Botanical Journal of the Linnean Society, 2024, **204**, 1–22
https://doi.org/10.1093/botlinnean/boad034
Advance access publication 23 September 2023
Original Article

OXFORD

Original Article

Genomic data resolve phylogenetic relationships of Australian mat-rushes, *Lomandra* (Asparagaceae: Lomandroideae)

Bee F. Gunn[1], Daniel J. Murphy[1], Neville G. Walsh[1], John G. Conran[2], J. Chris Pires[3], Terry D. Macfarlane[4], Michael D. Crisp[5], Lyn G. Cook[5] and Joanne L. Birch[6,*]

[1]Royal Botanic Gardens Victoria, Birdwood Avenue, Melbourne, VIC 3004, Australia
[2]The University of Adelaide, School of Biological Sciences, Adelaide, SA 5005, Australia
[3]Colorado State University, Department of Soil and Crop Sciences, Fort Collins, CO, 80523, USA
[4]Western Australian Herbarium, Dept. of Biodiversity, Conservation and Attractions, Locked Bag 104, Bentley Delivery Center, WA 6983, Australia
[5]The University of Queensland, School of Biological Sciences, Brisbane, QLD 4072, Australia
[6]The University of Melbourne, School of BioSciences, Parkville, VIC 3010, Australia

[*]Corresponding author. The University of Melbourne, School of BioSciences, Parkville, VIC 3010, Australia. E-mail: joanne.birch@unimelb.edu.au

ABSTRACT

Lomandra is the largest genus in Asparagaceae subfamily Lomandroideae and possesses economic, ecological, and ethnobotanical significance in Australia. *Lomandra* comprises four sections, *L.* section *Capitatae*, *L.* section *Macrostachya*, *L.* section *Typhopsis* and *L.* section *Lomandra*, the latter comprising series *Lomandra* and series *Sparsiflorae*, all recognized based solely on morphology. In this study, phylogenetic relationships were estimated for 79 Lomandroideae individuals, including 45 *Lomandra* species and subspecies (*c.* 63% of species and subspecies diversity). We generated genome-scale plastome sequence data and used maximum likelihood and Bayesian inference criteria for phylogenetic estimation. *Lomandra* was non-monophyletic, with *Xerolirion divaricata* nested within it. Two major clades were recovered: *Capitatae–Macrostachya* (CM) and *Lomandra–Typhopsis* (LT). The CM clade included a monophyletic *Lomandra* section *Capitatae* with a base chromosome number $x = 7$, and *L.* section *Macrostachya* ($x = 8$); the LT clade included *L.* sections *Typhopsis* and *Lomandra*, both $x = 8$. Section *Lomandra* series *Lomandra* and series *Sparsiflorae* were both recovered as non-monophyletic. Morphological characters were assessed to identify combinations of characters that characterize clades. A base chromosome number of $x = 8$ was plesiomorphic for *Lomandra*. The largest number of *Lomandra* species occupy the Mediterranean ecoregion and occupancy of sclerophyll vegetation was reconstructed as ancestral for the genus.

Keywords: base chromosome number; ecoregions; Mediterranean forest and woodlands; molecular phylogeny; plastome; polyploidy; temperate broadleaf forest; temperate grasslands, savannah and shrub; tropical and subtropical moist broadleaf forest; tropical grasslands, savannah and shrub

INTRODUCTION

Lomandra Labill. (mat-rushes) is the largest genus in Asparagaceae Juss., subfamily Lomandroideae Thorne & Reveal, including 60 species and 11 subspecies[1] [Australian Plant Census, IBIS database, Centre for Australian National Biodiversity Research, Council of Heads of Australasian Herbaria, viewed (19 April 2023), https://chah.gov.au/council-of-heads-of-australasian-herbaria/; VicFlora online: https://vicflora.rbg.vic.gov.au viewed (22 September 2022)]. Australia is the centre of diversity for *Lomandra*, with only two species, *L. banksii* (R.Br.) Lauterb. and *L. multiflora* (R.Br.) Brittan from tropical northern Australia also occurring in Papua New Guinea and *L. banksii* (sometimes treated as the separate species *L. insularis* Schltr.) in New Caledonia (Lee and Macfarlane 1986). *Lomandra* consists of perennial tufted or tussock-forming dioecious herbs that can sometimes attain shrub or treelet stature [*L. ordii* (F.Muell.) Schltr. and *L. banksii*], or even grow as rainforest vines (*L. insularis* in New Caledonia) (Fig. 1). The genus occupies a vast range of habitats including temperate sclerophyll forests, grasslands, mallee, coastal dunes, xeric

[1]*Lomandra longifolia* subsp. *exilis* A.T. Lee is recognized in VicFlora (2022) but is a synonym of *L. longifolia* Labill. in the Flora of Australia and in the Australian Plant Census.

Received 20 December 2022; revised 12 May 2023; accepted 9 June 2023

Figure 1. *Lomandra* habit, leaf, inflorescence, and floral diversity. A, *L. glauca*, B, *L. densiflora*, C, *L. hastilis*, D, *L. hystrix*, E, *L. banksii* (sometimes recognized as *L. insularis* Schltr.), F, *L. juncea*, G, *L. leucocephala* subsp. *robusta*, H, *L. micrantha*, and I, *L. pauciflora*. Photo credits: J. Conran.

Table 1. *Lomandra* taxa and infrageneric classification history based on Bentham (1878), Stevens (1978), and Macfarlane and Lee (1986) and placement of taxa in clades recovered in this study.

***Lomandra* spp.**	**Bentham (1878)**	**Stevens (1978)**	**Macfarlane & Lee (1986)**	**Gunn et al. (this study)**
acicularis M.D.Barrett	NR	NR	NR	NR
altior Jian Wang ter	NR	NR	NR	NR
banksii (R.Br.) Lauterb.	Sect. Euxerotes, ser. Glomeratae[1]	Group B	Sect. Lomandra ser. Lomandra	1
bracteata A.T.Lee	NR	Group A	Sect. Lomandra ser. Sparsiflorae	2A
brevis A.T.Lee	NR	Group A	Sect. Lomandra ser. Sparsiflorae	2A
breviscapa Jian Wang ter	NR	NR	NR	NR
brittanii T.S.Choo	NR	NR	Sect. Lomandra ser. Sparsiflorae	NR
caespitosa (F.Muell. ex Benth.) Ewart	Sect. Euxerotes, ser. Sparsiflorae[1]	NR	Sect. Lomandra ser. Sparsiflorae	2A
collina (R.Br.) Ewart	Sect. Cephalogyne[1]	Sect. Cephalogyne (as *L. glauca* subsp. *collina*)	Section Capitatae	1A
confertifolia (F.M.Bailey) Fahn subsp. *confertifolia*	NR	Group B	Sect. Lomandra ser. Lomandra	4A
confertifolia subsp. *leptostachya* A.T.Lee	NR	NR	Sect. Lomandra ser. Lomandra	NR
confertifolia subsp. *pallida* A.T.Lee	NR	NR	Sect. Lomandra ser. Lomandra	4A
confertifolia subsp. *rubiginosa* A.T.Lee	NR	NR	Sect. Lomandra ser. Lomandra	NR
confertifolia subsp. *similis* A.T.Lee	NR	NR	Sect. Lomandra ser. Lomandra	NR
confertifolia (glaucous)	NR	NR	NR	2A
confertifolia (scrambling)	NR	NR	NR	4A
cylindrica A.T.Lee	NR	Group A	Sect. Lomandra ser. Sparsiflorae	NR
decomposita (R.Br.) Jian Wang ter & A.R.Bean	NR	NR	NR	NR
densiflora J.M.Black	NR	Group A	Sect. Lomandra ser. Sparsiflorae	2A
drummondii (Benth.) Ewart	Sect. Euxerotes, ser. Glomeratae[1]	NR	Sect. Lomandra ser. Sparsiflorae	2B
effusa (Lindl.) Ewart	Sect. Euxerotes, ser. Sparsiflorae[1]	Group A	Sect. Lomandra ser. Sparsiflorae	2C
elongata (Benth.) Ewart	Sect. Cephalogyne[1]	Sect. Cephalogyne	Sect. Capitatae	NR
fibrata J.M.Black	NR	Group A	Sect. Lomandra ser. Sparsiflorae	2A
filiformis subsp. *coriacea* A.T.Lee	NR	NR	Sect. Lomandra ser. Sparsiflorae	2A
filiformis (Thunb.) Britten subsp. *filiformis*	Sect. Euxerotes, ser. Sparsiflorae[1]	Group A	Sect. Lomandra ser. Sparsiflorae	2A
filiformis subsp. *flavior* A.T.Lee	NR	NR	Sect. Lomandra ser. Sparsiflorae	NR
filiformis Grampians	NR	NR	NR	2A
filiformis Moggs Creek	NR	NR	NR	2A
fluviatilis (R.Br.) A.T.Lee	NR	NR	Sect. Lomandra ser. Lomandra	NR
glauca (R.Br.) J.F.Macbr.	Sect. Cephalogyne[1]	Sect. Cephalogyne (as *L. glauca* subsp. *collina*)	Sect. Capitatae	1A

Table 1. Continued

Lomandra spp.	Bentham (1878)	Stevens (1978)	Macfarlane & Lee (1986)	Gunn et al. (this study)
glauca sp. nov..	NR	NR	NR	1A
gracilis (R.Br.) A.T.Lee	NR	NR	Sect. Lomandra ser. Sparsiflorae	NR
grayi Jian Wang ter	NR	NR	NR	NR
hastilis (R.Br.) Ewart	Sect. Macrostachya[1]	Sect. Macrostachya	Sect. Macrostachya	1B
hermaphrodita (C.R.P.Andrews) C.A.Gard.	NR	NR	Sect. Lomandra ser. Sparsiflorae	3A
hispidula Jian Wang ter	NR	NR	NR	NR
hystrix (R.Br.) L.R.Fraser & Vickery	NR	NR	Sect. Lomandra ser. Lomandra	4A
integra T.D.Macfarl.	Sect. Euxerotes, ser. Glomeratae[1] (as *X. endlicheri*)	Group A (as *L. endlicheri*)	Sect. Lomandra ser. Sparsiflorae	NR
juncea (F.Muell.) Ewart	Sect. Schoenoxeros[1]	Sect. Typhopsis	Sect. Typhopsis	3C
laxa (R.Br.) A.T.Lee	NR	NR	Sect. Lomandra ser. Sparsiflorae	3A
leucocephala (R.Br.) Ewart subsp. *leucocephala*	Sect.Typhopsis[1]	Sect. Typhopsis	Sect. Typhopsis	NR
leucocephala subsp. *robusta* A.T.Lee	NR	NR	Sect. Typhopsis	NR
longifolia Labill. subsp. *longifolia*	Sect. Euxerotes, ser. Glomeratae[1]	Group B	Sect. Lomandra ser. Lomandra	4A
longifolia subsp. *exilis* A.T.Lee	NR	NR	NA	4A
marginata T.D.Macfarl. & Conran	NR	NR	NR	2B
maritima T.S.Choo	NR	NR	Sect. Lomandra ser. Sparsiflorae	3A
micrantha (Endl.) Ewart subsp. *micrantha*	Sect. Euxerotes, ser. Sparsiflorae[1]	Group A	Sect. Lomandra ser. Sparsiflorae	2B
micrantha subsp. *teretifolia* J.Everett	NR	NR	Sect. Lomandra ser. Sparsiflorae	2B
micrantha subsp. *tuberculata* J.Everett	NR	NR	Sect. Lomandra ser. Sparsiflorae	2B
montana (R.Br.) L.R.Fraser & Vickery	NR	Group B	Sect. Lomandra ser. Lomandra	NR
mucronata (R.Br.) A.T.Lee	NR	Sect. Capitatae	Sect. Capitatae	1A
multiflora (R.Br.) Britten subsp. *multiflora*	Sect. Euxerotes, ser. Fasciculatae[1] (as *X. multiflora*)	Group B (as *L. multiflora*)	Sect. Lomandra ser. Lomandra	NR
multiflora subsp. *dura* (F.Muell.) T.D.Macfarl.	Sect. Euxerotes, ser. Glomeratae[1](as *X. dura*)	Group B (as *L. dura*)	Sect. Lomandra ser. Lomandra	3B
nana (A.T.Lee) A.T.Lee	NR	NR	Sect. Capitatae	NR
nigricans T.D.Macfarl.	NR	NR	Sect. Lomandra ser. Sparsiflorae	2C
nutans T.D.Macfarl.	NR	NR	Sect. Lomandra ser. Sparsiflorae	NR
obliqua (Thunb.) J.F.Macbr.	Sect. Cephalogyne[1] (as *X. flexifolia*)	Sect. Cephalogyne	Sect. Capitatae	1A
odora (Endl.) Ewart	Sect. Euxerotes, ser. Glomeratae[1]	NR	Sect. Lomandra ser. Sparsiflorae	NR

Table 1. Continued

Lomandra spp.	Bentham (1878)	Stevens (1978)	Macfarlane & Lee (1986)	Gunn et al. (this study)
ordii (F.Muell.) Schltr.	NR	Group B	Sect. Lomandra ser. Lomandra	1
oreophila B.J.Conn & Quirico	NR	NR	NR	2C
patens A.T.Lee	NR	Group B	Sect. Lomandra ser. Lomandra	3B
pauciflora (R.Br.) Ewart	Sect. Euxerotes, ser. Sparsiflorae[1]	Group A	Sect. Lomandra ser. Sparsiflorae	3A
preissii (Endl.) Ewart	Sect. Euxerotes, ser. Fasciculatae[1]	Group A	Sect. Lomandra ser. Sparsiflorae	2C
purpurea (Endl.) Ewart	Sect. Euxerotes, ser. Fasciculatae[1]	Group A	Sect. Lomandra ser. Sparsiflorae	NR
ramosissima Wang Jian ter	NR	NR	NR	NR
rigida Labill.	Sect. Euxerotes, ser. Glomeratae[1]	Group B	Sect. Lomandra ser. Lomandra	NR
rupestris (Endl.) Ewart	Sect. Cephalogyne[1]	Sect. Cephalogyne	Sect. Capitatae	1A
sericea (Endl.) Ewart	Sect. Euxerotes, ser. Fasciculatae[1]	NR	Sect. Lomandra ser.Lomandra	3C
sonderi (F.Muell.) Ewart	Sect. Euxerotes, ser. Glomeratae[1]	NR	Sect. Lomandra ser. Lomandra	1B
sororia (F.Muell. ex Benth.) Ewart	Sect. Euxerotes, ser. Fasciculatae[1]	Group A	Sect. Lomandra ser. Sparsiflorae	2A
sp. Bamaga	NR	NR	NR	2A
sp. Stannary	NR	NR	NR	2A
sp. Watsonville	NR	NR	NR	2A
spartea (Endl.) Ewart	Sect. Schoenoxeros[1]	Group B	Sect. Lomandra ser. Lomandra	3C
spicata A.T.Lee	NR	Group B	Sect. Lomandra ser. Lomandra	4A
suaveolens (Endl.) Ewart	Sect. Cephalogyne[1]	Sect. Cephalogyne	Sect. Capitatae	1A
teres T.D.Macfarl.	NR	NR	Sect. Macrostachya	1B
tropica A.T.Lee	NR	NR	Sect. Capitatae	1A
whicherensis Keighery	NR	NR	NR	NR

[1] as synonym Xerotes

NR = Not represented

NA = Not accepted name in *Flora of Australia*

shrublands, subalpine woodland, and rainforest vegetation and includes widespread species (e.g. *L. filiformis* (Thunb.) Britten, *L. leucocephala* (R.Br.) Ewart), narrow-range endemics (e.g. *L. ordii* (F.Muell.) Schltr., *L. elongata* (Benth.) Ewart), and threatened species (e.g. *L. fluviatilis* (R.Br.) A.T.Lee). It is an economically, ecologically, and ethnobotanically significant genus in Australia that is in widespread use for horticulture, waste-water treatment, and stabilizing banks of waterways and roadsides (Conran 1998, Lismore City Council 2016).

LOMANDRA SYSTEMATICS AND TAXONOMY

Lomandra is placed in Asparagaceae subfamily Lomandroideae, supported by recent molecular phylogenetic evidence (Chen *et al.* 2013, APG IV 2016, Gunn *et al.* 2020). Generic boundaries in Lomandroideae have been informed by morphological and anatomical data (Fahn 1954, Rudall *et al.* 1997) and taxonomic clarification has been achieved through the circumscription of *Lomandra* section *Chamaexeros* (Benth.) Kuntze, and *L.* section *Acanthocarpus* (Lehm.) Kuntze as *Chamaexeros* Benth. and *Acanthocarpus* Lehm., respectively, and the transfer of *Lomandra papuana* Lauterb. into *Romnalda* P.Stevens (Stevens 1978, George 1986a). The monotypic genus *Xerolirion* (*Xerolirion divaricata* A.S.George) possesses similarities to *Lomandra* in that both are rhizomatous herbs with distichous leaves closely sheathing the main stem but was kept separate from *Lomandra* due to the divaricate habit, caducous leaves, terminal flowers, males in cymes, and females solitary (George 1986b). The nested

position of *Xerolirion divaricata* within *Lomandra* was identified by Donnon (2009) and confirmed by Gunn *et al.* (2020). But, *Xerolirion* nomenclature has not yet been revised.

Lomandra was first described by de Labillardière (1805). Delimitation of *Lomandra* species based solely on morphological characters is challenging. Bentham (1878) noted that widespread *Lomandra* (as *Xerotes* R.Br.) species, were often 'very variable and difficult to define' and accurate species delimitations were difficult due to 'difference[s] in habit, especially on the inflorescence, between the two sexes'. *Lomandra* contains four sections: *L.* section *Capitatae* (G.Don) A.T.Lee, *L.* section *Macrostachya* (Benth.) Engl., *L.* section *Typhopsis* (Benth.) Engl., and *L.* section *Lomandra*. Section *Lomandra* is the largest, containing 37 species divided into two series [*L.* section *L.* series *Lomandra* and *L.* section *L.* series *Sparsiflorae* (Benth.) A.T.Lee]. The remaining sections collectively comprise only 13 species (Table 1). Ten species remain unplaced at sectional rank.

Classification of *Lomandra* relies heavily on inflorescence characters, including arrangement (flowers single or in whorls) and complexity (unbranched or branched) (Bentham 1878, Stevens 1978, Lee and Macfarlane 1986). Inflorescence bract characters (presence, position, and apex) are considered informative for sectional placement (Lee and Macfarlane 1986). However, Stevens (1978) noted extensive variation of these bracts, referred to as 'cluster bracts' and 'bracteoles' (Stevens 1978) or 'cluster bracts', 'intermediary bracts', and 'outer or inner bracts' (Lee and Macfarlane 1986), which makes the identification of homologous structures challenging, particularly for species with reduced inflorescences. In this study we followed the terminology according to Lee and Macfarlane (1986). 'Cluster bracts' are prophylls subtending subunits of the inflorescence, 'intermediary bracts' subtend inflorescence branches, positioned between cluster bracts and bracts, and 'bracts' are associated with the flower and occur in pairs (inner and outer bracts) and are arranged in one of two ways: paired, opposite, and imbricate around the bud or one bract (outer) subtending the flower, and the other (inner) inside and lateral to the outer bract. Cluster bracts may be conspicuous only in the early stages of inflorescence development (e.g. *L.* section *Macrostachya*) or only in the basal floral clusters or lower nodes (e.g. *L. sericea* (Endl.) Ewart, *L. spartea* (Endl.) Ewart). Inflorescences may be arranged along the axis as individual-flowered per node (e.g. *L. filiformis*), multiple/clustered-flowered with two or more flowers (e.g. *L. multiflora*), or tightly clustered (e.g. *L. juncea*). As *Lomandra* is dioecious and often sexually dimorphic, many floral characters are unavailable for both staminate and pistillate plants, unless multiple samples are available. Difficulty in the accurate assessment of the homology of morphological characters in staminate and pistillate flowers adds complexity to the use of inflorescence characters for taxonomic circumscriptions.

Leaf, stem, and root anatomy have also been investigated in search of diagnostic morphological characters for *Lomandra* (Fahn 1954, 1961, Donnon 2009). Chanda and Ghosh (1976) proposed the exclusion of *L. micrantha* (Endl.) Ewart, *L. leucocephala* (R.Br.) Ewart subsp. *leucocephala*, and *L. endlicheri* (F.Muell.) J.F.Macbr. [and a close relationship of those taxa with *Aphyllanthes* L. (Asparagaceae: Aphyllanthoideae Lindl.)]. Stevens (1978) considered that relationships inferred based on vegetative, anatomical (Fahn 1954, 1961) and palynological (Chanda and Ghosh 1976) data were incongruent. To alleviate the challenges associated with the dependence on the use of reproductive characters for *Lomandra* species delimitation, Donnon (2009) investigated 26 leaf morphology and anatomy characters (e.g. leaf cuticle and mid-leaf cross-section). He combined morphological and molecular datasets and conducted phylogenetic analyses to infer relationships. He concluded that, while useful for species delimitation, leaf micro-morphological, flower and inflorescence characters were 'unreliable indicators' of relationships in *Lomandra* (Donnon 2009).

Understanding of *Lomandra* species relationships informed by phylogenetic analyses of DNA sequence data is limited. Gunn *et al.* (2020) inferred the relationships of *Lomandra* sections and series based on phylogenetic analyses of plastome data. Results of that study suggested that neither section *Lomandra* nor series within it, series *Lomandra* and series *Sparsiflorae*, were monophyletic. *Lomandra* sections were also non-monophyletic in phylogenetic analyses of plastid (*trn*L–F) and nuclear (Internal Transcribed Spacer, *ITS2*) data (Donnon 2009). Relationships inferred from phylogenetic analyses of morphological and genetic data were incongruent (Donnon 2009).

Two species complexes have historically been recognized within section *Lomandra* series *Sparsiflorae.* The first, *L. filiformis,* shows extensive variation in habit (tussocks sparse or forming dense mats), leaves (flat, folded, or inrolled; flexible, firm, or rigid), and inflorescences (various sizes, staminate inflorescences a raceme or panicle and pistillate inflorescences more or less reduced) (Lee 1962, Lee and Macfarlane 1986). *Lomandra filiformis* subspecies *filiformis*, subsp. *coriacea*, and subsp. *flavior* are more or less geographically distinct but can be difficult to distinguish (Lee and Macfarlane 1986; VicFlora, accessed 22 September 2022). Two additional Victorian entities, *L. filiformis* Grampians and *L. filiformis* Moggs Creek, tend morphologically towards *L. filiformis* subsp. *coriacea,* but with broader leaves and longer inflorescences. The second complex is that of *Lomandra. micrantha*, which comprises three subspecies (*micrantha*, *teretifolia*, and *tuberculata*). Conn and Quirico (1994) segregated part of *Lomandra micrantha* subsp. *tuberculata* Everett as a distinct species, *L. oreophila* Conn and Quirico, based on morphology. Morphology also indicates a close relationship of *L. oreophila* with *L. drummondii* (Conn and Quirico 1994). A well-resolved phylogeny will provide a context for assessing the monophyly of *Lomandra* species complexes and determining affinities of entities [e.g. *Lomandra filiformis* Grampians and *L. filiformis* Moggs Creek, *L.* sp. Bamaga (from Cape York), *L.* sp. Watsonville (from the Atherton Tablelands), and *L.* Stannary (from central Queensland)] that are currently recognized informally due to uncertainty around their relationships.

In *Lomandra*, accurate identification of species is important as many species [e.g. *L. fluviatilis* (R.Br.) A.T.Lee] and *L. longifolia* Labill. are used for ecological restoration (Cromer 2007, French 2010). Inability to accurately identify these taxa undermines the efficiency of restoration efforts, as incorrectly identified individuals may not provide the expected ecosystem services desired for restoration efforts. The genus includes multiple species that are the focus of monitoring and conservation efforts including *L. multiflora* subsp. *dura* (F.Muell.) T.D.Macfarl., which is a keystone species of iron-grass natural temperate grasslands in South Australia, an Australian Nationally Threatened Ecological

Community (Turner 2012). Greater understanding of *Lomandra* morphology is required for identification of morphological characters that distinguish infrageneric taxa or are informative for taxon identifications.

Cytotaxonomy, base chromosome number

Cytotaxonomic characters can be informative of lineage evolution and generic relationships (Pires *et al.* 2006, García *et al.* 2014). Chromosome counts are available for many *Lomandra* (Keighery 1984, Briggs 1986, Lee and Macfarlane 1986) but they have not yet been considered in a phylogenetic context. Lomandroideae genera exhibit a range of base chromosome numbers from $x = 4$ in *Sowerbaea* Sm. to $x = 11$ in *Cordyline* Comm. ex R.Br. and polyploidy is common and widespread. Genera in the sister clade to *Lomandra* (Gunn *et al.* 2020) have base numbers of $x = 8$ (*Acanthocarpus* Lehm. and *Romnalda* P.F.Stevens) and $x = 7$ (*Chamaexeros* Benth). Within *Lomandra*, two base chromosome numbers are documented: $x = 7$ for *Lomandra* section *Capitatae* (as syn. *Lomandra* section *Cephalogyne* Stevens) and $x = 8$ for *Lomandra* sections *Lomandra* and *Typhopsis* (Briggs 1986). Tetraploids are currently known from three of the four *Lomandra* sections, excluding section *Typhopsis*. Additionally, infraspecific polyploidy has been documented for multiple species, including *L. gracilis* (R.Br.) A.T.Lee ($2n = 16$, 32), *L. glauca* (R.Br.) Ewart ($2n = 14, 28$), *L. longifolia* (2n = 16, 32), and *L. leucocephala* ($2n = 16$, 24–28) (Briggs 1986).

Ecology

Lomandra is a widespread lineage that occupies diverse habitats in Australia. Most species occupy sclerophyll woodland, shrubland, or forest; although exceptions include *L. spicata* and *L. laxa* (R.Br.) A.T.Lee, which are found in tropical rainforests in northern Queensland, *L. patens* A.T.Lee, which grows on rocky hills or ranges, and a small number of species e.g. *L. fluviatilis*, *L. hystrix*, and *L. ordii* that are found in riparian vegetation. Multiple taxa exhibit east-west disjunct distributions e.g. *L. collina*, *L. effusa* (Lindl.) Ewart, and *L. micrantha* (Endl.) Ewart subsp. *micrantha* and multiple eastern Australian taxa have north-south disjunctions, e.g. *L. confertifolia* and *L. hystrix*. Reconstruction of the *Lomandra* phylogeny would enable investigation of vegetation occupancy patterns and evolutionary transitions in vegetation occupancy across the lineage.

In this study we used genomic data from the plastome to resolve the phylogenetic relationships of *Lomandra* to:

i) test the monophyly of *Lomandra* infrageneric taxa and species complexes and place informally recognized taxa in a phylogenetic context;
ii) investigate the morphological characters that characterize *Lomandra* clades; and
iii) investigate the evolutionary histories of base chromosome number, occupancy of ecological regions, and vegetation types.

MATERIALS AND METHODS

Taxon sampling

Taxon sampling was based on species of *Lomandra* recognized in the *Flora of Australia* (Lee and Macfarlane 1986) and the online Australian Plant Census (Council of Heads of Australasian Herbaria, 2006). The plastome dataset comprised 53 individuals of *Lomandra* (representing 45 species or subspecies and eight informally recognized entities), *Xerolirion divaricata*, 21 additional individuals of Lomandroideae (representing 19 species and two informally recognized entities) to assess the monophyly of *Lomandra*, plus one asparagoid and three nolinoid species as outgroups giving a total of 79 individuals. Comprehensive species coverage of sections and series was achieved as follows: *L.* section *Macrostachya* (2 of 2 species), *L.* section *Capitatae* (7 of 9 species), section *Typhopsis* (1 of 2 species), section *Lomandra* series *Lomandra* (11 of 14 species), and section *Lomandra* series *Sparsiflorae* (18 of 23 species) (Table 1). Taxon names, voucher information, and accession numbers are provided in Table 2.

DNA isolation and quantification

Genomic DNA was isolated from silica-dried leaf material or from material sampled (with permission) from dried herbarium specimens. DNA extractions were carried out using the Qiagen DNeasy Plant Mini-kit (Valencia, CA, USA) following the manufacturer's protocol with minor modifications as per (Gunn *et al.* 2020). Total genomic DNA was quantified using the Invitrogen Qubit Fluorometric Quantification (Carlsbad, CA, USA) assay to prepare the library for downstream whole genome skimming.

High-throughput sequencing library preparation

For each sample, ~3000 ng of genomic DNA was sheared in a Covaris S220 sonicator (Woburn, MA, USA) to obtain 500–600 bp fragments. Library construction for genome skimming for Illumina high-throughput sequencing followed protocols adapted from Gnirke *et al.* (2009), Fisher *et al.* (2011), Faircloth *et al.* (2012), Rohland and Reich (2012), and especially Schuster *et al.* (2018). DNA fragments were purified using Solid Phase Reversible Immobilization (SPRI) magnetic beads coated with carboxyl. Fragments of target size (500–600 bp) were blunt-end repaired and ligated with phosphothioate linkage-protected hairpin adapters with 6 bp barcodes (Rohland and Reich 2012). The yield of the size-captured fragments was increased by amplifying the adaptor-ligated fragments with KAPA Hi-Fi PCR kit (Kapa Biosystems, Cape Town, South Africa) using unique paired indices (TruSeq compatible) as primers. We carried out real-time PCR using the Bio-Rad CFX quantitative PCR instrument to identify the number of cycles for which exponential amplification of the templates occurred (Cq-value). Equimolar volumes of the indexed enriched samples were pooled and SPRI bead purified. Fragment size distributions were quantified using the Agilent Tape Station. High-throughput paired-end sequencing was performed with 150 bp paired-end reads loaded onto a single lane of the Illumina NextSeq 500 platform.

Plastome assembly

The paired-end sequence reads were de-multiplexed and quality control reports of the sequences were provided using GVL v.4.0.0 (Genomics Virtual Laboratory, Melbourne Bioinformatics, Australia). Geneious Prime v. 2019.2.1 (Biomatters Ltd, Auckland, New Zealand) and *the BBDuk v.37.25 plugin implemented in BBMap* (Bushnell 2014) were used to group the paired-end reads. Low quality (below Phred 20) bases at both ends of reads and reads with lengths <50 bp were removed. The

Table 2. Taxon name, herbarium specimen voucher and collection data, and GenBank accession numbers for taxa and individuals included in this study.

Ingroup taxa	Collector	Collection no.	Locality	Collection date	Herbarium voucher	GenBank No.
Lomandra banksii (R.Br.) Lauterb.	Crisp, M.D.	Crisp 10269	Cook, QLD.	11/11/06	CANB 743422.1	OL938759
Lomandra bracteata A.T.Lee	Crawford, I.	3474	Cooma-Monaro, NSW.	8/12/95	CBG 9611226.1	OR241497
Lomandra brevis A.T.Lee	Robertson, D.R.	s.n.	Blue Mountains, NSW.	10/2/05	NSW 793883	OR398310
Lomandra caespitosa (F.Muell. ex Benth.) Ewart	Macfarlane, T.D.	TDM 1218	Kwinana, Fremantle, WA.	13/8/83	PERTH 1991582	OR241498
Lomandra collina (R.Br.) Ewart	Crisp, M.D.	Crisp 10661	Marino Rocks, Marino Cons. Res., SA.	31/12/09	CANB 786044.1	OR241509
Lomandra cf conferfolia (F.M.Bailey) Fahn *(scrambling)*	Gunn, B.	BG 1289	Noosa National Park, Sunshine Coast, QLD.	14/12/17	MEL	OR241500
Lomandra confertifolia (F.M.Bailey) Fahn subsp. *confertifolia*	Crisp, M.D.	Crisp 9817	Cultiv. at the Aust. Nat. Bot. Gdns., ACT.	NA	CBG 770207	OL938760
Lomandra confertifolia (F.M.Bailey) Fahn (glaucous)	Walsh, N.G.	NG 8750	Walshs Pyramid, Wooroonooran NP. QLD.	11/12/17	MEL 2495017A	OR241499
Lomandra *confertifolia* subsp. *pallida* A.T.Lee	Crisp, M.D.	Crisp 10181	Carnarvon Gorge, Central Highlands, QLD.	8/9/06	CANB 743334.2	OR241501
Lomandra densiflora J..M.Black	Crisp, M.D.	Crisp 10663	Marino Rocks, Marino Cons. Reserve, SA.	31/12/09	CANB 786046.1	OR241502
Lomandra drummondii (Benth.) Ewart	Macfarlane, T.D.	TDM 6676	Vasse Highway, Busselton, WA.	26/10/17	PERTH 09491708	OR241503
Lomandra effusa (Lindl.) Ewart	Crisp, M.D.	Crisp 10467	Yathong, Cobar, NSW.	2/7/09	CANB 785434.1	OL938761
Lomandra fibrata J.M.Black	Crisp, M.D.	Crisp 10658	Mt Lofty summit, Adelaide Hills, SA.	30/12/09	CANB 786041.1	OR241505
Lomandra filiformis (Thunb.) Britten subsp. *coriacea* A.T.Lee	Gunn, B.	BG 1174	Arthurs Creek, Nillumbik, VIC.	23/3/17	MEL 2450921A	OR241496
Lomandra filiformis (Thunb.) Britten Grampians	Walsh, N.G.	Walsh 8324	Rose Track, Grampians, VIC.	12/10/15	MEL 2392583	OR398312
Lomandra filiformis (Thunb.) Britten Moggs Creek	Walsh, N.G.	Walsh 8810	Old Neuk Rd, Moggs Creek, VIC.	3/5/18	MEL 2450908A	OR241506
Lomandra filiformis (Thunb.) Britten subsp. *filiformis*	Crisp, M.D.	Crisp 10677	Mallacoota Inlet, East Gippsland, VIC.	16/5/10	CANB 790483.1	OR241495
Lomandra filiformis (Thunb.) Britten subsp. *filiformis*	Crisp, M.D.	Crisp 10481	Wondul National Park, Toowoomba, QLD.	21/8/09	CANB 785446.1	OR241504
Lomandra aff. glauca (R.Br.) Ewart *sp. nov*.	Copeland, L.M.	3550	Rock of Gibraltar, Tenterfield, NSW.	18/1/03	AD 162661	OR241508
Lomandra glauca (R.Br.) Ewart	Crisp, M.D.	Crisp 9857	Jervis Bay, Act.	13/8/05	CANB 669157.1	OR241507

Table 2. Continued

Ingroup taxa	Collector	Collection no.	Locality	Collection date	Herbarium voucher	GenBank No.
Lomandra hastilis (R.Br.) Ewart	Crisp, M.D.	Crisp 10866	Irwin, WA.	25/9/10	CANB 794159.1	OL938762
Lomandra hermaphrodita (C.R.P.Andrews) C.Gardner	Crisp, M.D.	Crisp 10936	Norwood Reserve, Kalamunda, WA.	2/10/10	CANB 794229.1	OR241533
Lomandra hystrix (R.Br.) L.R.Fraser & Vickery	Gunn, B.	BG 1266	Mt. Lewis National Park, Cairns, QLD.	8/12/17	MEL 2494956A	OR241494
Lomandra juncea (F. Muell.) Ewart	Walsh, N.G.	Walsh 7351	Little Desert Nat. Park, West Wimmera, VIC.	9/4/11	MEL 2346385A	OL938763
Lomandra laxa (R.Br.) A.T.Lee	Crisp, M.D.	Crisp 10447	White Rock Conservation Park, QLD.	31/7/08	CANB 785422.1	OR241510
Lomandra longifolia Labill.	Crisp, M.D.	Crisp 10216	Atherton Tablelands, QLD.	13/9/06	CANB 743369.1	OL938764
Lomandra longifolia subsp. *exilis* A.T. Lee	Crisp, M.D.	Crisp 10678	Mallacoota Inlet, East Gippsland, VIC.	16/5/10	CANB 790484.1	OR398311
Lomandra marginata T.D.Macfarl. & Conran	Macfarlane, T.D.	TDM 6596	Rothsay, WA.	6/10/67	PERTH 09491716	OR241511
Lomandra maritima T.S.Choo	Choo, R.	68122	Cambridge, WA.	6/10/67	PERTH 01442147	OR241512
Lomandra micrantha (Endl.) Ewart subsp. *micrantha*	Macfarlane, T.D.	TDM 6670	Preston Beach, WA.	26/10/17	PERTH 09491686	OL938765
Lomandra micrantha subsp. t*eretifolia* Everett	Crisp, M.D.	Crisp 9901	Esperance, Mt. Ragged, WA.	10/9/05	CANB 673406.1	OR241513
Lomandra micrantha subsp. *tuberculata* Everett	Crisp, M.D.	Crisp 10662	Marino Rocks, Marino Cons. Reserve, SA.	31/12/09	CANB 786045.1	OR241514
Lomandra mucronata (R.Br.) A.T.Lee	Crisp, M.D.	Crisp 11049	Esperance (S), WA.	11/10/10	CANB 794344.1	OL938766
Lomandra multiflora subsp. *dura* (F.Muell.) T.D.Macfarl.	Crisp, M.D.	Crisp 10659	Mt Lofty summit, Adelaide Hills, SA	30/12/09	CANB 786042.1	OR241515
Lomandra nigricans T.D.Macfarl.	Macfarlane, T.D.	TDM 6694	Little Mount Lindesay, Denmark, WA.	27/10/17	PERTH 09491759	OR241516
Lomandra obliqua (Thunb.) J.F.Macbr.	Crisp, M.D.	Crisp 10180	Carnarvon Gorge, Central Highlands, QLD.	8/9/06	CANB 743333.1	OR241517
Lomandra ordii (F.Muell.) Ewart	Macfarlane, T.D.	TDM 6685	Inlet River, Walpole, WA.	27/10/17	PERTH 09491740	OR241518
Lomandra oreophila B.J.Conn & Quirico	Walsh, N.G.	NG 8282	Mt. Buller, Alpine National Park, VIC.	20/9/15	MEL 2388679A	OR241519
Lomandra patens A.T.Lee	Crisp, M.D.	Crisp 10465	Yathong, Cobar, NSW.	1/7/09	CANB 785432.1	OL938767
Lomandra pauciflora (R.Br.) Ewart	Macfarlane, T.D.	TDM 6687	Inlet River, Walpole, WA.	27/10/17	PERTH 09491732	OR241520
Lomandra preissii (Endl.) Ewart	Macfarlane, T.D.	TDM 6665	Cataby, WA.	25/10/17	PERTH 09491767	OR241521
Lomandra rupestris (Endl.) Ewart	Crisp, M.D.	Crisp 10997	Albany, Pallinup River, WA.	6/10/10	CANB 794290.1	OR241522

Table 2. Continued

Ingroup taxa	Collector	Collection no.	Locality	Collection date	Herbarium voucher	GenBank No.
Lomandra sericea (Endl.) Ewart	Macfarlane, T.D.	TDM 6646	Regans Ford, WA.	25/10/17	PERTH 09491775	OR241523
Lomandra sonderi (F.Muell.) Ewart	Hortin, CA	2036	Goode Beach, Mistaken Island, WA.	8/10/04	PERTH 6910351	OR241524
Lomandra sororia (F.Muell. ex Benth.) Ewart	Walsh, N.G.	NG 8310	Pyrenees, Ben Major Nature Reserve, VIC.	12/10/15	MEL 2392570A	OR241525
Lomandra sp. Bamaga	Gray, B.	BGray 9947	Cultiv (Bruce Gray's Gdn.), Atherton, QLD.	12/12/17	MEL 2495057A	OR241526
Lomandra sp. Stannary Hill	Gray, B.	BGray 9246	Cultiv (Bruce Gray's Gdn.), Atherton, QLD.	12/12/17	MEL 2495056A	OR241527
Lomandra sp. Watsonville	Gray, B.	BGray 9948	Cultiv (Bruce Gray's Gdn.), Atherton, QLD.	12/12/17	MEL 2495058A	OR241528
Lomandra spartea (Endl.) Ewart	Macfarlane, T.D.	TDM 6630	Karagullen, WA.	24/10/17	PERTH 09491783	OR241529
Lomandra spicata A.T.Lee	Foreman	2113	Bellingen, NSW.	17/2/98	MEL 2044074A	OL938768
Lomandra suaveolens (Endl.) Ewart	Macfarlane, T.D.	TDM 6671	Preston Beach, WA.	26/10/17	PERTH 09491678	OR241530
Lomandra teres T.D.Macfarl.	Ballingall, ME	2652	Salvator Rosa NP., Central Highlands, QLD.	18/9/90	BRI AQ0501134	OR241531
Lomandra tropica A.T.Lee	Crisp, M.D.	Crisp 10416	Mt Elizabeth Stn., Wyndham-E Kimberley, WA.	11/10/07	CANB 760050.1	OR241532
Acanthocarpus canaliculatus A.S.George	Philips, LA	73	Dowerin (S), WA.	27/3/08	PERTH 7790716	OL938718
Acanthocarpus humilis A.S.George	Markey, A	1894	Shark Bay (S), WA.	4/10/97	PERTH 5270340	OL938719
Acanthocarpus sp. Ajana (C.A.Gardner 8596)	Keighery, G.J.	1467	Northampton (S), WA.	27/7/08	PERTH 8509409	OL938724
Acanthocarpus sp. Cooloomia (S.D.Hopper 3301)	Hislop, M.	3451	Northampton (S), WA.	7/6/05	PERTH 7293208	OL938725
Acanthocarpus verticillatus A.S.George	Godfrey, N.	NG 143/15	Ashburton (S), WA.	29/7/15	PERTH 08752990	OL938726
Arthropodium cirratum (G.Forst.) R.Br.	Birch, J.L.	863	Cult., Royal Botanic Gardens VIC.	30/10/13	MEL 2377039A	OL938727
Arthropodium curvipes S.Moore	Gibson, N.	5114	Coolgardie (S), WA.	3/9/11	PERTH 8503222	OL938728
Arthropodium strictum R.Br.	Gunn, B.	BG1305	Cultivated, Royal Botanic Gardens VIC.	14/2/17	MEL	OL938734
Chamaexeros longicaulis T.D.Macfarl.	Middleton, E.D.	EDM 411	Manjimup (S), WA.	1/9/01	PERTH 6575757	OL938735
Chamaexeros macranthera Kuchel	Smith, B. H.	1335	Mount Marshall (S), WA.	9/3/90	MEL 2016942A	OL938736
Cordyline indivisa (G.Forst.) Endl.						NC035998
Cordyline manners-suttoniae F.Muell.	Gunn, B.	BG 1274	Atherton Tablelands, QLD.	12/12/17	MEL 2494963A	OL938740
Eustrephus latifolius R.Br.						NC025305

Table 2. Continued

Ingroup taxa	Collector	Collection no.	Locality	Collection date	Herbarium voucher	GenBank No.
Laxmannia gracilis R.Br.	Forster, P.I.	PIF43697	North Burnett (R), QLD.	30/12/15	MEL 2412901A	OL938749
Laxmannia orientalis Keighery	Stasjsic, V.	7507	Beaumaris, VIC.	10/4/15	MEL 2393413A	OL938753
Romnalda grallata R.J.F.Hend.	Forster, P.I.	27701	Atherton Tablelands, QLD.	1/11/01	MEL 2281957A	OL938769
Romnalda ophiopogonoides Conran, P.I.Forst. & Donnon	Zich, F.A.	639	Cairns, QLD.	9/12/09	CNS 130812.1	OL938770
Romnalda strobilacea R.J.F.Hend. & Sharpe	Forster, P.I.	PIF41722	Sunshine Coast (R), QLD.	25/11/14	BRI AQ 837680	OL938771
Sowerbaea laxiflora Lindl.	Herbarium W.A.	WAH 85	Woodanilling (S), WA.	16/10/12	PERTH 8571503	OL938774
Thysanotus multiflorus R.Br.	Macfarlane, T.D.	TDM 6608	Byford, WA.	23/10/17	PERTH 09491724	OL938780
Thysanotus patersonii R.Br.	Gunn, B.	BG1233	Strathbogie (S), VIC.	13/10/17	MEL 2416801A	OL938781
Xerolirion divaricata A.S.George	Markey, A.	3834	Blue Hills Range, Perenjori, WA.	18/9/05	PERTH 7454856	OL938786
OUTGROUP TAXA ((Asparagaceae subf. Nolinoideae)						
Asparagus officinalis L.						NC034777.1
Maianthemum bicolor (Nakai) Cubey						NC035970.1
Polygonatum stenophyllum Maxim.						NC035995.1
Polygonatum verticillatum (L.) All.						NC028523.1

reads were error-corrected and normalized with target coverage level of 30 and minimum depth of 6 (kmer depth) using BBNorm v.37.25 plugin (Bushnell 2014).

De novo assemblies were performed using CLC Genomic Workbench v.10.0.1 (CLC bio, Aarhus, Denmark) and Geneious Prime v.2019.2.1. Using Geneious Prime, contigs were mapped to a plastome of *Lomandra* assembled in this study (*L. micrantha* subsp. *micrantha*) or to a published reference plastome of a closely related Asparagales taxon: *Agave attenuata* Salm-Dyck (NC032696) downloaded from GenBank. A consensus sequence was generated and the inverted repeat regions (IR) locations were identified using the 'Find Repeats' plugin (for perfect repeats > 70 bp), following the method outlined by Gibbs (2016) using Geneious Prime v.2019.2. In most cases, a single complete copy of the IR (*c.* 25 000 bp) and the truncated ends of a second IR were recovered. The truncated ends of the second IR and any flanking terminal sequence were deleted. To reconstruct a circular plastome genome, the single complete IR region was extracted, reverse complemented, and saved to serve as the second IR region. The consensus sequence (with truncated ends removed) and the inverted second IR region were then concatenated and circularized to generate the draft *de novo*-derived consensus sequence. The error-corrected and normalized reads were then mapped back to the *de novo*-derived draft plastome for validation and the final consensus plastome sequence was constructed. Annotations were transferred from the reference sequence and verified using GeSeq (Tillich *et al.* 2017). One of the IRs was removed before alignment for phylogenetic analyses.

Plastome sequence alignment and phylogenetic analyses

The assembled plastomes ranged from 146 000 to 154 000 base pairs (bp) in length. Alignment was performed using MAFFT v.6.822 using the Galaxy High Performance Computing (HPC) Platform release v.21.09 (Afgan *et al.* 2018). The resulting alignment was imported into Geneious Prime and gaps with 50% missing sequence data were masked and trimmed. The alignment was processed through BMGE (Criscuolo and Gribaldo

2010) with default settings to remove divergent and ambiguously aligned regions.

Maximum likelihood analyses were carried out using IQTree v.2.1.2 (Nguyen *et al.* 2015, Chernomor *et al.* 2016). The best-fit model was selected using ModelFinder in IQTree with the AIC (Akaike information criterion) implemented (Kalyaanamoorthy *et al.* 2017). We obtained branch supports with the ultrafast bootstrap (UFbs) in IQTree (Hoang *et al.* 2017) executed on the Galaxy HPC Platform. The resulting maximum likelihood consensus tree was visualized in FigTree v.1.4.4 (Rambaut 2014).

MrBayes was run on The University of Melbourne HPC facility (Spartan), for 15 000 000 generations per run for four independent runs. The GTR + I + G model was chosen for the analysis with six rate categories as this model was closest to, although more complex than, the model selected to support maximum likelihood analyses. Convergence was reached when the average standard deviation of split frequencies across the two runs was < 0.01 and the Effective Sample Size values quantified using Tracer v.1.7.1 (Rambaut *et al.* 2018) were > 200. The 50% majority-rule consensus tree was generated in MrBayes after removal of 25% of trees that were generated during the burnin period. The tree was visualized in FigTree v.1.4.4 (Rambaut 2014). Posterior probabilities (PP) were calculated to estimate internal branch support of Bayesian inference phylogenetic reconstructions.

Morphology, cytology, and ecoregion occupancy

Morphological characters, chromosome counts, and ploidy determinations were obtained from published literature (Bentham 1878, Doley 1973, Keighery 1984 Briggs 1986, Lee and Macfarlane 1986, Conn and Quirico 1994, Tamura 1995, Macfarlane and Conran 2014). Chromosome counts and ploidy of 28 *Lomandra* species and subspecies were available for character state reconstruction.

Ancestral character states of base chromosome number (7, 8) were inferred from the Bayesian phylogeny trimmed to include only those taxa of *Lomandra* for which chromosome numbers are known (Doley 1973, Keighery 1984, Briggs 1986, Lee and Macfarlane 1986). Ancestral character states were reconstructed using a maximum likelihood method for binary discrete characters and visualized on a set of trees generated under stochastic character mapping using Markov Chain Monte Carlo process for generating 1000 trees of likely character histories conducted in the package 'phytools' v.1.0-3 (Revell 2012) in R v.3.5.3 (R Core Team 2019). We computed the AIC and AIC weights (AICw) tests to evaluate the best-fit model of evolution, whether transitions between states occurred at equal rates (ER) or at all different rates (ARD).

Taxa were assigned as occupying one or more of the Australian Terrestrial Ecoregion/s (Olson *et al.* 2001; https://www.dcceew.gov.au/sites/default/files/env/pages/5b3d2d31-2355-4b60-820c-e370572b2520/files/terrestrial-ecoregions.pdf), based on georeference data from the Atlas of Living Australia (https://www.ala.org.au, last accessed 1 November 2019). Taxon distributions were manually checked against those in the Flora of Australia (Lee and Macfarlane 1986). No georeference data were available for *Lomandra glauca* sp. nov. Therefore, this taxon was excluded from the dataset and the terminal was pruned from the phylogeny for subsequent analyses. Base chromosome number and occupation of Australian ecoregions were placed onto the terminals of the maximum likelihood tree using the 'ape' package v.5.6.1 (Paradis and Schliep 2019) in RStudio v.1.2.5042 (Posit team, 2022). Vegetation type occupancy of terminal taxa was obtained from the literature (Lee and Macfarlane 1986, Macfarlane and Conran 2014: Western Australian Herbarium 1998, accessed 22 September 2022; PlantNET, accessed 22 September 2022: VicFlora, accessed 22 September. 2022) and was coded according to a vegetation classification scheme derived, with modification, from Specht (1970) as 'grasslands', 'mallee', 'rainforest', and 'sclerophyll' (including 'forest', 'woodland', 'heathland', and 'shrubland'). This character was reconstructed onto the maximum likelihood phylogeny using the package 'ape' v.5.7 (Paradis and Schliep 2019) in RStudio 2022.12.0 + 353 (Posit team, 2022). We used a likelihood test between models to evaluate the best-fit model of evolution, whether transitions between states occurred at equal rates (ER), were symmetrical (SYM), or varied at all different rates (ARD).

RESULTS

Sequence data and tree reconstructions

The plastome data alignment was 101 907 bp and the resulting trimmed alignment included 25 259 parsimony-informative and 55 773 constant sites. The best-fit model was TVM + F + R4, which was selected based on lowest consistent AIC scores. The Bayesian inference and maximum likelihood analyses resulted in similar tree topologies with no differences in strongly supported relationships. The Bayesian inference phylogenetic tree of *Lomandra* is presented in Figure 2 and the maximum likelihood topology is available in the Supporting Information (Fig. S1).

Lomandra relationships

Lomandra is paraphyletic as *Xerolirion* is nested within it. *Lomandra* section *Capitatae* is monophyletic. *Lomandra* section *L.* series *Lomandra* and series *Sparsiflorae* are non-monophyletic. *Lomandra* comprises two major clades: the *Capitatae–Macrostachya* (CM) clade (100% UFbs: 1.00 PP) and the *Lomandra–Typhopsis* (LT) clade (100% UFbs: 1.00 PP) (Fig. 2), as discussed next.

Capitatae–Macrostachya (CM) Clade (13 spp.)

The CM clade of *Lomandra*, comprises all sampled taxa from *L.* section *Capitatae* (Clade 1A) and *L.* section *Macrostachya* (Clade 1B), as well as three species from *L.* section *L.* series *Lomandra*: *L. sonderi* (F. Muell.) Ewart, *L. ordii*, and *L. banksii* (Fig. 2). *Lomandra* section *Capitatae* is well supported as monophyletic in the Bayesian (1.00 PP), and maximum likelihood topologies (100% UFbs) [UFbs values of > 95% indicate strong support for a clade (Bui, Nguyen, and Von Haeseler 2013)]. *Lomandra* section *Macrostachya* is monophyletic (100% UFbs; 1.00 PP) and is sister to *Lomandra sonderi* (98% UFbs; 1.00 PP).

Lomandra–Typhopsis (LT) Clade (41 spp.)

The LT clade of *Lomandra* is highly speciose and contains: (i) the 'individual-flowered' *Lomandra* (section *Lomandra* series *Sparsiflorae* (2A–C, 3A)); (ii) the remaining 'multiple/clustered-flowered' *Lomandra* species [section *Lomandra* series

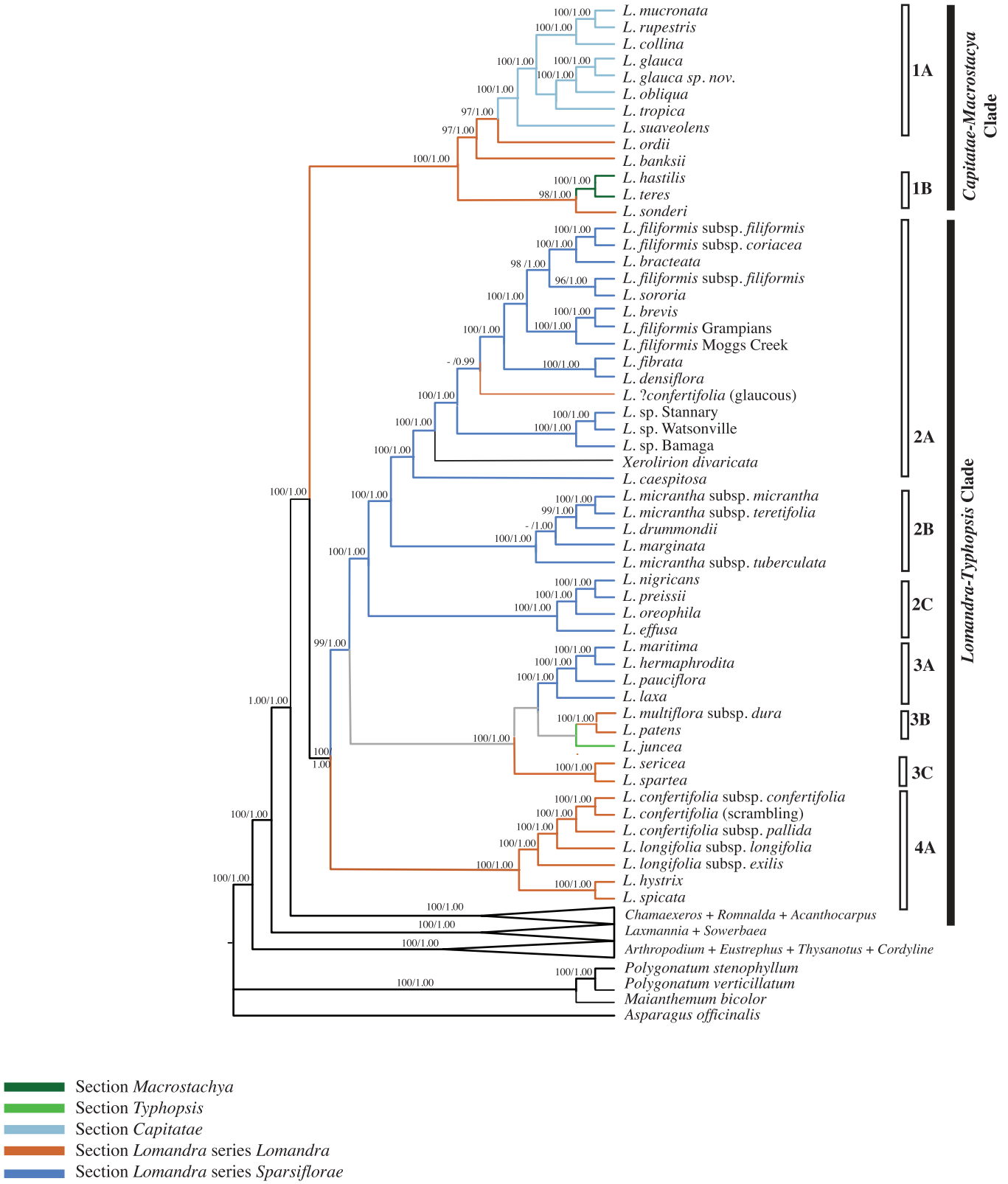


Figure 2. Bayesian 50% majority-rule consensus tree of *Lomandra* relationships inferred from the plastome dataset. Numbers above the branches are support values: ultrafast bootstrap (UFbs) values from the maximum likelihood topology and posterior probabilities (PP) of the Bayesian topology, respectively. Support values are provided only where either UFbs are ≥ 95% or PP are ≥ 0.95.

Lomandra (3B, 3C, 4A), section *Typhopsis*, and *X. divaricata*]. The monophyly of *Lomandra* section *Typhopsis* was not tested as it was represented by a single species [*L. juncea* (F.Muell.) Ewart].

Lomandra series *Sparsiflorae*: 'individual-flowered' clades

The species rich 'individual-flowered' *L.* section *Lomandra* series *Sparsiflorae* is represented here by 21 species and subspecies

plus five informally recognized taxa and those taxa are placed in four clades (clades 2A–C, and 3A). Clades 2A–C, referred to here as *Lomandra* series *Sparsiflorae s.s.*, contain most taxa in that series (100% UFbs: 1.00 PP). *Lomandra* section *Lomandra* series *Sparsiflorae* is non-monophyletic: *Xerolirion divaricata* is nested (100% UFbs: 1.00 PP) within it and *L. maritima*, *L. hermaphrodita*, *L. pauciflora*, and *L. laxa* (Clade 3A) are not closely related to other *Lomandra* series *Sparsiflorae s.s.* taxa. Neither *Lomandra micrantha* nor *L. filiformis* are monophyletic. Rather, *L. bracteata*, *L. sororia*, and *L. brevis* are each placed sister to *L. filiformis* individuals/clades (100% UFbs: 1.00 PP, 96 UFbs: 1.00 PP, 100% UFbs: 1.00 PP, respectively).

Lomandra series *Lomandra*: 'multiple/clustered -flowered' clades

The species rich 'multiple/clustered-flowered' *L.* section *Lomandra* series *Lomandra* is represented here by 13 species and subspecies plus two informally recognized taxa. Section *Lomandra* series *Lomandra* is non-monophyletic. The *Lomandra* series *Lomandra* species in Clade 4A are characterized by inflorescences with conspicuous and pungent cluster bracts (*L. confertifolia* subspp. *Confertifolia* and *pallida*, *L. longifolia* and *L. longifolia* subsp. *Exilis*, *L. hystrix*, and *L. spicata*) and are hereafter, referred to as section *Lomandra* series *Lomandra s.s.* (100% UFbs; 1.00 PP). *L. multiflora* subsp. *dura* is sister to *L. patens* (100% UFbs: 1.00 PP) in Clade 3B, *L. sericea* is sister to *L. spartea* (100% UFbs; 1.00 PP) in Clade 3C and *L. ordii*, *L. banksii*, and *L. sonderi* are placed the CM clade.

Evolution of base chromosome number

Within the CM clade, the base chromosome number of *L.* section *Capitatae* is $x = 7$ and among the remaining taxa for which this datum is known (*Lomandra ordii*, *L. hastilis* (R.Br.) Ewart, and *L. sonderi*) the base chromosome number is $x = 8$. The base chromosome number for all members of the LT clade is $x = 8$ with a single exception—that of *L. pauciflora*, which has a base chromosome number of $x = 7$. Ancestral reconstruction of base chromosome number estimated that the Most Recent Common Ancestor of *Lomandra* was $x = 8$ (> 95% probability) and that there were two independent transitions to $x = 7$ in *L.* section *Capitatae* and *L. pauciflora*, respectively (Fig. 3A). The ER was selected as the best-fit model (AIC = 21.074; AICw = 0.690) for the ancestral reconstruction of base chromosome numbers over the ARD (AIC = 22.666; AICw = 0.311). On average, trees had 2.53 changes between $x = 8$ and $x = 7$ from MCMC stochastic mapping across 1000 trees (Fig. 3, Table 3).

Ecological diversification

Most species in *Lomandra* section *Capitatae* and *L.* section *Lomandra* series *Sparsiflorae* occupy the Mediterranean ecoregion and more species are exclusive to that ecoregion than to any other (Fig. 4). Conversely, species of *L.* section *Lomandra* series *Lomandra* primarily occupy non-Mediterranean ecoregions, with only *L. spartea* and *L. sericea* (Clade 3C) occupying the Mediterranean ecoregion. A similar number of *Lomandra* species occupy the temperate (including Montane) and Tropical ecoregions and only *L. collina* and *L. patens* extend into the Desert/xeric shrubland ecoregion (Fig. 4). For reconstruction of vegetation type occupancy, the ARD (−lnL = 31.93) model was selected as the best-fit model using a likelihood test, over the ER model (−lnL = 40.77, d.f. = 11, $P = 0.09$) and the SYM (−lnL = 39.48, d.f. = 6, $P = 0.02$) models. Occupancy of 'sclerophyll' vegetation was reconstructed as ancestral for *Lomandra* (Fig. 6). Four transitions into occupancy of mallee vegetation, two independent transitions into grassland vegetation, two transitions, both from 'sclerophyll' ancestors, into rainforest vegetation were estimated (Fig. 6).

DISCUSSION

In this study, we sequenced and resolved phylogenetic relationships among 63% of *Lomandra* species and subspecies (Table 1) and used genome-scale plastome data to reconstruct evolutionary relationships within *Lomandra*. This study provides a phylogenetic framework to assess current taxon concepts, characterize morphological and ecological (ecoregion and vegetation type occupancy) diversity, and infer the evolutionary history of base chromosome number for the lineage.

Plastome data

Despite representing an entire genome, these data represent a single marker, as chloroplasts are uniparentally inherited and non-recombining (Wicke *et al.* 2011, but see also Gonçalves *et al.* 2019). This study represents a significant first step in erecting a phylogenetic hypothesis for a genus in which determination of relationships based on sequence data from a small number of plastome markers has previously had limited success (e.g. Donnon 2009). A full taxonomic revision of *Lomandra* will be improved by additional molecular data from the nuclear genome, along with morphological and ecological data to inform an accurate infrageneric classification. We used whole aligned plastomes, including non-coding regions, which has been shown in other studies (Parks, Cronn and Liston 2009, Givnish *et al.* 2018) to increase support for and resolution of phylogenetic relationships.

Relationships within *Lomandra*

This study inferred close relationships of *Lomandra* sections *Capitatae* and *Macrostachya* (CM clade) and of *L.* sections *Typhopsis* and *Lomandra* (LT clade). *Lomandra* section *Capitatae* was recovered as monophyletic, a result that is consistent with the uniform inflorescence morphology noted for taxa in that section (Stevens 1978). Our analyses indicated that a base chromosome number of $x = 7$ is synapomorphic for this clade. All *L.* section *Capitatae* taxa also have sexually dimorphic inflorescences with staminate flowers typically in whorls and pistillate plants having flowers in terminal head-like clusters. Cluster bracts are present (Stevens 1978).

All taxa in the CM clade share whorled inflorescence arrangement, opposite and imbricate floral bracts, and have either ellipsoid or campanulate flowers (Fig. 5). The close relationship of the CM clade taxa, including *L.* sections *Capitatae* and *Macrostachya* has, to our knowledge, not previously been inferred. *Lomandra banksii* and *L. ordii* were considered, based on morphological features, to be closely related to *L. multiflora* (placed in Clade 3B in these analyses) and *L. sonderi*, whose morphological affinities are unclear, had been placed as the first species of *L.* section *Lomandra* series *Lomandra* (Lee and

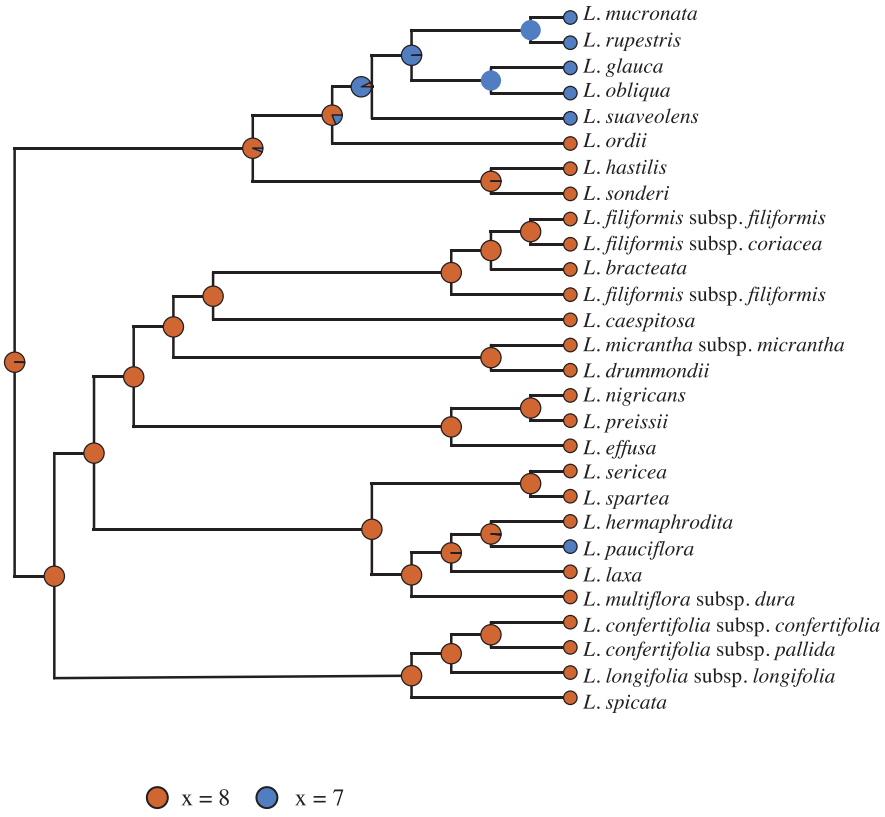


Figure 3. Ancestral state reconstructions of base chromosome numbers reconstructed using maximum likelihood (MCMC) method and the best-fit equal-rates model on the Bayesian 50% majority-rule consensus tree inferred from the plastome dataset trimmed to include only those taxa for which base chromosome number is known. Base chromosome number states were $x = 7$ (blue) and $x = 8$ (orange).

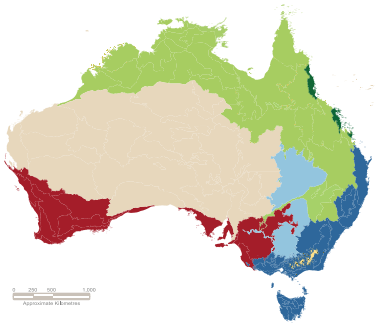
Table 3. Model selection for ancestral state reconstructions of chromosome numbers log-likelihoods using AIC and AICw between equal rates (ER) and ARD models.

Ancestral state reconstruction (maximum likelihood, Mk)	log-likelihood	Average changes between states on trees	X -> Y types of changes between states	AIC	AICw	LR	Pchisq
Chromosome base numbers			eight, seven -> seven, eight				
Equal-rates model (ER)	-9.537	2.53	2.18 -> 0.39	21.074	0.690	0.408	0.523
All-rates-different model (ARD)	-9.333	5.15	2.26-> 2.89	22.666	0.311		($P = 0.7698$)

P value is not significant for $P < 0.05$

Macfarlane 1986). Donnon (2009) also recovered *Lomandra banksii*, *L. ordii*, and *L. sonderi* in a clade with members of *L.* section *Capitatae* (and a small number of other taxa) *in* the Bayesian inference phylogeny inferred from combined plastid and nuclear sequence data. *Lomandra banskii*, *L. ordii*, and *L. sonderi* share inflorescence complexity (whorled branches and flowers in clusters or crowded) and floral bract arrangement with *L.* sections *Capitatae* and *Macrostachya* (Fig. 5.). A close relationship of *L. sonderi* and *L.* section *Macrostachya* is not evident based on morphology; the former has branched inflorescences in staminate plants and unbranched in pistillate plants, bearing ellipsoidal flowers that scarcely open and have united sepals and petals, while the latter has both staminate and pistillate plants with branched, narrowly cylindrical inflorescences, and campanulate flowers with free sepals and petals.

Clade 2A–C contains section *Lomandra* series *Sparsiflorae* along with *Xerolirion divaricata*. Member taxa have narrow leaves (< 5 mm wide), flowers that are arranged alternately on the rachis, floral bracts that do not completely enclose the flower or pedicel, and an inner floral bract that is lateral to the outer bract (Lee and Macfarlane 1986). *Xerolirion divaricata* shares these leaf and flower features but is otherwise morphologically distinct in Clade 2A; it has divaricate branching, reduced distichous leaves, and campanulate flowers and it is the only taxon in Clade 2A with sepals and petals that are united at the base. *Lomandra sororia* has previously been considered closely related to *L. pauciflora* (Lee and Macfarlane 1986): its placement in Clade 2A is consistent with floral morphology and suggests that morphologically similar *L. brittanii* Choo and *L. nutans* T.D.Macfarl., which were not represented in this study, may also be placed in



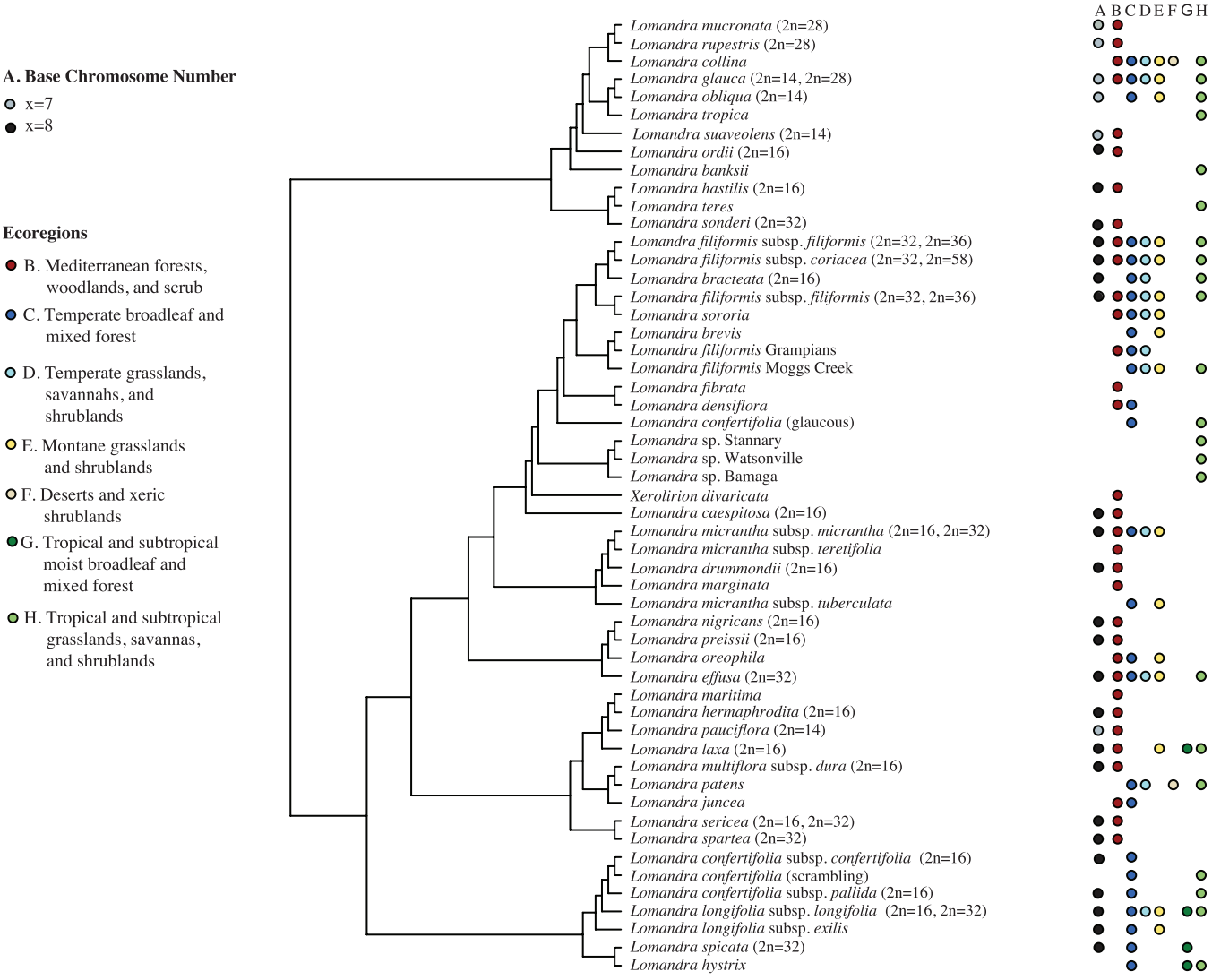
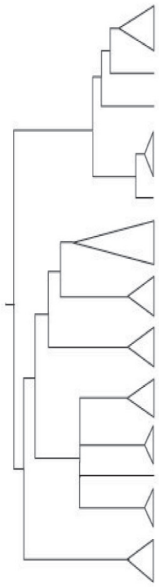


Figure 4. Base chromosome number and occupation of taxa in Australian ecoregions placed onto the terminals of the maximum likelihood tree inferred from the plastome dataset. Georeference data were not available for *L. glauca* sp. nov. and this terminal was removed from the dataset and pruned from the phylogeny. Chromosome counts (where available in the literature) are provided standardised as diploid (2n), following the taxon name. Character states are as follows: 1. Base chromosome number states were $x = 7$ (grey) and $x = 8$ (black). 2. Ecoregions were Mediterranean forests, woodlands, and scrub (maroon), Temperate broadleaf and mixed forest (dark blue), Temperate grasslands, savannas, and shrublands (turquoise), Montane grasslands and shrublands (yellow), Deserts and xeric shrublands (biege), Tropical and subtropical moist broadleaf and mixed forest (dark green), Tropical and subtropical grasslands, savannas, and shrublands (light green).

this clade. Clade 2C taxa are morphologically distinct from taxa in Clade 2A as they lack an inner floral bract (see Fig. 5). Based on morphological affinities, Western Australian taxa *L. purpurea* (Endl.) Ewart, *L. integra* T.D.Macfarl., and *L. odora* (Endl.) Ewart are likely placed in this clade. *Lomandra effusa* is morphologically distinct in Clade 2C, with strongly two-toothed leaves, flowers that are not clustered, and elongated sepals and petals in contrast to other taxa in Clade 2C, which have leaves with



Taxon	Leaf tip	Inflorescence arrangement	Male and female inflorescences	Intermediary bracts	Cluster bracts	Floral bracts	Sepals and petals size/shape	Sepals or petals fusion	Flower shape	Flower colour
1A	Acute, 1-toothed	**Male: whorled or alternate Female: whorled**[4]	**Different**	**Absent**	**Conspicuous**	**Opposite and imbricate**[8]	Similar	Staminate: United Pistillate: Free	**Ellipsoid**[11]	**Yellow or flushed with purple**
L. ordii	Rounded	**Whorled**	Similar[5]	Present	Inconspicuous	**Opposite and imbricate**[8]	Similar	United	**Campanulate**	White
L. banskii	Minutely 3-toothed	**Whorled**	Similar[5]	Present	Inconspicuous	**Opposite and imbricate**[8]	Different (sepals shorter)	United	**Campanulate**	White
1B	Rounded/ obtuse	**Whorled**	Similar[5]	Sometimes present	Inconspicuous[6]	**Opposite and imbricate**[8]	Similar	Free	**Campanulate**	Purple black
L. sonderi	Rounded	**Whorled**	Similar[5]	Present	Inconspicuous	**Opposite and imbricate**[8]	Similar	United	**Ellipsoid**	Cream
2A	Acute, 1–3 toothed[1]	**Alternate**	Similar[5]	**Absent**	Present or absent	**Inner lateral to outer**[9]	Different	**Free**	Globular[12]	Yellow[14]
2B	Rounded[2]	**Alternate**	Similar[5]	**Absent**	Present or absent	**Inner lateral to outer**[9]	Similar	**Free**	Campanulate	Greenish yellow flecked with dark red[15]
2C	Rounded[3]	**Alternate or whorled**	Similar[5]	**Absent**	Present or absent	**Inner typically absent**	Similar	**Free**[10]	Campanulate[13]	White with purple[16]
3A	Acute–rounded	**Alternate or whorled**	Similar[5]	**Absent**	**Absent**	Inner lateral to outer[9]	Different (sepals shorter, thinner)	Free	Globular	Yellow with purple sepals[17]
3B	Rounded	**Whorled**	Similar[5]	Present	**Inconspicuous**	Opposite and imbricate[9]	Similar	United	Campanulate	Yellow with purple sepals
L. juncea	Pungent	**Whorled**[4]	Similar[5]	Mass of crinkly hairs	**Inconspicuous**[6]	Modified, deeply fringed	Different	Sepals: free Petals united	Campanulate	White
3C	Rounded–pointed	**Whorled**	Similar[5]	Present	**Inconspicuous**[7]	Opposite and imbricate[9]	Similar	United	Ellipsoid	Purple
4A	**Acute, 2–3 toothed**	Whorled	Similar[5]	Present	**Conspicuous**	Opposite and imbricate[9]	Similar	Free[10]	Ellipsoid	Yellow or cream[18]

[1] except *Xerolirion divaricata* with obtuse-acute apex
[2] except *L. micrantha* subsp. *teretifolia* with sinuate apex and *L. marginata* with rounded or minutely 2–3 toothed apex
[3] except *L. gracilis* with long, acute apex and *L. effusa* with 2-toothed apex
[4] terminal head
[5] pistillate sometimes smaller or with reduced complexity
[6] except when young
[7] except at base of inflorescence or lower nodes only
[8] enclosing pedicel and flower base
[9] not enclosing pedicel and flower base
[10] sometimes united at base by filaments
[11] except *L. collina* and *L. nana* with globular flowers
[12] except *X. divaricata* and *L. densiflora* with campanulate flowers
[13] except *L. gracilis* with globular flowers
[14] except *X. divaricata* with white and *L. densiflora* with pale green flowers
[15] except *L. marginata* with white flowers
[16] except *L. purpurea* with purple and *L. gracilis* with yellow flowers
[17] except *L. nutans* and *L. whicherensis* with greenish-cream petals and red-flecked sepals
[18] except *L. confertifolia* subspecies *leptostachya* and *rubiginosa* with yellow petals and purple sepals

Figure 5. *Lomandra* leaf, inflorescence, and floral diversity relative to *Lomandra* clades or terminal taxa in the Bayesian 50% majority-rule consensus tree inferred from the plastome dataset. Text in bold shows morphological character states that characterise the taxa in that clade.

rounded apices, flowers in whorls, and short petals and sepals (Fig. 5).

Neither *Lomandra filiformis* nor *L. filiformis* subsp. *filiformis* are recovered as monophyletic, rather *Lomandra filiformis* individuals are sister to *L. bracteata*, *L. brevis*, or *L. sororia*. Lee (1966) considered both *Lomandra brevis* and *Lomandra bracteata* to be part of the *L. filiformis* complex. *Lomandra filiformis* and *L. brevis* share thin, inrolled leaves and a small narrow panicle and Lee (1966) noted that *L. brevis* 'superficially [..] resembles the smallest plants of *L. filiformis* subsp. *filiformis*'. However, our results indicate that *L. brevis* is sister to *L. filiformis* Grampians, which tends morphologically towards the larger and more robust *L. filiformis* subsp. *coriacea*. The placement of *Lomandra sororia* sister to *L. filiformis* was not anticipated due to the morphological differences of those species; *L. sororia* has typically unbranched staminate and pistillate inflorescences that are similar in size and complexity, while *L. filiformis* has branched staminate and pistillate inflorescences that differ in size and complexity. However, these species share globular to ellipsoidal male flowers with thick petals and shorter, thinner sepals. *Lomandra nutans* and *L. brittanii*, which were not represented in this study have similar flowers and may also be closely related to *L. filiformis*. Further taxonomic study and the inclusion of *L. filiformis* subsp. *flavior* A.T.Lee in the phylogeny is required to understand the morphological and genetic diversity of *L. filiformis*. The *Lomandra micrantha* species complex (Clade 2B) was also recovered as polyphyletic. Our results are consistent with Conn and Quirico (1994), who recognized *Lomandra oreophila* as a distinct species. The distant placement of *L. oreophila* (Clade 2C) relative to the *L. micrantha* Clade (Clade 2B) was unanticipated, and further investigation is warranted to identify morphological characters that might support the close relationship of *Lomandra oreophila* with *L. preissii* and *L. nigricans* that was inferred here.

Clade 3 is well supported and most members share whorled infloresences and cluster bracts that are inconspicuous or absent. Relationships among Clade 3 subclades (3A, 3B, and 3C) are poorly resolved. Within each subclade, taxa share morphological character states: Clade 3A taxa lack cluster bracts, possess an inner floral bract that is lateral to the outer bract, and have short, free sepals, and fleshy petals, species in Clades 3B and 3C have entire leaf apices, inconspicuous cluster bracts (at maturity or in all except the lower nodes), and sepals and petals that are basally united, and *L. juncea* has rigid leaves, pungent leaf apices, and modified floral bracts that are fringed (Fig. 5). Clade 3 taxa were recovered as a clade by Donnon (2009) in the Bayesian inference analysis of the combined chloroplast and nuclear dataset. However, that clade also included other taxa that were placed in distinct clades (*L. collina*, *L. confertifolia. sp. aff.*, *L. priessii*,

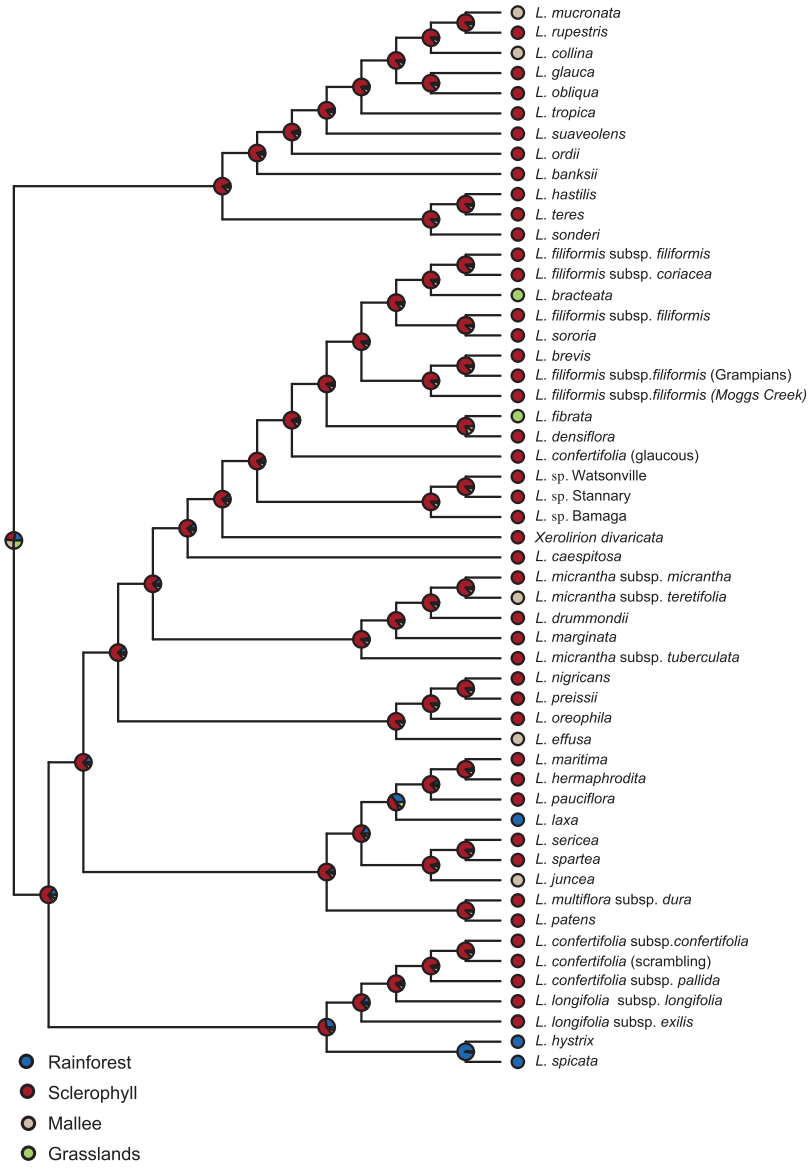


Figure 6. Ancestral state reconstruction of vegetation type occupancy onto the maximum likelihood tree inferred using the best-fit all-rates-different (ARD) model inferred from the plastome dataset. Vegetation type occupancy data were not available for *L. glauca* sp. nov. and this terminal was removed from the dataset and pruned from the phylogeny. Vegetation type was coded according to a vegetation classification scheme derived, with modification, from Specht (1970) as 'grasslands' (light green), 'mallee' (beige), 'rainforest' (dark blue), and 'sclerophyll' (including 'forest', 'woodland', 'heathland', and 'shrubland'; maroon).

L. suaveolens) or not represented (*L.leucocephala*, *L. multiflora* subsp. *multiflora*, *L. nutans*, *L. odora*) in this study. The sister lineage of *Lomanda juncea* remains equivocal. *Lomandra juncea* shares morphological features with taxa in each of the other Clade 3 subclades including different sepals and petals with taxa in Clade 3A, inconspicuous cluster bracts with taxa in Clades 3B and 3C, and campanulate flowers with taxa in Clade 3B. The presence of intermediary bracts that are a mass of crinkly hairs and deeply fringed floral bracts distinguish section *Typhopsis* taxa, including *L. juncea*.

Taxa within Clade 4A typically have a robust habit, broad leaves with toothed leaf apices, and conspicuous cluster bracts. Flowers have opposite and imbricate bracts, free sepals and petals, and the glossy, thin, and tough sepals are unique to this clade (Fig. 5). This clade contains taxa that were included in the traditional *L. longifolia* group (Lee 1962). Donnon (2009) also recovered a clade including *L. confertifolia*, *L. hystrix*, *L. longifolia*, and *L. spicata*, along with *L. fluviatilis*, *L. montana*, *L. rigida* (which were not represented in this study) and two unrelated species (*L. cylindrica* and *L. glauca*) in Bayesian inference analyses of the combined molecular and morphological dataset.

While revision of *Lomandra* subgenera and species is warranted, more data are required to inform such revisions.

Specifically, the inclusion of nuclear data would enable assessment of concordance among plastome and nuclear genomes for inference of *Lomandra* relationships. The expansion of *L.* section *Capitatae* to include *L.* section *Macrostachya* would achieve monophyly; a combination of morphological character states that characterize Clade 1 are identified here. Revision of *L.* sections *Lomandra* and *Typhopsis* are also warranted. One option would be to recognize Clades 2A–C plus *X. divaricata* as *L.* section *Lomandra* series *Sparsiflorae s.s.*, Clade 4A as series *Lomandra s.s*; a combination of morphological character states that characterize each of Clades 2 and 4 are identified here. Finally, Clade 3 plus *L. juncea* could potentially be recognized as a distinct series. However, taxonomic revision awaits inclusion of taxa that are currently considered to be closely related to Clade 3 taxa (e.g. *Lomandra* multiflora subsp. *multiflora* and *Lomandra leucocephala*) that were not represented in this study, resolution of the relationships among Clade 3 lineages, and further study to identify the morphological characters that characterize Clade 3.

Morphology of *Lomandra*

Habit and leaf characters

Habit is extremely labile in *Lomandra*; growth form has evolved multiple times in taxa placed in distinct sections and clades e.g., long-stemmed herbs with erect or decumbent woody stems (e.g. *L. banksii*) or vine-like with stems 3–4 m long (e.g. *L. insularis*), a scrambling or decumbent habit (e.g. *L. obliqua*; *L. confertifolia* subspp. *confertifolia* and *pallida*; *L. pauciflora*) and lawn-like rhizomatous colonies (e.g. *L. fibrata*). Leaf shape and venation characters for *Lomandra* do not appear to be informative of relationships. Donnon (2009) noted that while informative for taxonomic determinations, leaf cross-section was homoplasious, with multiple instances of sister taxa with distinct leaf cross-section state (e.g. flat leaves of *L. sericea* and terete leaves of *L. spartea*; see also Choo, (1969)); a conclusion supported by our phylogenetic analyses (Fig. 2, Clade 3C). The presence of conspicuous marginal bands on the leaf surfaces [e.g. *L. longifolia*, *L. drummondii* (F.Muell. ex Benth.) Ewart, *L. preissii*, and *L. oreophila*] also appears homoplasious. However, the presence of three-toothed leaf apices is a synapomorphy for Clade 4A (Fig. 2). In *L. hystrix* and *L. spicata* the middle tooth is prominent and the two laterals are much reduced while in *L. longifolia* and all *L. confertifolia* subspecies except *pallida* (teeth are equal in length), the middle tooth is reduced, and laterals are longer (Fig. 5).

Inflorescence characters

Inflorescence branching and arrangement of flowers are broadly informative of clades. Observed variation of inflorescence branching and arrangement of flowers in *L.* section *Sparsiflorae* is consistently partitioned into the clades that are recognized here (2A–C, 3A) so that taxa in each of these clades share a character state (Fig. 5). Within clades, sister species often vary in the extent of inflorescence branching (e.g. the spicate and paniculate inflorescences of *L. spicata* and *L. hystrix*, respectively). Condensed or head-like inflorescences have evolved multiple times, in distinct CM (*L.* section *Capitatae*) and LT (*L.* section *Typhopsis*) clades (Fig. 5).

Inflorescence bracts are informative of *Lomandra* relationships. Presence or absence of intermediary bracts is fairly consistent in the clades recognized here; intermediary bracts (subtending inflorescence branches) are lacking in *L.* section *Capitatae* (Clade 1A), Clades 2A–C, and in Clade 3A taxa (Fig. 5). As previously recognized by Lee and Macfarlane (1986), cluster bracts, those subtending subunits of the inflorescence, are also potentially informative; in the CM clade, they are conspicuous only in *L.* section *Capitatae* (Clade 1A) and in the LM clade, they are conspicuous only in Clade 4A, *L.* section *Lomandra* series *Lomandra s.s.*

Flower characters

Flower characters including the presence and type of floral bracts, sepal, and petal shape and fusion are broadly informative of relationships within *Lomandra*. Many *Lomandra* possess two opposite, imbricate floral bracts that enclose the flowers (Fig. 5), although these bracts may be reduced to hairs in *L.* section *Macrostachya* (Stevens 1978). Clades 2A–B and 3A possess an outer bract with an inner bract positioned laterally to it and in Clade 2C, the inner bract is reduced or has been lost entirely in some taxa (e.g. *L. preissii*). Sepals and petals are united for CM clade members (excluding Clade 1B) and are free for most LT clade members (excluding Clades 3B, 3C, and *L. juncea*) (Fig. 5). Flower shape and colour show significant lability; they are consistent within some clades and, conversely, may vary between closely related taxa e.g. *L. densiflora* J.Black and *L. fibrata* (although the female flowers in these two species are much more similar than the males) (Fig. 5).

Evolution of base chromosome number

A base chromosome number of $x = 8$ was inferred as ancestral for *Lomandra* (Fig. 3) with two independent transitions to $x = 7$ in *Lomandra* section *Capitatae* and in *L.* section *Lomandra* series *Sparsiflorae* (*L. pauciflora*). While the mechanism/s involved in the evolution of dysploidy in *Lomandra* are unknown, one possible mechanism could be Robertsonian fusion or fission (see Tamura 1995, Pires *et al.* 2006). Robertsonian rearrangements, have been documented for other Asparagales lineages, including Alliaceae Borkh., Iridaceae Juss., and Orchidaceae Juss. (Jones 1998). *Lomandra* chromosomes are small to medium sized and form a graded series in size (Doley 1973, Briggs 1986, Tamura 1995) noted that heterochromatic bands or constrictions were evident in some *Lomandra* chromosomes. However, the small size of many *Lomandra* chromosomes means their morphology and C-band patterns can be difficult to discern. Karyological characterization, potentially including chromosome painting using FISH techniques, and genome size estimations at population levels will be required to identify what mechanism(s) may have led to the loss of a single chromosome in the two *Lomandra* lineages with a base chromosome number of $x = 7$: in Clade 1A (*Lomandra* section *Capitatae*) and in Clade 3A (*L. pauciflora* belonging to section *Lomandra* series *Sparsiflorae*).

Polyploidy is common in *Lomandra* with approximately 23% of *Lomandra* taxa documented as tetraploids (Doley 1973, Keighery 1984, Briggs 1986). Chromosome counts for taxa that are not represented in this phylogeny, appear consistent with relationships inferred based on morphology, including for *L. integra* ($2n = 16$) and *L. odora* ($2n = 16$), which are morphologically similar to *L. nigricans* ($2n = 16$). Multiple *Lomandra* species are recorded as having both diploid and tetraploid individuals (Doley 1973; Briggs 1986; Keighery 1984). Briggs (1986) noted

that 'infraspecific polyploidy was found in *L. gracilis*, *L. glauca*, *L. longifolia* and probably *L. leucocephala* i.e. in over a third of the species sampled from more than a single site'. *Lomandra* species complexes with extensive morphological diversity e.g. *L. filiformis*, *L. micrantha*, and *L. longifolia* (Lee and Macfarlane 1986) appear to represent diploid-polyploid complexes (Fig. 4). Informally recognized entities may align with stable cytotypes [e.g. *L. longifolia* 'tufted' ($2n = 16$) and *L. longifolia* 'decumbent' ($2n = 32$) (Doley 1973)]. Alternatively, mixed-ploidy populations may be the result of recurring polyploidization events (Soltis and Soltis 1999; Duchoslav *et al.* 2020). The presence of *Lomandra filiformis* subsp. *coriacea* with tetraploid ($2n = 32$) individuals and an aneuploid heptaploid individual ($2n = 58$) suggests multiple polyploidization origins for that taxon. Distinct cytotypes can undergo different patterns of niche change. For example, in *Allium oleraceum* (Alliaceae) niche expansion and innovation was evident for tetraploids compared to triploids, while a trend of increasing unfilling of tetraploid niche was evident for higher ploidy levels (Duchoslav *et al.* 2020). Further documentation of ploidy for *Lomandra* diploid-polyploid complexes across their geographic ranges and quantification of cytotype frequencies, is warranted to quantify cytotype diversity within populations and would subsequently enable the investigation of potential habitat or niche differentiation among cytotypes in mixed-ploidy *Lomandra* populations.

Ecological diversification

The crown group of *Lomandra* is estimated to have diversified *c.* 24.5–9.70 Mya (95% highest posterior density; Gunn *et al.* 2020) during the increasing dry and cool conditions from the mid-Miocene onwards. These conditions were also accompanied by the contraction of rainforests and expansion of open forest and woodlands in Australia (Byrne *et al.* 2011). Most *Lomandra* species occupy sclerophyllous vegetation, including forests, woodlands, and heath, and this vegetation type is reconstructed as ancestral for the lineage. *Lomandra* possesses multiple traits that are considered adaptive to the typically low-nutrient and low-moisture conditions of sclerophyllous vegetation, including drought-tolerant coriaceous leaves and thickened epidermal cuticles (Donnon 2009). Transitions into other vegetation types have occurred in all clades and taxa occupying non-sclerophyllous vegetation are not clustered, indicating that multiple transitions into each of these other vegetation types have occurred during the evolution of *Lomandra*.

The largest number of *Lomandra* species occupy the Mediterranean ecoregion; 19 species are exclusively Mediterranean, six species occupy exclusively tropical ecoregions, and no species are exclusive to temperate ecoregions. Only two taxa occupy the Desert and xeric shrubland ecoregion (*L. collina*, *L. patens*) and neither are exclusive to that ecoregion. All *Lomandra* Clades (1–4) include widespread taxa that occupy multiple ecoregions. Gunn *et al.* (2020) demonstrated that polyploidy and biome occupancy transitions are correlated in Lomandroideae and suggested that polyploidy may generate novel phenotypes that can tolerate broader climatic ranges and soil types, which is potentially adaptive for expansion into different habitats. This may also be the case for *Lomandra*, with numerous examples of polyploid taxa that occupy multiple ecoregions (e.g. *L. effusa*, *L. longifolia*) sister to diploid taxa that occupy fewer ecoregions (e.g. *L.oreophila* + *L. preissii* + *L. nigricans*, *L. confertifolia*, respectively). The *Lomandra* species that occupy multiple ecoregions (four taxa for which chromosome counts are known occupy five or more ecoregions) are all polyploid taxa or are of mixed ploidy. Polyploidy may confer a selective advantage under changing environmental conditions and in marginal habitats with limited resources (Van De Peer *et al.* 2017), which would potentially be advantageous for establishment and persistence in novel and expanding habitats. Divergence dating analyses and reconstruction of ancestral areas for *Lomandra* would enable identification of the climatic and geological conditions that were in place during lineage diversification towards identification of the potential drivers of transitions among ecoregion or habitat occupancy in *Lomandra*.

CONCLUSIONS

This study is the first to provide a well-resolved phylogeny with extensive sampling of *Lomandra* species. *Lomandra* sections *Capitatae* and *Macrostachya* were recovered as monophyletic. Section *Lomandra* series *Sparsiflorae* and series *Lomandra* were not monophyletic. Relationships of *Lomandra* species were estimated, most of those were recovered with strong support, and the monophyly of *Lomandra* species complexes were assessed. Of the morphological characters assessed, inflorescence branching, arrangement of flowers, presence and arrangement of cluster and floral bracts, sepal and petal fusion, and flower shape serve, in combination to distinguish members of Clades 1–4. The study provides a valuable contribution towards understanding cytological evolution in *Lomandra*. A base chromosome number of $x = 8$ was inferred as ancestral for *Lomandra* and was identified as informative of relationships among the CM and LT clades. Base chromosome number ($x = 7$) is synapomorphic for Clade 1A whereas all members of the LT clade, with the exception of *Lomandra pauciflora*, have a base chromosome number of $x = 8$. *Lomandra* has a centre of species diversity in the Mediterranean ecoregion of Australia and sclerophyllous vegetation (including forests, woodlands, and heath) was reconstructed as ancestral for the lineage. *Lomandra* species have evolved to occupy multiple ecoregions and in doing so have adapted to the diverse climatic and soil environments within those ecoregions. Polyploidy is prevalent in *Lomandra*, which may have promoted diversification in ecoregion occupancy.

SUPPLEMENTARY DATA

Supplementary data are available at the *Botanical Journal of the Linnean Society* online.

ACKNOWLEDGEMENTS

We thank the Royal Botanic Gardens Victoria and The University of Melbourne for organisational support and the following herbaria for providing loans and plant material from herbarium vouchers for DNA extractions: AD, BRI, CANB, CNS, DNA, HO, NE, NSW, MEL, MELU, and PERTH. Many thanks to the Department of Biodiversity, Conservation, and Attractions, Western Australia, the Queensland Government, Environmental Protection Agency, Parks Victoria and the Department of Environment, Land, Water and Planning, Victoria for plant collecting permits. Thanks to Stuart Warboys (CNS), Bruce Gray, Enid Mayfield, Janet Dennis, and Suzy Duncan for fieldwork

assistance in Queensland. Chris French, Perth, provided access to his photographic collection. We thank Frank Zich and Katharina Nargar at the Australian Tropical Herbarium (CNS) and Gillian Brown at the Queensland Herbarium (BRI) for herbarium access. We thank S. Wilcox (Walter and Eliza Hall Institute, Melbourne) for assistance with Illumina sequencing, R. Fowler, T. McLay, T. Schuster, and M. Bayly (The University of Melbourne) for assistance with library preparation protocols, C. Jackson (Royal Botanic Gardens Victoria) for assistance with bioinformatics. High-Performance Computing facilities used included Spartan HPC at The University of Melbourne, Galaxy Australia, and CIPRES Science Gateway. Thanks to Brendan Lepschi and curation staff at CANB for hosting B. Gunn at the herbarium (2021–2023). This research was supported by The University of Melbourne's Research Computing Services and the Petascale Campus Initiative.

FUNDING

This research has been supported by the Australian Biological Resources Study, NTRGP grant no. RFL216-37 (PI: J.L. Birch and Co-PIs: D.J. Murphy, J.C. Pires, J.G. Conran, T.D. Macfarlane), Hermon Slade Foundation grant no. HSF 16/8 (J.L. Birch, D.J. Murphy, J.C. Pires, J.G. Conran), and the Australasian Systematic Botany Society, Marlies Eichler Postdoctoral Fellowship 2017–2018 (B. Gunn).

DATA AVAILABILITY

The DNA sequence data underlying this article are available in the GenBank Nucleotide Database and can be accessed with the accession numbers provided in Table 2.

REFERENCES

Afgan E, Baker D, Batut B *et al*. Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. *Nucleic Acids Research* 2018;**46**:W537–44.

APG IV. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society* 2016;**181**:1–20.

Australian Plant Census, IBIS Database. *Centre for Australian National Biodiversity Research, Council of Heads of Australasian Herbaria*, 2006. available online: https://biodiversity.org.au/nsl/services/search/taxonomy; viewed [19 Apr. 2023].

Bentham G. Juncaceae. In: Bentham G, ed. *Fl. Australiensis: a Description of the Plants of the Australian Territory VIII*. London: Reeve and Co., 1878:92–132.

Briggs B. Chromosome numbers in *Lomandra* (Dasypogonaceae). *Telopea* 1986;**2**:741–4.

Bui QM, Nguyen MAT, Von Haeseler A. Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution* 2013;**30**:1188–95.

Bushnell B. *BBMap: A Fast, Accurate, Splice-Aware Aligner*. 2014:1–2.

Byrne M, Steane DA, Joseph L *et al*. Decline of a biome: evolution, contraction, fragmentation, extinction and invasion of the Australian mesic zone biota. *Journal of Biogeography* 2011;**38**:1635–56.

Chanda S, Ghosh K. Pollen morphology and its evolutionary significance in the Xanthorrhoeaceae. In: Ferguson LK, Muller J, eds. *The Evolutionary Significance of the Exine*. London: Linn. Soc. Symp. Ser. 1 xii, 1976:527–559.

Chen S, Kim DK, Chase MW *et al*. Networks in a large-scale phylogenetic analysis: Reconstructing evolutionary history of Asparagales (Lilianae) based on four plastid genes. *PLoS ONE* 2013;**8**:e59472–18.

Chernomor O, von Haeseler A, Bui QM. Terrace aware data structure for phylogenomic inference from supermatrices. *Systematic Biology* 2016;**65**:997–1008.

Choo TS. A study of the Western Australian species of *Lomandra* Labill. (Xanthorrhoeaceae) with reference to their anatomy, taxonomy and phylogeny. *Unpublished M.Sc.Thesis*, Perth, WA: The University of Western Australia. 1969.

Conn BJ, Quirico AL. *Lomandra oreophila* (Lomandraceae) - a new species in the *L. micrantha* group. *Muelleria* 1994;**8**:123–32.

Conran JG. Lomandraceae. In: Kubitzki K, Huber H, Rudall PJ, Stevens PS, Stützel T, eds. *The Families and Genera of Vascular Plants. Volume 3. Flowering plants. Monocotyledons: Lilianae (except Orchidaceae)*. Berlin: Springer Verlag, 1998:354–365.

Criscuolo A, Gribaldo S. BMGE (Block Mapping and Gathering with Entropy): a new software for selection of phylogenetic informative regions from multiple sequence alignments. *BMC Evolutionary Biology* 2010;**10**:210–21.

Cromer EL. *Seed germination and research records from Alcoa's Marrinup Nursery*. Marrinup, Australia: ALCOA. 2007.

Doley JP. 1973. Sex ratios and their interpretation in Queensland angiosperms and gymnosperms. *Unpublished D. Phil. Thesis*, Brisbane, QLD: The University of Queensland.

Donnon MJ. Molecular systematics of the *Lomandra Labill*. complex (Asparagales: Laxmanniaceae). *Unpublished D. Phil. Thesis*, Adelaide, SA: The University of Adelaide. 2009.

Duchoslav M, Jandová M, Kobrlová L *et al*. Intricate distribution patterns of six cytotypes of *Allium oleraceum* at a continental scale: niche expansion and innovation followed by niche contraction with increasing ploidy level. *Frontiers in Plant Science* 2020;**11**:1–23.

Fahn A. The anatomical structure of the Xanthorrhoeaceae Dumort. *Botanical Journal of the Linnean Society* 1954;**55**:158–84.

Fahn A. The anatomical structure of Xanthorrhoeaceae Dumort and its taxonomic position. *Recent Advances in Botany* 1961;**1**:155–60.

Faircloth BC, McCormack JE, Crawford, Nicholas G, Harvey, MG, Brumfield RT, Glenn TC. Ultraconserved elements anchor thousands of genetic markers spanning multiple evolutionary timescales. *Systematic Biology* 2012;**61**:717–26.

Fisher S, Barry A, Abreu J *et al*. A scalable, fully automated process for construction of sequence-ready human exome targeted capture libraries. *Genome Biology* 2011;**12**:R1–15.

French K. *A framework to guide ecological restoration: coastal foredune scrub and temperate littoral rainforest*. Wollongong, NSW: South Coast of New South Wales, University of Wollongong. 2010.

García N, Meerow AW, Soltis DE *et al*. Testing deep reticulate evolution in Amaryllidaceae Tribe Hippeastreae (Asparagales) with *ITS* and chloroplast sequence data. *Systematic Botany* 2014;**39**:75–89.

George AS. *Chamaexeros*. In: George AS, ed. *Flora of Australia. Volume 46, Iridaceae to Dioscoreaceae*. Canberra: Australian Government Publishing Service, 1986a:90–91.

George AS. *Xerolirion*. In: George AS, ed. *Flora of Australia. Volume 46, Iridaceae to Dioscoreaceae*. Canberra: Australian Government Publishing Service, 1986b:98–99.

Gibbs MD. *De novo* assembly and reconstruction of complete circular chloroplast genomes using Geneious. Application Note, Geneious. 2016.

Givnish TJ, Zuluaga A, Spalink D *et al*. Monocot plastid phylogenomics, timeline, net rates of species diversification, the power of multi-gene analyses, and a functional model for the origin of monocots. *American Journal of Botany* 2018;**105**:1888–910.

Gnirke A, Melnikov A, Maguire J *et al*. Solution hybrid selection with ultra-long oligonucleotides for massively parallel targeted sequencing. *Nature Biotechnology* 2009;**27**:182–9.

Gonçalves DJP, Simpson BB, Ortiz EM *et al*. Incongruence between gene trees and species trees and phylogenetic signal variation in plastid genes. *Molecular Phylogenetics and Evolution* 2019;**138**:219–32.

Gunn BF, Murphy DJ, Walsh NG *et al*. Evolution of Lomandroideae: multiple origins of polyploidy and biome occupancy in Australia. *Molecular Phylogenetics and Evolution* 2020;**149**:1–16.

Hoang DT, Chernomor O, Haeseler AV *et al*. UFBoot2: improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution* 2017;**35**:518–22.

Jones K. Robertsonian fusion and centric fission in karyotype evolution of higher plants. *The Botanical Review* 1998;**64**:273–89.

Kalyaanamoorthy S, Minh BQ, Wong TKF *et al*. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* 2017;**14**:587–91.

Keighery GJ. Chromosome counts of Australian Liliaceae. *Feddes Repertorium* 1984;**95**:523–32.
Labillardiere JJ. *Novae Hollandiae Plantarum Specimen*. In: Huzard, D. ed. Tomus primus. Paris: ex typographiá. 1805.
Lee AT. Notes on *Lomandra* in New South Wales. *Contributions from the New South Wales National Herbarium* 1962;**3**:151–64.
Lee AT. Notes on *Lomandra* in New South Wales. *Contributions from the New South Wales National Herbarium* 1966;**4**:16–42.
Lee AT, Macfarlane TD. *Lomandra*. In: George AS, ed. *Flora of Australia, Volume 46, Iridaceae to Dioscoreaceae*. Canberra: Australian Government Publishing Service, 1986:100–141.
Lismore City Council. *Rural Landholder Initiative, Book 01: Healthy Landscapes and Waterways*. Lismore City Council, Lismore, NSW. 2016.
Macfarlane TD, Conran JG. *Lomandra marginata* (Asparagaceae), a shy-flowering new species from south-western Australia. *Australian Systematic Botany* 2014;**27**:421–6.
Nguyen LT, Schmidt HA, von Haeseler A *et al*. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* 2015;**32**:268–74.
Olson DM, Dinerstein E, Wikramanayake ED *et al*. Terrestrial ecoregions of the world: a new map of life on Earth. *Bioscience* 2001;**51**:933–8.
Paradis E, Schliep K. Ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* 2019;**35**:526–8.
Parks M, Cronn R, Liston A. Increasing phylogenetic resolution at low taxonomic levels using massively parallel sequencing of chloroplast genomes. *BMC Biology* 2009;**7**:84.
Pires JC, Sytsma KJ, Seberg O *et al*. Phylogeny, genome size, and chromosome evolution of Asparagales. *Aliso* 2006;**22**:287–304.
PlantNET (The NSW Plant Information Network System). 2022. *Royal Botanic Gardens and Domain Trust, Sydney*. Available online: https://plantnet.rbgsyd.nsw.gov.au. (accessed 22 September, 2022).
Posit team (2022). RStudio: Integrated Development Environment for R. Posit Software, PBC, Boston, MA. Available online: http://www.posit.co/.
R Core Team. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for statistical computing. 2019. Available online: (https://CRAN.r-project.org/).
Rambaut A. FigTree v.1.4.2, a graphical viewer of phylogenetic trees. 2014. Available online:(http://tree.bio.ed.ac.uk/software/figtree/).
Rambaut A, Drummond AJ, Xie D *et al*. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* 2018;**67**:901–4.
Revell LJ. phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* 2012;**3**:217–23.
Rohland N, Reich D. Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture. *Genome Research* 2012;**22**:939–46.
Rudall PJ, Furness CA, Chase MW *et al*. Microsporogenesis and pollen sulcus type in Asparagales (Lilianae). *Canadian Journal of Botany* 1997;**75**:408–30.
Schuster TM, Setaro SD, Tibbits JFG *et al*. Chloroplast variation is incongruent with classification of the Australian bloodwood eucalypts (genus *Corymbia*, family Myrtaceae). *PLoS ONE* 2018;**13**:e0195034–28.
Soltis DE, Soltis PS. Polyploidy: recurrent formation and genome evolution. *Trends in Ecology and Evolution* 1999;**14**:348–52.
Specht R. Vegetation. In: Leeper GW, ed. *The Australian Environment*. Melbourne: CSIRO-Melbourne University Press, 1970:44–67.
Stevens PF. Generic limits in Xeroteae. *Journal of the Arnold Arboretum* 1978;**59**:129–55.
Tamura MN. A karyological review of the orders Asparagales and Liliales (Monocotyledonae). *Feddes Repertorium* 1995;**106**:83–111.
Tillich M, Lehwark P, Pellizzer T *et al*. GeSeq – versatile and accurate annotation of organelle genomes. *Nucleic Acids Research* 2017;**45**:W6–W11.
Turner J. *National Recovery Plan for the Iron-grass Natural Temperate Grassland of South Australia ecological community 2012*. Adelaide. 2012. Available online: http://www.environment.gov.au/biodiversity/threatened/recovery-plans/national-recovery-plan-iron-grass-natural-temperate-grassland-sa.
Van de Peer Y, Mizrachi E, Marchal K. The evolutionary significance of polyploidy. *Nature Reviews Genetics* 2017;**18**:411–24.
VicFlora. *Flora of Victoria, Royal Botanic Gardens Victoria*. 2022. Available online: https://vicflora.rbg.vic.gov.au. (accessed on: 23 Jul. 2022 and 22 Sep. 2022).
Wicke S, Schneeweiss GM, dePamphilis CW *et al*. The evolution of the plastid chromosome in land plants: gene content, gene order, gene function. *Plant Molecular Biology* 2011;**76**:273–97.
Western Australian Herbarium. *Florabase––The Western Australian Flora*. Department of Biodiversity, Conservation and Attractions. 1998–. Available online: https://florabase.dpaw.wa.gov.au, (accessed on 22 Sep. 2022).