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Phylogenomic incongruence leads to different spatial phylogenetic patterns: an Australian continental scale case study with *Isopogon* and *Petrophile* (Proteaceae)

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ABSTRACT

Spatial phylogenetics complement existing biodiversity metrics of species richness and endemism by incorporating evolutionary information from phylogenies with spatial distribution data. These complementary metrics of phylogenetic diversity (PD), phylogenetic endemism (PE), and categorical analysis of neo- and paleo-endemism (CANAPE) have largely been obtained from phylogenies constructed with a few genetic markers from different genomic datasets. Incongruent topologies may affect subsequent spatial phylogenetic results, leading to uncertainty for conservation outcomes. Here, we examined the effects of topological conflicts between plastid and nuclear phylogenies of two Australian plant genera – *Isopogon* R.Br. ex Knight and *Petrophile* R.Br. ex Knight (Proteaceae). We constructed the first densely sampled and generally well-resolved nuclear and plastid phylogenies of the two genera using a hybrid capture high throughput sequencing approach. We show that incongruent phylogenies from different molecular datasets have an important effect on downstream spatial phylogenetic analyses. Significant areas of PD and PE and neo- and paleo-endemism for *Isopogon* and *Petrophile* include south-west Western Australia, Kangaroo Island and the Sydney Basin. These areas are located along the mesic fringes of the Australian continent, congruent with the peripheral vicariance hypothesis in biogeography. However, these significant areas differ depending on whether the analyses were made with a nuclear or plastid topology. We caution against combining different datasets as is commonly done without detailed assessment of potential incongruence between datasets.

Keywords: Australian flora, biodiversity hotspot, Isopogon, Petrophile, phylogenetic diversity, phylogenomics, Proteaceae, reticulate evolution, spatial phylogenetics, SWAFR.

Introduction

Spatial phylogenetics has been increasingly used to provide additional diversity metrics that incorporate the evolutionary history of selected biotic groups (Mishler 2023). These metrics include phylogenetic diversity (Faith 1992), phylogenetic endemism (Rosauer *et al.* 2009) and associated methods to distinguish different patterns across landscapes (relative phylogenetic diversity, RPD; relative phylogenetic endemism, RPE; and categorical analysis of neo- and paleo-endemism, CANAPE; Mishler *et al.* 2014). These spatial metrics have been applied across different scales from global (Safi *et al.* 2011; Fritz and Rahbek 2012; Rosauer and Jetz 2015; Qian *et al.* 2023; Tietje *et al.* 2023), regional and continental (e.g. Forest *et al.* 2007; Thornhill *et al.* 2016, 2017; Hu *et al.* 2020), to local scales (e.g. Arroyo-Rodríguez *et al.* 2012; Costion *et al.* 2015; Cardoso *et al.* 2021). Incorporating an evolutionary dimension into biodiversity studies has allowed researchers to determine the drivers for phylogenetic and species richness disparity across space, e.g. climate-driven regional extinctions in the Cenozoic across temperate Northern Hemispheric regions resulted in phylogenetic clustering of tree lineages (Eiserhardt *et al.* 2015; Saladin *et al.* 2020).

The argument for spatial phylogenetic studies has often been linked to optimising conservation outcomes (e.g. Faith 1992; Winter *et al.* 2013; Pollock *et al.* 2015;

Millar *et al.* 2017; Aguilar-Tomasini *et al.* 2021; Yang *et al.* 2022, to name a few). In many cases PD and PE hotspots match those of known species-richness hotspots and overlap with protected areas; however, there are also others that do not show this trend (Forest *et al.* 2007; Daru *et al.* 2015; Quan *et al.* 2018). Prioritising PD and PE metrics over species-richness and endemism areas for conservation has been increasingly promoted to conserve more evolutionary potential across different lineages (Rodrigues and Gaston 2002; Rosauer *et al.* 2009; Winter *et al.* 2013; Palmer and Fischer 2022).

Importantly, underpinning these spatial phylogenetic results are the input data of phylogenetic trees that contain evolutionary information. Phylogenetic uncertainty of poorly supported or unresolved nodes can be accounted for in spatial phylogenetic analyses (Mishler 2023). In addition, these uncertainties are found to have minimal effects on the results (González-Orozco *et al.* 2016; Thornhill *et al.* 2017; Allen *et al.* 2019). Most spatial phylogenetic studies for plants only used a few (1–10) genetic markers and usually combined genetic data from different genomes (nuclear, plastid and mitochondrial; Table 1). Most of these studies prioritised taxonomic rather than molecular sampling, i.e. trying to include as many taxa as possible over number of genes per taxon (Table 1). Combining different molecular datasets may lead to erroneous results if these datasets are in conflict. Indeed, incongruence across different phylogenetic datasets due to different analytical approaches (e.g. concatenated *v.* coalescent) or genomic datasets (nuclear *v.* organellar) has often been detected across the Tree of Life (e.g. Chong *et al.* 2016; French *et al.* 2016; García *et al.* 2017; Nge *et al.* 2021*a*; McLay *et al.* 2023; Stull *et al.* 2023). In the current phylogenomic era, we now have access to hundreds or even thousands of genetic loci providing unprecedented phylogenetic resolution but also uncovering gene-tree to species tree conflicts (Smith *et al.* 2015, 2020; Nge *et al.* 2024). The effects of phylogenetic conflicts have been commonly investigated for species delimitation, often with differing results across datasets affecting conservation outcomes (Jacobs *et al.* 2018; Weber *et al.* 2019; Firneno *et al.* 2021). By contrast, the effects of topological conflicts have not been fully explored for spatial phylogenetics.

Incongruent nuclear and plastic topologies did not substantially affect PD and PE results in one study (Toro-Núñez and Lira-Noriega 2020). In another, discordance between nuclear and plastid datasets did not affect the PD results across genera in Chloranthaceae but presented significantly different results within *Hedyosmum* Sw. at the species level (Zhang Q. *et al.* 2015). Elliott *et al.* (2018) considered differences in spatial phylogenetic results between phylograms and chronograms across four different plant groups and found substantial differences between the two types of phylogenies. This finding is congruent with another study showing that even with identical topologies, whether a phylogeny is time calibrated (chronogram) or not (phylogram) may result in significantly different spatial results (Allen *et al.* 2019). These studies show that, in some taxa at least, input phylogenetic data can affect spatial phylogenetic results depending on the specific data and phylogenetic methodology used in the study.

Several studies on Australian plants have been conducted on spatial phylogenetic patterns across the continent (Table 1). These studies generally highlight scattered pockets of high PD, PE and significant CANAPE levels across mesic fringes of the continent. Most of these studies utilised few selected plastid markers, and only several of these studies included additional nuclear markers. In all cases, studies that used both nuclear and plastid data combined these together and did not assess for potential topological conflicts and associated effects on their results.

Here, we focus on two Australian plant genera – *Isopogon* R.Br. ex Knight and *Petrophile* R.Br. ex Knight (Proteaceae). *Isopogon* and *Petrophile* are species-rich, charismatic Australian plant genera that are found across southern temperate Australia (Fig. 1). Both *Isopogon* and *Petrophile* have most of their species diversity in the South-west Australian Floristic Region (SWAFR), with 79% (30/38 spp.) and 88% (58/66 spp.) of species confined to the SWAFR respectively (Prentice *et al.* 2017). The Australian genera *Isopogon*, *Petrophile*, *Franklandia* R.Br. and *Adenanthos* Labill. comprise a clade along with several South African genera in the subfamily Proteoideae, with the clade being sister to *Protea* L. plus *Faurea* Harv. and *Beauprea* Brongn. & Gris (Sauquet *et al.* 2009). *Isopogon* is sister to *Adenanthos* and the African subtribe Leucadendrinae, *Franklandia* is sister to the tribe Leucadendreae (i.e. *Isopogon* + *Adenanthos* + subtribe Leucadendrinae), and *Petrophile* is sister to the African genus *Aulax* P.J.Bergius, based on the one nuclear (*ITS*) and seven plastid marker phylogeny from Sauquet *et al.* (2009). However, the topology of Sauquet *et al.* (2009) differs from that of a recent angiosperm-wide phylogenomic study based on 353 low copy nuclear markers (Zuntini *et al.* 2024). Species-level phylogenies are lacking for the Australian genera in subfamily Proteoideae, with the exception of *Adenanthos* (Nge *et al.* 2021*a*).

In this study, we present a phylogenomic nuclear and plastid dataset for *Isopogon* and *Petrophile*. We used this as a basis to (1) assess for phylogenomic incongruence between nuclear and plastid datasets for the two genera, (2) conduct spatial analyses of PD, PE and CANAPE to infer areas of high PD and PE, concentrated areas of neo- and palaeo-endemism, and (3) assess for potential effects of phylogenomic incongruence on these spatial patterns in an Australian context.

Materials and methods

Sampling and sequencing

We sampled 89 taxa from vouchered herbarium specimens across both *Petrophile* (72%; 48/66 spp.) and *Isopogon*

Table 1. Examples of spatial phylogenetic studies, associated study taxa, region and number of genetic loci used.

Study	Study group	Region	Nuclear genes	Plastid genes	Mitochondrial genes	Combined datasets
Schmidt-Lebuhn *et al.* (2015)	Asteraceae	Australia	1 (*ITS*)	3	0	Yes
Thornhill *et al.* (2016)	Angiosperm genus level	Australia	1 (*ITS*)	6	0	Yes
Hammer *et al.* (2021)	*Ptilotus* (Amaranthaceae)	Australia	1 (*ITS*)	1 (*matK*)	0	Yes
Miller *et al.* (2023)	*Banksia* (Proteaceae)	Australia	0	4	0	NA
Nagalingum *et al.* (2015)	ferns genus level	Australia	0	3	0	NA
Lee and Mishler (2024)	Conifers	Australia	0	2	0	NA
Mishler *et al.* (2014)	*Acacia* (Fabaceae)	Australia	2	4	0	Yes
González-Orozco *et al.* (2016)	eucalypts (Myrtaceae)	Australia	2	2	0	Yes
Forest *et al.* (2007)	Angiosperm genus-level	Cape South Africa	0	1 (*rbcL*)	0	NA
Scherson *et al.* (2017)	Vascular plants genus level	Chile	2	5	0	Yes
Thornhill *et al.* (2017)	Vascular plants	California, USA	4	5	0	Yes
Hu *et al.* (2020)	Vascular plants	China	0	4	1 (*matR*)	Yes
Qian *et al.* (2019)	Seed plants	China	Unspecified number	Unspecified number	0	Yes
Millar *et al.* (2017)	Vascular plants genus level	New Zealand	0	1 (*rbcL*)	0	NA
Qian *et al.* (2023)	Angiosperm	Africa and South America	Unspecified number	Unspecified number		
Tietje *et al.* (2023)	Vascular plants	Global	Unspecified number	Unspecified number	0	Yes
Faurby *et al.* (2016)	Palms (Arecaceae)	Global	4	9	0	Yes

Fig. 1. (*Caption on next page*)

Fig. 1. Representative floral and vegetative diversity of *Petrophile* and *Isopogon* (top left and across, down). (*a*) *Petrophile squamata*, (*b*) *Petrophile teretifolia*. (*c*) *Petrophile prostrata*. (d) *Petrophile diversifolia*. (*e*) *Petrophile fastigiata*. (*f*) *Petrophile recurva*. (*g*) *Isopogon dubius*. (*h*) *Isopogon asper*. (*i*) *Isopogon pruinosus* subsp. *glabellus*. (*j*) *Isopogon teretifolius* subsp. *teretifolius*. (*k*) *Isopogon formosus*. (*l*) *Isopogon sphaerocephalus* subsp. *lesueurensis*. (*m*) *Isopogon divergens*. (*n*) *Isopogon ceratophyllus*. (*o*) *Isopogon anemonifolius*. (*p*) *Isopogon baxteri*. Photographs: *a*–*e*, *k*–*p* by F. J. Nge, *f*–*j* by K. R. Thiele.

(94%; 36/38 spp.), including a putative hybrid of *Petrophile* – *P. ericifolia* × *seminuda* (Rye *et al.* 2011) (see Supplementary Table S1 for voucher details). All eastern Australian species of *Petrophile* (6 spp.) and *Isopogon* (8 spp.) were sampled, with multiple accessions for *P. shirleyae* F.M.Bailey, *P. canescens* A.Cunn. ex R.Br. and *I. anemonifolius* (Salisb.) Knight. Seven outgroup samples were included (*Aulax*, *Protea*, *Leucadendron* R.Br., *Franklandia* [2] and *Conospermum* Sm. [2]), selected based on the topology of Sauquet *et al.* (2009).

We adopted a high-throughput sequencing (HTS) approach using the OzBaits custom bait set (OzBaits; Biffin *et al.* 2025) that targets ~100 single-copy nuclear loci for angiosperms, following the success of the bait set in Proteaceae (Nge *et al.* 2021*a*) and other plant groups (Foster *et al.* 2021; Nge *et al.* 2021*b*, 2022, 2023; Simpson *et al.* 2022). We also used the OzBaits plastid baits (Biffin *et al.* 2025) that target ~60 chloroplast coding regions and three tRNAs (trnG-UCC, trnL-UAA and trnM-UAC) across seed plants. A quantity of ~20 mg of silica-dried leaf material sourced from herbarium or field-collected specimens was used for DNA extractions performed by Intertek Group plc (Adelaide, Australia) using sbeadex magnetic bead chemistry. Library preparation steps followed Biffin *et al.* (2025). Briefly, extracted DNA was sheared using a Diagenode Bioruptor Pico sonicator to fragment lengths of ~400–600 bp, DNA libraries were constructed using JetSeq Flex DNA Library preparation kit (Bioline, Cincinnati, OH, USA), hybrid capture was performed following manufacturer's instructions (Arbor Biosciences, Ann Arbor, MI, USA), and libraries were pooled in equimolar concentrations and sent for Illumina paired-end sequencing (2 × 150) on a lane of a HiSeqX Ten at the Garvan Institute for Medical Research in Sydney.

Bioinformatic pipelines

The raw reads were imported and processed (demultiplexed, barcodes and adaptors removed and quality trimmed using a phred score of 20) in CLC Genomics Workbench (ver. 20.0.2, see https://digitalinsights.qiagen.com/). To recover the targeted nuclear genes, trimmed reads were processed using the *SECAPR* pipeline (ver. 2.2.5, see http://www.github.com/AntonelliLab/seqcap_processor; Andermann *et al.* 2018). *SECAPR* uses *de novo* assembly and a *BLAST*-like algorithm to recover sequences from each individual that match the targeted genes at a specified level of similarity, and multiple sample sequence alignments are generated for each of the targeted genes. We used *SPAdes* (ver. 3.15, see https://github.com/ablab/spades; Bankevich *et al.* 2012) with default k-mer values to generate the assemblies. We used *LASTZ* (ver. 1.04.52, see https://github.com/lastz/lastz; Harris 2007) with a similarity value of 0.9 to extract the 'on target' nuclear loci with the '--keep_paralogs' flag disabled (i.e. samples with two or more hits to a given target are removed for that locus), using a reference comprising the corresponding OzBaits gene targets sourced from the 1KP (One Thousand Plant Transcriptomes Initiative 2019) sample for *Hakea drupacea* (C.F.Gaertn.) Roem. & Schult. (sample code: SIIK). The extracted loci were aligned using *MUSCLE* (ver. 3.8.425, see http://www.drive5.com/muscle/) and imported into *Geneious* (ver. 2022.0.1, see https://www.geneious.com; Kearse *et al.* 2012) for manual checking and curation.

We used the Yang and Smith (2014) pipeline as implemented by Jackson *et al.* (2023) to resolve putatively paralogous sequences for the target loci into ortholog groups. Briefly, the *de novo* assemblies generated by *SECAPR* were imported into *Geneious* and converted into a *BLAST* database. We used the *Hakea* target file (above) to query this database using *blastn* (see https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=tblastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome) with an expect (e) value of *1e-100* and keeping 500 sequences per query. For each query, the *BLAST* hits were filtered to remove short and poorly aligned hits (Yang and Smith 2014) using a query coverage cut-off of 30%. The locus specific 'paralog' files were compiled and input into the *paragone-nf* pipeline (ver. 1.0.0, https://github.com/chrisjackson-pellicle/paragone-nf) for orthology inference using the RT (rooted ingroups) algorithm of Yang and Smith (2014) and generating locus alignments using *MUSCLE*. We retained all alignments with >5 samples (248 ortholog groups in total) for downstream analyses.

To generate the plastid dataset, we used the chloroplast genome sequence of *Macadamia integrifolia* Maiden & Betche (GenBank accession: KF862711) as an initial mapping reference (Nock *et al.* 2014). The sequence reads from *Conopsermum glumaceum* Lindl. (FN631) were mapped to the *Macadamia* reference using CLC Genomics Workbench with a length and similarity fraction of 0.7 and otherwise default parameters. A set of consensus sequences was extracted using a low coverage threshold of 50× and splitting into separate sequences around low coverage regions. We retained a total of 43 consensus sequences with length greater than 300 bp and ranging from 341 to 5558 bp in length that were used as a mapping reference for all other samples. For this step, the length and similarity fractions were set to 0.85 with otherwise default parameters and following read mapping, consensus sequences were extracted for each sample using a low coverage threshold of 8, inserting Ns in low coverage regions and conflicts were resolved using a noise threshold of 0.3. The resulting contigs were imported into *Geneious* and aligned using the *MUSCLE* plugin (Edgar 2004) with default parameters, and the individual alignments were subsequently concatenated to generate the final dataset.

Phylogenetic reconstruction

We generated concatenated (hereafter, CON) and multi-species coalescent (hereafter, MSC) phylogenies for both nuclear (-NU) and plastid (-P) datasets. The concatenated datasets were analysed using *IQ-TREE* (ver. 2.1.3, see https://github.com/iqtree/iqtree2; Minh *et al.* 2020*b*), and alignments partitioned across all 82 nuclear genes used in this study. The optimum partition model was selected for each locus in *IQ-TREE* using MFP + MERGE, and clade support assessed by 1000 replicates of ultra-fast bootstrap (-B, *UFBoot2*, see http://www.iqtree.org/; Hoang *et al.* 2018), gene (-gCF) and site (-sCF) concordance factors (Minh *et al.* 2020*a*). For coalescent phylogenetic analyses, we implemented weighted ASTRAL (*wASTRAL*, ver. 1, see https://github.com/chaoszhang/Weighted-ASTRAL.data; Zhang and Mirarab 2022) that has been shown to improve species tree support by reducing noise from both low support and long terminal branches. The hybrid function command in *wASTRAL* was utilised as this considers both node support and branch lengths. The input gene trees for *wASTRAL* were constructed individually with *RAxML* (ver. 8.2.9, see https://github.com/stamatak/standard-RAxML; Stamatakis 2014) using the GTRGAMMA substitution model (Abadi *et al.* 2019). Nodes with very low bootstrap support (<10) were collapsed using *Newick Utilities* (ver. 1.6, see https://github.com/tjunier/newick_utils; Junier and Zdobnov 2010) to further improve the accuracy of MSC inference in *wASTRAL* (Zhang C. *et al.* 2017). Conflicts between different topologies were visualised as a tanglegram using 'cophylo' function in the *R* package *phytools* (ver. 1.2.0, see https://CRAN.R-project.org/package=phytools/; Revell 2012) implemented in *R* (ver. 4.0, R Foundation for Statistical Computing, Vienna, Austria, see https://www.r-project.org/). The phylogenies were rooted using *Franklandia* as this is the earliest diverging sampled outgroup in subfamily Proteoideae (that includes *Isopogon* and *Petrophile*). The phylogenetic plcaement of *Franklandia* is based on the Plant and Fungal Trees of Life (PAFTOL, see https://treeoflife.kew.org) and an unpublished Australian Angiosperm Tree of Life (AAToL, that includes *Franklandia*, *Conospermum*, *Isopogon* and *Petrophile*), with both datasets utilising the same nuclear markers from the Angiosperms353 bait kit (Johnson *et al.* 2019).

Spatial phylogenetics

To investigate spatial phylogenetic patterns for the two genera, distribution records and phylogenies were required. The *IQ-TREE* topologies were used for both nuclear and plastid datasets. The *wASTRAL* trees were not included as the branch lengths in these trees were not biologically meaningful (Zhang C. *et al.* 2018). Outgroups were pruned from the phylogenies prior to analysis, and *Isopogon* and *Petrophile* analysed separately. For species with multiple accessions sequenced, the best sequenced representative (greatest DNA coverage) was selected for each taxon. We also conducted sensitivity analyses by pruning out samples that were only successfully sequenced in one dataset but not the other, resulting in identical but reduced sampling of taxa across both datasets. These pruned topologies were subsequently used to conduct spatial analyses and compared with our main results (see Supplementary Materials for more detail).

Occurrence records based on vouchered herbarium specimens for the two genera were downloaded from the Australasian Virtual Herbarium (see http://avh.chah.org.au, accessed September 2022) that comprises specimens housed in Australasian herbaria. These records were manually cleaned in *OpenRefine* (ver. 3.4, see https://openrefine.org). Records of cultivated or introduced plants and erroneous records (e.g. locations located in oceans or outside the known distributional range) were trimmed. Search terms as 'botanical gardens', 'cultivated' and 'planted' were used to search for cultivated specimens. Records with more than 10 km of uncertainty associated with their geo-coordinates were excluded from our final dataset to improve precision of downstream analyses. Occurrence records of infraspecific taxa (e.g. subspecies or varieties) were merged up to the species level.

The co-ordinates of each spatial dataset were converted to Albers Equal (EPSG: 3577) 15- × 15-km grid cells (see Thornhill 2017 for justification of cell size). Diversity and endemism metrics were calculated using *Biodiverse* (ver. 3.0, see https://shawnlaffan.github.io/biodiverse/; Laffan *et al.* 2010) with the randomisations and figures completed using an *R* pipeline.

Five diversity metrics were calculated, two of which were empirical (PD and PE) and three randomised (PDR, RPD and CANAPE). The method and meaning of these metrics has previously been described in Mishler *et al.* (2014) and Thornhill *et al.* (2016). As raw PD values are heavily influenced by species richness, PDR accounts for this by identifying areas that contain diversity or endemism greater or less than that expected by chance (by comparing the observed richness against 999 randomised results, null expectation).

Results

Sequencing stats

Our plastid dataset included 79 samples with an aligned length of 60,977 base pairs (bp) across 30 genes for the full dataset. We used the full plastid dataset for all our downstream analyses as this contains more information than only the coding sequences (Supplementary Table S2). Our nuclear dataset (OzBaits) included 79 samples with an alignment length of 146,652 bp across 82 genes.

Phylogenetic relationships within *Isopogon* and *Petrophile*

Both *Isopogon* and *Petrophile* were recovered as monophyletic with high support in both nuclear (CON-NU) and plastid (CON-P) *IQ-TREE* topologies (Fig. 2). *Aulax* is sister to *Petrophile*, and *Leucadendron* sister to *Isopogon* of the genera included in our sampling. Infrageneric relationships of *Petrophile* were recovered with high support across both CON-NU and CON-P topologies, except for the sister relationship between *P. shirleyae* and *P. canescens* that had a bootstrap (BS) support value of 58 in CON-NU. Infrageneric relationships of the south-western Australian (SWA) clade within *Isopogon* were moderately well supported in general, with some weakly supported nodes noted across both datasets (Fig. 2). The *Isopogon spathulatus* R.Br. clade within the larger SWA clade was retrieved with high support across both datasets.

The south-eastern Australian (SEA) clades of both genera were recovered as monophyletic with high support and no topological conflict across all datasets. For *Isopogon*, the SEA clade was retrieved as sister to all SWA taxa that also formed a clade. In *Petrophile*, the SEA clade was strongly nested within SWA, being sister to *P. phylicoides* R.Br. and *P. ericifolia* R.Br., with high support (Fig. 2). All three species with multiple accessions (*P. shirleyae*, *P. canescens*, *I. anemonifolius*) were recovered as monophyletic with high support across all analyses.

Extensive topological conflicts between CON-NU and CON-P were noted for *Isopogon*, but not *Petrophile* (Fig. 2). Only two instances of incongruence were detected for *Petrophile* – *P. striata* and *P. scarbiuscula* Meisn. with *P. drummondii* Meisn. For *Isopogon*, *I. alcicornis* Diels was sister to *I. trilobus* R.Br. in CON-NU but shown to be nested in the SWA clade excluding *I. trilobus*, *I. sphaerocephalus* Lindl. and *I. spathulatus* clades in CON-P. Similarly, different topological placements were noted for *I. uncinatus* R.Br., *I. villosus* Mesin., *I. scabriusculus* Meisn., *I. adenanthoides* Meisn., *I. robustus* Foreman ex N.Gibson, *I. divergens* R.Br., *I. inconspicuous* (Meisn.) Foreman and *I. gardneri* Foreman.

The CON-NU and MSC-NU topologies were also in conflict, in particular for SWA *Isopogon* clades that were weakly supported in MSC-NU (Supplementary Fig. S1). The overall topology of CON-NU was better supported than MSC-NU. In *Petrophile*, *P. acicularis* R.Br. was sister to a clade consisting of *P. prostrata*, *P. filifolia* R.Br. and *P. longifolia* R.Br. in MSC-Nu, whereas *P. prostrata* Rye & Hislop was sister to *P. acicularis* and the other two species in CON-NU. Two other conflicts were noted in the genus across the two datasets, namely the position of *P. glauca* Foreman and *P. pedunculata* R.Br. (Supplementary Fig. S1). In *Isopogon*, conflicting phylogenetic positions were noted for *I. robustus*, *I. gardneri*, *I. divergens*, *I. adenanthoides* and *I. inconspicuus* (Meisn.) Foreman albeit with generally low support in MSC-NU (Supplementary Fig. S1).

Spatial phylogenetics

Significant areas based on our spatial phylogenetic analyses are highlighted in Fig. 3. The SWAFR contained the highest PD for both *Isopogon* and *Petrophile* across all datasets (plastid and nuclear) (Fig. 4, 5). For *Petrophile*, high PD was found throughout the SWAFR except for the south-west corner with the highest rainfall, whereas *Isopogon* had highest PD in the northern (Geraldton Sandplains) sub-region and Stirling Range within the SWAFR. For *Isopogon*, the Sydney Basin region was a secondary hotspot for PD across both plastid and nuclear topologies (Fig. 4).

Different results were obtained between plastid and nuclear topologies for randomised PD (PDR). For *Petrophile*, the semi-arid region adjacent to the SWAFR and northern New South Wales had significantly lower PDR in the plastid but not nuclear dataset. Similarly, the southern Queensland area had significantly higher PDR (>0.975, blue) in the nuclear but not plastid dataset, due to a sampling artefact as *P. shirleyae* was excluded from the plastid dataset due to low plastome recovery. For *Isopogon*, the north-west corner of the SWAFR had significantly low PDR (<0.01, red) based on the plastid topology, but this pattern was not present in our nuclear results (Fig. 4). Similarly, other regions such as the Sydney Basin region and south-west corner of the SWAFR had significantly low PDR, but these areas were only identified based on our nuclear and not plastid dataset (Fig. 4). Despite these differences, for *Petrophile* both plastid and nuclear datasets showed the Sydney Basin region as having significantly lower PDR than expected (<0.01, red), when compared to other regions across Australia (Fig. 5).

Different spatial phylogenetic patterns were also obtained for our CANAPE analyses in identifying hotspots for phylogenetic endemism. For *Petrophile*, despite three main areas identified as significant across both plastid and nuclear datasets (south-west SWAFR, northern sandplains and adjacent semi-arid SWAFR, and Kangaroo Island), different grid cells in these areas were identified across the SWAFR (Fig. 5). Kangaroo Island was noted as a paleo-endemic centre, as this area contains an endemic species (*P. multisecta* F.Muell.) that is sister to all other remaining eastern Australian species in both phylogenies. For *Isopogon*, the northern sandplains subregion of the SWAFR was identified as a hotspot of mixed PE, with five grid cells identified from that area for the nuclear dataset but only one grid cell for the plastid dataset (Fig. 4). The southern wheatbelt subregion of the SWAFR was identified as significant across both plastid and nuclear data, though for plastid data the area contained primarily neo-endemics compared to our nuclear topology that indicated a mixed hotspot (containing both neo- and paleo-endemics).

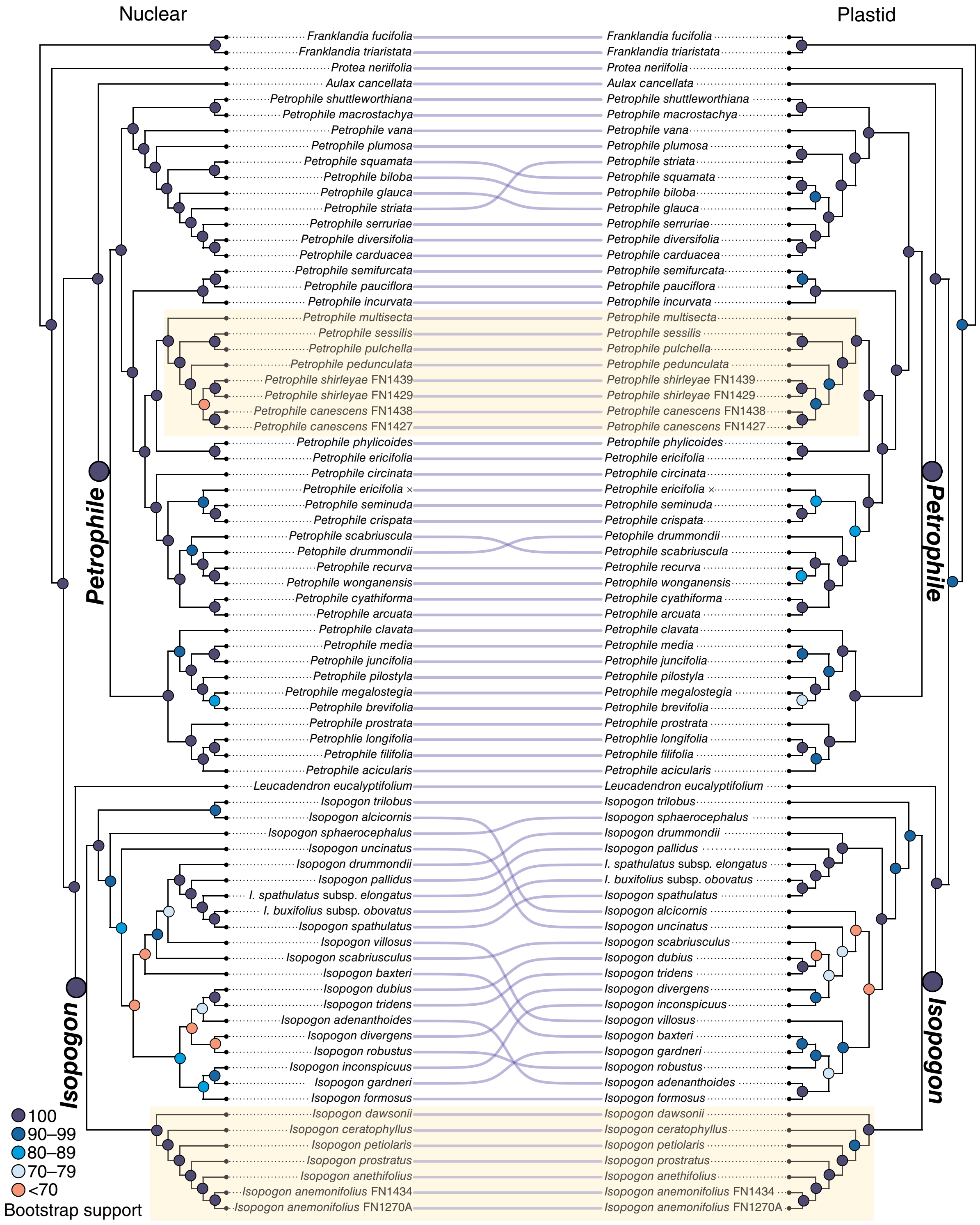

Fig. 2. Tanglegram showing nuclear (left, CON-NU) and plastid (right, CON-P) phylogenies of *Isopogon* and *Petrophile* (Proteaceae) estimated with concatenated datasets using *IQ-TREE*. The crown nodes of *Isopogon* and *Petrophile* are indicated with larger circles. Eastern Australian clades are highlighted in yellow. Support values are indicated by coloured circles from high to low support (blue–red). Note: '*Petrophile ericifolia* ×' represents the hybrid *Petrophile ericifolia* × *seminuda*.

Two other regions were identified as significant from the CANAPE results based on the plastid but not nuclear dataset: south-west corner of the SWAFR and Sydney Basin region (Fig. 4). These differences between nuclear and plastid topologies for both PDR and CANAPE analyses were also consistent in our sensitivity analyses (with exact matching taxa across

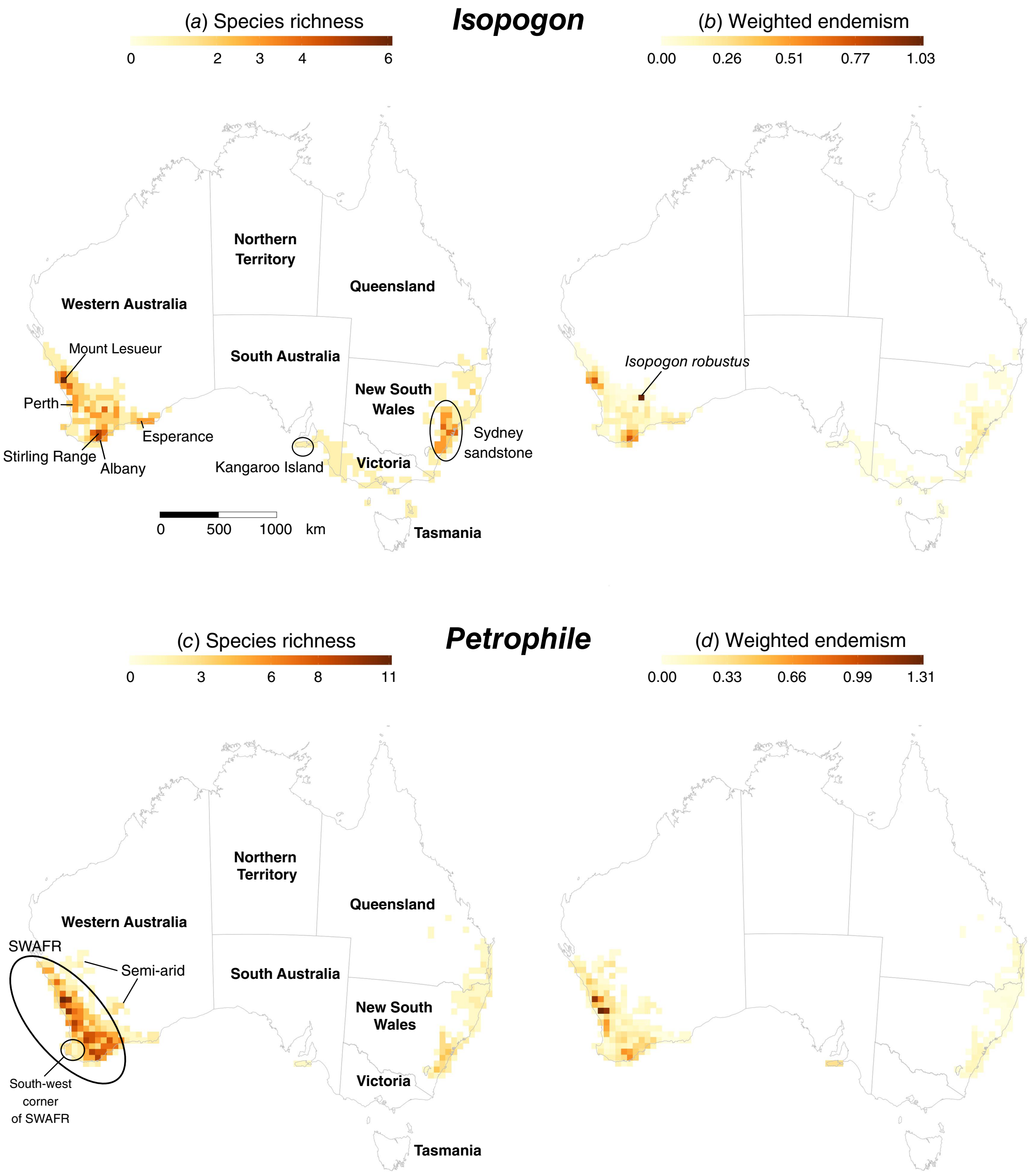

Fig. 3. Species richness and weighted endemism maps for *Isopogon* (*a*, *b*), and *Petrophile* (*c*, *d*). Maps show the states and territories, and geographical areas mentioned in text.

datasets), thus ruling out sampling artefact as a contributing factor (Supplementary Fig. S2, S3).

Discussion

Differences in spatial phylogenetic patterns between plastid and nuclear datasets

We present the first densely sampled and generally well-resolved nuclear and plastid phylogenies of *Isopogon* and *Petrophile*. We show that incongruent phylogenies from different molecular datasets have an effect on downstream spatial phylogenetic analyses. We document differing spatial phylogenetic results for the two genera depending on whether the spatial analyses were based on the nuclear or plastid data.

Incongruent spatial phylogenetic patterns in this study have important implications for conservation and policy decisions. Focusing on phylogenetic diversity and endemism for conservation has often been championed over species richness metrics (e.g. Winter *et al.* 2013; Pollock *et al.* 2015; Carvalho *et al.* 2017; Millar *et al.* 2017, 2018;

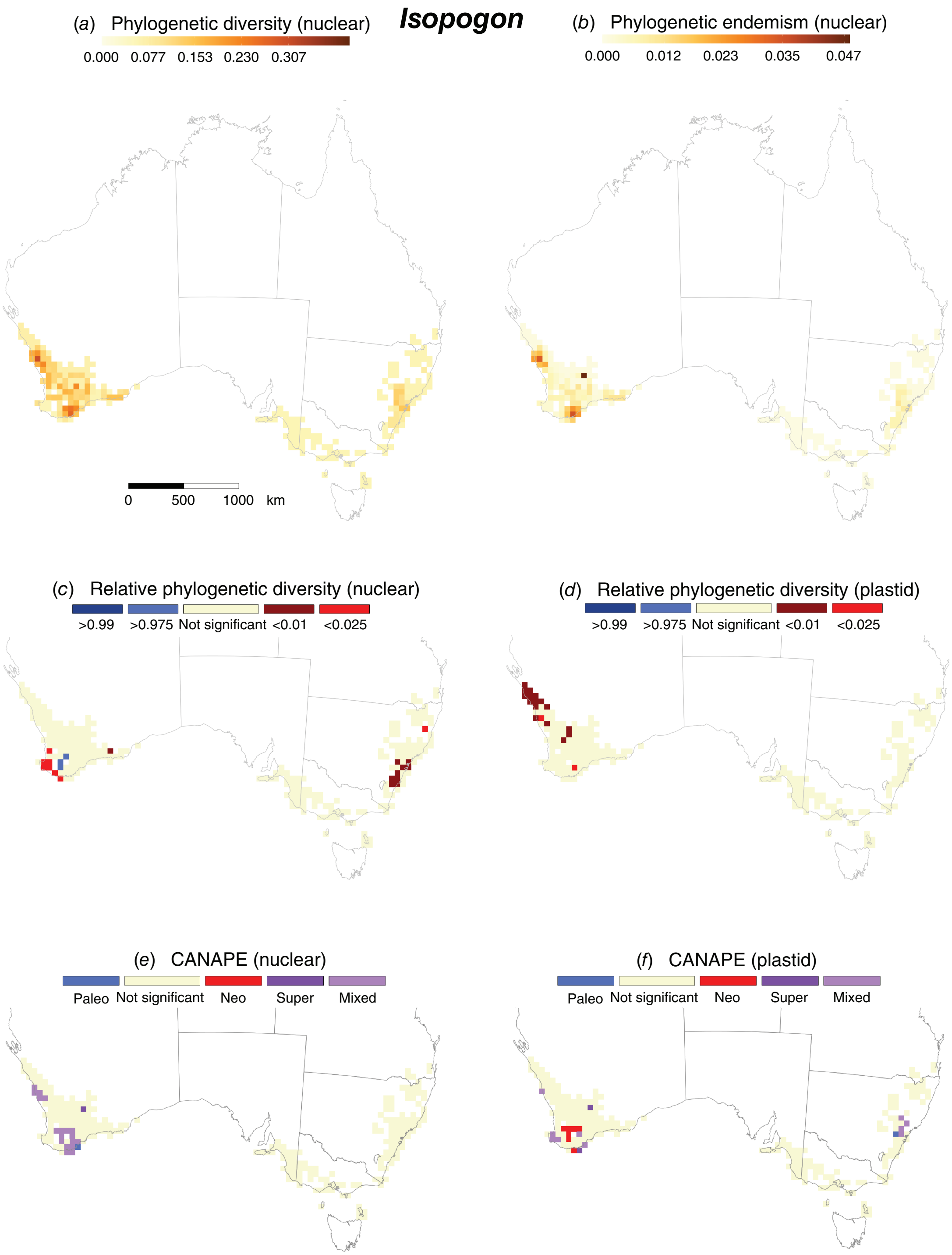

Fig. 4. Spatial phylogenetic results for *Isopogon* based on nuclear and plastid topologies. (*a*) Phylogenetic diversity. (*b*) Phylogenetic endemism, and randomisation test of relative phylogenetic diversity based on (*c*) nuclear and (*d*) plastid topologies, and endemism hotspots from CANAPE based on (*e*) nuclear and (*f*) plastid topologies.

Aguilar-Tomasini *et al.* 2021; Yang *et al.* 2022). However, most of these studies of plants combined nuclear and plastid datasets in their analyses (Table 1). Few of these studies explicitly tested for conflict across their datasets prior to conducting spatial phylogenetic analyses. In contrast to our study, Toro-Núñez and Lira-Noriega (2020) showed that phylogenetic discordance between nuclear and plastid datasets did not significantly affect spatial phylogenetic endemism results for Brassicaceae in the Atacama Desert. The presence and severity of phylogenetic conflict is likely

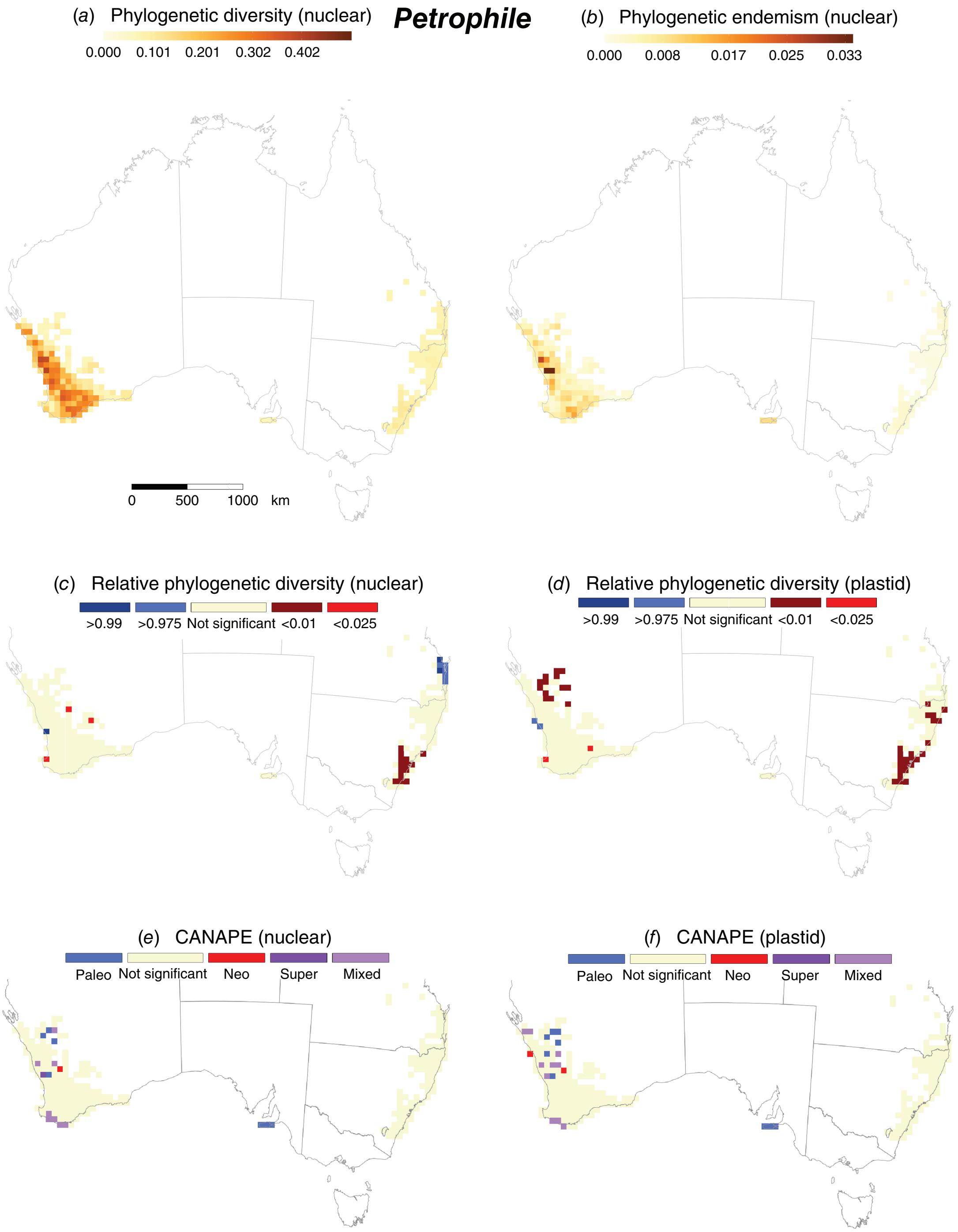

Fig. 5. Spatial phylogenetic results for *Petrophile* based on nuclear and plastid topologies. (*a*) Phylogenetic diversity. (*b*) Phylogenetic endemism, and randomisation test of relative phylogenetic diversity for (*c*) nuclear and (*d*) plastid topologies, and endemism hotspots from CANAPE based on (*e*) nuclear and (*f*) plastid topologies.

dependent on the individual biotic group and region of interest, due to their idiosyncratic evolutionary histories. Nevertheless, substantial phylogenetic conflict across different molecular datasets is present and being increasingly documented across a wide range of biotic groups (e.g. Smith *et al.* 2015; Vargas *et al.* 2017; Stull *et al.* 2020; Kao *et al.* 2022). We recommend that assessment of phylogenetic conflict across different datasets be conducted prior to spatial phylogenetic analyses. If substantial conflict is detected, we further recommend that the different datasets be treated separately and not combined in downstream analyses.

Different spatial phylogenetic patterns across nuclear and plastid datasets raise relevant questions about optimising conservation of areas of high PD and PE. One approach would be to prioritise the overlapping regions with high PD and PE in both plastid and nuclear datasets for *Isopogon* and *Petrophile* (i.e. most congruent option). Another would be to include all regions identified across all datasets (most inclusive option). Other approaches include regions that are identified as significant from one dataset but not the other (e.g. nuclear *v*. plastid results). The latter approaches might be justified based on specific conservation aims, e.g. conserving plastid haplotype diversity or using plastid data to identify refugial areas and geographic breaks. Indeed, some studies have shown that geographic patterns are more pronounced in plastid datasets for several reasons: maternal inheritance, smaller effective population size and no recombination (e.g. Currat *et al.* 2008; Worth *et al.* 2010; French *et al.* 2016; Biffin *et al.* 2020). Further research would be required to adequately assess the merits of each different approach. Making this information transparent, as we have done here, would allow for conservation policy makers to account for differences across datasets and organismal groups, in the hope of achieving better conservation outcomes (Pollock *et al.* 2015).

Significant areas of spatial phylogenetic diversity and endemism

Several regions across Australia were identified as having significant PD and PE/CANAPE levels for both *Petrophile* and *Isopogon*, despite group-specific differences and topological conflicts between plastid and nuclear datasets. Significant areas include the SWAFR, Sydney Basin and Kangaroo Island of South Australia that generally correspond to areas of high species richness and endemism, corroborating other studies of a general positive association between PD and PE measures and standard species richness metrics (Tucker and Cadotte 2013; Thornhill *et al.* 2016; Andrikou-Charitidou *et al.* 2020). These areas of significantly high PD and PE are not specific to *Isopogon* or *Petrophile*, but are a recurring pattern seen throughout the Australian flora (Rosauer *et al.* 2009; Mishler *et al.* 2014; González-Orozco *et al.* 2016; Thornhill *et al.* 2016; Miller *et al.* 2023). These regions also contain high plant species richness and endemism (Crisp *et al.* 2001; González-Orozco *et al.* 2011, 2014; Stevenson *et al.* 2012; Guerin *et al.* 2016; Prentice *et al.* 2017).

Recurring high species richness, endemism and significant spatial phylogenetic patterns in the SWAFR point to shared evolutionary histories of the flora. Indeed, Nge *et al.* (2020) have shown that the regional flora of the SWAFR was buffered from extinction in contrast to other regions, driven by large-scale global cooling in the Eocene–Oligocene boundary (*c*. 33 Ma) and in more recent Quaternary climatic cycles (Byrne 2008; Sniderman *et al.* 2013). Emerging consensus from our study and others (mentioned above) points to the SWAFR as an important region for the diversification and long-term persistence of many Australian plant lineages (Nge and Skeels 2025), not observed only from the high local species richness but also significant PD and PE levels.

In the SWAFR, areas of high PD for *Isopogon* are the northern sandplains and Stirling Range, both of which are dominated by kwongan heath vegetation. This spatial phylogenetic pattern matches the species richness and endemism patterns of *Isopogon* and wider flora more broadly (Gioia and Hopper 2017). Another study showed that the northern sandplains was the ancestral area for most extant species of *Cryptandra* Sm. (Rhamnaceae) in the SWAFR (Nge *et al.* 2023), reinforcing the evolutionary significance of this subregion. The high species and phylogenetic richness of kwongan vegetation has been attributed to a combination of environmental, ecological and evolutionary factors; namely climatic stability, mosaic of different environmental conditions (soil nutrients, fire), and adaptations allowing different lineages to persist in oligotrophic environments (Mucina *et al.* 2014; Zemunik *et al.* 2015; Tsakalos *et al.* 2019).

The northern sandplains of the SWAFR and adjacent semi-arid regions were noted as having significantly lower PD than expected for both *Isopogon* and *Petrophile* (plastid data). Phylogenetic clustering in these subregions of the SWAFR suggests that either habitat filtering (ecological) or evolutionary radiations have occurred. The CANAPE results support the latter, with mixed neo- and paleo-endemism for both genera noted in those regions, similar to results in another study on *Daviesia* Poir. (Fabaceae; Rosauer *et al.* 2009). In other words, high endemicity (CANAPE) suggests that *in-situ* radiations had occurred in the region. Similar spatial patterns have also been recorded for other plant groups such as *Banksia* L.f. (fig. 2b, c in Miller *et al.* 2023), and for angiosperms at the genus level (fig. 3 and 4 in Thornhill *et al.* 2016). Miller *et al.* (2023) argued that soil and climatic factors played a significant role in phylogenetic structure and turnover of *Banksia* in the region, more so than other Australian plant groups such as *Acacia* or eucalypts (Bui *et al.* 2014, 2017). Prentice *et al.* (2017) also demonstrated similar results for *Isopogon* and *Petrophile*, showing that soil factors (phosphorus and pH) were main determinants for species turnover. Whether these factors also play a role in explaining phylogenetic turnover across the SWAFR and Australia for these genera remains to be tested.

The Sydney Basin bioregion was also noted to have significantly lower PD than expected for both *Isopogon* (nuclear and plastid) and *Petrophile* (nuclear data only). Our CANAPE results also did not identify the Sydney Basin as a significant neo- or paleo-endemism centre in contrast to the northern sandplains of the SWAFR. Taken together, these results could be largely explained by the fact that only one clade is represented in SEA for both genera, in contrast to SWA. Other plant groups such as ferns and

Acacia Mill. (Fabaceae) also show similar spatial phylogenetic patterns of significantly lower PD in the region (Mishler *et al.* 2014; Nagalingum *et al.* 2015). Many narrow endemics are confined to sandstone substrates in the Sydney Basin, including those that are recently described (e.g. Coleby and Druitt 2019; Renner *et al.* 2022; Wilson *et al.* 2023; Barrett *et al.* 2024). Indeed, two rare endemic *Isopogon* species (*I. dawsonii* R.T.Baker and *I. fletcheri* F.Muell.) occur exclusively on or near sandstone cliff faces in the region (Benson and McDougall 2000). There has been extensive research interest on the ecological determinants of relative high plant species richness and endemism patterns in the Sydney Basin bioregion (e.g. Rice and Westoby 1983; Le Brocque and Buckney 1995; Auld 1996; Myerscough 1998; Mokany and Adam 2000; Auld 2001; Weston *et al.* 2005). By contrast, evolutionary drivers apart from population genetic refugia patterns are less well known (Rossetto *et al.* 2018; Rutherford *et al.* 2018; Wilson *et al.* 2022). Interestingly, assembly of the present-day Sydney Basin endemic flora occurred relatively recently, with most lineages diverging from sister lineages in other regions *c.* 5 Ma in the Pliocene (F. J. Nge, unpubl. data). Phylogenies from other plant groups also show endemic Sydney Basin lineages to be phylogenetically dispersed, i.e. the lineages are not from one clade. These include *Pomaderris brunnea* N.A.Wakef., *P. cotoneaster* N.A.Wakef. and *P. sericea* N.A.Wakef. in the genus-wide phylogeny of Nge *et al.* (2021*b*). Additional spatial phylogenetic studies are required to test whether other plant groups also show similar patterns of PD and PE in the Sydney bioregion.

Paleo-endemism for *Petrophile multisecta* on Kangaroo Island could be linked to the island acting as an important refugium – one that can be expanded to other plants based on their distributions (Crisp *et al.* 2001). Indeed Guerin and Lowe (2013) demonstrated that the Adelaide Geosyncline (that includes Kangaroo Island, and Mount Lofty and Flinders Ranges) is an arid refugium for different plant lineages across the region. The presence of paleo-endemics on Kangaroo Island is noteworthy as this region has experienced severe climatic and tectonic shifts, including recent sea-level changes in the Quaternary (McLaren and Wallace 2010; McGowran and Hill 2015; Bourman *et al.* 2016). Southern Australia has also experienced repeated marine transgressions particularly around the Eucla and Murray Basins from the Eocene to the present (McGowran *et al.* 2004; McGowran and Hill 2015). These repeated shifts in the tectonic, climatic and associated vegetation of southern Australia in contrast to the relatively stable SWAFR suggest that the *P. multisecta* lineage may have origniated from other regions of Australia. As the climate became progressively more arid in the Miocene and Pliocene, lineages retreated to the mesic fringes of the Australian continent (Byrne *et al.* 2011) (see the peripheral vicariance hypothesis; Nge *et al.* 2021*b*, 2023). A dated phylogeny would be required to estimate when *P. multisecta* diverged from other eastern Australian species. Interestingly, a leaf macrofossil with *Petrophile* affinities from the mid-Eocene was discovered at Maslin Bay (R. S. Hill, pers. comm.). *Petrophile* could possibly have been present in the region since at least the Eocene based on this fossil record. Biogeographic models and the incorporation of scenarios simulating the aridification of Australia would be required to test these questions and whether the paleo-endemic *P. multisecta* endemic to Kangaroo Island is the result of peripheral vicariance across southern Australia.

It is worth noting that results presented in this study are based on phylograms (i.e. not time calibrated). Therefore the different branch lengths between the plastid and nuclear topologies may also be contributing factors to the different spatial phylogenetic patterns documented in this study. Our plastid data show larger variation in root-tip distances than the nuclear data and several unusually long terminal branches, and this may have driven at least some of the differences in inferred spatial patterns between the two datasets. The significant PD, PE and CANAPE hotspots presented here are reflective of genetic differences (from phylograms) rather than by time of colonisation or divergence across regions (chronogram). Some studies have shown that spatial phylogenetic patterns do not differ significantly between phylograms and chronograms (Thornhill *et al.* 2017) whereas others show the opposite result (Elliott *et al.* 2018). Future studies using divergence dating methods on *Isopogon* and *Petrophile* would provide further insights on this topic. Although it is beyond the scope of our current study that focused on plastid and nuclear incongruences, further studies should compare spatial phylogenetic results derived from concatenated *v.* coalescent approaches (e.g. using *ASTRAL-IV* to explicitly compute branch lengths in substitution-per-site units; Zhang C. *et al.* 2025).

Phylogenomics

We show that the sister species relationships across *Petrophile* and *Isopogon* are well resolved, across both plastid and nuclear topologies. The backbone of *Isopogon* remains inadequately resolved in contrast to *Petrophile*, despite the inclusion of more than 50 nuclear and plastid loci per sample. The inclusion of additional genomic data in future works may provide better resolution of the backbone topology (i.e. crown radiation of *Isopogon*). However some recalcitrant nodes may never be resolved even with the presence of thousands of loci as shown for some other plant groups (Koenen *et al.* 2020).

The lack of substantial branch divergence and associated unresolved backbone topologies of *Isopogon* suggest that a rapid crown radiation occurred in the evolutionary history of this genus. Similar patterns of unresolved and conflicting topologies coinciding with evolutionary radiations have been documented for many other organismal groups (Parins-Fukuchi *et al.* 2021; Stull *et al.* 2021; Zhou *et al.* 2022). Interestingly, rapid radiations have also been documented for close relatives of *Isopogon* and *Petrophile*, such as the

Australian *Adenanthos* (Nge *et al.* 2021*a*) and South African *Paranomus* Salib., *Sorocephalus* R.Br., *Spatalla* Salisb., *Leucospermum* R.Br. and *Mimetes* Salisb. genera within the subtribe Leucadendrinae (Sauquet *et al.* 2009). These South African genera were shown to have elevated diversification rates indicative of rapid radiations (Sauquet *et al.* 2009). *Isopogon* and *Adenanthos* were shown to not have significantly elevated diversification rates compared to other Proteaceae genera in Sauquet *et al.* (2009). However, diversification rates of the two genera were calculated from stem divergence in this study. Denser taxonomic sampling in our study and Nge *et al.* (2021*a*) revealed rapid radiations of *Isopogon* and *Adenanthos*. Rapid radiations of *Isopogon* and closely related genera in Leucadendrinae suggest that radiations in Proteaceae are clade-specific (Kozak and Wiens 2016) and may be driven by shared evolutionary traits (Jordan *et al.* 2008; Onstein *et al.* 2016; Hayes *et al.* 2021).

Similarly, substantial incongruence was noted between nuclear and plastid topologies for *Isopogon* but not *Petrophile*. Some of these topological conflicts are poorly supported (soft conflict) due to the unresolved backbone, but others are strongly supported (hard conflict; see Fig. 2). Deep reticulate evolution has been shown for *Adenanthos* (Nge *et al.* 2021*a*) and may also be the case for the closely related genus *Isopogon*. However further analyses will be required to confirm this phenomenon for *Isopogon* (Joyce *et al.* 2025).

Very few extant hybrids have been documented for *Petrophile*, with two observed for *Isopogon* to our knowledge (Bate and Trickett 2019). Fewer than 10 putative hybrids have been observed in the field based on morphology (Supplementary Table S3). Only one putative hybrid for *Petrophile* (*P. ericifolia* × *seminuda*, sampled in this study) located in the SWAFR has been published (Rye *et al.* 2011). We show that this hybrid is sister to *P. seminuda* Lindl. and *P. crispata* R.Br. in both nuclear (concatenated) and plastid topologies. *Petrophile ericifolia* formed another distinct clade with *P. phylicoides* that is sister to the eastern Australian *Petrophile* clade, across both nuclear and plastid trees. The hybrid is possibly a backcross with *P. seminuda*, resulting in closer phylogenetic affinity with this than *P. ericifolia*. However, further population genetic studies are required to determine the taxonomic status of *P. ericifolia* × *seminuda*.

Summary

We presented the first comprehensive phylogenies of *Isopogon* and *Petrophile*, utilising both nuclear and plastid genomic data. We found incongruent topologies between the two datasets that substantially affected spatial phylogenetic results. Wider awareness is needed, and we caution against combining different datasets as is commonly practice without detailed assessment of potential incongruence in different datasets. Significant areas of PD and PE and neo- and paleo-endemism for *Isopogon* and *Petrophile* include the SWAFR, Kangaroo Island and the Sydney Basin. These areas are located along the mesic fringes of the continent, congruent with the peripheral vicariance hypothesis in biogeography. Further biogeographic studies are required to test this hypothesis. In addition, further spatial studies of different groups utilising different molecular data sources would shed light on the spatial evolution of the Australian flora, and impacts of phylogenetic incongruence on these patterns.

Supplementary material

Supplementary material is available online.

References

Abadi S, Azouri D, Pupko T, Mayrose I (2019) Model selection may not be a mandatory step for phylogeny reconstruction. *Nature Communications* **10**, 934. doi:10.1038/s41467-019-08822-w

Aguilar-Tomasini MA, Martin MD, Speed JD (2021) Assessing spatial patterns of phylogenetic diversity of Mexican mammals for biodiversity conservation. *Global Ecology and Conservation* **31**, e01834. doi:10.1016/j.gecco.2021.e01834

Allen JM, Germain-Aubrey CC, Barve N, Neubig KM, Majure LC, Laffan SW, Mishler BD, Owens HL, Smith SA, Whitten WM, Abbott JR, Soltis DE, Guralnick R, Soltis PS (2019) Spatial phylogenetics of Florida vascular plants: the effects of calibration and uncertainty on diversity estimates. *IScience* **11**, 57–70. doi:10.1016/j.isci.2018.12.002

Andermann T, Cano Á, Zizka A, Bacon C, Antonelli A (2018) *SECAPR*—a bioinformatics pipeline for the rapid and user-friendly processing of targeted enriched Illumina sequences, from raw reads to alignments. *PeerJ* **6**, e5175. doi:10.7717/peerj.5175

Andrikou-Charitidou A, Boutsis G, Karadimou E, Kallimanis A (2020) Untangling the positive association of phylogenetic, functional, and taxonomic diversity with alien bird species richness. *Ecosphere* **11**, e03007. doi:10.1002/ecs2.3007

Arroyo-Rodríguez V, Cavender-Bares J, Escobar F, Melo FP, Tabarelli M, Santos BA (2012) Maintenance of tree phylogenetic diversity in a highly fragmented rain forest. *Journal of Ecology* **100**, 702–711. doi:10.1111/j.1365-2745.2011.01952.x

Auld TD (1996) Ecology of the Fabaceae in the Sydney region: fire, ants and the soil seedbank. *Cunninghamia* **4**, 531–551.

Auld TD (2001) The ecology of the Rutaceae in the Sydney region of south-eastern Australia: poorly known ecology of a neglected family. *Cunninghamia* **7**, 213–239.

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA (2012) *SPAdes*: a new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology* **19**, 455–477. doi:10.1089/cmb.2012.0021

Barrett RL, Clugston JA, Albrecht DE, Elkan L, Hosking JR, Jobson PC, McCune SF, Orme AE, Palsson RL, Renner MA (2024) Revision of the *Pultenaea setulosa* species complex (Fabaceae: Mirbelieae) including 14 new species. *Australian Systematic Botany* **37**, SB23014. doi:10.1071/SB23014

Bate C, Trickett P (2019) Introducing a new hybrid: *Isopogon* 'Coaldale Cracker'. *Isopogon & Petrophile Study Group Newsletter* **25**, 8–11.

Benson D, McDougall L (2000) Ecology of Sydney plant species: part 7b, Dicotyledon families Proteaceae to Rubiaceae. *Cunninghamia* **6**, 1016–1202.

Biffin E, van Dijk K, Waycott M (2020) The evolutionary significance of range disjunctions in South Australian eucalypts. (Australian Flora Foundation: Sydney, NSW, Australia) Available at https://aff.org.au/wp-content/uploads/EdBiffin_final_report.pdf

Biffin E, Waycott M, Hammer TA, van Dijk K (2025) A comparison of two universal angiosperm bait sets and the phylogenomics of

Alismatales. *bioRxiv* **2025**, 2025.09.15.676180. [Preprint, published 16 September 2025] doi:10.1101/2025.09.15.676180

Bourman RP, Murray-Wallace CV, Harvey N (2016) Kangaroo Island. In 'Coastal Landscapes of South Australia'. (Eds RP Bourman, CV Murray-Wallace, N Harvey) pp. 355–386. (University of Adelaide Press)

Bui EN, González-Orozco CE, Miller JT (2014) *Acacia*, climate, and geochemistry in Australia. *Plant and Soil* **381**, 161–175. doi:10.1007/s11104-014-2113-x

Bui EN, Thornhill AH, González-Orozco CE, Knerr N, Miller JT (2017) Climate and geochemistry as drivers of eucalypt diversification in Australia. *Geobiology* **15**, 427–440. doi:10.1111/gbi.12235

Byrne M (2008) Evidence for multiple refugia at different time scales during Pleistocene climatic oscillations in southern Australia inferred from phylogeography. *Quaternary Science Reviews* **27**, 2576–2585. doi:10.1016/j.quascirev.2008.08.032

Byrne M, Steane DA, Joseph L, Yeates DK, Jordan GJ, Crayn D, Aplin K, Cantrill DJ, Cook LG, Crisp MD (2011) Decline of a biome: evolution, contraction, fragmentation, extinction and invasion of the Australian mesic zone biota. *Journal of Biogeography* **38**, 1635–1656. doi:10.1111/j.1365-2699.2011.02535.x

Cardoso MR, Matos FA, Almeida SM, Silva LC, Cerqueira PV, Santos MP, Edwards DP (2021) Connected riparian reserves retain high avian phylogenetic diversity in Amazonian oil palm. *Biological Conservation* **259**, 109171. doi:10.1016/j.biocon.2021.109171

Carvalho SB, Velo-Antón G, Tarroso P, Portela AP, Barata M, Carranza S, Moritz C, Possingham HP (2017) Spatial conservation prioritization of biodiversity spanning the evolutionary continuum. *Nature Ecology & Evolution* **1**, 0151. doi:10.1038/s41559-017-0151

Chong JP, Harris JL, Roe KJ (2016) Incongruence between mtDNA and nuclear data in the freshwater mussel genus *Cyprogenia* (Bivalvia: Unionidae) and its impact on species delineation. *Ecology and Evolution* **6**, 2439–2452. doi:10.1002/ece3.2071

Coleby D, Druitt R (2019) *Eucalyptus cunninghamii* (Myrtaceae) in the blue mountains of New South Wales-an unexpected connection between botany and geology. *Proceedings of the Linnean Society of New South Wales* **141**, 81–95.

Costion CM, Edwards W, Ford AJ, Metcalfe DJ, Cross HB, Harrington MG, Richardson JE, Hilbert DW, Lowe AJ, Crayn DM (2015) Using phylogenetic diversity to identify ancient rain forest refugia and diversification zones in a biodiversity hotspot. *Diversity and Distributions* **21**, 279–289. doi:10.1111/ddi.12266

Crisp MD, Laffan S, Linder HP, Monro A (2001) Endemism in the Australian flora. *Journal of Biogeography* **28**, 183–198. doi:10.1046/j.1365-2699.2001.00524.x

Currat M, Ruedi M, Petit RJ, Excoffier L (2008) The hidden side of invasions: massive introgression by local genes. *Evolution* **62**, 1908–1920. doi:10.1111/j.1558-5646.2008.00413.x

Daru BH, van der Bank M, Davies TJ (2015) Spatial incongruence among hotspots and complementary areas of tree diversity in southern Africa. *Diversity and Distributions* **21**, 769–780. doi:10.1111/ddi.12290

Edgar RC (2004) *MUSCLE*: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**, 1792–1797. doi:10.1093/nar/gkh340

Eiserhardt WL, Borchsenius F, Plum CM, Ordonez A, Svenning JC (2015) Climate-driven extinctions shape the phylogenetic structure of temperate tree floras. *Ecology Letters* **18**, 263–272. doi:10.1111/ele.12409

Elliott MJ, Knerr NJ, Schmidt-Lebuhn AN (2018) Choice between phylogram and chronogram can have a dramatic impact on the location of phylogenetic diversity hotspots. *Journal of Biogeography* **45**, 2190–2201. doi:10.1111/jbi.13399

Faith DP (1992) Conservation evaluation and phylogenetic diversity. *Biological Conservation* **61**, 1–10. doi:10.1016/0006-3207(92)91201-3

Faurby S, Eiserhardt WL, Baker WJ, Svenning J-C (2016) An all-evidence species-level supertree for the palms (Arecaceae). *Molecular Phylogenetics and Evolution* **100**, 57–69. doi:10.1016/j.ympev.2016.03.002

Firneno TJ Jr, O'Neill JR, Itgen MW, Kihneman TA, Townsend JH, Fujita MK (2021) Delimitation despite discordance: evaluating the species limits of a confounding species complex in the face of mitonuclear discordance. *Ecology and Evolution* **11**, 12739–12753. doi:10.1002/ece3.8018

Forest F, Grenyer R, Rouget M, Davies TJ, Cowling RM, Faith DP, Balmford A, Manning JC, Procheş S, van der Bank M, Reeves G, Hedderson TA, Savolainen V (2007) Preserving the evolutionary potential of floras in biodiversity hotspots. *Nature* **445**, 757–760. doi:10.1038/nature05587

Foster NR, van Dijk K, Biffin E, Young JM, Thomson VA, Gillanders BM, Jones AR, Waycott M (2021) A multi-gene region targeted capture approach to detect plant DNA in environmental samples: a case study from coastal environments. *Frontiers in Ecology and Evolution* **9**, 726. doi:10.3389/fevo.2021.735744

French PA, Brown GK, Bayly MJ (2016) Incongruent patterns of nuclear and chloroplast variation in *Correa* (Rutaceae): introgression and biogeography in south-eastern Australia. *Plant Systematics and Evolution* **302**, 447–468. doi:10.1007/s00606-016-1277-7

Fritz SA, Rahbek C (2012) Global patterns of amphibian phylogenetic diversity. *Journal of Biogeography* **39**, 1373–1382. doi:10.1111/j.1365-2699.2012.02757.x

García N, Folk RA, Meerow AW, Chamala S, Gitzendanner MA, Oliveira RS, Soltis DE, Soltis PS (2017) Deep reticulation and incomplete lineage sorting obscure the diploid phylogeny of rain-lilies and allies (Amaryllidaceae tribe Hippeastreae). *Molecular Phylogenetics and Evolution* **111**, 231–247. doi:10.1016/j.ympev.2017.04.003

Gioia P, Hopper SD (2017) A new phytogeographic map for the Southwest Australian Floristic Region after an exceptional decade of collection and discovery. *Botanical Journal of the Linnean Society* **184**, 1–15. doi:10.1093/botlinnean/box010

González-Orozco CE, Laffan SW, Miller JT (2011) Spatial distribution of species richness and endemism of the genus *Acacia* in Australia. *Australian Journal of Botany* **59**, 601–609. doi:10.1071/BT11112

González-Orozco CE, Thornhill AH, Knerr N, Laffan S, Miller JT (2014) Biogeographical regions and phytogeography of the eucalypts. *Diversity and Distributions* **20**, 46–58. doi:10.1111/ddi.12129

González-Orozco CE, Pollock LJ, Thornhill AH, Mishler BD, Knerr N, Laffan SW, Miller JT, Rosauer DF, Faith DP, Nipperess DA (2016) Phylogenetic approaches reveal biodiversity threats under climate change. *Nature Climate Change* **6**, 1110. doi:10.1038/nclimate3126

Guerin GR, Lowe AJ (2013) Multi-species distribution modelling highlights the Adelaide Geosyncline, South Australia, as an important continental-scale arid-zone refugium. *Austral Ecology* **38**, 427–435. doi:10.1111/j.1442-9993.2012.02425.x

Guerin GR, Biffin E, Baruch Z, Lowe AJ (2016) Identifying centres of plant biodiversity in South Australia. *PLoS One* **11**, e0144779. doi:10.1371/journal.pone.0144779

Hammer TA, Renton M, Mucina L, Thiele KR (2021) Arid Australia as a source of plant diversity: the origin and climatic evolution of *Ptilotus* (Amaranthaceae). *Australian Systematic Botany* **34**(6), 570–586. doi:10.1071/SB21012

Harris RS (2007) 'Improved pairwise alignment of genomic DNA.' (The Pennsylvania State University)

Hayes PE, Nge FJ, Cramer MD, Finnegan P, Fu P, Hopper SD, Oliveira RS, Turner BL, Zemunik G, Zhong H, Lambers H (2021) Traits related to efficient acquisition and use of phosphorus drive diversification in Proteaceae in phosphorus-impoverished landscapes. *Plant and Soil* **462**, 67–88. doi:10.1007/s11104-021-04886-0

Hoang DT, Chernomor O, Von Haeseler A, Minh BQ, Vinh LS (2018) *UFBoot2*: improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution* **35**, 518–522. doi:10.1093/molbev/msx281

Hu HH, Liu B, Liang YS, Ye JF, Saqib S, Meng Z, Lu LM, Chen ZD (2020) An updated Chinese vascular plant tree of life: phylogenetic diversity hotspots revisited. *Journal of Systematics and Evolution* **58**, 663–672. doi:10.1111/jse.12642

Jackson C, McLay T, Schmidt-Lebuhn AN (2023) *hybpiper-nf* and *paragone-nf*: containerization and additional options for target capture assembly and paralog resolution. *Applications in Plant Sciences* **11**, e11532. doi:10.1002/aps3.11532

Jacobs SJ, Kristofferson C, Uribe-Convers S, Latvis M, Tank DC (2018) Incongruence in molecular species delimitation schemes: what to do when adding more data is difficult. *Molecular Ecology* **27**, 2397–2413. doi:10.1111/mec.14590

Johnson MG, Pokorny L, Dodsworth S, Botigué LR, Cowan RS, Devault A, Eiserhardt WL, Epitawalage N, Forest F, Kim JT, Leebens-Mack JH, Leitch IJ, Maurin O, Soltis DE, Soltis PS, Wong GK-S, Baker WJ, Wickett NJ (2019) A universal probe set for targeted sequencing of 353 nuclear genes from any flowering plant designed using k-medoids clustering. *Systematic Biology* **68**, 594–606. doi:10.1093/sysbio/syy086

Jordan GJ, Weston PH, Carpenter RJ, Dillon RA, Brodribb TJ (2008) The evolutionary relations of sunken, covered, and encrypted stomata to dry habitats in Proteaceae. *American Journal of Botany* **95**, 521–530. doi:10.3732/ajb.2007333

Joyce EM, Schmidt-Lebuhn AN, Orel HK, Nge FJ, Anderson BM, Hammer TA, McLay TGB (2025) Navigating phylogenetic conflict and evolutionary inference in plants with target capture data. *Australian Systematic Botany* **38**, SB24011. doi:10.1071/SB24011

Junier T, Zdobnov EM (2010) The *Newick utilities*: high-throughput phylogenetic tree processing in the UNIX shell. *Bioinformatics* **26**, 1669–1670. doi:10.1093/bioinformatics/btq243

Kao TT, Wang TH, Ku C (2022) Rampant nuclear–mitochondrial–plastid phylogenomic discordance in globally distributed calcifying microalgae. *New Phytologist* **235**, 1394–1408. doi:10.1111/nph.18219

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) *Geneious Basic*: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**, 1647–1649. doi:10.1093/bioinformatics/bts199

Koenen EJM, Ojeda DI, Steeves R, Migliore J, Bakker FT, Wieringa JJ, Kidner C, Hardy OJ, Pennington RT, Bruneau A, Hughes CE (2020) Large-scale genomic sequence data resolve the deepest divergences in the legume phylogeny and support a near-simultaneous evolutionary origin of all six subfamilies. *New Phytologist* **225**, 1355–1369. doi:10.1111/nph.16290

Kozak KH, Wiens JJ (2016) Testing the relationships between diversification, species richness, and trait evolution. *Systematic Biology* **65**, 975–988. doi:10.1093/sysbio/syw029

Laffan SW, Lubarsky E, Rosauer DF (2010) Biodiverse, a tool for the spatial analysis of biological and related diversity. *Ecography* **33**, 643–647. doi:10.1111/j.1600-0587.2010.06237.x

Le Brocque AF, Buckney RT (1995) Vegetation and environmental patterns on soils derived from Hawkesbury Sandstone and Narrabeen substrata in Ku-ring-gai Chase National Park, New South Wales. *Australian Journal of Ecology* **20**, 229–238. doi:10.1111/j.1442-9993.1995.tb00534.x

Lee AC, Mishler B (2014) Phylogenetic diversity and endemism: metrics for identifying critical regions of conifer conservation in Australia. *Berkeley Scientific Journal* **18**(2), 48–58. doi:10.5070/bs3182022486

McGowran B, Hill RS (2015) Cenozoic climatic shifts in southern Australia. *Transactions of the Royal Society of South Australia* **139**, 19–37. doi:10.1080/03721426.2015.1035215

McGowran B, Holdgate G, Li Q, Gallagher S (2004) Cenozoic stratigraphic succession in southeastern Australia. *Australian Journal of Earth Sciences* **51**, 459–496. doi:10.1111/j.1400-0952.2004.01078.x

McLaren S, Wallace MW (2010) Plio-Pleistocene climate change and the onset of aridity in southeastern Australia. *Global and Planetary Change* **71**, 55–72. doi:10.1016/j.gloplacha.2009.12.007

McLay TG, Fowler RM, Fahey PS, Murphy DJ, Udovicic F, Cantrill DJ, Bayly MJ (2023) Phylogenomics reveals extreme gene tree discordance in a lineage of dominant trees: hybridization, introgression, and incomplete lineage sorting blur deep evolutionary relationships despite clear species groupings in *Eucalyptus* subgenus *Eudesmia*. *Molecular Phylogenetics and Evolution* **187**, 107869. doi:10.1016/j.ympev.2023.107869

Millar TR, Heenan PB, Wilton AD, Smissen RD, Breitwieser I (2017) Spatial distribution of species, genus and phylogenetic endemism in the vascular flora of New Zealand, and implications for conservation. *Australian Systematic Botany* **30**, 134–147. doi:10.1071/SB16015

Miller JT, Jolley-Rogers G, Mishler BD, Thornhill AH (2018) Phylogenetic diversity is a better measure of biodiversity than taxon counting. *Journal of Systematics and Evolution* **56**, 663–667. doi:10.1111/jse.12436

Miller JT, Prentice E, Bui EN, Knerr N, Mishler BD, Schmidt-Lebuhn AN, González-Orozco C, Laffan S (2023) *Banksia* (Proteaceae) contains less phylogenetic diversity than expected in southwestern Australia. *Journal of Systematics and Evolution* **61**, 957–966. doi:10.1111/jse.13019

Minh BQ, Hahn MW, Lanfear R (2020*a*) New methods to calculate concordance factors for phylogenomic datasets. *Molecular Biology and Evolution* **37**, 2727–2733. doi:10.1093/molbev/msaa106

Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, Von Haeseler A, Lanfear R (2020*b*) *IQ-TREE 2*: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution* **37**, 1530–1534. doi:10.1093/molbev/msaa015

Mishler BD (2023) Spatial phylogenetics. *Journal of Biogeography* **50**, 1454–1463. doi:10.1111/jbi.14618

Mishler BD, Knerr N, González-Orozco CE, Thornhill AH, Laffan SW, Miller JT (2014) Phylogenetic measures of biodiversity and neo-and paleo-endemism in Australian *Acacia*. *Nature Communications* **5**, 4473. doi:10.1038/ncomms5473

Mokany K, Adam P (2000) The biogeographical attributes of the threatened flora of New South Wales. *Cunninghamia* **6**, 873–892.

Mucina L, Laliberte L, Thiele KR, Dodson J, Harvey J (2014) Biogeography of Kwongan: Origins, diversity, endemism and vegetation patterns. In 'Plant life on the sandplains in southwest Australia'. (Ed. H Lambers) pp. 35–80. (UWA Publishing: Perth, WA, Australia)

Myerscough PJ (1998) Ecology of Myrtaceae with special reference to the Sydney region. *Cunninghamia* **5**, 787–807.

Nagalingum NS, Knerr N, Laffan SW, González-Orozco CE, Thornhill AH, Miller JT, Mishler BD (2015) Continental scale patterns and predictors of fern richness and phylogenetic diversity. *Frontiers in Genetics* **6**, 132. doi:10.3389/fgene.2015.00132

Nge FJ, Skeels A (2025) Diversification patterns of the southwest Australian biodiversity hotspot reveal a novel macroevolutionary pathway to plant hyperdiversity. *New Phytologist* **248**, 953–967. doi:10.1111/nph.70330

Nge FJ, Biffin E, Thiele KR, Waycott M (2020) Extinction pulse at Eocene–Oligocene boundary drives diversification dynamics of two Australian temperate floras. *Proceedings of the Royal Society of London – B. Biological Sciences* **287**, 20192546. doi:10.1098/rspb.2019.2546

Nge FJ, Biffin E, Thiele KR, Waycott M (2021*a*) Reticulate evolution, ancient chloroplast haplotypes, and rapid radiation of the Australian plant genus *Adenanthos* (Proteaceae). *Frontiers in Ecology and Evolution* **8**, 616741. doi:10.3389/fevo.2020.616741

Nge FJ, Kellermann J, Biffin E, Waycott M, Thiele KR (2021*b*) Historical biogeography of *Pomaderris* (Rhamnaceae): continental vicariance in Australia and repeated independent dispersals to New Zealand. *Molecular Phylogenetics and Evolution* **158**, 107085. doi:10.1016/j.ympev.2021.107085

Nge FJ, Biffin E, Waycott M, Thiele KR (2022) Phylogenomics and continental biogeographic disjunctions: insight from the Australian starflowers (*Calytrix*). *American Journal of Botany* **109**, 291–308. doi:10.1002/ajb2.1790

Nge FJ, Kellermann J, Biffin E, Thiele KR, Waycott M (2023) Rise and fall of a continental mesic radiation in Australia: spine evolution, biogeography, and diversification of *Cryptandra* (Rhamnaceae: Pomaderreae). *Botanical Journal of the Linnean Society* **204**, 327–342. doi:10.1093/botlinnean/boad051

Nge FJ, Chaowasku T, Damthongdee A, Wiya C, Soule VRC, Rodrigues-Vaz C, Bruy D, Mariac C, Chatrou LW, Choo LM, Dagallier L-PMJ, Erkens RHJ, Johnson DM, Leeratiwong C, Lobao AQ, Lopes JC, Martínez-Velarde MF, Munzinger J, Murray NA, Neo WL, Rakotoarinivo M, Ortiz-Rodriguez AE, Sonké B, Thomas DC, Wieringa JJ, Couvreur TLP (2024) Complete genus-level phylogenomics and new subtribal classification of the pantropical plant family Annonaceae. *Taxon* **6**, 1341–1369. doi:10.1002/tax.13260

Nock CJ, Baten A, King GJ (2014) Complete chloroplast genome of *Macadamia integrifolia* confirms the position of the Gondwanan early-diverging eudicot family Proteaceae. *BMC Genomics* **15**(Suppl 9), 13. doi:10.1186/1471-2164-15-S9-S13

One Thousand Plant Transcriptomes Initiative (2019) One thousand plant transcriptomes and the phylogenomics of green plants. *Nature* **574**, 679–685. doi:10.1038/s41586-019-1693-2

Onstein RE, Jordan GJ, Sauquet H, Weston PH, Bouchenak-Khelladi Y, Carpenter RJ, Linder HP (2016) Evolutionary radiations of Proteaceae are triggered by the interaction between traits and climates in open habitats. *Global Ecology and Biogeography* **25**, 1239–1251. doi:10.1111/geb.12481

Palmer C, Fischer B (2022) Should global conservation initiatives prioritize phylogenetic diversity? *Philosophia* **50**, 2283–2302. doi:10.1007/s11406-021-00422-7

Parins-Fukuchi C, Stull GW, Smith SA (2021) Phylogenomic conflict coincides with rapid morphological innovation. *Proceedings of the National Academy of Sciences* **118**, e2023058118. doi:10.1073/pnas.2023058118

Pollock LJ, Rosauer DF, Thornhill AH, Kujala H, Crisp MD, Miller JT, McCarthy MA (2015) Phylogenetic diversity meets conservation policy: small areas are key to preserving eucalypt lineages. *Philosophical Transactions of the Royal Society of London – B. Biological Sciences* **370**, 20140007. doi:10.1098/rstb.2014.0007

Prentice E, Knerr N, Schmidt-Lebuhn AN, González-Orozco CE, Bui EN, Laffan S, Miller JT (2017) Do soil and climate properties drive biogeography of the Australian proteaceae? *Plant and Soil* **417**, 317–329. doi:10.1007/s11104-017-3261-6

Qian H, Deng T, Jin Y, Mao L, Zhao D, Ricklefs RE (2019) Phylogenetic dispersion and diversity in regional assemblages of seed plants in China. *Proceedings of the National Academy of Sciences* **116**(46), 23192–23201. doi:10.1073/pnas.1822153116

Qian H, Zhang J, Jiang M (2023) Global patterns of taxonomic and phylogenetic diversity of flowering plants: biodiversity hotspots and coldspots. *Plant Diversity* **45**, 265–271. doi:10.1016/j.pld.2023.01.009

Quan Q, Che X, Wu Y, Wu Y, Zhang Q, Zhang M, Zou F (2018) Effectiveness of protected areas for vertebrates based on taxonomic and phylogenetic diversity. *Conservation Biology* **32**, 355–365. doi:10.1111/cobi.12986

Renner MA, Barrett RL, Clarke S, Clugston JA, Wilson TC, Weston PH (2022) Morphological and molecular evidence refute a broad circumscription for *Pultenaea glabra* (Fabaceae: Mirbelieae), with implications for taxonomy, biogeography, and conservation. *Australian Systematic Botany* **35**, 127–179. doi:10.1071/SB21030

Revell LJ (2012) *phytools*: an *R* package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* **3**, 217–223. doi:10.1111/j.2041-210X.2011.00169.x

Rice B, Westoby M (1983) Species richness in vascular vegetation of the West Head, New South Wales. *Australian Journal of Ecology* **8**, 163–168. doi:10.1111/j.1442-9993.1983.tb01603.x

Rodrigues AS, Gaston KJ (2002) Maximising phylogenetic diversity in the selection of networks of conservation areas. *Biological Conservation* **105**, 103–111. doi:10.1016/S0006-3207(01)00208-7

Rosauer DF, Jetz W (2015) Phylogenetic endemism in terrestrial mammals. *Global Ecology and Biogeography* **24**, 168–179. doi:10.1111/geb.12237

Rosauer D, Laffan SW, Crisp MD, Donnellan SC, Cook LG (2009) Phylogenetic endemism: a new approach for identifying geographical concentrations of evolutionary history. *Molecular Ecology* **18**, 4061–4072. doi:10.1111/j.1365-294X.2009.04311.x

Rossetto M, Beaumont L, Das S, Yap S (2018) Bioclimatic discordance: combining molecular and environmental data to identify floristic refugia and corridors. Final report. (NSW Office of Environment and Heritage). Available at https://www.climatechange.environment.nsw.gov.au/sites/default/files/2021-06/Bioclimatic%20discordance_floristic%20refugia%20and%20corridors.PDF

Rutherford S, Rossetto M, Bragg JG, McPherson H, Benson D, Bonser SP, Wilson PG (2018) Speciation in the presence of gene flow: population genomics of closely related and diverging *Eucalyptus* species. *Heredity* **121**, 126–141. doi:10.1038/s41437-018-0073-2

Rye BL, Hislop M, Shepherd KA, Hollister C (2011) New south-western Australian members of the genus *Petrophile* (Proteaceae: Petrophileae), including a hybrid. *Nuytsia* **21**, 35–67. doi:10.58828/nuy00570

Safi K, Cianciaruso MV, Loyola RD, Brito D, Armour-Marshall K, Diniz-Filho JA (2011) Understanding global patterns of mammalian functional and phylogenetic diversity. *Philosophical Transactions of the Royal Society of London – B. Biological Sciences* **366**, 2536–2544. doi:10.1098/rstb.2011.0024

Saladin B, Pellissier L, Graham CH, Nobis MP, Salamin N, Zimmermann NE (2020) Rapid climate change results in long-lasting spatial homogenization of phylogenetic diversity. *Nature Communications* **11**, 4663. doi:10.1038/s41467-020-18343-6

Sauquet H, Weston PH, Anderson CL, Barker NP, Cantrill DJ, Mast AR, Savolainen V (2009) Contrasted patterns of hyperdiversification in Mediterranean hotspots. *Proceedings of the National Academy of Sciences* **106**, 221–225. doi:10.1073/pnas.0805607106

Scherson RA, Thornhill AH, Urbina-Casanova R, Freyman WA, Pliscoff PA, Mishler BD (2017) Spatial phylogenetics of the vascular flora of Chile. *Molecular Phylogenetics and Evolution* **112**, 88–95. doi:10.1016/j.ympev.2017.04.021

Schmidt-Lebuhn AN, Knerr NJ, Miller JT, Mishler BD (2015) Phylogenetic diversity and endemism of Australian daisies (Asteraceae). *Journal of Biogeography* **42**(6), 1114–1122. doi:10.1111/jbi.12488

Simpson J, Conran JG, Biffin E, Waycott M (2022) The *Crinum flaccidum* (Amaryllidaceae) species complex in Australia. *Australian Systematic Botany* **35**, 395–402. doi:10.1071/SB21038

Smith SA, Moore MJ, Brown JW, Yang Y (2015) Analysis of phylogenomic datasets reveals conflict, concordance, and gene duplications with examples from animals and plants. *BMC Evolutionary Biology* **15**, 150. doi:10.1186/s12862-015-0423-0

Smith SA, Walker-Hale N, Walker JF, Brown JW (2020) Phylogenetic conflicts, combinability, and deep phylogenomics in plants. *Systematic Biology* **69**, 579–592. doi:10.1093/sysbio/syz078

Sniderman JMK, Jordan GJ, Cowling RM (2013) Fossil evidence for a hyperdiverse sclerophyll flora under a non-Mediterranean-type climate. *Proceedings of the National Academy of Sciences* **110**, 3423–3428. doi:10.1073/pnas.1216747110

Stamatakis A (2014) *RAxML* version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**(9), 1312–1313. doi:10.1093/bioinformatics/btu033

Stevenson LA, González-Orozco CE, Knerr N, Cargill DC, Miller JT (2012) Species richness and endemism of Australian bryophytes. *Journal of Bryology* **34**, 101–107. doi:10.1179/1743282012Y.0000000004

Stull GW, Soltis PS, Soltis DE, Gitzendanner MA, Smith SA (2020) Nuclear phylogenomic analyses of asterids conflict with plastome trees and support novel relationships among major lineages. *American Journal of Botany* **107**, 790–805. doi:10.1002/ajb2.1468

Stull GW, Qu X-J, Parins-Fukuchi C, Yang Y-Y, Yang J-B, Yang Z-Y, Hu Y, Ma H, Soltis PS, Soltis DE, Li DZ, Smith SA, Yi TS (2021) Gene duplications and phylogenomic conflict underlie major pulses of phenotypic evolution in gymnosperms. *Nature Plants* **7**, 1015–1025. doi:10.1038/s41477-021-00964-4

Stull GW, Pham KK, Soltis PS, Soltis DE (2023) Deep reticulation: the long legacy of hybridization in vascular plant evolution. *The Plant Journal* **114**, 743–766. doi:10.1111/tpj.16142

Thornhill AH, Mishler BD, Knerr NJ, González-Orozco CE, Costion CM, Crayn DM, Laffan SW, Miller JT (2016) Continental-scale spatial phylogenetics of Australian angiosperms provides insights into ecology, evolution and conservation. *Journal of Biogeography* **43**, 2085–2098. doi:10.1111/jbi.12797

Thornhill AH, Baldwin BG, Freyman WA, Nosratinia S, Kling MM, Morueta-Holme N, Madsen TP, Ackerly DD, Mishler BD (2017) Spatial phylogenetics of the native California flora. *BMC Biology* **15**, 96. doi:10.1186/s12915-017-0435-x

Tietje M, Antonelli A, Forest F, Govaerts R, Smith SA, Sun M, Baker WJ, Eiserhardt WL (2023) Global hotspots of plant phylogenetic diversity. *New Phytologist* **240**, 1636–1646. doi:10.1111/nph.19151

Toro-Núñez O, Lira-Noriega A (2020) Discordant phylogenetic endemism patterns in a recently diversified Brassicaceae lineage from the Atacama Desert: when choices in phylogenetics and species distribution information matter. *Journal of Biogeography* **47**, 1792–1804. doi:10.1111/jbi.13846

Tsakalos JL, Renton M, Dobrowolski MP, Veneklaas EJ, Macintyre PD, Broomfield SJ, Mucina L (2019) Composition and ecological drivers of the kwongan scrub and woodlands in the northern Swan Coastal Plain, Western Australia. *Austral Ecology* **44**, 906–916. doi:10.1111/aec.12759

Tucker CM, Cadotte MW (2013) Unifying measures of biodiversity: understanding when richness and phylogenetic diversity should be congruent. *Diversity and Distributions* **19**, 845–854. doi:10.1111/ddi.12087

Vargas OM, Ortiz EM, Simpson BB (2017) Conflicting phylogenomic signals reveal a pattern of reticulate evolution in a recent high-Andean diversification (Asteraceae: Astereae: *Diplostephium*). *New Phytologist* **214**, 1736–1750. doi:10.1111/nph.14530

Weber AA-T, Stöhr S, Chenuil A (2019) Species delimitation in the presence of strong incomplete lineage sorting and hybridization:

lessons from *Ophioderma* (Ophiuroidea: Echinodermata). *Molecular Phylogenetics and Evolution* **131**, 138–148. doi:10.1016/j.ympev.2018.11.014

Weston PH, Perkins AJ, Entwisle TJ (2005) More than symbioses: orchid ecology, with examples from the Sydney region. *Cunninghamia* **9**, 1–15.

Wilson TC, Rossetto M, Bain D, Yap JS, Wilson PD, Stimpson ML, Weston PH, Croft L (2022) A turn in species conservation for hairpin banksias: demonstration of oversplitting leads to better management of diversity. *American Journal of Botany* **109**, 1652–1671. doi:10.1002/ajb2.16074

Wilson TC, Rutherford S, Yap J-YS, Douglas SM, Lee E, Rossetto M (2023) *Eucalyptus cryptica* (Myrtaceae): a critically endangered new species. *Australian Systematic Botany* **36**, 386–400. doi:10.1071/SB22031

Winter M, Devictor V, Schweiger O (2013) Phylogenetic diversity and nature conservation: where are we? *Trends in Ecology & Evolution* **28**, 199–204. doi:10.1016/j.tree.2012.10.015

Worth JRP, Jordan GJ, Marthick JR, McKinnon GE, Vaillancourt RE (2010) Chloroplast evidence for geographic stasis of the Australian bird-dispersed shrub *Tasmannia lanceolata* (Winteraceae). *Molecular Ecology* **19**, 2949–2963. doi:10.1111/j.1365-294X.2010.04725.x

Yang Y, Smith SA (2014) Orthology inference in nonmodel organisms using transcriptomes and low-coverage genomes: improving accuracy and matrix occupancy for phylogenomics. *Molecular Biology and Evolution* **31**, 3081–3092. doi:10.1093/molbev/msu245

Yang X, Qin F, Xue T, Xia C, Gadagkar SR, Yu S (2022) Insights into plant biodiversity conservation in large river valleys in China: a spatial analysis of species and phylogenetic diversity. *Ecology and Evolution* **12**, e8940. doi:10.1002/ece3.8940

Zemunik G, Turner BL, Lambers H, Laliberté E (2015) Diversity of plant nutrient-acquisition strategies increases during long-term ecosystem development. *Nature Plants* **1**, 15050. doi:10.1038/nplants.2015.50

Zhang C, Mirarab S (2022) Weighting by gene tree uncertainty improves accuracy of quartet-based species trees. *Molecular Biology and Evolution* **39**, msac215. doi:10.1093/molbev/msac215

Zhang C, Sayyari E, Mirarab S (2017) *ASTRAL-III*: increased scalability and impacts of contracting low support branches. In 'Proceedings of the Comparative Genomics: 15th International Workshop, RECOMB CG 2017', 4–6 October 2017, Barcelona, Spain. (Eds J Meidanis, L Nakhleh) Lecture Notes in Computer Science, vol. 10562, pp. 53–75. (Springer: Cham, Switzerland) doi:10.1007/978-3-319-67979-2_4

Zhang C, Rabiee M, Sayyari E, Mirarab S (2018) *ASTRAL-III*: polynomial time species tree reconstruction from partially resolved gene trees. *BMC Bioinformatics* **19**, 153. doi:10.1186/s12859-018-2129-y

Zhang C, Nielsen R, Mirarab S (2025) *ASTER*: a package for large-scale phylogenomic reconstructions. *Molecular Biology and Evolution* **42**, msaf172. doi:10.1093/molbev/msaf172

Zhang Q, Feild TS, Antonelli A (2015) Assessing the impact of phylogenetic incongruence on taxonomy, floral evolution, biogeographical history, and phylogenetic diversity. *American Journal of Botany* **102**, 566–580. doi:10.3732/ajb.1400527

Zhou B-F, Yuan S, Crowl AA, Liang Y-Y, Shi Y, Chen X-Y, An Q-Q, Kang M, Manos PS, Wang B (2022) Phylogenomic analyses highlight innovation and introgression in the continental radiations of Fagaceae across the Northern Hemisphere. *Nature Communications* **13**, 1320. doi:10.1038/s41467-022-28917-1

Zuntini AR, Carruthers T, Maurin O, Bailey PC, Leempoel K, Brewer GE, Epitawalage N, Françoso E, Gallego-Paramo B, McGinnie C, Negrão R, Roy SR, Simpson L, Toledo Romero E, Barber VMA, Botigué L, Clarkson JJ, Cowan RS, Dodsworth S, Johnson MG, Kim JT, Pokorny L, Wickett NJ, Antar GM, DeBolt L, Gutierrez K, Hendriks KP, Hoewener A, Hu A-Q, Joyce EM, Kikuchi IABS, Larridon I, Larson DA, de Lírio EJ, Liu J-X, Malakasi P, Przelomska NAS, Shah T, Viruel J, Allnutt TR, Ameka GK, Andrew RL, Appelhans MS, Arista M, Ariza MJ, Arroyo J, Arthan W, Bachelier JB, Bailey CD, Barnes HF, Barrett MD, Barrett RL, Bayer RJ, Bayly MJ, Biffin E, Biggs N, Birch JL, Bogarín D, Borosova R, Bowles AMC, Boyce PC, Bramley GLC, Briggs M, Broadhurst L, Brown GK, Bruhl JJ, Bruneau A, Buerki S, Burns E, Byrne M, Cable S, Calladine A, Callmander MW, Cano Á, Cantrill DJ, Cardinal-McTeague WM, Carlsen MM, Carruthers AJA, de Castro Mateo A, Chase MW, Chatrou LW, Cheek M, Chen S, Christenhusz MJM, Christin P-A, Clements MA, Coffey SC, Conran JG, Cornejo X, Couvreur TLP, Cowie ID, Csiba L, Darbyshire I, Davidse G, Davies NMJ, Davis AP, van Dijk K, Downie SR, Duretto MF, Duvall MR, *et al.* (2024) Phylogenomics and the rise of the angiosperms. *Nature* **629**, 843–850. doi:10.1038/s41586-024-07324-0

Data availability. Paired sequenced reads from this study were uploaded onto the sequence read archive (SRA) at NCBI (BioProject PRJNA887684; submission number SUB13310338, for Ozbaits).

Conflicts of interest. Andrew Thornhill and Michelle Waycott are Associate Editors of *Australian Systematic Botany*, however these authors took no part in the review and acceptance of this manuscript, in line with the publishing policy. The authors declare that they have no further conflicts of interest.

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