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RESEARCH

# Conservation genetics of Mahogany Gliders and their complex evolutionary relationship with Squirrel Gliders

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## Abstract

Squirrel Gliders (*Petaurus norfolcensis*) are widely distributed throughout the woodlands of eastern Australia, while the similar but larger Mahogany Glider (*Petaurus gracilis*) inhabits the coastal woodlands of the Wet Tropics in northeastern Queensland. The Mahogany Glider is an Endangered species due to habitat loss and fragmentation. This study used single nucleotide polymorphism markers (SNPs) from field and museum-derived samples to investigate genetic relationships within the Squirrel/Mahogany Glider complex and conduct a conservation genetics assessment for the Mahogany Glider. Analyses of genetic structure, phylogenomics, and outlier loci identified four genetic groups: Mahogany Glider and three distinct groups in Squirrel Gliders (North Queensland, Cape Cleveland, and mid-eastern/south-eastern Queensland). We found genetic admixture between these groups, but whether the admixture is historic or current remains unclear. Squirrel Gliders from North Queensland were genetically more similar to Mahogany Gliders in some analyses than to the other two Squirrel Glider groups. Morphological analysis confirmed that Mahogany Gliders are distinguishable from other gliders primarily by their larger body size but did not provide clear differences for the other three genetic groups. We hypothesize that the four genetic groups represent four subspecies of Squirrel Glider, of which the Mahogany Glider is one. This hypothesis can be tested with further field sampling of genetics and morphology, particularly in identified areas of potential contact. When assessing Mahogany Gliders alone, we found a clear north–south split in genetic structuring, with the southern cluster being more structured than the northern cluster. Genetic diversity within Mahogany Gliders was generally comparable to that of Squirrel Gliders, but some sampling localities indicated loss of genetic diversity and low effective population size. Regardless of whether Mahogany Gliders are classified as a species or subspecies, their Endangered status underscores the need for targeted conservation efforts. The genetic findings offer practical pathways for on-ground management to enhance population recovery and connectivity.

**Keywords** Habitat fragmentation · *Petaurus* gliders · Threatened species · Genomics · SNPs · Genetic structure · Genetic diversity · Taxonomic uncertainty

## Introduction

Habitat destruction poses a significant threat to wildlife species, with estimates suggesting this threat impacts nearly 90% of threatened species and is the primary driver of extinction (Hogue and Breon 2022). Loss of habitat often leads to fragmentation, hence transforming once-interconnected habitats into smaller, isolated patches. In these fragmented landscapes, wildlife populations decline and become isolated, suffering from harmful edge effects, habitat degradation, and a loss of connectivity (Bender et al. 1998; Laurance et al. 2007; Didham 2010). Small, isolated populations are more vulnerable to genetic stochasticity, which can lead to the loss of genetic diversity and inbreeding depression (Willi et al. 2007; Frankham et al. 2017; Lino et al. 2019). Ultimately, loss of genetic diversity diminishes a species' adaptive potential to environmental changes (Hedrick 2000; Charlesworth and Willis 2009) and can lead to extinction (Frankham 2005).

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Integrating genetic tools into conservation efforts enhances our understanding of habitat loss and fragmentation impacts, facilitating targeted management strategies (Luikart et al. 2003; Kohn et al. 2006). One powerful tool is the analysis of Single Nucleotide Polymorphisms (SNPs), which uses thousands of specific genome positions with nucleotide substitutions to provide high-resolution information on individual differences, population structure, and genetic diversity (Marth et al. 1999; Schork et al. 2000; Primmer 2009; Steiner et al. 2013). The results can then be used to target conservation efforts, such as identifying areas for revegetation to improve population connectivity (e.g., Bertola et al. 2023) and detecting local adaptation to environment change (e.g., McCulloch and Waters 2023; Giska et al. 2022). Importantly, genomic tools can also reveal cryptic species (Dufresnes et al. 2019) and help resolve taxonomic uncertainty, which is crucial because conservation efforts are focussed on formally designated taxa (Dufresnes et al. 2019, 2023).

The Squirrel Glider (*Petaurus norfolcensis*) and the Mahogany Glider (*Petaurus gracilis*) are both medium-sized gliding possums inhabiting eucalyptus open forests in eastern Australia. Squirrel Gliders are distributed from Victoria to North Queensland, while Mahogany Gliders are restricted to a 120 km stretch of wet sclerophyll lowland forests in North Queensland between Ingham and Tully (Van Dyck et al. 2013). The distributions of the two species are not known to overlap (Van Dyck 1993; Jackson and Claridge 1999; Goldingay and Jackson 2004; Sharpe and Goldingay 2010). Initially considered a subspecies of the Squirrel Glider, the Mahogany Glider was reclassified as a distinct species due to morphological differences (Van Dyck 1993). The Mahogany Glider is larger, with a body length of 215–265 mm and weight of 255–500 g, compared to the Squirrel Glider's 180–244 mm length and 173–300 g weight. It also has a longer, less fluffy tail (300–390 mm vs. 220–300 mm) (Van Dyck 1993; Jackson 2011; Jackson and Schouten 2012). In skull morphology, the Mahogany Glider has a narrower interorbital width but larger skull length, rostral height, and zygomatic width (Van Dyck 1993; Jackson 2011).

Previous studies have observed morphological and body size variation in Squirrel Gliders but data from gliders in northeastern Queensland is limited (Stobo-Wilson et al. 2020). The genetic differentiation between Mahogany and Squirrel Gliders also remains unresolved. Earlier research using two mitochondrial genes (ND2 and ND4) and two nuclear markers (ω-globin and ApoB gene) showed low sequence divergence (1.8–2.2%) between the species, and the phylogeny displayed a single admixed clade (Malekian et al. 2010; Ferraro 2012). No detailed genomic assessment of genetic differentiation and relatedness between the two species has been conducted to date, thereby creating taxonomic uncertainty that hinders accurate species identification and effective conservation strategies.

The Mahogany Glider is listed as Endangered under the Environment Protection and Biodiversity Conservation (EPBC) Act of 1999. Within its small distribution, the glider relies on mature lowland forests with large, diverse trees that provide essential tree hollows and year-round flower nectar (Jackson 2000). Agricultural deforestation, particularly for sugarcane and cattle farming, has reduced its habitat by approximately 40% (Jackson et al. 2011, 2019). Furthermore, suitable habitat is now fragmented and includes more than 400 habitat patches smaller than 1 $km^2$ (Chang et al. 2022). Habitat loss and severe fragmentation can reduce and structure genetic diversity and may have resulted in poor genetic consequences in some isolated, small populations (Frankham et al. 2017). Understanding genetic diversity across populations of the Mahogany Glider is a key priority in the recovery plan (Parson and Latch 2006) and other conservation assessments (Curtis 2012; Burbidge et al. 2014). Despite the recognition of the importance of population genetic assessments to efficiently manage threatened species (e.g., Frankham et al. 2017), none has been conducted for the Mahogany Glider.

In this study, we present the first detailed genetic assessment of Squirrel Glider and Mahogany Glider populations across Queensland. Our aims were to: (1) assess genetic structuring of the populations to identify any genetic groups; (2) examine morphological differences among identified genetic groups; and (3) assess genetic health of the Endangered Mahogany Glider. At the outset of the study, our hypotheses for aims 1 and 2 were that Mahogany Gliders in the known range would represent a discrete genetic and phenotypic group compared to all Squirrel Gliders, which would be a genetic group with lesser genetic sub-structure across Queensland (and minimal morphological variation). This was based primarily on the published phenotypic differences for Mahogany Gliders compared to Squirrel Gliders. And our hypothesis for aim 3 was that Mahogany Glider populations would show fine-scale structuring and poor genetic health (low heterozygosity, high inbreeding), due to the documented recent history of habitat loss and fragmentation across their small range. Our results ultimately provide a framework for resolving taxonomic uncertainty in this group, and for informing continued conservation efforts for Mahogany Gliders.

## Methods

### Fieldwork — surveys and sample collection

A total of 16 trapping surveys were conducted to collect tissue samples from Mahogany Gliders (*Petaurus gracilis*) and Squirrel Gliders (*P. norfolcensis*) at 14 distinct sites

between 15th April 2021 and 31st August 2022, for a total of 1,525 trap nights (Fig. 1; Appendix S1). We also collected tissue samples from Krefft's Gliders (*P. notatus*), a smaller glider species that is sympatric with Mahogany and Squirrel Gliders.

At each site, we strapped 20 wire cage traps (56 × 20 × 20 cm) to trees 2.5–4 m above the ground and 100–400 m apart depending on habitat area and suitability. The back half of each cage trap was covered with waterproof plastic sheeting for rain shelter. Each trap contained a bait ball made of peanut butter, honey and oats to attract gliders to the traps (Jackson 2001; Knipler et al. 2021). We squeezed additional honey on top of the bait ball to increase bait smell and keep the bait moist. We also sprayed a solution of water, raspberry cordial and honey above the trap as a scent lure. Traps were baited and opened just before sunset (5 pm) and checked at 11 pm and each morning before sunrise (5 am). Captured individuals were weighed, sexed, and measured (head length and width, body length, and tail length). We collected a tissue biopsy sample from the edge of an ear using a small ear punch and preserved the sample in 90% ethanol. The glider was then released at the point of capture. All equipment was sterilized using 70% ethanol after each capture.

A total of 44 Mahogany Gliders, 6 Squirrel Gliders and 9 Krefft's Gliders were trapped and sampled in the field surveys (Appendix S1). Five of the 16 surveys conducted yielded no captures. For the sites with catching success, trapping rates were generally low, ranging from 0.8 to 15%. The highest trapping rates were observed in the northern section of Paluma Range National Park, particularly at Bambaroo, Easter Creek, and Allendale (Fig. 1; Appendix S1). Additional samples were obtained from five rescued Mahogany Gliders (via wildlife-carer Daryl Dickson, MGDD01−05, Appendix S2) and from a previous trapping survey during 2008 and 2010 conducted by Queensland Parks and Wildlife Service (via Mark Parsons, MGMP01−08, Appendix S2). We also included historical samples of 10 Mahogany Glider and 43 Squirrel Glider from the Queensland Museum (Appendix S2). The museum samples, collected between 1989 and 2017, were sourced from fur, skin, liver, or muscle specimens that were obtained from field-specimens, rescued individuals, or deceased animals. In total, the Mahogany Glider samples came from the extent of the known range and the Squirrel Glider samples extended from southeastern to far north Queensland (Fig. 1).

## SNP genotyping and filtering

A total of 125 tissue samples were genotyped: 67 from Mahogany Gliders, 49 from Squirrel Gliders, and 9 from Krefft's Gliders (Appendix S2; Appendix S5). DNA extraction and SNP genotyping were performed at Diversity Arrays Technology (DArTseq) in Canberra, using the DArTseq method with PstI and Sphlv4 restriction enzymes. Genomic DNA was extracted using the Macherey-Nagel NucleoMag

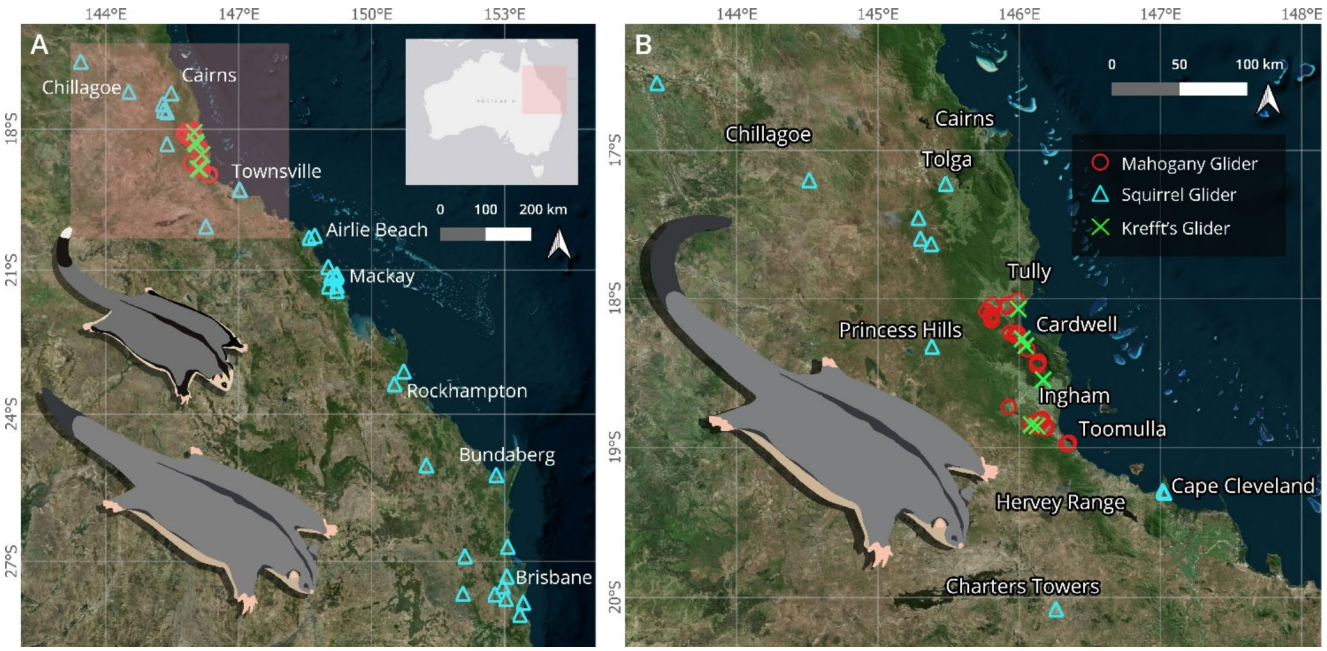



**Fig. 1** Geographical distribution of sampling localities in (**A**) Queensland and (**B**) the Wet Tropics region. Symbols and colours represent different glider species: Squirrel Glider (blue triangles); Mahogany Glider (red circles); Krefft's Glider (green crosses). The illustrations of the glider, from the smallest to the largest, are Krefft's Glider (map A), Squirrel Glider (map A), and Mahogany Glider (map B). The illustrations were created by Marine Lechene

Plant kit and subjected to high-density sequencing (2.5 million reads per individual) on an Illumina NovaSeq 6000 S2 flow cell, referencing the *Petaurus* DArTseq (1.0) genomic library (Jaccoud et al. 2001; Kilian et al. 2012). Single Nucleotide Polymorphism (SNP) calling was performed using the DArTsoft14 algorithm within the KDCompute pipeline developed by Diversity Arrays Technology (http://www.kddart.org/kdcompute.html). Raw sequence data are deposited in the NCBI Short Read Archive under BioProject ID PRJNA1234690.

We performed SNP quality control using a customized R script (Appendix S5) and dartR v2.7.2 (Gruber et al. 2018) to ensure standardized sequence quality (R v4.2.2; R Development Core Team 2022; Rstudio team 2023). Individuals with more than 35% missing genotypes (i.e., a low call rate) and single nucleotide polymorphisms (SNPs) with more than 10% missingness were removed. To ensure reliable and consistent genotyping results, SNPs with extreme read depth (< 10 & >50) and reproducibility (consistency of SNPs calling result) lower than 0.99 were also removed. Secondary SNPs (i.e., SNPs called from the same locus) were removed by retaining the SNP with the higher reproducibility.

We further applied minor allele count, linkage disequilibrium, and outlier loci filters to both the complete dataset and the species-specific datasets. Briefly, singleton SNPs (minor allele count equals to one) were removed (O'Leary et al. 2018). The loci under linkage disequilibrium were filtered out with a threshold of 0.9 using PLINK v1.90 (Purcell et al. 2007) and bigsnpr v1.11.6 (Purcell et al. 2007; Prive et al. 2018), retaining only one of the linked markers with higher call rate.

To identify loci under selection, we conducted outlier analysis using three distinct methods: OutFLANK v0.2 (Whitlock and Lotterhos 2015), Bayescan v2.1 (Foll and Gaggiotti 2008), and pcadapt v4.3.3 (Luu et al. 2017). The results from OutFlank and Bayescan did not reveal any outlier loci. Using pcadapt, we discovered the outlier loci that significantly contribute to the genetic structure. We performed principal component analyses with a false discovery rate of 0.01. Outlier loci were then filtered based on their significance using two thresholds: the highly conservative Bonferroni method and the moderately conservative Benjamini-Hochberg method (Luu et al. 2017). To maximize the information retained, we created two distinct datasets based on the outlier filter: (1) a dataset containing only neutral loci, with outlier loci removed using the Bonferroni method, for genetic structure analysis; (2) a separate dataset comprising solely outlier loci, identified through the Benjamini-Hochberg method, for signatures of selection analysis.

First-degree relatives and potentially duplicated samples were identified using the KING method of moments in SNPRelate v1.32.0 (Manichaikul et al. 2010; Zheng et al. 2012). For first-degree relatives, only the individual with the highest call rate was retained for genetic structure and diversity analyses.

Downstream analyses were conducted in two steps. First, to better understand the evolutionary relationship between Mahogany and Squirrel Gliders, population genetic structure was assessed with a dataset including all three species (Appendix S5). For each of the identified genetic clusters, we then evaluated genetic distance, genetic diversity, morphological characteristics, and signatures of selection. Second, we conducted a conservation genetic assessment focusing on the Mahogany Glider.

Different datasets were used based on the assumptions and information required for each analysis (Funk et al. 2012) (Table 1). Phylogenetic analyses were performed on all loci, including both neutral and outlier loci. Genetic structure and diversity analyses were conducted on neutral loci only, while signature of selection analysis was investigated with outlier loci only. Therefore, the SNPs number varied across datasets. An overview of the analysis workflow is presented in Table 1, and the detailed steps to produce the different datasets are summarised in Appendix S5.

## Genetic and morphological assessment of Mahogany and Squirrel Gliders

### Interspecific population structure

To identify genetically distinct populations, we analysed genetic structure with a dataset of neutral loci for all three *Petaurus* species sampled ($N = 86$).

NetView uses the k-nearest neighbours (kNN) approach to visualize genetic distance matrices (Neuditschko et al. 2012). These matrices were computed using three distinct methods: Euclidean distance applied on allele frequency within individuals (eucl) (Jombart and Ahmed 2011), pairwise difference on number of loci for which individuals differ (nLoci) (Paradis and Schliep 2019), and number of allelic differences between two individuals (nAllele) (Kamvar et al. 2014).

We used the Discriminant Analysis of Principal Components (DAPC) and the unsupervised membership grouping in adegenet v2.1.8 to visualize genetic clustering patterns without assumptions about sampling localities (Jombart and Collins 2015). DAPC reduces the dimensionality of genetic data to identify the underlying population structure (Jombart et al. 2010). The unsupervised membership grouping on the sampling localities were compared and visualized using K-means clustering. In response to recent critiques of PCA in genetic analyses (Elhaik 2022), we reported the variance explained by the first two PCs and used additional methods to confirm the population structure.

**Table 1** Overview of the genetic analyses presented in this study, highlighting the dataset used. The table details the dataset used for each type of analysis for (i) all gliders, (ii) Mahogany and Squirrel Gliders together (MG-SQ), (iii) Mahogany Gliders only (MG), and (iv) Squirrel Gliders only (SQ)

| Analysis | Species/Loci subset | | | |
|---|---|---|---|---|
| | All gliders | MG-SQ | MG | SQ |
| DAPC, STRUCTURE | Neutral Loci | Neutral Loci | Neutral Loci | |
| NetView | Neutral Loci | | | |
| AMOVA | All Loci<br>Neutral Loci<br>Outlier Loci | Neutral Loci<br>Outlier Loci | | |
| Mantel test (Isolation by distance) | | | Neutral Loci | Neutral Loci |
| Genetic differentiation ($F_{ST}$) | | Neutral Loci | Neutral Loci | |
| Genetic diversity | Neutral Loci | Neutral Loci | Neutral Loci | Neutral Loci |
| Phylogenetic Tree | All Loci | | | |
| Signature of Selection | Outlier Loci | Outlier Loci | | |

To investigate genetic structure and admixture jointly, we used the Bayesian clustering method of STRUCTURE v2.3.4 (Pritchard et al. 2000; Falush et al. 2003, 2007; Hubisz et al. 2009). Ten replicates for each K value ranging from 1 to 10 were performed and the results were extracted using pophelper v2.3.1 (Francis 2017). The optimal K value was determined by identifying the peak of ΔK in the Evanno plots (Evanno et al. 2005).

Isolation by distance for each of Mahogany and Squirrel Gliders was investigated by testing correlations between geographical Euclidean distance and pairwise individual genetic distance (proportion of alleles shared) using Mantel tests (Gruber et al. 2018). The results of Mantel tests were then visualized using MASS v.7.3 (Kemp 2002).

Genetic differentiation ($F_{ST}$) analyses were performed to assess the extent of variation explained between the four genetic groups based on neutral loci. These analyses were performed using the bootstrapped method (hierfstat v0.5, Goudet 2005) and Analysis of Molecular Variance (AMOVA) (poppr v2.9.3, Kamvar et al. 2014).

### Phylogenomics

To clarify the phylogenetic relationships between Mahogany and Squirrel Gliders, we constructed a maximum likelihood phylogenetic tree using IQ-TREE v2.2.2.2 (Minh et al. 2020). The tree was built using both neutral and outlier loci with monomorphic loci and missing data removed ($N$=6,501) (Appendix S5). We used ModelFinder Plus in IQ-TREE to identify the substitution model (Kalyaanamoorthy et al. 2017). A maximum likelihood tree was then computed using the selected substitution model (TVM+F+I+G4), in conjunction with the ultrafast bootstrap method with 30,000 replicates. The resulting phylogenetic tree was visualized using iTOL (Letunic and Bork 2021), with Krefft's Gliders rooted as the outgroup based on available literature (Malekian et al. 2010; Cremona et al. 2020) and the population structure analyses of this study.

### Analysis of signatures of selection among consensus genetic groups

Individual loci that significantly deviate from average genome-wide population divergence patterns may indicate the presence of selection. Therefore, we assessed potential local adaptation of the identified genetic groups using the outlier loci dataset derived from the SNP genotyping and filtering section above. We then used DAPC in R package adegenet v2.1.8 to assess clustering patterns of individuals based on the outlier loci (Jombart and Collins 2015).

### Morphological assessment of consensus genetic groups

Once the consensus genetic groups were identified across the above analyses, we assessed morphological differences among them. We measured body length (snout-vent length) and tail length on live individuals captured in the field and on specimens housed in the Queensland Museum (Brisbane). The museum specimens included both wet (spirit) and dry (skin) specimens. Additionally, head length and head width were taken when specimens contained skulls. We conducted a Factor Analysis of Mixed Data (FAMD) for the total of 118 Mahogany Glider and 79 Squirrel Gliders. The FAMD analysis integrated both categorical (specimen type, sex, tail character) and continuous data (body, tail, head length and width) into a principal component analysis (Kassambara 2016). To address missing values, the regression method from R package missMDA v1.19 was applied (Husson and Josse 2023). The analysis was conducted using the FactoMineR v2.9 (Lê et al. 2008) and factoextra v1.07 (Kassambara and Mundt 2020).

### Conservation genetic analyses of Mahogany Gliders

The genetic analyses in this section were based on the Mahogany Glider-only data. Genetic diversity was assessed based on neutral loci and the data was filtered to remove markers monomorphic for Mahogany Gliders. Population genetic structure was assessed following the methodology described in the interspecific population structure section above. Additionally, the effective population size for each sampling locality was estimated using the linkage disequilibrium method (and assuming a monogamous mating system) in NeEstimator v2.1 (Jackson 2000; Do et al. 2014). Only Mahogany Glider samples collected between 2017 and 2022 were used, to prevent overlapping generations.

To evaluate the conservation genetics of Mahogany Gliders, we compared genetic diversity of Mahogany Gliders to that of the consensus genetic groups in Squirrel Gliders. We quantified individual genetic diversity using observed and expected heterozygosity ($H_o/H_e$) and standardized multilocus heterozygosity (sMLH), as per the methodology in dartR v2.9.7 (Gruber et al. 2018) and inbreedR v0.3.3 (Stoffel et al. 2016), respectively. The genetic diversity of each sampling locality, consensus genetic group, and species was assessed using several indices, including averaged sMLH, $H_o/H_e$, Wright's inbreeding index $F_{IS}$ (Gruber et al. 2018), and allelic richness (Ar) corrected using the rarefaction method (Adamack and Gruber 2014). To ensure the accurate estimation of heterozygosity, any loci with missing data were excluded (Schmidt et al. 2021). Additionally, we included monomorphic loci to examine their effect on the estimation of genetic diversity (Schmidt et al. 2021).

## Results

### SNP genotyping and filtering

DArTSeq genotyping identified 67,261 single nucleotide polymorphisms (SNPs) across 115 individuals from all three species (Appendix S5). For nine samples, DNA extraction was unsuccessful. The quality control process, which considered call rate, reproducibility, secondary loci, and read depth, filtered out low-quality loci and removed an additional 18 low-quality samples (16 from museums and 2 from old field collections). Most of the failed museum samples were fur samples (Appendix S6). After these steps, 97 individuals remained with 10,408 SNPs.

Filtering based on minor allele count and linkage disequilibrium excluded 3,455 loci from the all-species dataset, 948 loci from the Mahogany Glider dataset, 1,598 loci from the Squirrel Glider dataset, and 2,514 loci from the Krefft's Glider dataset. Outlier analysis identified 349, 37, and 32 loci as outliers in the all-species dataset, Mahogany Glider dataset, and Squirrel Glider dataset, respectively (Table 1). No outliers were identified in the Krefft's Glider dataset because of the low sample size of nine individuals.

Kinship analyses revealed the presence of three pairs of duplicates (kinship coefficient > 0.354, (Manichaikul et al. 2010) indicating three individuals were sampled in the field twice. Additionally, six pairs of first-degree relatives (kinship coefficient > 0.16) were identified among the Mahogany Glider samples in Bambaroo and Easter Creek, as well as one triplet of first-degree relatives among Squirrel Glider samples from near Airlie Beach. Among the first-degree relatives, the individual with the highest call rate was retained for genetic structure and diversity analyses.

The number of SNPs filtered after quality control was: 9,258 for all-species dataset ($N=97$), 9,651 for Mahogany-Squirrel Glider dataset ($N=88$), 9,719 for Mahogany Glider dataset ($N=58$), and 8,941 for Squirrel Glider dataset ($N=30$) (Appendix S5).

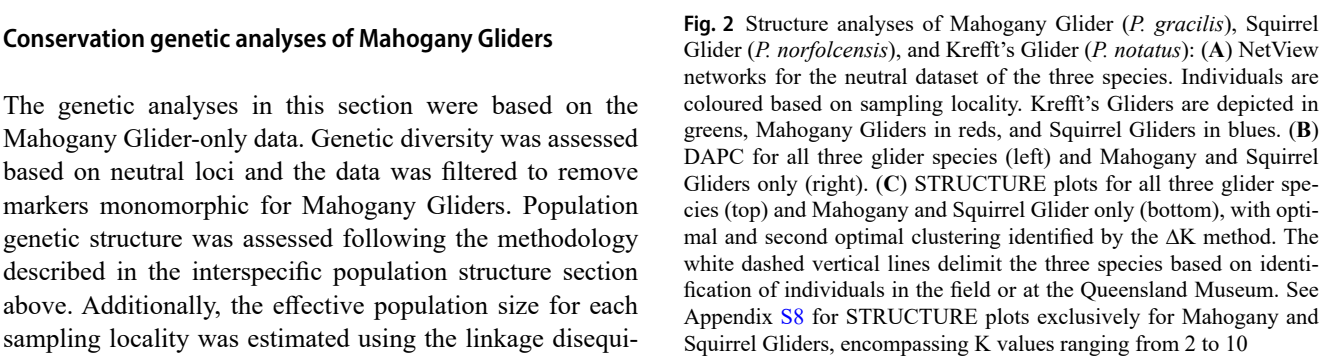
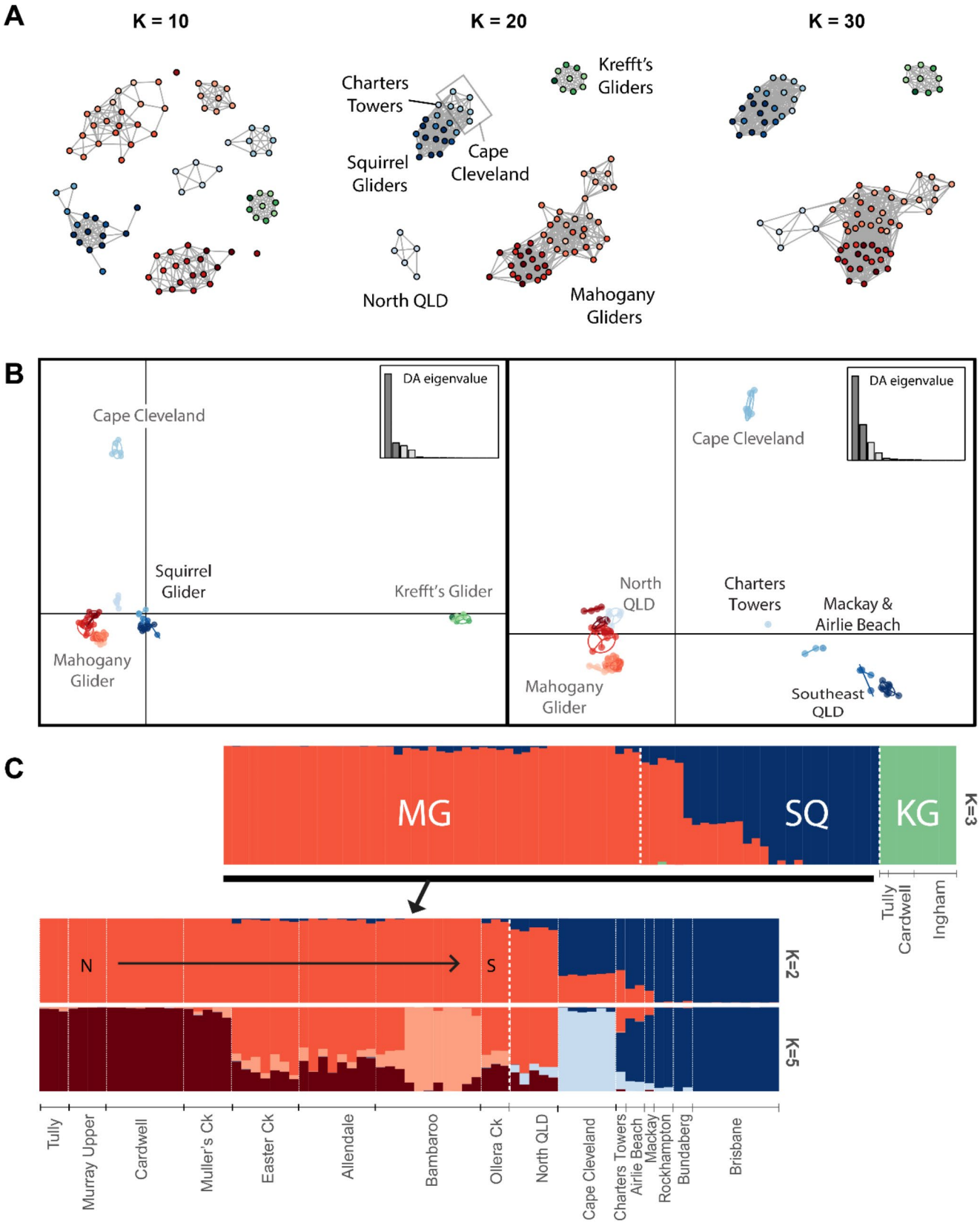


**Fig. 2** Structure analyses of Mahogany Glider (*P. gracilis*), Squirrel Glider (*P. norfolcensis*), and Krefft's Glider (*P. notatus*): (**A**) NetView networks for the neutral dataset of the three species. Individuals are coloured based on sampling locality. Krefft's Gliders are depicted in greens, Mahogany Gliders in reds, and Squirrel Gliders in blues. (**B**) DAPC for all three glider species (left) and Mahogany and Squirrel Gliders only (right). (**C**) STRUCTURE plots for all three glider species (top) and Mahogany and Squirrel Glider only (bottom), with optimal and second optimal clustering identified by the ΔK method. The white dashed vertical lines delimit the three species based on identification of individuals in the field or at the Queensland Museum. See Appendix S8 for STRUCTURE plots exclusively for Mahogany and Squirrel Gliders, encompassing K values ranging from 2 to 10

### Genetic and morphological assessment of Mahogany and Squirrel Gliders

#### Interspecific population structure

In all analyses, Krefft's Gliders from the Wet Tropics formed a highly distinct group compared to Mahogany and Squirrel Gliders. In the NetView analyses, Krefft's Gliders were not joined to any other species, even when the nearest neighbours (kNN) parameter was set to 30 (Fig. 2A; Appendix S7). In the DAPC analyses, Krefft's Gliders were identified as a highly distinct group that is well-distinguished from the other two species with a high eigenvalue (7608 with all loci, 2927 with neutral loci) (Fig. 2B). In the STRUCTURE analyses, Krefft's Glider also emerged as genetically distinct, showing no evidence of genetic admixture with other species (Fig. 2C). Results of AMOVA also showed that the outlier loci ($N=542$) explained 70% of variation when Krefft's gliders were included (Appendix S11). Therefore, below we present results based on neutral loci of Mahogany and Squirrel Glider only.


A
K = 10
K = 20
K = 30
Charters Towers
Krefft's Gliders
Squirrel Gliders
Cape Cleveland
North QLD
Mahogany Gliders
B
DA eigenvalue
Cape Cleveland
Squirrel Glider
Krefft's Glider
Mahogany Glider
North QLD
Charters Towers
Mackay & Airlie Beach
Southeast QLD
C
MG
SQ
KG
K=3
Tully
Cardwell
Ingham
N
S
K=2
K=5
Tully
Murray Upper
Cardwell
Muller's Ck
Easter Ck
Allendale
Bambaroo
Ollera Ck
North QLD
Cape Cleveland
Charters Towers
Airlie Beach
Mackay
Rockhampton
Bundaberg
Brisbane

The genetic structure of Mahogany Gliders exhibits some degree of differentiation from Squirrel Gliders, although the extent of this differentiation varies across different analyses. NetView analyses utilizing Euclidean distance matrices (eucl) consistently delineated Mahogany and Squirrel Gliders into two distinct groups (Appendix S7), maintaining separation even up to a kNN value of 42. Conversely, the other two distance matrices (nLoci and nAllele) demonstrated a convergence between Squirrel and Mahogany Gliders at kNN values of 15 and 25, respectively. DAPC distinguished Mahogany and Squirrel Gliders with a high eigenvalue of 1890 (Fig. 2B), although only 37% of total variance was explained. STRUCTURE analyses also suggested an optimal clustering at K = 2, demarcating the Mahogany Glider from the Squirrel Glider (Fig. 2C; Appendix S9).

Three distinct groups of Squirrel Gliders were identified: (1) gliders from the mid-eastern and south-eastern regions of Queensland, extending from Brisbane to Charters Towers (SQ, *N* = 19), (2) gliders from Cape Cleveland (CC, *N* = 6), and (3) gliders from Chillagoe, Tolga, and Princess Hills north of Townsville (NQ, *N* = 5) (Fig. 2). The SQ group exhibited considerable variation and consistently formed its own cluster, distinct from the Mahogany Gliders (Fig. 2). The assignment of the CC group varied across analyses. In most structure analyses, these gliders were grouped with the Squirrel Gliders (Fig. 2A; Appendix S8A), but in some analyses, they formed their own distinct cluster (Fig. 2B, C; Appendix S8A, B). The NQ samples, which comprise a broad distribution from Townsville to Chillagoe in north Queensland, were particularly interesting. Unlike the SQ group, these samples were grouped with Mahogany Gliders in most of the structure analyses, rather than with Squirrel Gliders (Fig. 2A, B; Appendix S8A).

Despite the clear genetic distinction between Mahogany Gliders and Squirrel Gliders, evidence of introgression between the two species is evident. A gradient of admixture is observed in Squirrel Gliders from north of Mackay to the southern and northwestern range of the Mahogany Glider (Fig. 2C). In the STRUCTURE analysis (K = 2), minor introgression is detected between Mahogany Gliders (MG) and Squirrel Gliders north of Mackay, but more than half of the genetic composition of the CC and NQ groups originates from Mahogany Gliders (Fig. 2C). The NQ group, identified as Squirrel Gliders based on morphology and collection localities, were connected with Mahogany Gliders in the NetView analysis at kNN = 30 (Fig. 2A). This pattern persisted in the unsupervised membership grouping, where these samples consistently clustered with Mahogany Gliders (Appendix S8). Even in the STRUCTURE analysis (K = 2 and K = 5), the NQ samples predominantly displayed genetic components from Mahogany Gliders, with some admixture from CC (Fig. 2C).

### Phylogenomic analysis further supports recognition of four genetic groups

The maximum likelihood tree based on the all-species dataset using both neutral and outlier loci conforms with the population genetic results presented above (Fig. 3; Appendix S5). Krefft's Gliders form a distinct and divergent group, while the relationships among Mahogany and Squirrel Gliders are complex. All Mahogany Glider samples cluster into a single clade, yet this clade is nested within the broader Squirrel Glider clade. Within this broader clade, the North Queensland (NQ) Squirrel Gliders are the most divergent group, forming a sister clade to the clade that includes the subclades of Mahogany Glider, Cape Cleveland Squirrel Gliders (CC), and mid-eastern/south-eastern Queensland Squirrel Gliders (SQ). These clades and subclades have high bootstrap support (98–100; Fig. 3). Additionally, the Mahogany Glider clade is divided into two groups: a northern group (North MG) and a southern group (South MG), separated by the Cardwell Range.

### Genetic differentiation among consensus genetic groups

Pairwise genetic differentiation ($F_{ST}$) was calculated based on neutral loci among the four genetic groups (Table 2). The $F_{ST}$ estimates are moderate between groups, ranging from 0.14 to 0.19. The exception to this is the lower estimate of 0.07 between the NQ Squirrel Gliders and Mahogany Gliders (Table 2). Notably, the $F_{ST}$ estimates between Mahogany Gliders and the three Squirrel Glider groups (NQ, CC, and SQ) were lower (0.07–0.14) compared to the differentiation observed among the three Squirrel Glider groups themselves (0.17–0.19; Table 2).

### Signatures of selection

In the analysis of molecular variance (AMOVA) of the all-species dataset, the outlier loci explained around 65% of the variance between species, whereas only about 10% of the variance was attributed to the four consensus genetic groups (Appendix S11). However, in the AMOVA focusing only on Mahogany and Squirrel Gliders, the variance explained between species became negative. Instead, nearly 75% of the genetic variation was explained by the outlier loci within the four genetic groups (Appendix S11).

The DAPC plot based on 101 outlier loci explained 82% of the total variance among the genetic groups (Fig. 4). PC1 (x-axis) had a high eigenvalue of 1908 and primarily differentiated CC Squirrel Gliders from Mahogany Glider and NQ Squirrel Gliders. PC2 (y-axis), with an eigenvalue of 691, further distinguished mid-eastern/south-eastern Queensland (SQ) Squirrel Gliders from the other three genetic groups.

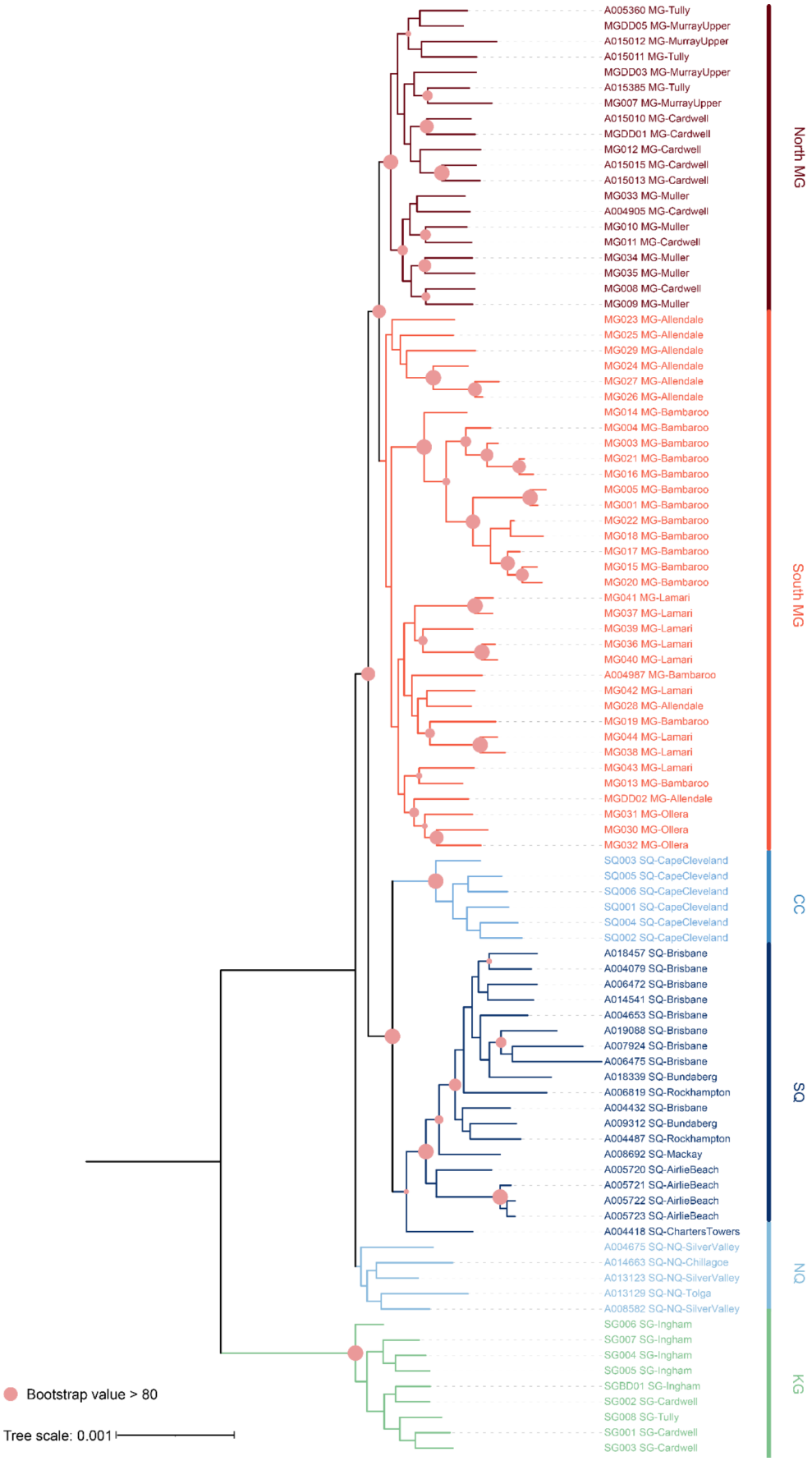



**Fig. 3** The maximum likelihood phylogenetic tree of all glider samples. Krefft's Gliders form a distinct clade, and four subclades were found in Mahogany and Squirrel Glider clade: North Queensland (NQ), Mahogany Gliders (MG), Cape Cleveland (CC) and mid-eastern/south-eastern Queensland individuals (SQ). The Mahogany Glider clade is further split into northern (brown) and southern (orange) individuals. The unit for the tree scale represents the number of substitutions per site. The tree was built using IQ-TREE v2.2.2.2 (Minh et al. 2020) with 30,000 bootstrap replicates and was rooted with Krefft's Glider in iTOL (Letunic and Bork 2021)

**Table 2** Pairwise $F_{ST}$ calculated based on neutral loci (lower unshaded diagonal) between the four consensus genetic groups as determined by the results of population genetic structure and phylogenetic analyses. These groups are Mahogany Gliders (MG), the North Queensland individuals (NQ), the Cape Cleveland individuals (CC), and the remaining Squirrel Gliders (SQ)

| $F_{ST}$/ Group | MG | NQ | CC | SQ |
|---|---|---|---|---|
| MG | NA | | | |
| NQ | 0.07 | NA | | |
| CC | 0.14 | 0.19 | NA | |
| SQ | 0.14 | 0.19 | 0.17 | NA |

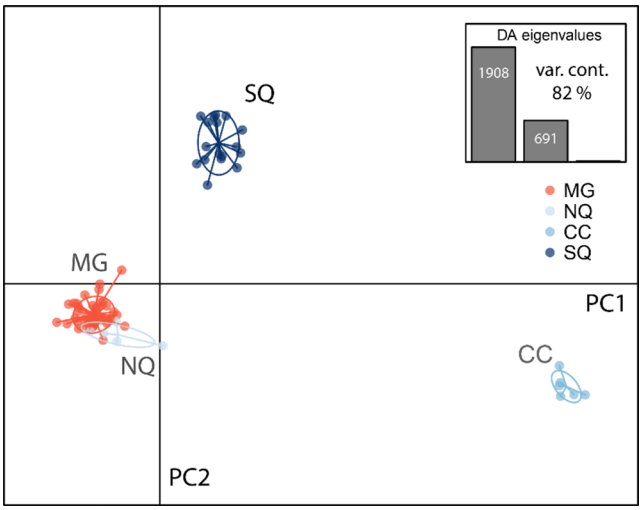



**Fig. 4** The Discriminant Analysis of Principal Components (DAPC) of outlier loci from Mahogany and Squirrel Gliders. The individuals are marked by the four consensus genetic groups based on genetic structure. Mahogany Gliders (MG) are represented in red, North Queensland individuals (NQ) and Cape Cleveland individuals (CC) in light blue, and mid-eastern/south-eastern Queensland individuals (SQ) in dark blue. The eigenvalues and the total variance contribution (var. cont.) of the DAPC plot are displayed in the top right corner

The plot also shows distinct genetic clustering of CC and SQ, while MG and NQ show significant overlap (Fig. 4).

### Morphological assessment among consensus genetic groups

The Mahogany Glider was originally described as a larger glider, with a relatively longer and more slender tail, compared to Squirrel Gliders. The measurements in this study generally confirmed these morphological differences (Fig. 5A). MG individuals were larger, with longer bodies and longer tails compared to NQ, CC, and SQ (Fig. 5A). However, they are not distinct for relative tail length, with the mean tail-to-body length ratio for NQ and CC being similar to that of Mahogany Gliders (Fig. 5A). Compared to the other groups, SQ have relatively shorter tails. However, larger sample sizes are required for NQ and CC.

The Factor Analysis of Mixed Data (FAMD) revealed that individuals identified as Mahogany versus Squirrel Gliders could be distinguished by a combination of body length, tail length, head length, head width, and tail base thickness (slender versus wide/fluffy tail base); however, there was some overlap (Fig. 5B). The first dimension of the FAMD accounted for 36.5% of the variation, with body length, head length, and tail length contributing 26.25%, 26.15%, and 24.65%, respectively. Nevertheless, it is important to interpret the results cautiously due to different specimen types and limited numbers of individuals for NQ and CC.

## Conservation genetics of Mahogany Gliders

After confirming that Mahogany Gliders are a moderately distinct genetic and phenotypic group through the analyses above, we conducted a genetic assessment of this Endangered taxon.

### Genetic structure analysis

Two distinct genetic clusters were identified within Mahogany Gliders. These clusters correspond to the sampling localities north of the Herbert River/Cardwell Range (Muller's Creek, Cardwell, Murray Upper, and Tully) versus the sampling localities south of the Herbert River (Ollera Creek, Bambaroo, Allendale, and Easter Creek) (Figs. 1 and 6). In the Discriminant Analysis of Principal Components (DAPC), K-means clustering identified two clusters as optimal, explaining 44% of total variation. The northern and southern clusters were separated by the first eigenvalue (811.8), while the second eigenvalue (106) showed some discrimination among localities within each of the northern and southern clusters (Fig. 6A). The Evanno plots generated during the STRUCTURE analysis supported the identification of two clusters (K) as optimal, aligning with the clustering observed in the DAPC analysis (Appendix S9). However, $F_{ST}$ between the northern and southern clusters was relatively low ($F_{ST}$ = 0.054, 95% CI: 0.051–0.058), and the unsupervised membership grouping only identified one cluster within Mahogany Gliders.

The northern cluster demonstrated greater genetic homogeneity, while the southern cluster showed more genetic substructure (Fig. 6B). Upon identifying the optimal two clusters (K = 2) in the STRUCTURE analysis, the southern cluster demonstrated genetic admixture from the northern cluster, though not reciprocally. At the second optimal clustering (K = 6), the northern cluster remained virtually homogenous, but the southern cluster showed more substructure. Interestingly, the Bambaroo site displayed its own genetic subcluster and had the least genetic admixture compared to other sampling localities in the southern cluster

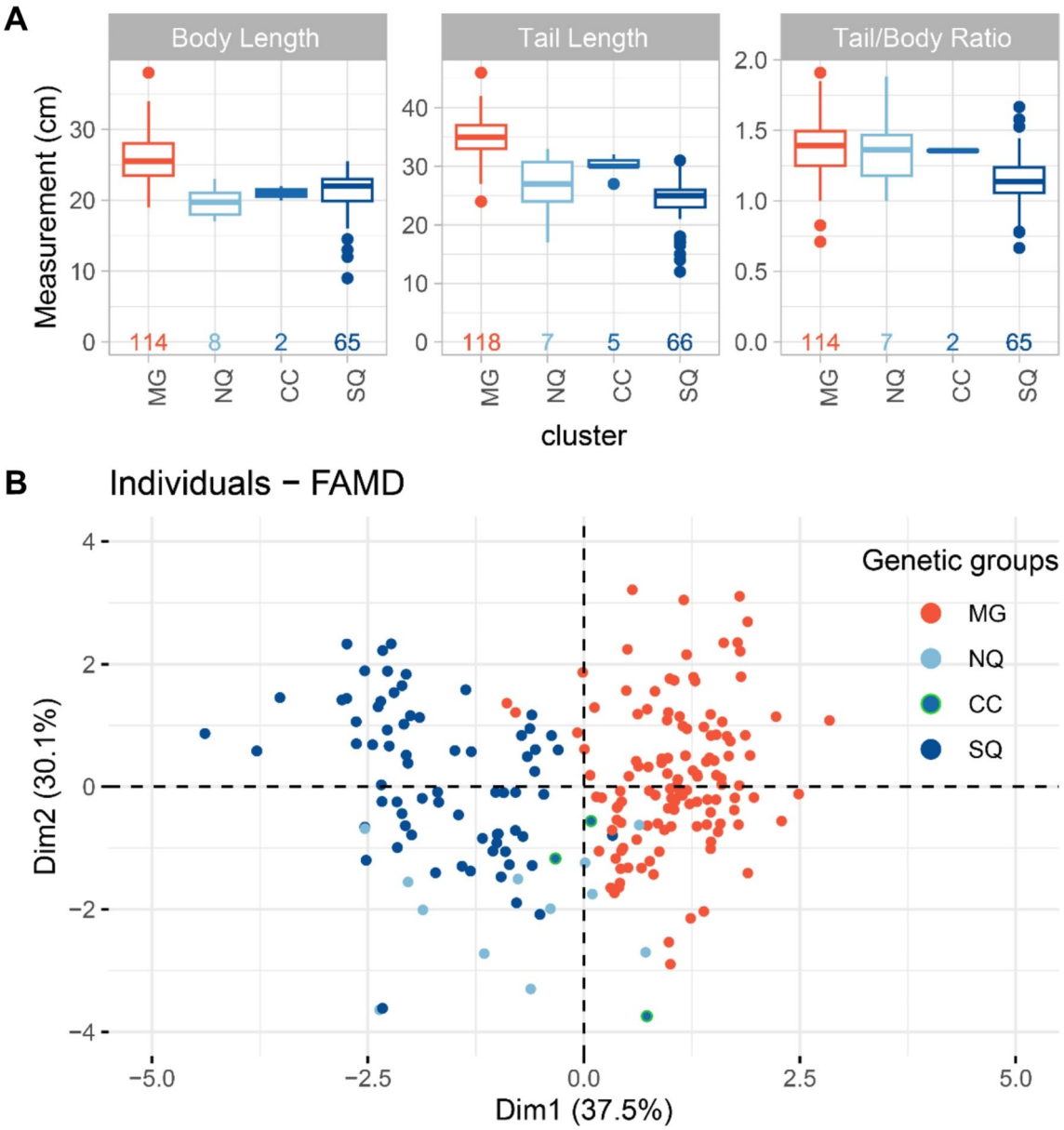



**Fig. 5** Boxplots (**A**) and Factor Analysis of Mixed Data (FAMD) (**B**) for morphological traits for Mahogany and Squirrel Glider consensus genetic groups. The boxplots illustrate body length (cm), tail length (cm), and tail-to-body length ratio for the four consensus genetic groups: Mahogany Gliders (MG), North Queensland individuals (NQ), Cape Cleveland individuals (CC), and mid-eastern/south-eastern Queensland Squirrel Gliders (SQ). Sample sizes for each measurement are indicated under the boxplot. The FAMD plot (B) demonstrates the four genetic groups based on five morphological measurements: body length, tail length, head length, head width, and tail character

(Fig. 6B). NetView analyses yielded similar results — while all Mahogany Gliders form a distinct cluster when the nearest neighbours were set to 20 (kNN = 20), at kNN = 10, three distinct groups emerged: northern, southern, and Bambaroo groups (Appendix S7).

The Mantel test results showed a statistically significant but weak isolation by distance, with only 12.6% ($R^2 = 0.126$) of the variation explained by geographical distance among the Mahogany Glider samples (Appendix S13A). Notably, after grouping the gliders into northern and southern clusters based on genetic structure analysis, genetic distances within the northern cluster exhibit a stronger correlation with geographical distance ($R^2 = 0.236$) (Appendix S13C).

### Genetic diversity and effective population size estimates

The neutral genetic diversity between Mahogany Gliders and Squirrel Gliders is similar. Mahogany Gliders exhibit a standardized multi-locus heterozygosity (sMLH) of 1.13, while Squirrel Gliders have a sMLH of 0.895. The observed

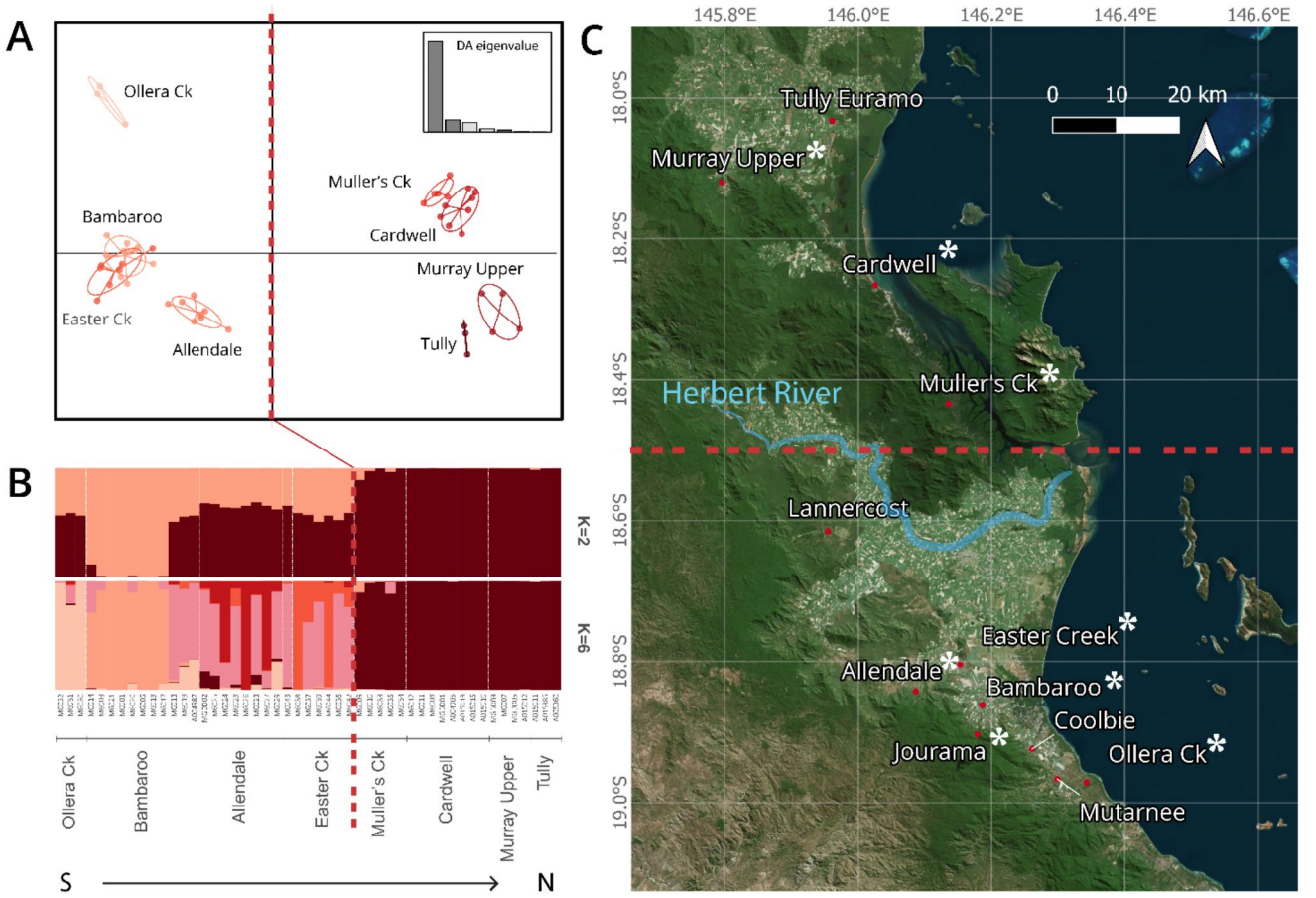



**Fig. 6** Population genetic structure of Mahogany Gliders. (**A**) Discriminant Analysis of Principal Components (DAPC) across all sampling localities, with the first DA eigenvalue (PC1, x-axis) explaining most of the variation. (**B**) STRUCTURE analysis illustrating the optimal (K = 2) and second optimal (K = 6) clustering, with sampling localities arranged from south (left) to north (right). (**C**) Map of sampling localities, with the Herbert River marked in blue. The red dashed line in plots A, B, and C signifies the separation between northern and southern populations based on optimal clustering results. Asterisks on the map in panel C show sampling sites with trapping success (Appendix S1)

heterozygosity ($H_o$) is 0.116 for Mahogany Gliders and 0.089 for Squirrel Glider, and the expected heterozygosity (He) is 0.13 for Mahogany Gliders and 0.118 for Squirrel Glider (Appendix S3). Note that the inbreeding coefficient ($F_{IS}$) is higher in Squirrel Gliders in comparison to Mahogany Gliders, likely due to the mixture of structured populations (Wahlund effect) (De Meeûs 2018).

Within Mahogany Gliders (MG), the sMLH ranges from 0.78 to 1.06, with an average of 0.98. The $H_o$ varies from 0.14 to 0.19, averaging at 0.17, and the $F_{IS}$ ranged from 0.01 to 0.20, with an average of 0.07 (Table 3; Appendix S3). The northern cluster showed lower genetic diversity indices compared to the southern cluster, with individuals from Murray Upper—the northernmost site of their current known range—exhibiting the lowest genetic diversity (sMLH = 0.776, FIS = 0.201). Bambaroo, despite its small size and isolation, displayed a wide range of sMLH (0.75–1.26) and $H_o$ (0.13–0.22) values, with a low $F_{IS}$ of 0.05 (Appendix S12; Table 3).

The effective population size could only be estimated for the three sampling localities with more than six samples: Allendale, Bambaroo, and Easter Creek (Table 3; Appendix S4). In Allendale, the effective population size was low, with a mean of 37 individuals (parametric CI: 35.4–38.8, Jackknife CI: 7.8–infinite). Bambaroo also exhibited a low effective population size, with a mean of 27.6 individuals (parametric CI: 27.1–28.1, Jackknife CI: 19–45.1). Conversely, Easter Creek displayed a notably high effective population size, with a mean of 431.2 individuals (parametric CI: 324.4–640.6, Jackknife CI: 45.8–infinite).

## Discussion

We conducted the first detailed genetic assessment for the Queensland populations of Squirrel and Mahogany Gliders. At the outset of this study, Squirrel Glider populations were assumed to be relatively uniform across their extensive and connected range in eastern Queensland. Mahogany Gliders,

**Table 3** Genetic diversity metrics and effective population size estimates for Mahogany Gliders, including South and North populations, and sampling localities. Metrics provided are the number of individuals (nInd), standardized multilocus heterozygosity (sMLH), observed heterozygosity ($H_o$), expected heterozygosity ($H_e$), inbreeding coefficient (FIS), and effective population size estimates (Ne). Values of $H_o$ that are lower than he by more than 1% are highlighted in red. Refer to Appendices S3 and S4 for the complete dataset, including standard deviations, confidence intervals, and corresponding metrics for Squirrel Glider genetic groups

| Dataset | Group | nInd | sMLH | $H_o$ | $H_e$ | FIS | Ne |
|---|---|---|---|---|---|---|---|
| All-species | MG | 49 | 1.13 | 0.116 | 0.13 | 0.114 | - |
| Mahogany Glider | South MG | 29 | 1.04 | 0.184 | 0.204 | 0.111 | - |
| | North MG | 20 | 0.942 | 0.167 | 0.186 | 0.124 | - |
| | Allendale | 8 | 1.063 | 0.188 | 0.189 | 0.068 | 64.7 |
| | Bambaroo | 11 | 1.028 | 0.182 | 0.184 | 0.054 | 27.6 |
| | Cardwell | 8 | 0.973 | 0.172 | 0.177 | 0.085 | - |
| | Easter Ck | 7 | 1.044 | 0.185 | 0.186 | 0.077 | 431.2 |
| | Muller Ck | 5 | 1.039 | 0.184 | 0.175 | 0.053 | - |
| | Murray Upper | 4 | 0.776 | 0.138 | 0.151 | 0.201 | - |
| | Ollera Ck | 3 | 1.014 | 0.18 | 0.158 | 0.053 | - |
| | Tully | 3 | 0.921 | 0.163 | 0.149 | 0.085 | - |

on the other hand, were subject to taxonomic uncertainty due to ambiguous genetic results from a limited number of loci. However, they were generally considered a distinct species from all Squirrel Gliders based on morphological differences, particularly their larger body size and relatively longer tail. Our results show that the situation is much more complex. Individuals identified as Squirrel Gliders form three genetic groups — southern and eastern Queensland (SQ), Cape Cleveland near Townsville (CC), and North Queensland (NQ). Mahogany Gliders, sampled across their known range, also form a genetic group; however, this group is nested within the Squirrel Glider groups. Different analyses yield varying levels of support for these four consensus genetic groups and their relationships. Importantly, evidence of introgression is found between them. Morphological analyses support some of the recognised differences for Mahogany Gliders and show limited differences among the three Squirrel Glider groups. We hypothesize that the four genetic groups represent subspecies of Squirrel Gliders (discussed below), with Mahogany Gliders being one. Conservation genetic analysis of Mahogany Glider populations supported the prediction of fine-scale structuring, particularly between northern and southern populations. However, despite very low effective population size estimates in some areas, heterozygosity and inbreeding metrics were generally better than expected.

## Genetic and morphological assessment of Mahogany and Squirrel Gliders

### Genetic relationship and morphological differences between Mahogany and Squirrel Gliders

Mahogany Gliders are generally distinct from the other three genetic groups in the analyses but with genetic admixture evident with the NQ and CC genetic groups (Fig. 2). The genetic admixture between Mahogany and Squirrel Gliders suggests that historical or contemporary introgression has occurred between these species. The complexity of relationships between Mahogany and Squirrel Gliders has been previously suggested in phylogenetic studies on the Petaurid gliders. For instance, Malekian et al. (2010) revealed a genetic difference of only 1.8–2.2% between Mahogany and Squirrel Gliders for two mitochondrial genes (ND2 and ND4) and one nuclear marker (ω-globin). A different phylogenetic study, based on ND2 mitochondrial gene and ApoB1 nuclear gene, clustered Mahogany Glider samples with Squirrel Glider samples from Hervey Range (west of Townsville) and Einasleigh Uplands (west of Atherton Tablelands) (Ferraro 2012). The phylogenomic tree we present here, based on thousands of SNPs, shows Mahogany Gliders as a highly supported distinct clade but nested among the Squirrel Glider clades (Fig. 3). The morphological analyses found that Mahogany Gliders could be separated based on a combination of body length, tail length, head length, head width and tail base thickness (FAMD analysis; Fig. 5b). However, in terms of the two key traits typically used to distinguish Mahogany Gliders, larger body size and relatively longer tail, only size separates them from NQ and CC gliders on a univariate basis (Fig. 5A). NQ and CC gliders also have relatively long tails (albeit based on small sample sizes), which is interesting because these populations have not previously been specifically compared with Mahogany Gliders (e.g., Van Dyck (1993).

### The genetics and morphology of North Queensland gliders

We found substantial introgression between Mahogany Gliders and the gliders from North Queensland (NQ) (samples collected near Princess Hills, Atherton Tablelands, and Chillagoe) (Fig. 2C). The NQ gliders also group closely to Mahogany Gliders in the NetView, DAPC and Structure

analyses based on neutral loci (Fig. 2), and in the analysis of outlier loci (Fig. 4). The low $F_{ST}$ value between NQ gliders and Mahogany Gliders further support the close relationship between them (Table 2). Interestingly, in the SNPs-based phylogeny (Fig. 3), the NQ samples are divergent to a monophyletic group of Mahogany, CC and SQ gliders, rather than being clustered within it (Fig. 3). The morphology is interesting for the NQ gliders — they are of similar body size to Squirrel Gliders, but their relative tail length is more akin to Mahogany Gliders (albeit based on a small sample size) (Fig. 5). Overall, the results suggest a close genetic relationship between NQ gliders and Mahogany Gliders, and some level of historic or current introgression that requires further sampling and analyses to resolve.

#### Distinctiveness of Cape Cleveland gliders

The gliders from Cape Cleveland (CC) are identified as a genetically distinct group in most of the analyses, and are highly distinct in some (e.g., Figs. 2B and 4). Across the analyses, they appear to be more closely related to Squirrel Gliders but show signs of introgression with Mahogany Gliders (Fig. 2C). As for NQ gliders, they appear to have a relatively long and slender tail, similar to that of Mahogany Gliders (Fig. 5). Additionally, CC samples exhibit a unique signature of selection that differs from both Mahogany and Squirrel Gliders (analysis of outlier loci; Fig. 4). It is possible that the gliders at Cape Cleveland are adapted to their local coastal habitat, a peninsula of tropical lowland eucalyptus woodlands, rainforest, and wetlands. It is unclear whether they are currently isolated on the peninsula from adjacent mainland Squirrel Glider populations (in the Mt Elliot/Giru area). It is also worth noting that despite being apparently restricted to a very small area, we recorded high glider density at Cape Cleveland, with a catch rate of 15.4%. This high density, along with their greater genetic diversity compared to other Squirrel Gliders (Table 3), indicates that the CC population is genetically healthy.

#### Taxonomic hypothesis for Squirrel and Mahogany Gliders

We hypothesize that the four genetic groups represent four subspecies of Squirrel Glider, representing: (1) the main range of Squirrel Gliders up to at least Proserpine and Charters Towers (SQ), (2) north Queensland (NQ) from the western edge of the Wet Tropics and north, (3) Cape Cleveland (CC), and (4) the Mahogany Glider populations through the coastal Wet Tropics. The hypothesis of four subspecies is based on the consistent appearance of these groups across the genetic analyses herein, with each of them being highly distinct in at least some analyses. Amongst these analyses is the SNP phylogeny (Fig. 3), in which they all form monophyletic groups. More detailed morphological sampling is required for NQ, CC and SQ across the range to better test for phenotypic differences. There is no universally accepted definition of a subspecies, but we propose this classification because the four groups are genetically distinct yet not sufficiently divergent to suggest complete or near-complete reproductive isolation (i.e., Biological Species Concept; *sensu* Mayr 1963), either where they currently overlap or if they come into contact in the future.

Genetic introgression was detected between these groups, but whether it is historical or ongoing remains unclear. As the NQ and CC groups were previously unknown, our sampling was not designed to assess hybridization in detail. Future research should focus on the broad introgression zones identified and conduct detailed contact zone sampling to evaluate the current extent of genetic isolation (e.g., Hoskin et al. 2005; Harrison and Larson 2016; Malinsky et al. 2018; Caeiro-Dias et al. 2021). Key sampling areas are located at the southern and western edge of the Mahogany Glider distribution (for contact with NQ and CC), and the Charters Towers–Townsville–Ayr region (for contact between NQ, SQ and CC).

### Conservation genetics of Mahogany Gliders

At the outset of this study, we hypothesized that the Mahogany Glider would form a distinct genetic group separate from all Squirrel Gliders. However, the results are more complex, showing genetic admixture between the Mahogany Glider and other genetic groups. We conclude that the Mahogany Glider likely represents a subspecies of the Squirrel Glider rather than a distinct species. In Australia, subspecies receives the same conservation status as species under the EPBC Act 1999 (Threatened Species Scientific Committee 2023), therefore the taxon should remain of conservation relevance. To guide future conservation efforts, we assessed genetic diversity and connectivity of populations across the known distribution.

#### Comparative genetic diversity

Genetic diversity of the Mahogany Glider is generally comparable to the non-threatened Squirrel Gliders analysed in this study. Comparisons of heterozygosity based on SNPs, employing similar filtering methods, indicate that the observed heterozygosity ($H_o$) of the Mahogany Glider is comparable to other threatened marsupials listed in the EPBC Act 1999 (Threatened Species Scientific Committee 2023), such as the Koala (Phascolarctos cinereus), Northern Bettong *(Bettongia tropica*), Western Barred Bandicoot (*Perameles bougainville*) and Greater Glider (*Petauroides volans*) (Table 4). However, this range of

**Table 4** Observed SNPs heterozygosity ($H_o$) ranges of selected Australian marsupials with EPBC conservation status

| Species | Scientific name | EPBC Status | $H_o$ (range) | Reference |
|---|---|---|---|---|
| Mahogany Glider | *Petaurus gracilis* | Endangered | 0.12 (0.16–0.19) | This study |
| Koala | *Phascolarctos cinereus* | Endangered | (0.22–0.29) | Kjeldsen et al. 2019 |
| Northern Bettong | *Bettongia tropica* | Endangered | (0.15–0.22) | Todd et al. 2023 |
| Western Barred Bandicoot | *Perameles bougainville* | Endangered | (0.14–0.22) | White et al. 2018 |
| Greater Glider (south) | *Petauroides volans* | Endangered | 0.14 (0.09–0.21) | Knipler et al. 2023 |
| Greater Bilby | *Macrotis lagotis* | Vulnerable | 0.26 | White et al. 2018 |
| Burrowing Bettong | *Bettongia lesueur* | Vulnerable | (0.18–0.31) | White et al. 2018 |
| Long-nosed potoroo | *Potorous tridactylus* | Vulnerable | 0.34 | Mulvena et al. 2020 |
| Golden Bandicoot (mainland) | *Isoodon auratus* | Vulnerable | (0.28–0.31) | Rick et al. 2023 |
| Red-tailed Phascogale | *Phascogale calura* | Vulnerable | (0.19–0.20) | Pierson et al. 2023 |
| Squirrel Glider | *P. norfolcensis* | Least Concern | 0.18 | Knipler et al. 2021 |
| Sugar Glider | *P. breviceips* | Least Concern | (0.15–0.16) | Knipler et al. 2022 |

observed heterozygosity is lower when compared to species with a vulnerable status, such the Greater Bilby (*Macrotis lagotis*), Burrowing Bettong (*Bettongia lesueur*), Long-nosed Potoroo (*Potorous tridactylus*), and Golden Bandicoot (*Isoodon auratus*) (Table 4). Caution should be taken when comparing genetic indices across species, as these indices are heavily dependent on the evolutionary history and population genetics of each species and thus can be influenced by biases introduced through different filters, thresholds, and sample sizes (Schmidt et al. 2021). For instance, despite being categorized as Least Concern in terms of conservation status, the Sugar Glider (*P. breviceps*) and Krefft's Glider consistently exhibit low observed heterozygosity (Knipler et al. 2022), as also seen for Krefft's Gliders in our study here (Appendix S3).

### Genetic clustering of populations

Structure analyses revealed two distinct genetic clusters within Mahogany Gliders — the northern and the southern cluster (Fig. 6). The Cardwell Range, situated between these two clusters, appears to serve as a natural barrier. Interestingly, despite this geographical division, genetic admixture persists between the northern and southern groups. This admixture could be a result of natural gene flow along the coastal strip at the eastern base of the Cardwell Range or through the Herbert River catchment, and the significant northern genetic component found in the southern cluster may suggest asymmetrical movement and/or introgression.

The northern cluster is characterized by lower genetic diversity compared to the southern cluster, a more homogeneous genetic structure (Fig. 8B), and moderate isolation by distance (24% of genetic variation in this cluster is explained by isolation by distance; Appendix S13). Some connectivity of populations may exist through this area, but perhaps only until recently. The catch rate at Muller's Creek was recorded at 7.5–15% in 1995–1996 (Jackson 2000) and around 11.5% in 2008 (personal communication with Mark Parsons, Queensland Government 2020). These high catch rates suggest high glider density in good quality habitat. In contrast, our catch rate at Muller's Creek, using similar field techniques, was lower —2% in 2021 and 3% in 2022 (Appendix S1). Furthermore, individuals from Murray Upper, the northernmost known population of the species, exhibit noticeably low individual heterozygosity ($H_o$ and sMLH; Table 3), indicating the population is inbred to some degree. The extensive habitat clearing and sugar cane farming in the Tully region from 1880 to 1905 removed and fragmented much suitable habitat (QImaginary 1957; Bolton 1970; The University of Queensland 2018), a situation exacerbated by further clearing over the following decades and widespread habitat damage during Cyclone Yasi (Holloway 2013). However, ongoing forest thickening due to changes in fire regimes (Stanton et al. 2014a, b) may be a key issue in reducing habitat suitability in recent times in the northern half of the range (Chang et al. 2022).

The southern cluster of the Mahogany Glider is genetically more structured across different sampling localities (Fig. 6), and only 10% of genetic variation is explained by isolation by distance (Appendix S13). Therefore, factors other than distance are likely contributing to the population structure. The most likely explanation is habitat fragmentation and hence restricted movement between populations. Catch rates at some southern sites were high, suggesting considerable abundance within the habitat fragments. Catch rates were high at Bambaroo, Allendale and Easter Creek (Appendix S1), and these sites had higher genetic diversity than sites in the northern cluster (Table 3). These sites are intriguing given how small these fragments are and the low Ne estimates for Bambaroo (Ne=27.6) and Allendale (Ne=64.7). This discrepancy may reflect a delay in the loss of neutral genetic diversity which can lag behind population decline and isolation (Pinto et al. 2023). The connectivity between Bambaroo and adjacent coastal forest was lost in 1988 (QImaginary 1951; Google Earth imagery through time) and Easter Creek only became fragmented during extensive logging from 1987 to 1997 (QImaginary 1993; Google Earth imagery through time). Given population isolation only

occurred in the past 40 years, the full impact of habitat loss and fragmentation on the density and genetic diversity of the Mahogany Gliders in this area is yet to be seen.

## Management recommendations

The genetic results support the ongoing conservation focus on addressing the primary threats of habitat loss and fragmentation. Management actions for the northern genetic cluster should aim to increase population size and genetic diversity through habitat restoration, including both replanting to increase patch size and connectivity, and trialling the use of fire to reduce rainforest thickening in some areas. For the southern genetic cluster, it is important to enhance functional connectivity between fragmented populations via habitat corridor revegetation, particularly along creek lines, and assisted gene flow (translocation of individuals) for some isolated patches. In addition, long-term genetic monitoring of populations, especially those with low genetic diversity, is critical for tracking genetic health and guiding future conservation actions. Future genetic work needs to be coupled with detailed surveys to refine estimates of Mahogany Glider distribution and local densities. One limitation of our study is the small and scattered sample sizes. Glider sampling requires significant effort; for example, we trapped at 14 sites over 1,525 trap nights, resulting in 44 Mahogany Glider, 6 Squirrel Glider, and 9 Krefft's Glider samples (with more samples added from the Queensland museum and other previous sampling; Fig. 1; Appendix S1). More sampling is required across parts of the range to find isolated populations that may be small, have poor genetic health, and require connectivity and genetic management. This should focus on areas of highly fragmented habitat and peripheral areas of the distribution in the north, south and coastal fringe.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s10592-025-01700-7.

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**Author contributions** CJH and YC conceived and designed the study. Material preparation, data collection and analysis were performed by YC, with input from all authors. The first draft of the manuscript was written by YC, with input from CJH and LVB, and all authors commented on subsequent versions of the manuscript. All authors read and approved the final manuscript. YC and CJH responded to the reviewers' comments and revised the manuscript to the final publication.

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**Data availability** The datasets generated and/or analysed during the current study are available in FigShare (https://doi.org/10.6084/m9.figshare.26952436.v1). Appendices are provided in the Electronic supplementary material. Raw sequence data are deposited in the NCBI Short Read Archive under BioProject ID PRJNA1234690.

## Declarations

**Ethical approval** The research was conducted in accordance with Queensland animal permits for scientific purposes (protected areas: P-PTUKI-100021853; non-protected areas: WA0025939) and animal ethics under James Cook University (A2699).

**Competing interests** The authors declare no competing interests.



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