Big trees of small baskets: phylogeny of the Australian genus Spyridium (Rhamnaceae: Pomaderreae), focusing on biogeographic patterns and species circumscriptions

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Big trees of small baskets: phylogeny of the Australian genus *Spyridium* (Rhamnaceae: Pomaderreae), focusing on biogeographic patterns and species circumscriptions

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ABSTRACT

Spyridium Fenzl is a genus of ~45 species endemic to south-western and south-eastern Australia. This study provides the most comprehensive phylogenies of *Spyridium* to date, analysing both entire chloroplast genomes and the nuclear ribosomal array (*18S–5.8S–26S*). There was substantial incongruence between the chloroplast and nuclear phylogenies, creating phylogenetic uncertainty, but some clear relationships and biogeographic patterns could be established. Analyses support the monophyly of *Spyridium*, identifying an early east–west split at the base of the nuclear phylogeny and deep divergences of New South Wales and Tasmanian endemic clades. We also found evidence of more recent dispersal events between eastern and western Australia and between Tasmania and the mainland. Eleven taxa were found to be monophyletic in the nrDNA phylogeny and two were clearly polyphyletic (*S. eriocephalum* Fenzl and *S. phylicoides* Reissek). Although the polyphyly of *S. eriocephalum* correlates with the two varieties, suggesting distinct taxa, further research is required on *S. phylicoides*.

Keywords: biogeography, chloroplast genome, molecular phylogeny, next-generation sequencing, nuclear ribosomal DNA, Rhamnaceae, species delimitation, *Spyridium*.

Introduction

Spyridium Fenzl is a member of the tribe Pomaderreae Reissek ex Endl., which includes 10 genera and ~230 species endemic to Australia and New Zealand (Kellermann *et al.* 2008). *Spyridium* is derived from the Greek word *spyridion* meaning 'a small basket'; a reference to the flowerheads surrounded by leafy bracts (Perrin 2018). The tribe is easily distinguished within the family Rhamnaceae and is characterised by species with stellate hairs on at least some of the vegetative or floral parts.

Generic boundaries within Pomaderreae have been difficult to define (Thiele and West 2004). According to Bentham (1863, p. 410):

> …most [genera], even the most natural ones, are difficult to characterize. The differences in their flowers and fruits are very trifling; they often pass into one another by the finest gradations, and habit, foliage and inflorescence must often be relied upon for fixing generic limits.

Consequently, numerous species within the tribe have been transferred from one genus to another (and in some cases back), including species from *Cryptandra* Sm. and *Spyridium*.

The distinction between *Cryptandra* and *Spyridium* had traditionally been the presence of a floral tube in the former and its absence (or close to it) in the latter. However, for some species from both genera, the floral tube grades from effectively absent to very much present, blurring these traditional generic boundaries. Recently, more distinct

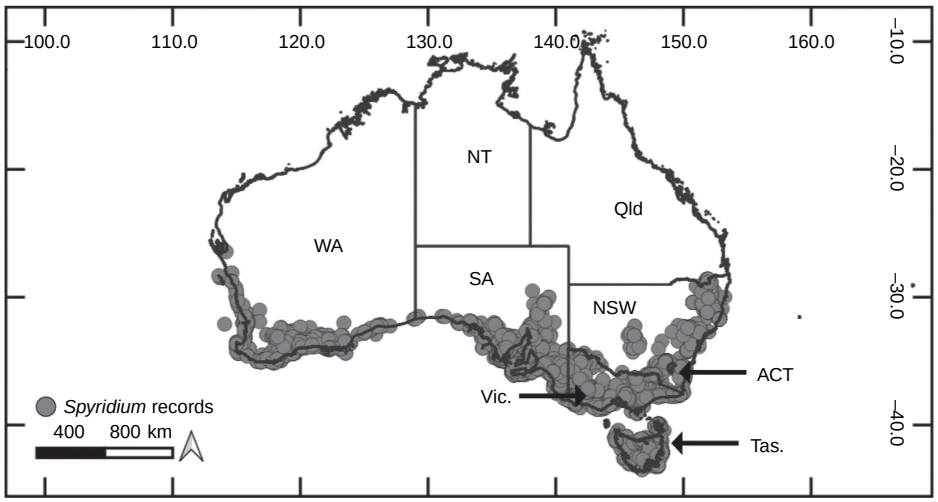

Fig. 1. Distribution of *Spyridium* in Australia. Dots represent filtered records accessed from Atlas of Living Australia (2020). States and territories are also highlighted as follows: WA, Western Australia; SA, South Australia; NT, Northern Territory; Qld, Queensland; NSW, New South Wales; ACT, Australian Capital Territory; Vic., Victoria and Tas., Tasmania.

characters between the two genera have been described (including disc, stipule and inflorescence characters), resulting in the transfer of several species of *Cryptandra* to *Spyridium*, including *S. scortechinii*[1] and *S. buxifolium* (Thiele and West 2004). In one extreme example, *S. waterhousei* has been moved to several genera within the tribe (including *Cryptandra* and *Stenanthemum* Reissek) before being re-instated as *Spyridium waterhousei* (Kellermann 2007) on the basis of its clear placement in molecular phylogenetic analyses (Kellermann *et al.* 2005).

The genus *Spyridium* currently includes ~45 species (Kellermann and Barker 2012) distributed in semi-arid to temperate regions of southern Australia (Fig. 1), including at least six potentially undescribed species identified as part of work towards a *Flora of Australia* treatment (Kellermann *et al.* 2022). The genus has two hotspots of local endemism, including ~20 species in southern South Australia (SA) and ~15 species in south-western Western Australia (WA). While the majority of species endemic to the south-west are confined to that region, two species have disjunct distributions across the Nullarbor Plain (*S. tricolor* and *S. subochreatum*). In south-eastern Australia, many of the species have narrow distributions, but several taxa (*S. parvifolium*, *S. vexilliferum* var. *vexilliferum*, *S. eriocephalum* var. *eriocephalum*) occur widely in this area (Coates and Kirkpatrick 1999; Kellermann and Barker 2012).

The monophyly of *Spyridium* has been confirmed by multiple molecular phylogenies (Richardson *et al.* 2004; Kellermann *et al.* 2005; Kellermann and Udovicic 2007; Hauenschild *et al.* 2016, 2018). The first nuclear DNA-based phylogeny (ITS) to focus on the tribe Pomaderreae was published by Kellermann *et al.* (2005), including 15 species of *Spyridium*. *Spyridium* was strongly supported as monophyletic, and four geographically based clades were identified, with one being endemic to the eastern mainland, one from Tasmania, one being endemic to the south-west, and one including species from south-eastern Australia more generally. In addition, two species were moved from other genera to *Spyridium* (*S. daltonii* from *Trymalium* and *S. waterhousei* from *Cryptandra*). A chloroplast DNA phylogeny (*trn*L–*trn*F) of the tribe, which included the same (now) 17 species of *Spyridium* (Kellermann and Udovicic 2007), did not resolve monophyly of the genus, instead species of *Spyridium* formed part of a large polytomy at the base of the tree. But, like the findings of the ITS phylogeny, species endemic to the east and Tasmania formed separate clades. Finally, Hauenschild *et al.* (2018) included 18 species of *Spyridium* in their study on Gondwanan biogeography of ziziphoid Rhamnaceae, on the basis of nuclear, plastid and mitochondrial markers, and found that eastern endemics diverged first, followed by Tasmanian endemics and, finally, those endemic to the south-west. These studies have provided valuable information about relationships in *Spyridium* and suggest early divergence of endemic groups from the south-west, east and Tasmania. However, more than half of the species of *Spyridium* have not yet been included in a molecular phylogeny.

The aim of this study is to produce a comprehensive molecular phylogeny for the genus *Spyridium*, by including all described species and analysing the full chloroplast genome (cpDNA) and the *18S–5.8S–26S* array of nuclear rDNA (nrDNA). We use these phylogenies to investigate broad biogeographic patterns within *Spyridium*, to explore vicariance, dispersal and diversification in the genus and to assess the monophyly and relationships within and among currently accepted species, as well as some proposed but so far undescribed taxa.

[1]Author names for all taxa at species level and lower are shown in Table 1; vouchers and authors of phrase-name taxa are in Table 2.

Materials and methods

Taxon sampling

In total, 143 samples of *Spyridium* were analysed in this study (Table 1). All species, subspecies and varieties of *Spyridium* recognised in the Australian Plant Census (APC; CHAH 2020) were included with at least one sample, except for *S. bifidium* var. *bifidum*, which was excluded because of sequencing issues associated with low DNA yield and quality. Of the 56 taxa included in this study, 45 (when counted at both specific and intraspecific levels) were represented by more than one accession. For species with wide distributions, multiple samples from across the geographic range were included where possible. Samples of six proposed but so far undescribed taxa were also included (Table 2). Four samples from three other genera (*Pomaderris* Labill., *Cryptandra* and *Trymalium*) from the tribe Pomaderreae were included as outgroups.

For most samples, fresh leaf material was collected and dried in silica gel, along with a voucher specimen. Eight samples were obtained from existing herbarium collections at the WA Herbarium (PERTH). Herbarium voucher details are given in Table 1; many of the vouchers deposited at the University of Melbourne Herbarium (MELU) also have duplicates deposited variously at the National Herbarium of Victoria (MEL), the Tasmanian Herbarium (HO), PERTH or the State Herbarium of South Australia (AD).

DNA extraction

Total genomic DNA was extracted from ~60 mg of silica-dried leaf material, or ~30 mg of herbarium material, following a modified cetyl trimethylammonium bromide (CTAB) protocol (Shepherd and McLay 2011; McLay 2017) based on Doyle and Doyle (1987). Where possible, young leaf material from stem tips or dried floral leaves was selected for extraction. DNA quality and quantity were recorded using a Nanodrop 2000 (NanoDrop Products) and Qubit 2.0 fluorometer (Invitrogen) and used to inform library preparation.

Library preparation and DNA sequencing

Genomic DNA was prepared for multiplexed sequencing by using the library preparation and sequencing protocol of Schuster *et al.* (2018), with a few modifications. In total, 100 μL of each sonicated sample was transferred to a PCR plate and cleaned with solid-phase reversible immobilisation (SPRI) beads, by using a beads:sample ratio targeted to retain fragments of >300 bp (Rohland and Reich 2012). Following incubation and bead capture on a 96S super magnet plate (Alpaqua), the supernatant-free sample was washed with 100 μL of 80% ethanol. All subsequent 80% ethanol washes were also performed with 100 μL. Final q-PCRs were performed with 20 μL per reaction.

Sequence assembly

Quality filtering and base calling was conducted at Walter and Eliza Hall Institute of Medical Research (WEHI) with Illumina pipeline software (ver. 1.7) and pre-processed with custom scripts, as in Schuster *et al.* (2018). De-multiplexed reads were imported into Geneious (ver. 10.2, Dotmatics, see https://www.geneious.com/; Kearse *et al.* 2012) and trimmed using an error probability limit of 0.05. Paired-end reads were set by name and contigs were built in CLC Genomics Workbench (ver. 10.0.1, QIAGEN, see https://digitalinsights.qiagen.com/) by using default *de novo* settings. Contigs less than 1800 bp were discarded and remaining contigs were trimmed, removing 150 bp from each end.

Nuclear rDNA (*18S–5.8S–26S,* including both internal transcribed spacers and partial external and non-transcribed spacers) sequences were initially assembled by mapping contigs to the reference sequence for *Helianthus annuus* L. (GenBank number: KF767534), because there was no suitable extended (5′ETS–3′ETS + NTS) nrDNA reference for *Spyridium* or close relatives. Although *Helianthus* is not closely related to *Spyridium*, the highly conserved nature of sections of nrDNA (Jobes and Thien 1997), and success building long contigs spanning the region, resulted in successful mapping. Contigs were separated where required to assist mapping and, once completed, annotations were transferred from the reference and a draft consensus sequence was generated, with gaps preserved and the reference sequence used where data were missing. Paired reads were mapped to the draft nrDNA sequence for quality-control purposes, with a base calling threshold of 75% employed. The consensus sequence was manually adjusted where required and a final consensus sequence generated. Once the first nrDNA sequence of *Spyridium* was finalised (CC211; Table 1), this new sequence was used as the reference for subsequent sequence building.

The cpDNA genome for each sample was assembled using the same methodology as for the nrDNA sequences but using a different reference sequence (*S. parvifolium* var. *parvifolium*, GenBank accession MH234313; Clowes *et al.* 2018) and with the threshold for base calls in the final consensus sequence set at 50%. In addition, contigs and paired reads could map to multiple best-fit locations to enable the assembly of the inverted repeats.

Phylogenetic analyses

Sequences were aligned using the MAFFT (ver. 7.308, see https://mafft.cbrc.jp/alignment/software/; Katoh *et al.* 2002; Katoh and Standley 2013) Geneious plugin under default settings. Aligned sequences were reviewed in Geneious, base pairs were re-aligned by eye where required and ambiguous regions were excluded from the final alignment. In addition, inverted repeat A (IRA) was excluded from the cpDNA alignment at this stage.

Aligned sequences were partitioned before model testing and phylogenetic analyses. For the nrDNA, the alignment

Table 1. Voucher information for samples included in this study.

Species	Collecting number	Voucher	Location	Nuclear rDNA GenBank accession number	Chloroplast genome GenBank accession number
Cryptandra amara Sm.	CC377	MELU	Cultivated; origin south-eastern Australia[A]	OK481389	OK624202
Pomaderris rotundifolia[B] (F.Muell.) Rye	L.S.J. Sweedman 7822	PERTH08703434	Cape Arid NP; Mallee; WA	OK481390	OK624203
Pomaderris vacciniifolia Reissek	CC378	MELUD117648a	Cultivated; South Eastern Highlands; origin Vic.	OK481391	OK624204
Spyridium bifidum subsp. *wanillae* Kellermann & W.R.Barker	JK587	AD272790	Wanilla; Eyre Yorke Block; SA	OK481392	OK624205
Spyridium bifidum subsp. *wanillae*	RMF374	MELUD122295a	Wanilla; Eyre Yorke Block; SA	OK481393	OK624206
Spyridium burragorang K.R.Thiele	CANB606 176[C]	CBG9611161	Cultivated; origin NSW	OK481394	OK624346
Spyridium burragorang	CC567[D]	MELUD122292a	Cessnock; Sydney Basin; NSW	OK481395	OK624347
Spyridium buxifolium (Fenzl) K.R.Thiele	CC504	MELUD117685a	Denman; Sydney Basin IBRA; NSW	OK481396	OK624207
Spyridium buxifolium	CC568[C]	MELUD122291a	Goulburn River NP; Sydney Basin; NSW	OK481397	OK624208
Spyridium cinereum N.A.Wakef.	CC428	MELUD117664a	Grampians NP; Victorian Midlands; Vic.	OK481398	OK624209
Spyridium cinereum	CC498	MELUD117683a	Croajingolong NP; South East Corner; Vic.	OK481399	OK624210
Spyridium coactilifolium Reissek	CC322	MELUD117633a	Victor Harbor; Kanmantoo; SA	OK481400	OK624211
Spyridium coactilifolium	CC562	MELUD122276a	Newland Head CP; Kanmantoo; SA	OK481401	OK624212
Spyridium coalitum Kellermann & W.R.Barker	CC554	MELUD122269a	Kangaroo Island; Kanmantoo; SA	OK481402	OK624213
Spyridium coalitum	JK521	AD232557	Kangaroo Island; Kanmantoo; SA	OK481403	OK624214
Spyridium cordatum (Turcz.) Benth.	CC528	MELUD117699a	Gairdner, Esperance Plains; WA	OK481404	OK624215
Spyridium cordatum	CC530	MELUD117701a	Jerdacuttup; Esperance Plains; WA	OK481405	OK624216
Spyridium cordatum	GFC10247	MELUD122286a	Ravensthorpe; Esperance Plains; WA	OK481406	OK624217
Spyridium cordatum	JK370	AD213501	Ravensthorpe; Esperance Plains; WA	OK481408	OK624219
Spyridium cordatum	JK361	AD213497	Lake King; Mallee; WA	OK481407	OK624218

(*Continued on next page*)

Table 1. (*Continued*)

Species	Collecting number	Voucher	Location	Nuclear rDNA GenBank accession number	Chloroplast genome GenBank accession number
Spyridium daltonii (F.Muell.) Kellermann	CC513	MELUD117686a (CC505)	Grampians NP; Victorian Midlands; Vic.	OK481409	OK624220
Spyridium daphnoides (Reissek) Kellermann	CC550	MELUD122265a	Kangaroo Island; Kanmantoo; SA	OK481410	OK624221
Spyridium daphnoides	CC561	MELUD122275a	Deep Creek CP; Kanmantoo; SA	OK481411	OK624222
Spyridium daphnoides	JK721	AD283745	Lock; Eyre Yorke Block; SA	OK481412	OK624223
Spyridium eriocephalum Fenzl var. *eriocephalum*	CC515	MELUD117687a	Long Forest Nature Conservation Reserve; Victorian Midlands; Vic.	OK481413	OK624224
Spyridium eriocephalum var. *eriocephalum*	WAP01	MELUD122301a	East Risdon NR; Tasmanian South East; Tas.	OK481414	OK624225
Spyridium eriocephalum var. *glabrisepalum* J.M.Black	CC551	MELUD122266a	Kangaroo Island; Kanmantoo; SA	OK481415	OK624226
Spyridium eriocephalum var. *glabrisepalum*	JK484	AD232614	Kangaroo Island; Kanmantoo; SA	OK481416	OK624227
Spyridium erymnocladum W.R.Barker	JK729	AD283738	E of Hincks Wilderness Protection Area; Eyre Yorke Block; SA	OK481417	OK624228
Spyridium fontis-woodii Kellermann & W.R.Barker	CC563	MELUD122277a	Coorong; Naracoorte Coastal Plain; SA	OK481418	OK624229
Spyridium fontis-woodii	JK441	AD213808	Coorong; Naracoorte Coastal Plain; SA	OK481419	OK624230
Spyridium furculentum W.R.Barker & Kellermann	MJB2558	MELUD122313a	Little Desert; MDD; Vic.	OK481420	OK624231
Spyridium glaucum Rye	CC534	MELUD117705a	Ravensthorpe; Esperance Plains; WA	OK481421	OK624232
Spyridium globulosum (Labill.) Benth.	CC517	MELUD117688a	Woodman Point Recreation Reserve; Swan Coastal Plain; WA	OK481422	OK624233
Spyridium globulosum	EMS2413	MELUD122282a	Torndirrup NP; Warren; WA	OK481423	OK624234
Spyridium globulosum	GFC10245	MELUD122284a	Hopetoun; Esperance Plains; WA	OK481424	OK624235
Spyridium gunnii (Hook.f.) Benth.	WAP12	MELUD122305a	Renison Bell; Tasmanian West; Tas.	OK481425	OK624236
Spyridium gunnii	WAP23	MELUD122323a	Molesworth Conservation Area; Tasmanian South East; Tas.	OK481426	OK624237

(*Continued on next page*)

Table 1. (*Continued*)

Species	Collecting number	Voucher	Location	Nuclear rDNA GenBank accession number	Chloroplast genome GenBank accession number
Spyridium halmaturinum F.Muell. ex Benth.	CC548	MELUD122263a	Kangaroo Island; Kanmantoo; SA	OK481427	OK624238
Spyridium halmaturinum	JK497	AD232543	Kangaroo Island; Kanmantoo; SA	OK481428	OK624239
Spyridium halmaturinum	JK533	AD232599	Kangaroo Island; Kanmantoo; SA	OK481429	OK624240
Spyridium halmaturinum	JK536	AD232602	Kangaroo Island; Kanmantoo; SA	OK481430	OK624241
Spyridium lawrencei (Hook.f.) Benth.	CC237	MELUD117616a	Royal George; Tasmanian Northern Midlands; Tas.	OK481431	OK624242
Spyridium lawrencei	WAP20	MELUD122311a	Three Thumbs State Reserve; Tasmanian South East; Tas.	OK481432	OK624243
Spyridium leucopogon (F.Muell. ex Reissek) F.Muell.	JK572	AD272772	Ungarra; Eyre Yorke Block; SA	OK481433	OK624244
Spyridium majoranifolium (Fenzl) Rye	CC521	MELUD117692a	Lake Pleasant View NR; Jarrah Forest; WA	OK481434	OK624245
Spyridium majoranifolium	CC529	MELUD117700a	Stirling Range NP; Esperance Plains; WA	OK481435	OK624246
Spyridium majoranifolium	CC538	MELUD117709a	Ravensthorpe; Esperance Plains; WA	OK481436	OK624247
Spyridium majoranifolium	CC540	MELUD117711a	Jerdacuttup Lakes NR; Esperance Plains; WA	OK481437	OK624248
Spyridium majoranifolium	EMS2412	MELUD122281a	Torndirrup NP; Warren; WA	OK481438	OK624249
Spyridium microcephalum (Turcz.) Benth.	CC527	MELUD117698a	Gairdner; Esperance Plains; WA	OK481439	OK624250
Spyridium microcephalum	KRM826	MELUD122289a	Cape Arid NP; Esperance Plains; WA	OK481440	OK624251
Spyridium minutum Rye	CC542	MELUD117713a	Scaddan; Mallee; WA	OK481441	OK624252
Spyridium minutum	G.J. Keighery & N. Gibson 4183	PERTH07624697	Grass Patch; Mallee; WA	OK481442	OK624253
Spyridium montanum Rye	DAR 206	PERTH08742278	Stirling Range NP; Esperance Plains; WA	OK481443	OK624254
Spyridium montanum	S. Barrett 962	PERTH06045588	Stirling Range NP; Esperance Plains; WA	OK481444	OK624255

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Table 1. (*Continued*)

Species	Collecting number	Voucher	Location	Nuclear rDNA GenBank accession number	Chloroplast genome GenBank accession number
Spyridium mucronatum Rye subsp. *mucronatum*	CC522	MELUD117693a	Kojaneerup South; Esperance Plains; WA	OK481445	OK624256
Spyridium mucronatum subsp. *mucronatum*	CC526	MELUD117697a	Needilup; Mallee; WA	OK481446	OK624257
Spyridium mucronatum subsp. *recurvum* Rye	CC523	MELUD117694a	Kojaneerup South; Esperance Plains; WA	OK481448	OK624259
Spyridium mucronatum subsp. *recurvum*	CC532	MELUD117703a	Cheadanup; Mallee; WA	OK481449	OK624260
Spyridium mucronatum subsp. *recurvum*	GFC10244	MELUD122283a	Ravensthorpe; Esperance Plains; WA	OK481450	OK624261
Spyridium mucronatum subsp. *multiflorum* Rye	S. Barrett 1427	PERTH07354827	Fitzgerald River NP; Esperance Plains; WA	OK481447	OK624258
Spyridium nitidum N.A.Wakef.	JK477	AD232608	Kangaroo Island; Kanmantoo; SA	OK481451	OK624262
Spyridium nitidum	JK584	AD272784	Wanilla; Eyre Yorke Block; SA	OK481452	OK624263
Spyridium obcordatum (Hook.f.) W.M.Curtis	CC239	MELUD117618a	Greens Beach; Furneaux; Tas.	OK481453	OK624264
Spyridium obcordatum	CC284	MELUD117626a	Shearwater; Furneaux; Tas.	OK481454	OK624265
Spyridium obovatum (Hook.) Benth. var. *obovatum*	CC235	MELUD117614a	Apsley Conservation Area; Tasmanian South East; Tas.	OK481455	OK624266
Spyridium obovatum var. *obovatum*	WAP17	MELUD122309a	Douglas-Apsley NP; Tasmanian South East; Tas.	OK481456	OK624267
Spyridium obovatum var. *velutinum* (F.Muell. ex Reissek) Benth.	CC236	MELUD117615a	Friendly Beaches; Tasmanian South East; Tas.	OK481457	OK624268
Spyridium obovatum var. *velutinum*	WAP08	MELUD122303a	Snug; Tasmanian South East; Tas.	OK481458	OK624269
Spyridium obovatum var. *velutinum*	WAP15	MELUD122306a	Tasman NP; Tasmanian South East; Tas.	OK481459	OK624270
Spyridium obovatum var. *velutinum*	WAP16	MELUD122307a	Orford; Tasmanian South East; Tas.	OK481460	OK624271
Spyridium obovatum var. *velutinum*	WAP19	MELUD122310a	Sandspit River Forest Reserve; Tasmanian South East; Tas.	OK481461	OK624272
Spyridium oligocephalum (Turcz.) Benth.	CC536	MELUD117707a	Hopetoun; Esperance Plains; WA	OK481462	OK624273

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Table 1. (*Continued*)

Species	Collecting number	Voucher	Location	Nuclear rDNA GenBank accession number	Chloroplast genome GenBank accession number
Spyridium parvifolium (Hook.) F.Muell.	CC057	MELUD117595a	Brisbane Ranges; Victorian Midlands; Vic.	OK481463	OK624274
Spyridium parvifolium	CC174	MELUD117609a	Dandenong Ranges NP; South Eastern Highlands; Vic.	OK481464	OK624275
Spyridium parvifolium	CC211	MELUD155066a	Rocky Cape NP; King; Tas.	OK481465	MH234313
Spyridium parvifolium	CC273	MELUD117623a (CC265)	Flinders Island; Furneaux; Tas.	OK481466	OK624276
Spyridium parvifolium	CC433	MELUD117665a	Grampians NP; Victorian Midlands; Vic.	OK481467	OK624277
Spyridium parvifolium	CC490	MELUD117682a (CC484)	Cann River; South East Corner; Vic.	OK481468	OK624278
Spyridium parvifolium	MJB2239E	MELUD118621a	Mount Buffalo NP; South Eastern Highlands; Vic.	OK481469	OK624279
Spyridium parvifolium	MJB2288A	MELUD118616a	Burrinjuck Waters State Park; Southern Highlands; NSW	OK481470	OK624280
Spyridium phlebophyllum (F.Muell.) F.Muell.	RMF388	MELUD122296a	Ikara–Flinders Ranges NP; Flinders Lofty Block; SA	OK481471	OK624281
Spyridium phylicoides Reissek	CC557	MELUD122272a	Kangaroo Island; Kanmantoo; SA	OK481472	OK624282
Spyridium phylicoides	CC565	MELUD122279a	Coorong; Naracoorte Coastal Plain; SA	OK481473	OK624283
Spyridium phylicoides	JK487A2	AD232583	Kangaroo Island; Kanmantoo; SA	OK481474	OK624284
Spyridium phylicoides	JK593	AD273231	Coffin Bay NP; Eyre Yorke Block; SA	OK481475	OK624285
Spyridium polycephalum (Turcz.) Rye	CC525	MELUD117696a	Needilup; Mallee; WA	OK481476	OK624286
Spyridium polycephalum	CC535	MELUD117706a	Ravensthorpe; Esperance Plains; WA	OK481477	OK624287
Spyridium riparium Rye	CC518	MELUD117689a	Mount Lindesay NP; Jarrah Forest; WA	OK481478	OK624288
Spyridium riparium	CC519	MELUD117690a	Kentdale; Warren; WA	OK481479	OK624289
Spyridium scabridum (Tate) Kellermann & W.R.Barker	CC553	MELUD122268a	Kangaroo Island; Kanmantoo; SA	OK481480	OK624290

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Table 1. (*Continued*)

Species	Collecting number	Voucher	Location	Nuclear rDNA GenBank accession number	Chloroplast genome GenBank accession number
Spyridium scabridum	JK502	AD232548	Kangaroo Island; Kanmantoo; SA	OK481481	OK624291
Spyridium scabridum	JK527	AD232565	Kangaroo Island; Kanmantoo; SA	OK481482	OK624292
Spyridium scortechinii (F.Muell.) K.R.Thiele	CC503	MELUD117684a	Tilba Tilba; South East Corner; NSW	OK481483	OK624293
Spyridium scortechinii	CC569[E]	MELUD122290a	Kurrajong Heights; Sydney Basin; NSW	OK481484	OK624294
Spyridium scortechinii	JRH3933A	MELUD122298a	Woodsreef; Nandewar; NSW	OK481485	OK624295
Spyridium sp. Dwarf (J.Kellermann 579) Kellermann	JK489	AD232593	Seal Bay CP, Kangaroo Island; Kanmantoo; SA	OK481486	OK624296
Spyridium sp. Dwarf (J.Kellermann 579)	JK579	AD272779	Lincoln NP; Eyre Yorke Block; SA	OK481487	OK624297
Spyridium sp. Dwarf (J.Kellermann 579)	JK740	AD283757	Kangaroo Island; Kanmatoo; SA	OK481488	OK624298
Spyridium sp. Finniss (J.Kellermann 653 & F. Nge) Kellermann	JK653	AD283669	Bullock Hill CP; Kanmantoo; SA	OK481489	OK624299
Spyridium sp. Finniss (J.Kellermann 653 & F. Nge)	JK661	AD283668	Currency Creek; Kanmantoo; SA	OK481490	OK624300
Spyridium sp. Jerdacuttup (A.Williams 332) WA Herbarium	CC533	MELUD117704a	Jerdacuttup; Esperance Plains; WA	OK481491	OK624301
Spyridium sp. Jerdacuttup (A.Williams 332)	KRM738	MELUD122287a	WA36183 NR; Esperance Plains; WA	OK481492	OK624302
Spyridium sp. Kangaroo Island (W.R.Barker 7560) Kellermann	JK510	AD232574	Kangaroo Island; Kanmantoo; SA	OK481495	OK624305
Spyridium sp. Kangaroo Island (W.R.Barker 7560)	JK511	AD232575	Kangaroo Island; Kanmantoo; SA	OK481496	OK624306
Spyridium sp. Kangaroo Island (W.R.Barker 7560)	JK518	AD232582	Kangaroo Island; Kanmantoo; SA	OK481497	OK624307
Spyridium sp. Kangaroo Island (W.R.Barker 7560)	JK504A	AD232552	Kangaroo Island; Kanmantoo; SA	OK481494	OK624304
Spyridium sp. Kangaroo Island (W.R.Barker 7560)	JK526A	AD232561	Kangaroo Island; Kanmantoo; SA	OK481498	OK624308

(*Continued on next page*)

Table 1. (*Continued*)

Species	Collecting number	Voucher	Location	Nuclear rDNA GenBank accession number	Chloroplast genome GenBank accession number
Spyridium sp. Kangaroo Island (W.R.Barker 7560)	CC556	MELUD122271a	Kangaroo Island; Kanmantoo; SA	OK481493	OK624303
Spyridium sp. Red dots (J.Kellermann 689) Kellermann	JK689	AD283725	Warramboo; Eyre Yorke Block; SA	OK481499	OK624309
Spyridium sp. Wollar (E.F.Constable *s.n.*, NSW 16590) Kellermann	CC566[F]	MELUD122293a	Wollar; Sydney Basin; NSW	OK481500	OK624310
Spyridium spadiceum (Fenzl) Benth.	CC520	MELUD117691a	Gull Rock NP; Jarrah Forest; WA	OK481501	OK624311
Spyridium stenophyllum subsp. *renovatum* Kellermann & W.R.Barker	CC345	MELUD117636a	Cowell; Eyre Yorke Block; SA	OK481503	OK624313
Spyridium stenophyllum subsp. *renovatum*	CC348	MELUD117638a	Campoona; Eyre Yorke Block; SA	OK481504	OK624314
Spyridium stenophyllum subsp. *renovatum*	RMF256	MELUD122299a	Wharminda; Eyre Yorke Block; SA	OK481507	OK624317
Spyridium stenophyllum subsp. *renovatum*	BSB838–173	AD261711	Hiltaba NR; Gawler; SA	OK481502	OK624312
Spyridium stenophyllum subsp. *renovatum*	CC546	MELUD117717a	Kimbra; Eyre Yorke Block; SA	OK481505	OK624315
Spyridium stenophyllum subsp. *renovatum*	JK561	AD273243	Cowell; Eyre Yorke Block; SA	OK481506	OK624316
Spyridium stenophyllum subsp. *renovatum*	RMF372	MELUD122294a	Rudall; Eyre Yorke Block; SA	OK481508	OK624318
Spyridium stenophyllum (Reissek) Kellermann & W.R.Barker subsp. *stenophyllum*	PSF46	MELUD122297a	Mangalo; Eyre Yorke Block; SA	OK481509	OK624319
Spyridium subochreatum (F.Muell.) Reissek	JK601	AD273246	Campoona; Eyre Yorke Block; SA	OK481514	OK624324
Spyridium subochreatum	CC371	MELUD117643a	Billiatt Wilderness Protection Area; MDD; SA	OK481511	OK624321
Spyridium subochreatum	MJB2557C	MELUD122314a	Little Desert NP; MDD; Vic.	OK481515	OK624325
Spyridium subochreatum	E.D. Adams 21/0907	PERTH08067805	Nuytsland NR; Esperance Plains; WA	OK481513	OK624323
Spyridium subochreatum	CC349	MELUD117639a	Campoona; Eyre Yorke Block; SA	OK481510	OK624320

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Table 1. (*Continued*)

Species	Collecting number	Voucher	Location	Nuclear rDNA GenBank accession number	Chloroplast genome GenBank accession number
Spyridium subochreatum	CC372	MELUD117644a	Ngarkat CP; MDD; SA	OK481512	OK624322
Spyridium thymifolium Reissek	CC298	MELUD117627a (CC285)	Mount Billy Conservation Reserve; Kanmantoo; SA	OK481516	OK624326
Spyridium tricolor W.R.Barker & Rye	CC545	MELUD117716a	Watraba; Eyre Yorke Block; SA	OK481517	OK624327
Spyridium ulicinum (Hook.) Benth.	WAP03C	MELUD122304a	Glenlusk; Tasmanian South East; Tas.	OK481519	OK624329
Spyridium ulicinum	WAP04C	MELUD122315a	Collinsvale; Tasmanian South East; Tas.	OK481520	OK624330
Spyridium ulicinum	WAP05B	MELUD122316a	Neika; Tasmanian South East; Tas.	OK481521	OK624331
Spyridium ulicinum	WAP06	MELUD122317a	Lower Longley; Tasmanian South East; Tas.	OK481522	OK624332
Spyridium ulicinum	WAP09B	MELUD122318a	Snug Tiers Nature Recreation Area; Tasmanian Southern Ranges; Tas.	OK481523	OK624333
Spyridium ulicinum	WAP10B	MELUD122319a	Ouse; Tasmanian Southern Ranges; Tas.	OK481524	OK624334
Spyridium ulicinum	WAP11C	MELUD122320a	Ouse; Tasmanian Southern Ranges; Tas.	OK481525	OK624335
Spyridium ulicinum	CC283	MELUD117625a (CC282)	Cataract Gorge Reserve; Tasmanian Northern Midlands; Tas.	OK481518	OK624328
Spyridium vexilliferum var. *latifolium* Benth.	CC464	MELUD117670a	Kingston SE; Naracoorte Coastal Plain; SA	OK481526	OK624336
Spyridium vexilliferum var. *latifolium*	CC473	MELUD117675a	Desert Camp Conservation Reserve; Naracoorte Coastal Plain; SA	OK481527	OK624337
Spyridium vexilliferum (Hook.) Reissek var. *vexilliferum*	DJM738	MEL	Grampians NP; Victorian Midlands; Vic.	OK481528	OK624338
Spyridium vexilliferum var. *vexilliferum*	WAP07G	MELUD118619a	Tom Gibson NR; Tasmanian Northern Midlands; Tas.	OK481529	OK624339
Spyridium vexilliferum var. *vexilliferum*	WAP13	MELUD122321a	Arthur Pieman Conservation Reserve; King; Tas.	OK481530	OK624340

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Table 1. (*Continued*)

Species	Collecting number	Voucher	Location	Nuclear rDNA GenBank accession number	Chloroplast genome GenBank accession number
Spyridium vexilliferum var. *vexilliferum*	WAP14	MELUD122322a	Freycinet NP; Tasmanian South East; Tas.	OK481531	OK624341
Spyridium villosum (Turcz.) Benth.	S. Barrett 2200	PERTH08773874	Stirling Range NP; Esperance Plains; WA	OK481533	OK624343
Spyridium villosum	S. Barrett 950	PERTH06045596	Stirling Range NP; Esperance Plains; WA	OK481532	OK624342
Spyridium waterhousei F.Muell.	CC558	MELUD122273a	Kangaroo Island; Kanmantoo; SA	OK481534	OK624344
Trymalium elachophyllum Rye	CC531	MELUD117702a	Cheadanup; Mallee; WA	OK481535	OK624345

Names of described taxa included in this study follow the Australian Plant Census (CHAH 2020), the exception being *S. daphnoides,* which is a recent name change not yet updated in the Census (Kellermann 2021). Phrase names were formed according to Barker (2005). Authorities have been provided with the first reference to each taxon. Collectors are as follows: CC, Catherine Clowes; JK, Juergen Kellerman; L.S.J. Sweedman, Luke Sweedman; RMS, Rachael Fowler; CANB, Australian National Botanic Gardens (living collection number); GFC, Gillian Craig; WAP, Mark Wapstra; MJB, Michael Bayly; EMS, Libby Sandiford; KRM, Kenneth Mills; G.J. Keighery, Gregory Keighery; N. Gibson, Neil Gibson; DAR, Damien Rathbone; Barrett, Sarah Barrett; BSB, Juergen Kellerman; JRH, John Hosking; PSF, Patrick Fahey. To standardise location details, for samples collected from mainland Australia, either the reserve name is provided or the nearest town (where the sample was collected outside a reserve system). Location details provided for samples collected on islands include the island name. States have also been provided for all samples. For all samples, Interim Biogeographic Regionalisation for Australia 7 (IBRA 7) regions have also been provided in location information (Department of Agriculture Water and the Environment 2020). Location abbreviations are as follows: SA, South Australia; WA, Western Australia; Vic., Victoria; Tas. Tasmania; NSW, New South Wales; NP, National Park; NR, Nature Reserve; CP, Conservation Park. Nuclear rDNA and chloroplast genome GenBank accession numbers are provided. A voucher has not yet been lodged for *S. vexilliferum* var. *vexilliferum* (DJM738), but it is accessible at the National Herbarium of Victoria upon request from Daniel J. Murphy.

[A]The state of origin for this sample was unknown, but the distribution of this species is known to be south-eastern Australia.

[B]This sample was lodged with PERTH as *Spyridium tricolor,* but was determined by Juergen Kellerman to be *Pomaderris rotundifolia* on receipt from PERTH.

[C]This number is the accession for the plant in the Australian National Botanic Gardens Living Collections.

[D]This sample was collected by Dr Stephen Bell (Eastcoast Flora Survey). A unique collecting number was not provided, so this collecting number was generated by Catherine Clowes.

[E]This sample was collected by Dr Mathew Dell (Southeast Botanical Consulting). A unique collecting number was not provided, so this collecting number was generated by Catherine Clowes.

[F]This sample was collected by Dr Stephen Bell (Eastcoast Flora Survey). A unique collecting number was not provided, so this collecting number was generated by Catherine Clowes.

Table 2. Distribution and affinities of phrase-name taxa included in this study.

Phrase name	Distribution	Morphological affinities
Spyridium sp. Kangaroo Island (W.R. Barker 7560) Kellermann [A]	Sandy soils on Kangaroo Island	Morphologically similar to *S. vexilliferum* var. *vexilliferum* and *S. thymifolium*
Spyridium sp. Dwarf (J. Kellermann 579) Kellermann[A]	Southern Eyre Peninsula (SA) and Kangaroo Island on sand over limestone	Prostrate to upright shrub with similarities to *S. phylicoides*
Spyridium sp. Red Dots (J. Kellermann 689) Kellermann[A]	Eyre Yorke Block on limestone	Like *S. phylicoides*, but with a much smaller habit
Spyridium sp. Finniss (J. Kellermann 653 & F. Nge) Kellermann[A]	North-eastern Fleurieu Peninsula	A robust shrub which has similarities to *S. eriocephalum* var. *eriocephalum* and var. *glabrisepalum*
Spyridium sp. Wollar (E.F. Constable *s.n.*; NSW16590) Kellermann[A]	Only found in an area north-west of Sydney near Dubbo; on sandy soils	Similar to both *S. eriocephalum* var. *eriocephalum* and *S. scortechinii*
Spyridium sp. Jerdacuttup (A.Williams 332) WA Herbarium[B]	Jerdacuttup region of WA	Similar to *S. cordatum* (Wilkins *et al.* 2011)

[A]Phrase name used as part of collaborative work towards a *Flora of Australia* treatment (Kellermann *et al.* 2022); published here for the first time.
[B]Phrase name currently used in Australian Plant Census (CHAH 2020).

was partitioned as follows: partial external transcribed spacer (5′ETS), *18S*, internal transcribed spacer 1 (ITS1), *5.8S*, internal transcribed spacer 2 (ITS2), *26S*, and partial non-transcribed spacer (3′ETS + NTS). The four cpDNA partitions, based on annotations from the reference sequence of *S. parvifolium* var. *parvifolium* (GenBank accession MH234313) were as follows: gene-coding sequence (CDS); transfer ribonucleic acid (tRNA); ribosomal ribonucleic acid (rRNA); and all remaining sequences, including introns and intergenic spacers (referred to in the partition as spacers).

Both nrDNA and cpDNA alignments were analysed using Bayesian inference (BI) and maximum likelihood (ML) methods. For the BI analyses, model testing was performed for each partition following Akaike's information criterion (AIC) using MrModelltest2 (ver. 2.4, J. A. A. Nylander, see https://github.com/nylander/MrModeltest2) for nrDNA (selected models: 5′ETS GTR + G, *18S* GTR + I + G, ITS1 SYM + G, ITS2 K80 + G, *26S* GTR + I + G and 3′ETS + NTS HKY + G; with *5.8S* alignment being excluded from analyses because all sequences were identical) and cpDNA (selected models: CDS GTR + I + G, tRNA K80 + I, rRNA HKY and spacers GTR + I + G). Bayesian inference analyses were undertaken in MrBayes XSEDE (ver. 3.2.6, C. Zhang, J. Huelsenbeck, P. van der Mark, F. Ronquist and M. Teslenko, see https://github.com/NBISweden/MrBayes/releases; Ronquist and Huelsenbeck 2003) by using the CIPRES portal (Miller *et al.* 2010). For the nrDNA alignment (6367 bp), two independent analyses with four chains (Markov-chain Monte Carlo) were run for 5 000 000 generations, sampling every 1000 steps, with a burnin of 25%. For the cpDNA alignment (168 343 bp), chains were run for 2 500 000 generations, sampling every 500 steps. Output files were viewed in Tracer (ver. 1.6, A. Rambaut, A. J. Drummond and M. Suchard, see https://github.com/beast-dev/tracer), checking convergence (<0.01 standard deviation of split frequencies). The 50% majority-rule consensus trees were visualised in FigTree (ver. 1.4.2, A. Rambaut and A. J. Drummond, see https://github.com/rambaut/figtree) with posterior probabilities (PP) of ≥0.95 being viewed as fully supported, and those lower considered unsupported.

Maximum likelihood analysis was performed with IQ-Tree using default settings (ver. 1.6.12, L. T. Nguyen, H. A. Schmidt, A. von Haeseler and B. Q. Minh, see https://iqtree.org/; Nguyen *et al.* 2015). IQ-Tree automatically employs ModelFinder (Kalyaanamoorthy *et al.* 2017) to select models for each partition by using Bayesian information criterion (BIC) as default. For nrDNA analysis, the models were as follows: 5′ETS TPM2 + F + G4, *18S* K2P + I + G4, ITS1 TIM2e + G4, *5.8S* JC, ITS2 TNe + G4, *26S* TIM3 + F + I and 3′ETS + NTS TN + F + G4; for cpDNA analysis, the models were as follows: CDS TVM + F + I, tRNA K2P, rRNA HKY + F and spacers TVM + F + I + G4. In total, 261 parsimony-informative characters were reported for the nrDNA ML analysis and 3129 were reported for the cpDNA ML analyses. The bootstrap consensus trees were viewed in FigTree and exported to TreeGraph 2 (ver. 2.14.0-771 beta, Bio10, see https://treegraph.bioinfweb.info/; Stöver and Müller 2010) where branches with <50% ultrafast bootstrap (UFBS) support where collapsed to allow for easier comparison with the BI tree. UFBS values of ≥95% were viewed as supported and those below 95% were considered unsupported.

The results of the nrDNA phylogeny were mapped using QGIS (ver. 3.18.3, QGIS Development Team, see http://qgis.org/en/site/index.html). Species distributions were accessed from Atlas of Living Australia (2020) and these data filtered removing samples lacking collection dates. Outliers were reviewed and removed where errors in location or identification were suspected. Taxa were mapped by clades according to the nrDNA phylogeny.

All sequences included in this study were uploaded to GenBank (Table 1). Text files containing the alignment,

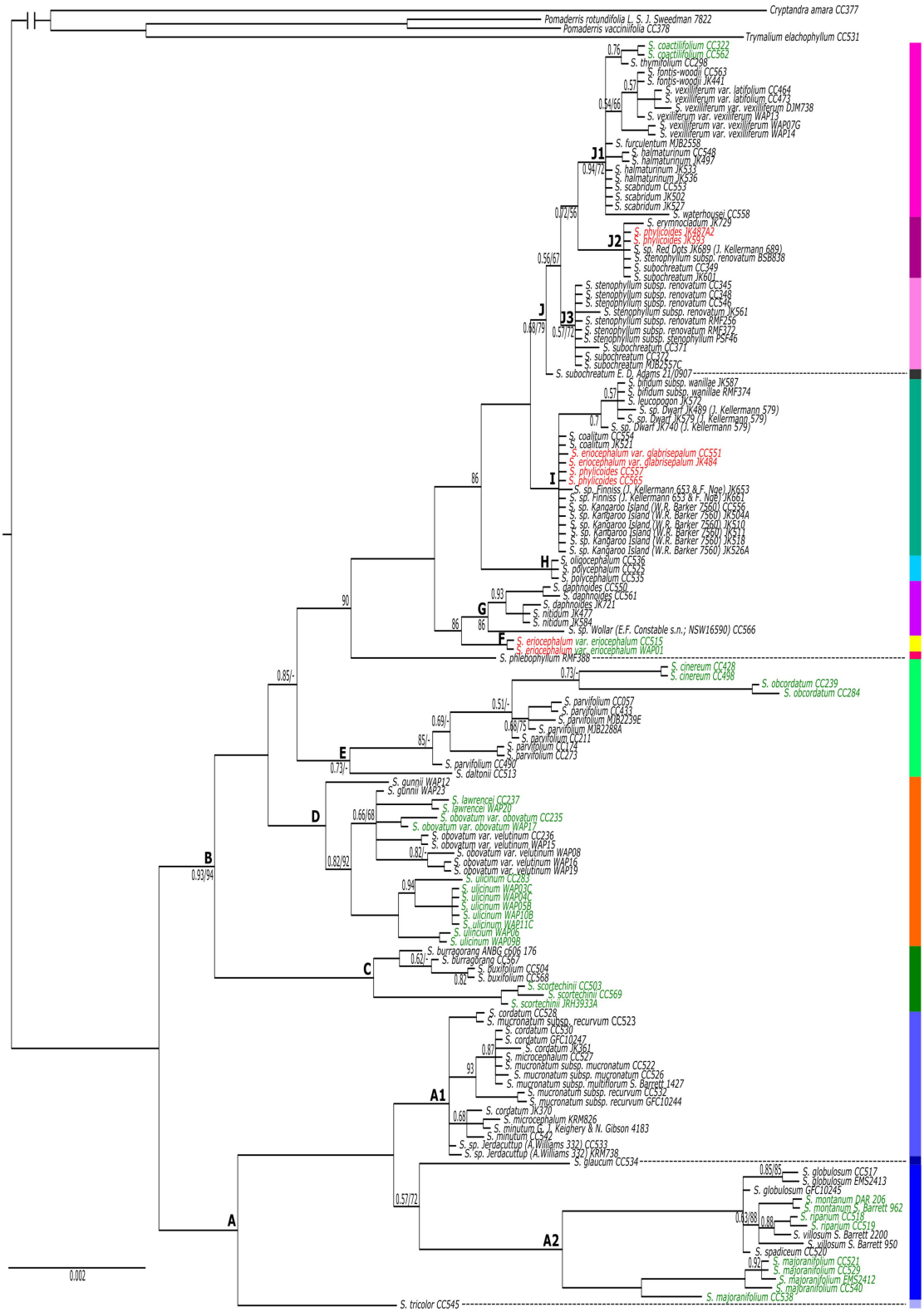

Fig. 2. (*Caption on next page*)

Fig. 2. Nuclear rDNA (nrDNA) phylogeny of *Spyridium*, based on Bayesian inference (BI) analysis. Bayesian posterior probabilities (PP) and ultrafast bootstrap (UFBS) values are shown at nodes when <95%; values ≥95% are not shown. Where one value for a node is supported (≥95%) and the other for that node is unsupported (<95%), only the unsupported value is shown. Where a hyphen (-) is provided at a node, this node varied in resolution in the ML tree and was therefore not transferable to the BI phylogeny. Colour coding of clades and taxa in the bar to the right of the tree matches that used on maps in Fig. 3, 4. Labels are given for some clades (A–J) and subclades (A1–J3) discussed in text. Species polyphyletic across clades are highlighted in red text. Monophyletic taxa with supported nodes are highlighted in green text. Note: *S. eriocephalum* is polyphyletic, but var. *eriocephalum* is monophyletic (and therefore coloured half red and half green). Dashed lines associated with *S. tricolor*, *S. glaucum*, *S. phlebophyllum* and *S. subochreatum* E.D.Adams 21/0907 are provided as reference points connecting taxa to the sidebar.

partitions, BI and ML trees for each dataset (nrDNA and cpDNA) were submitted to TreeBase (accession number 28815).

Results

Nuclear rDNA phylogeny

The topologies of BI and ML nrDNA trees were largely congruent, and only the BI tree is shown here, with ML bootstrap values mapped onto it (Fig. 2). A key supported difference in the ML tree was in the placement of *S. daltonii* as divergent from the *S. parvifolium* clade (UFBS = 95), with *S. obcordatum* and *S. cinereum* being unsupported as successive sisters to a clade of *S. parvifolium* samples (UFBS = 85 for the former and UFBS = 65 for the latter). Another supported difference was that *S. burragorang* formed a clade, sister to *S. buxifolium* (UFBS = 98).

Spyridium was resolved as monophyletic (PP = 1.0, UFBS = 100), with a distinct spilt at the base of the tree between largely south-western Australian endemics (Clade A) and largely south-eastern Australian species (Clade B). Clade A (PP = 1.0, UFBS = 96) grouped species endemic to south-western Australia together with one species (*S. tricolor*) that includes outlying populations disjunct across the Nullarbor Plain (Fig. 3*a*), whereas Clade B (PP = 0.93, UFBS = 94) included species from south-eastern Australia, one that occurs in both eastern and western Australia (*S. subochreatum*, Clade J; Fig. 2, 3*j*), plus two south-western endemics (*S. oligocephalum* and *S. polycephalum*, Clade H; Fig. 2, 3*h*) nested among the south-eastern taxa. Within the largely eastern clade (B), two early diverging lineages were a clade (PP = 1.0, UFBS = 100) of primarily New South Wales (NSW and southern Queensland[2]) endemics (Clade C; Fig. 2, 3*b*) and a clade (PP = 1.0, UFBS = 100) of Tasmanian endemics (Clade D; Fig. 2, 3*b*). The only other Tasmanian endemic, *S. obcordatum*, was placed in Clade E with *S. cinereum*, *S. parvifolium* and *S. daltonii* (PP = 0.73; Fig. 2, 3*d*).

Of the 45 species, subspecies or varieties represented by more than one accession, 11 (24.4%) were monophyletic and 11 (24.4%) were polyphyletic (Fig. 2, Table 3). One species polyphyletic across disparate clades was *S. eriocephalum*, although var. *eriocephalum* was monophyletic (PP = 1.0, UFBS = 100; Clade F), whereas var. *glabrisepalum* samples were placed in a polytomy (PP = 100, UFBS = 97; Clade I). The other example of a species polyphyletic across disparate clades was *S. phylicoides*, with the four accessions of this species divided among two clades (two samples in Clade I and two samples in Clade J2; Fig. 2, 4). Owing to a lack of support (BI or ML) for many branches at the tips of the nrDNA phylogeny, the remaining 23 taxa represented by more than one accession (51.1% of included taxa), were unresolved, i.e. with their monophyly neither supported nor strongly refuted.

Chloroplast genome phylogeny

The topologies of BI and ML cpDNA trees were largely congruent, and only the BI tree is shown here, with ML bootstrap values being mapped onto it (Fig. 5). One unsupported difference in the ML tree was that *S. burragorang* formed a clade (UFBS = 92), sister to *S. buxifolium* (UFBS = 100). *Spyridium* was resolved as monophyletic (PP = 1.0, UFBS = 100). Clades dominated by Tasmanian endemics (K; PP = 1.0, UFBS = 100) and NSW endemics (L; PP = 1.0, UFBS = 100) diverged early in the cpDNA phylogeny (Fig. 5). *Spyridium phlebophyllum* also diverged early on a branch between Clades K and L (PP = 1.0, UFBS = 98). Clade N predominantly contained taxa endemic to WA, as well as a SA sample of *S. tricolor* (PP = 1.0, UFBS = 100), a species found in WA and in SA that is disjunct on either side of the Nullarbor Plain (Fig. 3*a*, 5). One Tasmanian endemic species (*S. obcordatum*) was sister to *S. parvifolium* and *S. daltonii* in Clade O (PP = 1.0, UFBS = 100; Fig. 5). *Spyridium polycephalum* and *S. oligocephalum*, both WA endemics, were resolved in Clade M6 (PP = 1.0, UFBS = 100; Fig. 3*h*, 5) as sister to a WA sample of the widespread, polyphyletic *S. subochreatum* (Fig. 3*j*, 5, Table 1). These samples were found nested within the

[2]*Spyridium scortechinii* has a distribution that extends approximately 20 km into southern Queensland. However, this species is largely restricted to NSW. For ease of reading, for the remainder of the text, we will refer to this clade (and the equivalent clade in the cpDNA phylogeny) as NSW endemics.

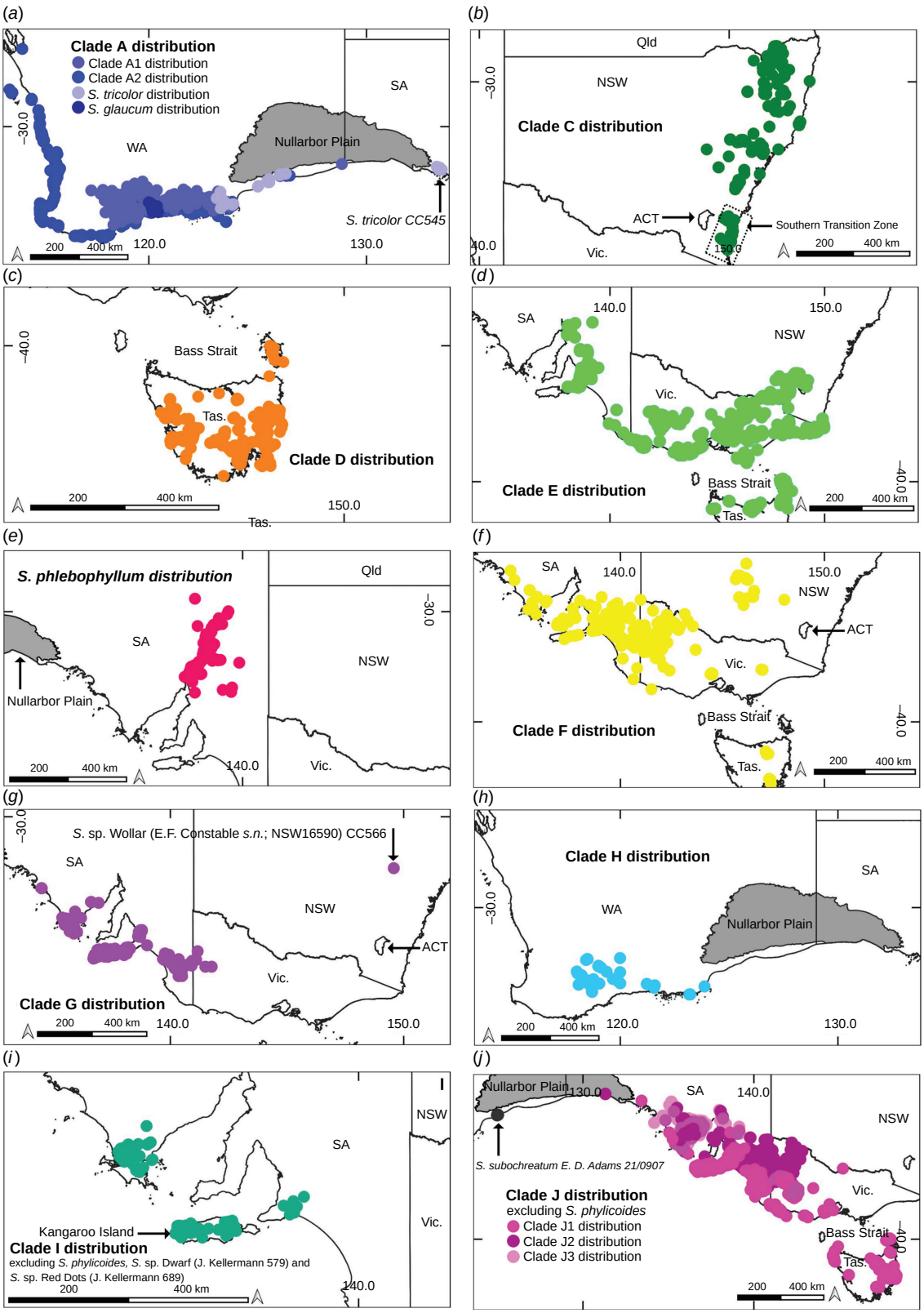

Fig. 3. (*Caption on next page*)

Fig. 3. Distributions of nrDNA clades of *Spyridium*, colour-coded to match groups shown in Fig. 2. Clade distributions are based on those of included species, using records in the Atlas of Living Australia (2020). Distributions of *S. phylicoides*, S. sp. Dwarf (J.Kellermann 579) and *S.* sp. Red Dots (J.Kellermann 689) have been omitted from these maps and are provided in Fig. 4. (*a*) Distribution of Clade A1 (mid blue), Clade A2 (royal blue), *S. tricolor* (light blue) and *S. glaucum* (dark blue). The location of sample CC545 (*S. tricolor*) is highlighted. (*b*) Distribution of Clade C. The general location of the southern transition zone is also highlighted. (*c*) Distribution of Clade D. (*d*) Distribution of Clade E. (*e*) Distribution of *S. phlebophyllum*. (*f*) Distribution of *S. eriocephalum* var. *eriocephalum* from Clade F. (*g*) Distribution of Clade G. The location of sample CC566 (*S.* sp. Wollar) is highlighted. (*h*) Distribution of Clade H. (*i*) Distribution of Clade I, excluding *S. phylicoides* and *S.* sp. Dwarf (J.Kellermann 579). (*j*) Distribution of Clade J1 (bright pink), Clade J2 (deep pink) and Clade J3 (light pink). The location of sample E.D.Adams 21/0907 (*S. subochreatum*) is highlighted (dark grey). *Spyridium phylicoides* and *S.* sp. Red Dots (J.Kellermann 689) have been excluded from this map.

eastern Australian-dominated Clade M (PP = 1.0, UFBS = 100; Fig. 5). Of the 45 taxa that included more than one accession, nine (20.0%) were monophyletic and 30 (66.7%) were polyphyletic (Fig. 5, Table 3), two (4.4%) were paraphyletic (*S. parvifolium* with respect to *S. daltonii* in Clade O1 and *S. polycephalum* with respect to *S. oligocephalum* in Clade M6) and four (8.8%) were unresolved.

Incongruence between the nrDNA and cpDNA phylogenies

Although the nrDNA and cpDNA trees included some nodes in common, there was substantial incongruence between the two phylogenies (Fig. 2, 5). This included differences in the order of divergence, in species relationships (within and between clades) and between the support for species circumscriptions (e.g. monophyly).

Key clades in common between the two trees were the clade of Tasmanian endemic species (nrDNA Clade D; cpDNA Clade K), and the clade of NSW endemic species (nrDNA Clade C; cpDNA Clade L); cpDNA Clade N also included most members of the WA endemic nrDNA Clade A, but excluding *S. globulosum*, *S. spadiceum* and one sample of *S. majoranifolium* that were placed in Clade M4 (Fig. 5). In the clade of NSW endemics, both trees showed similar relationships among species; however, within the Tasmanian and WA clades, relationships varied substantially.

Apart from this, there were many differences in the order of divergence between the two phylogenies, with these differences being particularly apparent at the base of each tree. For example, the base of the nrDNA phylogeny showed an early east–west split (Clade A–Clade B; Fig. 2), a split not supported by the cpDNA phylogeny (Fig. 5). The cpDNA phylogeny, instead, showed the Tasmanian (Clade K) and NSW (Clade L) endemics diverging first (Fig. 5).

There were many differences in species relationships between the two phylogenies, with this incongruence being particularly apparent from Clade M to Clade Q in the cpDNA phylogeny (Fig. 5). For example, *S. cinereum* is found in the *S. parvifolium*-dominated clade in the nrDNA phylogeny (Clade E; Fig. 2), but is nested within the SA endemic Clade M1 in the cpDNA tree (Fig. 5). In another example, *S. daphnoides* and *S. nitidum* were found to be sister to *Spyridium* sp. Wollar (E.F.Constable *s.n.*, NSW 16590) Kellermann and *S. eriocephalum* var. *eriocephalum* in the nrDNA phylogeny (Clades F and G), but were distantly related in the cpDNA tree (in Clades M1, M5 and P; Fig. 5).

There were also many differences relating to support for species circumscriptions between the two trees (Fig. 2, 5; Table 3). For example, *S. majoranifolium* was supported as monophyletic in the nrDNA phylogeny (PP = 1.0, UFBS = 100; clade A2) but found to be polyphyletic across Clades M5, N1 and N2 in the cpDNA tree. In another example, *S.* sp. Kangaroo Island (W.R.Barker 7560) was unresolved in the nrDNA phylogeny (Clade I; PP = 1.0, UFBS = 97; Fig. 2), but polyphyletic across Clades M2 and Q2 in the cpDNA tree (Fig. 5).

Discussion

In this study, we have presented the most comprehensive molecular phylogeny of *Spyridium* to date, including all recognised species, subspecies and varieties (excluding *S. bifidum* var. *bifidum*), as well as six undescribed, phrase-name taxa. These results provide an understanding of biogeographic patterns within the genus, support some species circumscriptions, call others into question, and provide support for recognition of some currently undescribed taxa. However, incongruence between the nrDNA and cpDNA phylogenies means that some relationships within the genus were unable to be resolved by these results alone.

Incongruencies: introgression or incomplete lineage sorting

Although the nrDNA and cpDNA phylogenies contained similarities, there were many differences between the two trees. These were apparent both in the relationships among major clades and when comparing implications for the monophyly, paraphyly or polyphyly of species and infraspecific taxa (Table 3). If the results of the cpDNA tree were viewed alone, as an indication of phylogenetic relationships, revising circumscriptions of half of the species in the genus might seem required. However, conversely, many of these polyphyletic taxa were found to be monophyletic or

unresolved in the nrDNA tree (Table 3), providing at least some support for, or not contradicting, most of the current circumscriptions of species.

Two key processes potentially leading to incongruence between nuclear and chloroplast gene trees are introgression, including chloroplast capture (Rieseberg and Soltis 1991; Tsitrone *et al*. 2003), and incomplete lineage sorting (ILS; Wiley and Lieberman 2011). Distinguishing the relative influence of these processes can be difficult, a problem commonly discussed in plant phylogenetic studies (e.g. Meudt and Bayly 2008; French *et al*. 2016; Barrett *et al*. 2018; Schuster *et al*. 2018). Other explanations for nuclear and chloroplast incongruence could include incomplete taxon sampling, which might result in artefacts of long branch attraction in one dataset over another; however, this is unlikely, given the almost complete taxon sampling in our study.

In our analyses, we have not attempted to distinguish potential instances of introgression from ILS, or the relative importance of each, but we expect, as outlined below, that both processes have potentially had a greater influence on the cpDNA results than on the nrDNA. As such, much of the species polyphyly reported in the cpDNA phylogeny might result from either chloroplast capture or ILS, and the nrDNA tree could better match the species tree for the genus.

In angiosperms, introgression of chloroplast genomes can be more apparent than nrDNA introgression (Rieseberg and Soltis 1991; Tsitrone *et al*. 2003). This is because chloroplasts are (generally) maternally inherited, not recombinant or subject to concerted evolution that can obscure signals of introgression in nrDNA (Álvarez and Wendel 2003), and have lower mutation rates (Palmer 1987), meaning that, in the absence of selective pressure or stochastic events, the signal of past cpDNA introgression is preserved. Potential introgression has been inferred in other studies of Rhamnaceae, including in *Pomaderris* (Nge *et al*. 2021*c*), *Discaria* Hook. (Aagesen *et al*. 2005; Medan *et al*. 2012) and *Ceanothus* L. (Hardig *et al*. 2002; Burge *et al*. 2013). One possible example of introgression contributing to incongruent results in the current study may be *S*. sp. Kangaroo Island (W.R.Barker 7560) Kellermann, which was unresolved in the nrDNA phylogeny (Clade I; Fig. 2), but was polyphyletic across disparate clades (M2 and Q2) in the cpDNA tree (Fig. 5). *Spyridium* sp. Kangaroo Island (W.R.Barker 7560) has an overlapping distribution, with several species found in the same subclades in the cpDNA phylogeny (e.g. *S. coalitum,* clade M2; and *S. thymifolium,* clade Q2); therefore, chloroplast capture through introgression is a possible explanation of this incongruence.

Another potential explanation for differences between cpDNA and nrDNA phylogenies is ILS. This occurs when the coalescence for alleles at a locus present in different species pre-dates the speciation event that gave rise to these species, and it can result in a gene tree that is incongruent with the species tree (Wiley and Lieberman 2011) or with other gene trees. The usual expectation is that ILS has a greater confounding influence on phylogenetic analyses of nuclear data than of chloroplast data, because the effective population size of haploid chloroplast markers is only one quarter that of diploid nuclear markers, and this should lead to quicker coalescence of chloroplast sequences than of nuclear alleles (Birky *et al*. 1989). However, for nrDNA markers, as used in our study, the processes of concerted evolution (Arnheim 1983), which homogenise rDNA sequences within the genomes of organisms, can reduce the effective population size of rDNA markers relative to other nuclear sequences, by as much as 200-fold (Buckler and Holtsford 1996); this leads to substantially shorter coalescence times, and reduces the influence of ILS on nrDNA phylogenies.

For the reasons outlined above, in the following discussions of biogeography and taxonomy we place most emphasis on the nrDNA results but also incorporate additional details from cpDNA trees where relevant.

There is substantial scope to further explore the potential influence of introgression and ILS in the history of *Spyridium*. Multi-locus nuclear datasets, for instance, would be more amenable to statistical analyses of introgression (Joly *et al*. 2009; Joly 2012; García *et al*. 2017) than is nrDNA, and could give a stronger phylogenetic signal to assess incongruence between nuclear and plastid gene trees. Nonetheless, the data presented here highlight that nuclear and chloroplast incongruence is conspicuous in the genus.

Broad biogeographic patterns in *Spyridium*

This study has provided the first comprehensive (all species) phylogenetic assessment of biogeographic patterns in *Spyridium*. Our inferences (below) are based on the molecular phylogenies and insight from other studies of biogeography in southern Australian. We focus on broad (continent scale) patterns in general terms. The work presented here could be extended by additional analyses, such as, for example, using dated trees or probabilistic biogeographic modelling (e.g. Ree and Smith 2008; Matzke 2013), although these are not explored here.

An early split in *Spyridium* has been identified in the nrDNA phylogeny, between the east and west of Australia across the Nullarbor Plain (Clades A and B; Fig. 2, 3*a–j*). There are many examples of east–west divergences in plants distributed across southern Australia, including *Phebalium* Vent. (Mole *et al*. 2004), *Eucalyptus* L'Hér. subgenus *Eucalyptus* (Ladiges *et al*. 2012), Goodeniaceae R.Br. (Jabaily *et al*. 2014), *Xanthorrhoea* Sol. ex Sm. (McLay *et al*. 2021), *Adenanthos* Labill. (Nge *et al*. 2021*a*) and *Pomaderris* (Nge *et al*. 2021*c*). The Nullarbor Plain disjunction in a range of plant groups has been related to vicariance associated with uplifting and climatic cooling in the mid-Miocene (Crisp and Cook 2007), including in *Pomaderris* (Nge *et al*. 2021*c*), a close relative of

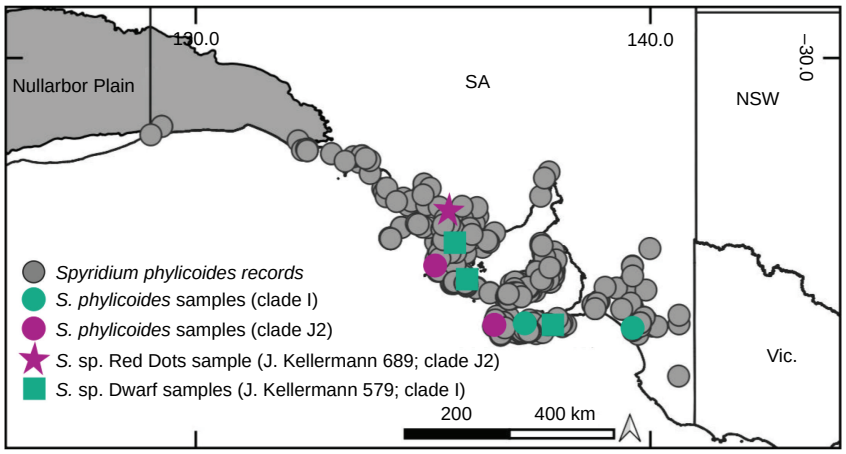

Fig. 4. Distributions of samples of *S. phylicoides*, *S.* sp. Red Dots (J.Kellermann 689) and *S.* sp. Dwarf (J.Kellermann 579) used in this study. For *S. phylicoides*, samples are coloured by the clades in which they are placed in the nrDNA tree (Fig. 2), with the distribution of the species, on the basis of the records in the Atlas of Living Australia (2020), also shown (grey dots).

Spyridium in the tribe Pomaderreae. Nge *et al.* (2021*c*) concluded that *Pomaderris* was widespread throughout southern and eastern Australia until *c.* 14 Ma, when the Nullarbor Plain uplift occurred, with subsequent rapid 'within region' diversification in eastern Australia from *c.* 10 Ma, and little movement across biomes since. Although we did not use dated trees to determine diversification dates (as per Nge *et al.* 2021*c*), a similar explanation for the early east–west divergence in *Spyridium* could be inferred from our results.

Assuming the deep east–west divergence in *Spyridium* relates to formation of the Nullarbor Plain, the nrDNA tree suggests that up to three lineages in the genus have potentially dispersed across the plain subsequent to this early east–west divergence (Fig. 2, 3*a*, *h*, *j*). An east-to-west dispersal of *S. subochreatum* is inferred from both nrDNA and cpDNA trees, because the species is nested within eastern taxa and its distribution extends to just west of the Nullarbor Plain (Fig. 3*j*). Conversely, for *S. tricolor*, a west-to-east dispersal could be inferred, because the SA sample of this species (CC545; Fig. 3*a*) groups with western taxa. However, because it is sister to other western taxa in Clade A in the nrDNA phylogeny (Fig. 2), a reverse scenario could not be ruled out. Like *S. subochreatum*, *S. tricolor* grows on sandy soils and limestone (FloraBase – the Western Australian Flora, see https://florabase.dpaw.wa.gov.au/, accessed 7 May 2021), suggesting it could have made use of land connections south of the Nullarbor Plain that have been exposed at times of lower sea level since the late Pliocene (Nelson 1974; Wright and Ladiges 1997). An alternative explanation, that *S. tricolor* was widespread across this region and became disjunct during the Nullarbor Plain uplift, would require retention of morphological resemblance, such that it is recognised as a single species, despite a considerable geographic disjunction, for a substantial period of time since the mid-Miocene. Population-level sampling of *S. tricolor* using variable genomic markers could provide a greater insight into geographic history of the species and be used to further test its taxonomic circumscription.

The third lineage for which dispersal over the Nullarbor Plain might possibly be inferred is that including *S. polycephalum* and *S. oligocephalum* (Fig. 2, 3*h*, 5). A deep east-to-west dispersal event, before the diversification of the two species, is inferred from both the nrDNA and cpDNA trees, because this clade was nested within species from east of the Nullarbor Plain. Evidence of early east–west vicariance across southern Australia, followed by subsequent dispersal events such as these have been inferred in studies of other plant groups, such as, for example, in *Eucalyptus* subgenus *Eucalyptus* (Wright and Ladiges 1997), *Thelymitra* J.R.Forst. & G.Forst. (Nauheimer *et al.* 2018), *Calytrix tetragona* Labill. (Nge *et al.* 2021*b*) and *Pomaderris* (Nge *et al.* 2021*c*). Despite this, an alternative explanation of vicariance to account for the Western Australian distribution of the *S. polycephalum*–*S. oligocephalum* clade cannot be immediately discounted on the basis of our data. Although a vicariance explanation is less parsimonious because it would infer extinction of multiple lineages in western Australia, such reasoning assumes that multiple extinctions are less probable than is a single dispersal, which might not be true (Sanmartín and Meseguer 2016), for example, in the face of substantial climatic change in Australia since the mid-Miocene. A robust time-calibrated phylogeny for *Spyridium* could help corroborate one of these alternative scenarios.

Within the eastern Australian branch of the nrDNA phylogeny (Clade B, Fig. 2), an early NSW divergence is inferred (Fig. 3*b*). This deep divergence of NSW endemics from other south-eastern Australian taxa occurs near a broadly defined area that has been termed the southern transition zone (STZ; Fig. 3*b*) by Milner *et al.* (2012). The STZ is found east of the Great Dividing Range (GDR) and north of the Victoria–NSW border and is identified as a region where genetic or distributional discontinuities are seen in a range of taxa, but with the exact position of the discontinuities being dependent on habitat requirements of individual species and potentially different timescales of divergence (Milner *et al.* 2012). Other plant taxa showing genetic breaks across the STZ include *Hardenbergia violacea* (Schneev.) Stearn (Larcombe *et al.*

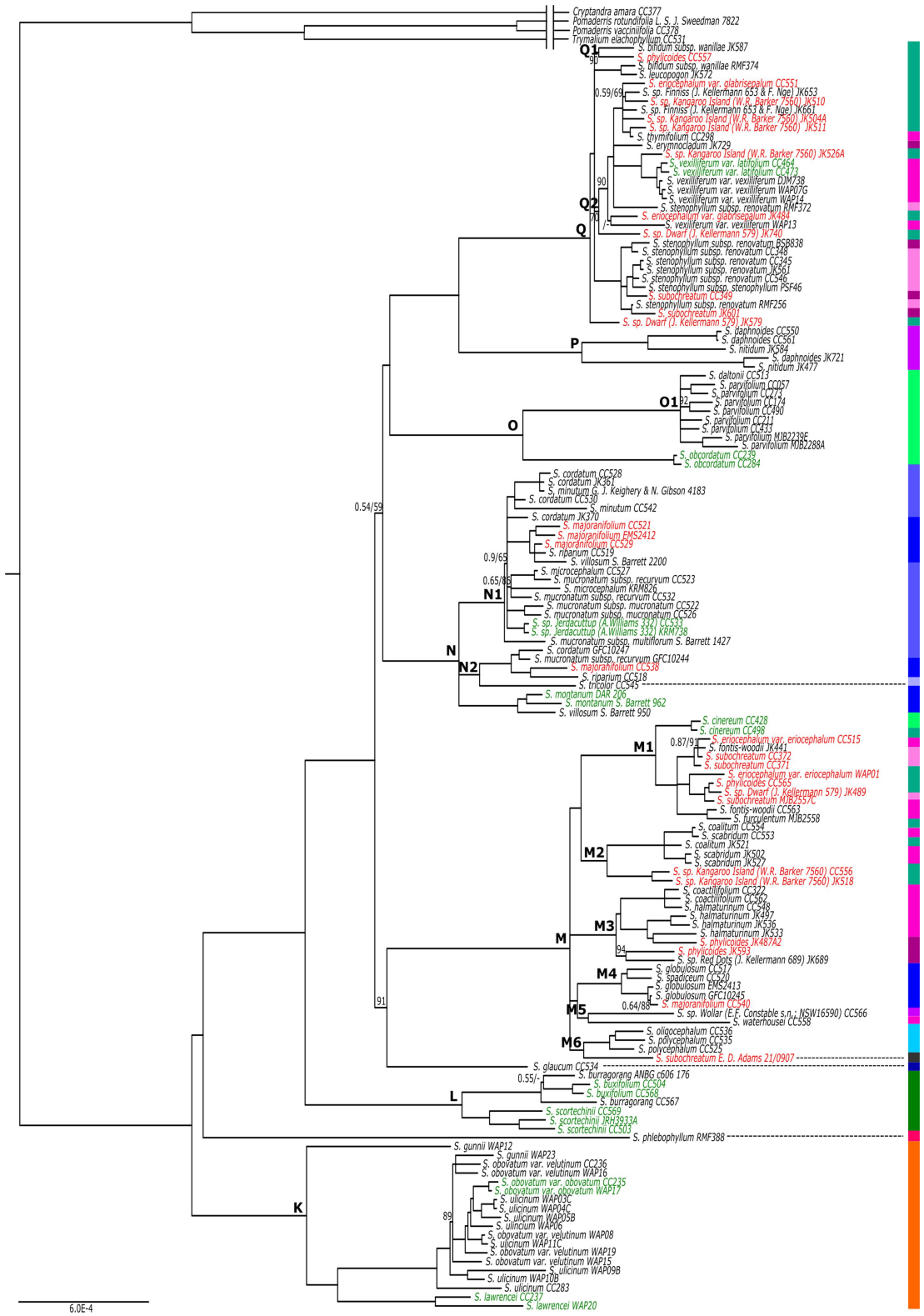

Fig. 5. (*Caption on next page*)

Fig. 5. Chloroplast genome (cpDNA) phylogeny of *Spyridium*, based on Bayesian inference (BI) analysis. Bayesian posterior probabilities (PP) <0.95 and ultrafast bootstrap (UFBS) values are shown at nodes when <95%; values ≥95% are not shown. Where one value for a node is supported (≥95%) and the other for that node is unsupported (<95%), only the unsupported value is shown. Where a hyphen (-) is provided at a node, this node varied in resolution in the ML tree and was therefore not transferable to the BI phylogeny. Coloured bar to the right of the tree indicates placement of samples in the nrDNA phylogeny (i.e. matching the coloured bar on Fig. 2). Labels are given for some clades (K–Q) and subclades (M1–Q2) discussed in text. Species polyphyletic across clades are highlighted in red text. Monophyletic taxa with >0.95 PP support are highlighted in green. Dashed lines associated with *S. tricolor*, *S. glaucum*, *S. phlebophyllum* and *S. subochreatum* E.D.Adams 21/0907 are provided as reference points connecting taxa to the sidebar.

2011), *Lomatia* R.Br. (Milner *et al.* 2012), *Callitris rhomboidea* R.Br. ex Rich. & A.Rich. (Worth *et al.* 2018) and *Xanthorrhoea* (McLay *et al.* 2021). *Spyridium* provides a further example of this pattern, although potential drivers of the divergence are unclear in this case.

Tasmanian endemics (excluding *S. obcordatum*) were found in a single, early diverging clade separate from their mainland counterparts in the nrDNA tree (Clade D, Fig. 2, 3*c*), suggesting early vicariance of *Spyridium* across Bass Strait. This early divergence and diversification of Tasmanian endemics is also supported by the cpDNA phylogeny (Clade K, Fig. 5) and the findings of Kellermann *et al.* (2005) and Hauenschild *et al.* (2018). The continued barriers to dispersal and gene-flow are likely to be the inundation of Bass Strait during interglacial periods (Galloway and Kemp 1981) and the semi-arid climate of the land-bridge exposed during glacial periods (Kirkpatrick and Fowler 1998). Major glacial and interglacial fluctuations occurred throughout the Quaternary (*c.* 2.2 Ma to *c.* 10 000 years ago; Hope 1994; Quilty 1994) and their resulting climatic extremes have been inferred to contribute to the limited distribution of narrow-range endemism in *Spyridium* in Tasmania (Coates and Kirkpatrick 1999).

Recent dispersal or gene flow between Victoria and Tasmania are here inferred for the lineage represented by *S. obcordatum*, the only endemic Tasmanian species not placed in Clades D or K (Fig. 2, 3*d*, 5), and several widespread taxa, including *S. eriocephalum* var. *eriocephalum*, *S. vexilliferum* var. *vexilliferum* and *S. parvifolium* (Fig. 2, 3*d*, *f*, *j*). Accessions of each of these widespread taxa collected from Tasmania (and Flinders Island for *S. parvifolium*) were found within the same clade as samples of the same taxa from Victoria (Table 1). Similar patterns of recent gene-flow between Victoria and Tasmania have been inferred in other plant groups, including *Eucalyptus globulus* Labill. (Freeman *et al.* 2001), *Hardenbergia violacea* (Larcombe *et al.* 2011), *Correa* Andrews (French *et al.* 2016), *Zieria veronicea* Sm. (Neal *et al.* 2019), *Xanthorrhoea* (McLay *et al.* 2021) and a range of other species (Worth *et al.* 2017). Evidence suggests that at least some areas of the Bassian Plain were covered in eucalypt woodland habitat (Hope 1978, 1994; Kirkpatrick and Fowler 1998) which could have been suitable for *S. parvifolium*, *S. vexilliferum* var. *vexilliferum* and *S. eriocephalum* var. *eriocephalum* (VicFlora 2018), i.e. potentially facilitating over-land rather than over-water dispersal between Victoria and Tasmania.

Review of circumscriptions of species

The molecular phylogenies support the circumscriptions of several *Spyridium* species, but raise questions about others. A quarter of the taxa represented by more than one accession were identified as monophyletic in the nrDNA phylogeny, with approximately one-third being resolved as polyphyletic, and the remainder being unresolved (Fig. 2, Table 3). Of the monophyletic taxa resolved in the nrDNA tree, several were also found to be monophyletic in the cpDNA phylogeny (e.g. *S. obcordatum*, *S. scortechinii* and *S. montanum*), providing additional support for these circumscriptions of species (Fig. 5, Table 3). Of the polyphyletic taxa in the nrDNA phylogeny, two of the most notable were distributed across disparate clades, namely, *S. eriocephalum* and *S. phylicoides* (Fig. 2). Given that both of these species were also resolved in separate clades in the cpDNA tree (Fig. 5), they are discussed in more detail below, along with several associated phrase-name taxa.

Spyridium eriocephalum

Spyridium eriocephalum is polyphyletic and requires taxonomic revision, because its two varieties were found in distinct clades in both nrDNA and cpDNA phylogenies (Clades F and I, Fig. 2; Clades M1 and Q2, Fig. 5). *Spyridium eriocephalum* var. *eriocephalum* is monophyletic (albeit with limited sampling, but from separated localities) and geographically distinct from other taxa in the nrDNA tree (Fig. 2, 3*f*). *Spyridium eriocephalum* var. *glabrisepalum* is unresolved in a polytomy in the nrDNA phylogeny (Clade I, Fig. 3) with several other taxa (Fig. 2, 3*i*). The two varieties of *S. eriocephalum* are for the most part geographically distinct, with the exception being some overlap on Kangaroo Island (J. Kellermann, unpubl. data). The typical variety is widespread in south-eastern Australia (SA, Victoria, NSW and Tasmania), whereas var. *glabrisepalum* is restricted to Kangaroo Island. The two varieties are also morphologically distinguished by the presence of woolly sepal hairs (var. *eriocephalum*) versus hairless sepals that are instead glabrous-viscid (var. *glabrisepalum*; Canning 1986). Although the two taxa appear distinct (from each other) in both phylogenies, given that the two samples of var. *glabrisepalum* are placed with some other Kangaroo Island endemic taxa (e.g. *S. coalitum*) in the nrDNA tree, it

Table 3. Summary of resolution of taxa comparing the nrDNA phylogeny to the cpDNA trees.

Taxona	nrDNA phylogeny (Fig. 2)	cpDNA phylogeny (Fig. 5)	Congruent (in both trees)
S. burragorang	Unresolved	Unresolved	Unresolved
S. buxifolium	Unresolved	Monophyletic	–
S. cinereum	Monophyletic	Monophyletic	Monophyletic
S. coactilifolium	Monophyletic	Unresolved	–
S. coalitum	Unresolved	Polyphyletic	–
S. cordatum	Polyphyletic	Polyphyletic	Polyphyletic
S. daphnoides	Unresolved	Polyphyletic	–
S. eriocephalum	Polyphyletic	Polyphyletic	Polyphyletic
S. eriocephalum var. *eriocephalum*	Monophyletic	Polyphyletic	–
S. eriocephalum var. *glabrisepalum*	Unresolved	Polyphyletic	–
S. fontis-woodii	Unresolved	Polyphyletic	–
S. globulosum	Unresolved	Polyphyletic	–
S. gunnii	Unresolved	Polyphyletic	–
S. halmaturinum	Unresolved	Polyphyletic	–
S. lawrencei	Monophyletic	Monophyletic	Monophyletic
S. majoranifolium	Monophyletic	Polyphyletic	–
S. microcephalum	Polyphyletic	Polyphyletic	Polyphyletic
S. minutum	Polyphyletic	Polyphyletic	Polyphyletic
S. montanum	Monophyletic	Monophyletic	Monophyletic
S. mucronatum	Polyphyletic	Polyphyletic	Polyphyletic
S. mucronatum subsp. *mucronatum*	Unresolved	Unresolved	Unresolved
S. mucronatum subsp. *recurvum*	Polyphyletic	Polyphyletic	Polyphyletic
S. nitidum	Unresolved	Polyphyletic	–
S. obcordatum	Monophyletic	Monophyletic	Monophyletic
S. obovatum	Unresolved	Polyphyletic	–
S. obovatum var. *obovatum*	Monophyletic	Monophyletic	Monophyletic
S. obovatum var. *velutinum*	Unresolved	Polyphyletic	–
S. parvifolium	Unresolved	Paraphyletic (with respect to *S. daltonii*)	–
S. phylicoides	Polyphyletic	Polyphyletic	Polyphyletic
S. polycephalum	Unresolved	Paraphyletic (with respect to *S oligocephalum*)	–
S. riparium	Monophyletic	Polyphyletic	–
S. scabridum	Unresolved	Polyphyletic	–
S. scortechinii	Monophyletic	Monophyletic	Monophyletic
S. sp. Dwarf (J. Kellermann 579)	Unresolved	Polyphyletic	–
S. sp. Finniss (J. Kellermann 653 & F. Nge)	Unresolved	Unresolved	Unresolved
S. sp. Jerdacuttup (A.Williams 332)	Unresolved	Monophyletic	–
S. sp. Kangaroo Island (W.R. Barker 7560)	Unresolved	Polyphyletic	–
S. stenophyllum	Polyphyletic	Polyphyletic	Polyphyletic
S. stenophyllum subsp. *renovatum*	Polyphyletic	Polyphyletic	Polyphyletic
S. subochreatum	Polyphyletic	Polyphyletic	Polyphyletic
S. ulicinum	Monophyletic	Polyphyletic	–
S. vexilliferum	Unresolved	Polyphyletic	Polyphyletic
S. vexilliferum var. *latifolium*	Unresolved	Monophyletic	–
S. vexilliferum var. *vexilliferum*	Polyphyletic	Polyphyletic	Polyphyletic
S. villosum	Unresolved	Polyphyletic	–
Totals (45 taxa total)			
Monophyletic	11	9	6
Paraphyletic	0	2	1
Polyphyletic	11	30	12
Unresolved	23	4	4

Only taxa represented by more than one accession are included (=45 taxa). Only supported branches were considered when determining the resolution of the taxa (e.g. *S. gunnii* was unresolved in the nrDNA phylogeny because branches between the two accessions were not supported, resulting in an unresolved polytomy for the species). In the column 'Congruent (in both trees)', a dash (–) indicates that the resolution between the phylogenies was incongruent.

is possible that introgression may be influencing this placement (Fig. 2). However, placement of var. *glabrisepalum* in the cpDNA phylogeny is somewhat incongruent although supported, with samples being placed with accessions representing other taxa collected from a range of sites from SA to Tasmania (Fig. 5). Additional morphological or molecular work is recommended to re-assess these taxa and their relationships.

Spyridium phylicoides, *S.* sp. Dwarf (J.Kellermann 579) and *S.* sp. Red Dots (J.Kellermann 689)

Spyridium phylicoides is polyphyletic and requires taxonomic revision. In both phylogenies, samples of *S. phylicoides* were found in two clades (Clades I and J2, Fig. 2; Clades M1, M3 and Q1, Fig. 5). There is no biogeographic pattern to these (Fig. 4) and perhaps further unidentified forms exist within *S. phylicoides*, in addition to the two forms already given phrase names, namely, *S.* sp. Dwarf (J.Kellermann 579) Kellermann and *S.* sp. Red Dots (J.Kellermann 689) Kellermann (Table 2). *Spyridium* sp. Dwarf (J.Kellermann 579) is distinguished from *S. phylicoides* by generally smaller leaves and low-growing, almost prostrate habit (J. Kellermann, unpubl. data). However, the distribution of samples of *S.* sp. Dwarf (J.Kellermann 579; Fig. 5) overlaps with that of many species found in SA (Fig. 3*f*, *g*, *i*, *j*); therefore, it is possible that the incongruent result for this taxon (unresolved in the nrDNA phylogeny and polyphyletic in the cpDNA tree; Fig. 2, 5) may be attributed to chloroplast capture or ILS. Only one accession of *S.* sp. Red Dots (J.Kellermann 689) was included in this study and support for this taxon as distinct from *S. phylicoides* in either phylogeny is limited (Clade J2, Fig. 2; Clade M3, Fig. 5). We recommend further investigation into both phrase-name taxa, particularly *S.* sp. Dwarf (J.Kellermann 579), and a more detailed investigation into the circumscription of *S. phylicoides*.

Conclusions

Here we have presented the first comprehensive phylogenies of the genus *Spyridium*, representing all described species and utilising both nrDNA and whole chloroplast genomes. Most incongruencies between the two trees could relate to introgression and chloroplast capture or ILS.

We found evidence of an early east–west split at the base of the nrDNA phylogeny and early diverging clades dominated by Tasmanian and NSW endemics. Our trees provide evidence of two subsequent within-species dispersal events across the Nullarbor Plain (*S. subochreatum* and *S. tricolor*) as well as a possible dispersal and diversification of a lineage including the south-western Australian endemics *S. polycephalum* and *S. oligocephalum* (although a vicariance explanation is also plausible in that case). In Tasmania, we found *S. obcordatum* to be the result of a recent dispersal and subsequent diversification event, and evidence of recent gene-flow between Tasmania and Victoria in several widespread taxa (including *S. vexilliferum* var. *vexilliferum*).

Eleven taxa were supported as monophyletic in the nrDNA phylogeny and the following two were polyphyletic across disparate clades, requiring taxonomic review: *S. eriocephalum* (with two genetically distinct varieties) and *S. phylicoides*.

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Data availability. The data that support this study are available in TreeBASE at https://www.treebase.org/treebase-web/home.html (Accession number 28815).

Conflicts of interest. Michael J. Bayly is an Associate Editor for *Australian Systematic Botany*. Despite this relationship, he did not at any stage have Associate Editor-level access to this manuscript while in peer review, as is the standard practice when handling manuscripts submitted by an editor to this journal. *Australian Systematic Botany* encourages its editors to publish in the journal and they are kept totally separate from the decision-making process for their manuscripts. The authors declare that they have no further conflicts of interest.

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