Population genetics of rainforest mountain frogs (Anura: Limnodynastidae: *Philoria*) severely impacted by the Australian megafires

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Abstract

The 2019/20 Australian megafires impacted numerous species, including six of the seven montane frog species in the genus *Philoria*, which are confined to isolated rainforest habitats across high-altitude areas in eastern Australian Gondwonan rainforest. Using single nucleotide polymorphisms, we examined the genetic structure and diversity of the six northern *Philoria* species to inform conservation management and assess their capacity for post-fire recovery. Narrow-range species were confirmed as a single population for management purposes, while *P. kundagungan*, *P. loveridgei*, and *P. sphagnicolus* exhibit marked genetic differentiation between populations, indicating strong allopatric differentiation among populations isolated on separate mountaintops, suggesting limited natural dispersal ability. We further identify high-value genetic populations in these structured species. Populations that were heavily impacted by the fires, such as *P. pughi* and *P. knowlesi*, may face longer-term threats due to potential declines in adaptive capacity. We recommend prioritizing in situ management, genetic rescue, and translocation efforts to bolster resilience in isolated populations. Updated conservation planning and targeted fire buffer management are crucial for the survival of these ancient, regionally endemic frogs in a rapidly changing climate.

Keywords: phylogenetic analysis, bushfire impacts, genetic conservation management, captive breeding, short-range endemic

Introduction

In recent decades, climate change has made Australia increasingly hotter and drier (Yu et al., 2020), and climate projections suggest temperatures could rise by 2-4 °C by 2100 (Watterson et al., 2015). Climate change is also causing large wildfires to be more common (Liu et al., 2010), and the time between large wildfires is decreasing. At the time, 2019 was the hottest and driest year in Australia's recorded history (Yu et al., 2020), and consequently, the 2019/20 bushfire season was the worst bushfire disaster Australia has ever suffered. These fires, now known as the Black Summer megafires (Davey & Sarre, 2020; Kemter et al., 2021) burned more than 18.6 million hectares of land, killed more than one billion animals, and severely damaged a wide area of Australia's rainforest ecosystems (Roff & Aravena, 2020; Van Eeden et al., 2020). Specifically, 53% of Gondwanan rainforest burned on Australia's east coast, destroying or altering the habitat of its endemic fauna (Collins et al., 2021).

A Wildlife and Threatened Species Bushfire Recovery Expert Panel, convened by Australia's Minister for Environment, compiled a list of 119 animal species requiring urgent management interventions following the 2019/20 megafires (Legge et al., 2020). Four of the 16 amphibian species on the list belonged to the genus *Philoria* (Family Limnodynastidae) – a relatively poorly studied group of seven species of montane leaf-litter frogs from eastern and southern Australia. *Philoria* diverged from its sister genus *Adelotus* approximately 30 MY (Brennan et al. 2023), and the divergence between individual *Philoria* species is >2 MYA (Knowles et al. 2004), suggesting a long persistence of individual species on isolated mountain tops. Except for the most southern species, *P. frosti*, which occurs in alpine Victoria, all other species occupy mountain-top Gondwanan rainforests in north-eastern New South Wales and south-eastern Queensland (Fig. 1; Mahony et al., 2022; Anstis, 2018; Knowles et al., 2004). Each species has a highly fragmented habitat and is susceptible to environmental change due to low fecundity and terrestrial breeding (Bolitho et al., 2022; Anstis, 2018).

Climate change, resulting in prolonged drought and the increasing frequency and severity of bushfires, is the main threat to *Philoria* (Bolitho and Newell, 2022; Heard et al., 2023; Beranek et al., 2023; Mahony et al., 2023; Abram et al., 2021). The 2019/20 megafires

affected many *Philoria* habitats in north-eastern New South Wales and south-eastern Queensland (Mahony et al. 2023). A post-fire impact assessment found that megafires burned 30% of the habitat of *P. kundagungan* and 21% of the potential habitat of *P. richmondensis*. The megafires impacted over 90% of suitable habitats for *P. pughi* (Heard et al., 2021), making it one of the most fire-impacted species across all taxa affected by the megafires (Legge et al. 2020). Fire in *Philoria* habitat destroys ground cover and woody debris, changes hydrology, and promotes access for feral pigs that can destroy breeding habitats (Heard et al. 2023). Given the additional risk of extinction of these species due the megafires, more information on the demography and genetic structure of rainforest *Philoria* is needed to guide their recovery.

Ideally, conservation management of amphibians following a major disturbance should be undertaken with an understanding of mortality rates at embryonic, larval and juvenile life stages, and the age at maturity (Biek et al. 2002). Although several studies exist on *Philoria* species delimitation, population structure (Knowles et al., 2004; Mahony et al., 2022), and species distributions (Abram et al., 2021; Bolitho et al., 2019), little is known about species demography. Demographic studies in the field are challenging due to the species' rarity, limited accessibility due to mountaintop distributions, and fossoriality. Count data exist from patch occupancy studies for *P. richmondensis* and *P. kundagungan* (Bolitho et al. 2019; Heard et al. 2021) and show that extremely low abundances of calling males characterise all populations. Capture of males at breeding sites presents an ethical challenge; disruption may damage a nest and compromise egg viability. Adult females are extraordinarily difficult to find; and so little information on female age at maturity or habitat use is available. However, individuals currently being raised to adulthood from eggs are still not sexually mature at three years of age (DN, unpubl. data), suggesting long-lived frogs.

When demographic data are limited, population genetics can provide important information for conservation planning. For example, information on population structure provides an understanding of species' ecological limits (Sexton et al. 2009) and the level of migration between areas (Bergl & Vigilant 2007; Meirmans & Hedrick 2011; Abdellaoui et al. 2013). Genetic diversity estimates can help identify isolated populations undergoing declines, which

should be a target for conservation actions such as relocations or supplementation (Ewen et al. 2012; Sheean et al. 2012; Kissel et al. 2014).

This study focuses on the population genetics of the six northern *Philoria* species, aiming to clearly define their population genetic structure and diversity and to infer dispersal processes that may impact their recovery from the 2019-20 megafires. Firstly, we are interested in whether analysis of phylogenetic and population genetic structure can inform whether individuals can move between suitable habitat areas, suggesting an ability to recolonise habitats. Secondly, we seek to assess whether populations are at elevated risk of population decline due to low genetic diversity levels and inbreeding, which is relevant for planning conservation interventions such as translocations. Thirdly, we aim to identify high-value genetic populations that should be primary targets for protection and management, and to determine whether high-value populations are covered by current conservation plans for these species (e.g. Assets of Intergenerational Significance (AIS) in NSW). Fourthly, given that *Philoria* species were among those most impacted by the 2019/20 megafires (Legge et al. 2020), there is value in analysing the potential impacts of the fires on each species, such as whether fires burned populations that had higher genetic diversity and/or a higher frequency of unique alleles. Guided by these new insights, we then make management recommendations to better protect this ancient radiation of regionally endemic frog species.

Materials and Methods

Study species

Philoria species occur on high-elevation mountains almost entirely within protected areas (Bolitho et al. 2019). Due to their fossorial habit, adults are challenging to find during the non-breeding season (Knowles et al. 2004; Hoskin et al. 2009; Willacy et al. 2015), but males are conspicuous when calling during the breeding season from muddy seepages within rainforest headwater streams (Knowles et al. 2004; Anstis 2018; Bolitho et al. 2023). Unlike many other frogs that disperse as tadpoles, *Philoria* larvae remain in the nest until completing metamorphosis (Hollis 2004; Knowles et al. 2004; Mahony et al. 2022).

The Mountain frog*, Philoria kundagungan,* occurs in the Main Range National Park Queensland (Qld) and the Koreelah, Mount Clunie, and Tooloom National Parks in New South Wales (NSW), with around eleven square kilometres of suitable habitat (Bolitho et al. 2019). Pugh's Mountain frog, *P. pughi*, is found only in the Gibraltar Ranges, Timbarra Plateau and New England Ranges in northern NSW, in both national parks and reserves and on land managed for forestry. The Mount Ballow Mountain frog, *P. knowlesi*, has one of the smallest distributions, occurring only in Mount Barney and Mt Ballow National Parks in Qld and on Mount Nothofagus and Levers Plateau in the eastern Border Ranges National Park NSW (Mahony et al. 2022). Loveridge's Mountain Frog, *P. loveridgei,* occurs in the Lamington and Springbrook National Parks in Qld., and the Border Ranges, Mount Warning and Nightcap National Parks in NSW. The Richmond Range sphagnum frog, *P. richmondensis,* mainly occurs in the Yabbra, Richmond Range, and Toonumbar National Parks in NSW. The sphagnum frog, *P. sphagnicolus*, is the most widespread *Philoria* species, and occurs from Guy Fawkes River National Park in the north to the Tapin Tops National Park in the south in NSW. It appears to be naturally absent from the Oxley Wild Rivers National Park and was recognised previously as having northern and southern populations (Knowles et al. 2004; Murray & Hose 2005).

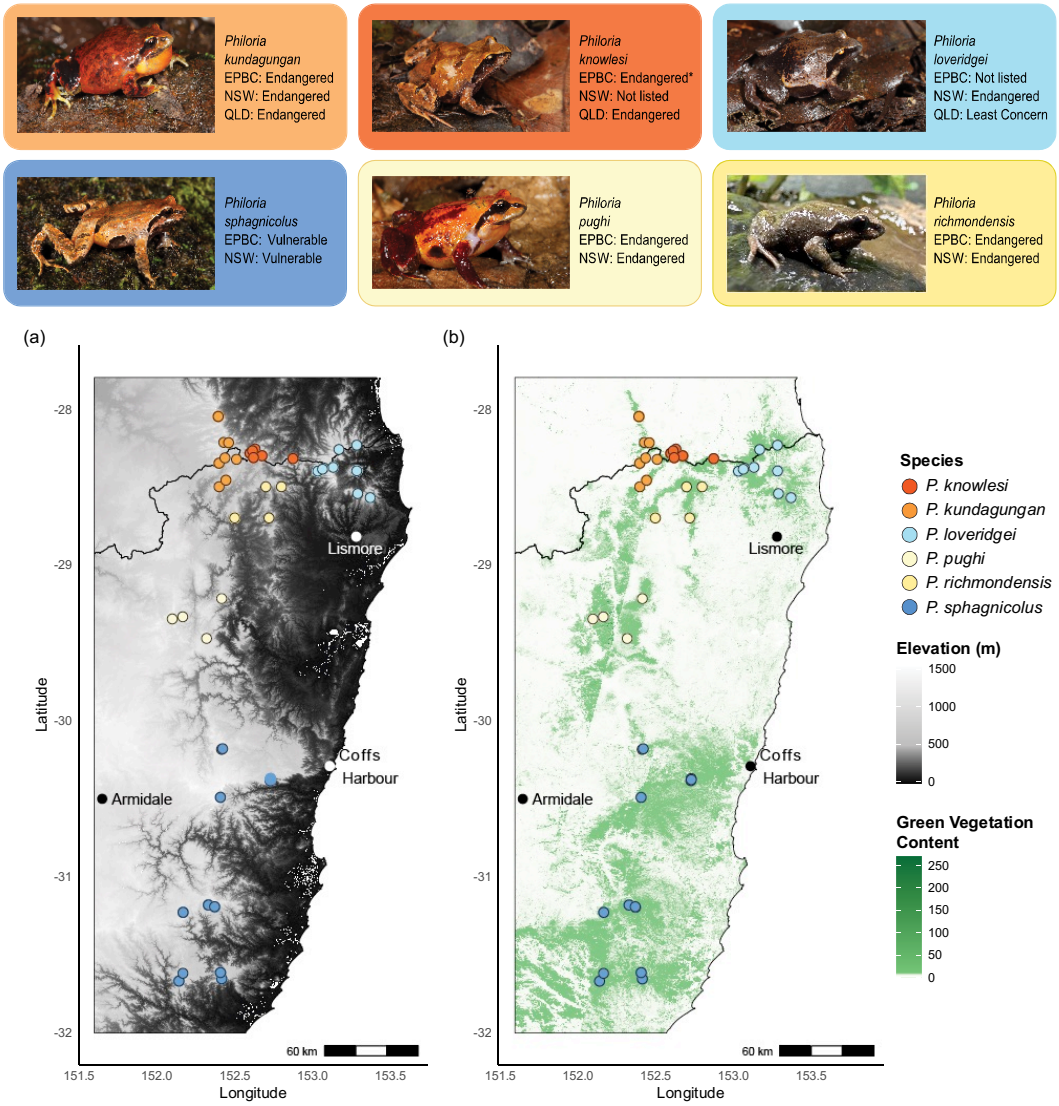

Figure 1. Genetic samples of *Philoria* species in northern NSW and QLD. Each species (coloured boxes) shows the listing status federally under the Environment Protection and Biodiversity Conservation (EPBC) Act and in relevant states. Samples are shown over elevation (a) and Green Vegetation Content (b; https://pid.geoscience.gov.au/dataset/ga/74350), highlighting the restriction of *Philoria* species to high elevation rainforest habitats. The asterisk for *P. knowlesi* represents the preliminary EPBC status. Photos by Liam Bolitho (*P. richmondensis*) and Stephen Mahony (all others).

DNA Sequencing and SNP data generation

A total of 163 tissue samples (*P. knowlesi*=17, *P. kundagungan* = 32, *P. loveridgei* = 39, *P. pughi* = 16, *P. richmondensis* = 12, *P. sphagnicolus* = 47) from the Australian Biological Tissue Collection (ABTC) were used for DNA extraction and sequencing, and represented all *Philoria* tissue samples available after the 2019-20 megafires (details in Appendix S1). All tissue samples were submitted to Diversity Arrays Technology Pty Ltd (Canberra) for commercial DNA extractions, library preparation, and DArT-seq 1.0 high-density sequencing following the proprietary methods outlined in Kilian et al. (2012) and Sansaloni et al. (2011) papers. In brief, the *PstI-SphI* restriction enzyme combination was used for DNA digestion and all other laboratory steps followed by Georges et al. (2018).

SNP data generation

We used the *Stacks* v2.6.4 (Catchen et al. 2011; Catchen et al. 2013) for quality filtering, identifying loci in individuals, and genotyping each locus. We first ran *process_radtags* to remove barcodes and quality filter raw reads. We then used the Trimmomatic v0.39 (Bolger et al. 2014) to trim all obtained reads to 68 bp with the script: "java -jar trimmomatic-0.39.jar SE -phred64 ILLUMINACLIP: TruSeq3-SE.fa:2:30:10 LEADING:5 SLIDINGWINDOW:4:5 CROP:68 MINLEN:68". Then we ran the de novo pipeline with *Stacks* v2.6.4, using default settings for *ustacks, cstacks*, *sstacks*, *tsv2bam*, and *gstacks*.

We ran *gstacks* and *populations* steps multiple times using different popmap files and settings as required for the theoretical requirements of different analyses. For the population genetic structure analysis, we used the single popmap file for each species with the following settings: "--min-samples-overall" to 0.75, "--min-mac" to 3, "--min-maf" to 0.05, and "--max-obs-het" to 0.5. To generate a PHYLIP file for the phylogenetic analysis, we used a popmap file containing all the individuals after filtering and the following settings: "--min-samples-overall" to 0.75, "--min-mac" to 3, "--min-maf" to 0.05, and "--max-obs-het" to 0.5. We used the popmap files with only one and each species for the genetic diversity analyses. We set the "--min-samples-overall" to 0.75 and "--max-obs-het" to 0.5 to calculate autosomal heterozygosity and F_{IS}.

The VCF files were filtered in *R* V4.3.2 (R Core Team, 2023) using the *dartR* package (Gruber et al. 2018) to filter the loci and individuals for each species. The key settings for the filtering process are outlined in Appendix S2.

Phylogenetic analysis

We used the *IQ-TREE2* v2.2.2.6 (Minh et al. 2020) to generate the maximum likelihood (ML) phylogenetic tree, with 10000 ultrafast bootstrap (Hoang et al. 2017) replicates to provide approximately unbiased branch support values. *ModelFinder* v1.0 (Kalyaanamoorthy et al. 2017) was used to find the best nucleotide substitution model (-m TEST+ASC).

Population genetic structure analysis

We first ran the Principal Coordinates Analysis (PCoA) for each species using the *dartR* package. Then, we used the *snmf* (sparse Non-Negative Matrix Factorization algorithms) function from the R LEA package (Frichot & François 2015) to estimate the number of genetic clusters (K) for each *Philoria* species. We used 5% of the final data with K from 1 to 6 to find the optimal tolerances and alpha values setting (Frichot et al. 2014), with the best tolerances and alpha settings used to run the full dataset for each K value, and repeated 100 times. The cross-entropy criterion was used to determine the best K-value (Frichot et al. 2014). Pairwise F_{ST} and Nei's genetic distances were calculated in R using the *hierfstat* package (Goudet 2005).

Genetic diversity and inbreeding analysis

The number of private alleles (N_P), allelic richness (A_R), observed and expected heterozygosity (H_O and H_E), and the inbreeding coefficient (F_{IS}) for each species and subpopulation (as identified in the population structure analyses) were calculated in R using the *dartR* and *hierfstat* packages.

Bushfires dataset and potential impacts analysis

Due to the lack of tissue samples available after the megafires, we classified all individuals into "Burnt" and "Unburnt" groups based on whether the 2019/20 megafires burned the sampling location. By comparing the number of private alleles and allelic richness of these groups, we assessed whether the megafires could impact genetic diversity by assessing the diversity held within burnt populations versus unburnt populations. To classify the individual locations as unburnt or burned, we used the National Indicative Aggregated Fire Extent Datasets (NIAFED, https://fed.dcceew.gov.au/datasets/dc651afe7ec944d0a22e6c1f120f3a15), which mapped all the areas that burned during the 2019/20 megafires.

Results

After filtering for population genetic structure analyses, we retained 15 individuals with 1,273 SNPs for *P. knowlesi*, 23 individuals with 4,621 SNPs for *P. kundagungan*, 29 individuals with 2,980 SNPs for *P. loveridgei*, 11 individuals with 758 SNPs for *P. pughi*, 11 individuals with 949 SNPs for *P. richmondensis*, and 37 individuals with 4,117 SNPs for *P. sphagnicolus*.

Phylogenetic analyses

Our maximum likelihood phylogeny (Fig. 2) with additional sampling for *P. kundagungan, P. pughi, P. richmondensis* and *P. sphganicolus,* was consistent with previous studies of northern *Philoria* (Mahony et al., 2022). The southernmost species, *P. sphagnicolus*, was set as the sister lineage to the other two major clades following Mahony et al. (2022). The second clade comprises the sister species *P. knowlesi* and *P. loveridgei*. The third clade comprises three closely related species: *P. pughi, P. richmondensis,* and *P. kundagungan.* The interspecific relationships between species are strongly supported (posterior probability ≥ 0.99, bootstrap support = 100).

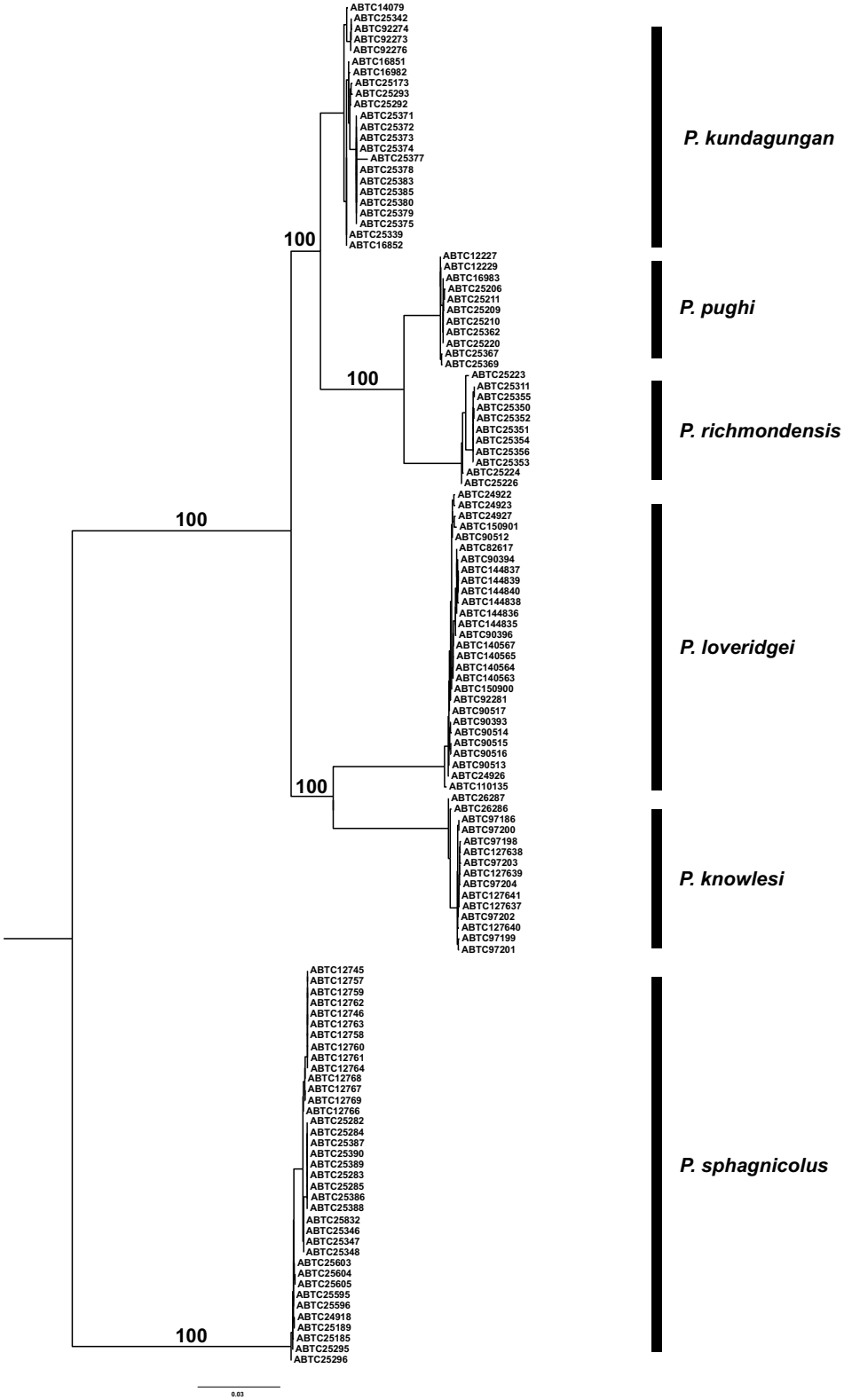

Figure 2. Maximum likelihood phylogeny of northern *Philoria* frogs. Values represent bootstrap support at each node.

Analysis of population genetic structure

The population genetic structure analysis identified between 1-3 clusters for each *Philoria* species. For *P. knowlesi* and *P. richmondensis,* the optimal genetic cluster number (K) was 1 (Figure 3).

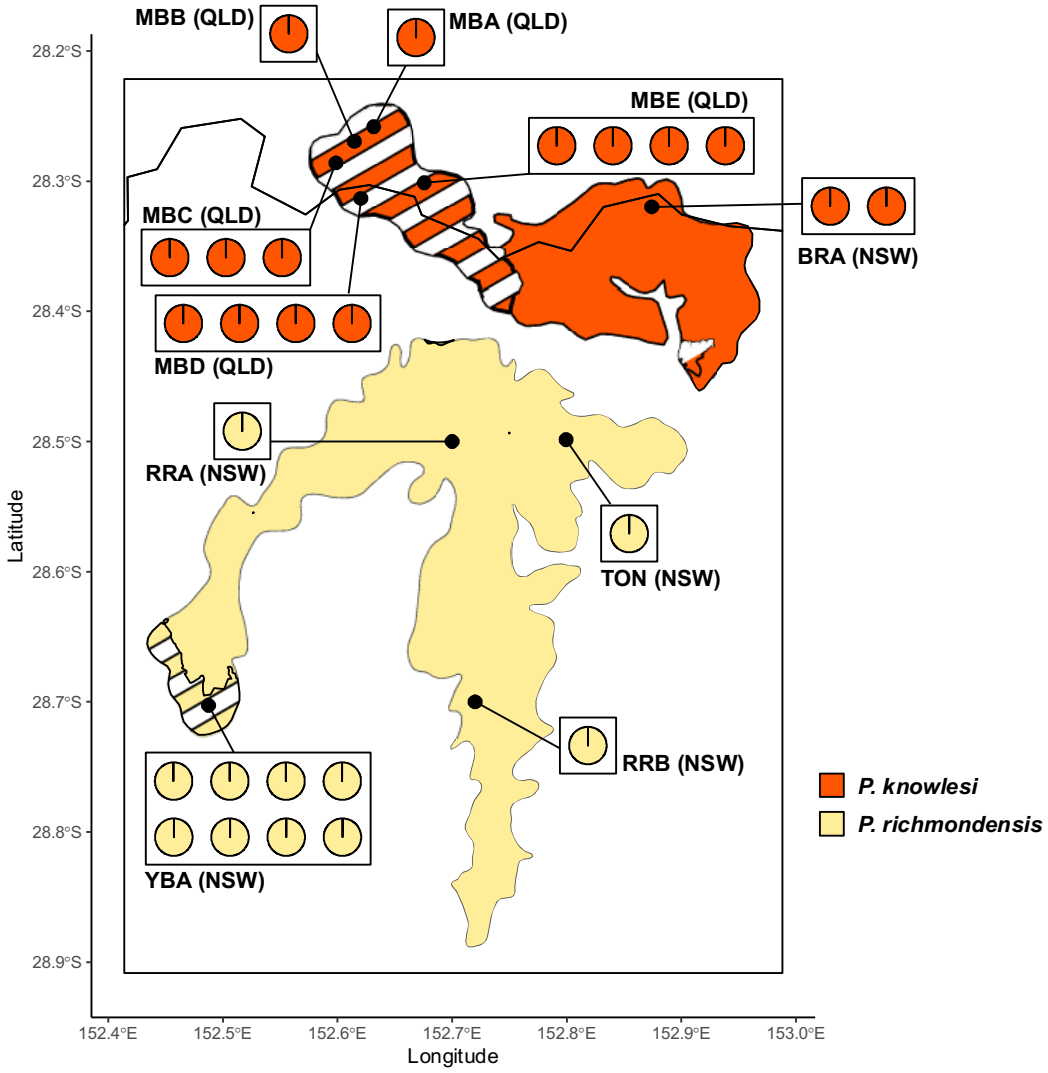

Figure 3. Distribution maps and population genetic structure for *P. knowlesi* (red) and *P. richmondensis* (yellow). Location abbreviations are available in Appendix S2. The striped areas on the map show areas burnt in the 2019/20 megafires.

Two genetic clusters were identified in *P. kundagungan* and *P. loveridgei* (Figure 4a, 4b). For *P. kundagungan*, one genetic cluster encompasses the northern end of the Main Range

National Park (KUN.P1), while the second cluster comprises the rest of the species range in the southern end of the Main Range National Park, Koreelah National Park, Mount Clunie National Park, and Tooloom National Park (KUN.P2) (Figure 4c). For *P. loveridgei*, the genetic clusters comprise sampling sites from the eastern side of the Border Ranges National Park, Mount Warning National Park, and Nightcap National Park (LOV.P2), and sampling site on the northern side of the Border Ranges National Park, Lamington National Park, and Springbrook National Park (LOV.P1) (Figure 4c). For both species, some admixture exists between the genetic clusters at the edge of their ranges.

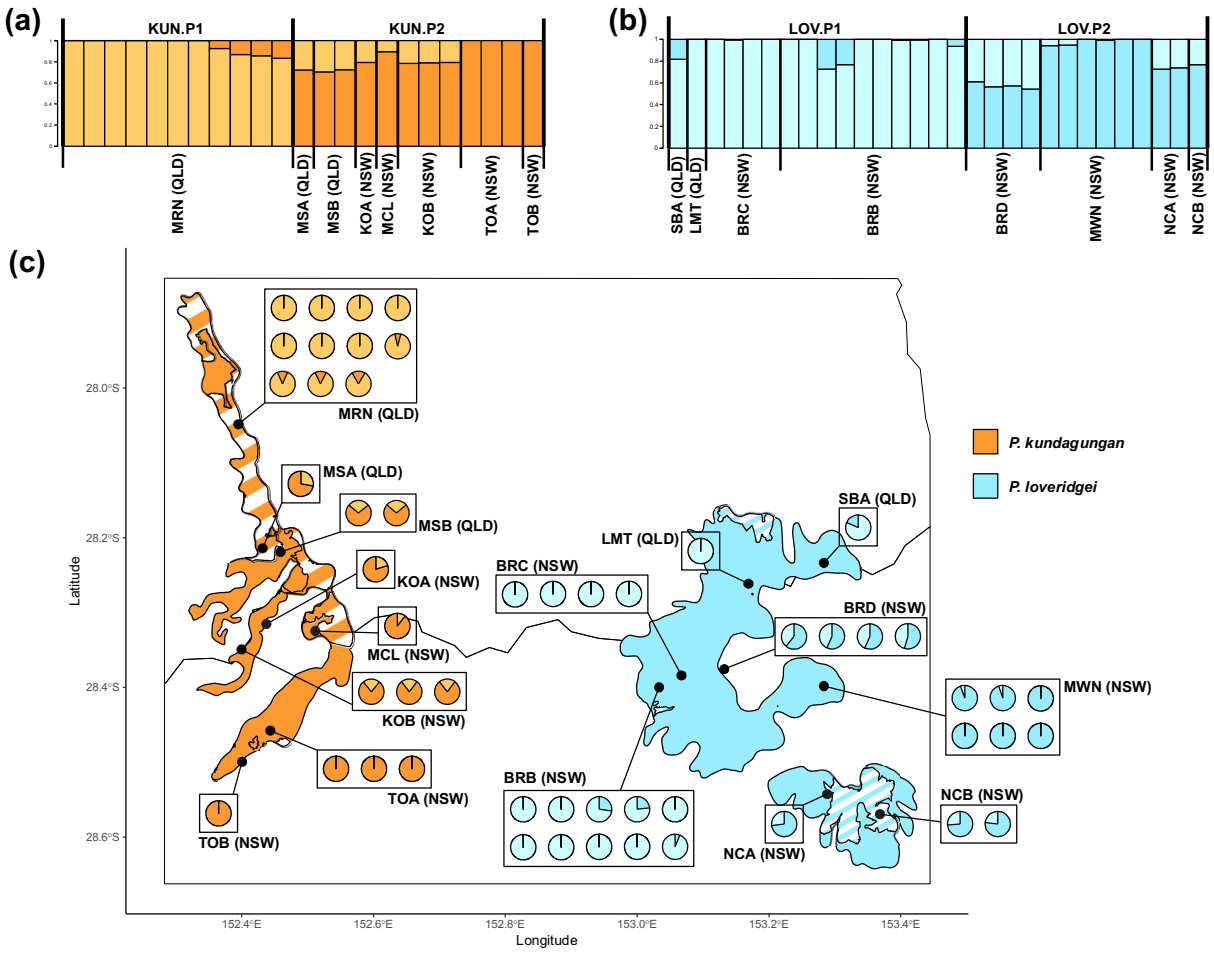

Figure 4. Population genetic structure (a, b) and the distribution maps (c) for *P. kundagungan* (orange) and *P. loveridgei* (blue). Location abbreviations are available in Appendix S2. The striped areas on the map show areas burnt in the 2019/20 megafires.

We identified one genetic cluster in *P. pughi* and three in *P. sphagnicolus* (Figure 5). For *P. sphagnicolus*, these three genetic clusters include samples from Mount Hyland Nature Reserve, Dorrigo National Park, New England National Park (SPH.P1); samples from Werrikimbe National Park, Mount Boss State Forest (SPH.P2); and samples from Boorganna Nature Reserve, Killabakh Nature Reserve, and Dingo State Forest (SPH.P3). Admixture occurs between adjacent clusters at the edges of the distribution range of each subpopulation.

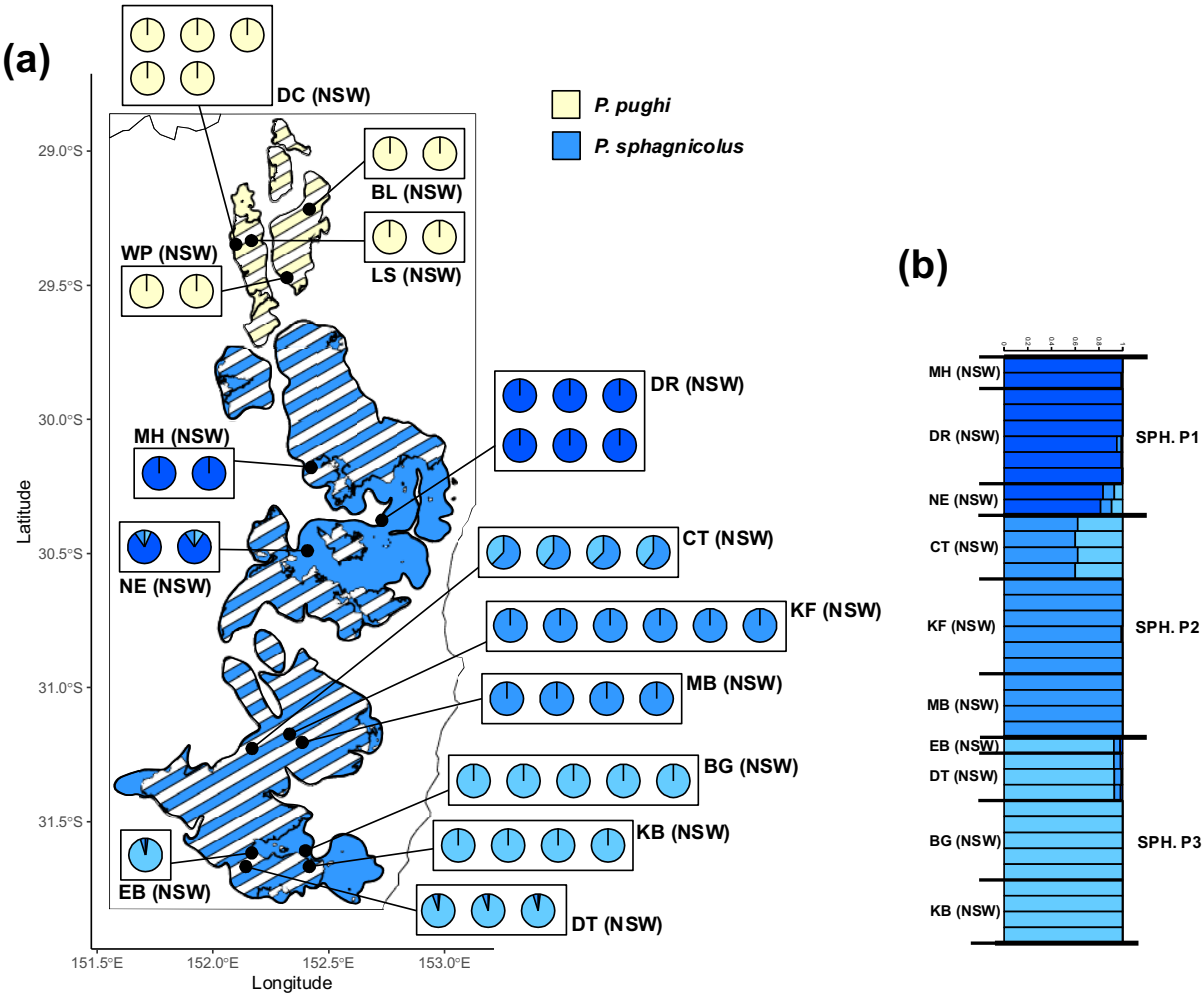

Figure 5. Distribution maps (a) and population genetic structure (b) for *P. pughi* (pale yellow) and *P. sphagnicolus* (shades of blue). Location abbreviations are available in Appendix S2. The striped areas on the map show areas burnt in the 2019/20 megafires.

We identified relatively high pairwise F_{ST} and Nei's genetic distance values (>0.5 for several comparisons) between genetic clusters of *Philoria* species, particularly within *P. kundagungan* and *P. sphagnicolus* (Figure 6). The pattern of differentiation aligns with

limited gene flow among geographically separated populations, likely reflecting historical isolation and restricted dispersal across unsuitable habitats.

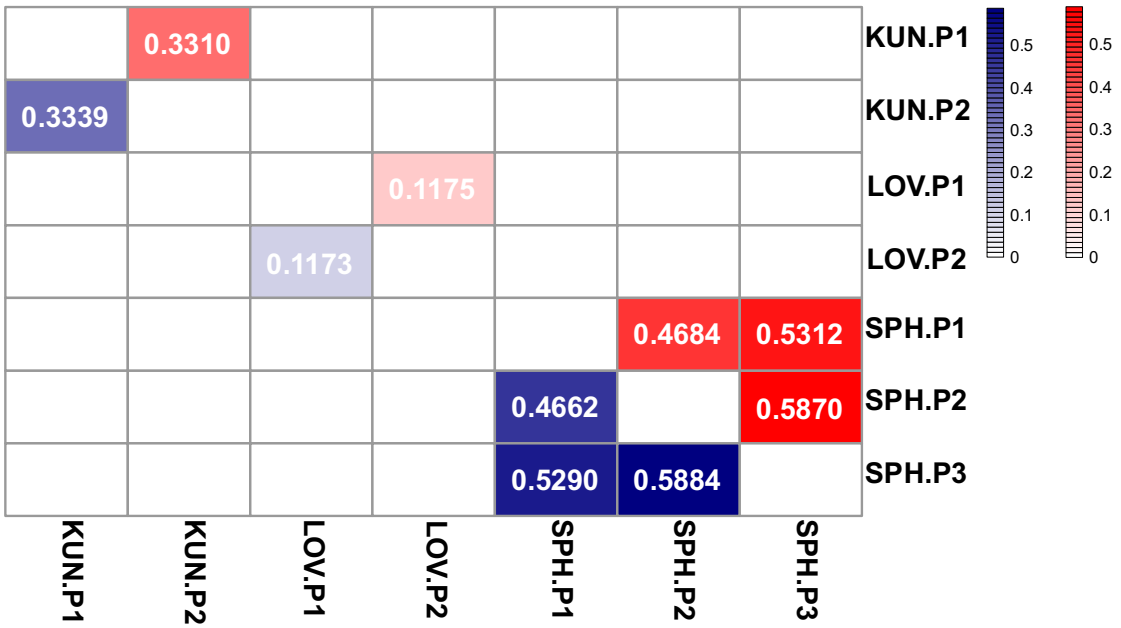

Figure 6. Pairwise F_{ST} and Nei's genetic distance among *P. kundagungan, P. loveridgei* and *P. sphagnicolus* subpopulations. Values are presented as a heatmap, with the range shown in the upper right. The blue colour represents the $F_{ST,}$ and the red colour represents the Nei's distance.

Genetic diversity

Genetic diversity varied among the six *Philoria* species studied here (Table 1). Both *P. pughi* and *P. richmondensis* had a relatively high F_{IS} value (F_{IS} > 0.23). For *P. kundagungan*, the southern genetic cluster (*P. kundagungan* P2) had more private alleles and higher allelic richness than the northern genetic cluster (*P. kundagungan*. P1). The southern cluster (*P. kundagungan* P2) had a high F_{IS} value (F_{IS} = 0.3210), and the northern cluster (*P. kundagungan* P1) had a negative F_{IS} value. The western cluster of *P. loveridgei* (P1) had a higher number of private alleles and allelic richness than the eastern cluster (*P. loveridgei P2*), but there was no significant difference in F_{IS} (p-value = 0.6955). The northern cluster (*P. sphagnicolus* P1) had a higher number of private alleles and allelic richness than the other two clusters. The southern cluster (*P. sphagnicolus* P3) had the lowest F_{IS} value (F_{IS} =

0.1096), while the middle cluster (*P. sphagnicolus* P2) had the highest F_{IS} value (F_{IS} = 0.1972) within the species.

Table 1. Population genetic diversity summary statistics: number of private alleles (N_p), allelic richness (A_R), observed heterozygosity (H_O), expected heterozygosity (H_E), and inbreeding coefficient (F_{IS}). Private alleles can only be reported for species with more than one population. Standard errors are given in parentheses.

Species/cluster	N	N_P	A_R	H_O	H_E	F_{IS}
P. knowlesi	15	-	2.0000 (0.0000)	0.3494 (0.0029)	0.4336 (0.0018)	0.1837 (0.0071)
P. kundagungan						
P. kundagungan P1	11	633	1.7296 (0.0063)	0.4312 (0.0036)	0.3825 (0.0024)	-0.1061 (0.0051)
P. kundagungan P2	12	1186	1.8468 (0.0050)	0.2451 (0.0022)	0.3909 (0.0022)	0.3210 (0.0055)
P. loveridgei						
P. loveridgei P1	16	322	1.9591 (0.0029)	0.3023 (0.0026)	0.3456 (0.0024)	0.1087 (0.0051)
P. loveridgei P2	13	66	1.8821 (0.0057)	0.3010 (0.0030)	0.3471 (0.0027)	0.1117 (0.0059)
P. pughi	11	-	2.0000 (0.0000)	0.3656 (0.0047)	0.4807 (0.0015)	0.2308 (0.0105)
P. richmondensis	11	-	2.0000 (0.0000)	0.3677 (0.0042)	0.4858 (0.0013)	0.2365 (0.0091)
P. sphagnicolus						
P. sphagnicolus P1	10	1474	1.6900 (0.0070)	0.3563 (0.0034)	0.4195 (0.0023)	0.1296 (0.0067)
P. sphagnicolus P2	14	475	1.4681 (0.0073)	0.2494 (0.0036)	0.3203 (0.0030)	0.1972 (0.0076)
P. sphagnicolus P3	13	289	1.2755 (0.0068)	0.3524 (0.0061)	0.3985 (0.0038)	0.1096 (0.0117)

The potential impacts of megafires on genetic diversity

The 2019-20 megafires had different potential impacts on genetic diversity for the four *Philoria* species that had both burnt and unburnt populations (Table 2). In general, the proportion of individuals occupying burnt locations was higher than those from unburnt locations (Figure 3, 4, 5, Table 2). Correspondingly, individuals from burnt locations tended

to have more private alleles and greater allelic richness than those from unburnt locations (Table 2).

Table 2. Genetic diversity among burnt and unburnt regions of *Philoria* species* impacted by the 2019-2020 megafires

Genetic cluster	N	N_P	A_R
P. knowlesi			
Burnt	13	773	1.4237 (0.0023)
Unburnt	2	0	1.2219 (0.0079)
P. kundagungan			
Burnt	14	762	1.9217 (0.0026)
Unburnt	9	100	1.8190 (0.0054)
P. richmondensis			
Burnt	8	340	1.7726 (0.0057)
Unburnt	3	1	1.5236 (0.0133)
P. sphagnicolus			
Burnt	29	1273	1.6917 (0.0051)
Unburnt	8	262	1.6622 (0.0070)

N number of individuals, N_p number of private alleles, A_R allelic richness (standard error)
**Philoria pughi* not included as all sequenced individuals were impacted by the fires, while *P. loveridgei* was not included as no populations were impacted.

Discussion

Mountaintop species often show strong signatures of allopatric speciation, driven by historical geographic isolation as populations become confined to high-altitude habitats (Catchen et al. 2013). Such isolation can result in significant genetic differentiation between populations on separate mountaintops, with limited or no gene flow occurring across unsuitable lowland habitats. This pattern is common in montane species with poor dispersal abilities (Bell et al. 2010; Velo-Antón et al. 2013; Mahony et al. 2021; Mahony & Donnellan 2022). As climate change continues, the inability of these species to maintain gene flow across increasingly fragmented landscapes has crucial conservation implications. Populations that have experienced declines are unlikely to be rescued by dispersal by individuals from neighbouring mountaintops. This lack of gene flow exacerbates the risk of inbreeding and

genetic drift (Furlan et al. 2012) and reduces the potential for recolonisation (Driscoll 1997), making these species particularly vulnerable to extinction. For taxa such as *Philoria* that have diversified through allopatric speciation on mountaintops, population supplementation after declines and managing genetic diversity across isolated populations will be vital to reduce their risk of extinction in the face of environmental change (Sheean et al. 2012; Kissel et al. 2014).

Our results based on genomic data are consistent with the hypothesis that allopatric speciation drove diversification in *Philoria* species as they retracted to rainforests at higher elevations (Hollis 2004; Bolitho et al. 2019). Our phylogenetic analyses found no overlap in the geographic ranges of any species (Fig. 1, 3,4,5) and strongly support the monophyly of the six *Philoria* species we analysed here, with *P. sphagnicolus* being the sister taxa to the five extant northern species. Sister species are always geographically proximate, supporting the hypothesis that habitat contraction over millions of years led to allopatric speciation, likely due to poor dispersal ability in combination with the retraction of wet forests (Morgan et al. 2008; Byrne et al. 2011; Chapple et al. 2011).

Our analyses of within-species population genetic structure showed that populations are geographically separated by lowland valleys that create breaks in rainforest habitat, especially for *P. kundagungan* and *P. sphagnicolus* (Fig 4,5; Byrne et al., 2011; Chapple et al., 2011). Conservation managers focussing on *Philoria kundagungan*, *P. loveridgei*, and *P. sphagnicolus* should treat each genetic cluster (subpopulation) as a management unit (MU) given the apparent lack of gene flow between rainforest blocks. The other three *Philoria* species analysed here should be considered as one MU for conservation actions. Initiatives such as captive breeding (Fraser 2008; Ralls & Ballou 2013; Harley et al. 2018), should be considered for each management unit to maintain or increase genetic diversity, ideally supported by structured decision-making to evaluate the relative costs and benefits of ex-situ and in-situ actions (Rout et al. 2023).

Our results support the hypothesis that topography and habitat play an ongoing role in driving the population structure of *Philoria* species due to poor dispersal through unsuitable lowland open forest. Population genetics studies of other montane species living in the Gondwana

rainforests (especially frogs) have also shown that topography and habitat are crucial drivers of population structure (Mahony & Donnellan, 2022; Mahony et al., 2021). However, the movement ecology of *Philoria* species, and the habitats they use during the non-breeding season, is a critical knowledge gap (Heard et al. 2023). Studies on the Victorian species *P. frosti* have shown that males and females disperse small distances (< 85 metres) from breeding sites (Hollis 2004). Observations by the authors in the field (DN & MM) indicate use of rainforest habitat outside breeding areas and possible movement between headwaters within a rainforest fragment. However, it is likely that the northern species have limited dispersal between rainforest fragments, which requires testing by appropriate field studies. *Philoria* species likely require specific temperatures and high humidity for survival (Anstis 2018; Heard et al. 2023), thus the hotter and drier valleys between mountain-top habitats may prevent their dispersal. Consequently, overcoming dispersal limitations for declining populations will be key to species persistence, and genetic rescue may be a suitable option (Willi et al. 2022).

As most species occur as small, isolated populations, they are at risk of further decline due to habitat degradation, leading to elevated inbreeding, genetic drift, and an increased risk of extinction (Hedrick & Kalinowski 2000; Fahrig 2003; Kramer et al. 2008; Pavlova et al. 2017; Wang et al. 2017). Reduced genetic diversity can lessen the capacity to adapt to environmental change and lead to species extinction (Barrett & Schluter, 2008; Frankham, 2005). We found that all subpopulations of *P. kundagungan*, *P. pughi*, *P. richmondensis*, and the middle subpopulation of *P. sphagnicolus* (*P. sphagnicolus* P2) had comparatively low genetic diversity and/or comparatively high levels of inbreeding relative to other populations. Hence as these populations have an elevated risk of extinction, management actions that increase their genetic diversity and reduce inbreeding depression should be a priority, such as biobanking (Howell et al. 2021) and translocations (Ewen et al. 2012; Sheean et al. 2012). In parallel, actions that can increase population sizes, such as habitat restoration (Beranek et al. 2020) or head-starting (Mendelson III & Altig 2016) would be similarly beneficial.

The identification of populations with the highest genetic diversity and lowest inbreeding (hereafter ‘high-value populations’) is key to maintaining species over the longer term (Booy et al. 2000; Frankham 2005; Hughes et al. 2008). High-value populations should be primary

424 targets for threat management, including protective fire buffer management, disease
425 management, and invasive species control such as feral pig management (Acevedo-
426 Whitehouse 2009; Moskwa et al. 2016; Gerber et al. 2018). For the six *Philoria* species
427 (Table 1), the entire ranges of *P. knowlesi*, *P. pughi*, and *P. richmondensis* should be
428 considered high-value populations. The southern subpopulation of *P. kundagungan* (*P.*
429 *kundagungan* P2) is the highest value within the species, noting that the diversity in the Main
430 Range portion of this population is much higher than the most southern sites such as
431 Tooloom. The western subpopulation of *P. loveridgei* (*P. loveridgei* P1), and the northern
432 subpopulation of *P. sphagnicolus* (*P. sphagnicolus* P1).
433
434 When fires cause population declines, the resulting loss of genetic diversity can have lasting
435 impacts on species' resilience and adaptive potential, and therefore understanding fire impact
436 relative to pre-existing diversity is vital. Heard et al. (2023) assessed the impacts of the
437 megafires on *Philoria* by quantifying the extent of habitat affected and assessing changes in
438 the patterns and abundance of calling males. Heard et al.'s study found that 30% and 12% of
439 the potential habitats of *P. kundagungan* and *P. richmondensis* were impacted by the
440 megafires. For *P. kundagungan*, site occupancy and the number of calling males after
441 megafires decreased by 19% and 40%, respectively, compared to before the megafires. In
442 contrast, the effects of the megafires on *P. richmondensis* were less pronounced. The post-fire
443 site occupancy and the number of calling males reduced by 10% and 14%, respectively,
444 compared to pre-fire, but the effect of the megafires was less apparent due to the low
445 numbers of males detected in the preceding drought (D. Newall, unpublished observation).
446 More recently, Beranek et al. (2023) reported that the 2019/20 megafires had a very
447 significant negative impact on *P. pughi,* which drastically reduced the number of occupied
448 sites and the average probable occupancy levels.
449
450 These findings of consistent declines post fire mean that actions to improve population
451 persistence should be implemented, which is supported by our new understanding of how
452 genetic diversity may have also been impacted. Our comparison of pre-fire genetic diversity
453 in burnt and unburnt habitats (Table 2) shows that for all species except *P. loveridgei*,
454 megafires burned areas that had high genetic diversity before the fires. Fire impacts are likely
455 to have significantly lowered the genetic diversity of species identified as a single genetic

population (*P. knowlesi, P. richmondensis,* and *P. pughi*). Fortuitously for species with more genetic structure (*P. kundagungan* and *P. sphagnicolus*), the "high value" subpopulations were least affected by the fires. However, it is important to note that our understanding the impacts of the 2019-20 megafires on the genetic diversity of *Philoria* is rudimentary, as we lacked post-fire tissue samples. Collection of these within the first 6 year would be beneficial, given current understanding of generation time. In addition, changes in the genetic diversity of populations following fire are not expected to arise immediately (Legge et al. 2020), as changes in diversity take one or more generations to accrue.

Conclusion

The ecological niche of *Philoria* species is narrow and contracting due to climate change (Bolitho & Newell 2022; Mahony et al. 2022). More dire predictions from ecological niche models show that the current ranges of *P. kundagungan* and *P. richmondensis* would shrink by 64% and 50% in the future (2055) under a low-warming scenario and by 91% and 85% under a high-warming scenario (Bolitho & Newell 2022). The occurrence of stochastic catastrophic events such as the megafires, primed by a proceeding extensive drought, overlain on the shallower decline trajectory due to climate change, has major ramifications for this revictual lineage of frogs (Heard and Bolitho et al. 2023). Genetic management is essential in the context of escalating threats, and we encourage managers to use this new information on genetic diversity, genetic heath and contracting niches to implement targeted conservation plans that can help *Philoria* species persist in the wild.

Statements and Declarations

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Author Contributions

RAC, DN, NJM, SCD, and MM conceptualised the study and developed its overarching objectives. SRAC led the project, designed the methodology, and provided supervision throughout. Samples were collected by DN, LB, SCD, and MM. SL conducted the data analysis and drafted the initial version of the manuscript. DN, NJM, SCD, MM, LJB, and RAC provided critical feedback, contributing to the review and refinement of the manuscript. All authors reviewed and approved the final version for submission.

Data Availability

The datasets generated analysed during the current study will be available on the sequence read archive after acceptance.

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Population genetics of rainforest mountain frogs (Anura: Limnodynastidae: *Philoria*) severely impacted by the Australian megafires

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Table S1. Sample information. All samples are from the Australian Biological Tissue Collection (ABTC). Location coordinates have been rounded to hide sensitive information.

Sample ID	Species	Latitude	Longitude	Locality code
ABTC26286	*P. knowlesi*	-28.32	152.87	BRA (NSW)
ABTC26287	*P. knowlesi*	-28.32	152.87	BRA (NSW)
ABTC97186	*P. knowlesi*	-28.26	152.63	MBA (QLD)
ABTC97198	*P. knowlesi*	-28.30	152.68	MBE (QLD)
ABTC97199	*P. knowlesi*	-28.30	152.68	MBE (QLD)
ABTC97200	*P. knowlesi*	-28.30	152.68	MBE (QLD)
ABTC97201	*P. knowlesi*	-28.30	152.68	MBE (QLD)
ABTC97202	*P. knowlesi*	-28.29	152.60	MBC (QLD)
ABTC97203	*P. knowlesi*	-28.29	152.60	MBC (QLD)
ABTC97204	*P. knowlesi*	-28.29	152.60	MBC (QLD)
ABTC127637	*P. knowlesi*	-28.27	152.61	MBB (QLD)
ABTC127638	*P. knowlesi*	-28.31	152.62	MBD (QLD)
ABTC127639	*P. knowlesi*	-28.31	152.62	MBD (QLD)
ABTC127640	*P. knowlesi*	-28.31	152.62	MBD (QLD)
ABTC127641	*P. knowlesi*	-28.31	152.62	MBD (QLD)
ABTC14079	*P. kundagungan*	-28.32	152.51	MCL (NSW)
ABTC16851	*P. kundagungan*	-28.35	152.40	KOB (NSW)
ABTC16852	*P. kundagungan*	-28.35	152.40	KOB (NSW)
ABTC16982	*P. kundagungan*	-28.35	152.40	KOB (NSW)
ABTC25173	*P. kundagungan*	-28.22	152.43	MSA (QLD)
ABTC25292	*P. kundagungan*	-28.22	152.46	MSB (QLD)
ABTC25293	*P. kundagungan*	-28.22	152.46	MSB (QLD)
ABTC25339	*P. kundagungan*	-28.32	152.44	KOA (NSW)
ABTC25342	*P. kundagungan*	-28.50	152.40	TOB (NSW)
ABTC25371	*P. kundagungan*	-28.05	152.39	MRN (QLD)

ABTC25372	*P. kundagungan*	-28.05	152.39	MRN (QLD)
ABTC25373	*P. kundagungan*	-28.05	152.39	MRN (QLD)
ABTC25374	*P. kundagungan*	-28.05	152.39	MRN (QLD)
ABTC25375	*P. kundagungan*	-28.05	152.39	MRN (QLD)
ABTC25377	*P. kundagungan*	-28.05	152.39	MRN (QLD)
ABTC25378	*P. kundagungan*	-28.05	152.39	MRN (QLD)
ABTC25379	*P. kundagungan*	-28.05	152.39	MRN (QLD)
ABTC25380	*P. kundagungan*	-28.05	152.39	MRN (QLD)
ABTC25383	*P. kundagungan*	-28.05	152.39	MRN (QLD)
ABTC25385	*P. kundagungan*	-28.05	152.39	MRN (QLD)
ABTC92273	*P. kundagungan*	-28.46	152.44	TOA (NSW)
ABTC92274	*P. kundagungan*	-28.46	152.44	TOA (NSW)
ABTC92276	*P. kundagungan*	-28.46	152.44	TOA (NSW)
ABTC24922	*P. loveridgei*	-28.40	153.03	MWN (NSW)
ABTC24923	*P. loveridgei*	-28.40	153.03	MWN (NSW)
ABTC24926	*P. loveridgei*	-28.40	153.03	MWN (NSW)
ABTC24927	*P. loveridgei*	-28.40	153.03	BRB (NSW)
ABTC25313	*P. loveridgei*	-28.26	153.17	BRB (NSW)
ABTC82617	*P. loveridgei*	-28.54	153.29	BRB (NSW)
ABTC90393	*P. loveridgei*	-28.39	153.06	BRB (NSW)
ABTC90394	*P. loveridgei*	-28.57	153.37	BRB (NSW)
ABTC90396	*P. loveridgei*	-28.57	153.37	BRB (NSW)
ABTC90512	*P. loveridgei*	-28.40	153.03	BRB (NSW)
ABTC90513	*P. loveridgei*	-28.40	153.03	BRB (NSW)
ABTC90514	*P. loveridgei*	-28.40	153.03	BRB (NSW)
ABTC90515	*P. loveridgei*	-28.40	153.03	BRB (NSW)
ABTC90516	*P. loveridgei*	-28.40	153.03	NC (NSW)
ABTC90517	*P. loveridgei*	-28.40	153.03	NCB (NSW)
ABTC92281	*P. loveridgei*	-28.39	153.06	NCB (NSW)
ABTC110135	*P. loveridgei*	-28.23	153.28	SBA (QLD)
ABTC140563	*P. loveridgei*	-28.38	153.13	LMT (QLD)
ABTC140564	*P. loveridgei*	-28.38	153.13	BRD (NSW)
ABTC140565	*P. loveridgei*	-28.38	153.13	BRD (NSW)
ABTC140567	*P. loveridgei*	-28.38	153.13	BRD (NSW)

ABTC144835	*P. loveridgei*	-28.40	153.28	BRD (NSW)
ABTC144836	*P. loveridgei*	-28.40	153.28	BRC (NSW)
ABTC144837	*P. loveridgei*	-28.40	153.28	BRC (NSW)
ABTC144838	*P. loveridgei*	-28.40	153.28	BRC (NSW)
ABTC144839	*P. loveridgei*	-28.40	153.28	BRC (NSW)
ABTC144840	*P. loveridgei*	-28.40	153.28	MWN (NSW)
ABTC150900	*P. loveridgei*	-28.38	153.07	MWN (NSW)
ABTC150901	*P. loveridgei*	-28.38	153.07	MWN (NSW)
ABTC12227	*P. pughi*	-29.22	152.42	BL (NSW)
ABTC12229	*P. pughi*	-29.22	152.42	BL (NSW)
ABTC16983	*P. pughi*	-29.33	152.17	LS (NSW)
ABTC25206	*P. pughi*	-29.35	152.10	LS (NSW)
ABTC25209	*P. pughi*	-29.35	152.10	DC (NSW)
ABTC25210	*P. pughi*	-29.35	152.10	DC (NSW)
ABTC25211	*P. pughi*	-29.35	152.10	DC (NSW)
ABTC25220	*P. pughi*	-29.33	152.17	DC (NSW)
ABTC25362	*P. pughi*	-29.35	152.10	DC (NSW)
ABTC25367	*P. pughi*	-29.47	152.32	WP (NSW)
ABTC25369	*P. pughi*	-29.47	152.32	WP (NSW)
ABTC25223	*P. richmondensis*	-28.50	152.80	TON (NSW)
ABTC25224	*P. richmondensis*	-28.70	152.72	RRA (NSW)
ABTC25226	*P. richmondensis*	-28.50	152.70	RRB (NSW)
ABTC25311	*P. richmondensis*	-28.70	152.50	YBA (NSW)
ABTC25350	*P. richmondensis*	-28.70	152.50	YBA (NSW)
ABTC25351	*P. richmondensis*	-28.70	152.50	YBA (NSW)
ABTC25352	*P. richmondensis*	-28.70	152.50	YBA (NSW)
ABTC25353	*P. richmondensis*	-28.70	152.50	YBA (NSW)
ABTC25354	*P. richmondensis*	-28.70	152.50	YBA (NSW)
ABTC25355	*P. richmondensis*	-28.70	152.50	YBA (NSW)
ABTC25356	*P. richmondensis*	-28.70	152.50	YBA (NSW)
ABTC12745	*P. sphagnicolus*	-31.18	152.33	KF (NSW)
ABTC12746	*P. sphagnicolus*	-31.18	152.33	KF (NSW)
ABTC12757	*P. sphagnicolus*	-31.18	152.33	KF (NSW)

ABTC12758	*P. sphagnicolus*	-31.18	152.33	KF (NSW)
ABTC12759	*P. sphagnicolus*	-31.18	152.33	KF (NSW)
ABTC12760	*P. sphagnicolus*	-31.18	152.33	KF (NSW)
ABTC12761	*P. sphagnicolus*	-31.19	152.37	MB (NSW)
ABTC12762	*P. sphagnicolus*	-31.19	152.37	MB (NSW)
ABTC12763	*P. sphagnicolus*	-31.19	152.37	MB (NSW)
ABTC12764	*P. sphagnicolus*	-31.19	152.37	MB (NSW)
ABTC12766	*P. sphagnicolus*	-31.23	152.17	CT (NSW)
ABTC12767	*P. sphagnicolus*	-31.23	152.17	CT (NSW)
ABTC12768	*P. sphagnicolus*	-31.23	152.17	CT (NSW)
ABTC12769	*P. sphagnicolus*	-31.23	152.17	CT (NSW)
ABTC24918	*P. sphagnicolus*	-30.49	152.41	NE (NSW)
ABTC25185	*P. sphagnicolus*	-30.38	152.73	DR (NSW)
ABTC25189	*P. sphagnicolus*	-30.49	152.41	NE (NSW)
ABTC25282	*P. sphagnicolus*	-31.65	152.42	KB (NSW)
ABTC25283	*P. sphagnicolus*	-31.65	152.42	KB (NSW)
ABTC25284	*P. sphagnicolus*	-31.65	152.42	KB (NSW)
ABTC25285	*P. sphagnicolus*	-31.65	152.42	KB (NSW)
ABTC25295	*P. sphagnicolus*	-30.18	152.42	MH (NSW)
ABTC25296	*P. sphagnicolus*	-30.18	152.42	MH (NSW)
ABTC25346	*P. sphagnicolus*	-31.67	152.14	DT (NSW)
ABTC25347	*P. sphagnicolus*	-31.67	152.14	DT (NSW)
ABTC25348	*P. sphagnicolus*	-31.67	152.14	DT (NSW)
ABTC25386	*P. sphagnicolus*	-31.61	152.41	BG (NSW)
ABTC25387	*P. sphagnicolus*	-31.61	152.41	BG (NSW)

ABTC25388	*P. sphagnicolus*	-31.61	152.41	BG (NSW)
ABTC25389	*P. sphagnicolus*	-31.61	152.41	BG (NSW)
ABTC25390	*P. sphagnicolus*	-31.61	152.41	BG (NSW)
ABTC25595	*P. sphagnicolus*	-30.38	152.73	DR (NSW)
ABTC25596	*P. sphagnicolus*	-30.38	152.73	DR (NSW)
ABTC25603	*P. sphagnicolus*	-30.37	152.73	DR (NSW)
ABTC25604	*P. sphagnicolus*	-30.37	152.73	DR (NSW)
ABTC25605	*P. sphagnicolus*	-30.38	152.73	DR (NSW)
ABTC25832	*P. sphagnicolus*	-31.62	152.17	EB (NSW)

Table S2.

Parameter	Setting
Genotyping Quality	25
Minimum and maximum average locus read depth	Min=15, Max=85
Minimum average SNP read count	4
Maximum frequency of heterozygosity	0.6
Max difference in read depth between REF and SNP alleles	0.6
Individuals call rate	0.6
Proportion of populations not in HWE	0.1
Reproducibility	98
MAF	0.005
Individuals call rate	0.9
Loci call rate	0.9
MAF	3 / individuals' number

Table S3. Abbreviations and full names for subpopulations of each species.

Abbreviation	**Full name**
BG	Boorganna Nature Reserve
BL	Bililimbra State Forest
BRA	Border Ranges National Park Group A

BRB	Border Ranges National Park Group B
BRC	Border Ranges National Park Group C
BRD	Border Ranges National Park Group D
CT	Cobcroft Trail, Werrikimbe National Park
DC	Dingo Creek Flora Reserve
DR	Dorrigo National Park
DT	Dingo Tops State Forest
EB	Bulga SF near Blue Knob Forest Road (Ellenborough River at Pole Bridge Rd crossing)
KB	Killabakh Nature Reserve
KF	King Fern Falls, Werrikimbe National Park
KOA	Koreelah National Park Group A
KOB	Koreelah National Park Group B
LMT	Lamington National Park
LS	Forest Land State Forest
MB	Mt Boss State Forest
MBA	Mount Barney National Park Group A
MBB	Mount Barney National Park Group B
MBC	Mount Barney National Park Group C
MBD	Mount Barney National Park Group D
MBE	Mount Barney National Park Group E
MCL	Mount Clunie National Park
MH	Mount Hyland Nature Reserve
MRN	Main Range National Park North Group
MSA	Mount Superbus Group A
MSB	Mount Superbus Group B
MWN	Mount Warning National Park
NCA	Nightcap National Park Group A
NCB	Nightcap National Park Group A
NE	New England National Park
RRA	Richmond Range National Park Group A
RRB	Richmond Range National Park Group B
SBA	Springbrook National Park Group A

TOA	Tooloom National Park Group A
TOB	Tooloom National Park Group B
TON	Toonumbar National Park
WP	Washpool National Park
YBA	Yabbra National Park Group A