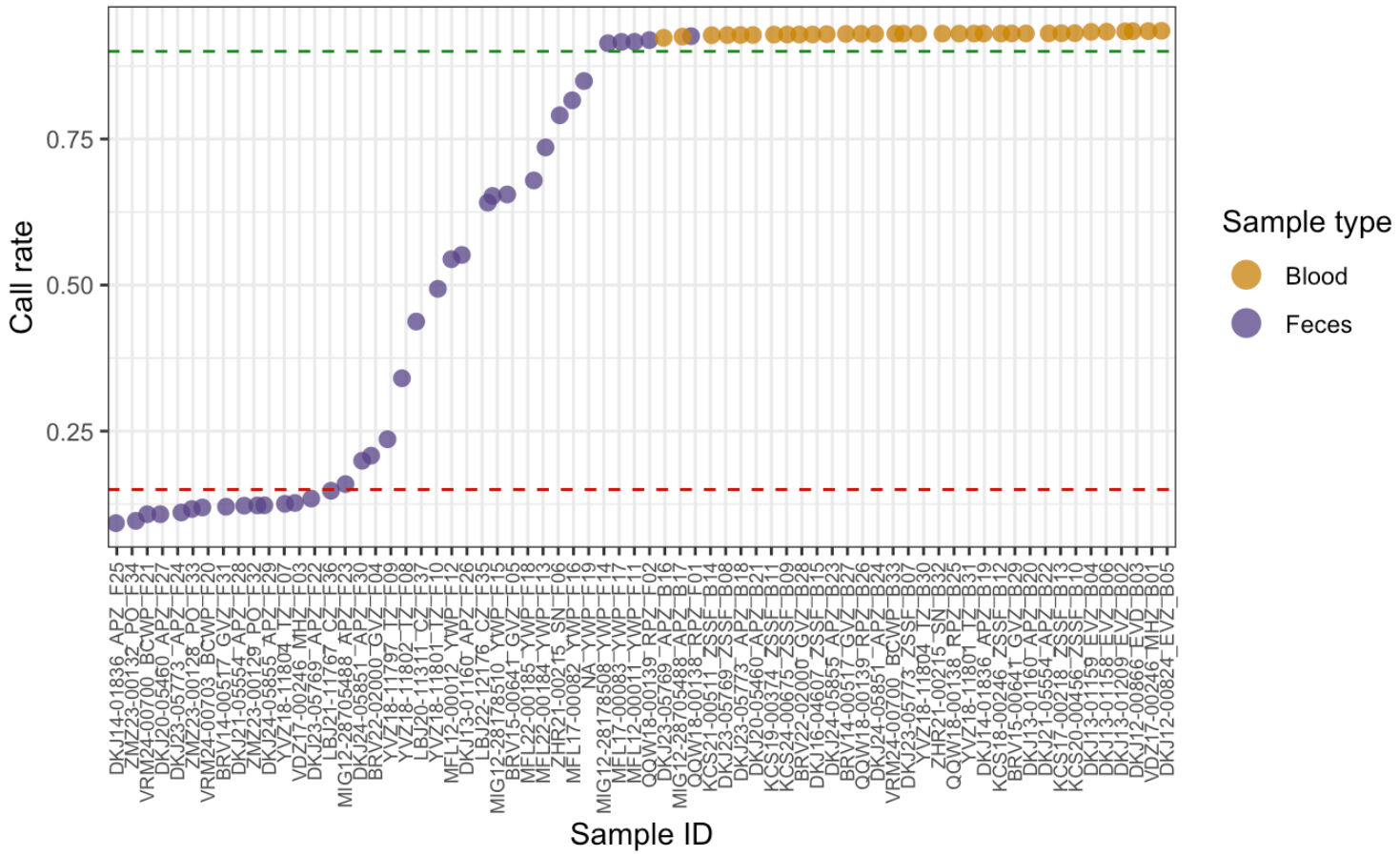


Figure 1

Call rates for samples obtained from whole blood (dark yellow) and feces (purple). Quality control thresholds are indicated with dashed lines: green (>0.90) for high quality samples and red (<0.15) for failed samples.

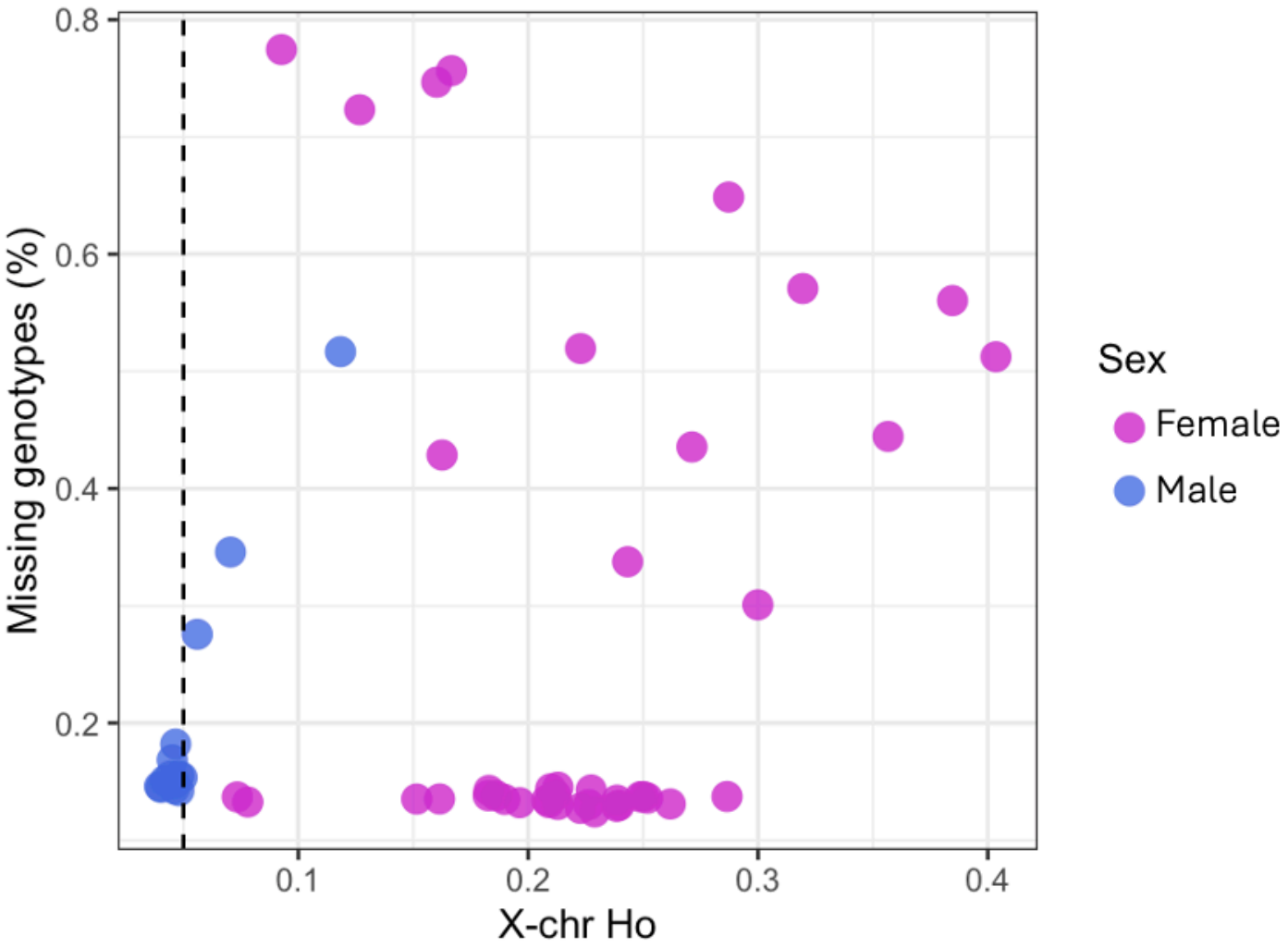


Figure 2

Observed heterozygosity of SNPs located in the X chromosome. Actual individual sex is coloured in pink for females and blue for males. The dashed line indicates the threshold used for molecular sex assignment. Samples with H_O values below 0.05 are categorized as males, whereas samples with H_O values between 0.05 and 0.5 are categorized as females.

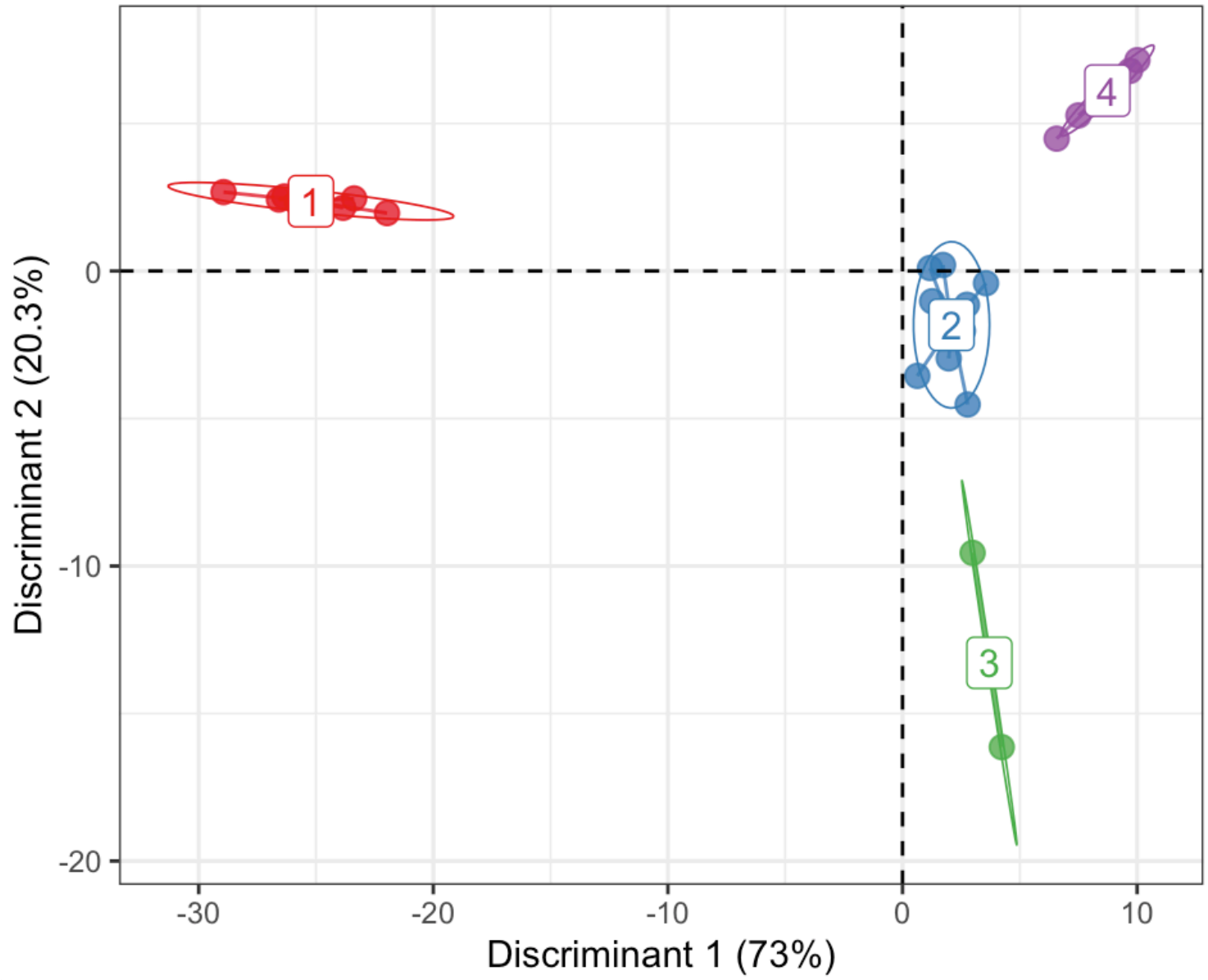


Figure 3

Discriminant Analysis of Principal Components (DAPC) for four genetic clusters identified in the dataset.

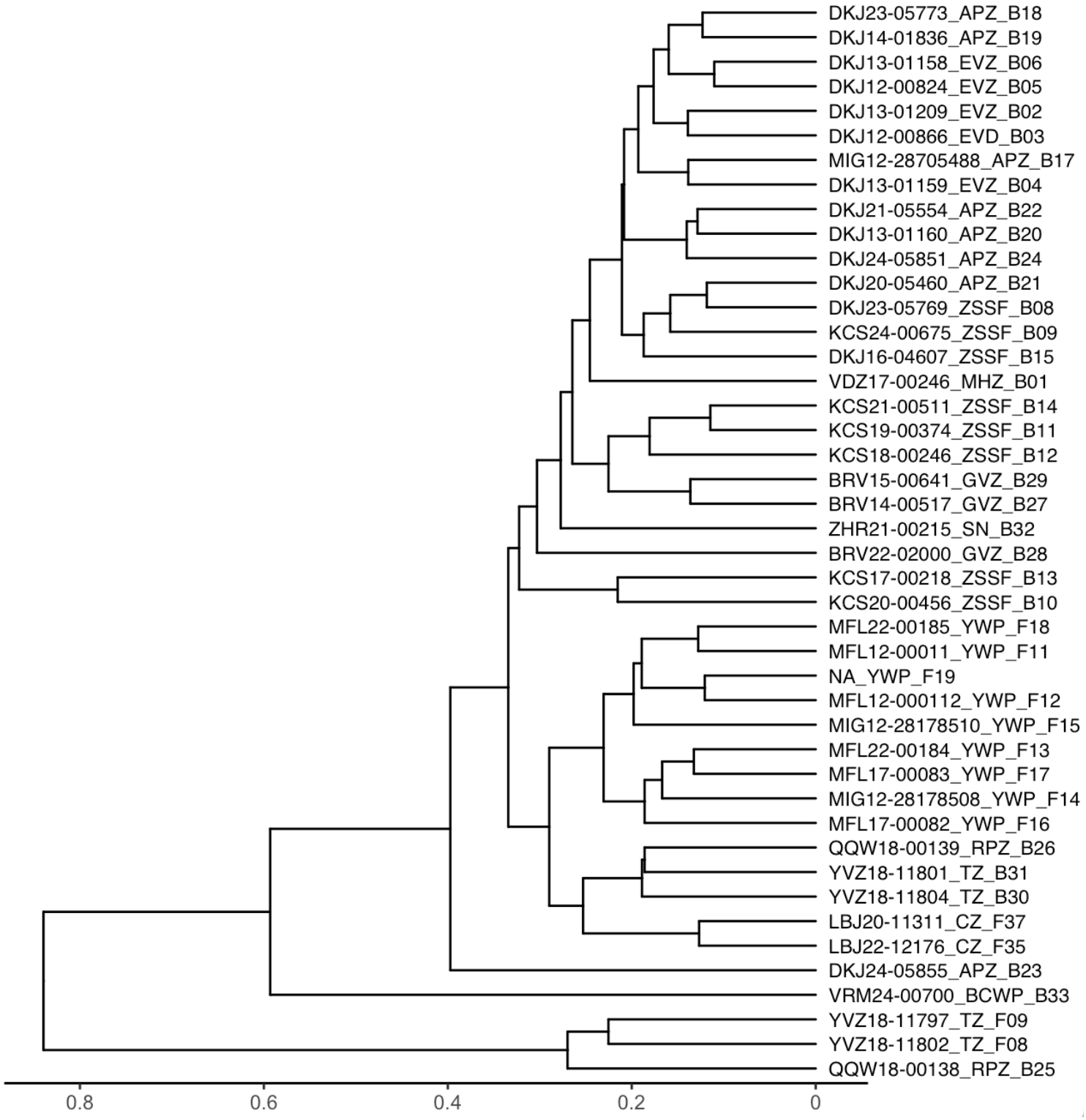


Figure 4

Cladogram of pairwise kinship between 44 caribou / reindeer housed in Canadian zoos. Branch lengths represent genetic distances, which were calculated as 0.5 – kinship. Closely related individuals are clustered together and have shorter branch lengths.

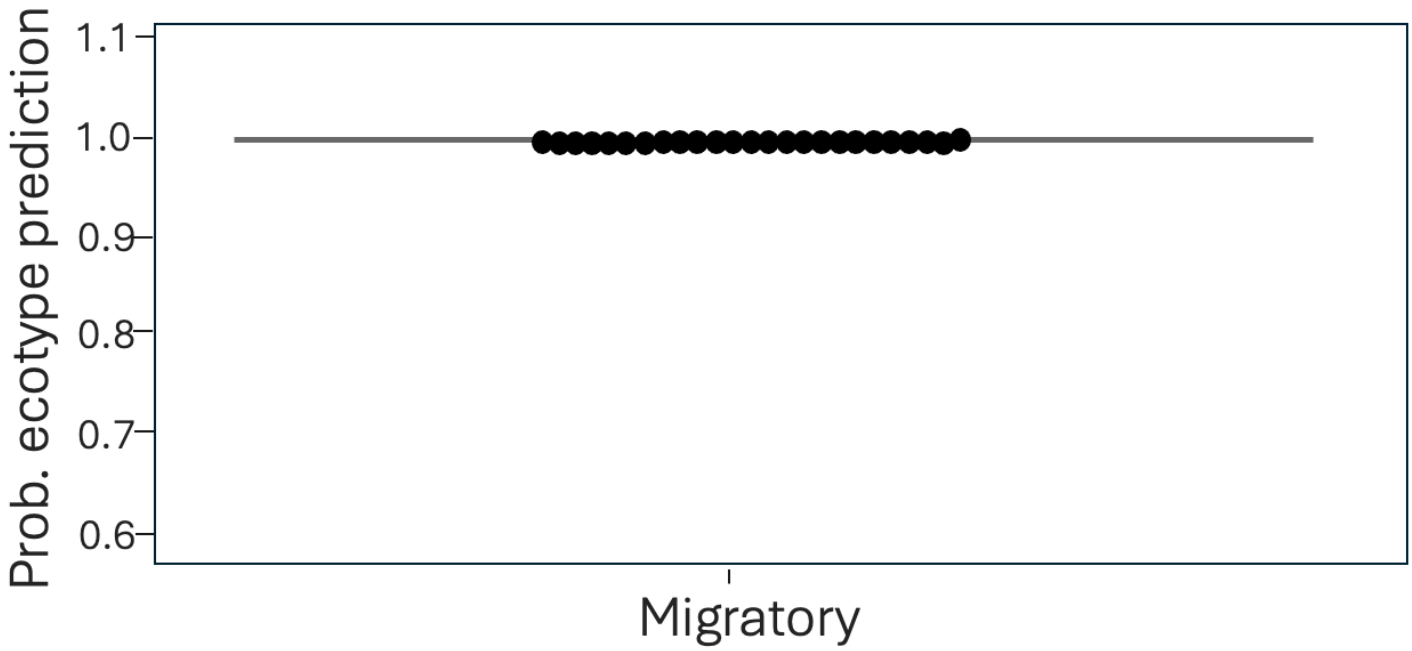


Figure 5

Results of ecotype assignment for all samples in our dataset, generated by Genovalia.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- LuzuriagaAveigaetal2026SupplementaryInformationApril27.pdf