BOTANIC GARDENS OF SYDNEY

Telopea

Journal of Plant Systematics

ISSN 2200-4025 (Online)

A genetic investigation of relationships and species boundaries between *Eucalyptus dalrympleana* and allied taxa in New South Wales

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Abstract

A revision of the classification of taxa in the *Eucalyptus dalrympleana* complex within *E.* series *Viminales* from New South Wales using population level genetic data is presented here. It remains unclear if the complex is a natural grouping to the exclusion of other related species, and hybridisation between members of the complex and *E. mannifera*, *E. viminalis* and *E. elliptica* is recorded. In line with observations from field surveys, the two taxa from the North Tablelands region, *E. dalrympleana* subsp. *heptantha* and *E. rubida* subsp. *barbigerorum* formed a single genetic lineage and the latter is therefore considered a synonym of the former, given this lineage is most closely related to *E. dalrympleana* subsp. *dalrympleana*. The threatened narrow-range endemic taxon *E. canobolensis* is shown to be nested within the more widespread *E. rubida* subsp. *rubida*, and therefore the original circumscription of this taxon as *E. rubida* subsp. *canobolensis* is supported.

Introduction

Eucalyptus rubida H.Deane & Maiden, *Eucalyptus dalrympleana* Maiden and *Eucalyptus canobolensis* (L.A.S.Johnson & K.D.Hill) J.T.Hunter form a complex of morphologically similar taxa in which it has historically proven difficult to draw species boundaries (here termed the *E. dalrympleana* complex), although it is widely recognised the three species form a natural grouping (Brooker *et al.* 2015; Nicolle 2024). A previous phylogenetic study of *Eucalyptus* sect. *Maidenaria* and relatives, including multiple representatives of all three species in the complex, was unable to resolve species level relationships within series *Viminales* (Jones *et al.* 2016), but provided moderate support for the three taxa being each other's closest relatives. In that study a sample of *E. darympleana* from Tasmania showed affinities to *E. viminalis,* possibly due to hybridisation, and a sample of *E. rubida* from Tasmania and *E. dalrympleana* from South Australia formed part of a polytomy near the base of series *Viminales*. All remaining samples of the three species (n = 17) formed an unsupported grouping in the phylogeny. In their follow-up publication to Jones *et al.* (2016), Nicolle and Jones (2018) provided a revised classification of *E.* section *Maidenaria*, which recognises ~40 species as members of *E.* ser. *Viminales*, which in turn consists of two subseries, *E.* subser. *Circulares* and *E.* subser. *Lanceolatae*. The *E. dalrympleana* complex was placed in *E.* subser. *Circulares*, although the particular relationship of the complex to other members of this subseries remains unclear.

Fahey PS, Rossetto M, Bruhl JJ, Andrew R, Andrew D (2024) A genetic investigation of relationships and species boundaries between *Eucalyptus dalrympleana* and allied taxa in New South Wales. *Telopea* 27: 177–196. doi:10.7751/telopea18544

Received: 31 May 2024
Accepted: 20 September 2024
Published: 22 November 2024

Eucalyptus rubida was the first taxon described in the complex by Deane and Maiden (1899), with *E. dalrympleana* following shortly after, being described by Maiden and Flockton (1922). This two taxa system lasted 42 years before a new subspecies was erected within *Eucalyptus dalrympleana, E. dalrympleana* subsp. *heptantha* L.A.S.Johnson, which separated the populations of the Northern Tablelands of New South Wales and southern Queensland that typically have seven flowers per umbel from the typical three-flowered form that occurs south of these regions. More recently Nicolle (2022) recognised two new subspecies within *E. dalrympleana*, *E. dalyrmpleana* subsp. *lutruwita* Nicolle, which is endemic to Tasmania and includes all the populations from the island state, and *E. dalrympleana* subsp. *poliophylla* Nicolle from the highest elevations (>1200 m) of the Victorian Alps. This leaves *E. dalrympleana* subsp. *dalrympleana* with a distribution stretching from the Central Tablelands of NSW south through north-eastern Victoria, as well as isolated populations in the southern Mount Lofty Ranges in South Australia.

In the case of *E. rubida*, Hill and Johnson (1991) recognised three new subspecies based upon morphological and geographic variation:

- *Eucalyptus rubida* subsp. *septemflora* K.D.Hill & L.A.S.Johnson from the Kiewa River valley of Victoria was segregated based upon its seven flowered inflorescences, differing from the three flowered inflorescences otherwise typical of the species. However, as the seven-flowered form occurs amongst three flowered populations, most authorities today consider this taxon to represent occasional hybrids or a rare morphological variant of the typical subspecies, and therefore do not consider it a valid taxon (Brooker *et al.* 2015; Council of Heads of Australasian Herbaria 2016; Nicolle 2022, 2024).
- *Eucalyptus rubida* subsp. *canobolensis* K.D.Hill & L.A.S.Johnson was erected to recognise a population of trees on the upper slopes of Mount Canobolas in the Central Tablelands of NSW that exhibit a stunted growth form, larger leaves, and larger buds and fruit than the typical form of the species, as well as square stems on the seedlings and juvenile growth. Based upon comments by Maiden (1917) on the resemblance of *E. gunnii* Hook.f. from Tasmania and the Mt Canobolas population, and a morphological phylogenetic study by Chappill and Ladiges (1996) that suggested a closer relationship between these two taxa than between either and *Eucalyptus rubida* subsp. *rubida*, Hunter (1998) raised the population to the species level as *Eucalyptus canobolensis*. While this taxon is broadly accepted as a distinct species today, it is believed to be much more closely related to *Eucalyptus rubida* than to *Eucalyptus gunnii* (Brooker *et al.* 2015; Nicolle 2024).
- *Eucalyptus rubida* subsp. *barbigerorum* K.D.Hill & L.A.S.Johnson was erected to include scattered populations in the Northern Tablelands of NSW that exhibit elliptical juvenile leaves and a basal stocking of rough bark. It was noted in the description of this taxon that extensive intergradation occurred with *Eucalyptus dalrympleana* subsp. *heptantha*, and recent authors have suggested this could reflect the true affinity of the taxon (Hunter 2017).

In this study we use population-level sampling and reduced representation genetic data to investigate the relationships between *E. dalrympleana*, *E. rubida* and *E. canobolensis,* and subspecies thereof within NSW. To allow for a full understanding of the complex's relationship to other members of *E.* series *Viminales*, sampling of other taxa native to NSW within the group is also undertaken, which leads to some wider insights into the series' evolutionary history. This represents the first use of population-level genetic data in the *E. dalrympleana* complex and highlights the power of population genetics approaches in resolving shallow evolutionary relationships.

Methods

Sampling

Sampling for this study was limited to New South Wales and so affinities of populations outside the state cannot be commented on. *Eucalyptus rubida* was collected from 25 sites across NSW, including eight populations of *E. rubida* subspecies *barbigoreum* (Table 1 and Fig. 1a) while samples of *E. canobolensis* were collected from the only known population of the species. *Eucalyptus dalrympleana* was collected from 19 sites, split between 5 *E. dalrympleana* subsp. *dalrympleana* and 14 *E. dalrympleana* subsp. *heptantha* sites (Table 1 and Fig. 1b). The type populations of *E. dalrympleana* subsp. *heptantha*, *E. rubida* subsp. *barbigerorum* and *E. canobolensis* were all sampled to allow for the unambiguous taxonomic conclusions to be drawn.

Samples of several other members of *E.* series *Viminales* as defined by Nicolle and Jones (2018) were sampled as outgroups for the entire *E. dalrympleana* complex, focussing on species native to the Northern Tablelands of NSW (Table 1). Figure 2 shows the spatial spread of outgroup sampling. In all cases leaf samples were collected and placed into envelopes before drying either in silica gel or by freeze-drying. Voucher specimens were collected from a single sampled individual at most sites to confirm field identifications.

Where samples collected at a site did not show expected relationships, voucher identifications were reassessed and if a misidentification could be confirmed the new identification was employed. The two instances where this occurred are highlighted in Table 1, including a site where all samples were identified as *E. rubida* subsp. *rubida* in the field but that was subsequently determined to be *E. dalrympleana* subsp. *dalrympleana*, and a site where all samples were identified as *E. rubida* subsp. *rubida* in the field, but that genetic data showed that one sample, which happened to be the vouchered sample, was actually a misidentified *E. viminalis* individual, with the remaining samples all within the *E. rubida* subsp. *rubida* clusters in genetic analyses suggesting that field identifications were correct.

Table 1. Sample and collection location information for *Eucalyptus* series *Viminales* taxa genotyped in this study. All samples of a species collected within 5 km of one another are grouped into a site, with the number of samples which met data quality thresholds for each site shown.

Taxon and classification per Nicolle (2024)	Site	Samples in final dataset	GPS locations	Representative herbarium voucher collected from site
E. rubida subsp. *rubida* (series *Viminales* subseries *Circulares*)	Bucky Springs Rd - ~2.8 km SE of Bombala, NSW	NSW1197181	-36.92281, 149.26110	
		NSW1197186	-36.92277, 149.26113	
		NSW1197137	-36.92277, 149.26109	
		NSW1197112	-36.92275, 149.26113	
		NSW1197107	-36.92265, 149.26103	
E. rubida subsp. *rubida*	Barry Way - ~5.5 km S of Grosses Plain, NSW	NSW1197251	-36.61317, 148.49158	NSW1197135
		NSW1197226	-36.61405, 148.49197	
		NSW1197231	-36.61375, 148.49177	
		NSW1197236	-36.61354, 148.49187	
		NSW1197241	-36.61344, 148.49175	
		NSW1197246	-36.61327, 148.49156	
E. rubida subsp. *rubida*	Snowy Mountains Hwy - ~5.7 km WNW of Adaminaby, NSW	NSW1197200	-35.98312, 148.72607	NSW1197151
		NSW1197195	-35.98346, 148.72636	
		NSW1197270	-35.98335, 148.72618	
		NSW1197235	-35.98332, 148.72614	
		NSW1197265	-35.98312, 148.72589	
		NSW1197117	-35.98284, 148.72577	
E. rubida subsp. *rubida*	Namadgi National Park, ~5.7 km from Boboyan Rd along Nass Valley Firetrail, ACT	JJB3613	-35.85031, 149.04436	NE 110462
E. rubida subsp. *rubida*	Coppabella Rd, Opposite Rosewood Cemetery, Rosewood, NSW	NSW1197095	-35.69046, 147.86218	NSW1197357
		NSW1197274	-35.69256, 147.86168	
		NSW1197080	-35.69252, 147.86161	
		NSW1197085	-35.69135, 147.86129	
		NSW1197090	-35.69079, 147.86153	
		NSW1197275	-35.69064, 147.86171	
E. rubida subsp. *rubida*	Burra Rd, Tinderry Nature Reserve, NSW	NSW1197188	-35.62512, 149.21671	NSW1197130
		NSW1197190	-35.62540, 149.21670	
		NSW1197189	-35.62532, 149.21661	
		NSW1197182	-35.62524, 149.21674	
		NSW1197187	-35.62523, 149.21693	
		NSW1197177	-35.62518, 149.21662	
E. rubida subsp. *rubida*	Tidbinbilla Rd, Greenway, ACT	JJB3612	-35.45678, 149.01114	NE 108977
E. rubida subsp. *rubida*	Doctors Flat Rd, ~1 km SE of Waddys Plain, NSW			NSW1176522 [sequence failed]
		NSW1176526	-35.10250, 148.75647	
		NSW1144548	-35.10248, 148.75647	
		NSW1176531	-35.10241, 148.75631	
		NSW1144358	-35.10237, 148.75633	
		NSW1144359	-35.10234, 148.75634	
E. rubida subsp. *rubida*	Marked Tree Rd, Mcleods Creek Nature Reserve, NSW	NSW1147789	-35.01687, 149.28573	NSW1144397
		NSW1147794	-35.01735, 149.28542	
		NSW1147793	-35.01733, 149.28531	
		NSW1147788	-35.01718, 149.28553	
		NSW1147799	-35.01713, 149.28545	
		NSW1147798	-35.01712, 149.28544	

Taxon and classification per Nicolle (2024)	Site	Samples in final dataset	GPS locations	Representative herbarium voucher collected from site
				NSW1144540 - [sequence failed]
E. rubida subsp. *rubida*	Targo TSR, ~10 km NE of Kenmore, NSW	NSW1144542	-34.61778, 149.79907	
		NSW1144557	-34.61776, 149.79894	
		NSW1144552	-34.61772, 149.79854	
		NSW1144620	-34.61772, 149.79892	
		NSW1144567	-34.61763, 149.79905	
E. rubida subsp. *rubida*	Lachlan Valley Way, ~7.4 km NW of Kangiara, NSW	NSW1176466	-34.58313, 148.73704	NSW1176453
		NSW1176460	-34.58339, 148.73723	
		NSW1176465	-34.58296, 148.73706	
		NSW1176476	-34.58296, 148.73693	
		NSW1176471	-34.58294, 148.73687	
		NSW1176470	-34.58290, 148.73690	
E. rubida subsp. *rubida*	Berrima Reserve Campground	NSW1144483	-34.48497, 150.33227	NSW1144498
		NSW1144489	-34.48503, 150.33223	
		NSW1144484	-34.48498, 150.33216	
		NSW1144499	-34.48483, 150.33262	
		NSW1144494	-34.48482, 150.33264	
		NSW1144495	-34.48473, 150.33258	
E. rubida subsp. *rubida*	Funny Hill TSR, ~4.4 km NNW of Binda, NSW	NSW1175697	-34.28660, 149.34723	NSW1175692
		NSW1175702	-34.28662, 149.34720	
		NSW1175707	-34.28672, 149.34731	
		NSW1175712	-34.28687, 149.34722	
		NSW1175717	-34.28718, 149.34710	
		NSW1175722	-34.28725, 149.34736	
E. rubida subsp. *rubida*	Hyde Park Bushland Reserve, Hartley, NSW	NSW1197333	-33.53312, 150.18869	NSW1197284
		NSW1197279	-33.53290, 150.18872	
		NSW1197289	-33.53304, 150.18857	
		NSW1197290	-33.53287, 150.18838	
		NSW1197294	-33.53308, 150.18865	
		NSW1197299	-33.53293, 150.18869	
E. rubida subsp. *rubida*	Carcoar Rd, ~2.5 km NNW Browns Creek, NSW	DDA143	-33.50472, 149.13228	NE 109691
		DDA143a	-33.50497, 149.13236	
		DDA143b	-33.50489, 149.1321	
		DDA143c	-33.50408, 149.13178	
		DDA143d	-33.50300, 149.13192	
E. rubida subsp. *rubida*	Ophir Rd, ~7.5 km N of Orange, NSW	DDA139	Site GPS: -33.23475, 149.13244	NE 109687
		DDA139a		
		DDA141a	-33.21317, 149.13669	NE 109689
		DDA141b	-33.21239, 149.13711	
E. rubida subsp. *rubida*	Long Point Rd, ~5 km NE of Mullions Creek, NSW	NSW1175640	-33.11138, 149.14818	NSW1175641
		NSW1175634	-33.11179, 149.14801	
		NSW1175638	-33.11178, 149.14802	
		NSW1175639	-33.11137, 149.14820	
		NSW1175703	-33.11178, 149.14802	

Taxon and classification per Nicolle (2024)	Site	Samples in final dataset	GPS locations	Representative herbarium voucher collected from site
E. rubida subsp. *barbigerorum* (series *Viminales* subseries *Circulares*)	Forest Way, Nundle State Forest, NSW	DDA255	-31.43883, 151.40906	NE 111727
		DDA255a	-31.43817, 151.40958	
		DDA255c	-31.43853, 151.40964	
		DDA255d	-31.43903, 151.40903	
E. rubida subsp. *barbigerorum*	Thungatti Campground, New England National Park, NSW	DDA259	-30.50197, 152.38767	NE 111731
		DDA259a	-30.50167, 152.38833	
		DDA259b	-30.50192, 152.38817	
		DDA259c	-30.50214, 152.38722	
		DDA259d	-30.50194, 152.38822	
E. rubida subsp. *barbigerorum*	Moredun TSR, Wandsworth, NSW			NSW1187967 [sequence failed]
		NSW1188090	-30.00180, 151.47598	
		NSW1192862	-30.00002, 151.47556	
		NSW1192863	-30.00002, 151.47556	
		NSW1192864	-30.00025, 151.47542	
		NSW1192865	-30.00054, 151.47559	
E. rubida subsp. *barbigerorum*	**Type population:** Wandsworth TSR, Wandsworth, NSW	DDA204	-29.96872, 151.43569	NE 110896
		DDA204a	-29.96897, 151.46861	
		DDA204c	- 29.96894, 151.46950	
E. rubida subsp. *barbigerorum*	Flaggy Creek, Pinkett, NSW		-29.91025, 151.96967	NE 110977
		DDA221a	-29.91000, 151.96986	
		DDA221b	-29.91017, 151.97003	
		DDA221c	-29.91033, 151.97000	
		DDA221d	-29.91042, 151.96969	
E. rubida subsp. *barbigerorum*	Strathbogie road, ~11.7km NW of Glen Innes, NSW	DDA49	-29.66793, 151.64515	NE 108224
		DDA50	-29.66825, 151.64562	NE 108227
		DDA51	-29.66665, 151.64545	NE 108230
		DDA54	-29.66723, 151.64445	NE 108326
E. rubida subsp. *barbigerorum*	Near eastern entrance road to Gibraltar House, Gibraltar Range National Park, NSW		-29.51367, 152.31311	NE 111246
		DDA227a	-29.51175, 152.31342	
		DDA227b	-29.51300, 152.31222	
		DDA227c	-29.51261, 152.31300	
		DDA227d	-29.51444, 152.31300	
		DDA227e	-29.51469, 152.31211	
E. rubida subsp. *barbigerorum*	Chaelundi Campground, Chaelundi National Park, NSW		Site GPS: -30.06830, 152.33300	NE 114906
		DDA313a		
		DDA313b		
		DDA313c		
		DDA313d		
		DDA313e		
E. canobolensis (series *Viminales* subseries *Circulares*)	**Type population:** Junction of Old Canobolas Rd and Towac Way, Mount Canobolas, NSW			NSW1196982 - [sequence failed]
		NSW1196988	-33.34232, 148.97787	
		NSW1196993	-33.34230, 148.97772	
		NSW1196998	-33.34226, 148.97767	
		NSW1197003	-33.34226, 148.97767	

Taxon and classification per Nicolle (2024)	Site	Samples in final dataset	GPS locations	Representative herbarium voucher collected from site
E. dalrympleana subsp. *dalrympleana*	Boboyan Rd, Namadgi National Park, ACT	JJB3614	Site GPS: -35.83525, 149.00969	NE 110463
		JJB3614a		
		JJB3614b		
		JJB3614c		
		JJB3614d		
E. dalrympleana subsp. *dalrympleana* [collected as *E. rubida* subsp. *rubida*]	Bushland next to Hobbys Yards Tip, ~3 km S from Hobby Yards, NSW	NSW1175762	-33.73216, 149.33515	NSW1175751
		NSW1175745	-33.73223, 149.33578	
		NSW1175750	-33.73224, 149.33579	
		NSW1175755	-33.73222, 149.33589	
		NSW1175756	-33.73222, 149.33595	
		NSW1175761	-33.73228, 149.33600	
E. dalrympleana subsp. *dalrympleana*	Kerl Rd, ~12 km S of Orange, NSW	DDA146	-33.38272, 149.04375	NE 109694
		DDA146a	-33.38275, 149.04358	
		DDA146b	-33.38272, 149.04339	
		DDA146c	-33.38267, 149.04333	
		DDA146d	-33.38264, 149.04311	
E. dalrympleana subsp. *dalrympleana*	Gum Ridge Trail, Mt Canobolas, NSW	DDA117	-33.35625, 148.95997	NE 109667
		DDA117a	-33.35581, 148.96031	
		DDA117b	-33.35564, 148.96039	
		DDA117c	-33.35531, 148.96044	
		DDA117d		
E. dalrympleana subsp. *heptantha* (series *Viminales* subseries *Circulares*)	Forest Rd, Coolah Tops National Park, NSW	DDA247	-31.73356, 150.02917	NE 111507
		DDA247a	-31.73353, 150.02922	
		DDA247b	-31.73344, 150.02897	
		DDA247c	-31.73361, 150.02869	
		DDA247d	-31.73353, 150.02803	
E. dalrympleana subsp. *heptantha*	Blue Mountain Rd, ~15 km NE of Walcha, NSW	DDA216	-30.88217, 151.68350	NE 110972
		DDA216a	-30.88278, 151.68333	
		DDA216b	-30.88233, 151.68325	
		DDA216c	-30.88169, 151.68319	
		DDA216d	-30.88122, 151.68314	
E. dalrympleana subsp. *heptantha*	Gostwyck Rd, ~2.5 km W of Mihi, NSW	MWH36	-30.71065, 151.64570	NE 113376
E. dalrympleana subsp. *heptantha*	Woodridge Fossicking and Recreation Area, ~3 km WNW of Uralla, NSW	JWC20	-30.62833, 151.47194	NE 109727
		JWC20ai	Cultivated from JWC20a	
		JWC20aiii	Cultivated from JWC20a	
		JWC20b		
		JWC20ii	Cultivated from JWC20	
E. dalrympleana subsp. *heptantha*	Point Lookout Rd, ~6 km E of intersection with Waterfall Way, NSW	DDA260	-30.49161, 152.34892	NE 111732
		DDA260a	-30.49167, 152.34826	
		DDA260b	-30.49176, 152.34852	
		DDA260c	-30.49190, 152.34905	

Taxon and classification per Nicolle (2024)	Site	Samples in final dataset	GPS locations	Representative herbarium voucher collected from site
E. dalrympleana subsp. *heptantha*	Kanoona road, Armidale, NSW	DDA100	-30.48669, 151.75902	NE 109152
		DDA100a	-30.48672, 151.75875	
		DDA100b	-30.48654, 151.75900	
		DDA101	-30.48683, 151.75889	
		DDA101a	-30.48688, 151.75897	
		DDA101b	-30.48696, 151.75892	
E. dalrympleana subsp. *heptantha*	Boorolong Rd, ~10 km NW of Armidale, NSW	MWH25	-30.45265, 151.56907	NE 112964
E. dalrympleana subsp. *heptantha*	Newholme, ~1.4 kms East of Dumaresq Dam, Armidale, NSW	DDA29	-30.42617, 151.61425	NE 107949
		DDA30	-30.42714, 151.61528	NE 107950
		DDA31	-30.42753, 151.61558	NE 107951
E. dalrympleana subsp. *heptantha*	Newholme Rd, ~3.5 km W of intersection with New England Highway, NSW	GC28	-30.42292, 151.64719	NE 108301
		GC28b	-30.42286, 151.64714	
		GC28c	-30.42294, 151.64714	
E. dalrympleana subsp. *heptantha*	~12.5 km NW of Armidale, NSW	NSW1043530	Site GPS: -30.40175, 151.62558	
		NSW1043567		
		NSW1043587		
		NSW1043588		
		NSW1043622		
		NSW1043628		
E. dalrympleana subsp. *heptantha*	Avondale State Conservation Area, NSW	DDA88	-30.35419, 152.00319	NE 109129
		DDA88a	-30.35411, 152.00311	
		DDA88b	-30.35425, 152.00303	
		DDA88c	-30.35428, 152.00300	
		DDA88d	-30.35419, 152.00289	
		DDA88e	-30.35408, 152.00289	
E. dalrympleana subsp. *heptantha*	Wards Mistake Rd TSR, Bald Blair, NSW	DDA219	-30.14789, 151.81983	NE 110976
		DDA219a	-30.14772, 151.82056	
		DDA219b	-30.14800, 151.82039	
		DDA219c	-30.14817, 151.82003	
		DDA219d	-30.14847, 151.82000	
E. dalrympleana subsp. *heptantha*	Guyra Rd, ~13 km NW of Guyra, NSW	MWH28	-30.14183, 151.58034	NE 112967
E. dalrympleana subsp. *heptantha*	Guyra Rd, ~15km NW of Guyra, NSW	NSW1043606	Site GPS: -30.13578, 151.55108	NSW1043606
		NSW1043547		
		NSW1044799		
		NSW1044805		
E. dalrympleana subsp. *heptantha*	**Type population:** Wandsworth TSR, ~26 km NW of Guyra, NSW	MWH40a	-30.03396, 151.50117	NE 113379
		DDA203	-30.03217, 151.50114	NE 110895
		DDA203a	30.03233, 151.50117	
		DDA203b	-30.03233, 151.50147	
		DDA203c	-30.03256, 151.50167	
		DDA203d	-30.03281, 151.50197	
		DDA203e	-30.03325, 151.50200	
E. cinerea subsp. *cinerea* (series *Viminales* subseries *Circulares*)	Cultivated at Australian Botanic Gardens Mount Annan, NSW	NSW1175008		ABG living collection: A2001-0815/5A
		NSW1175009		ABG living collection: 2004-0413/5

Taxon and classification per Nicolle (2024)	Site	Samples in final dataset	GPS locations	Representative herbarium voucher collected from site
E. lactea (series *Viminales* subseries *Circulares*)	Hidden Trail, Gardens of Stone National Park	NSW1047486	-33.22054, 150.02715	NSW1048580
		NSW1047485	-33.22072, 150.02746	NSW1048582
		NSW1047492	-33.21969, 150.02488	NSW1048588
		NSW1045236	-33.22035, 150.02592	
		NSW1045237	-33.22035, 150.02592	
		NSW1045238	-33.21950, 150.02497	
		NSW1045373	-33.22035, 150.02672	
E. lactea	Coricudgy Rd, Wollemi National Park, NSW	NSW1045227	Site GPS: -32.85563, 150.22936	
		NSW1045228		
		NSW1045229		
E. elliptica (series *Viminales* subseries *Circulares*)	New England Highway TSR, ~15 km NE of Bendemeer, NSW	DDA239	-30.78764, 151.27736	NE 111498
		DDA239b	-30.78782, 151.27729	
		DDA239c	-30.78740, 151.27800	
		DDA239d	-30.787440, 151.27809	
E. elliptica	Dawson's Springs Nature Trail, Mt Kaputar National Park, NSW	DDA238	Site GPS: -30.27933, 150.16489	NE 111497
		DDA238a		
		DDA238b		
		DDA238c	-30.28133, 150.16372	
		DDA238d	-30.28164, 150.16350	
		DDA238e	-30.28164, 150.16372	
E. acaciiformis (series *Viminales* subseries *Lanceolatae*)	Rockvale road, c.1 km SE of Boundary Creek NSW	DDA306	Site GPS: -30.31200, 151.96500	NE 113625
		DDA306a		
		DDA306b		
		DDA306c		
		DDA306d		
E. dorrigoensis (series *Viminales* subseries *Lanceolatae*)	**Type population:** Coramba road, Megan, NSW	DDA228	-30.28278, 152.77603	NE 111247
		DDA228a	-30.28272, 152.77608	
		DDA228b	-30.28281, 152.77622	
		DDA228c	-30.28275, 152.77700	
		DDA228d	-30.28261, 152.77750	
E. dorrigoensis	London Bridge Trail, Oakwood Flora Reserve, NSW	NSW1018019	-29.92043, 152.09882	NSW1022169
		NSW1018079	-29.91693, 152.09788	
E. mannifera subsp. *mannifera* (series *Viminales* subseries *Lanceolatae*)	Burra Rd, ~3 km S of Burra, NSW	NSW1030053	-35.58522, 149.22930	NSW1029689
		NSW1030049	-35.58500, 149.22931	
		NSW1030050	-35.58509, 149.22925	
		NSW1030051	-35.58543, 149.22931	
		NSW1030052	-35.58537, 149.22934	
		NSW1030054	-35.58500, 149.22919	
E. nicholii (series *Viminales* subseries *Lanceolatae*)	New Valley Fire Trail, Single National Park, NSW	DDA242	-30.01800, 151.38844	NE 111503
		DDA242a	-30.01775, 151.38836	
		DDA242b	-30.01797, 151.38825	
		DDA242c	-30.01839, 151.38825	
		DDA242d	-30.01794, 151.38847	

Taxon and classification per Nicolle (2024)	Site	Samples in final dataset	GPS locations	Representative herbarium voucher collected from site
E. nobilis (series *Viminales* subseries *Lanceolatae*)	Wild Cattle Creek Rd, Nowendoc State Forest, NSW	DDA208	-31.41000, 151.57936	NE 110921
		DDA208a	-31.41000, 151.57936	
		DDA208b	-31.41008, 151.57947	
		DDA208c	-31.41008, 151.57928	
		DDA208d	-31.41022, 151.58006	
		DDA208e	-31.41050, 151.58069	
E. nobilis	Point Lookout Rd TSR, Ebor, NSW		-30.48653, 152.32831	NE 109130
		DDA89a	-30.48650, 152.32825	
		DDA89b	-30.48678, 152.32853	
		DDA89c	-30.48689, 152.32833	
		DDA89d	-30.48717, 152.32914	
		DDA89e	-30.48722, 152.32953	
		DDA89f	-30.48697, 152.32983	
E. nobilis	Summit Trail, Duval Nature Reserve, NSW	DDA20	-30.40481, 151.63142	NE 107558
		DDA21	-30.40472, 151.63147	NE 107559
		DDA22	Site GPS: -30.40839, 151.64206	NE 107942
		DDA22a		
		DDA22c		
		DDA22d		
		DDA22e		
E. nobilis	Wards Mistake Rd TSR, Bald Blair, NSW	DDA220	-30.14294, 151.82450	NE 110975
		DDA220a	-30.14264, 151.82464	
		DDA220b	-30.14244, 151.82478	
		DDA220c	-30.14336, 151.82450	
E. nova-anglica (series *Viminales* subseries *Lanceolatae*)	Guyra Rd, ~6.5 km W of Wongwibinda, NSW	NSW1193132	-30.28201, 152.12193	NSW1187924
		NSW1193150	-30.28188, 152.12262	
		NSW1193151	-30.28193, 152.12230	
		NSW1193152	-30.28210, 152.12177	
		NSW1193153	-30.28173, 152.12222	
		NSW1193154	-30.28161, 152.12263	
E. nova-anglica	Oakey Creek Pullover, Waterfall Way, NSW	NSW1193200	-30.49984, 152.25358	NSW1089654
		NSW1188049	-30.50034, 152.25347	
		NSW1188051	-30.50086, 152.25346	
		NSW1193134	-30.50019, 152.25321	
		NSW1193201	-30.50044, 152.25331	
		NSW1193202	-30.50037, 152.25294	
E. nova-anglica	Guyra Ebor road T.S.R, ~10 km E of Guyra, NSW	DDA201	-30.22610, 151.77720	NE 110901
		DDA201a	-30.22613, 151.77727	
		DDA201b	-30.22606, 151.77713	
E. nova-anglica	**Type population:** Bukeiro road, Moona Plains, NSW	DDA309	Site GPS: -30.99630, 151.89360	NE 113768
		DDA309a		
		DDA309b		
		DDA309c		
		DDA309d		

Taxon and classification per Nicolle (2024)	Site	Samples in final dataset	GPS locations	Representative herbarium voucher collected from site
E. viminalis subsp. *viminalis* (series *Viminales* subseries *Lanceolatae*	Mountain Creek Rd, Tidbinbilla Nature Reserve, ACT	JJB3608	-35.45881, 148.89617	NE 108971
		JJB3609	-35.45906, 148.89406	NE 108972
		JJB3610	-35.45839, 148.89181	NE 108973
		JJB3607	-35.45931, 148.89775	NE 108970
		JJB3611	-35.45678, 148.88953	NE 108976
E. viminalis subsp. *viminalis* [collected as *E. rubida* subsp. *rubida*]	Bucky Springs Rd, ~2.8 km SE of Bombala, NSW	NSW1197127	-36.92263, 149.26104	NSW1197125
E. viminalis subsp. *viminalis*	Kowmung River Trail at Kowmung River crossing, NSW	NSW1032935	-33.95630, 149.97893	NSW1029295
		NSW1032926	-33.95617, 149.97868	
		NSW1032927	-33.95597, 149.97756	
		NSW1032928	-33.95568, 149.97687	
		NSW1032930	-33.95602, 149.97825	
E. viminalis subsp. *viminalis*	Jenolan, NSW	NSW1027202	-33.81580, 150.02214	NSW1028616
		NSW1027199	-33.81544, 150.02127	
		NSW1027200	-33.81436, 150.02086	
		NSW1027201	-33.81584, 150.02260	
		NSW1027203	-33.81605, 150.02248	
		NSW1027204	-33.81557, 150.02162	
E. viminalis subsp. *viminalis*	Katoomba View Lookout, Jenolan, NSW	NSW1033192	-33.79050, 150.03232	
		NSW1033193	-33.79060, 150.03232	
		NSW1033194	-33.79058, 150.03202	
		NSW1033195	-33.79057, 150.03153	
E. viminalis subsp. *viminalis*	Gosling Creek Reserve, Orange, NSW	DDA149	Site GPS: -33.32975, 149.08297	NE 109698
		DDA149a		
		DDA149b		
		DDA149c		
		DDA149e		
E. viminalis subsp. *viminalis*	Cox's Creek Rd, Rylston, NSW	DDA114	-32.75272, 150.07375	NE 109664
		DDA114a	-32.75258, 150.07422	
		DDA114b	-32.75257, 150.07399	
		DDA114c	-32.75252, 150.07393	
		DDA114d	-32.75263, 150.07375	
		DDA114e	-32.75314, 150.07361	
E. viminalis subsp. *viminalis*	Brackendale road, ~30.0km SSE of Walcha, NSW	DDA222	-31.24158, 151.71003	NE 110978
		DDA222a	-31.24172, 151.70983	
		DDA222b	-31.24117, 151.70986	
		DDA222c	-31.24094, 151.70944	
		DDA222d	-31.24142, 151.70947	
E. viminalis subsp. *viminalis*	Jalna Rd TSR, ~3 km SW of Bendemeer, NSW	DDA213	-30.90614, 151.13039	NE 110969
		DDA213a	-30.90561, 151.12967	
		DDA213b	-30.90597, 151.13050	
		DDA213c	-30.90633, 151.13164	
		DDA213d	-30.90644, 151.13242	
		DDA213e	-30.90686, 151.13550	

Taxon and classification per Nicolle (2024)	Site	Samples in final dataset	GPS locations	Representative herbarium voucher collected from site
E. viminalis subsp. *viminalis*	Hill View Rd, ~3.5km W of intersection with Thunderbolts Way, NSW	DDA217	-30.80314, 151.57814	NE 110973
		DDA217a	-30.80331, 151.57842	
		DDA217b	-30.80342, 151.57853	
		DDA217c	-30.80342, 151.57872	
		DDA217d	-30.80325, 151.57822	
E. viminalis subsp. *viminalis*	Gostwyck Rd, ~2.5 km W of Mihi, NSW	MWH35	-30.71138, 151.64625	NE 113375
		MWH37	-30.70890, 151.64819	NE 11337
E. viminalis subsp. *viminalis*	Waterfall Way, ~5 km E of Armidale, NSW	MWH43a	-30.52706, 151.70217	NE 113383
E. viminalis subsp. *viminalis*	University of New England, Armidale, NSW	MWH22b	-30.48536, 151.63775	NE 112521
		DDA102	-30.48206, 151.63975	NE 109158
		DDA102a	-30.48186, 151.63978	
		DDA102b	-30.48159, 151.64021	
		DDA102c	-30.48164, 151.64050	
		DDA102d	-30.48172, 151.64011	
		DDA102e	-30.48212, 151.63990	
E. viminalis subsp. *viminalis*	Cachs Creek TSR, ~34 km WNW of Armidale, NSW	DDA207	-30.40331, 151.31903	NE 110899
		DDA207a	-30.40339, 151.31908	
		DDA207b	-30.40317, 151.31964	
		DDA207c	-30.40342, 151.31919	
		DDA207d	-30.40328, 151.31789	
		DDA207e	-30.40367, 151.31681	
E. viminalis subsp. *viminalis*	3003 Rockvale Rd, Thalgarrah, NSW	DDA263	-30.39597, 151.92928	NE 112253
		DDA263a	-30.39556, 151.92894	
		DDA263b	-30.39575, 151.92872	
		DDA263c	-30.39669, 151.92853	
		DDA263d	-30.39722, 151.92883	
E. viminalis subsp. *viminalis*	Guyra-Ebor Rd TSR, Green Hills, NSW	DDA200a	Site GPS: -30.22609, 151.77685	NE 110900
		DDA200b		
		DDA200c		
		DDA200d		
		DDA200e		
		DDA200f		
E. viminalis subsp. *viminalis*	Frazer's Creek TSR, Nullamanna, NSW	DDA223	-29.64586, 151.23325	NE 111239
		DDA223a	-29.64581, 151.23300	
		DDA223b	-29.64586, 151.23292	
		DDA223c	-29.64628, 151.23311	
		DDA223d	-29.64722, 151.23336	
		DDA223e	-29.64658, 151.23300	
E. viminalis subsp. *viminalis*	Nullamanna Rd TSR, Wellingrove, NSW	DDA224	-29.51875, 151.39828	NE 111240
		DDA224a	-29.51875, 151.39725	
		DDA224b	-29.51889, 151.39689	
		DDA224c	-29.51847, 151.39808	
		DDA224d	-29.51808, 151.39881	

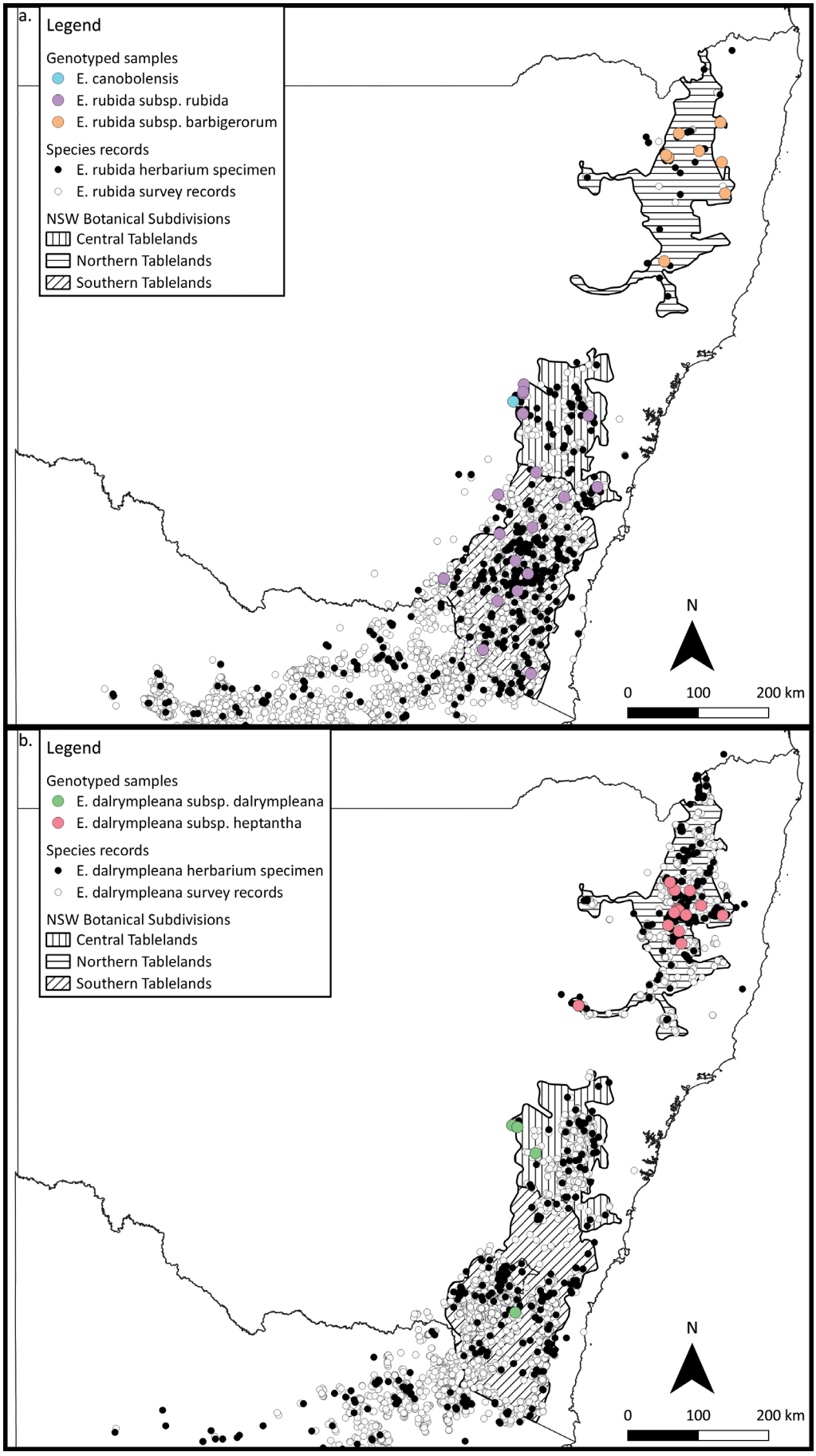

Figure 1. Maps showing geographic locations of collections of (a) *E. rubida* and *E. canobolensis;* and (b) *E. dalrympleana* overlaid upon herbarium specimen collection localities and survey records downloaded from the Atlas of Living Australia (Atlas of Living Australia 2024). Also shown are the three botanical subdivision of NSW they occur in, the Northern Tablelands (horizontal hashing), Central Tablelands (vertical hashing), and Southern Tablelands (diagonal hashing).

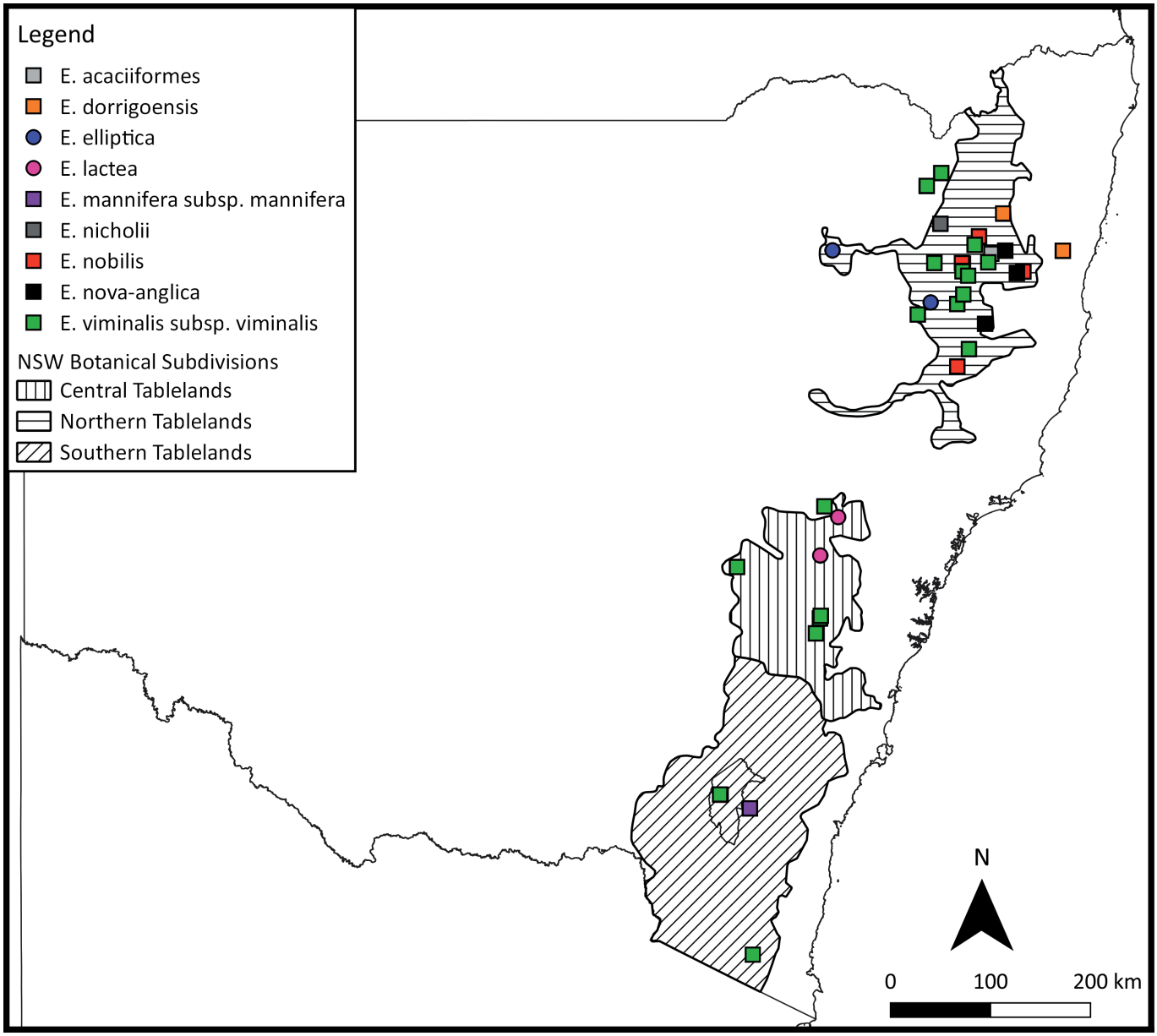

Figure 2. Map showing the collecting localities of outgroup samples included in this study. Points are coloured by taxon and shapes indicated subseries assignment in Nicolle and Jones' (2018) classification: circles indicate members of *E.* subser. *Circulares* and squares indicate members of *E.* subser. *Lanceolatae*. Also shown are the three botanical subdivision of NSW they occur in, the Northern Tablelands (horizontal hashing), Central Tablelands (vertical hashing), and Southern Tablelands (diagonal hashing).

DArTseq data generated and analysis.

Small amounts (~10 mg) of dried leaf tissue from each sample were sent to Diversity Array Technologies Pty Ltd (Canberra) for DNA extraction and sequencing using the DArTseq platform, generating tens of thousands of single nucleotide polymorphisms (SNPs) across the sampled individuals (Sansaloni *et al.* 2010; Sansaloni *et al.* 2011; Kilian *et al.* 2012). The returned *SNP_mapping* formatted SNP dataset was filtered using the *RRtools* package (Rossetto *et al.* 2019) in *R* (R Development Core Team 2016) to remove any individuals with > 50% missing data, along with SNPs with call rates < 80% and minor allele frequencies of < 0.05. SNPs were then subsampled to include only one SNP per sequenced locus meaning the final dataset contained only SNPs with high probability of being unlinked within the genome. A matrix of Euclidean genetic distances between all samples was then calculated using the *dist* function and exported to a nexus file. This file was used to visualise a phylogenetic network in the *splitstree 4* program (Huson and Bryant 2006) using default parameters, to test if the *E. dalrympleana* complex formed a single genetic cluster to the exclusion of all other sampled taxa and to identify any potentially hybrid samples for removal before further analyses.

A secondary dataset was also created by first removing all samples not belonging to the *E. dalrympleana* complex and then performing that same filtering steps as used for the complete dataset. A PCA was then performed on this SNP dataset using the *glPca* function from the *adegenet* package (Jombart 2008; Jombart and Ahmed 2011). In parallel to this, the *dartR* package (Gruber *et al.* 2018) was used to read the *SNP* formatted file provided by DArT, removing all samples with > 50% missing data, and non-*E. dalrympleana* complex samples. The same loci filtering parameters as used previously were also applied, namely a call rate threshold of 80%, reproducibility threshold

of 0.96, and subsampling to one SNP per sequenced locus. This dataset was then randomly subsampled to 5000 SNP to decrease computational requirements for downstream analysis and written to a *STRUCTURE* formatted file. Five replicate runs of *STRUCTURE* (Pritchard *et al.* 2000) were then performed on this dataset for values of K (cluster number) of one through six. All replicates were run for 50 000 burnin replications, and a further 100 000 MCMC replications, and MCMC chains were checked for parameter convergence, which was reached during the burnin in all cases. *StructureHarvester* (Earl and vonHoldt 2011) was then used to collate replicates and investigate the performance of different values of K by inspecting posterior probabilities and employing the *Evanno* method (Evanno *et al.* 2005).

Results

Data quality and filtering

From the raw dataset of 66 087 SNPs and 415 samples, a total of 17 165 SNPs passed quality filtering, with 384 samples included in the filtered series *Viminales* dataset, and 201 samples in the *E. dalrympleana* complex dataset. All datasets are available on request from the authors.

Broader phylogenetic network of series Viminales

Figure 3 shows the *splitstree* network including all samples of species in series *Viminales*. Broadly the clustering corresponds to the classification of Nicolle (2024), however *E. mannifera* subsp. *mannifera* appears to be a member of subseries *Circulares* most closely related to *E. cinerea* and *E. lactea*, the latter of which is considered *E. mannifera* subsp. *praecox* by the APC (Council of Heads of Australasian Herbaria 2016). Of the subseries *Lanceolatae* species, close relatives *E. nicholii* and *E. acaciiformis* formed a cluster with *E. nova-anglica*, and *E. viminalis* subsp. *viminalis*, *E. nobilis* and *E. dorrigoensis* each formed discreet clusters. It was not clear that the *E. dalrympleana* complex formed a natural grouping in our genetic data, as *E. elliptica,* including a sample that appeared to be a putative *E. dalrympleana* × *elliptica* hybrid, clustered closely with the two subspecies of *E. dalrympleana* and *E. rubida* subsp. *barbigerorum*. When a test *splitstree* analysis was run without this hybrid sample in the dataset the remaining *E. elliptica* samples formed a more distinct cluster. Conversely, *E. rubida* subsp. *rubida,* with *E. canobolensis* clustered within it, formed a discreet cluster well removed from this cluster. The most genetically divergent population of *E. rubida* subsp. *rubida* from NW of Yass in the Southern Tablelands region was identified as putatively having experienced gene-flow with *E. mannifera* subsp. *mannifera,* which also occurs in the collection location. Additionally, a *E. viminalis* subsp. *viminalis* sample collected from Katoomba View, Jenolan was putatively identified as a *E. dalrympleana* × *viminalis* hybrid.

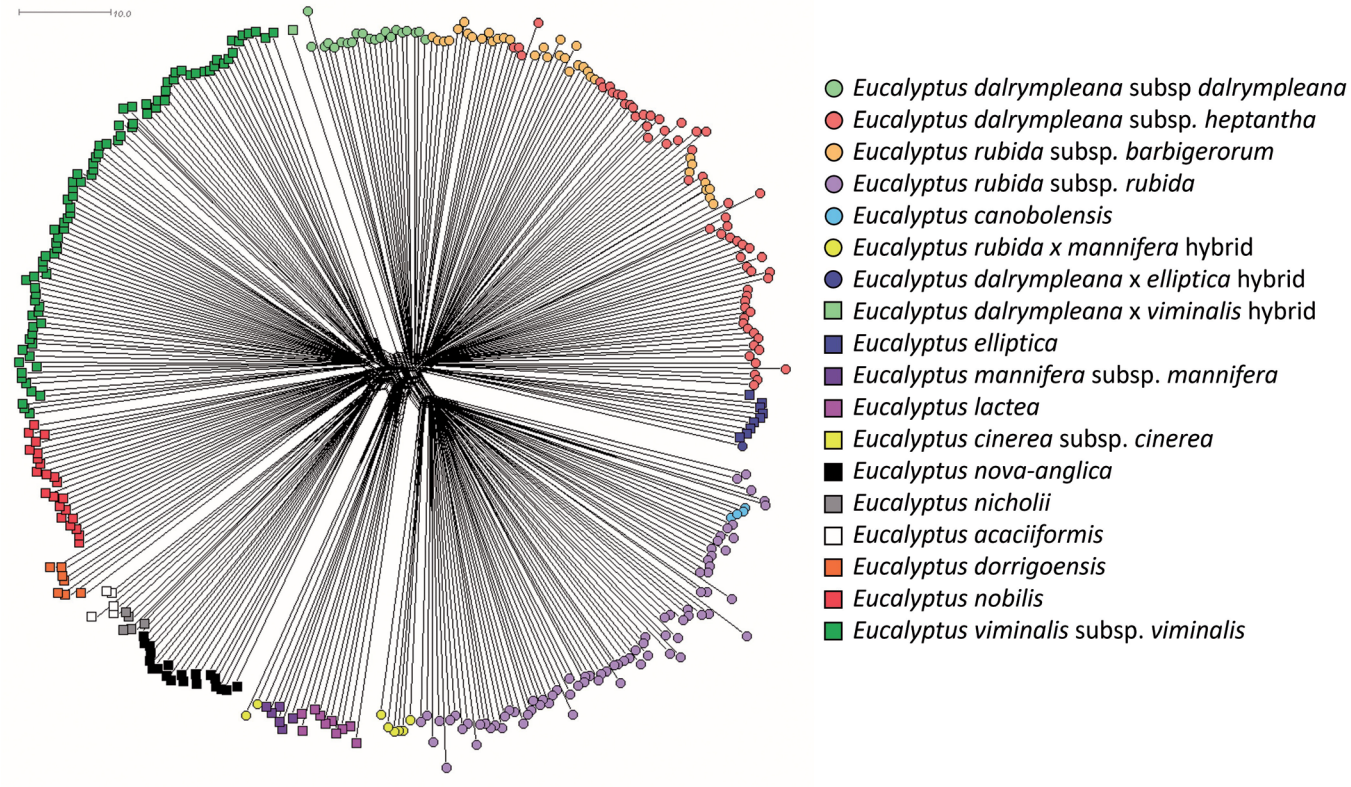

Figure 3. *SplitsTree* network of all *Eucalyptus* series *Viminales* species sampled for this study calculated using Euclidean genetic distances. Tip node colouration corresponds to species, with members of the *E. dalrympleana* complex indicated with circular node tips and all other taxa having square node tips.

Eucalyptus dalrympleana *complex analyses*

In the PCA of the *E. dalrympleana* complex (Fig. 4) PC1 explained ~8.4% of the variation in the data, and broadly separated the samples into three clusters; with the *E. rubida* subsp. *rubida* and *E. canobolensis* cluster being more distinct from the *E. dalrympleana* subsp. *dalrympleana*, and *E. dalrympleana* subsp. *heptantha* and *E. rubida* subsp. *barbigerorum* clusters than the latter were from one another. *Eucalyptus dalrympleana* subsp. *heptantha* and *E. rubida* subsp. *barbigerorum* showed none of the separation in the PCA that would be expected if there were distinct taxa. PC2 (~2% of variation explained) primarily separated the two *E. dalrympleana* s.l. clusters; however, several samples of *E. rubida* subsp. *barbigerorum* from Nundle State Forest and New England National Park and a single sample of *E. dalrympleana* subsp. *dalrympleana* from Hobby's Yard in the Central Tablelands were intermediate between the two *E. dalrympleana* subspecies. The populations of *E. rubida* subsp. *barbigerorum* from Nundle State Forest and New England National Park were noted as having a form resembling *E. dalympleana* subsp. *dalrympleana* in the field.

The results of the STRUCTURE analyses were congruent with those of the PCA (Fig. 5). In the two-cluster analysis *E. rubida* subsp. *rubida* and *E. canobolensis* were assigned to one cluster and *E. dalrympleana* subsp. *heptantha* and *E. rubida* subsp. *barbigerorum* were primarily assigned to the second. Meanwhile, *E. dalrympleana* subsp. *dalrympleana* was partially assigned to both clusters. When the cluster number was increased to three, *E. dalrympleana* subsp. *dalrympleana* was assigned to the third cluster, separating it from *E. dalrympleana* subsp. *heptantha* and *E. rubida* subsp. *barbigerorum*, with the samples of the latter from Nundle State Forest and New England NP showing partial assignment to both clusters. All tested values of K above three showed poor fit to the data, and so are not shown

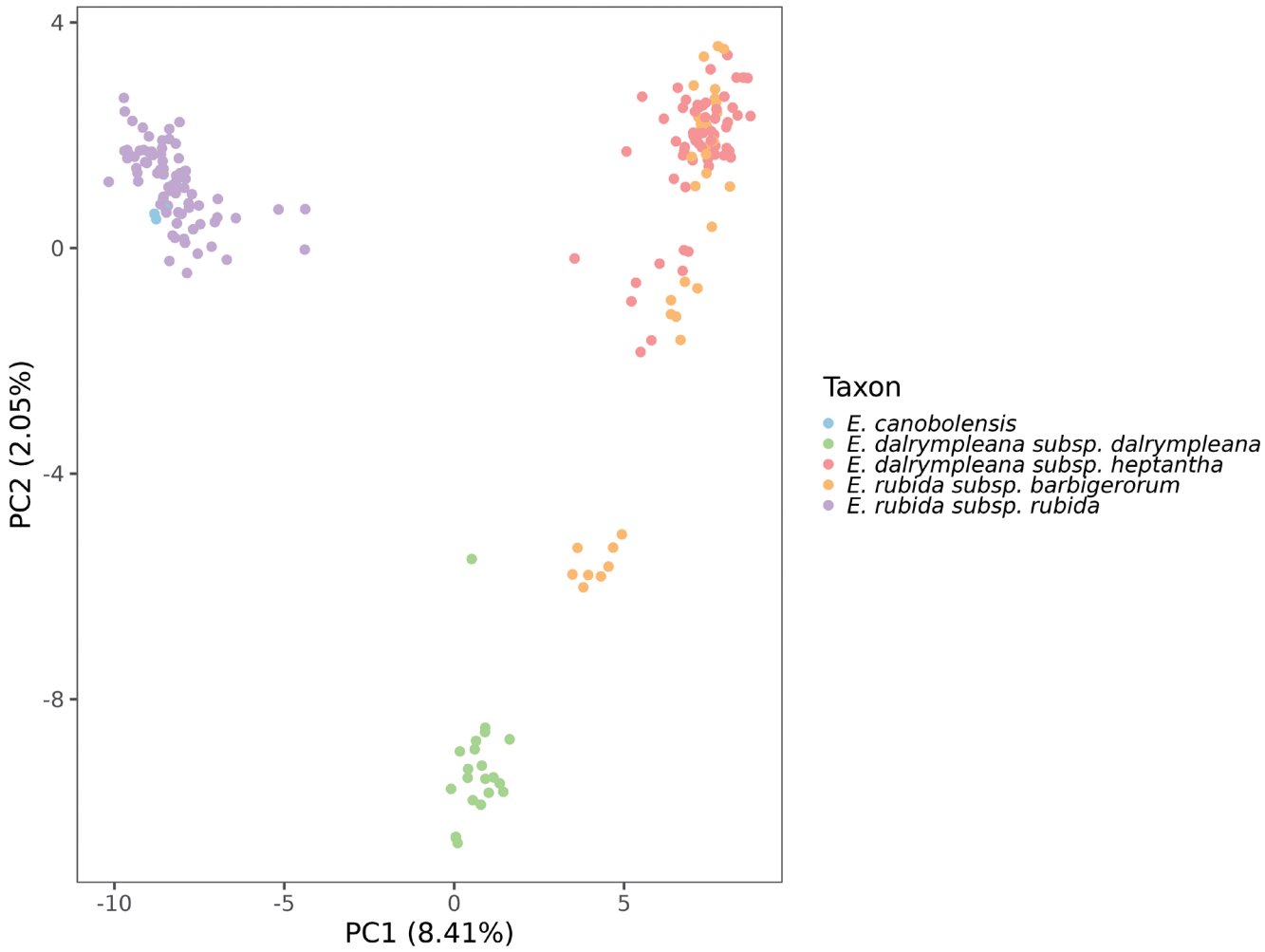

Figure 4. The first two axes of a principal component analysis of SNP data for members of the *E. dalrympleana* complex, with points representing genotyped individuals coloured to match their field identification. *Eucalyptus rubida* subsp. *rubida* and *E. canobolensis* cluster separately to both *E. dalrympleana* subspecies and *E. rubida* subsp. *barbigerorum* on axis one (~8.4% of variation), with *E. dalrympleana* subsp. *dalrympleana* separating from *E. dalrympleana* subsp. *heptantha* and *E. rubida* subsp. *barbigerorum* on axis 2 (~2 % of variation).

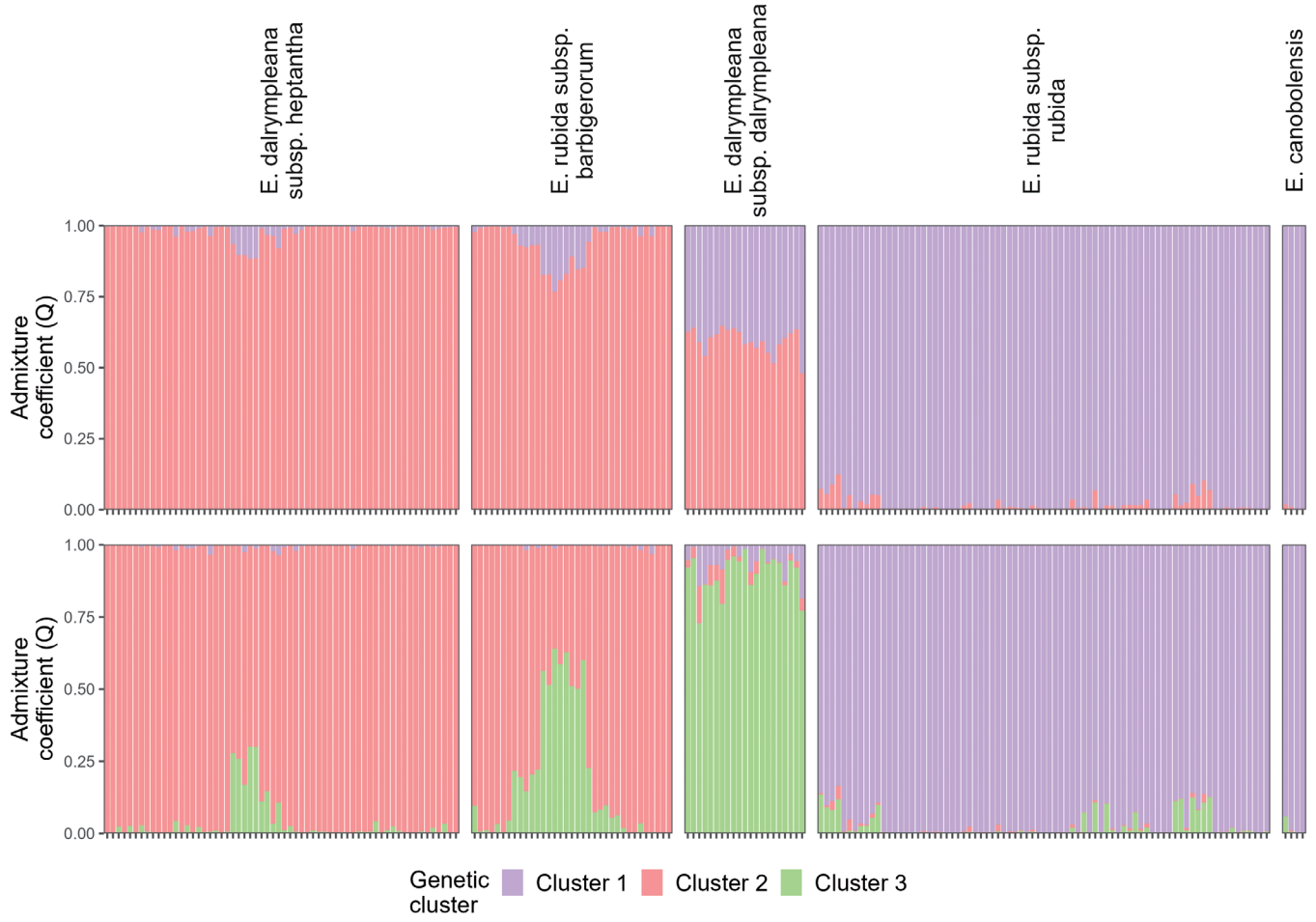

Figure 5. Bar plot summarising STRUCTURE analysis of the *Eucalyptus dalrympleana* complex, showing the two best performing values of K (2 and 3), each run with five replicates. Samples are grouped by existing species definitions, with clustering at both K= 2 and 3 showing *E. rubida* subsp. *barbigerorum* sharing a genetic cluster with *E. dalrympleana,* and *E. canobolensis* forming a single cluster with *E. rubida* subsp. *rubida*.

Discussion

Bar the close relationship between *E. mannifera* subsp. *mannifera* (subseries *Lanceolatae*) and members of subseries *Circulares*, the classification of series *Viminales* by Nicolle and Jones (2018) is broadly congruent with our findings. Our finding of a close relationship between *E. mannifera* subsp. *mannifera* and *E. cinerea* and *E. lactea* aligns with the topology of the phylogeny presented by Jones et al. (2016), although this relationship lacked statistical support in their analysis. Therefore, we suggest that the placement *of E. mannifera* in subser. *Lanceolatae* may need reconsideration to ensure subseries reflect evolutionary relatedness. Despite this, we find support for *E. lactea* being considered a distinct species rather than a subspecies of *E. mannifera* due to close relationship between *E. cinerea* and *E. mannifera* subsp. *mannifera*. Our phylogenetic network (Fig. 3) does not return the *E. dalrympleana* complex as a natural grouping to the exclusion of *E. elliptica*, however we hypothesis this is due to the inclusion of a *E. dalrympleana* × *elliptica* hybrid in our dataset and not a reflection of true evolutionary relatedness.

Based upon the genetic relationships presented in Figures 3, 4 and 5, we suggest that the distinctiveness of *E. canobolensis* is significantly overemphasised by its recognition as a distinct species. Contrary to the hypothesis put forward by Hunter (1998) based upon morphology, the genetic evidence shows with certainty that the nearest relatives of the Mount Canobolas population are the surrounding *E. rubida* subsp. *rubida* populations (Fig. 5). However, the morphological distinctiveness and unique habitat of this population has long been recognised (Maiden 1917; Chappill and Ladiges 1996; Hunter 1998) and reciprocal monophyly is not a necessary, nor even expected, evolutionary pattern for subspecies (Patten 2015). Therefore, in the absence of more detailed morphological and genetic study, we believe it appropriate to take the conservative approach of maintaining this taxon at the subspecies level as *E. rubida* subsp. *canobolensis* per Hill and Johnson (1991). This change does not impact the conservation assessment of the population which remains under threat (Phillips and Zimmer 2022), however as a subspecies of an otherwise non-threatened species, this may allow for different management techniques such as genetic supplementation to assist in adaptation to climate change that threaten to send the population to extinction (Bragg *et al.* 2022).

Our sampling of *E. rubida* subsp. *barbigerorum* includes samples from both the type population north-west of Wandsworth and from additional sites that were assessed by Hunter (2017) as being very likely to be genetically pure/lack introgression from E. *dalrympleana* subsp. *heptantha*. Despite this, all *E. rubida* subsp. *barbigerorum* samples are clearly more closely related to E. *dalrympleana* than to *Eucalyptus rubida* subsp. *rubida*. This matches the field observations of Hunter (2017) and warrants transferring this taxon out of *E. rubida* and into *E. dalrympleana*.

There are two approaches that could be taken with this transfer, either erecting a new subspecies within *E. dalrympleana* to accommodate these populations or to only recognise a single taxon in the *E. dalrympleana* complex in the New England Tablelands of NSW. Given the very low genetic divergence observed in this study, the widespread morphological intergradation observed during systematic field surveys of the taxon, and the high amount of phenotypic plasticity in the morphological traits used to delimit *E. rubida* subsp. *barbigerorum* and *E. dalrympleana* subsp. *heptantha* (Hunter 2017) (namely the number of flowers per inflorescences and extent of rough bark on the trunk) in related taxa, we conclude that *E. rubida* subsp. *barbigerorum* should be considered a taxonomic synonym of *E. dalrympleana* subsp. *heptantha*.

This does raise questions around the distinctness of the two *E. dalrympleana* subspecies in NSW, as they are differentiated morphologically by the number of flowers in each inflorescence, a distinction that is weakened by the synonymising of the three flowered *E. rubida* subsp. *barbigerorum* with the seven flowered *E. dalrympleana* subsp. *heptantha*. Additionally, the identification of populations genetically intermediate between *E. dalrympleana* subsp. *heptantha* and *E. dalrympleana* subsp. *dalrympleana* in the eastern parts of the Northern Tablelands complicates any taxonomic resolutions. Given the level of genetic divergence between most populations of *E. dalrympleana* subsp. *heptantha* s.l. in the Northern Tablelands, and those of *E. dalrympleana* subsp. *dalrympleana* in the Central and Southern Tablelands of NSW we support the recognition of these subspecies based on genetic divergence and geography at this point. However, further work is needed to investigate reliable morphological features that differentiate these subspecies and the identity of *E. dalrympleana* populations on the eastern edge of the Northern Tablelands which show some genetic affinity to southern populations of the species.

Overall, we believe our study highlights the power of using population level sampling and population genetic analytical approaches in resolving the relationships and taxonomy of closely related eucalypt taxa, which has long been complicated by their propensity to hybridise and high variability across species ranges.

Taxonomy

Eucalyptus dalrympleana Maiden, *Proc. Linn. Soc. New South Wales* 23: 164 (1898). *Type*: New South Wales: Yarrangobilly, Peppercorn Plain, January 1920, *W.A.W. de Beuzeville* 1, 2, 3 (syn: NSW).

Notes: No samples of *E. dalrympleana* from outside NSW were investigated in this genetic study so the recent taxonomic changes proposed by Nicolle (2022) are not tested here, but should be considered in future studies.

Eucalyptus dalrympleana subsp. ***dalrympleana***

Distribution: Widespread in the Central and Southern Tablelands of New South Wales through eastern Victoria, with outlying populations in the Mount Lofty Ranges of South Australia (Fig. 6).

Notes: Populations genetically intermediate between this subspecies and *E. dalrympleana* subsp. *heptantha* appear to exist on the eastern edge of the Northern Tablelands, with further morphological study needed to investigate where these populations fit. Here these populations are treated under *E. dalrympleana* subsp. *heptantha*.

Eucalyptus dalrympleana subsp. ***heptantha*** L.A.S.Johnson, *Contr. New South Wales Natl Herb.* 3: 110 (1962). *Type*: New South Wales: Wandsworth to Moredun Ck, 3000 ft, 23 May 1957, *L.A.S.Johnson* (holo: NSW 41849; iso: FRI, K).

Eucalyptus rubida subsp. *barbigerorum* L.A.S.Johnson & K.D.Hill, *Telopea* 4: 240 (1991). *Type*: New South Wales: Northern Tablelands: 14.1 km from Wandsworth towards Tingha, 29 Aug. 1986, *K.D.Hill 2117* (holo: NSW200439; iso: BRI, CANB, CBG, MEL).

Distribution: Widespread in the Northern Tablelands of New South Wales and adjacent areas of southern Queensland (Fig. 6).

E. dalrympleana subsp. ***lutruwita*** D.Nicolle, *Native Eucalypts Vict. & Tasman., S.-E. Austral.* 180–181 (2023). *Type:* Tasmania: Lakes Highway [Highland Lakes Rd], 26.4 km NW of Bothwell, 4 June 1979, *A.M.Gray 374* (holo: AD 97943538; iso: CANB, HO, MEL, NSW, PERTH).

Distribution: Widespread across central and eastern Tasmania (Fig. 6).

E. dalrympleana subsp. ***poliophylla*** D.Nicolle, *Native Eucalypts Vict. & Tasman., S.-E. Austral.* 182–183 (2023). *Type:* Victoria: Howmans Gap Alpine Resort, c. 4 km N from Falls Creek Village, 12 January 2001, *N.GWalsh 5293* (holo: MEL 2104852; iso: CANB, NSW).

Distribution: Restricted to higher elevation (>1200 m) regions of eastern Victoria (Fig. 6).

Eucalyptus rubida H.Deane & Maiden, *Proc. Linn. Soc. New South Wales* 24: 456 (1899). *Type:* New South Wales: Jindabyne, Jan. 1898, *J.H.Maiden s.n.* (lecto: NSW325637, NSW325636), first step lectotypification (restricting the type to a single collection at NSW, but mounted on two sheets) designated by J.H.Maiden, Crit. Rev. genus Eucalyptus 3(6) 110–122 (1916).

Eucalyptus rubida subsp. ***rubida***

Eucalyptus *rubida* subsp. *septemflora* L.A.S.Johnson & K.D.Hill, *Telopea* 4: 241 (1991). *Type*: Victoria: 3 km N of Mt Beauty North on Wodonga road, 20 Feb. 1986, *K.D.Hill 1430 & L.A.S.Johnson* (holo: NSW205444).

Distribution: Widespread in hilly and tableland country from the Central and Southern Tablelands of New South Wales, eastern Victoria, and Tasmania (Fig. 7).

Eucalyptus rubida subsp. ***canobolensis*** L.A.S.Johnson & K.D.Hill, *Telopea* 4: 239 (1991). *Eucalyptus canobolensis* (L.A.S.Johnson & K.D.Hill) J.T.Hunter, *Telopea* 8: 157 (1998). *Type*: New South Wales: Central Tablelands: Mt Canobolas, Orange, Jan. 1908, *J.L. Boorman s.n.* (holo: NSW325550).

Distribution: Endemic to higher elevations on Mount Canobolas, Orange, New South Wales (Fig. 7)

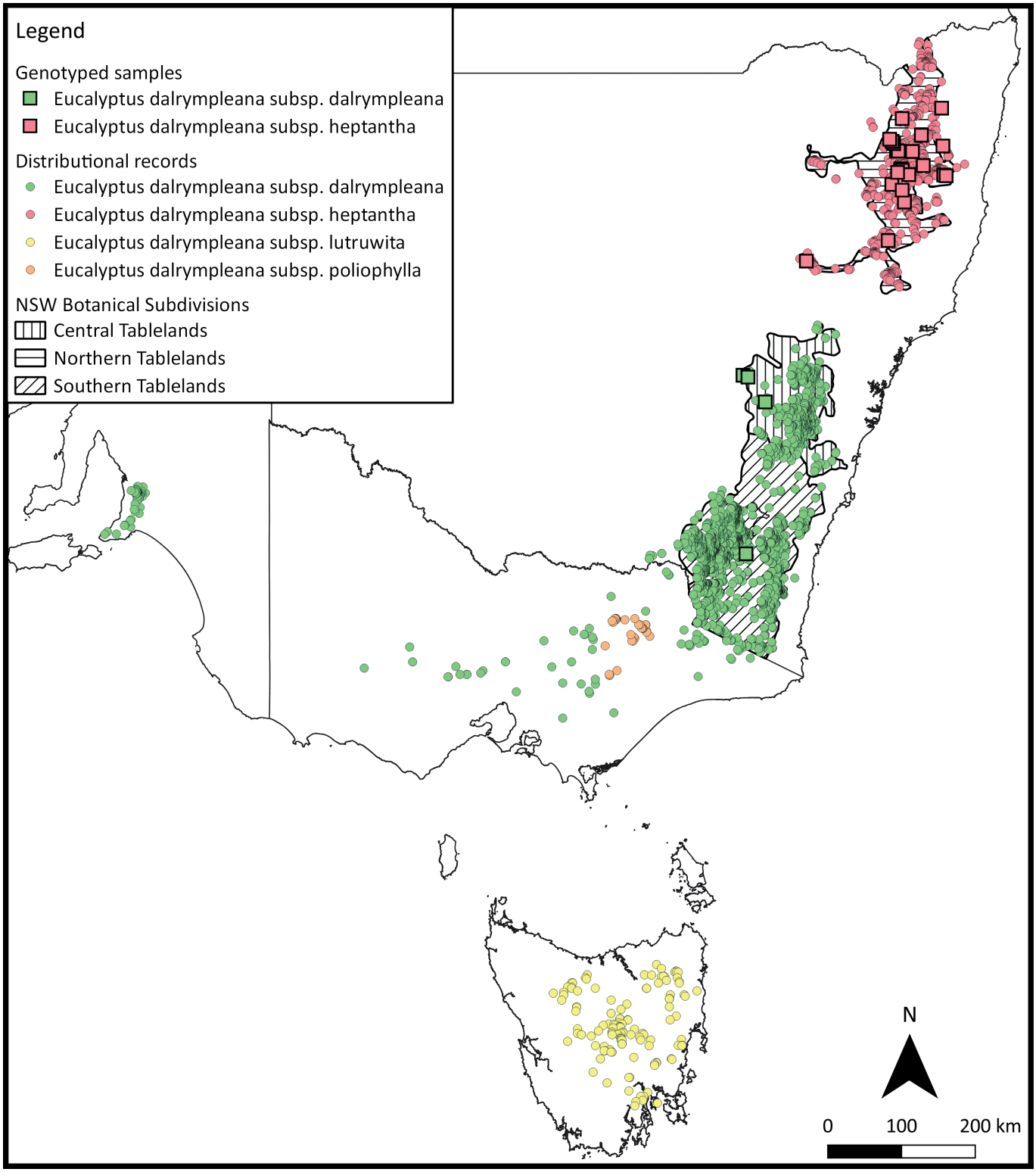

Figure 6. The distribution of the subspecies of *Eucalyptus dalrympleana* based upon the findings of our genetic study and the most recent taxonomic literature on the species. Genotyped samples are shown on the map using large squares and coloured by the subspecies assignment best supported by genetic data. Atlas of Living Australia (2024) records are shown with circular points and coloured by subspecies assignment based upon the understanding of their distributions from the literature and our genetic data. Also shown are the botanical regions of New South Wales in which the species is considered to naturally occur.

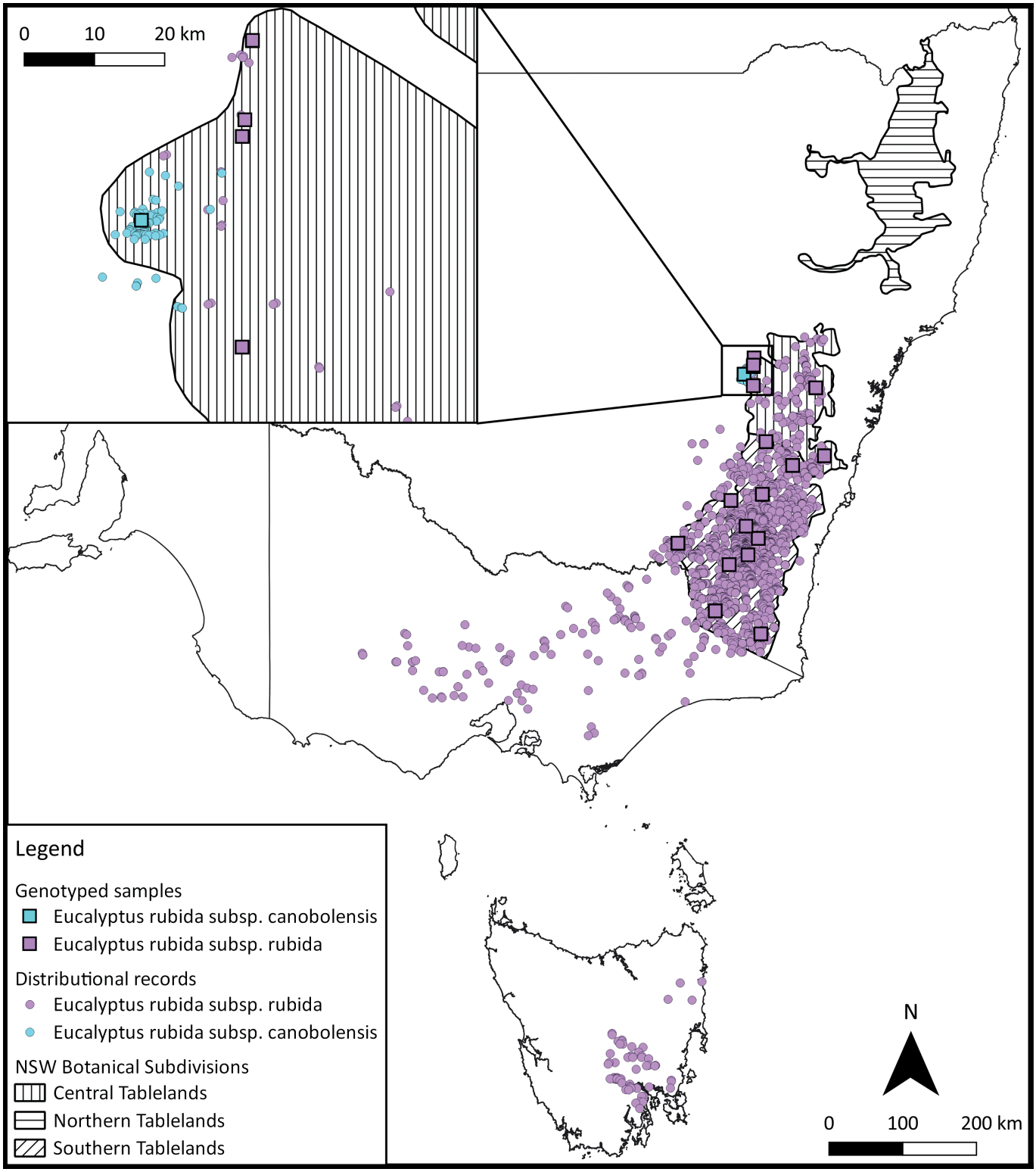

Figure 7. The distribution of the subspecies of *Eucalyptus rubida* based upon the findings of our genetic study and the most recent taxonomic literature on the species. Genotyped samples are shown on the map using large squares and coloured by the subspecies assignment best supported by genetic data. Atlas of Living Australia (2024) records are shown with circular points and coloured by subspecies assignment based upon the understanding of their distributions from the literature and our genetic data. Also shown are the botanical regions of New South Wales in which the species is considered to naturally occur.

References

Atlas of Living Australia (2024) Atlas of Living Australia. Available at URL [Accessed 5 April 2024].

Bragg, JG, van der Merwe, M, Yap, J-YS, Borevitz, J, Rossetto, M (2022) Plant collections for conservation and restoration: Can they be adapted and adaptable? *Molecular Ecology Resources* **22**, 2171–2182. DOI

Brooker, MIH, Slee, AV, Connors, JR, Duffy, S, West, J (2015) EUCLID: Eucalypts of Australia. Available at URL

Chappill, J, Ladiges, P (1996) Phylogenetic analysis of *Eucalyptus* informal subgenus *Symphyomyrtus* section *Maidenaria*. *Australian Systematic Botany* **9**, 71–93. DOI

Council of Heads of Australasian Herbaria (2016) *Australian Plant Census*, IBIS database. Centre for Australian National Biodiversity Research, Australian Government, Canberra.

Deane, H, Maiden, JH (1899) Observations on the eucalypts of New South Wales. Part V. *Proceedings of the Linnean Society of New South Wales* **24**, 448–471.

Earl, DA, vonHoldt, BM (2011) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* **4**, 359–361. DOI

Evanno, G, Regnaut, S, Goudet, J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14**, 2611–2620. DOI

Gruber, B, Unmack, PJ, Berry, OF, Georges, A (2018) *DARTR*: An R package to facilitate analysis of SNP data generated from reduced representation genome sequencing. *Molecular Ecology Resources* **18**, 691–699. DOI

Hill, KD, Johnson, LAS (1991) Systematic studies in the eucalypts – 3. New taxa and combinations in *Eucalyptus* (Myrtaceae). *Telopea* **4**, 223–267. URL

Hunter, JT (1998) *Eucalyptus canobolensis* (Myrtaceae), a new combination for a former subspecies of *Eucalyptus rubida*. *Telopea* **8**, 157–159. URL

Hunter, JT (2017) Monitoring of *Eucalyptus rubida* subsp. *barbigerorum*. Document Prepared by. Dr John T. Hunter. Hewlett Hunter Pty Ltd. For 'Saving Our Species' PDF

Huson, DH, Bryant, D (2006) Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution* **23**, 254–267. DOI

Jombart, T (2008) adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* **24**, 1403–1405.

Jombart, T, Ahmed, I (2011) adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. *Bioinformatics* **27**, 3070–3071. DOI

Jones, R, Nicolle, D, Steane, D, Vaillancourt, R, Potts, B (2016) High density, genome-wide markers and intra-specific replication yield an unprecedented phylogenetic reconstruction of a globally significant, speciose lineage of *Eucalyptus*. *Molecular Phylogenetics and Evolution* **105**, 63–85. DOI

Kilian, A, Wenzl, P, Huttner, E, Carling, J, Xia, L, Blois, H, Caig, V, Heller-Uszynska, K, Jaccoud, D, Hopper, C, Aschenbrenner-Kilian, M, Evers, M, Peng, K, Cayla, C, Hok, P, Uszynski, G (2012) Diversity Arrays Technology: a generic genome profiling technology on open platforms. *Data Production and Analysis in Population Genomics* **888**, 67–89. DOI

Maiden, JH (1917) *A critical revision of the genus* Eucalyptus *Vol. III*. (Government Printer: Sydney)

Maiden, JH, Flockton, M (1922) *The forest flora of New South Wales*. (W.A. Gullick: Sydney)

Nicolle, D (2022) *Native Eucalypts of Victoria and Tasmania, South-eastern Australia*. (Bloomings Books: Adelaide)

Nicolle, D (2024) Classification of the eucalypts (*Angophora*, *Corymbia* and *Eucalyptus*) Version 7. Available at PDF

Nicolle, D, Jones, R (2018) A revised classification for the predominantly eastern Australian *Eucalyptus* subgenus *Symphyomyrtus* sections *Maidenaria*, *Exsertaria*, *Latoangulatae* and related smaller sections (Myrtaceae). *Telopea* **21**, 129–145. DOI

Patten, MA (2015) Subspecies and the philosophy of science. *The Auk* **132**, 481–485. DOI

Phillips, GP, Zimmer, H (2022) Conservation assessment of *Eucalyptus canobolensis* (L.A.S.Johnson & K.D.Hill) J.T.Hunter. NSW Threatened Species Scientific Committee.

Pritchard, JK, Stephens, M, Donnelly, P (2000) Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–959. DOI

R Development Core Team (2016) *R: A language and environment for statistical computing*. (R Foundation for Statistical Computing: Vienna, Austria)

Rossetto, M, Bragg, J, Kilian, A, McPherson, H, van der Merwe, M, Wilson, PD (2019) Restore and Renew: a genomics-era framework for species provenance delimitation. *Restoration Ecology* **27**, 538–548. DOI

Sansaloni, C, Petroli, C, Jaccoud, D, Carling, J, Detering, F, Grattapaglia, D, Kilian, A (2011) Diversity Arrays Technology (DArT) and next-generation sequencing combined: genome-wide, high throughput, highly informative genotyping for molecular breeding of *Eucalyptus*. *BMC Proceedings* **5**, P54. DOI

Sansaloni, CP, Petroli, CD, Carling, J, Hudson, CJ, Steane, DA, Myburg, AA, Grattapaglia, D, Vaillancourt, RE, Kilian, A (2010) A high-density Diversity Arrays Technology (DArT) microarray for genome-wide genotyping in *Eucalyptus*. *Plant Methods* **6**, 16. DOI