

Supplementary Materials

Title

Conservation genetics of Mahogany Gliders and insights into their evolutionary relationship with Squirrel Gliders

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Supplementary Tables

Table S1 Summary of trapping results and effort across sites, ordered from south to north of the Mahogany Glider range in the Wet Tropics. The gliders caught indicated Mahogany Glider except for the site at Cape Cleveland. The table includes information on the number of cages used, the duration of trapping in nights, the number of gliders caught, trapping effort calculated as the product of cages and nights, and the trapping rate expressed as a percentage, calculated as the total number of gliders caught by the total trapping effort for the site.

Region	Site	Cages	Nights	Gliders Caught	Trapping Effort [†]	Trapping rate (%)
Southern Paluma	Ollera Ck	20	5	3	100	3.0
	Mutarnee	20	5	0	100	0.0
Northern Paluma	Jourama	20	2	0	40	0.0
	Coolbie	20	5	0	100	0.0
	Jourama & Bamabroo	20	5	6	100	6.0
	Easter Ck	20	5	9	100	9.0
	Bambaroo	20	5	10	100	10.0
	Jourama + Allendale	20	5	7	100	7.0
Ingham	Lannercost SF	20	5	0	100	0.0
Cardwell	Murray Upper	21	6	1	126	0.8
	Cardwell SF	23	5	1	115	0.9
	Muller's Ck	20	5	2	100	2.0
	Seafarm	21	5	2	105	1.9
	Muller's Ck	20	5	3	100	3.0
Tully	Tully Euramo	20	5	0	100	0.0
Townsville	Cape Cleveland	13	3	6	39	15.4
Total			318	50	1525	

[†]Trapping effort = number of cages*number of nights

SF = State Forest; Ck = Creek

Table S2 Summary of genetic samples used in this study. The table comprises 67 Mahogany Gliders (44 from cage trapping, 13 from rescued and previously studied individuals, 10 sourced from Queensland Museum), 49 Squirrel Gliders (6 from cage trapping and 43 sourced from the Queensland Museum), and 9 Krefft's Gliders (all from cage trapping in the Wet Tropics). The table details the unique identification (ID), common species name, sex, sampling location, year of collection, specimen type, collection source, sampling latitude, longitude, and the associated accuracy of these coordinates.

ID	Species	Sex	Sampling Locality	Year	Type	Source	Lat	Lon	GPS Accuracy
MGDD01	Mahogany Glider	F	Cardwell	2021	fur	Acquired	-18.23	145.94	high
MGDD02	Mahogany Glider	M	Allendale	2021	ear	Acquired	-18.84	146.08	high
MGDD03	Mahogany Glider	F	Murray Upper	2021	fur	Acquired	-18.13	145.80	low
MGDD04	Mahogany Glider	F	Murray Upper	2021	fur	Acquired	-18.15	145.80	high
MGDD05	Mahogany Glider	F	Murray Upper	2022	skin	Acquired	-18.11	145.80	high
MGMP01	Mahogany Glider	NA	Muller's Ck	2008	ear	Acquired	-18.42	146.13	medium
MGMP02	Mahogany Glider	NA	Muller's Ck	2008	ear	Acquired	-18.43	146.14	medium
MGMP03	Mahogany Glider	NA	Muller's Ck	2008	ear	Acquired	-18.43	146.14	medium
MGMP04	Mahogany Glider	M	Cardwell	NA	ear	Acquired	-18.25	145.97	medium
MGMP05	Mahogany Glider	M	Ingham	2011	ear	Acquired	-18.86	146.13	low
MGMP06	Mahogany Glider	M	Tully	2011	ear	Acquired	-18.05	145.91	low
MGMP07	Mahogany Glider	F	Cardwell	2010	ear	Acquired	-18.23	145.98	medium
MGMP08	Mahogany Glider	F	Tully	2011	ear	Acquired	-18.05	145.91	low
SGBD01	Krefftt's Glider	M	Ingham	2021	muscle	Acquired	-18.55	146.17	low
MG001	Mahogany Glider	F	Bambaroo	2021	ear	Field	-18.87	146.18	high
MG002	Mahogany Glider	M	Bambaroo	2021	ear	Field	-18.86	146.18	high
MG003	Mahogany Glider	F	Bambaroo	2021	ear	Field	-18.86	146.19	high
MG004	Mahogany Glider	F	Bambaroo	2021	ear	Field	-18.86	146.19	high
MG005	Mahogany Glider	M	Bambaroo	2021	ear	Field	-18.87	146.18	high
MG006	Mahogany Glider	M	Bambaroo	2021	ear	Field	-18.87	146.19	high
MG007	Mahogany Glider	F	Murray Upper	2021	ear	Field	-18.11	145.81	high
MG008	Mahogany Glider	M	Cardwell	2021	ear	Field	-18.25	145.99	high
MG009	Mahogany Glider	M	Muller's Ck	2021	ear	Field	-18.45	146.13	high
MG010	Mahogany Glider	F	Muller's Ck	2021	ear	Field	-18.42	146.14	high
MG011	Mahogany Glider	F	Cardwell	2021	ear	Field	-18.34	146.06	high
MG012	Mahogany Glider	M	Cardwell	2021	ear	Field	-18.34	146.06	high
MG013	Mahogany Glider	M	Bambaroo	2021	ear	Field	-18.86	146.18	high
MG014	Mahogany Glider	M	Bambaroo	2021	ear	Field	-18.86	146.19	high
MG015	Mahogany Glider	M	Bambaroo	2021	ear	Field	-18.86	146.18	high
MG016	Mahogany Glider	M	Bambaroo	2021	ear	Field	-18.87	146.19	high
MG017	Mahogany Glider	F	Bambaroo	2021	ear	Field	-18.86	146.18	high
MG018	Mahogany Glider	M	Bambaroo	2021	ear	Field	-18.86	146.18	high
MG019	Mahogany Glider	M	Bambaroo	2021	ear	Field	-18.87	146.19	high
MG020	Mahogany Glider	M	Bambaroo	2021	ear	Field	-18.86	146.19	high
MG021	Mahogany Glider	F	Bambaroo	2021	ear	Field	-18.87	146.19	high
MG022	Mahogany Glider	M	Bambaroo	2021	ear	Field	-18.86	146.18	high
MG023	Mahogany Glider	M	Allendale	2022	ear	Field	-18.85	146.09	high
MG024	Mahogany Glider	F	Allendale	2022	ear	Field	-18.85	146.09	high
MG025	Mahogany Glider	M	Allendale	2022	ear	Field	-18.84	146.09	high
MG026	Mahogany Glider	F	Allendale	2022	ear	Field	-18.85	146.09	high
MG027	Mahogany Glider	M	Allendale	2022	ear	Field	-18.84	146.09	high
MG028	Mahogany Glider	F	Allendale	2022	ear	Field	-18.84	146.09	high
MG029	Mahogany Glider	F	Allendale	2022	ear	Field	-18.86	146.13	high
MG030	Mahogany Glider	M	Ollera Ck	2022	ear	Field	-18.97	146.34	high

MG031	Mahogany Glider	F	Ollera Ck	2022	ear	Field	-18.98	146.34	high
MG032	Mahogany Glider	M	Ollera Ck	2022	ear	Field	-18.97	146.35	high
MG033	Mahogany Glider	F	Muller's Ck	2022	ear	Field	-18.44	146.13	high
MG034	Mahogany Glider	F	Muller's Ck	2022	ear	Field	-18.44	146.13	high
MG035	Mahogany Glider	M	Muller's Ck	2022	ear	Field	-18.44	146.13	high
MG036	Mahogany Glider	M	Easter Ck	2022	ear	Field	-18.82	146.15	high
MG037	Mahogany Glider	M	Easter Ck	2022	ear	Field	-18.82	146.16	high
MG038	Mahogany Glider	M	Easter Ck	2022	ear	Field	-18.81	146.16	high
MG039	Mahogany Glider	M	Easter Ck	2022	ear	Field	-18.82	146.16	high
MG040	Mahogany Glider	M	Easter Ck	2022	ear	Field	-18.82	146.15	high
MG041	Mahogany Glider	M	Easter Ck	2022	ear	Field	-18.82	146.16	high
MG042	Mahogany Glider	F	Easter Ck	2022	ear	Field	-18.82	146.14	high
MG043	Mahogany Glider	F	Easter Ck	2022	ear	Field	-18.82	146.16	high
MG044	Mahogany Glider	F	Easter Ck	2022	ear	Field	-18.81	146.16	high
SG001	Krefftt's Glider	M	Cardwell	2021	ear	Field	-18.27	146.02	medium
SG002	Krefftt's Glider	F	Cardwell	2021	ear	Field	-18.27	146.02	medium
SG003	Krefftt's Glider	M	Cardwell	2021	ear	Field	-18.32	146.05	high
SG004	Krefftt's Glider	F	Ingham	2022	ear	Field	-18.85	146.09	high
SG005	Krefftt's Glider	M	Ingham	2022	ear	Field	-18.85	146.09	high
SG006	Krefftt's Glider	M	Ingham	2022	ear	Field	-18.85	146.09	high
SG007	Krefftt's Glider	M	Ingham	2022	ear	Field	-18.86	146.13	high
SG008	Krefftt's Glider	M	Tully	2022	ear	Field	-18.07	145.99	high
SQ001	Squirrel Glider	F	Cape Cleveland	2021	ear	Field	-19.30	147.03	high
SQ002	Squirrel Glider	F	Cape Cleveland	2021	ear	Field	-19.29	147.01	high
SQ003	Squirrel Glider	M	Cape Cleveland	2021	ear	Field	-19.29	147.02	high
SQ004	Squirrel Glider	M	Cape Cleveland	2021	ear	Field	-19.29	147.02	high
SQ005	Squirrel Glider	M	Cape Cleveland	2021	ear	Field	-19.29	147.02	high
SQ006	Squirrel Glider	M	Cape Cleveland	2021	ear	Field	-19.30	147.03	high
A004079	Squirrel Glider	NA	Brisbane	2006	muscle	Museum	-27.65	152.78	low
A004418	Squirrel Glider	NA	NQ-Chartres Towers	2000	muscle	Museum	-20.08	146.26	low
A004432	Squirrel Glider	NA	Brisbane	2006	muscle	Museum	-27.50	152.96	low
A004487	Squirrel Glider	NA	Rockhampton	2001	NA	Museum	-23.38	150.51	low
A004653	Squirrel Glider	NA	Brisbane	2008	muscle	Museum	-28.06	153.34	low
A004675	Squirrel Glider	NA	NQ-Silver Valley	2001	NA	Museum	-17.63	145.38	low
A004905	Mahogany Glider	NA	Cardwell	1996	liver	Museum	-18.04	145.91	low
A004987	Mahogany Glider	NA	Bambaroo	1995	muscle	Museum	-18.87	146.19	low
A005360	Mahogany Glider	NA	Tully	1989	liver	Museum	-18.04	145.81	low
A005720	Squirrel Glider	NA	Airlie Beach	2005	NA	Museum	-20.27	148.72	low
A005721	Squirrel Glider	NA	Airlie Beach	2005	NA	Museum	-20.31	148.59	low
A005722	Squirrel Glider	NA	Airlie Beach	2005	NA	Museum	-20.31	148.59	low
A005723	Squirrel Glider	NA	Airlie Beach	2005	NA	Museum	-20.31	148.59	low
A006472	Squirrel Glider	NA	Brisbane	2009	liver	Museum	-26.70	153.07	low
A006475	Squirrel Glider	NA	Brisbane	2010	muscle	Museum	-27.82	153.42	low
A006476	Squirrel Glider	NA	Brisbane	2010	muscle	Museum	-27.82	153.42	low
A006819	Squirrel Glider	NA	Rockhampton	2009	muscle	Museum	-23.11	150.72	low

A007924	Squirrel Glider	NA	Brisbane	2007	muscle	Museum	-27.75	153.03	low
A008582	Squirrel Glider	NA	NQ-Silver Valley	NA	NA	Museum	-17.60	145.30	low
A008692	Squirrel Glider	NA	Mackay	2013	muscle	Museum	-21.32	149.21	low
A009312	Squirrel Glider	NA	Bundaberg	2007	muscle	Museum	-25.05	151.23	low
A009916	Squirrel Glider	NA	Mackay	2013	muscle	Museum	-21.34	149.02	low
A013109	Mahogany Glider	NA	Ingham	2016	NA	Museum	-18.73	145.93	low
A013123	Squirrel Glider	NA	NQ-Silver Valley	2005	muscle	Museum	-17.45	145.29	low
A013129	Squirrel Glider	NA	NQ-Tolga	2012	muscle	Museum	-17.22	145.48	low
A013130	Squirrel Glider	NA	NQ-Princess Hills	1995	liver	Museum	-18.32	145.38	low
A014541	Squirrel Glider	NA	Brisbane	2014	muscle	Museum	-26.89	152.10	low
A014663	Squirrel Glider	NA	NQ-Chillagoe	2016	NA	Museum	-17.20	144.51	low
A015010	Mahogany Glider	NA	Cardwell	2012	skin	Museum	-18.24	145.97	low
A015011	Mahogany Glider	NA	Tully	2010	skin	Museum	-18.01	145.98	low
A015012	Mahogany Glider	NA	Murray Upper	2010	skin	Museum	-18.09	145.77	low
A015013	Mahogany Glider	NA	Cardwell	2017	skin	Museum	-18.23	145.98	low
A015015	Mahogany Glider	NA	Cardwell	2017	skin	Museum	-18.24	145.99	low
A015067	Squirrel Glider	NA	Mackay	2005	fur	Museum	-21.14	149.19	low
A015068	Squirrel Glider	NA	Mackay	2005	fur	Museum	-21.14	149.19	low
A015069	Squirrel Glider	NA	Mackay	2005	fur	Museum	-21.14	149.19	low
A015071	Squirrel Glider	NA	Mackay	2005	fur	Museum	-21.14	149.19	low
A015072	Squirrel Glider	NA	Mackay	2005	fur	Museum	-21.14	149.19	low
A015073	Squirrel Glider	NA	Mackay	2005	fur	Museum	-21.14	149.19	low
A015075	Squirrel Glider	NA	Mackay	2006	Fur	Museum	-21.16	149.06	low
A015078	Squirrel Glider	NA	Mackay	2006	fur	Museum	-21.08	149.21	low
A015079	Squirrel Glider	NA	Mackay	2006	fur	Museum	-21.43	149.20	low
A015081	Squirrel Glider	NA	Mackay	2006	fur	Museum	-21.43	149.20	low
A015082	Squirrel Glider	NA	Mackay	2006	fur	Museum	-21.08	149.21	low
A015088	Squirrel Glider	NA	Mackay	2006	fur	Museum	-21.16	149.06	low
A015089	Squirrel Glider	NA	Mackay	2006	fur	Museum	-20.93	149.02	low
A015245	Squirrel Glider	NA	NQ-Mitchell River	2003	skin	Museum	-16.54	143.43	low
A015246	Squirrel Glider	NA	NQ-Silver Valley	NA	skin	Museum	-17.60	145.30	low
A015385	Mahogany Glider	NA	Tully	2007	muscle	Museum	-18.04	145.91	low
A018339	Squirrel Glider	NA	Bundaberg	2006	NA	Museum	-25.25	152.81	low
A018457	Squirrel Glider	NA	Brisbane	2012	muscle	Museum	-27.65	152.06	low
A019042	Squirrel Glider	NA	Brisbane	2010	muscle	Museum	-27.82	153.42	low
A019088	Squirrel Glider	NA	Brisbane	2016	muscle	Museum	-27.30	153.05	low

Table S3 Calculated genetic diversity indices for each species, genetic groups, and sampling locality, with the number of individuals (nInd), loci (nloc), polymorphic loci (polyLoc), and monomorphic loci (monoLoc) displayed. Observed heterozygosity (H_o), expected heterozygosity (H_e), and inbreeding coefficient (F_{IS}) were calculated using dartR (Gruber et al. 2018). The confidence interval of F_{IS} was determined through bootstrapping using Hierfstat (Goudet 2005). The abbreviations used are: MG for Mahogany Glider, SQ for mid-eastern/south-eastern Queensland Squirrel Glider, CC Cape Cleveland Squirrel Glider, NQ for North Queensland Squirrel Glider, and KG for Krefft's Glider.

Grouping	Group	nInd	nLoc	polyLoc	monoLoc	sMLH	sMLH.SD	Ho	HoSD	He	HeSD	uHe	uHeSD	FIS	FIS_2.5Q	FIS_97.5Q
Species (All data)	Mahogany	49	2424	1834	590	1.13	0.14	0.116	0.131	0.13	0.142	0.131	0.144	0.114	0.104	0.125
	Squirrel	28	2424	1675	749	0.895	0.189	0.089	0.108	0.118	0.14	0.121	0.143	0.265	0.253	0.282
	Krefft's	9	2424	497	1927	0.572	0.042	0.061	0.145	0.07	0.152	0.074	0.161	0.17	0.15	0.207
MG-cluster	South MG	29	2877	2675	202	1.04	0.094	0.184	0.143	0.204	0.148	0.207	0.151	0.111	0.102	0.122
(MG data)	North MG	20	2877	2282	595	0.942	0.132	0.167	0.154	0.186	0.16	0.191	0.164	0.124	0.114	0.138
Sampling locality (MG data)	Allendale	8	2877	1974	903	1.063	0.053	0.188	0.19	0.189	0.169	0.202	0.181	0.068	0.054	0.095
	Bambaroo	11	2877	1964	913	1.028	0.137	0.182	0.189	0.184	0.173	0.193	0.181	0.054	0.041	0.076
	Cardwell	8	2877	1808	1069	0.973	0.08	0.172	0.187	0.177	0.172	0.188	0.183	0.085	0.072	0.108
	Easter Ck	7	2877	1882	995	1.044	0.052	0.185	0.196	0.186	0.171	0.2	0.184	0.077	0.065	0.104
	Muller	5	2877	1596	1281	1.039	0.055	0.184	0.22	0.175	0.181	0.194	0.201	0.053	0.033	0.081
	Murray Upper	4	2877	1277	1600	0.776	0.182	0.138	0.203	0.151	0.184	0.172	0.211	0.201	0.203	0.256
	Ollera Ck	3	2877	1246	1631	1.014	0.09	0.18	0.251	0.158	0.191	0.19	0.229	0.053	0.04	0.091
	Tully	3	2877	1173	1704	0.921	0.065	0.163	0.243	0.149	0.189	0.178	0.226	0.085	0.066	0.133
SQ-cluster (SQ data)	SQ	17	1912	1667	245	0.958	0.232	0.163	0.137	0.216	0.159	0.222	0.164	0.265	0.257	0.288
	CC	6	1912	1094	818	1.209	0.055	0.206	0.233	0.195	0.193	0.213	0.211	0.031	0.015	0.056
	NQ	5	1912	1037	875	0.891	0.129	0.152	0.195	0.178	0.186	0.198	0.206	0.232	0.226	0.284

*Interpretation of genetic diversity indices should be approached with caution when the sample size comprises fewer than 10 individuals.

Table S4 Estimated Effective Population Sizes for Mahogany Glider Sampling Localities. The effective population size is determined through the linkage equilibrium method and is reported at three critical values (0.05, 0.02, 0.01). Confidence intervals are provided using both parametric and jackknife (non-parametric) methods, offering a comprehensive view of the estimated variability (Do *et al.* 2014).

Sampling Locality	Sample Size	Crit.Value	Ne.est	Cl.para.low	Cl.para.high	Cl.jk.low	Cl.jk.high
Allendale	8	0.05	64.7	62	67.6	16.1	Infinite
		0.02	64.7	62	67.6	16.1	Infinite
		0.01	64.7	62	67.6	16.1	Infinite
Bambaroo	10	0.05	27.6	27.1	28.1	19	45.1
		0.02	27.6	27.1	28.1	19	45.1
		0.01	27.6	27.1	28.1	19	45.1
Cardwell	4	0.05	-21.2	Infinite	Infinite	Infinite	Infinite
		0.02	-21.2	Infinite	Infinite	Infinite	Infinite
		0.01	-21.2	Infinite	Infinite	Infinite	Infinite
Easter Creek	7	0.05	431.2	324.4	640.6	45.8	Infinite
		0.02	431.2	324.4	640.6	45.8	Infinite
		0.01	431.2	324.4	640.6	45.8	Infinite
Muller's Creek	5	0.05	-56.2	Infinite	Infinite	Infinite	Infinite
		0.02	-56.2	Infinite	Infinite	Infinite	Infinite
		0.01	-56.2	Infinite	Infinite	Infinite	Infinite
Murray Upper	3	0.05	-6.2	Infinite	Infinite	Infinite	Infinite
		0.02	-6.2	Infinite	Infinite	Infinite	Infinite
		0.01	-6.2	Infinite	Infinite	Infinite	Infinite
Ollera Creek	3	0.05	-5.6	Infinite	Infinite	Infinite	Infinite
		0.02	-5.6	Infinite	Infinite	Infinite	Infinite
		0.01	-5.6	Infinite	Infinite	Infinite	Infinite

Supplementary Figures

Fig. S1 A flowchart illustrating the customized filtering process in this study for the analysis of genetic structure and genetic diversity.

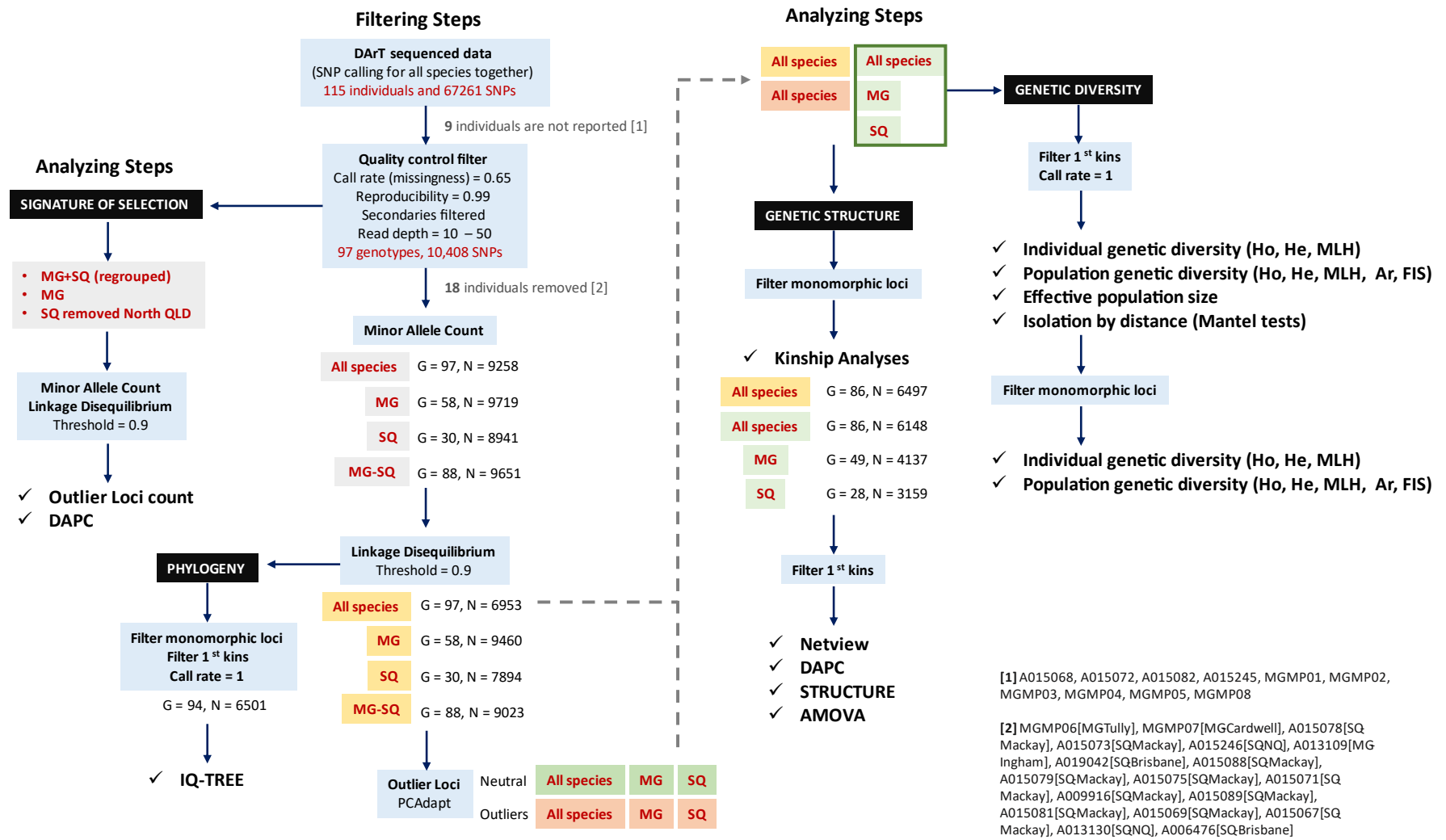


Fig. S2 Types of museum samples passing/failing SNP quality control filters

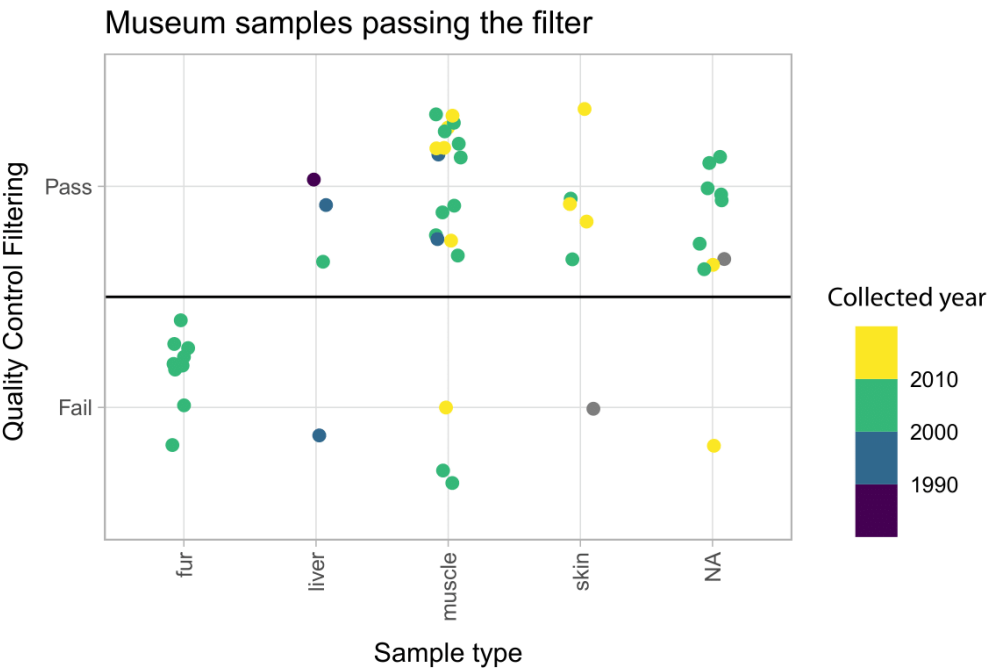


Fig. S3 Netview network on three different distance matrices connecting 10, 20, and 30 nearest neighbors. The three distance matrices presented Euclidean distance (eucl) applied on allele frequency within individuals (Jombart and Ahmed 2011), pairwise difference on number of loci for which individuals differ (nLoci) (Paradis and Schliep 2019), and number of allelic differences between two individuals (nAllele) (Kamvar *et al.* 2014).

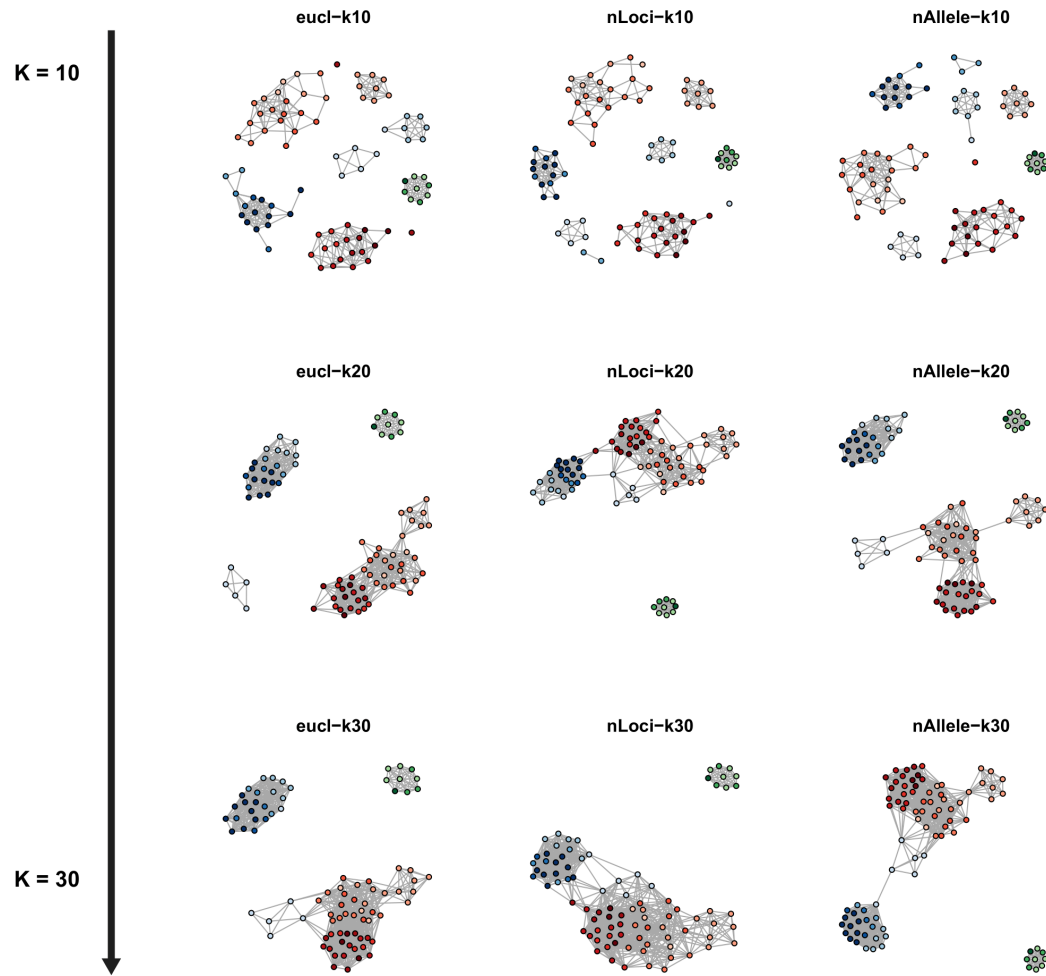


Fig. S4 Unsupervised K means clustering using R adegenet package (Jombart and Ahmed 2011) on all-species datasets (A) and two species datasets of neutral loci (B). The y-axis represents the original cluster, and the x-axis indicates the assigned cluster. The sampling localities identified as Mahogany Glider are shown red, Squirrel Gliders are shown in blue, and Krefft's Glider are shown in green.

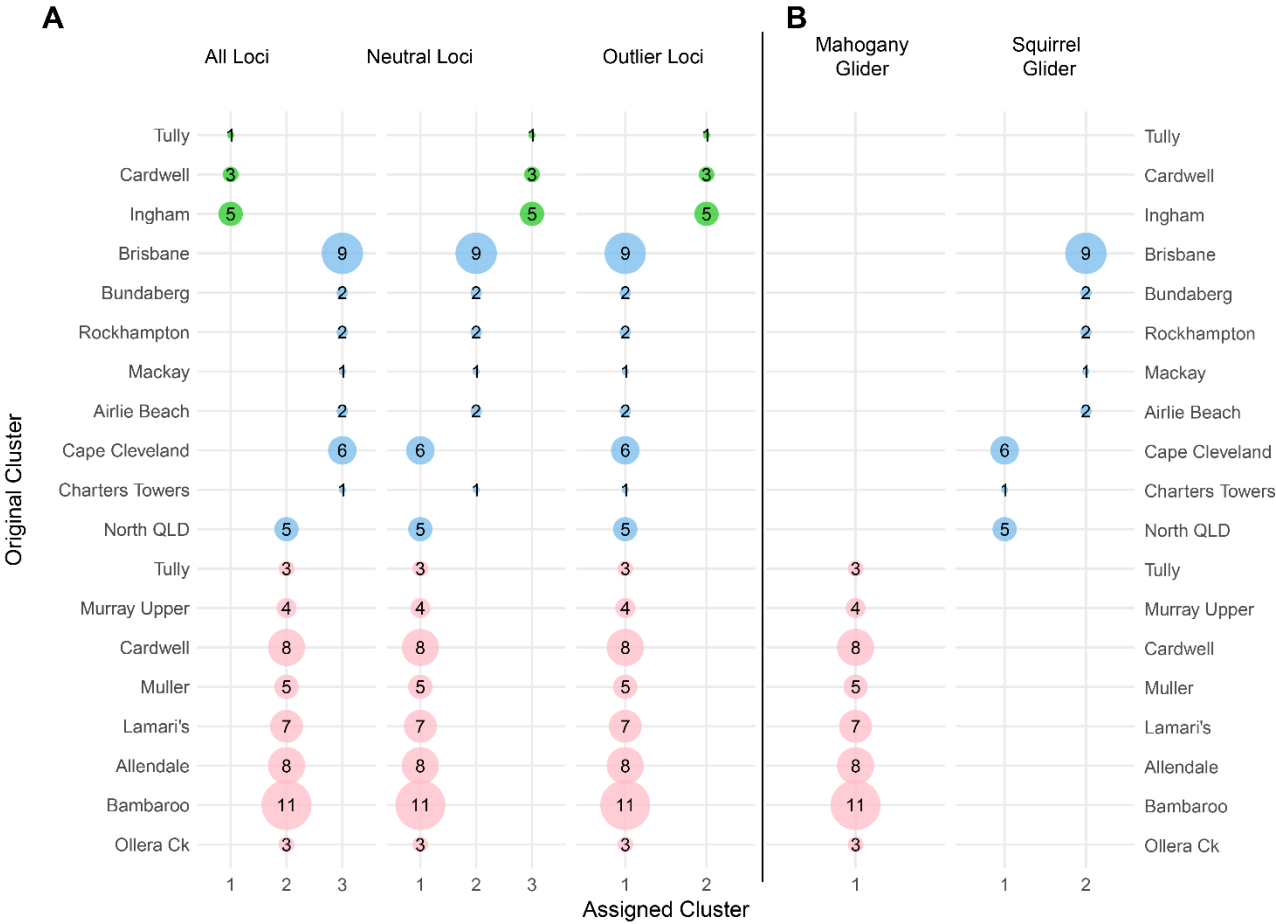


Fig. S5 Delta K (ΔK) in the Evanno plots (Evanno *et al.* 2005) determining the optimal cluster K in the STRUCTURE analyses for Mahogany and Squirrel Glider dataset (A) and Mahogany Glider only dataset (B)

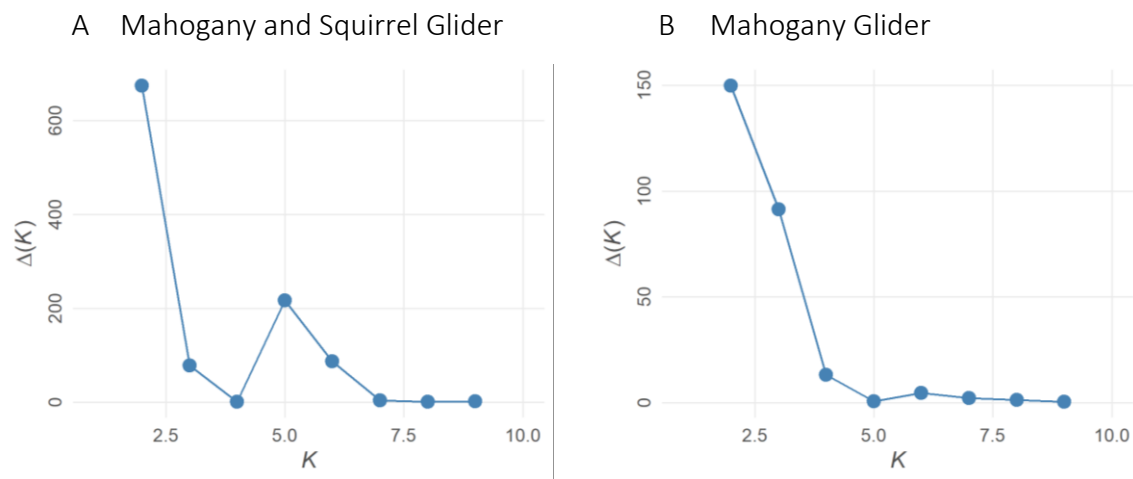


Fig. S6 Structure Plot of Mahogany and Squirrel Glider presenting the genetic components of Mahogany and Squirrel Gliders, with K values ranging from 2 to 10. The gliders are arranged geographically from north to south. The red lines separate the Squirrel Gliders and Mahogany Glider. The genetic component of the Mahogany Glider is represented in orange for the southern population and brown for the northern population. In contrast, the genetic component of the Squirrel Gliders is depicted in various shades of blue. The hypothetical optimal K and the second optimal K value for the Mahogany-Squirrel Glider cluster is determined to be K=2 and K=5, respectively (Fig. S5).

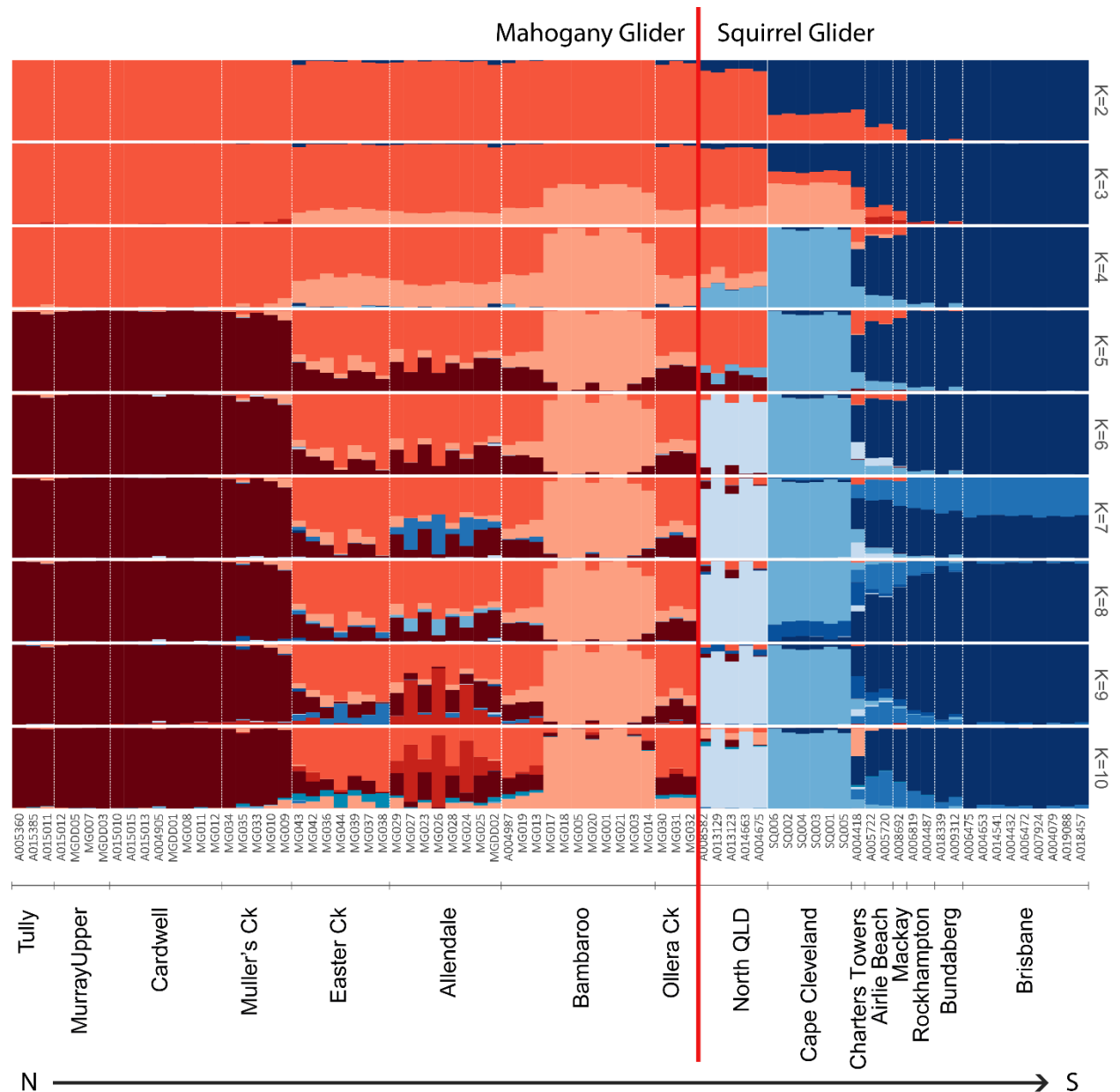


Fig. S7 Analysis of molecular variance (AMOVA) of all-species datasets (A) and Mahogany-Squirrel Glider only datasets. Bar plots depict variances contributing to population structure among species (Mahogany, Squirrel, and Krefft's Glider), within the four consensus genetic groups (MG, NQ, CC, SQ), and among and within samples. The percentage of explained variance was significant at all levels.

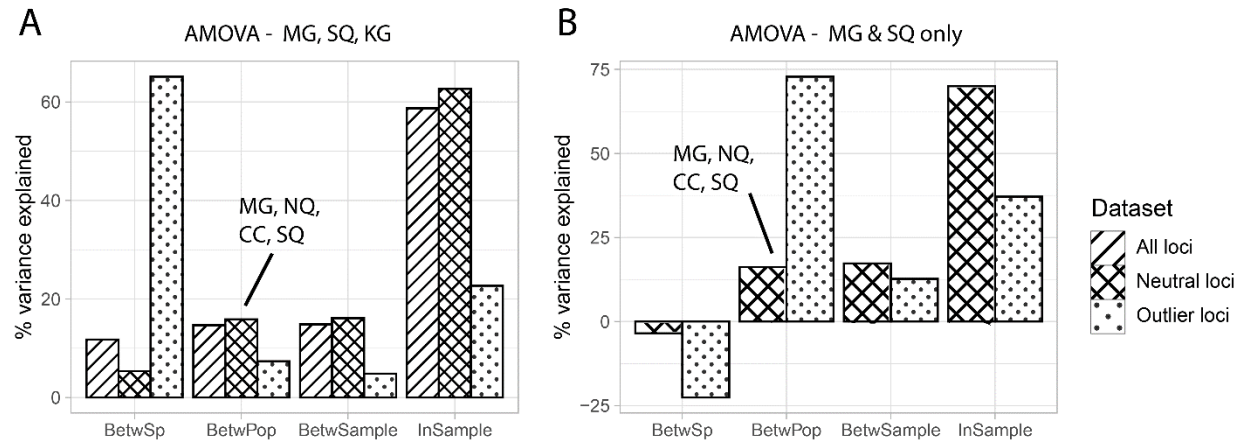


Fig. S8 Observed heterozygosity (H_o) and standardized multi-locus heterozygosity (sMLH) of individual Mahogany Glider and Squirrel Glider with monomorphic loci filtered. The populations of Mahogany Glider are arranged geographically from south (left) to north (right). The samples are colour-coded by the year of collection.

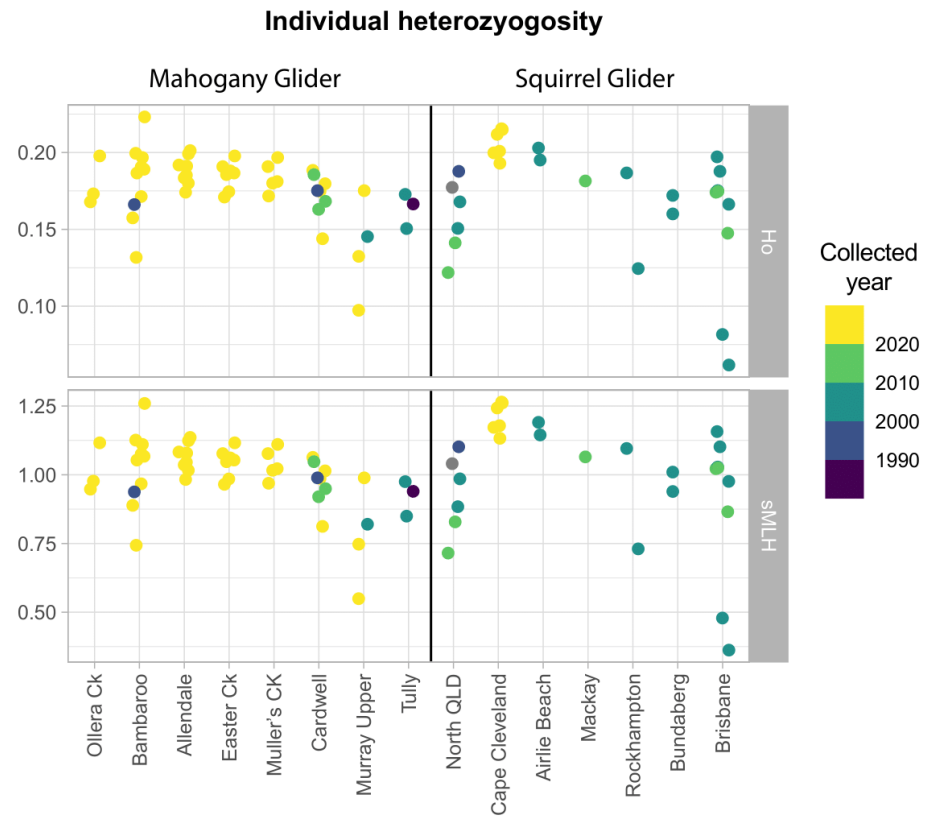


Fig. S9 Analysis of isolation by distance (IBD) based on the proportion of shared alleles among individual Mahogany Gliders (MG). The x-axis represents the Euclidean distance in kilometres, while the y-axis depicts the proportion of shared alleles between individuals. The colour gradient reflects the kernel density of individuals within the plot, and the trend lines illustrate the correlation. (A) The IBD for all Mahogany Gliders (B) The IBD for the southern cluster (C) The IBD for the northern cluster. Despite the Mantel tests for all groupings being statistically significant, the correlations are relatively weak

