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RESOURCE ARTICLE OPEN ACCESS

Design and Application of a Genome-Wide SNP Array to Improve Conservation Outcomes in the Critically Endangered Southern Corroboree Frog

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Received: 7 December 2024 | **Revised:** 21 October 2025 | **Accepted:** 6 November 2025

Keywords: amphibian | captive breeding | chytridiomycosis | genomic diversity | *Pseudophryne corroboree* | SNP chip

ABSTRACT

Species-specific genomic information has the potential to transform modern conservation management strategies through improved genomic assessment and management outcomes. Gaining genomic insights into genetic diversity, adaptability and potential resilience against infectious diseases is essential to enhance conservation efforts for threatened species. Here, we describe the development of the first custom SNP array for an amphibian, designed for the critically endangered *Pseudophryne corroboree*, which has experienced a near-total population collapse due to the amphibian chytrid fungus (*Batrachochytrium dendrobatidis, Bd*). The array comprises 48,386 SNPs, with an average density of 5.45 SNPs per Mb and was effective in genotyping multiple tissue types, including non-lethal buccal swabs. Of the SNPs, 82.1% were polymorphic across 910 captive-bred *P. corroboree* individuals derived from ~54 families. Population genetic analysis revealed evidence of ancestral inbreeding and two historic bottlenecks, one coinciding with the arrival of *Bd* in Australia. Notably, we demonstrate successful cross-species amplification of 21,077 (43.6%) polymorphic loci in three closely related anurans, highlighting the array's broader utility beyond *P. corroboree*. This tool represents a valuable resource for investigating the genetic basis of disease resistance and developing management strategies for improving reintroduction outcomes in *P. corroboree*, while also providing a foundation for advancing conservation genomics in other amphibian species.

1 | Introduction

Despite rapid advancements in genomic technologies, the development of species-specific resources for non-model wildlife species has progressed more slowly, largely reflecting the pace at which conservation priorities have come to incorporate genomic insights. As conservation management evolves to address emerging threats, there is a growing emphasis on understanding functional diversity, gene architecture and genome-wide effects, all of which require modern genotyping tools tailored to individual species. SNP arrays (or SNP chips), valued for their precision and reproducibility across samples, generations, and projects, have become increasingly popular. While low-density SNP arrays are relatively common in conservation genetics, only a few medium- to high-density arrays have been developed for wild non-model species, such as the bald eagle (*Haliaeetus leucocephalus*; Judkins et al. 2020), *Acropora* corals (Kitchen et al. 2020), *Ficedula* flycatchers (Kawakami et al. 2014), Florida scrub jay (*Aphelocoma coerulescens*; Chen et al. 2016), Antarctic fur seal (*Arctocephalus gazella*; Humble et al. 2020), great tit (*Parus major*; Kim et al. 2018), hihi (*Notiomystis cincta*; Lee et al. 2022) and house sparrow (*Passer domesticus*; Lundregan et al. 2018).

Their application has primarily been assessing population structure and diversity, with fewer efforts directed towards unravelling phenotype and genotype relationships. Furthermore, very few translate findings into applied conservation management strategies such as targeted genetic intervention using genomic associations to improve fitness traits, and none have been developed for an amphibian.

Medium- to high-density SNP arrays have routinely been used in agriculture and aquaculture to understand the genetic basis of animal production traits, and to facilitate selective breeding to modify commercially relevant phenotypes such as growth rate and disease resistance (Tsai et al. 2015; Seabury et al. 2017; Lillehammer et al. 2020; Peñaloza et al. 2021; Fraslin et al. 2022). Until recently, these resources were not required in wildlife conservation, as genetic studies were primarily focused on population monitoring. However, with the shift towards more proactive conservation strategies, there is a growing need for these genetic resources to support targeted interventions. Increasing threats, such as climate change and infectious diseases, are driving declines in many species, and the establishment of captive assurance colonies has created opportunities to study the genetic basis of fitness traits and allow for targeted genetic interventions (i.e., selective breeding and targeted gene flow) to support the management of threatened populations (Khan and Murn 2011; McKnight et al. 2017; Kosch et al. 2022; Humble et al. 2023).

Beyond their primary application in the target species, SNP arrays can also be applied to closely related taxa, providing an efficient means to generate genome-wide data for multiple species. Although ascertainment bias—where SNPs identified in a single reference species may underrepresent rare or lineage-specific variants and tend to capture older, shared polymorphisms—remains a known limitation, these arrays still offer substantial value (Miller et al. 2012; Lachance and Tishkoff 2013). In many conservation contexts resources to develop species-specific genomic tools are limited. Therefore, leveraging existing SNP arrays across related species can enable genomic approaches in threatened taxa that might otherwise lack such resources. A notable example is the use of canine SNP arrays, which have successfully been applied to genotype dingoes (Cairns et al. 2023), wolves and coyotes (Ke et al. 2011). This strategy is particularly relevant for groups like amphibians, where large and complex genomes make whole genome sequencing prohibitively expensive and technically challenging (Kosch et al. 2024). Leveraging SNP arrays across closely related species therefore offers a practical and cost-effective means to generate genome-wide data for multiple taxa, ultimately enhancing conservation outcomes across related species.

Managing animals in captivity, particularly those with small effective population sizes and low genetic diversity can pose significant challenges in preventing inbreeding and maintaining overall population health. Genomic datasets can aid in monitoring these risks by enabling the examination of population structure, particularly regarding the crossing of subpopulations that could inadvertently lead to inbreeding or outbreeding depression. Genome-wide datasets are particularly valuable because they provide comprehensive coverage across the genome, enabling more powerful and precise analyses, such as accurately estimating inbreeding by quantifying runs of homozygosity (ROH)—continuous homozygous segments that arise from inheritance of identical alleles from a common ancestor. The length of ROH can offer insights into the timing of inbreeding events, with longer segments indicating inbreeding across more recent generations (McQuillan et al. 2008). Additionally, high-density SNP datasets enable the assessment of linkage disequilibrium (LD), offering valuable insights into shared genome co-ancestry, which aids in the detection of quantitative trait loci (QTL) and supports the application of genomic selection in breeding programs (Meadows et al. 2008; Rabier et al. 2016). Additionally, LD assessments can improve estimations of historical changes in effective population size (Wang et al. 2016). In other taxa, understanding population diversity has been instrumental in shaping practical conservation strategies; for instance, genetic assessments have informed breeding strategies in the Californian condor (*Gymnogyps californianus*, Ralls and Ballou 2004; Moran et al. 2021) and the Florida panther (*Puma concolor coryi*, Johnson et al. 2010; Van De Kerk et al. 2019), leading to restored genetic diversity and improved population viability.

Amphibians are currently the most threatened group of vertebrates globally, with numerous threats having caused devastating population declines and extinctions (Scheele et al. 2019; Luedtke et al. 2023). Among these, one of the most severe threats is the amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*). In response to the global spread of *Bd*, the number of captive colonies for amphibians has surged, encompassing over 540 species (Jacken et al. 2020), including 213 managed for conservation purposes as of 2016 (Harding et al. 2016). However, with no effective solution to counter persistent threats like *Bd*, many species in these colonies are at risk of becoming functionally extinct in the wild unless alternative management strategies are developed (Luedtke et al. 2023; Berger et al. 2024).

The Critically Endangered southern corroboree frog (*Pseudophryne corroboree*; known as 'Gyack' by the Australian indigenous people; see Figure 2 inset), faces a high risk of extinction due to its susceptibility to *Bd* and the lack of effective strategies to mitigate the impact of the fungus in the wild (Hunter et al. 2010; Kosch et al. 2019; Davidson, Kosch, et al. 2025). In response, captive assurance colonies for the species were established in 1997, and now maintain around 200 living founding frogs across three Australian institutions–Melbourne Zoo, Healesville Sanctuary and Taronga Conservation Society–which serve as a refuge to prevent extinction, support reintroduction efforts and enable conservation research (McFadden et al. 2013; Davidson et al. 2022). Despite ongoing reintroduction efforts, recruitment in the wild remains minimal, likely due to the widespread presence of *Bd* and the presence of tolerant reservoir hosts (Brannelly et al. 2018). With only approximately 25 individuals remaining in the wild (pers. comm. David Hunter 2024), all at reintroduction sites, the captive population is believed to hold all the remaining genetic diversity of this species. Recently, selective breeding has been proposed as a strategy to reduce *Bd* susceptibility and improve reintroduction success in *P. corroboree* (Kosch et al. 2022; O'Connell et al. 2024; Davidson, Kosch, et al. 2025). Given the intensive management of *P. corroboree* breeding, this species presents a unique opportunity to investigate the genetic basis of disease susceptibility in a threatened, captively managed population,

potentially providing a model for evaluating the efficacy of selective breeding to improve conservation outcomes.

To advance the captive management of *P. corroboree* towards targeted breeding strategies, it is essential to develop genomic tools that facilitate the assessment of genetic diversity and improve our understanding of the species' genomic architecture. While previous studies have examined a subset of the genetic diversity in wild-caught individuals (Morgan et al. 2008; Kosch et al. 2019), there has yet to be an evaluation of the genome-wide genetic diversity in *P. corroboree* bred through the captive program. As we move towards more targeted breeding strategies in this species, understanding genetic variation, trait heritability, and the underlying genetic architecture will be crucial for ensuring the species' long-term survival in captivity and for the success of selective breeding efforts.

To address these needs, we developed a SNP array to enable cost-effective high-density genome-wide SNP genotyping of a large number of F1 *P. corroboree* from the captive population. Here, we describe the development of the *P. corroboree* 50 K SNP array, the first custom array for an amphibian. This *P. corroboree* array not only builds upon the existing genetic resources for this species, enabling the development of targeted conservation strategies guided by genomic data, but also offers broader potential value for other amphibians if markers prove cross-amplifiable, thus supporting conservation efforts at a wider taxonomic scale. A key long-term objective for *P. corroboree* is to better understand the genetic basis of survival from *Bd* infection and ultimately implement a selective breeding program for the captive population to improve conservation outcomes. To support this goal, we genotyped 910 captive-bred *P. corroboree* to assess the population and genomic structure within the breeding colony. Additionally, we explored the suitability of the array for genotyping non-lethal samples and species beyond the primary target. Genotyping data from this array will support management practices aimed at enhancing the survival and reintroduction success of this critically endangered amphibian.

2 | Materials and Methods

2.1 | Whole Genome Sequencing

Whole genome sequence (WGS) data from 23 *P. corroboree* was generated to identify high-quality SNPs for inclusion on the genotyping array. Animal information, including their source populations, sex and tissue types used for DNA isolation is outlined in Table S1. This included WGS previously generated from three frogs: a captive-bred individual used for genome size estimation and two wild-caught founders of the *P. corroboree* captive breeding program. Additionally, toe clips were sequenced from 20 wild-caught founding frogs that were likely parents of the F1 individuals in this study (see Section 2.4). These individuals represent 10 out of the 15 historical populations of *P. corroboree* (Figure S1).

The two samples used for genome assembly were sequenced by the Vertebrate Genomes Project (VGP) following methods outlined in Rhie et al. (2021). All other samples were processed in full by the Australian Genome Research Facility (AGRF), including extraction, library preparation and sequencing on the Illumina NovaSeq using their standard workflows (see: https://www.agrf.org.au), with sequencing aimed at achieving approximately 10× coverage. The combined sequencing data from the 23 frogs resulted in a total of 12,858,943,965 paired-end reads, with a median number of 494,029,117 reads per sample and a mean coverage of ~19× (Table S1).

2.2 | SNP Discovery

The WGS data was pooled and evaluated using *FastQC* (v0.11.9; Andrews 2010), followed by the removal of adaptors, polyG tails and low-quality score reads with *FastP* (v 0.23.2) with default settings (Chen et al. 2018). The trimmed and filtered reads were aligned to the *P. corroboree* genome (accession ID: GCA_028390025.1) using *BWA-MEM* (v 0.7.17) using default parameters (Li and Durbin 2009). Aligned paired-end reads were processed using *Samtools* (v1.12) to generate a coordinate-sorted BAM file (Danecek et al. 2021), and duplicate sequences were removed using *Picard* (v2.25.0; The Picard Toolkit 2019).

Variant calling was performed using *BCFtools* (v1.15; Danecek et al. 2021) with the multiallelic model (−m) and otherwise default parameters, resulting in 53,902,064 SNPs. The SNP dataset was filtered to remove low-quality sites using *BCFtools*. Specifically, SNPs were filtered out if they fell within unassigned contigs; had a Phred-scaled quality score of <20; or had a depth of coverage of <5 and >25. Individuals were then removed if they had $>70\%$ of their genotypes missing, and SNPs were removed, which had a fraction of missing genotypes $>20\%$. Low-complexity regions of the genome were identified using *DustMasker* (v1.0.0; Morgulis et al. 2006), and any SNPs that fell within those regions were discarded. Further filtering removed SNPs that were multiallelic or monomorphic; fell within 100 bp of indels; had a HWE threshold less than $p=10^{-6}$; or had a minor allele frequency of $<10\%$, corresponding to presence in at least three individuals. The remaining SNPs were checked for duplicates using *PLINK* (v1.90b6.21; Purcell et al. 2007) and any sites containing wildcard nucleotides (i.e., N) at the SNP position were removed. We then removed any SNPs that had another SNP in their flanking sequences (30 bp on either side of the SNP), as these can interfere with probe binding. Finally, SNPs were thinned by distance, keeping 1 SNP every Kb, to ensure even distribution across the genome, resulting in a total of 537,368 SNPs (see Table S2 for filtering pipeline).

2.3 | SNP Selection for the Array

The filtered dataset, comprising 537,368 SNPs, was submitted to Thermo Fisher Scientific for assessment of their suitability for inclusion on an Axiom myDesign 384HT Custom Array. After *in silico* scoring, 115,961 SNPs were recommended for tiling on the array. We discarded any SNP that did not fall into the 'recommended' category by Thermo Fisher Scientific (neutral recommendation = 47,132, or not recommended = 374,275), as they have a lower probability of converting genotypes. We further filtered the recommended SNPs to remove any with non-unique probes, and any SNP types that were A/T or G/C since they require two probes on the array. The remaining SNPs were

then ranked according to their design score and tiled ensuring an even distribution across the genome, resulting in a total of 48,386 SNPs included on the array.

2.4 | Samples for Genotyping

We genotyped a total of 1143 samples on the SNP array, the majority of which ($n = 995$, 87%) were collected in 2022 and 2023 from captive-bred, F1 juvenile *P. corroboree* (Table S3). These individuals were part of a separate study on the genetic basis of chytrid resistance (Davidson, Berger, et al. 2025), which was conducted under the approval of the University of Melbourne's Animal Ethics Committee (Application 2021-22144-24454-5), and Wildlife Act 1975 Research Authorisation permit number 10010261.

We were also interested in evaluating the use of buccal and skin swabs as a non-invasive method for collecting samples for genotyping. Thus, we included skin and buccal swabs from five F1 frogs, which also had tissue sequenced on the array. Additionally, we genotyped 118 *P. corroboree* samples of unknown preservation status, collected during past projects in our laboratory, referred to as 'past project samples' (Table S3 for sample information). These samples were included to evaluate the array's effectiveness at genotyping samples that are either not freshly preserved or have suboptimal or unknown preservation histories. If successful, this would enhance the usefulness of the array, as obtaining samples from endangered species can be challenging (Kosch et al. 2024) and it could also enable the use of museum specimens for sampling (Hahn et al. 2022).

Additionally, we investigated the ability of the array to perform cross-species amplification by genotyping samples from other amphibians. To evaluate SNP transferability, we selected three species within the Myobatrachidae family that range in phylogenetic distance to *P. corroboree* (Read et al. 2001). This resulted in a total of 18 additional samples, with six individuals from each of *P. pengilleyi*, *P. bibroni* and *Crinia signifera*. The tissue type, storage media and conditions varied between samples, as outlined in Table S3.

All 1131 tissue samples and 10 swab samples were submitted to the Garvan Institute of Medical Research for DNA extraction with their high throughput spin column extraction protocol (see: www.garvan.org.au). Samples were quantified and normalised to a concentration of 5–80 ng/μL per sample. Thirty-three samples fell below the recommended concentration threshold of 5 ng/μL for running on the array (all 10 swabs, 21 *P. corroboree* and 2 cross-species tissues). Corresponding DNA concentrations are available in the 'Overall-data.xlsx' document uploaded to DataDryad. Despite their suboptimal concentrations, these samples were included to evaluate the array's performance with low-quantity DNA samples.

2.4.1 | Statistical Analysis of Array Performance

To evaluate differences in genotyping success across the different tissue types, we modelled the proportion of successfully called SNPs per individual using a weighted generalised linear model (GLM) with a quasibinomial family to account for overdispersion, in R (v4.1.2) via the RStudio interface (RStudio Team 2020; R Core Team 2021). Estimated marginal means were obtained for each tissue type on the response scale using the emmeans package (Lenth 2022), and pairwise comparisons were performed with Tukey's adjustment.

To assess whether DNA concentration (measured in ng/μL) or DNA quality (measured by the 260/280 absorbance ratio) influenced the likelihood of a sample passing the 95% call rate threshold, a GLM (family = binomial) was used to model the binary pass/fail outcome.

2.5 | SNP Genotyping

A total of 1143 samples were submitted for genotyping on the SNP array at the Ramaciotti Centre for Genomics using the Axiom Propel XPRES 384HT Workflow (Applied Biosystems). Among these, two samples were duplicated across the three plates (384 samples per plate) to evaluate genotyping technical error rates, resulting in 1141 unique samples.

The resulting genotype data was analysed using the *Axiom Analysis Suite* (v5.3.0.45) following their Best Practices Workflow. Two workflows were applied to the data, the first to assess the performance of the array across all *P. corroboree* samples (F1 and past project tissue and swab samples), and the second to quantify rates of cross-species amplification. For the former, we excluded the three other amphibian species, and filtered out samples with a Dish QC (DQC) score lower than 0.90 and a call rate below 95%. This dataset was then exported from *Axiom Analysis Suite*, and *PLINK* was used for additional filtering to remove SNPs with minor allele frequency (MAF) of < 5%. The final *P. corroboree* exclusive dataset contained 39,701 polymorphic SNPs from 1087 samples, representing 54 captive-bred pedigree families.

For the dataset containing the other amphibian species, six randomly selected *P. corroboree* samples were run in conjunction with the three other species (*P. pengilleyi*, *P. bibroni* and *C. signifera*). No additional filtering was performed (e.g., DQC or call rate filtering), in order to retain as many samples as possible from the other species.

2.6 | Population Diversity and Structuring

To assess genome-wide genetic diversity of captive *P. corroboree* animals, the dataset was subsetted to include only the F1 *P. corroboree* that were used in our *Bd* disease challenge experiment (Davidson, Berger, et al. 2025) and passed quality control ($N = 910$). This F1 dataset was comprised of frogs from 54 pedigree families, including half- and full-siblings, as well as other co-ancestry relationships. To investigate population structure, we performed a principal component analysis (PCA) and visualised population structure using *pcadapt* (Luu et al. 2017). For a detailed assessment of family relationships, we applied PCA and *NETVIEW P* (Steinig et al. 2016) to the entire dataset to examine genetic relationships across the broader family structure within the captive breeding program.

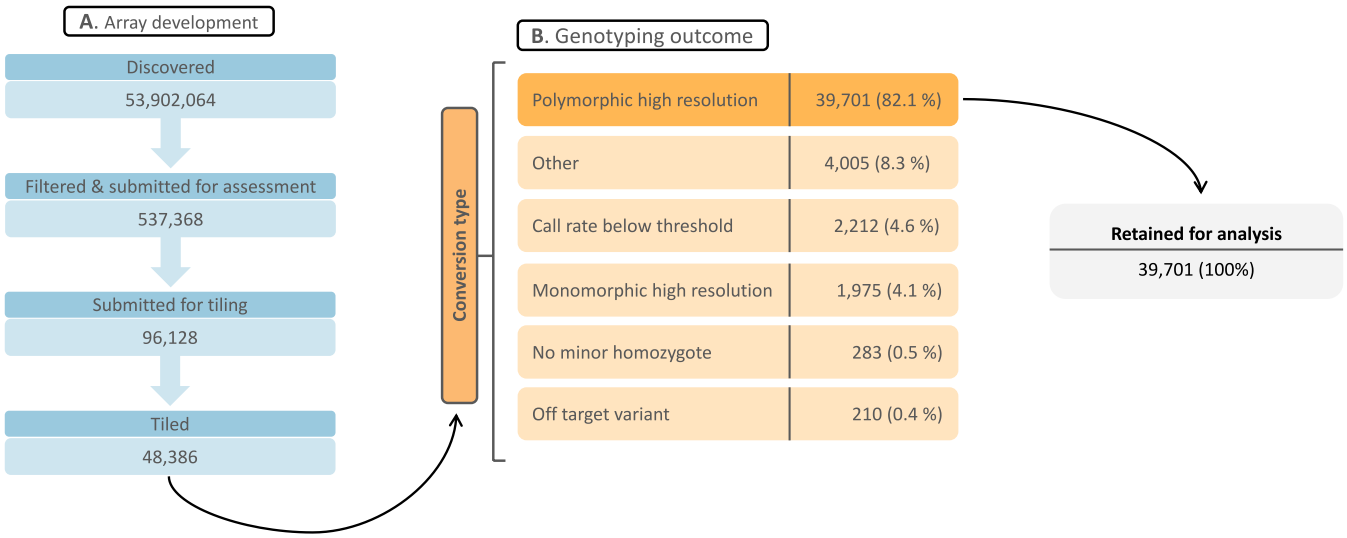

FIGURE 1 | Overview of the number of SNPs at each stage of the design, development, and implementation of the array. (A) The number of SNPs discovered, filtered, submitted for tiling and successfully tiled on the array; and (B) the classification outcome of converted SNPs, from 1115 *P. corroboree* tissue samples, and the final number of polymorphic SNPs used in the analysis.

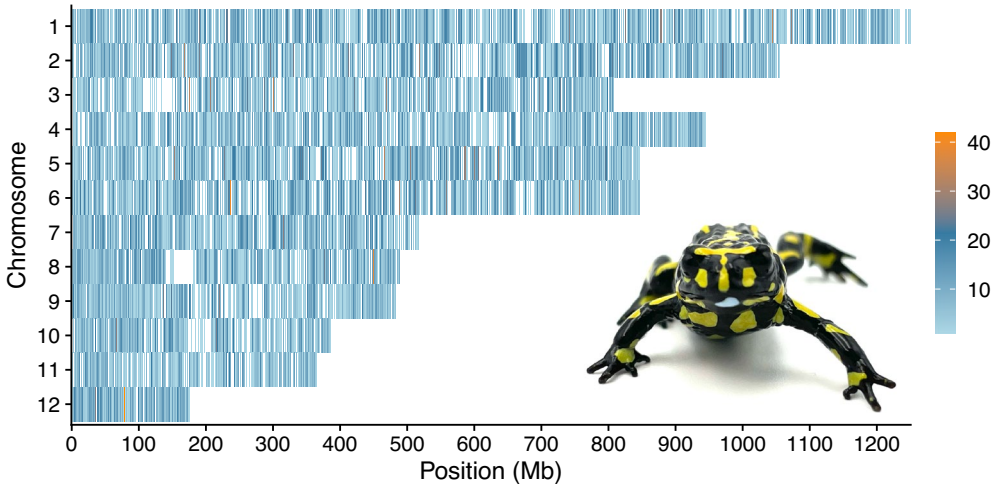

FIGURE 2 | Density of SNPs on the *P. corroboree* array distributed across the genome. Density is shown as the total number of SNPs per Mb window for the 48,386 SNPs tiled on the array. The colour gradient indicates SNP density, as shown in the figure legend, with an inset picture of an adult *P. corroboree*.

2.6.1 | Linkage Analysis and Effective Population Size

LD measured as r^2, was calculated between all pairs of syntenic SNPs in the F1 dataset using *PLINK*, applying a 5 Mb sliding window. LD decay was visualised in R using the *ggplot2* package (Wickham et al. 2016), with a LOESS-smoothed trend line fitted to illustrate the overall pattern of LD decay with increasing distance. We then estimated Ne using three complementary LD-based approaches. First, we used *SNP1101* (Sargolzaei 2014; available upon request from the corresponding author) to estimate Ne over historical time, based on patterns of LD decay across the genome. Given that *P. corroboree* typically reach sexual maturity between 3 and 5 years of age, we defined a generation length accordingly for temporal scaling. Second, we used *NeEstimator* (v2; Do et al. 2014), implemented via the *RLDNe* (v1) R package, to estimate contemporary Ne from LD among unlinked SNPs, applying a MAF threshold of 0.1, 0.05 and 0.02. Third, we used default thresholds in *SNeP* (v1.1; Barbato et al. 2015) to validate historical Ne estimates and compared estimates between the F1 dataset and 22 wild-caught F0 individuals. For the latter, we subsetted the WGS data to SNPs overlapping the array using *PLINK* before estimating Ne.

2.6.2 | Runs of Homozygosity and Inbreeding

Using the entire F1 dataset, we estimated inbreeding as the proportion of the genome in runs of homozygosity (F_{ROH}) by determining the sum of the detected ROH for each individual and dividing it by the total length of the genome, 8.87 Gb, using *PLINK*. ROH were calculated using a window-based approach, with a sliding window of 30 SNPs. Homozygous segments were called as an ROH when they had no more than five heterozygous sites, contained at least 20 SNPs, had at least one SNP per 100 Kb and were at least 300 Kb in length. The selection of a 300

Kb threshold for defining a ROH was based on previous studies conducted on other wild populations (Duntsch et al. 2021; Foote et al. 2021). This approach corrects for short ROH which arise from SNPs in LD, as no LD filtering was applied to this dataset (Meyermans et al. 2020). In addition, we estimated inbreeding as the inbreeding coefficient (F), based on the observed and expected heterozygosity for each individual using *VCFtools* (v1.16; Danecek et al. 2011).

3 | Results

3.1 | SNP Discovery, Filtering and Array Design

Variant calling of the WGS sequence data from the 23 *P. corroboree* (20 founders and 3 previously sequenced frogs) resulted in ~53 M putative SNPs. Downstream quality filtering was applied, resulting in 537,368 SNPs that passed our strict filtering thresholds (Figure 1A). Further selection based on quality metrics reduced this number to 96,128 SNPs, which were submitted to Thermo Fisher Scientific for tiling on the array (Figure 1A). The SNPs were ranked according to their design score and genome coverage, and tiled until the array was filled. This process resulted in the successful tiling of 48,386 SNPs onto a custom *Pseudophryne corroboree* SNP array (AXIOM PSCO SNP 384S384). The array has an average density of 4.6 SNPs per Mb (Figure 2). The number of SNPs tiled per chromosome, and the number that successfully converted polymorphic loci are listed in Table S4.

3.2 | Array Performance

To evaluate the array's performance, we genotyped a total of 1115 *P. corroboree* tissue samples across three plates. Of those, 26 samples fell below the call rate threshold of 95% (25 F1 samples, and one sample from a past project). The remaining 1087 *P. corroboree* samples successfully converted genotypes across 42,058 SNPs, representing 86.7% of the tiled loci. Successfully genotyped SNPs were polymorphic (82.1%), monomorphic (4.1%) or classified as 'no minor homozygous' (0.5%) which were samples that had good clustering resolution but produced no minor homozygous genotypes (Figure 1B). The remaining 13.3% of SNPs were excluded because they failed to convert genotypes, had a low call rate (4.6%), were off-target variants (0.4%), or were low-quality SNPs ('other' category, 8.3%; Figure 1B).

We examined the 26 *P. corroboree* tissue samples that failed to meet the call rate threshold but were unable to identify any consistent patterns explaining the genotype conversion failures. Notably, 75% of the failed SNPs in these samples occurred in three or fewer individuals, though these individuals varied across SNPs, suggesting that the failures were not associated with specific samples or DNA quality thresholds. Additionally, there was no evidence of individual relatedness between samples or batch effects that could account for the call rate failures.

To assess genotyping error rates across the array, we evaluated the performance of the two sets of duplicated samples. The duplicated samples achieved high reproducibility of 99.6%. These duplicate samples were then removed before subsequent analyses, resulting in a dataset comprised of 1085 *P. corroboree* samples genotyped across 39,701 polymorphic SNPs (Figure 1B).

3.3 | Sample Type, Quantity and Quality

Overall, tissue type had a significant impact on genotyping success, as reflected by the call rate per sample. Pairwise comparisons revealed that toe clips yielded significantly higher call rates compared to both head and liver tissue (Figure S2). Specifically, the odds of a SNP being called successfully were 2.83 times higher for toe clips compared to head tissue (OR = 0.353, 95% CI: 0.158–0.790, $p = 0.005$) and 3.87 times higher compared to liver tissue (OR = 0.258, 95% CI: 0.099–0.667, $p = 0.001$; Figure S2B). The difference observed between tissue types here is likely impacted by unequal sample sizes among tissue types (Table S3), and poor-performing outliers. Interestingly, the toe clips performed extremely well, even though they were older samples from past projects. Only one past project sample, a frozen liver sample, failed QC filtering, with a call rate of 72.55%.

DNA sample concentrations did not have a significant effect on genotyping success, measured as passing the call rate threshold of 95% (OR = 0.991, 95% CI: 0.969–1.013, $p = 0.44$; Figure S3A). All 21 *P. corroboree* tissue samples with DNA concentrations below the recommended threshold of 5 ng/μL achieved a call rate exceeding 95%. Conversely, the 26 samples that were excluded for failing to meet the call rate threshold had DNA concentrations ranging from 6.4 to 73.4 ng/μL, and no sample exceeded the maximum recommended DNA input of 80 ng/μL. In contrast, DNA quality had a significant effect on the likelihood of samples passing the call rate threshold (OR = 0.00113, 95% CI: 0.0000141–0.0972, $p < 0.001$), as samples with higher 260/280 ratios had a lower probability of passing the call rate threshold (Figure S3B).

Additionally, we evaluated the efficacy of genotyping non-lethal samples using skin and buccal swabs. All five buccal swabs exceeded the DQC threshold, despite being below the DNA concentration threshold of < 1 ng/μL. However, they failed to pass the call rate threshold, with call rates ranging from 80.65% to 93.97% (average = 90.14%; Figure S4). In contrast, all skin swabs failed the DNA concentration and DQC thresholds, preventing call rate estimation for these samples.

3.4 | Cross-Species Amplification

All cross-species samples failed to meet the DQC filtering threshold, a measurement of interference between the AT and GC channel, based on non-polymorphic loci. However, when no DQC filtering threshold was applied, 36,372 SNPs successfully converted genotypes, representing 75% of the tiled loci. Of those SNPs that converted genotypes, there was a significant reduction in the number of polymorphic ($n = 21,077$, 43.6%) and monomorphic ($n = 900$, 1.8%) loci, whereas the number of no minor homozygous calls ($n = 14,395$, 29.8%) increased (Table S5) when compared to the *P. corroboree* only dataset. These samples also varied in tissue types (Table S3), but interestingly they all had similar average call rates (*C. signifera*, 92.2%; *P. bibroni*, 92.1%; and *P. pengilleyi* 92.7%; Table S6, Figure S5).

3.5 | Population Diversity and Structuring

PCA indicated that the first two components collectively explain 21% of the total genetic variance (Figure S6A). Fine-scale family structuring was observed across the first two principal components (Figure S6B), with the network analysis (Figure S7) further highlighting the division into distinct family groups.

3.5.1 | Linkage Disequilibrium and Effective Population Size

The LD estimates of the F1 animals were high and displayed minimal variation across chromosomes (Figure 3). The average r^2 between adjacent SNPs was 0.67 ± 0.34 for all chromosomes and ranged between 0.58 and 0.72 within the chromosomes (Table S7). On average, 82.1% of adjacent SNPs across the genome had an r^2 value greater than 0.2, and 50.4% had an r^2 value exceeding 0.8. The average distance between adjacent SNPs across the genome was 216 kb. As the physical distance between pairs of markers increased, the mean r^2 declined, with strong LD ($r^2 \geq 0.5$) decaying by 400 Kb, while moderate LD ($r^2 \geq 0.2$) diminished by approximately 900 Kb (Figure 3). Contemporary Ne estimates generated with unlinked SNPs ($n = 7743$) in *NeEstimator* were highly consistent across MAF thresholds of 0.1, 0.05 and 0.02, yielding values of 33.2, 33.4 and 33.4, respectively. Confidence intervals were narrow, varying by less than one decimal place across all estimates. These results are comparable to the contemporary estimates obtained using all SNPs in *SNP1101*, which were 29. Historical Ne estimates using the F1 dataset from *SNP1101*, spanning the past 500 generations suggest the population was undergoing a long-term decline until approximately 100 generations ago. At that point, Ne began to steadily increase from 57 to 101, before undergoing a dramatic decline 10 generations ago, leading to the current estimates of 29–33 (Figure 4). Estimates derived from *SNeP* cover a shorter timeframe (13–180 generations ago) and therefore do not capture the recent decline observed in *SNP1101*. Nonetheless, F1-based estimates from SNeP are broadly consistent with those from *SNP1101* (Figure S8). In contrast, estimates derived from the 22 wild-caught F0 founders show lower Ne values over the past 100 generations, ranging from 24 to 44, followed by a gradual increase to 69 approximately 200 generations ago, which follows a similar trend to that of the F1 across both methods (Figure S8). All Ne estimates across methods, and datasets remained below 100.

3.5.2 | Inbreeding

Among individuals, F_{ROH} ranged between 0.02 and 0.08 (mean = 0.04, Figure S8A) and consisted of 86–289 runs across each genome (Figure S9B). The frequency of ROH across the genome varied among the F1 cohort (Figure 5). A comparison between animals in the top 10% and bottom 10% for the number of ROH demonstrated significant variation in the number of ROH between the two groups (270 compared to 96, respectively; Figure S10). ROH length ranged from 305 to 12,000 Kb, with short runs (<4 Mb) comprising 94.3% of total ROH (Figure S11). The number of ROH decreased as the length increased, with 5.7% falling between 4 Mb and 12 Mb, and none exceeding 12 Mb (Figure S11).

The mean inbreeding coefficient (F) across all F1 samples was −0.018 (SD±0.064, range = −0.285–0.319; Figure S12), indicating that on average this population is slightly more heterozygous than expected.

4 | Discussion

We developed a custom 50 K SNP array for the Critically Endangered southern corroboree frog, the first SNP array for any amphibian and used it to characterise the species' genomic architecture. Genotyping data across 910 F1 *P. corroboree* samples, from 54 pedigree families, selected to represent the broad genetic diversity of the species in captivity, revealed evidence of ancestral inbreeding based on ROH, a low effective population size and high LD, indicative of long-term demographic declines and genetic bottlenecks in this species. With 39,701 tiled SNPs

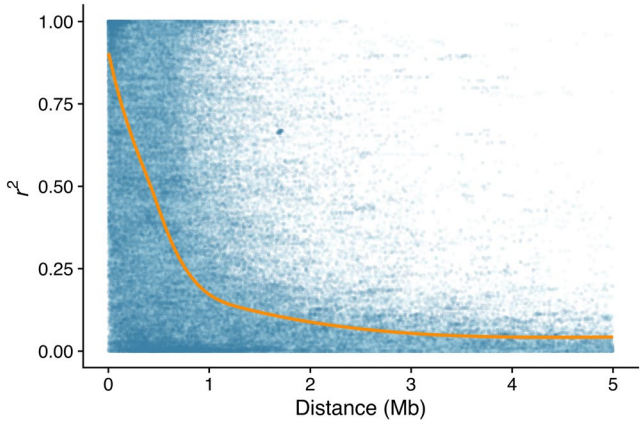

FIGURE 3 | Linkage disequilibrium, measured as r^2, between all syntenic pairs of SNPs across the *P. corroboree* genome. Blue points represent the observed pairwise r^2 values, while the solid orange line illustrates the expected decay of LD as estimated by nonlinear regression.

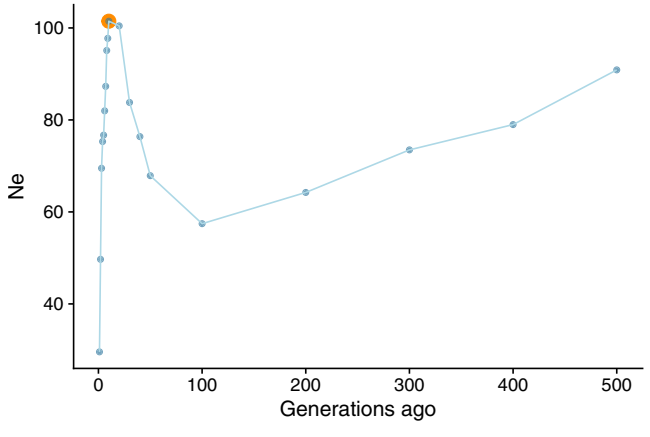

FIGURE 4 | Effective population size (Ne) in *P. corroboree* modelled as a function of generations. With an average generation time of 3–5 years, the orange point indicates the approximate generation when *Batrachochytrium dendrobatidis* was introduced to their home range in 1984.

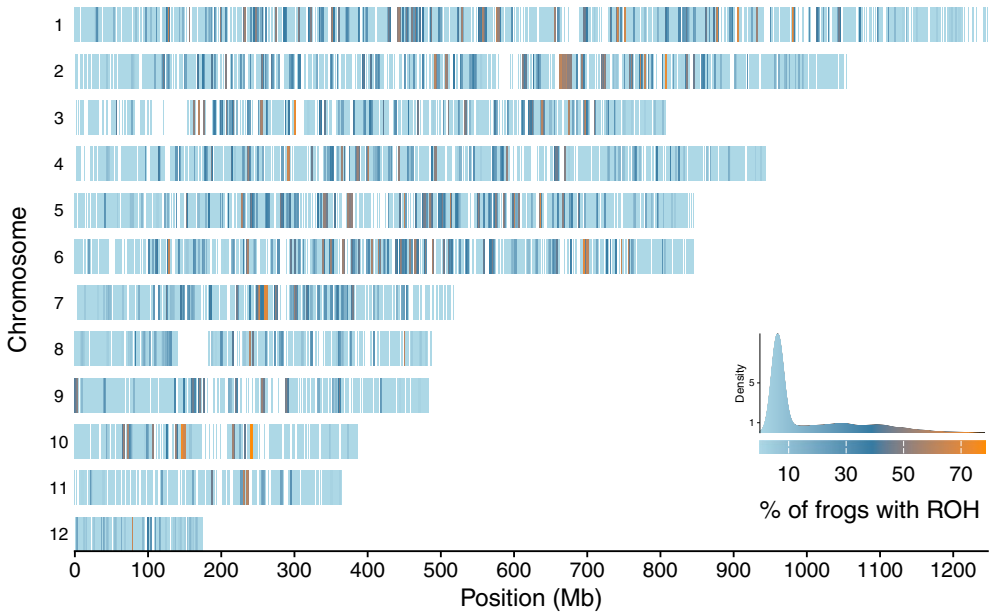

FIGURE 5 | Density of runs of homozygosity (ROHs) across the *P. corroboree* genome among 910 F1 frogs. ROHs were measured using non-overlapping 300 Kb windows. The colour gradient reflects ROH density, as indicated in the figure legend.

(82.1%) successfully converted into polymorphic genotypes, this array offers an invaluable tool to advance targeted conservation efforts in *P. corroboree* by enabling future studies on the genetic basis of disease resistance traits, and supporting breeding strategies that enhance genetic diversity and disease resilience, ultimately improving reintroduction success.

SNP genotyping arrays are a powerful genomic tool due to their targeted SNP approach, which enables controlled coverage across the genome with a consistent, high-quality set of SNPs. This approach results in high call rates with low error rates, even when working with degraded samples. However, in non-model species, the success of array design and SNP selection varies depending on the level of genomic resources available and investment in the design (Helyar et al. 2011; Hagen et al. 2013; Humble et al. 2020). Using low-coverage WGS and a fully phased reference genome, the majority of *P. corroboree* samples genotyped on the array exhibited call rates in excess of 99%, with an overall genotyping error rate of just 0.4%. While 82.1% of the total tiled SNPs were identified as polymorphic, incorporating monomorphic sites and SNPs with no minor homozygotes raised the overall genotyping success to 86.7%. These results are comparable to those observed in other non-model species such as the fur seal 90.1% (Humble et al. 2020), hihi 87.6% (Lee et al. 2022) and polar bear 90.3% (Miller et al. 2024). This pattern of incomplete conversion is commonly observed in SNP array development and typically results from a combination of technical factors (e.g., incomplete probe conversion or allelic dropout) and biological factors (e.g., reduced diversity or differing genetic backgrounds between the discovery panel and test population). Therefore, as additional unrelated families are genotyped on the array (i.e., ~40% of available *P. corroboree* founder animals evaluated to date), the number of polymorphic SNPs is anticipated to increase, since some of the currently monomorphic SNPs could prove polymorphic in other datasets. Consequently, the observed 82.1% polymorphic conversion rate likely represents the lower bound, while the number of polymorphic SNPs could rise towards the technical upper limit of 86.7% set by the SNPs that successfully converted genotypes.

Genotyping success can also be evaluated by the proportion of samples that produce high-quality genotypes. Notably, only 2.5% of 1115 *P. corroboree* tissue samples failed to meet genotyping QC thresholds. Utilising the same SNP genotyping array technology, the impact of DNA quantity on genotyping success has varied across non-model species, with no effect observed in fur seals (Humble et al. 2020), but a significant effect noted in the hihi (Lee et al. 2022) and house sparrows (Hagen et al. 2013). In our study, DNA quantity did not significantly affect genotyping success. However, there was a significant relationship between DNA quality and genotyping success. This contrasts with the findings from the hihi, where DNA quality did not affect genotyping outcomes (Lee et al. 2022). Interestingly the accepted DNA concentration threshold of 5 ng/μL in our study was markedly lower than those reported in the aforementioned studies. Nevertheless, similar to those studies, our findings indicate that samples falling below the recommended DNA concentration threshold were still equally likely to yield successful genotyping results.

To evaluate if different tissue types influenced genotyping success, we compared the performance of four *P. corroboree* tissue types on the array. Toe clips performed better than head and liver tissue, showing significantly higher call rates and no failed samples. Of the 26 failed tissue samples, 23 were from head tissue and the remaining three were liver samples. The uneven distribution of sample numbers may explain some of the variation in genotyping success, as head tissue comprised the majority of samples (953 head, 95 toe, and 56 liver samples; Table S3). Additionally, the use of crude tissue extracts from the head may have introduced variability in genotyping success due to the presence of tissue-specific inhibitors or contaminants present in these heterogeneous samples. It is worth noting that all toe samples were sourced from adult frogs in past projects; therefore,

future studies should evaluate toe samples from juvenile frogs, as this could establish a non-lethal genotyping method for this age class.

Tissue preservation methods can also affect genotyping success. We showed that past project samples, which consisted of mixed tissue types and preservation methods had excellent performance on the array with a 99% passing rate. Although we did not include any formalin-fixed or museum specimens in our analysis, testing these in the future would provide valuable insight into the array's performance in sampling degraded DNA (Pacheco et al. 2022). This exploration would be particularly beneficial, as access to samples from critically endangered species is often limited and opportunistic. We have demonstrated the array's robustness in genotyping across various sample types, so successfully genotyping historical specimens would further validate its applicability across a wide range of research scenarios. Notably, this could enable investigations into past genetic diversity and population structure, providing valuable insights to inform current and future conservation strategies.

In addition to testing the array's performance across various tissue types, we were particularly interested in assessing its utility for genotyping samples collected through non-lethal sampling methods. Skin and buccal swabs provide a minimally intrusive and more ethical approach for genotyping amphibians, which is particularly favourable when working with threatened species (Müller et al. 2013). Similar to their performance on other genotyping platforms, skin swabs were unsuccessful in converting genotypes. This was likely due to the challenges of obtaining sufficient host DNA and the presence of microbial contaminants on the skin (Prunier et al. 2012; Ringler 2018). In contrast, buccal swabs successfully generated genotypes, achieving call rates between 80.65% and 93.97%. Despite this, they did not reach our call rate threshold, likely due to their low DNA concentrations ($<1\,ng/\mu L$). Martin et al. (2024) found that DNA yield from amphibian buccal swabs is significantly influenced by factors such as swab type, storage conditions and DNA extraction methods. Therefore, future research should prioritise optimising these parameters for this species.

We evaluated the ability of the custom *P. corroboree* SNP array for genotyping different related species to determine its versatility and potential for broader applications. Since the cross-species transferability of SNPs is highly dependent on genetic divergence (Miller et al. 2012), we tested three species with increasing levels of divergence: *P. pengilleyi*, *P. bibronii* and *C. signifera*. Although divergence among *Pseudophryne* species has not yet been estimated, the myobatrachid genera *Pseudophryne* and *Crinia* last shared a common ancestor approximately 32 million years ago (Brennan et al. 2024). Across the three species, 36,000 loci were successfully cross-amplified, with 58% yielding polymorphic SNPs. Despite differences in divergence times, the variation in mean call rates between species was minimal (Table S6, Figure S5). Given the variation in divergence times across species we anticipated more variation in call rates. However, the limited variation might be attributed to the small sample size ($n=6$ per species) or differences in sample type and preservation method. Notably, *P. corroboree's* sister taxon *P. pengilleyi* was represented by historical formalin-fixed samples, which may have contributed to lower call rates observed in this species (i.e., due to degraded DNA quality resulting in poor probe binding). Despite this, over 21,000 polymorphic SNPs were amplified, highlighting the array's applicability for genotyping other species. It is important to acknowledge that SNP arrays are subject to ascertainment bias, since SNPs identified in a single reference species may fail to capture the full extent of genetic variation present in more distantly related taxa (Miller et al. 2012; Lachance and Tishkoff 2013). In the future, re-testing these species with standardised tissue types and preservation methods would be valuable. Additionally, expanding testing to include other species within the *Pseudophryne* genus (13 spp.) and other genera within the Myobatrachidae family (136 spp. in 22 genera) could further assess the array's utility.

We used the SNP array to examine the genomic structure and assess evidence of inbreeding of frogs bred through the captive breeding program. We were able to determine fine-scale family structure; however, there were no observed strong substructuring patterns among families (i.e., indicating discrete lineages), with most families showing similar levels of co-ancestry and relatedness. This observed pattern aligns with the captive breeding program design, which prioritises the maximum avoidance of inbreeding by crossing founders from different subpopulations derived from their historical geographic origins (Morgan et al. 2008; Lees et al. 2013; Figure S1). Additionally, relatedness among some founding frogs likely contributes to the observed overlap and lack of clear differentiation among certain family groups.

Two studies have examined the genetic structure of *P. corroboree* subpopulations: one using four microsatellite markers across five of the 15 subpopulations (Morgan et al. 2008), while the other employed 3245 SNPs across four subpopulations (Kosch et al. 2019). Both studies reported low genetic differentiation within and between subpopulations. For example, Morgan et al. (2008) reported between-population structure, although the level of differentiation was low, F_{ST} and Φ_{ST} values of 0.038 and 0.066 respectively. This evidence was used to support managing the subpopulations as a single unit, leading to the adoption of the current cross-subpopulation breeding strategy in the captive population (Morgan et al. 2008; Lees et al. 2013). Breeding of animals from genetically distinct subpopulations can result in a temporary surplus of heterozygotes, which we observed in the F1 study animals. These frogs exhibited slightly elevated levels of heterozygosity (mean $F=-0.018$), but not entirely unexpected for this captive breeding program design. The primary advantage of this approach is its ability to minimise inbreeding and maintain higher genetic diversity levels, which has been associated with increased fitness (Tallmon et al. 2004; Whiteley et al. 2015; Frankham 2016; Fitzpatrick et al. 2020). However, this approach can also lead to possible increases in outbreeding depression (Edmands 2007; Frankham 2015, 2016), which could compromise population-specific genetic adaptability (Woodworth et al. 2002; Tallmon et al. 2004), or potentially increase the risk of genetic incompatibilities (i.e., post-zygotic selection) as seen in sister species *P. bibronii* (Byrne and Silla 2020). Since these factors can negatively affect fitness and long-term population resilience, their potential effects should

also be explored in *P. corroboree*. Additionally, future studies should monitor subsequent generations for signs of outbreeding depression to ensure the long-term viability of the breeding strategy for this species.

In addition to assessing population structure, we assessed the level of inbreeding in the F1 captive population using F_{ROH}, revealing evidence of historic inbreeding. Individuals exhibited ROH comprising approximately 4% of each genome. Most of these ROH were relatively short, measuring less than 2 Mb, with none exceeding 12 Mb. Although comparable estimates are currently lacking for amphibians, reported F_{ROH} values in other wild vertebrates with small or isolated populations range from 11% to 21% in caribou (Dedato et al. 2022) to as high as 60% in Indian tigers and Florida pumas (Saremi et al. 2019; Khan et al. 2021). However, cross-taxa comparisons should be interpreted cautiously, as ROH are shaped by differences in the level of co-ancestry, demographic history and life-history traits such as dispersal capacity. Taken together, our findings support the presence of a historical bottleneck in this population, originating from ancestral origins rather than recent inbreeding events (Kirin et al. 2010; Ceballos et al. 2018).

Consistent with these findings, our LD-based estimate of Ne supports the occurrence of a historical bottleneck in this population. Approximately 100 generations ago (300–500 years), this population experienced a bottleneck following a prolonged period of decline, likely driven by factors such as population isolation or environmental stresses (Figure 4). This event was followed by a more recent bottleneck approximately 10 generations ago (30–50 years), during which Ne decreased from ~100 to the current estimates of 29–33. We acknowledge that the inclusion of a discrete generation of F1 individuals from a non-idealised captive population deviates from the assumptions of random mating and unrelated individuals typically required for Ne estimation. Such structure may downwardly bias Ne estimates due to relatedness and population structure (Waples and Do 2010; Waples et al. 2014). However, these offspring represent a significant portion of the captive founder cohort and therefore capture much of the remaining genetic diversity of the species. Moreover, estimates derived from the 22 wild-caught F0 founders–although based on fewer individuals spanning 10 historical populations–indicate lower Ne values, suggesting that low effective population size is not solely an artefact of captive management. Despite methodological deviations, estimates across approaches and datasets consistently indicate a long-term decline in Ne, with all values remaining below 100, providing evidence of reductions in the effective population size of the species. Nonetheless, these estimates should be interpreted with prudence given the potential influence of family structure and non-random mating. The decline in Ne coincides with the introduction of *Bd* to their home range, which occurred in 1984 (Scheele et al. 2017). Together, the patterns observed in ROH and the Ne estimates highlight the long-term impacts of population contractions on the genetic diversity in *P. corroboree*.

Our genome-wide SNP dataset indicates that the captive-bred F1 *P. corroboree* has high levels of LD across the genome, with an average r^2 value of 0.67 between adjacent SNPs. Additionally, LD decay in these F1 individuals is very slow, with moderate levels of LD ($r^2 = 0.2$) extending up to 900 Kb. This decay is slower than observed in other wild vertebrates. For example, previous genome-wide studies found that LD decayed to moderate levels by approximately 100 Kb in polar bears (Malenfant et al. 2015), 200 Kb in hihi (Lee et al. 2022) and 400 Kb in fur seals (Humble et al. 2018). While only one other study has measured genome-wide LD in amphibians, our findings align with those of Trumbo et al. (2023), who reported similarly high levels of genome-wide LD ($r^2 > 0.7$) extending over 1 Mb in *Anaxyrus boreas*, another endangered amphibian with a small Ne. LD tends to be higher in populations with small Ne, such as *P. corroboree*, due to the effects of reduced diversity, shared co-ancestry, genetic drift and inbreeding, all of which contribute to the persistence of LD.

Understanding the extent of LD in *P. corroboree* is essential for our goal of using genomic predictions to explore the genetic basis of fitness-related traits, such as resistance to chytridiomycosis. The extent of LD directly influences the ability to detect QTL and apply marker-assisted selection (MAS) breeding strategies, as it affects the ability to detect associations between genetic markers and specific traits (Goddard and Hayes 2009). Higher LD means that fewer markers are required to detect genetic associations, but it limits the precision of approaches such as fine mapping because high LD can obscure the specific location of causal variants within linked genomic regions. For genome-wide MAS applications (i.e., genomic selection), an LD threshold of $r^2 > 0.2$ is necessary to provide sufficient statistical power for reliable genetic merit predictions (Meuwissen et al. 2001; Meuwissen 2009). Based on the observed LD in *P. corroboree*, at least 10,000 markers are necessary for accurate genomic selection predictions among population members (calculated from the genome size of 8.8 Gb and a mean r^2 of 0.2 reached at 900 Kb (8.8 Gb/900 Kb); Karimi et al. 2020). Since the array contains nearly four times the number of polymorphic SNPs required, it serves as a powerful tool for genomic breeding strategies in this species.

The successful development and application of the *P. corroboree* SNP array makes it a valuable resource for advancing genomic research in this species and other amphibians. As the global amphibian crisis deepens (Luedtke et al. 2023), the need for genomic resources that support conservation efforts becomes increasingly essential. We have demonstrated the robustness of the *P. corroboree* array at generating high-quality genomic data across multiple sample types and applied it to investigate the genetic diversity of the animals in the captive breeding program. Crucially, we have shown that the array is well-powered to enable accurate genomic predictions in future studies for this species. Its application will facilitate the optimisation of captive management strategies by monitoring population genetic health, supporting our ongoing work investigating selective breeding aimed at increasing resistance to *Bd* and ultimately improving reintroduction outcomes in this threatened species.

Author Contributions

M.J.D., K.R.Z. and T.A.K. designed the study; M.J.D., L.B., T.A.K. and J.S.K. collected the samples; M.J.D. conducted the analysis with the help from K.R.Z. and T.A.K.; M.J.D. wrote the first draft; all authors revised and edited the manuscript.

Acknowledgements

We express our gratitude to Michael McFadden, Deon Gilbert and Damian Goodall as well as the *Pseudophryne corroboree* keepers at Melbourne Zoo and Taronga Conservation Society for providing the animals used in this study. We thank Phillip Byrne, Sarah May and Cambell Nalder for generously providing us with tissues. Special thanks to Ian Campelj for support with geospatial mapping, David Hunter for providing the historical location data and Amy Aquilina for assistance in collecting samples. We would also like to thank the Vertebrate Genomes Project for sequencing and assembling the *P. corroboree* genome. This study was funded by the Australian Research Council grants awarded to L.F.S., L.B. and K.R.Z. (FT190100462 and LP200301370).

Disclosure

Benefit-Sharing Statement: Benefits from this research accrue from sharing our data and results with the Corroboree Frog recovery team and Zoological institutes involved in their conservation and captive breeding, as well as being made accessible through public databases as described above.

Ethics Statement

This study was conducted under the approval of the University of Melbourne's Animal Ethics Committee (Application 2021-2214424454-5) and Wildlife Act 1975 Research Authorisation permit number 10010261.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data relating to this project is publicly available. The *Pseudophryne corroboree* genome (Accession GCA_028390025.1) and the whole genome sequencing data (BioProject PRJNA1088171) are available on NCBI. All scripts used in the design of the array, as well as scripts and data for the analysis are available on DataDryad (https://doi.org/10.5061/dryad.ngf1vhj4b). The array itself (AXIOM PSCO SNP 384S384) is publicly available for manufacturing from Thermo Fisher Scientific.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Appendix S1:** men70080-sup-0001-AppendixS1.pdf.